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APPLICATION OF RADIOBIOLOGIC PRINCIPLES TO RADIATION THERAPY

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Oxygen remains one of the most potent sensitizers of mammalian cells yet studied and, most important, it is maximumly sensitizing at physiologic concentrations. There is very good evidence that solid tumors of both experimental animals and man contain hypoxic and viable cells. In experimental animals hyperbaric oxygen does result in improvement of radiation therapy even when the treatment is highly fractionated and protracted over periods of up to 3 to 4½ weeks. This represents strong evidence that—at least in some human tumors—results of fractionated irradiation will be significantly improved by the addition of hyperbaric oxygen. At the same time it is admitted that results of clinical trials of hyperbaric oxygen and radiation therapy do not indicate any marked improvement by the addition of hyperbaric oxygen. However, the trials are early and based on small numbers; they may well show a modest but clinically important improvement as they mature. It is essential that the trials cover several tumor types. Hyperbaric oxygen may not be useful in radiotherapy for all tumors. Evidence from the tests of tourniquet hypoxia and radiotherapy indicate that, at least for osteosarcoma, the hypoxic cell per se may not be the dominant factor in deciding results of standard fractionated therapy.

Note: Wherever R appears it designates rads not roentgens.

MODERN ATTEMPTS TO APPLY RADIOBIOLOGIC principles to radiation therapy began with the important papers by Puck et al. in 1956 and 1957.[21, 22] Extensions and elaborations of varying degrees of complexity of that initial work has been presented by a number of investigators[10, 13, 16, 18, 19, 28, 36] Briefly stated, all of the theoretical efforts to apply principles developed from studies in cellular radiation biology to clinical radiotherapy have assumed for cells of tissues that cell killing follows one of the simple response laws, e.g., multi-target or multi-hit, and that values for the parameters D_0 and N (extrapolation number) for cells constituting tissues in vivo are the same as observed for individual cells cultured in vitro. With respect to tumors two critical assumptions are generally made: (1) *all* cells must be killed by irradiation to achieve permanent local control, and (2) the viable cell population is comprised of aerobic and hypoxic cells.

Because of the marked difference in sensitivity between aerobic and hypoxic cells, the presence of a small number of hypoxic cells within a tumor would determine the dose required to achieve tumor control, i.e., even though the aerobic cells were numerically dominant they would be incidental radiobiologically.[10] The concept that there are hypoxic cells in human tumors and that they account for many of the failures of radiotherapy to achieve local control of tumor has been generally accepted by the radiotherapy community for the past 12 to 15 years. Historically, two publications contributed powerfully to the acceptability of this concept: (1) the British Institute of Radiology symposium in 1953 entitled "The Concentration of Oxygen Dissolved in Tissue at the Time of Irradiation as a Factor in Radiotherapy" [11] and (2) the 1955 paper by Thomlinson and Gray[30] which described the excellent correlation between predicted and observed distances between capillaries and necrotic regions within tumors.

Since the initiation of the hyperbaric oxygen project at St. Thomas Hospital by Churchill-Davidson in 1955,[6] there have been a number of clinical efforts to overcome the effect of hypoxic tumor cells on tumor control probability (TCP). These investigations have been based on three approaches: (1) ir-

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radiation of tumors while patients respired oxygen at high pressure; (2) irradiations of tumors of the extremities under local conditions of hypoxia induced by application of a tourniquet above the treatment field; and (3) use of high LET radiation beams whose effectiveness is relatively independent of cellular pO_2.

This paper is a review of the laboratory and clinical support for the proposal that hypoxic tumor cells represent a frequent cause of failure of radiation therapy administered according to the usual fractionation schedule and that clinical trials of the combination of hyperbaric oxygen and radiotherapy, etc. are desirable. This review has been prompted, in part, by the recent statement of Hall[12] that the hypoxic cell is irrelevant to the day-to-day practice of radiation therapy.

Hall referred to earlier calculations which we had made demonstrating that the TCD_{90} (tumor control dose 90) for tumors comprised of 10^7 viable and aerobic cells [D_0=165 R and N=3] would be approximately 7290 R for radiation administered in 30 doses.[28] This value is reasonably close to dose levels employed with effectiveness in the clinic. However, if 10^5 or 10^6 cells within the tumor (1–10% of tumor cells) were hypoxic and there were no change in oxygen tension during the course of treatment, the TCD_{90} would be > 15,000 R [D_0=400 R, N=3], viz., in excess of doses used in human radiotherapy.*

Although previously untreated tumors of experimental animals and of man almost certainly contain viable and hypoxic cells, the critical question is: Do cells survive in an hypoxic state throughout a course of radiation therapy which extends over a 4- to 7-week period? If cells do not survive hypoxia for such prolonged times, perhaps cells move from the aerobic to the hypoxic compartment during the course of treatment. There is evidence from several laboratories demonstrating that following a first dose of radiation or during a course of treatments, many cells become aerobic that were hypoxic at the start of treatment.[14, 24, 25, 29, 34] No experiments have been described which indicate the reverse movements of oxygen tensions. Provided the transfer of cells from hypoxic to aerobic status were essentially complete by the end of the first two thirds of a course of treatment, the influence of the initially hypoxic cells on the TCP would be minor. Therefore, if in most human tumors the transfer of cells from the hypoxic to aerobic status were that rapid, attempts to improve efficacy of radiotherapy by hyperbaric oxygen, tourniquet hypoxia, or high LET particles would be unsuccessful.

Hyperbaric Oxygen and Fractionated Radiotherapy of Tumors of Experimental Animals

In our laboratory, four different animal tumor systems have been investigated to determine the effectiveness of respiration of oxygen at increased pressure at the time of irradiation to improve results of radiotherapy. These tumors were: (1) a C_3H mouse mammary carcinoma; (2) a DBA mouse mammary carcinoma; (3) a C_3H fibrosarcoma; and (4) a C_3H squamous-cell carcinoma. TCD_{50} values have been determined for 250 mm^3 tumors growing as early generation transplants in the thigh and treated under one of three conditions: (1) local tissue "hypoxia"; (2) "air" or normal respiration and circulation; or (3) "$O_2$30 psi" or respiration of pure oxygen at 3 atmospheres of pressure absolute. Radiation was administered either as a single dose (ν=1) or as a series of 10 equal doses (ν=10, t_1=24 hours, a 24-hour interval between fractions).

Results showed that "$O_2$30 psi" had a very modest effect on single dose irradiation but sharply increased effectiveness of fractionated irradiation (ν=10, t_1=24 hours). For example, in the latter circumstance, if the dose which would correspond to the TCD_{10} for "air" were administered under "$O_2$30 psi" conditions it would be the equivalent of a TCD_{32}–TCD_{90}. This greater efficacy of fractionated irradiation administered under "$O_2$30 psi" conditions was noted even though there was an improvement of oxygenation of tumor during the course of treatment. This latter was indicated by the increase in the ratio TCD_{50} Hypoxia/TCD_{50} Air of 0.93–1.08 for ν=1 to 1.05–1.48 for ν=10.

In a recent experiment the effectiveness of protracting as well as fractionating the radia-

* It should be pointed out that the calculated dose of > 15,000 R was based on the assumption that hypoxic cells do repair sublethal damage, e.g., N is taken to be about 3. If the hypoxic cells are less efficient in repairing radiation damage as has been found for two animal tumor systems,[1, 27] then the TCD_{90} values would be less than the 15,000 R. For example, if N were 2 instead of 3, the TCD_{90} would be ≈ 11,000 R.

tion dose was examined. In that study, ten dose fractions were protracted over an ≈ 4½ week period (ν=10, t_1=3–4 days) instead of a 9-day period (ν=10, t_1=1 day). For this experiment, radiation dose–tumor control response assays were performed on a slowly growing mammary carcinoma of the C_3H mouse, viz. a volume doubling time of about 10 to 11 days as compared with 2–3 days of the four tumors investigated earlier. Results are presented in Table 1.[26] Note that for ν=1 the TCD_{50} is approximately the same for "air" and "$0_2$30 psi" conditions as in earlier studies. However, for ν=10 the difference between "air" and "$0_2$30 psi" conditions is significant for both the 9-day and ≈ 4½-week treatment schedules. Therefore, in this tumor system there was a marked improvement in results of radiotherapy by employing hyperbaric oxygen even for greatly protracted treatment. That is, this greatly expanded treatment time was not accompanied by a significantly greater transfer of cells from hypoxic to aerobic status than occurred during the daily irradiation schedule.

The studies of DuSault, et al.[8, 9] on the spontaneous mammary carcinoma of C_3H mice are especially relevant. They irradiated the spontaneous tumors in mice respiring air or 95% oxygen and 5% carbon dioxide (the oxygen was respired for 30 minutes before each treatment) or while respiring pure oxygen at 30 psi and employed 6–20 dose fractions over 18 to 25 days. In all instances mice breathing the 95% 0_2–5% CO_2 mixture or "$0_2$30 psi" experienced higher tumor control rates than did air-breathing mice. Thus, in both series of experiments, where radiation dose was given in six or more fractions and over total times of 9 to 25 days, the addition of oxygen improved results. In summary, the hypoxic cell was a highly important factor in determining results of fractionated treatment of these murine neoplasms despite the fact that many cells transferred from a hypoxic to an aerobic status during treatment.

HYPERBARIC OXYGEN AND RADIATION THERAPY

Hyperbaric oxygen has been combined with radiation therapy at the clinical level in an attempt to improve results since 1955; even so, the efficacy of such treatments has not been established. The question is raised:

TABLE 1. Effect of Hyperbaric Oxygen and Dose Fractionation on TCD_{50} of a Slowly Growing Mammary Carcinoma (C_3H/He)

	TCD_{50} Air	TCD_{50} $O_2$30 psi	$\frac{TCD_{50}\ Air}{O_2 30\ psi}$
Single dose	4375 R	4000 R	1.09
10 equal doses t_i = 24 hours	7680 R	4570 R	1.68
10 equal doses t_i=3–4 days	11,670 R	7760 R	1.50

Does the available evidence from clinical trials in progress warrant a continuation or an expansion of this line of research?

The plan of a clinical trial which would yield results most readily interpreted would feature at least two groups of patients: a control series and a test series. The control group should be treated according to a conventional dose fractionation schedule and a technique which would yield a tumor control probability (TCP) and necrosis probability (NP) which would be accepted by most radiotherapists as being excellent by current standards.

The test or hyperbaric oxygen group could be treated according to the dose fractionation schedule and technique deemed most likely to yield better figures. If the TCP were higher and or NP lower in the test series, then a better method of treatment would have been demonstrated. For such a study, results of treatment must be analyzed in terms of frequency of local control of tumor and of normal tissue complications. That is, follow up examination should give special attention to evaluation of results within the treated volume. Failure of therapy to achieve local control should be scored only when regrowth of tumor has been observed. Slow regression or persistent ulceration are not the equivalent of recurrence.

Survival figures are secondary end-points in this clinical experiment in radiobiology.

In Tables 2 and 3 results from several current trials are presented. Clearly, the numbers are small and observation periods short. Data from van den Brenk's series at Melbourne[31] and Chang's series at Columbia[4] are given in Table 2. In these trials the fractionation number was small, i.e., four and six respectively, and the control and test series within each trial were treated according to essentially the same fractionation schedule. Results refer to local control at 12 months, and in

TABLE 2. Hyperbaric Oxygen and Radiotherapy—Small Fractionation Number

Investigator	Anatomic site	End-point	"Air" 4 × 775 R	Hyperbaric oxygen 4 × 7 5 R $O_2$45 psi
van den Brenk[31]	Head and neck	≈1 year control		
	T 3–4	Primary	6/12	14/17
		Cervical node	2/12	9/13
	Urinary bladder	Primary control	6 × 550 R 2/8	6 × 500 R 5/6
Chu Chang[4]	Ora-pharynx	Primary control	7 × 600 R	6 × 600 R $O_2$30 psi
		1 year	3/9	7/10

both trials there is a suggestion of better control of tumor in the hyperbaric oxygen treated patients. Unfortunately, there was not a concurrent series treated with conventional radiation, so that results of the test series might be compared directly with those of standard therapy.

Chang does have a third group (not shown in Table 2) treated with 30 doses of 200 R; however, those patients experienced a much less severe mucous membrane reaction than did either group treated with six or seven fractions. Accordingly, those 30 dose fraction cases, as such, are not really controls.

In Table 3 are presented the results of three trials where conventional fractionation schedules were employed in both the air and hyperbaric oxygen group ($0_2$30 psi). The trial for stage III carcinoma of the cervix by Johnson is not a strict trial because the air group has been matched retrospectively to the hyperbaric oxygen series. In both the Johnson[15] and Dische[7] series there is a reasonably firm suggestion of an increase in local control or a more dramatic initial response of advanced cervical carcinomas when the patients received treatment while respiring oxygen at high pressure. However, in the treatment of advanced bladder carcinoma, neither the results of Cade and McEwen[3] nor Dische provide an indication of better result in the "$0_2$30 psi" groups. In summary, the evidence which is available from trials of hyperbaric oxygen and radiation therapy provide only a suggestion of an improvement in local results.

TABLE 3. Hyperbaric Oxygen ($O_2$30 psi) and Conventionally Fractionated Radiotherapy

Investigator	Anatomic site	End-point	Air	Hyperbaric oxygen
Johnson[5]	Uterine cervix Stage III	2-yr disease-free survival	3/16	9/17
Dische[7]	uterine cervix	Regression to less than 25% of orig. mass at complet. of therapy	5/14	10/11
	Stage IIB III, IV			
	Urinary bladder	Persistent local tumor	5/11	5/11
Cade[3]	Urinary bladder	1-yr survival	10/17	4/16

Clearly, the addition of hyperbaric oxygen to radiation therapy is not going to produce dramatic improvement in results. To my knowledge all centers employing hyperbaric oxygen have observed local regrowth of disease after treatments which produced moderately severe acute reactions in the normal tissue. However, a clinically useful improvement might result from the addition of hyperbaic oxygen to a standard fractionation schedule or perhaps a shorter one, such as that favored by van den Brenk. Note that even if a difference of 20 points existed between TCP of test and control series, a total of 60 to 90 patients would be required to detect such a difference ($\varrho < 0.05$)[2]; the actual number of patients would depend on the particular TCP of the control treatment. An improvement by 20 points would certainly warrant the extra effort involved in the hyperbaric oxygen treatment. Before any negative conclusions are permissible several trials will have to be continued to the point of accumulating at least the indicated number of cases of a given tumor, clinical stage, and anatomic site. This is especially so since the results are not inferior to those obtained with standard threapy; it is a relatively risk-free treatment method; most of the clinicians who have used hyperbaric oxygen have the impression that it improves results.[4, 5, 20, 31, 35]

RADIATION THERAPY FOR SARCOMAS OF THE EXTREMITIES TREATED UNDER TOURNIQUET-INDUCED HYPOXIA

Under this heading reference is made to the experiences at Melbourne by van den Brenk[33] and by ourselves at the M.D. Anderson[23] in the treatment of bone and soft tissue sarcomas using torniquet-induced hypoxia and radiotherapy.

Using conventional dose levels and fractionation schedules, Lee and Mac Kenzie[17] treated 119 patients with osteosarcoma.

Their policy was to administer a total dose of ≈ 6000 to 7000 R, employing a daily fractionation schedule, and then to perform an amputation some 6 months later. In their series, six patients refused amputation and have survived ≥5 years free of evident disease. From that limited experience one cannot estimate the TCP for conventional therapy of osteosarcoma, but it is at least 0.05 (the number of patients who developed local regrowth of tumor before amputation was not given). Since the shape of the radiation dose–tumor control response curve is likely to be fairly steep and standard therapy is at the ≈0.05 point, then moderate increases in dose should increase TCP sharply.*

If the clinically observed TCP is determined by the presence of a small fraction of cells which are hypoxic, e.g., 0.01, the effect of making all of the tumor cells hypoxic should be to increase modestly the dose yielding a TCP of 0.05. Provided the dose–response curve were relatively steep, then the total dose required to increase TCP from 0.05 to 0.90 should be rather small. In accordance with this thinking we administered 12,000 R and 14,000 R ($\nu = 12$ and $\nu = 14$) over total times of 36 to 43 days to 16 human osteosarcomas with the expectation that local control of tumor would be regularly achieved in view of the great increase in total dose and the sharp reduction in fractionation number. This may be considered in quantitative terms by employing the simple tumor model described earlier.[28] For example, if the total number of viable cells in the tumor were 10^7 (all being hypoxic because of the tourniquet) then the TCD_{90} for $\nu = 12$ would be ≈ 12,240 R (assume multitarget response law; $D_0 = 400$ R, $N = 3$). Because of the heavy sedation required for control of discomfort associated with the tourniquet, treatment was given only two times per week. It is of interest to note that had ν been 30, the TCD_{90} for the same population of cells would have been ≈ 17,700 R. Therefore, our dose of 12,000 to 14,000 R should be the equivalent of an increase of standard dose of 6000 to 7000 R ($\nu \approx 30$) by a factor of ≥ 2.5.

Unfortunately, experience has not confirmed our optimistic expectation. Nine patients died at less than 12 months because of distant metastases but with local control. However, in five of the remaining seven patients, local regrowth of tumor was observed: in two of three cases after 12,000 R and in three of four cases after 14,000 R. The dose was further increased: three osteosarcomas received 16,000 R in 16 fractions over a 50-day period and local regrowth has not been observed in a minimum follow-up period of 12 months.

However, an amputation was performed on one patient at 9 months because of massive soft tissue necrosis. In the surgical specimen there was a persistent tumor mass which measured 15 × 8 cm. Microscopically, fields of intact and apparently proliferating tumor cells were seen.

Another patient received 16,000 R in 50 days for a chondrosarcoma of the distal femur. An amputation was required at 24 months because of a painful limb (apparently due to late fibrosis in the peripheral nerve). A 4-cm residual tumor was found at the site of the primary. Histologically, this also showed areas of intact cells with mitotic figures (Fig. 1). The presence of areas of apparently intact and proliferating tissue suggests that these two tumors were in the process of recurring following 16,000 R ($\nu = 16$). In our series of eight patients with primary sarcomas of bone who were treated with 16,000 R, no instance of clinically evident local regrowth of tumor has occurred. This good control rate is not of clinical value because seven of the eight patients have had major complications. At the slightly lower dose of 14,000 R, a prohibitive frequency of local recurrence (three of four) and one serious complication developed.

Van den Brenk treated 11 osteosarcomas by a similar tourniquet technique but employed three dose fractions of 2000 to 2700 R delivered over a 21- to 28-day period. Five patients died at less than 12 months because of distant metastasis but with local control. Three of the remaining six developed local regrowth of tumor and one had amputation at 24 months because of painful limb.[32] Thus, in both the Melbourne and the M.D. Anderson series local recurrence of osteosarcoma was observed following the administration of radiation therapy under tourniquet technique with doses sufficiently high to produce a significant incidence of serious complications. Although neither series is suf-

* This cannot be done using conventional treatment techniques because normal tissues cannot tolerate higher doses.

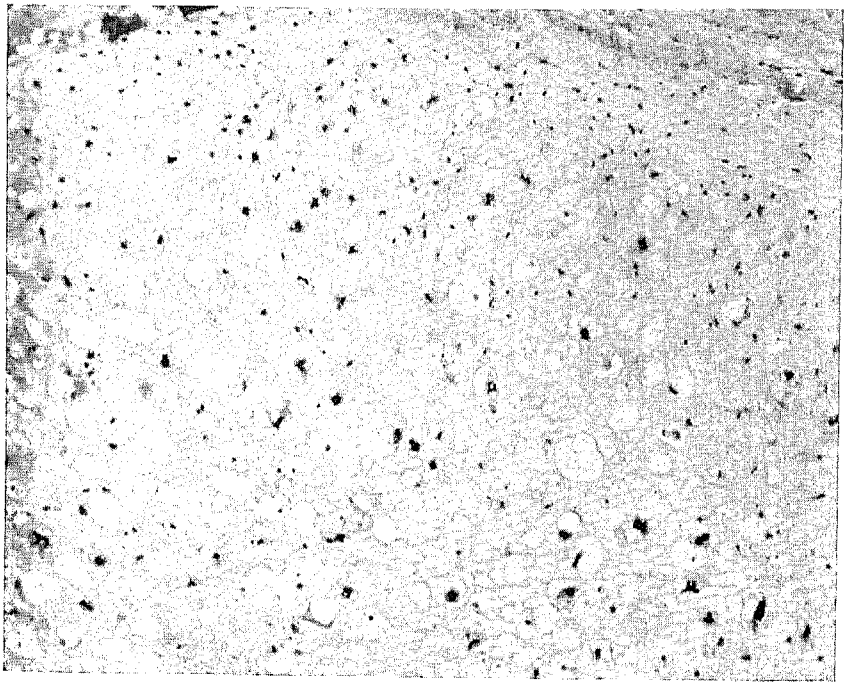

FIG. 1. Histological section of residual chrondrosarcoma at 2 years after treatment to 16,000 rads in 16 fractions over 50 days. These slides have been reviewed by Dr. James Butler of the Department of Pathology of our hospital. In his opinion, the section shown is an area of actively growing tumor; this was taken from a grossly viable portion of tumor at the edge of a large necrotic area. This tumor is a well-differentiated chondrosarcoma and does not differ in appearance from the pretreatment biopsy specimen.

ficiently large to permit a good estimate of TCP to be made, it is clear that the tourniquet technique as presently employed does not represent a successful method of treatment for osteosarcoma. In view of these results my conclusion is that in this tumor system the hypoxic cell is not the dominant factor in deciding results of standard fractionated therapy.

Van den Brenk and we have used the tourniquet technique in treatment of soft tissue sarcoma and obtained better results. However, in our series not a single recurrence has been observed in patients treated either with conventional fractionation schedule (nontourniquet) ≥ 7000 R ($v \approx 30$) in 7 weeks or with tourniquet technique using 14,000 R ($v=14$) in 43 days. Our experience is based almost entirely on small lesions or postexcision cases.

REFERENCES

1. Belli, J. A., Dicus, G. J., and Bonte, F. J.: Radiations response of mammalian tumor cells—I. Repair of sublethal damage in vivo. *J. Nat. Cancer Inst.* 38:673-682, 1967.

2. Boag, J. W.: Statistical problems which arise in cancer therapy. *Clin. Radiol.* 11:150, 1960.

3. Cade, I. S., and McEwen, J. B.: Megavoltage radiotherapy in hyperbaric oxygen—A Controlled Trial. *Cancer* 20:817-821, 1967.

4. Chang, Chu: Personal communication, 1968.

5. Churchill-Davidson, I.: Hyperbaric oxygen and radiotherapy—Clinical experiences. Proceedings First Annual San Francisco Cancer Symposium, 1966; pp. 207-214.

6. Churchill-Davidson, I., Sanger, C., and Thomlinson, R. H.: Oxygenation in radiotherapy—II. Clinical application. *Brit. J. Radiol.* 30:406, 1957.

7. Dische, S.: Progress report on hyperbaric oxygen trial. Mount Vernon Hospital, 1967.

8. DuSault, L. A.: The effect of oxygen on the response of spontaneous tumours in mice to radiotherapy. *Brit. J. Radiol.* 36:749-754, 1963.

9. ———, Eyler, W. R., and Dobben, G. D.: The combination of oxygen and optimum fractionation in radiation therapy of adenocarcinoma. *Am. J. Roentgen.* 85:688-692, 1959.

10. Gray, L. H.: Radiobiologic basis of oxygen as a modifying factor in radiation therapy. *Am. J. Roentgen.* 85:805, 1961.

11. ———, Conger, A. D., Ebert, M., Hornsey, S., and O. C. A. Scott: The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Brit. J. Radiol.* 26:638-647, 1953.

12. Hall, E. J.: The oxygen effect—Pertinent or irrelavent to clinical radiotherapy. *Brit. J. Radiol.* 40: 874-875, 1967.

13. Hewitt, H. B.: Fundamental aspects of the radiotherapy of cancer. The Scientific Basis of Medicine Annual Reviews, 1962.

14. Howes, A.: Personal communication, 1967.

15. Johnson, R. J. R.: Personal communication, 1968.

16. Lajtha, L. G., and Oliver, R.: Some radiobiological considerations in radiotherapy. *Brit. J. Radiol.* 34: 252-257, 1961.

17. Lee, E. S., and MacKenzie, D. H.: Osteosarcoma —A study of the value of preoperative megavoltage radiotherapy. *Brit. J. Surg.* 51:252-274, 1964.

18. Maruyama, Y.: Current concepts of radiobiology relevant to radiotherapy. Lancet 86:169, 1966.

19. Munro, T. R., and Gilbert, C. W.: The relation between tumour lethal doses and the radiosensitivity of tumour cells. *Brit. J. Radiol.* 34:246-251, 1961.

20. Plenk, H. P., and Card, R. Y.: Hyperbaric radiation therapy—Salt Lake City experience. Proceedings First Annual San Francisco Cancer Symposium, 1966; pp. 257-274.

21. Puck, T. T.: and Marcus, P. I.: Action of x-rays on mammalian cells. *J. Exp. Med.* 103:653, 1956.

22. ———, Morkovin, D., Marcus, P. I., and Cieciura, S. J.: Action of x-rays on mammalian cells II. Survival curves of cells from normal human tissues. *J. Exp. Med.* 106:485-500, 1957.

23. Suit, H. and Lindberg, R.: Radiation therapy administered under conditions of tourniquet-induced local tissue hypoxia. *Am. J. Roentgen.* 102:27-37, 1968.

24. ———, and Maeda, M.: Hyperbaric oxygen and radiobiology of a C_3H mouse mammary carcinoma. *J. Nat. Cancer Inst.* 39:639-652, 1967.

25. ———, and Schiavone, J. V.: Effect of a single dose of radiation on proportion of hypoxic cells in a C_3H mouse mammary carcinoma. *Radiology* 20:325-328, 1968.

26. ———, and Schiavone, J. V.: Hyperbaric oxygen and radiotherapy of a slowly growing C_3H mouse mammary carcinoma. In preparation, 1968.

27. ———, and Urano, M.: Kinetics of repair and recovery in an irradiated mouse mammary carcinoma. In preparation.

28. ———, Wette, R., and Lindberg, R.: Analysis of tumor-recurrence times. *Radiology* 88:311-321, 1967.

29. Thomlinson, H.: Radiation-induced change of oxygenation in tumor, J. M. Vaeth, ed. *Frontiers of Radiation Therapy and Oncology* Basel, Karger, vol. 3. 1968.

30. Thomlinson, R. H., and Gray, L. H.: The histological structure of some human lung cancers and possible implications for radiotherapy. *Brit. J. Cancer* 9:539-549, 1955.

31. Van den Brenk, H. A. S.: Hyperbaric oxygen in radiation therapy: an investigation of dose-effect relationships in tumor response and tissue damage. *Am. J. Roentgen.* 102:8-26, 1968.

32. Van den Brenk, H. A. S.: Personal communication, 1967.

33. Van den Brenk, H. A. S., Madigan, J. P., Kerr, R. C., and Richter, W.: The treatment of malignant disease of the extremities by megavoltage irradiation under tourniquet anoxia. *J. Coll. Radiol. Aust.*, 7:142-158, 1963.

34. Van Putten, L. M., and Kallman, R. F.: Effect of preirradiation on the ratio of oxygenated and anoxic cells in a transplanted mouse tumor. Proceedings First Annual San Francisco Cancer Symposium, 1966; pp. 29-38.

35. Wildermuth, O.: The Seattle experience with hybaroxic radiotherapy. Proceedings First Annual San Francisco Cancer Symposium, 1966; pp. 233-248.

36. Wilson, C. W.: Possible implications of recent radiobiological observations for tumour-dose-fractionation schedules. *Radiology* 77:940-947, 1961.