Combination Therapy of Malignant Tumors with Ionizing Radiations and Chemicals: A Review

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Since the discovery of the damaging effects of ionizing radiations on cells, attempts have been made to increase the radiation curability of malignant neoplasms by the simultaneous administration of various chemical agents. Three major avenues of approach will be reviewed: (a) combining radiation with agents which themselves have antitumor properties in an attempt to gain a synergistic action; (b) sensitizing the tumor to radiation by means of a noncarcinostatic agent; (c) introducing into the tumor a compound which will increase the radiation dose to the tumor by secondary radiation or by nuclear disintegration. A review of previous experience in these fields is needed because of the discovery of better chemotherapeutic agents, the increasing knowledge of fundamental radiobiology, and the advent of biologically applicable beams of electrons, neutrons, and heavy particles.

The literature is extensive, and it is impossible to include reference to all work that has been done, but an effort has been made to include all the major trends of experimentation. The authors have exercised critical discretion as to the material which is included. Whenever possible, examples of investigations with human tumors, animal tumors, and in vitro technics have been cited.

Reference has been omitted to agents which have a systemic effect, such as the sex hormones (16) and cortisone (15) which may alter tumors by indirect pathways, and to radioactive isotopes such as P³², I¹³¹, and Au¹⁹⁸ which are of proved value (5).

CARCINOSTATIC AGENTS

The alterations in the malignant cell following irradiation represent a combination of direct and indirect effects of radiation. The direct effects comprise a more or less prolonged reduction in the number of cells entering mitosis, abnormalities in the mechanism of cell division, and changes in cell metabolism. The indirect effects are those induced in the tumor bed, more especially in the intra- and peritumoral capillaries, lymphatics, and surrounding connective tissue. If the radiation dose is small, the direct effect is of a temporary nature only and is followed by recovery (131). When the radiation dose is increased, after temporary inhibition of mitotic activity, degenerate cells appear upon resumption of mitosis. Many cells break down in the premitotic phase or at the end of prophase, some in telophase, and a few at other stages of mitosis.

The majority of carcinostatic chemicals exert their action directly on the tumor cells. Many chemicals which produce no visible effects on a cell during interphase can interrupt the mitotic cycle. Some drugs cause the degeneration of dividing or premitotic cells leading to the formation of pyknotic nuclei; others arrest mitosis in certain phases (62, 82).

Colchicine.—Colchicine was the first mitotic inhibitor used in combination with radiation for treatment of malignant tumors. Colchicine temporarily arrests mitotic division of animal and plant cells in metaphase, suppresses spindle-fiber formation, and inhibits separation of the daughter cells after division (87). The therapeutic value of colchicine in the treatment of malignant diseases is not great, since tumors can be only temporarily inhibited, and the drug is too toxic to allow its prolonged use at higher dose levels. In 1951, Levine (88) reviewed the literature on the combined use of colchicine and x-rays for the treatment of animal and human neoplasms. Results obtained on mouse and rat tumors were inconclusive, though in some cases better effects were produced by colchicine and x-rays than when either agent was used alone. Clinical work reviewed by Levine was carried out in advanced stages of malignant disease and with a small number of patients.

Additional clinical information has been ob-
therapeutically effective doses than colchicine, has been found to be much less toxic and to the mouth. The tumors were also irradiated with treatment of two patients with advanced cancer of travenous dose levels of 20-30 mg/day in the ed with either agent alone.

Observed in animals receiving combined treatment with either agent alone. The percentage of mice showing complete regression of the tumors was approximately 50 per cent less than that of untreated controls, while an x-ray dose of 600 r per cent inhibition. A dose of 1.35 mg/kg of nitrogen mustard oxide combined with a single x-ray dose of 300 r to the tumor; 3 days later the mean weight of the tumors was approximately 50 per cent less than that of untreated controls, while an x-ray dose of 600 r per cent inhibition. A dose of 1.35 mg/kg of nitrogen mustard oxide caused about 40 per cent and a dose of 3.3 to 3.5 mg/kg about 70 per cent inhibition. Thus, there was no evidence of a synergistic effect by the combination of these two agents.

Triethylenemelamine (TEM) injected in a single dose of 1.25 mg/kg and combined with a single tumor dose of 750 r or 1000 r produced responses suggestive of synergism in mice bearing Sarcoma 180 or RC mammary carcinoma (140).

Clinical studies of combinations of alkylating agents and x-rays have been reported by several authors. The largest group of patients treated in this way was that of Gil y Gil (39) who reported good immediate results in some of 100 patients with different tumors in advanced stages treated with a combination of x-rays and HN3 (tris-2-chloroethylamine) or TEM. Results were of a palliative nature, and the observation time was too short to give a fuller evaluation of results. HN3 and x-rays produced better response in combination than when used alone in cases of Hodgkin’s
cases of lung cancer. No beneficial effects were reported in cases of tumors of the breast or of the female genitalia.

Satisfactory remissions of Hodgkin’s disease following the combined use of alkylating agents and radiotherapy were reported by Holy and Suchan (57), Linke and Lasch (88), Malaguzzi-Valeri and Di Raimondo (90), Pedro-Botel and Gaix (118), Truhaut (146), and others. Temporary improvement was observed in cases of tumors of the urinary tract (147), choriocarcinoma (4), and the urinary tract (147), choriocarcinoma (4), and bronchial carcinoma (84) treated with combinations of HN2 (methylbis[2-chloroethyl]amine) and x-rays.

In a review of 700 cases of malignant blood diseases and reticuloses, Wilkinson (158) cautions against the use of HN2 prior to or simultaneously with radiotherapy in the treatment of Hodgkin’s disease and of lymphatic and myelogenous leukemias. In his experience the use of such combinations leads to bone marrow damage and the production of severe refractory or aplastic anemias.

Phillips et al. (177) treated fourteen cases of liver metastases secondary to carcinoma of other organs with a single intravenous injection of 0.4 mg/kg of HN2 followed by super voltage x-ray therapy. The whole liver was irradiated to a tumor dose of 2000-3750 r in 8 days. There was no significant difference in the results of x-ray therapy alone compared with combined treatment; whereas ten out of fourteen patients treated with both modalities showed symptomatic improvement, sixteen out of 22 patients treated with x-ray alone reacted in the same way. Improvement in liver function tests was observed, but there was no difference between the two groups. The patients on combined therapy showed fewer side effects of treatment.

Urethan.—Urethan produces general disruption of most aspects of cell division in both isolated animal cells and in the tissues of intact animals (Corman [21]). A range of mitotic abnormalities is produced: multipolar mitoses, accumulation of metaphases, fragmentation of chromosomes, and, in some tissues (e.g., in the intestinal crypts), karyopyknosis. Urethan inhibits several experimental tumors, e.g., mouse leukemias, Sarcoma 180, Walker rat carcinosarcoma 256; it causes temporary remissions in chronic myelogenous leukemia in man but is less effective in chronic lymphatic leukemias (17).

Whitehead and Lanier (151) observed an enhancement of tumor-inhibitory effects of multiple x-ray doses of 150–300 r in mouse mammary carcinoma A-179985 and mouse leukemia L4946 when, in addition to radiotherapy, animals were treated with two to three doses of 1,3-dichloro-2-isopropyl-N-diethylcarbamate injected intraperitoneally in doses of 0.0019 cc. every 3d day. The degree of inhibition of tumors implanted into the leg muscles was determined by comparing tumor weights of treated and control mice at the end of treatment.

In 1949, Dustin (28) reported the results of concurrent and alternating treatment with urethan and x-rays of fourteen cases of chronic myelogenous and ten chronic lymphatic leukemias. Myelogenous leukemias are more sensitive to urethan alone than are lymphatic leukemias. Several patients became resistant to either urethan or x-rays; no cross-resistance was observed. Simultaneous therapy with both agents was used at some time in the course of treatment of eleven patients with myelogenous and of four with lymphatic leukemias. Remissions were obtained in these cases more rapidly and with smaller doses of these agents than with either agent alone. They were also observed in cases resistant to urethan or x-rays, and in those who did not tolerate high doses of urethan. Lings (92) reported that the treatment of patients with multiple myelomas with a combination of urethan and x-rays produced better clinical results than therapy with x-rays only.

Purine analogs.—8-Azaguanine is incorporated in small amounts into the nucleic acids of animal tumors (mouse Sarcoma 37, mouse Leukemia L1210) and normal tissues (Mandel [92], Skipper [127]). It appears that the inhibitory action of 8-azaguanine on tumor growth may be caused by the inability of nucleotides containing this purine analog to perform the still unknown functions of the natural purine nucleotides.

Carpender and Lanier (12) treated mouse adenocarcinoma E 0771 with a daily subcutaneous dose of 1 mg. of 8-azaguanine, combined with daily x-ray doses of 200–600 r to a total dose of 4600 r in 14 days. With the tumor weight used as a criterion of therapeutic effects, they found that combined treatment caused a greater degree of tumor inhibition than did treatment with either agent alone.

In experiments with Sarcoma 180, RC carcinoma, and Bashford mouse Carcinoma 63 (140), 8-azaguanine neither produced any increase in the number of tumor regressions caused by radiation alone nor altered the degree of the radiation-induced tumor inhibition 1 week after treatment.

Another purine derivative, 6-mercaptopurine, used in combination with x-rays, produced an enhancement of both tumor growth retardation and tumor regressions at 8 weeks in the case of Sarcoma 180. There was an increase in tumor inhibition
at 1 week in the case of the RC carcinoma.

In 1927, Chambacher and Rieder (13) reported great improvement of therapeutic results obtained with x-rays in a variety of human tumors when multiple doses of 0.05 mg. “sodium nucleinate” were injected subcutaneously prior to or simultaneously with irradiation.

**Antibiotics**.—Antibiotics have often been used in conjunction with radiation therapy of cancer with the aim of combating the infectious component of a tumor or of the postirradiation syndrome. In addition to this, aureomycin was used by Bateman et al. (8) in conjunction with radiation therapy of 39 various advanced human tumors, since it had been reported that aureomycin inhibits cell division in tissue cultures and retards certain small animal tumors. Regression of palpable tumors was observed in 22 patients, clinical improvement in 39, and healing of ulcerated tumors in twelve. Freedom of symptoms was recorded in nine out of nineteen patients observed for 4–19 months after the beginning of therapy. No comparison was made with x-ray treatment without aureomycin.

In 60 cases of Hodgkin’s disease, lower doses of x-rays were required to produce remissions when radiation was combined with actinomycin C therapy at dose levels of 250 mg/day for 3–6 weeks. Good results were observed in two cases of aleukemic lymphadenopathy, but carcinomas and leukemias responded poorly (Schulte and Lings [125]). Magnus and Zeitler (88) published additional observations on eleven cases of Hodgkin’s disease and three leukemic patients treated with actinomycin C (Sanamycin) and x-rays. Seven cases of Hodgkin’s disease and one myelogenous leukemia showed complete or partial remissions. No comparisons with the results of radiation therapy alone were presented.

**Miscellaneous compounds**.—Colchicine, urethane, alkylating agents, certain purine analogs, and antibiotics are chemicals the tumor-inhibiting activities of which have been well established. In addition to them, several chemicals have been used in combination with ionizing radiations for the treatment of human and animal tumors on the basis of some observed tumor-damaging properties or following only certain preconceived ideas about their possible deleterious effects on tumors.

Failla (33) postulated, on the basis of the fact that radiation-damaged cells and their nuclei swell, that radiation increases the intracellular osmotic pressure. According to this theory, it should be possible to increase the radiosensitivity of a tissue by decreasing the osmotic pressure of the surrounding extracellular fluids. To test this assumption Sugiuura (137) injected daily, for 4–5 days, 0.5 cc. of distilled water or Locke-Ringer solutions of different tonicity into 1-week-old implants of Sarcoma 180, previously irradiated with x-rays. Hypotonic saline and distilled water markedly enhanced the tumor damage caused by radiation, but water itself, injected intratumorally, did not affect the growth of the tumor.

Upon injection into tumor-bearing animals, bacterial endotoxins and certain polysaccharides obtained by their degradation produce hemorrhages in the tumors and occasionally cause their regressions. Lawrence and Duran-Reynals (81) treated mice of strain A bearing transplantable mammary carcinoma with E. coli, paratyphoid, and typhoid toxins plus x-radiation. Toxins did not increase prolongation of the survival time of tumor hosts over that obtained with x-rays alone.

**Megaphen**—(N-3-dimethylamino)-propyl-3-chloraphenothiazine—produces mitotic abnormalities in some biological systems. Peters et al. (114, 115) treated mice bearing the Elberfeld strain of Ehrlich ascites tumor with three injections of 12.5 mg/kg/day of Megaphen on the 5th, 7th, and 9th day after tumor inoculation. On the 6th day tumor-bearing mice were irradiated. This compound accentuated the radiation-induced depression of tumor cell mitoses and caused an accumulation of ana- and telophases due to the prolongation of mitotic time, but did not potentiate the tumor inhibition caused by radiation alone.

**Choline** (149) and its salts (150), isamine blue (9), arsenicals (126), and other chemicals were used with equivocal results in combination with x-rays in therapy of human tumors on the basis of such rather vague conceptions as those of stimulation of the reticulo-endothelial system, the general role of phospholipides in tumor cell metabolism, etc.

It appears from the preceding survey of tumor-inhibitory chemicals used in combination with ionizing radiations for the therapy of tumors that in certain instances real synergistic effects have been observed. Further progress in this field can be expected from more extensive testing of the reported and other tumor-inhibiting chemicals in combination with ionizing radiations on various animal tumors, including those occurring in the ascites form. Better understanding of the mechanisms responsible for the antitumor activity of both carcinostatic chemicals and ionizing radiations will permit investigation on a less empiric basis.

**Sensitizing Agents**

It is necessary to define the word sensitizer in order to avoid confusion between this class of com-
pounds and other agents used in combination with radiation. Quite often an agent is called a "sensitizer" when the actual action is one of direct cell toxicity. In this review the term radiation sensitizer will specifically include those drugs or treatments that produce a condition of hypersusceptibility to the action of ionizing radiation, but when administered alone do not have a carcinostatic effect.

**Oxygen sensitization.**—Prevailing theories of the mode of action of ionizing radiation on cells assume the state of oxygenation of the cell to be of prime importance. These theories have been reviewed by other authors (47, 112), and Packard (111) reviewed the earlier work concerning x-rays and respiration.

The relation of oxygenation of cancerous tissues to radiation sensitivity has been suspected for some time. Mottram and Eidinow (110) found that bleeding by cardiac puncture lessened the skin reactions to radiation and also lowered the radiation sensitivity of implanted Jensen rat sarcoma. They suggested that the endothelial cells of the blood vessels might be altered during irradiation so that some unspecified toxin entered the tissues and that bleeding reduced the reaction by diminishing the blood flow to the tissues. The increased capillary permeability produced by radiation is extremely transient (49, 95), and its role in altering the state of oxygenation of the tissue is not clear. More applicable is the observation (1) that any generalized peripheral hypotension, such as that produced by bleeding, will result in a reduction of tumor circulation and a subsequent decrease in its oxygenation.

Another approach was tried by other workers (28) who found that anerobiosis decreased, but cold (0°C.) increased, the radiation sensitivity of tumor fragments in vitro. Conversely, later work (31) showed that chilling newborn rats to 0°C. tended to protect the skin of these animals from the damaging effects of radiation. Following this lead, cold and compression were used in an attempt to protect the skin during therapeutic irradiation (39), but the results were slight and variable.

The apparent paradoxical action of cold in the in vitro and in vivo studies may be resolved if the physiology of the two systems is kept in mind. Cold, in vivo, tends to produce anerobiosis in the skin by vascular constriction. On the other hand, the in vitro system tends to have an increased oxygen content because of a lowered metabolic demand for oxygen as a result of chilling. The oxygen content of the media is only slightly affected in the in vitro system, since the physical parameters of oxygenation are only slightly dependent upon temperature.

Metabolic inhibitors may not be classified as sensitizers and yet, indirectly, may work as sensitizing agents by virtue of their ability to raise the oxygen tension locally by decreasing metabolic requirements for oxygen. In this manner KCN, iodoacetate, and KAg(CN)2 enhanced the action of x-rays on the mouse Sarcoma 37 in vivo (34). In vitro, cold (0°C.) and cyanide increased the radiosensitivity of mouse carcinoma fragments from an LD50 of 3000 r to below 1500 r when irradiated in air (53). Anoxia in conjunction with these agents prevented the sensitization. The combination of cold (0°C.) and NaCN does not increase sensitization much above the increment produced by either alone (52). This might imply that the maximum O2 level was reached with one agent so that the second agent had no further effect.

More significantly, Hollcraft et al. (56) varied the composition of the inspired air during irradiation of the tumors and found that tumor growth was retarded to a greater degree when irradiated in an atmosphere of 95 per cent O2 with 5 per cent CO2 than in N2 or air. Interrupting the blood supply to the tumor during irradiation had no effect.

Gray et al. (48) irradiated Ehrlich ascites carcinoma cells in vitro at various oxygen concentrations and then inoculated mice with the irradiated cells. Daily paracenteses were done, and the number of chromosome abnormalities at anaphase was taken as a measure of radiation damage. When the oxygen concentration was increased from 0 per cent to 21 per cent, the radiation sensitivity increased by a factor of three with only a slight further increase in sensitivity at 100 per cent oxygen. These results are in accord with earlier observations on nonmammalian systems. When the Ehrlich carcinoma in its solid form is irradiated in the leg of the mouse, 100 per cent oxygen inhalation at the time of irradiation with 1000 r results in as much tumor regression as does irradiation with 1500 r when the animal is breathing air. However, the latter results in more epilation and skin damage. Preliminary data suggest that there is no greater response when irradiation is carried out at three atmospheres of oxygen tension than at one atmosphere. These authors do not state the exact gas concentrations in the in vivo experiments, and it may be that some CO2 was present. In neither the in vitro nor in the in vivo situations does high oxygen tension increase the sensitivity to radiation with neutrons or α-rays.

Other investigators (27) found a marked increase of radiation sensitivity of the Ehrlich carcinoma when irradiation was carried out with the
mouse breathing 95 per cent O₂ plus 5 per cent CO₂ at two atmospheres of pressure. It was also found (25) that the percentage of mitotic inhibition in tumors given 830 r while the mouse was breathing air was 32 per cent, but it was 67 per cent when pure oxygen was employed. At higher doses, when the number of abnormalities approached 90 per cent, the difference was not so marked, but it was still present. Two strains of Ehrlich ascites carcinoma, designated as 0 and 22, have been irradiated in vivo (26) by giving the mouse 200 r total-body irradiation. Strain 0 was a radiation-sensitive strain and showed twice the number of chromosome fragments when irradiation was carried out with the animal breathing pure oxygen; however, there was no significant increase in the number of bridges, suggesting that the increased oxygen tension does not increase the number of chromosome breaks but interferes with the rejoining of fragments. Strain 22 was a radiation-resistant strain, and the number of chromosome fragments produced was unaffected by the oxygen tension of the inspired gas at the time of irradiation.

Reports on the clinical use of combined oxygen and radiation therapy are limited. Four patients were irradiated while breathing 100 per cent oxygen at atmospheric pressure (64); one half of the lesion was irradiated while the patient breathed room air and the other half while pure oxygen was breathed, and in each case there was greater regression with pure oxygen, but the skin reaction was also more pronounced. Eight patients were treated with x-rays while oxygen was administered at three atmospheres of pressure (17), and again one half of the same tumor was first irradiated while air was breathed at normal pressure. In six of the eight patients so treated, there was histological evidence of greater radiation damage in the part of the tumor irradiated during the positive pressure oxygen therapy.

It is obvious that the oxygen tension must be increased in the tissue and not merely in the inspired gas. Since no satisfactory method has been devised for the measurement of oxygen tension in the tissue, little is known about the factors determining the partial pressure of oxygen at the cell. Gray (48) has calculated that in normally respiring tumor the oxygen tension may fall from the normal value of 40 mm. of Hg found at the venous end of the capillary to 0 mm. of Hg within a distance of 150 μ.

Both normal and neoplastic cells of squamous epithelium grow in contact with one another, and they receive nutrient from capillaries in the stroma which do not penetrate between the cells. Bronchial carcinomas arising from these cells tend to grow in rods with central areas of necrosis so that histological sections cut at right angles to these strands show necrotic regions surrounded by a ring of tumor cells. Thomlinson and Gray (142) have measured the diameters of the areas of necrosis and the width of the tumor bands. The average width of these bands was found to be 109 μ and was independent of the diameter of the tumor strand; however, the diameter of the necrotic area increased linearly with the over-all diameter. This correlation with the theoretical value suggests that oxygen tension may be the controlling variable in the production of tumor necrosis, but these investigations do not rule out the possibility that catabolite accumulation may play a significant role.

The diffusion of oxygen into the tissue is directly related to the diffusion constant and to the environmental oxygen tension but inversely to the metabolic rate (52, 53). The time for establishing equilibrium between the oxygen in blood and in tissue must also be taken into account, and this time may be longer than has been appreciated. In addition to these local factors that influence O₂ concentrations, it should be remembered that hemoglobin carries the vast majority of the oxygen to the cells even when moderate pressures of a few atmospheres are used. It is necessary that the hemoglobin be able to transfer the oxygen to the cell. Perturbations of the oxygen-unloading mechanism may alter the tissue oxygen tension.

Many more factors no doubt control tissue oxygen tension, but it is clearly complex; however, its very complexity makes many variables available for experimental alteration.

Synkavit.—Originally, work was undertaken with synthetic vitamin K, tetra-sodium-2-methyl-1,4-naphthohydroquinone disphosphate (Synkavit), because it was thought that this material might modify nucleic acid metabolism. However, it was noticed that in conjunction with x-rays it potentiated the radiation damage in both chick fibroblast tissue cultures and in a human tumor (97, 105), but at that time no particular therapeutic value was claimed for this combination. A subsequent paper (96) analyzed the results of a larger group of 116 patients, all with advanced malignant tumors other than bronchial carcinomas. The treatment program was palliative irradiation alone, intramuscular Synkavit alone, or both together. At least 23 out of 73 patients receiving the combined treatment showed "an unexpectedly good palliative response" (44). In the combined treatment of inoperable bronchial carcinoma, the mean survival time was increased to 7.6 months, compared with 4.2 months for palliative radio-
therapy alone (97). Additional papers (68, 69, 99) reported a larger number of patients with subjective and objective improvement following therapy with x-rays and Synkavit. The effect of combined Synkavit and x-rays, with O₂ inhalation for 15 minutes before and during radiation, has been tried, and it was noted that the skin and general reactions were more severe than those after x-rays alone (100).

A more detailed study (101) was carried out on patients with inoperable carcinoma of the bronchus. Synkavit was given intravenously or intramuscularly, and patients who received the compound had a mean survival time of 8.7 months from the onset of treatment as compared with 3.8 months for those receiving x-ray therapy alone. Groups of patients receiving the drug by either route were considered together even though animal experiments had indicated that intramuscular administration was without effect. A similar series of patients (108) was observed in which the "controls" were given intramuscular Synkavit. The results were similar to those in the earlier report. The authors conclude (98) that the combination of x-ray and Synkavit results in a small increase in the survival time over that following x-ray alone in cases of carcinoma of the bronchus.

Synkavit plus x-rays reduced the mitotic figures in chick fibroblast cultures to 14 per cent of the number in untreated controls, with Synkavit alone exerting almost the same effect as x-rays alone (69.8 per cent versus 64.9 per cent) (105). With Walker rat carcinosarcoma 256 used as the test tumor, it was found that the combination therapy increased by about 25 per cent the number of regressions over those caused by 1100 r of x-ray alone (104). The optimum results were obtained with the intravenous injection of Synkavit about 30 minutes before irradiation (102). In addition, it was demonstrated that Synkavit plus O₂ plus x-rays slightly increased the radiosensitivity of the Walker 256 rat carcinosarcoma (108). As far as the mode of action of Synkavit is concerned, it is postulated (100) that the Synkavit is selectively concentrated by tumor cells, especially by the mitochondria, and that its action is exerted there.

Other investigators have not confirmed the above results with animal tumors. It was found that neither Synkavit nor its 2-methyl derivative had any significant effect on the growth of Jensen rat sarcoma alone, or in combination with x-rays (37). In an attempt to confirm Mitchell's thesis of radiation sensitization with Synkavit, with the use of Synkavit obtained from Professor Mitchell, the work was repeated with the Walker 256 carcinosarcoma (140). It could not be demonstrated in a large series of animals that Synkavit acts as a sensitizer to ionizing radiation.

**Dyes.**—Early attempts at sensitization came with the introduction of dyestuffs in combination with radiation. Dyes were utilized, because they were thought to concentrate in malignant tissue to a greater extent than in normal tissue. However, recent work (107) has cast some doubt upon this. Radioactively tagged dyes were found to be highly concentrated in kidney, liver, spleen, feces, and to some extent in tumor, so that the desired selective concentration is not obtained. The earlier impression of tumor concentration occurred because tumor tissue is usually light in color; and, therefore, when estimated visually, the dye was indicated out of proportion to its actual concentration. Previous workers were not aware of this fact and believed that the dyes concentrated specifically in the tumor. The concentration of radioactively tagged dye can be measured independently of tissue color. Clinical attempts were made to treat cancer more effectively by using "activated" fluorescein, i.e., fluorescein plus x-rays (19), on the assumption that the dye produced a "secondary" radiation that had a lethal effect on tumor cells. Later papers (20, 46) gave complete details regarding administration of the dye in patients with various diagnoses, including carcinoma of the breast and esophagus and soft-part sarcomas. The follow-up in many of these cases was inadequate and too short for general conclusions; however, in at least one case, one half of a lesion was given combined therapy and the other radium gamma irradiation alone with more marked regression in the portion given combined treatment. Other authors (141) did not obtain improved results with dyes.

A slow-growing rat sarcoma irradiated by radium gamma rays and exposed to fluorescein produced fewer tumor "takes" upon subsequent implantation than did the sarcoma treated with radium alone (109), indicating an increase in radiosensitivity. Fluorescein itself had no effect on the tumor. Only nine rats were used in this experiment, but the results were rather definite. Others found similar results (74, 123) with rat sarcoma F16, Jensen rat sarcoma, and Kato rabbit sarcoma, using various dyes and x-rays.

The experimental evidence in favor of combined tumor therapy with dyes and radiation is somewhat equivocal. Modern experimentalists do not seem inclined to follow these early leads, and little work appears in the contemporary literature.

**Inorganic salts.**—Following the increased interest in the biological effects of various salts upon protoplasm, it was suggested (9) that NaCl be administered prior to x-rays without any reasoning.
other than that it might “stimulate” the cells. However, no experimental observations were reported.

Subsequently, it was noticed (28) that the respiration of Jensen rat sarcoma in vitro decreased 20–30 per cent in phosphate Ringer’s solution compared with bicarbonate Ringer’s. Tumor pieces irradiated in phosphate Ringer’s produced fewer “takes” upon implantation than did pieces irradiated in bicarbonate Ringer’s, and the same effect was found with mouse carcinoma.

Others (29) attempted sensitization in vivo using various ions. Magnesium salts did not significantly increase the x-ray sensitivity of an unspecified rat sarcoma, but calcium chloride did. The number of regressions depends on the time elapsed from injection to irradiation, decreasing after 30 minutes subsequent to CaCl2 administration. The authors postulated that the radiosensitivity was affected in some “catalytic” manner. Other investigators (98) found that an increase in the number of regressions could be achieved with concomitant x-ray irradiation, and subsequent to CaCl2 administration. The authors postulated that the radiosensitivity was affected in some “catalytic” manner. Other investigators (98) found that an increase in the number of regressions could be achieved with concomitant x-ray irradiation and magnesium sulfate injection in the tumor.

Glucose and insulin.—An early clinical observation on tumor therapy with intravenous injections of 25 per cent dextrose solutions and x-rays seemed to indicate that the tumor is thereby sensitized to x-rays (94). Others (50) reported four cases that showed a better x-ray response with dextrose. Likewise, rat tumors showed an increased radiosensitivity with intraperitoneal dextrose (120). Increasing sensitivity was demonstrated with the Kato rabbit sarcoma when treated with dextrose or with dextrose and cesium iodide injections followed by irradiation (66). A regimen of dextrose and x-rays followed by insulin was tried clinically and in certain cases appeared to be of benefit (116).

The local application of insulin to a neoplastic ulceration of the breast resulted in prompt palliative relief. Subsequent x-ray treatment resulted in a much more favorable response than was obtained from a prior course of radiation before the local application of insulin (98). The claim was repeated subsequently without additional data (44). Encouraging clinical results were obtained by others who sought to enhance the radiation effect by insulin in addition to blood transfusions (42). Quantitatively, insulin injections increased the percentage of regressions of rat tumors from a value of 18.8 per cent for x-ray alone, to about 52 per cent (30). Another attempt at local application of insulin resulted in an increase in the radiosensitivity of several human tumors (48).

It is possible that changes in the carbohydrate and electrolyte metabolism of tumor tissue may increase the tumor radiosensitivity, improve the general resistance of the host or in some other way potentiate the effectiveness of radiotherapy. However, very little is known about the mechanisms involved, and the reports reviewed on the use of glucose, insulin, and electrolytes for increasing the effects of tumor radiotherapy are 15–20 years old, so that this whole field is obviously in need of a thorough re-investigation and re-evaluation.

CARRIERS, SECONDARY RADITORS, AND THERMAL NEUTRON CAPTURE

Many attempts have been made to increase selectively the dose delivered to the tumor. Three major lines of investigation have been used: (a) Heavy metals have been administered in the hope that these would concentrate in tumors and hence increase the tumor dose by soft secondary radiations produced by more energetic primary, external beams. (b) If compounds could be found which would be taken up more in tumors than in normal tissue, these could serve as carriers of radioactive isotopes. (c) With the advent of high intensity neutron beams, nuclear reactions within tumors can be achieved if a compound which will react with neutrons in the desired manner can be localized in the tumor.

Secondary radiators.—One of the earliest attempts to enhance radiation sensitivity of tumors by chemical means was the use of colloidal heavy metals. If tumors concentrated heavy metals, irradiation with an external beam would produce secondary radiations from these elements of high atomic number. Such secondary radiation would have low energy and would be absorbed within the tumor with a resultant increase in tumor dosage and no increase in dosage to surrounding normal tissue.

Many metal colloids have been used, including cobalt (75, 76), copper (14), bismuth (70), and lead (6–8, 130, 148). Clinical impressions varied as to the merits of this treatment but were, in general, favorable. The numbers of cases in all series were small, and no controls were used, so that no objective conclusion can be made.

However, Todd and Aldwinkle (145) reported in twelve cases which were given an extensive course of lead selenide therapy followed by x-ray or radium, the results were “nothing less than disastrous.” Radiation was stopped at one-quarter to one-half of what they considered to be a normal dose, at which time there was ulceration, fistula formation, and rapid progression of disease. They conclude that either the secondary radiation from the lead is so severe as to cause the side effects or, more probably, that the colloidal selenide par-
tiles are reduced in size by the radiation and become more toxic, both in the tumor and stromal cells. However, excellent results in two cases were reported by Gosse and Mottram (45).

Sperti and Norris (132) extensively treated the theoretical aspects of increasing the radiation dose to tumors by concentrating metals within them. Fragments of a mouse sarcoma were irradiated in vitro following the intravenous injection of CaI to the donor animals, and the number of subsequent successful transplants was used as a criterion of the extent of radiation damage. Fewer "takes" were found at every dose level of x-rays employed. Intravenous injection of colloidal lead enhanced the action of x-rays on the Flexner-Jobling rat carcinoma but showed no increased effect when rat Sarcoma 10 was used (153). It is interesting to note that part of this positive effect was interpreted as occurring because of thrombosis of the arteries and impairment of the blood supply. This concept is substantiated in part by the work of Mottram (108), who found that systemic administration of colloidal lead, followed by irradiation, showed no advantage over radiation alone and resulted only in a reduction in the growth rate of the tumor. However, postirradiation treatment with lead showed a marked effect, and some tumors regressed completely.

Other metals, ranging in atomic number from aluminum to uranium, have been used with equivocal results (65, 73, 89). Moreover, animal work confirmed the clinical experiments of Todd (145) that prior treatment with lead selenide was contraindicated. Indirect support of this thesis was reported by others (51) who used colloidal bismuth and platinum.

**Carriers.**—If compounds could be found which were selectively taken up by neoplastic tissue, radioactive isotopes could be incorporated into them, and, hence, large doses of radiation could be given to the tumors. Two important facets of this concept should be stressed. First, the chemical compound need not be carcinostatic in itself, though any tumor action it might have would be advantageous. Second, these compounds could also be taken up by distant metastases, whether clinically detectable or not. This latter point represents an advantage over other forms of combined therapy insofar as this type of treatment would include the tumor foci which lie outside the beam of external radiation.

Several investigators tried to inhibit tumor growth by the injection of naturally occurring radioactive substances such as RaE in combination with colloidal Bi (80), ThB (64), and radioactive Pb (86). None of the experiments showed any effect on the tumors, though autoradiographs showed uptake in the kidney, liver, and spleen.

Zahl and Waters (156) suggested that certain acid dyes that localize in tumor tissue to some degree might be used as carriers of radioactivity; however, they report no experimental work along these lines. Radioactive Br was incorporated into several diazo dyes by Tobin and Moore (144) in such a way that the bond was stable and nonionizable. They showed that these dyes concentrated in inflammatory lesions (106) and in tumors (107). The radioactive dyes were given intravenously to a large number of mice carrying several types of tumor. The quantity of dye present in the organs and tumor was determined by the radioactivity present. By this method the true amount of dye present is analyzed, and the quantity is not obscured by natural pigmentation of the organ. Results are expressed as the percentage of injected dose per gram of tissue. In tumor-free animals, the liver, colon, small intestine, bile, and feces accounted for about 50 per cent of the injected dose. Since tumor concentrations were seldom higher than the liver concentrations, the results of dibromo-trypan blue injection expressed as the tumor-liver ratio in C5H mice varied from 0.14 to 0.62 for different tumors tested. Tumor-liver ratios found after dibromo-Evans blue injection ranged from 0.29 to 0.66 in various mouse tumors.

The two dyes show about the same concentration in tumors, but the tumor-liver ratio is lower for dibromo-trypan blue because of greater liver concentration. On occasion, tumor-liver ratios greater than 1 are found. These cases occur in cachectic animals with large tumors where the liver uptake is small, presumably owing to impaired hepatic function.

Necrotic areas of tumors which were filled with clear fluid or which were caseous showed little staining, whereas hemorrhagic necrosis tended to pool the dye from the plasma. The authors conclude that their data cast doubt on the earlier enthusiastic reports of dye concentration, but the fact that two closely related dyes have a different uptake by the liver suggests the possibility that a more differentially concentrating substance can be found.

Similar experiments were performed by Stevens et al. (133) using I131-labeled trypan blue prepared by the method of Bloch and Ray (11), who synthesized a number of I131-iodinated dyes with the hope that they might selectively concentrate in neoplasms, particularly gastric cancer. The dye was injected intravenously into Swiss albino mice carrying two mammary carcinomas 15091a of different size. The I131 content of both tumors was pro-
portional to the amount injected, but no striking difference was found in the concentrations in the large and small tumors. As was reported by Moore et al. (106) for bromine, the concentrations of I\(^{131}\) were highest in the liver, kidney, spleen, and feces. The tumor:liver ratios were lower than those reported above.

Bloch and Ray (11) and Kremen and co-workers (78) studied the distribution of S\(^{34}\)-labeled methionine in normal and neoplastic tissue in mice following intraperitoneal injection. Distribution was studied in normal AK mice, AK mice with either transplanted lymphosarcoma or spontaneous leukemias, and in JJB mice bearing spontaneous mammary carcinoma. In all instances the concentration was highest in the liver and intestine than it was in the tumors.

The only therapeutic trial of radioactively tagged dyes is reported by Sloviter (129), who used I\(^{131}\)-iodinated Nile blue A, a basic oxazine dye, which diffusely stains the tumor cells themselves in contrast to the stromal staining properties of Evans blue and trypan blue. C3H mice were given inoculations of either a transplantable fibrosarcoma which had been originally induced with methylocholanthrene or of a transplantable mammary carcinoma. Average survival time of animals carrying the fibrosarcoma was 20 days for the controls and 51 days for the animals receiving the radioactive dye parenterally. Mice with the mammary carcinoma had average survival times of 25, 36, and 74 days, respectively, for controls, nonradioactive dye, and I\(^{131}\)-tagged dye. The dye was mixed with the food. It is unfortunate that the values for the control survival times are taken from the literature and that the amount of radioactivity administered is not stated, nor is it possible to calculate the dose of radiation because of incomplete knowledge of the time interval between the preparation of labeled dye and the beginning of therapy.

In 1950, Pressman and Eisen (118) described a technic for attaching one to two atoms of I\(^{131}\) per molecule of antibody of molecular weight 160,000. Using I\(^{131}\)-labeled antibodies, Pressman and Kornfeld (119) showed that there could be produced in rabbits antiseras that would localize in tumors, liver, and kidney of mice bearing the Wagner osteogenic sarcoma. They found some indication that by purification methods some of the tumor-localizing antibodies could be separated from the liver- and kidney-localizing antibodies. These same investigators (77) found similar results with the Murphy lymphosarcoma in the rat. While I\(^{131}\) was used only as a tracer in these studies to detect the presence of antibodies, an increase in the degree of iodination and specificity of these antibodies could provide a method of bringing therapeutically significant quantities of radiation to both the primary tumor and metastases.

**Nuclear reactions within tumors.**—Immediately after the discovery of the neutron in 1932, work was initiated on its biological effects. In 1936, Locher (86) pointed out that the absorption of low energy or thermal neutrons did not depend upon the atomic number, as with x-rays and gamma rays, but varied widely depending upon the nuclear structure of the absorbing element. While the absorption of neutrons by the common elements of tissue is small, he suggested that the highly absorbing elements might be introduced into or concentrated by tumors. Even at very low concentrations, this could result in the absorption of a large amount of energy of a thermal neutron beam within the tumor. Two of the elements which have high absorption properties are Li\(^+\) and B\(^{10}\); they react with neutrons according to the nuclear equations:

\[ ^{10}B(\alpha,\gamma) Li^7 + 2\alpha + 2.8 \text{ Mev} \]

and

\[ ^7Li(\alpha,\gamma) He^4 + 4.6 \text{ Mev} \]

It should be pointed out that it is these two isotopes of lithium and boron that have a high probability of interacting with neutrons. Li\(^{7}\) is only 7.5 per cent of normally occurring lithium, and B\(^{10}\) is 18.4 per cent of naturally occurring boron; however, methods are now available to concentrate these isotopes so that “enriched” lithium and boron containing up to 85 per cent of these isotopes can be prepared.

These physical considerations led Kruger (79) in 1940 to the irradiation of tumors in vitro. A spontaneous and differentiated mouse sarcoma, a mouse mammary carcinoma, and a mouse lymphoma were irradiated with a thermal neutron beam produced by a cyclotron. A control sample of tumor was exposed to a solution of H\(_2\)BO\(_3\) but was not irradiated. Two other tumor fragments also were placed in the boron solution. One of these was exposed directly to the thermal neutron beam, and this would be the sample which would receive the highest dose from the neutron-boron reaction. The other boron-containing tumor fragment was also exposed to the cyclotron beam but was shielded by a B\(_2\)C shield which effectively stops the thermal neutrons. However, the neutrons and gamma-ray contaminants of the beam are not stopped by such a shield, and, therefore, this sample served as a control to measure the damage produced by these higher energy radiations. In some instances a nonboron-containing sample of tumor was exposed to...
the thermal neutron beam. Irradiated tumors were then transplanted to appropriate hosts, and the failure to "take" was used as a criterion of radiation damage. Until very high doses of thermal neutrons were reached, there was no difference between the number of "takes" in the nonirradiated controls and the boron-shielded but boron-containing samples. Also, in those instances in which a nonboron-containing specimen was irradiated, the number of "takes" was the same as in the controls until very high doses were reached. The number of "takes" was greatly reduced, and this effect increased with increasing exposure. This showed quite clearly that the lethality of thermal neutrons to neoplastic cells could be greatly enhanced by boron.

Even in in vitro work the problem of dosimetry is very difficult, from both the biological and the physical point of view, and it is not completely solved today. With in vivo experiments, dosimetry becomes even more difficult because of the lack of knowledge of the boron concentration in the tumor at the time of exposure. Moreover, the α-particles, which produce the ionization, have very short ranges in tissue at these energies. Therefore, more exact information is needed to determine the intracellular-extracellular partition of boron compounds.

Zahl et al. exposed mice bearing Sarcoma 180 to sublethal doses of total-body irradiation with slow neutrons (155). Experimental groups had an oil suspension of finely pulverized lithium metaborate injected into the tumors. This technic kept the boron and lithium atoms localized in the tumor during the long exposure times used. Whether, with the short range of the secondary α-particles, there was a sufficient amount intracellularly to be effective is questionable. Boron-injected mice had some regressions of tumors without irradiation, but the irradiated animals had an even greater regression rate. With the use of the survival curves for animals treated with neutrons or x-rays and the data of Sugiura for the dose-response curve of Sarcoma 180 to local x-rays (186), the authors estimate the presence of boron and lithium had the effect of 350–600 r.

Theoretical considerations of whether Li, B, or the pure isotopes Li⁶ and B¹⁰ offer the best increase in differential between tumor and surrounding tissue have been discussed by Zahl and Cooper (154). They used lithium carmine, which had previously been shown to concentrate in tumors. When injected into mice, lithium was found at a maximum concentration of 0.08 per cent in the tumor 4 hours after injection. The appearance of lithium was more rapid and was found in greater concentration than would be predicted from the observed concentration of dye, suggesting a selective uptake of inorganic lithium. Using these theoretical considerations, they concluded that there would be a gain of 43 per cent in the dose to the tumor over the surrounding normal tissues. Unfortunately, they report no radiation experiments.

No further animal work, by these technics, has been reported. However, in 1951, Sweet (188) pointed out that the concentration of P³² and K⁴² in brain tumors was much higher than in either the gray matter or the white matter and that these isotopes might be used for diagnostic localization of these lesions. At this time, he suggested that the blood-brain barrier might be utilized for the concentration of boron in tumors for slow neutron therapy. Sweet and Javid (189) showed that 15 gm. of borax can be given intravenously without toxic effects, that concentration ratios of greater than 3:1 can be achieved in brain tumors compared with normal tissue, and that in some instances the ratio can be as high as 48:1. Tumor concentration at these doses is greater than 50 μg B/gm of tissue; it is 15 μg B/gm in the gray matter, and even less in the white matter. The maximum concentrations are achieved within 10 minutes. On the basis of these findings and according to certain assumptions about the relative biological effectiveness of alpha particles and gamma rays, Javid et al. (67) concluded that boron would account for 85 per cent of the radiation effect in gray matter, 79 per cent in white matter, and 94 per cent in tumor, if the incident beam is a pure thermal neutron one. Thus, with the concentration ratios observed earlier, at least a 3X higher dose could be delivered to the tumor than would be given to the gray matter.

Clinical trial of neutron capture therapy currently is being done at Brookhaven National Laboratories by Farr et al. (34). The physical setup and the dose distributions have been described by Stickley (134). Farr et al. (35) have reported preliminary clinical experience with ten cases of glioblastoma multiforme. The ten patients were given 20 gm. of B⁴⁰-enriched borax 10 or 15 minutes before being irradiated with a dose of approximately 10¹² neutrons/sq. cm. at the skin surface with a thermal neutron beam from the Brookhaven pile. Five of the patients received more than one irradiation. Of the 21 irradiations, eight resulted in temporary palliation, six patients showed questionable improvement, and seven showed no improvement. Eight of the ten cases have come to autopsy, and, in three of the five cases having multiple courses of radiation, some changes were seen in the normal brain; however, nothing could be said about radiation effects in the tumor itself (41).
Though not done on tumors, the work of Tobias et al. (143) deserves special mention, because it introduces a new concept. The capture of a thermal neutron by Li$^6$ or B$^{10}$ results in the release of 4.6 and 2.8 Mev of energy, respectively, most of which is given to the resultant $\alpha$-particles. Capture of a thermal neutron by U$^{238}$ results in the liberation of 159 Mev of energy distributed among the fission products which are much more densely ionizing and hence may have a relative biological effectiveness which is 3–4 times that of the $\alpha$-particles. UO$_2$ colloid prepared from U$^{238}$-enriched uranium, given by vein to mice, concentrated primarily in the liver and spleen. When these animals were given sublethal doses of thermal neutrons, all died within 3 weeks. Animals given comparable amounts of nonenriched UO$_2$ (not fissionable) and exposed to the same thermal neutron-flux were alive and well after 6 weeks. Similarly, animals given comparable doses of either enriched or regular UO$_2$ did not die within 6 weeks. To be sure that it was not the enriched UO$_2$ plus the stress of radiation which caused death, a group which had received the enriched UO$_2$ was given a comparable dose of x-rays, and no deaths occurred. The authors conclude that a dose of radiation from the fission products of U$^{238}$ was the cause of death.

Clinically, the animals which had received enriched UO$_2$ followed by irradiation showed the signs of the radiation syndrome. At autopsy the spleens were markedly shrunken and gray or black in color. The livers were pale, some having a mottled appearance, and some showed areas of necrosis or hemorrhage. None of the control animals showed similar effects.

**DISCUSSION**

Investigation of the effects of combined chemical and radiation therapy of cancer has been mainly empirical, and many of the theories which exist have been reached a posteriori.

Some alkylating agents (nitrogen mustards, TEM) in several instances have shown results indicative of potentiation of the radiation response. Purine antagonists and antifolics in combination with radiation have been explored very little, but the results obtained with 6-mercaptopurine seem to justify further work in this field (140). Combinations including colchicine and its derivative, N-deacetylthiocolchicine, have been studied much more extensively but did not produce significant improvement of the therapeutic results. Some possibilities of success may exist in the field of antibiotics, e.g., actinomycins, azaserine, 6-diazo-5-oxo-N-leucine (DON), but only a little work has been done in this direction. These empirical investigations should be continued as newer chemotherapeutic agents become available.

Information is being gained about the fundamental processes involved in the transfer of energy from the primary ionization produced by the incident radiation to the biologically significant portion of the cell. The a priori theories arising from this knowledge should result in a greater ability to alter the radiosensitivity of tumors than has been achieved previously. The role of the oxygen tension at the cellular level in determining radiation sensitivity has been studied in a wide variety of biological systems, including tumors, and has been found to be quantitatively the same. Preliminary results of treatment with x-rays and high oxygen tension in the inspired gases have been encouraging, and further investigations should be pursued. Methods of measuring the tissue oxygen tension are inadequate, and this represents one of the major difficulties. As was mentioned earlier in this paper, many factors influence the oxygen tension at the cellular level, and increased knowledge is needed about cellular permeability to oxygen, the vascular structure of tumors, and their hemodynamics.

Gray (47) points out that, though the radiomimetic drugs produce cytological damage that is similar to that from radiation, their toxicity is not a function of oxygen tension and their modes of action must be different. Thus, it is possible that both oxygen and carcinostatic agents in combination with radiation might have a marked effect on neoplasms without increased systemic reactions.

Secondary radiators are probably of little value, and certain colloids, such as lead selenide (145), are probably contraindicated. However, much of this work was done before modern methods of biological research had been developed, and some of the concepts may bear re-investigation. The use of B$^{10}$ and Li$^6$ with external thermal neutron beams shows promise, particularly if they can be incorporated into compounds which will selectively concentrate in tumors. Any compound which could do this, whether carcinostatic or not, could be used as a carrier of radioactive isotopes to both the primary growth and to the metastases. If any fissionable element could be incorporated into such a compound, the work of Tobias (143) offers interesting possibilities.

In general, while no spectacular results have emerged from the combined chemical and radiation therapy of malignant neoplasms, enough encouraging results have been obtained to warrant continued investigations, particularly in view of the increasing knowledge of fundamental radiobiology and the availability of new radiation sources.
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