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EXPERIMENTAL PARTICLE RADIATION THERAPY IN ANIMAL NEOPLASIA

I. Electron versus roentgen irradiation

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During the past decade the possibilities of using various charged 'heavy' particles for radiation therapy of human neoplasms has been carefully considered by a few investigators having access to accelerators producing beams of either protons, deuterons, alpha particles or negative pi mesons (FALKMER et coll. 1959, 1962, FOWLER & PERKINS 1961, LIPPINCOTT et coll. 1963 a, b, LAWRENCE & TOBIAS 1965). Some modalities have actually been tried experimentaliy in animals and in a limited number of human cases. The noncharged fast neutron is also under careful investigation (FOWLER et coll. 1972). The reasons for comparing the therapeutic efficiency (RBE) of these particles with conventional electromagnetic radiation are based upon the theoretical advantages deriving from their physical characteristics that determine the mode of energy deposition (LET). These desiderata include (FALKMER et coll. 1959) relatively low entry dose as compared to peak dose at the site of the neoplasm (FALKMER et coll. 1962). Essentially uniform ionization may be obtained in a selected volume by

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utilizing a series of consecutive (Bragg) peaks, and (FOWLER & PERKINS 1961) control of depth dose with little forward or lateral scatter.

Historically, the deuteron (TOBIAS et coll. 1957) was the first of the heavy charged particles to be used in radiation therapy in animal neoplasia. This was followed by employment of the proton in both animals and man. From these investigations have come the most significant data thus far obtained (FALKMER et coll. 1959, 1962, TOBIAS et coll. 1957). The alpha particle has been used but sparingly in in vivo work while for the negative pi meson, as yet, no uncontaminated beam has been available. The slowness of progress in the development of this field has been chiefly due to the limitation of the available beams which were designed principally for experiments in physics and not for biomedical research. Currently, some of the new beam sources are much more readily adaptable to making possible well designed therapeutic trial programs in animal neoplasia which then may serve as suitable models for man. Notable examples are the recently developed monoenergetic alpha particle beam at the Space Radiation Effects Laboratory (in Virginia) and the negative pi meson source under construction at Los Alamos, New Mexico. Many of the previous disadvantages of the earlier accelerators will be overcome with these devices. For example, the greatest advantage of the new alpha particle beam is that the primary beam can be degraded to any energy, then refocused and analyzed before final focusing onto the target. Such a final relatively monoenergetic beam is preferable to those that have been used in previous biomedical experiments in which it was necessary to use an initial simple degraded beam with resultant heavy scatter and contamination by the degrader.

It is well recognized that in any scheme of radiation therapy for malignant neoplasms the maximum number of rad that can be delivered therapeutically to a neoplasm is determined to a great extent by the dose that the normal structures in the path of irradiation can tolerate. Usually the total dose required to effectively destroy a given malignant neoplasm cannot be administered as a single dose from any type of radiation because the normal structures cannot tolerate it. For this reason, various plans have been devised so that the desired total effective dose is obtained by giving fractions at selected intervals over a given period of time. Whereas, roentgen and gamma rays deposit energy in tissue in electron tracks in which the energy absorption events are, on the average, relatively far apart (low LET), in the case of 'heavy' charged particle radiation the absorption events are close together (high LET). High LET radiation is considered more effective against anoxic tumor cells than low LET radiation (BERRY & ANDREWS 1963, RAJU et coll. 1972).

The purpose of the present investigation is to compare the therapeutic efficiency in animal neoplasia of low energy electrons with that of roentgen irradia-

Table

Experimental particle radiation therapy in animal neoplasia. 1.0 MeV electron versus 250 keV roentgen irradiation. Transplanted C_3H/HeJ neoplasms in mice

Dose (rad)	1.0 MeV electron irradiation			250 keV roentgen irradiation		
	No. irradiated	No. with positive growth after irradiation	Percent with positive growth after irradiation	No. irradiated	No. with positive growth after irradiation	Percent with positive growth after irradiation
1000	11	11	100.0	—	—	—
1500	12	10	83.33	11	10	90.91
2000	11	5	45.45	10	9	90.0
2500	12	3	25.0	12	7	58.33
3000	11	4	36.36	11	8	72.73
3500	10	1	10.0	9	7	77.78
4000	13	1	7.69	10	6	60.0
4500	12	1	8.33	7	2	28.57
5000				11	3	27.27

tion. The model system consists of implanting a one mm cube of a 100 per cent transplantable mammary neoplasm into the thigh of the mouse at a selected depth and then determining with a range of single doses which form of radiation is more effective in completely destroying the neoplasm. The fall-off in energy deposition from roentgen irradiation is only about 10 per cent from surface to exit in the mouse leg, whereas by selecting the proper energy for the electrons the depth of energy deposition may be controlled. The disadvantage with roentgen irradiation in this situation is that a much larger volume of normal tissue is irradiated as compared to electron irradiation. With these data as a background for subsequent investigations, a comparison can be made of the conventional modalities with the therapeutic efficiency of protons, alpha particles, negative pi mesons and probably heavy ions.

Methods and Materials. Monoenergetic 0.5, 1.0 and 1.3 MeV electrons were used for normal tissue tolerance studies and for irradiation of the implanted neoplasms. The doses reported are those on the skin surface. The dosimetry systems employed have been described by us before (LIPPINCOTT et coll. 1970). A tissue equivalent extrapolation ion chamber was used to measure tissue dose at various depths in tissue equivalent plastic under the same irradiation conditions as for the animal exposures. Thermoluminescent dosimeters and an NBS-calibrated 1 cm^3 tissue equivalent ion chamber were used in beam uniformity measurements

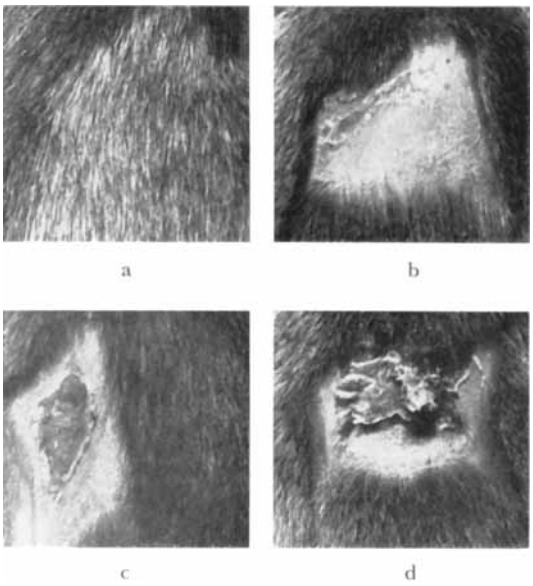

Fig. 1. Twenty-eight days post-irradiation with 0.5 MeV electrons (1.2 mm penetration). a) 1 320 rad. b) 5 400 rad. c) 6 500 rad. d) 12 500 rad.

and as reference monitors for the various exposures. A Faraday cup receiving the straight ahead electron beam was used to obtain consistency of performance of the electron accelerator.

The roentgen ray exposures were obtained using a Westinghouse therapy unit operated at 250 kV and 15 mA with one mm Cu and one mm Al added filtration. (First HVL is equal to 2.1 mm Cu.) At a distance of 70 cm from the target and under conditions of maximum back scatter the dose-rate was 75.2 R/min as measured with a Baldwin—Farmer dosimeter. Various doses were obtained by altering the exposure time. The mice were taped to lucite strips with the left hind leg bearing the tumor inoculum extended. The animals were then placed, seven at a time, on a turntable that was rotated at 6 rpm under the radiation beam. The animals were placed so that the tumor sites were 7 cm from the center of rotation of the turntable and were shielded by a two mm thick piece of lead which contained a one cm wide circular window. The lead shield was positioned so that a one cm strip, which included the tumor, was irradiated. In this situation, the whole body including the left hind leg beyond the tumor was shielded.

The range of doses employed and the number of animals with transplanted neoplasms in each group are shown in the Table. The size of the tumor inoculum was about a one mm cube. The solid plug of neoplasm was introduced just above the knee joint and extruded from the trochar at the mid point of the thigh in the

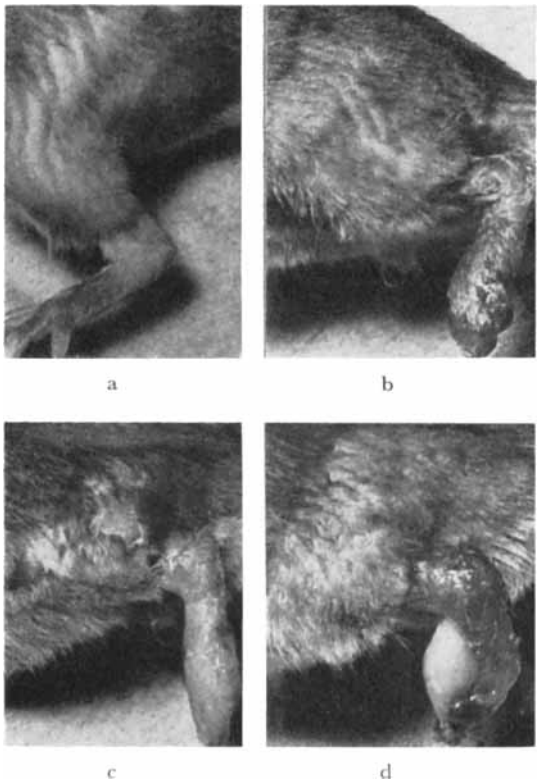

Fig. 2. Twelve days post-irradiation with 1.3 MeV electrons (2.8 mm penetration). Whole leg exposure. a) 870 rad. b) 1 980 rad. c) 4 500 rad. d) 6 650 rad.

dermis or in the outer thin layer of subcutaneous tissue. Occasionally, some tumor cells appeared to be deposited at about the point where the trochar was withdrawn, and growth took place there. Such neoplasms were outside of the one cm diameter field of irradiation and such animals could not be used. Direct inoculation in the mid-thigh at a right angle to the skin could not be used because the tumor might be deposited too deeply for the electron treated groups in which the maximum depth of energy deposition was chosen to be 2.8 mm.

Results

The tolerance of normal tissues to electron exposure at low energies is of importance in evaluating effective tumoricidal doses. In Fig. 1 0.5 MeV electrons were used to penetrate 1.2 mm, the exposed area being 2 cm $\times$ 2 cm. The doses were 1 320, 5 400, 6 500, and 12 500 rad respectively, twenty-eight days post-irradiation. Greying of hair appeared at the lowest dose while at the next dose

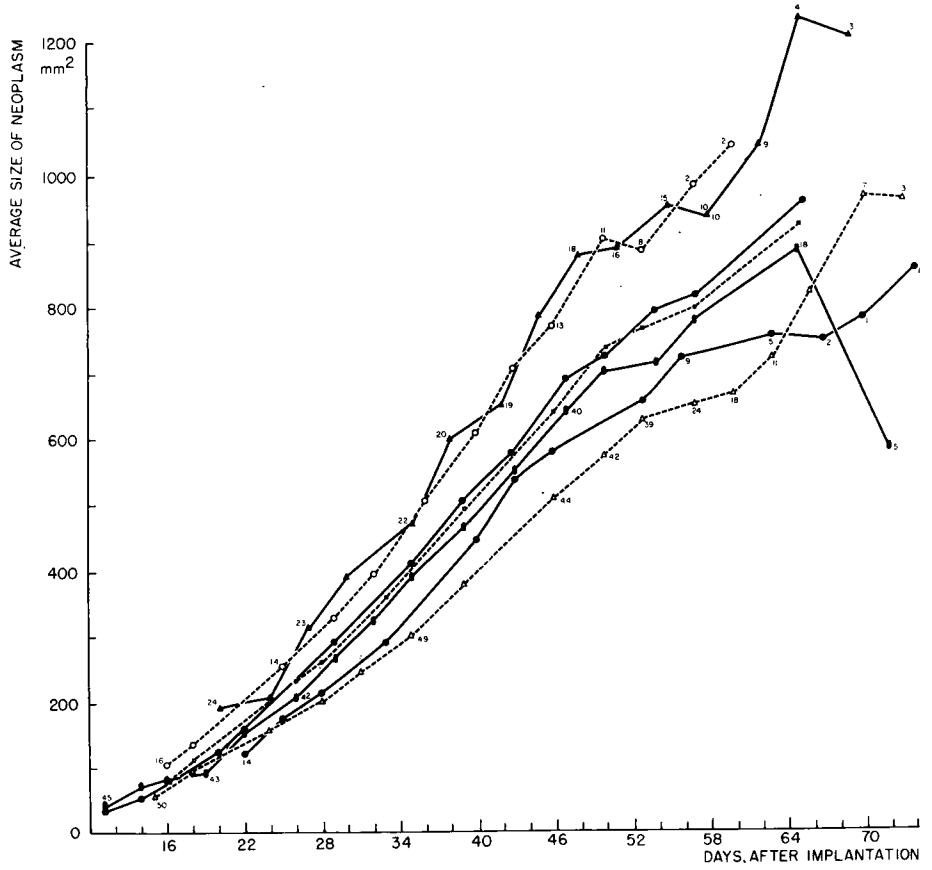

Fig. 3. Growth curves of transplanted mammary C3H/HeJ neoplasms in mice. ▲—▲ 2 mm³ implants; 25 mice. ■—■ 2 mm³ implants; 45 mice. ●—● 2 mm³ implants; 14 mice. △—△ 1 mm³ implants; 50 mice. ○—○ 1 mm implants; 16 mice. ⊙—⊙ Composite of 2 mm data. □—□ Composite of 1 mm data. Numbers indicate animals alive.

wet desquamation occurred. At the two highest doses deep ulceration resulted, with very slow subsequent healing. In Fig. 2 1.0 MeV electrons were used to penetrate 2.8 mm, with the whole leg exposed. The doses were 870, 1 980, 4 500 and 6 650 rad. Twelve days post-irradiation no edema was found at the lowest dose. It was marked at the next two doses and excessive at the highest dose. At each of these last three doses ulceration occurred, being maximum at the highest dose. With these results in mind, the size of area to be irradiated, depth of penetration, and range of doses were decided upon for the experimental therapeutic schedule.

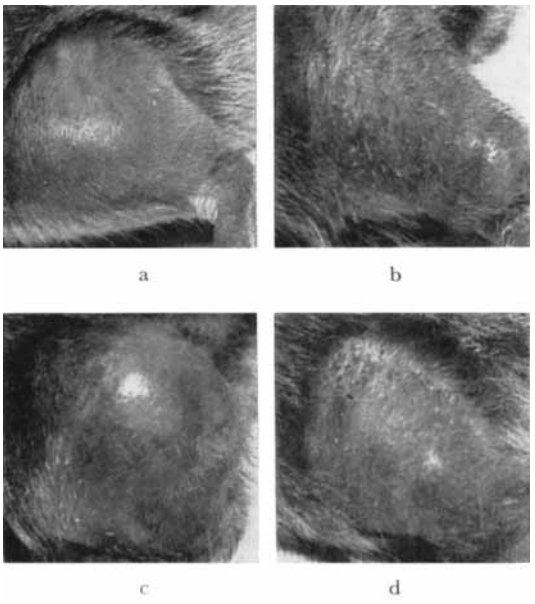

Fig. 4. Thirty days post-irradiation with 250 keV roentgen. a) No irradiation. b) 2 000 rad. c) 3 000 rad. d) 4 000 rad.

Before irradiating any neoplasms growth curves were established for non-irradiated transplanted mammary C_3H/HeJ neoplasms in mice (Fig. 3). This was essential for several reasons. It was necessary to learn how long an animal would live after tumor transplantation and what the rate of tumor growth was. This was determined for one mm and for two mm cube tumor transplants and for groups of animals of different sizes to establish how consistent the findings were. Composites of the one and two mm cube transplants were then made. In doses that did not completely destroy the neoplasm growth curves could then be compared, if desired, with non-irradiated tumor growth curves to learn whether a substantial palliative effect occurred. Upon a basis of the growth curves (Fig. 3) it was decided that by the end of the eleventh week all non-irradiated tumor bearing animals were dead and that the findings in tumor irradiated animals should be compared with that time schedule. It was also considered that one mm tumor transplants were desirable because with a selected energy of 1.0 MeV for electrons the energy deposition would be limited to 2.8 mm. This depth should readily be sufficient for irradiation of the entire neoplasm, while minimizing the amount of normal tissue that would be irradiated.

The tumor inoculum was delivered by a device designed to give a uniform volume, namely, as close as possible to a one mm cube. The implanted neoplasms were allowed to grow for approximately forty-eight hours before irradia-

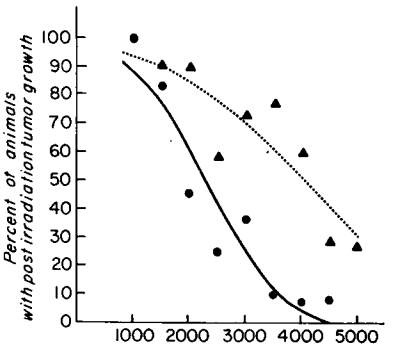

Fig. 5. Dose response curves fitted by probit analysis for electron and roentgen irradiation (dose in rad). ● Electron irradiation. ▲ Roentgen irradiation.

tion. This was to be sure that a viable tumor was growing when irradiation took place. Figure 4 a shows the usual size of growth of the non-irradiated neoplasm at 30 days post-inoculation. In the same figure following 250 keV roentgen irradiation examples of failure to destroy the neoplasm are seen following doses of 2 000, 3 000, and 4 000 rad. At death, all animals were autopsied and sections were taken at the site of irradiation in every exposed animal for histopathologic examination.

The results of irradiating one mm cubes of mammary carcinoma transplanted into the skin of the thigh of C_3H/HeJ mice is shown in the Table. The objective was to determine at similar doses the relative biologic efficiency (RBE) of low energy electrons compared to 250 keV roentgen rays. The endpoint for this was the 50 per cent effect dose (ED_{50}). Dose response curves were fitted to both the electron and roentgen irradiation data by means of a probit analysis (Fig. 5) assuming a normal (Gaussian) tolerance distribution. The chi square test of fit yielded values of the square with six degrees of freedom of 6.85 ($p > 0.25$) and 4.90 ($p > 0.25$) respectively for the electron and roentgen irradiation data, thus indicating that the curves fit the data well. The estimated median effective dose (ED_{50}) for electron treated animals was 2 326 rad with a standard error of 203 and the estimated ED_{50} for roentgen irradiated animals was 4 014 rad with a standard error of 555. Thus, the estimated relative biologic efficiency (RBE) of electron relative to roentgen irradiation therapy is 1.726.

Discussion

The C_3H mammary neoplasm has been used frequently to investigate the effects of various qualities of irradiation upon it. It is a very satisfactory mouse neoplasm with which to work because it is available both as a spontaneously

growing as well as a 100 per cent transplantable tumor. COHEN & COHEN (1954) presented data on the radiobiology of the C_3H mammary mouse tumor. They indicated that for roengten irradiation the LD_{50} for treatment of the neoplasms in situ was 5 700 rad, while for viable fragments irradiated prior to implant 2 000 to 2 500 rad constituted sublethal doses. These data helped us to form the range of doses selected for roentgen irradiation in our experiments. SUIT et coll. (1965) investigated cellular radiation sensitivity of the C3H mammary carcinoma by an analysis of 16 radiation dose-response experiments using as the endpoint the permanent local control of tumor. All irradiations were given as single doses under conditions of local tissue anoxia and with uniform distribution of radiation dose throughout the tumor. The D_{37} was 300 to 325 rad. These authors considered that the data were consistent with the proposal that the tumor cells responded to radiation randomly and on the basis of individual cellular radiation sensitivity.

FOWLER et coll. (1972) investigated the effectiveness of fractionation with roentgen rays and neutrons in mice bearing transplanted C_3H neoplasms. The rationale for the clinical use of fast neutrons is that the oxygen enhancement ratio (OER) for neutrons is less than for roentgen rays. A comparison was made of the TCD_{50} (dose to control 50 per cent of the tumors) with skin reactions produced in the feet of other mice, using roentgen rays or fast neutrons as single doses, or as five or nine fractions at three fractions per week. Roentgen rays given as five fractions in nine days were as effective as any of the neutron treatments, and were the most effective fractionation scheme tested with that system. Neutron treatmen was less dependent upon fractionation.

Using a transplantable mouse fibrosarcoma LIPPINCOTT et coll. (1971) bombarded one mm cubes of tumor with 20 MeV deuterons. Radiation of the entire neoplasm with essentially uniform ionization was achieved by delivering multiple successive Bragg peaks. This technique was accomplished by placing a rotating filter of variable thickness in the beam. The dose range was from 1 000 to 50 000 rad. A single exposure of 1 000 rad was sufficient to destroy all neoplastic growth. By the twenty-eighth post-irradiation day the site of inoculation of the neoplasm destroyed by the deuteron bombardment contained only fibrotic tissue.

In the strong A strain of mice TOBIAS et coll. (1957) reported the first experiments designed to localize the ionization of deuterons in transplanted mammary neoplasms. The ratio of peak ionization to ionization at the point of entry in Lucite, which is roughly equivalent to soft tissue, was found to be about 4: 1. Tumor bearing mice were treated with deuterons passing only through tumor and skin enclosing it. With a specific energy transfer of 6.7×10^5 erg/g complete tumor regression occurred in about one half of the animals, in agreement with an

earlier investigation of LAWRENCE et coll. (1965) for 200 kV roentgen rays. This result gave an estimated RBE of about 1.

In rabbits, FALKMER et coll. (1959) reported results with proton and roentgen radiation of V×2 carcinoma in rabbit ears. The RBE appeared to be about 1. No results have been found reported for proton or alpha particle bombardment in mouse mammary neoplasms. Thus, the above comparison of RBE for deuterons and protons is based on transplanted neoplasms in two different species. The sparsity of recorded experiments in the literature, using heavy particle radiation therapy in animals, forms the real basis for our systematic experiments using electrons and roentgen rays as the choice of types of electromagnetic radiation to compare with protons which are currently available at the Oak Ridge Isochronous Cyclotron and alpha particles now obtainable at the Space Radiation Effects Laboratory Accelerator. It is hoped that when the meson factory is completed at Los Alamos negative pi mesons may be added to this comparative series.

SUMMARY

In a comparison of the therapeutic efficiency of low energy electron versus roentgen irradiation of transplanted mammary neoplasm in mice it was found that the ED_{50} for the former was 2 326 rad (±203) and for the latter 4 014 rad (±555). Probit analysis was used to fit the dose response curves. The estimated relative biologic efficiency (RBE) of electron relative to roentgen irradiation at the 50 per cent effect (ED_{50}) was 1.726.

ZUSAMMENFASSUNG

In einem Vergleich zwischen der therapeutischen Effektivität von Elektronen mit niedriger Energie gegenüber Röntgenbestrahlung auf transplantierte Brustneoplasmen bei Mäusen wurde für Elektronen eine ED_{50} von 2 326 rad (±203) und für Röntgenstrahlen von 4 014 rad (±555) gefunden. Eine Wahrscheinlichkeitsanalyse wurde verwendet, um die Dosis-Effektkurven anzupassen. Die berechnete relative biologische Effektivität (RBE) der Elektronen gegenüber Röntgenstrahlung bei einem 50 %-igen Effekt (ED_{50}) betrug 1,726.

RÉSUMÉ

Une comparaison de l'efficacité thérapeutique des électrons de faible énergie et de l'irradiation Roentgen de cancer du sein transplanté chez des souris a montré que la ED_{50} est de 2 326 rad (±203) pour les électrons et de 4 014 rad (±555) pour les rayons de Roentgen. L'analyse des probits a été utilisée pour ajuster les courbes de réponse de dose. L'efficacité biologique relative estimée (RBE) des électrons par rapport à l'irradiation Roentgen pour l'effet 50 pour cent (ED_{50}) a été de 1,726.

REFERENCES

BERRY J. and ANDREWS J. R.: The effect of radiation ionization density (LET) upon the reproductive capacity of mammalian tumor cells irradiated and assayed in vivo. Brit. J. Radiol. 36 (1963), 49.

COHEN A. and COHEN L.: Radiobiology of the C_3H mammary carcinoma: Effect of attenuation of the tumour prior to implantation in F_1 hybrid hosts. Brit. J. Cancer 8 (1954), 522.

FALKMER S., LARSSON B. and STENSON S.: Effects of single dose proton irradiation of normal skin and Vx2 carcinoma in rabbit ears. Acta radiol. 52 (1959), 217.

— FORS B., LARSSON B., LINDELL A., NAESLUND J. and STENSON S.: Pilot study on proton irradiation of human carcinoma. Acta radiol. 58 (1962), 34.

FOWLER J. F., DENEKAMP P. J., PAGE A. L. and BEGG A. C.: Fractionation with X-rays and neutrons in mice: Response of skin and C3H mammary tumours. Brit. J. Radiol. 45 (1972), 237.

FOWLER P. H. and PERKINS D. H.: The possibility of therapeutic applications of beams of negative Pi mesons. Nature (Lond.) 189 (1961), 524.

LAWRENCE J. H. and TOBIAS C. A.: Heavy particles in medicine. Prog. Atom. Med. 1 (1965), 127.

LIPPINCOTT S. W., MOORE W. H., JESSEPH J. E., CALVO W. and FARR L. E. (a): Effects of heavy high energy charged particles. I. Literature review of effects of protons, deuterons and alpha particles in mammals with a theoretical consideration of the negative Pi meson. Arch. Path. 76 (1963), 497.

— CALVO W., BAKER C. P., JESSEPH J. E., JANSEN C. R. and FARR L. E. (b): Effects of heavy high energy charged particles. IV. The fate of giant cells produced in a neoplasm by 20 MeV deuteron bombardment. Arch. Path. 76 (1963), 543.

— BENDER R., FOELSCHE T., AZZAM R., MONTOUR J. and ROGERS C.: Early effects of monoenergetic low-energy electrons on pig skin. Arch. Path. 89 (1970), 416.

— BAKER C. and JANSEN C.: Experimental radiation therapy by deuteron bombardment of a transplanted neoplasm in mice. Radiat. Res. 47 (1971), 307.

RAJU M. R., GNANAPURANI M., MARTINS B., HOWARD J. and LYMAN J. T.: Measurement of OER and RBE of a 910 MeV helium ion beam using cultured cells (T-1). Radiology 102 (1972), 425.

SUIT H. D., SHALEK R. J. and WETTE R.: Radiation response of C3H mouse mammary carcinoma evaluated in terms of cellular radiation sensitivity. *In:* Cellular radiation biology, p. 514. William and Wilkins Co, Baltimore 1965.

TOBIAS C. A., ANGER H. O. and LAWRENCE J. H.: Radiological use of high energy deuterons and alpha particles. Amer. J. Roentgenol. 1 (1957), 67.