

Photoradiation Therapy for the Treatment of Malignant Tumors¹

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ABSTRACT

Administration of hematoporphyrin derivative i.v. followed by local exposure to red light has resulted in complete or partial response in 111 of 113 cutaneous or s.c. malignant lesions. Tumors treated have included carcinomas of the breast, colon, prostate, squamous cell, basal cell, and endometrium; malignant melanoma; mycosis fungoides; chondrosarcoma; and angiosarcoma. No type has been found to be unresponsive. In several cases complete clearing of chest wall metastasis has been achieved in treated areas. Deep-seated and pigmented tumors required a higher dose of drug for effective treatment than did the more superficial and nonpigmented lesions. A high therapeutic ratio between tumor and skin response has been obtained by allowing at least 3 days between drug injection and exposure to the therapeutic light for 2.5-mg/kg doses and at least a 4-day interval for 5.0-mg/kg doses.

INTRODUCTION

The combination of HPD³ and red light (photoradiation therapy) is curative for a number of experimental tumor systems. For example we have reported previously nearly 50% long-term cures in the isogenic DBA/2 Ha-SMT mammary tumor system in mice (5). The success of this technique is due to the ability of HPD to accumulate (and/or be retained) to a higher degree in malignant tissue than in most other tissues and its ability to produce the cytotoxic agent, singlet oxygen, when activated by red light (photodynamic process) (16). These properties of HPD have been known for some time. Hematoporphyrin and HPD have been used extensively to aid in identification and localization of human cancers since their red fluorescence can be observed readily upon activation with blue light. Apparent selective HPD uptake has been demonstrated in a wide range of human and animal tumors (7, 12, 17). The ability of hematoporphyrin and HPD to act as photodynamic agents has been demonstrated in several systems (13). We have shown recently that the cytotoxicity of activated HPD probably results from intracellular formation of singlet oxygen (a short-lived, highly reactive state of the oxygen molecule) when cells containing the dye are exposed to visible light (16).

The combined properties of tumor localization and photoactivated toxicity of certain porphyrins provide the possi-

bility of achieving a relatively localized tumoricidal effect with sparing of surrounding normal tissue.

The earliest attempt to use the photodynamic effect for treatment of human tumors was by Tappenier and Jesionek in 1903 (14). Although favorable results were reported with eosin as the photosensitizing dye, no further reports were forthcoming. Diamond *et al.* (1) reported in 1972 that the crude hematoporphyrin, activated by white light, caused regression of an experimental glioma in rats.

At about the same time, we reported that fluorescein, activated by 488 nm light, could be used in a similar fashion to treat an experimental mammary tumor in mice (2, 3). Later we reported that cures could be obtained in experimental tumor systems with the use of HPD activated by red light (5). Tomson *et al.* (15) reported in 1975 that acridine orange, activated by an argon laser, caused destruction of a mouse epithelial tumor. Kelly *et al.* (10) showed, in the next year, that specific destruction of a heterotransplant of human bladder carcinoma in mice could be obtained by local exposure to white light following HPD injection.

Because of the success in experimental animal tumor systems, we have initiated a clinical study of the efficacy of photoradiation in the treatment of various human tumors. We report here our results in the first group of 25 patients with cutaneous and s.c. tumors. A recent report of treatment of a single patient with bladder carcinoma by a method similar to ours has been reported by Kelly and Snell (9).

MATERIALS AND METHODS

HPD was prepared by a method similar to that of Lipson *et al.* (11). Hematoporphyrin hydrochloride (Roussel Corp., New York, N. Y.), 1 part, was dissolved in glacial acetic acid:sulfuric acid (19:1, by volume) and allowed to stand overnight at room temperature. The mixture was filtered by gravity through a Whatman No. 1 filter paper (1 to 3 hr depending on total volume) and neutralized to approximately pH 6.0 by addition of 3% sodium acetate solution. The precipitated material was recovered by filtration and washed extensively with distilled water until the washings were neutral. The solid was then dried in a vacuum to remove all traces of acetic acid and water and was stored in the dark in the solid state at -20°. This material had 3 components detectable by thin-layer chromatography (Brinkman Polygram SIL-N-HR plates; benzene:methanol, 4:1, by volume) in a ratio of 15, 30, and 55% in increasing R_F. The material with the lowest R_F (15%) corresponds to a major peak in the crude hematoporphyrin hydrochloride. The other 2 components have been identified as a monoacetate (30%) and diacetate (55%) by using [¹⁴C]acetic acid to

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³ The abbreviation used is: HPD, hematoporphyrin derivative.

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prepare HPD and by isolating the individual components by thin-layer chromatography.

An injectable solution of HPD was prepared by mixing 1 part HPD with 50 parts by volume of 0.1 N sodium hydroxide and stirring at room temperature for 1 hr. The pH was brought to 7.2 to 7.4 by addition of 0.1 N hydrochloric acid (approximately 15 parts), made isotonic by addition of sodium chloride, and finally brought to 200 parts total with 0.9% sodium chloride solution. The final solution of 5 mg/ml was sterilized by Millipore filtration and tested for sterility and pyrogenicity. The solution was stored in the dark at -20° until used. Thin-layer chromatography as previously done indicated that none of the acetates survived the sodium hydroxide treatment and that only a single component could be detected corresponding to the component with the lowest R_F. We are currently attempting to identify this material. If these procedures are carried out as indicated, a reproducible material of constant biological activity is obtained.

HPD blood concentration was determined spectrophotometrically (with the 500 nm absorption peak, common to all porphyrins, and a standard curve of absorbance at this wavelength versus concentration of HPD added to fresh human serum) and was found to have a clearance half-life of approximately 25 to 35 hr for a dose of 2.5 or 5.0 mg/kg body weight.

Measurement of Penetration of Red Light in Tissue. A solid piece of rhabdomyosarcoma tumor (8) (approximately 1 cu mm) was transplanted by trocar into the axilla of inbred female WAG/Rij rats and allowed to grow to 5 to 6 cm in diameter. These tumors are relatively nonnecrotic even at this size. The tumors were excised and kept moist in 0.9% sodium chloride solution while used. A fiber optic (0.8 mm; DuPont Crofon) was fed into an 18-gauge needle and then inserted to a depth of approximately 1 cm into the tumor. The protruding end of the fiber was aligned with the beam from a 2-milliwatt helium:neon laser (Spectra-Physics Corp., Mountain View, Calif.) emitting at 632.8 nm. A second fiber similarly arranged was inserted to abut as closely as possible to the first. The distal end of this detector fiber was fed into the detector head of an Oriel Model 7010 radiometer. This reading was taken as 100% transmission. The detector fiber was then withdrawn in known amounts, and readings were recorded as a function of distance between the inlet fiber and the detector fiber. The readings were made 3 or 4 times, and an average value was determined. The results of 4 separate experiments are shown in Chart 1. Such *in vitro* experiments discount the effects of circulatory blood.

In another series of experiments, an actinometric (photochemical) technique was developed based upon the consumption of 1,3-diphenylisobenzofuran in the presence of HPD activated by red light as we have previously described (4). The light source for these experiments was a 500-watt projector fitted with a Corning 2418 filter to pass wavelengths over 600 nm. The E₆₂₀ of HPD is 1500 in ethanol solution or water. The initial rate of consumption of the furan upon exposure of the solution to light was followed spectrophotometrically at 398 nm and was reproducibly constant at constant concentrations of HPD and furan,

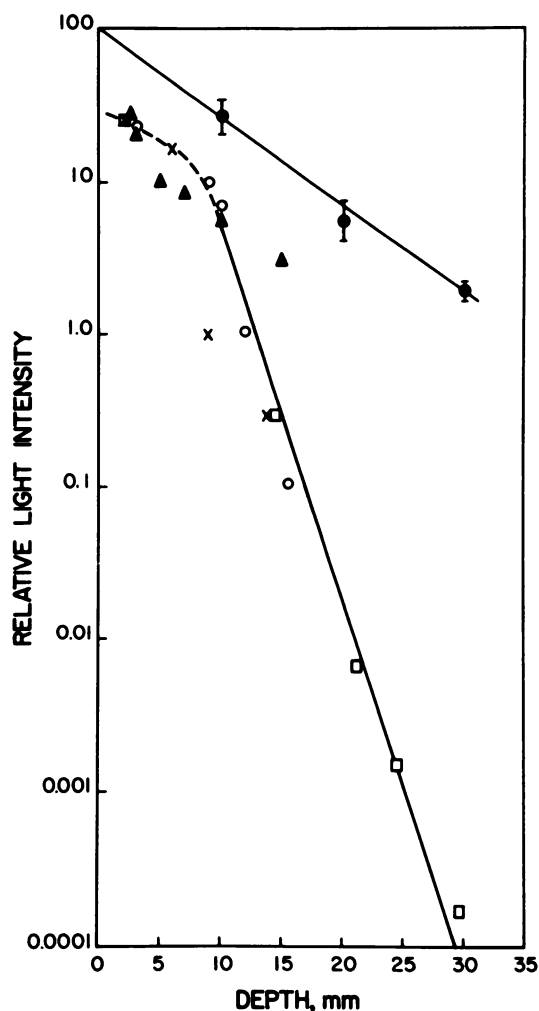


Chart 1. Relative light intensity of 632.8 nm light at various depths in excised tumor tissue (rhabdomyosarcoma grown in WAG/Rij rats). O, x, □, ▲, separate experiments with fiber optics imbedded into the tumor as described in the text. ●, actinometric results, 600 to 700 nm. See text for details. Bars, S.E.

varying only with light intensity as measured by means of the Oriel radiometer. In a typical experiment 1-mm (inside diameter) capillary tubes were filled with 10 μl of an ethanolic solution of the furan (1 mg/ml) and HPD (0.75 mg/ml) and placed at a known distance from the light. Samples taken periodically were diluted into 3 ml of ethanol, and the absorbance at 398 nm was determined. Furan consumption was directly proportional to light intensity. These control experiments indicated no furan consumption in the absence of light or in the absence of HPD. Capillary tubes containing the same solutions were then placed at various depths within the excised rat tumors, the tumor was exposed to the light, and again the initial consumption rate of furan was determined as a function of distance within the tumor. Since the furan loss was directly proportional to light intensity, its initial consumption rate within the tumors was assumed to be proportional to the total light flux at that point. The data, normalized to 100% light transmission at zero depth, are shown in Chart 1.

Therapeutic Light Source. A 5000-watt xenon arc lamp (Schoeffel Instrument Corp., Westwood, N. J.) was fitted

with a 6-inch water filter, an IR-reflecting mirror (Baird Atomic 34-01-2), 2 IR-absorbing filters (Corning CSI-75), and a red cutoff filter (Corning CS2-61). The spectral range of the emitted light was 600 to 700 nm. The light was directed downward through a 60° mirror and then through a pair of lenses to produce a uniform beam approximately 7 cm in diameter at a point 12 cm from the lens. The total intensity, *i.e.*, full emitted spectrum at this point as measured by a Coherent Radiation Model 210 thermopile, was 100 milliwatts/sq cm. Approximately 25% of the emitted spectrum occurs between 620 to 640 nm [corresponding to the HPD absorption spectrum when contained in tumor cells (15)], thus producing an effective intensity of 25 milliwatts/sq cm.

Procedure. This study evaluated the therapeutic effectiveness of *i.v.* HPD followed by local exposure to red light (wavelength >600 nm) in the treatment of a variety of cutaneous and *s.c.* recurrent and metastatic tumors. Twenty-five adult white patients who had not responded to conventional treatment and had progressive tumor were included in the study. There were 7 patients with malignant melanomas (metastatic), 3 patients with colon carcinomas (recurrences of primary lesions), 5 patients with metastatic breast carcinomas on the chest wall, 2 patients with basal cell carcinomas (recurrent), 3 patients with mycosis fungoides (recurrent), and 1 patient each with chondrosarcoma (metastatic), prostatic carcinoma (metastatic), squamous cell carcinoma of skin (metastatic), endometrial carcinoma (metastatic), and angiosarcoma (metastatic). All diagnoses were confirmed histologically. Before a patient was admitted to the study, the procedures were carefully explained, and full informed consent was obtained.

After HPD was administered (*i.v.*, at 2.5 or 5.0 mg/kg body weight), patients received treatment with the light (either 50 or 100 milliwatts/sq cm, full spectrum) to the area of known tumor including a margin when feasible. Ten patients (30 separate areas) received comparable treatment to an area of grossly normal skin.

Treatment with red light was given from 24 hr (2 patients) to 192 hr (3 patients) after injection. All other patients were treated at 48, 72, 96, 120, or 168 hr after injection (Table 2). Therapy was limited to multiple 5- x 5-cm areas. During the course of therapy, no topical medications or systemic chemotherapy was given.

Exposure times were generally for 20 min at 100 milliwatts/sq cm or its equivalent, *i.e.*, 40 min at 50 milliwatts/sq cm (full spectrum). In the early stages of the study, an exposure time as long as 60 min was used on 2 patients but was found to be unnecessarily long and resulted in skin necrosis. In general a single treatment or fractionated treatments were used.

Tumor response as well as normal tissue response were judged daily for at least 7 days following treatment and at least every 4 weeks thereafter when possible. In 4 cases posttreatment biopsies were performed.

Fluorescence was observed in a darkened room by illuminating the area with a Blak-Ray Model B-100A lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.). In the latter cases (15 patients), fluorescence was observed through laser goggles (Laser-Gard LGS-A) which increased sensitiv-

ity to the observation of the red fluorescence by filtering out the activating blue light.

RESULTS

The results of the combined effects of HPD and activating red light are summarized in Tables 1 and 2. In 98 of 113 tumors (21 of 24 patients), there was complete response (complete disappearance of palpable mass or biopsy-confirmed tumor necrosis within the light field) beginning within 24 hr after light treatment. This was manifested initially as local erythema and edema at the tumor area, progressing to necrosis, usually within 24 hr after light treatment. Since in the early stages of the study we often observed necrosis of skin overlying the tumors, we studied the effect of the treatment on normal skin remote from the tumor in 10 selected patients. In most other cases we were able to observe the effect in margin areas around the tumor sites. We found that several procedures involving fractionation of the light dose or increase of the time between HPD injection and light exposure allowed complete tumor response without adverse effects on normal overlying or surrounding skin (Tables 2 and 3). When the skin was grossly involved with tumor, however, necrosis usually occurred with all schedules. A few patients were treated with fractionation procedures to determine whether an improvement in therapeutic ratio could be obtained. In each case the total delivered dose (time × intensity) was held constant; where feasible, comparisons were made with a single exposure of the same dose delivered on the day of the last fraction.

The tumor response was predictable with apparent complete disappearance of the tumor mass to a depth of at least 2 cm as indicated by 4 cases in which histological examination of biopsy sections were carried out 1 week after treatment. Sixty-three of the tumors or tumor areas (multi-

Table 1
Tumor response to HPD followed by red light

Tumor type	No. of patients	No. of tumors or tumor sites (multiple nodules in field) treated			
		Complete response ^a	Partial response ^b	No response	Total
Basal cell	2	2			2
Malignant melanoma	7	6		1	7
Chondrosarcoma	1	1			1
Colon adenocarcinoma	3	2		1	3
Prostate	1	1			1
Mycosis fungoides	3	4	2		6
Endometrial carcinoma	1	6			6
Breast carcinoma	5	75	10		85
Angiosarcoma	1		1		1
Squamous cell carcinoma	1	1			1
Total patients	25				
Total tumors or tumor sites		98	13	2	113

^a Disappearance of measurable or palpable tumor within a treated field.

^b Reduction in mass by greater than 50% or apparent complete disappearance followed by recurrence in treatment field.

ple nodules) were 2 cm deep or less. As noted previously, we have used only red light for treatment since tissue penetration is greatest over 600 nm (4) (Chart 1). When the

tumor was limited to the apparent effective penetration depth of the red light, *i.e.*, 2 cm, relapse has not occurred (6 months), except in 2 patients where the periphery of the tumor apparently was not included in the light field. While selective HPD fluorescence could be seen in all patients with superficial tumors, no fluorescence was observable for *s.c.* tumors since the maximum penetration of the blue light used for fluorescence is no more than a few mm. HPD fluorescence was also observed at sites of acute inflammation (*i.e.*, recent surgical incision and abscess). After initial treatment was concluded, patients could be reinjected for additional treatment within 10 days. There was no observable difference in skin or tumor response when comparing 100 milliwatts/sq cm intensity for 20 min and 50 milliwatts/sq cm for 40 min. This is consistent with our *in vitro* data reported previously (4).

HPD *i.v.* followed by red light was well tolerated by the patients. There were no gastrointestinal symptoms. Hemoglobin, WBC and differential, blood urea nitrogen, bilirubin, aspartate aminotransferase, alkaline phosphatase, and clotting time were unchanged during and after therapy. The only toxicity that could be ascribed to the drug was moderate to severe generalized skin reaction when patients were exposed to direct or indirect sunlight up to 30 days after an initial HPD injection. This occurred in 6 of 25 patients. It was comparable to a second-degree sunburn on exposed areas lasting for 2 to 3 days. There are no long-term complications apparent at this time.

The 2 methods for determining penetration of red light through tumor tissue led to very different results as expected (Chart 1), since the use of cylindrical tubes containing photosensitive material takes into account scattered as well as directly transmitted light whereas the fiber detector excludes nearly all scattered light. The scattered light in tissue adds substantially to the total light flux in tissue as

Table 2

Photoradiation parameters for single treatments

Treatment time was 20 min. Light intensity was 100 milliwatts/sq cm (full spectrum). Since most patients had multiple tumors, these data are derived from treatment of well over 100 individual tumors and include all parameters tested to date for single treatments.

HPD dose (mg/kg)	Time interval (injection-light-exposure) (hr)	No. of tumors	Tumor response	Overlying or surrounding normal skin response
2.5	24	2	Complete	Necrosis
2.5	48	3	Complete	Partial necrosis
2.5	72	20	Complete	Erythema, edema
2.5	96	50	Complete	Slight erythema and edema
2.5	120	10	Complete	Slight erythema and edema
2.5	144	2	Complete	Slight erythema and edema
2.5	168	2	Partial	Slight erythema and edema
2.5	192	3	Slight or none	None
5.0	24	1	Complete	Necrosis
5.0	48	10	Complete	Necrosis
5.0	72	3	Complete	Partial necrosis
5.0	96	3	Complete	Erythema, edema
5.0	120	1	Complete	Erythema, edema
5.0	144	1	Complete	Slight erythema and edema
5.0	168	1	Complete	Slight erythema and edema
5.0	192	1	Complete	Slight erythema and edema

Table 3

Effect of treatment fractionation on tumor and normal tissue response

Tumor	HPD dose (mg/kg)	Time to 1st fraction (hr)	No. of fractions	Exposure time/fraction (min) ^a	Time interval between fractions (hr)	Normal skin response (adjacent to tumor)	Tumor response
Metastatic breast carcinoma ^b	2.5	48	6	3	24	Mild erythema	Partial
Metastatic breast carcinoma ^b	2.5	72	3	5, 7, 8	24	Mild erythema	Complete
Metastatic breast carcinoma ^b	2.5	96	2	10	24	Mild erythema	Complete
Metastatic breast carcinoma ^b	2.5	120	1	20		Mild erythema and edema	Complete
Metastatic breast carcinoma ^b	2.5	168	1	10		Mild erythema and edema	Complete
Metastatic breast carcinoma	2.5	96	3	6, 12, 18	24	Mild erythema	Complete
Endometrial carcinoma	5.0	96	2	10	72	None	Complete
Endometrial carcinoma	5.0	168	1	20		Slight erythema after 1st fraction	Complete

^a Light intensity was 50 or 100 milliwatts/sq cm (full spectrum). Times refer to 100 milliwatts/sq cm equivalent (*i.e.*, 10 min at 50 milliwatts/sq cm = 5 min at 100 milliwatts/sq cm).

^b Case 3.

we have pointed out previously (5). Thus, the actinometric method indicates only one-fourth of the attenuation of red light as that observed by determining transmission from only 1 direction. The actinometric results have been normalized to 100% at zero depth to cancel out constant loss factors, especially surface reflections from the cylindrical tubes. The results have been averaged for 4 different tumors all approximately 5 x 5 x 2 cm. The data from use of fiber optics represent 4 individual experiments. More refined light dosimeter techniques are currently being developed.

Selected Case Reports

Case 1. A 72-year-old white male had 3 basal cell carcinoma lesions of the nose approximately 1 to 1.5 cm in diameter and adenocarcinoma of the colon. The patient had been treated with surgery, radiation therapy, and 5-fluorouracil in order to control the colon carcinoma. The patient was given an injection of HPD, 5.0 mg/kg, and 96 hr later was given a 20-min light exposure at 100 milliwatts/sq cm to the tumor area on the side of the nose indicated in Fig. 1 with about a 0.5-cm margin. Prior to treatment the basal cell lesions were fluorescent and photographed with clear delineation of the 3 lesions (Fig. 1). This same light exposure was repeated at 120 hr postinjection. Within 2 days the tumor appeared necrotic and a scab formed. The area of normal skin in the light field showed mild erythema and some edema. When the patient was last seen 7 months after treatment, there was no evident tumor in the treated area, and complete healing with good cosmetic effect had occurred. Untreated tumors remained (Fig. 2).

Case 2. A 33-year-old white female with metastatic malignant melanoma had progressive disease in spite of previous *Bacillus Calmette-Guérin*, *trans*-1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, procarbazine, dimethyltrigonoimidazolecarboxamide, and *Corynebacterium parvum*. She was given an injection of HPD, 5.0 mg/kg, and 48 hr later a s.c. nodule 3 cm in diameter was exposed with a 75-mm margin for 30 min to the red light at 100 milliwatts/sq cm. The skin over the tumor area showed moderate fluorescence at this time. The next day the skin over the tumor showed extensive erythema and edema, while the skin in the margin showed only slight response (Fig. 3). After 7 days moderate mass reduction was apparent with moderate erythema still apparent in the overlying skin. Skin adjacent to the tumor, which was in the light field, appeared normal. The mass was biopsied 7 days after treatment, revealing complete coagulation necrosis within the area exposed to the light to a depth of approximately 2 cm and apparently viable tumor beyond this depth and outside the light field (Fig. 4). At 17 days after treatment, extensive necrosis of tumor and overlying skin was apparent (Fig. 5). Approximately 2 months after treatment, a scab had formed over the immediate treatment area, but tumor necrosis beyond the immediate light field and further reduction in mass were evident.

Case 3. A 53-year-old white female with metastatic breast carcinoma showed involvement of entire right chest wall as well as metastasis to lungs, liver, and rectum. She had had a right mastectomy 3 years previously. The chest wall had received a full course of radiation therapy after surgery.

Metastasis was not controlled by cyclophosphamide, prednisone, methotrexate, 5-fluorouracil, or 3-deazauridine. She was given HPD, 2.5 mg/kg and the entire upper right chest wall was treated in 10 separate sessions spanning over 16 days. Reinjection occurred after 12 days with a second 2.5-mg/kg dose of HPD. Three fractionated regimens and 5 single exposures were used, all of which proved to be effective in preserving the normal skin and causing tumor reduction. The total light dose (time × intensity) was held constant for both fractionated and single treatments. Light intensity was 50 or 100 milliwatts/sq cm. Results are shown in Table 3 (Lines 1 to 5). In addition to schedules in Table 3, single 20-min exposures were carried out at 72, 96, and 120 hr following the second injection of HPD. In all cases normal skin response was minimal (slight erythema), and tumor necrosis appeared to be complete within 24 to 48 hr after treatment. Fig. 6 shows a close-up of a single field prior to treatment. Figure 7 shows the same area 1 day after treatment (20 min, 100 milliwatts/sq cm, 5 days after injection) indicating tumor nodule necrosis and minimal normal skin damage.

DISCUSSION

The purpose of this study was to determine the efficacy and scope of photoradiation therapy utilizing HPD and activating red light in treating solid malignant tumors. Over 100 individual tumors comprising 10 different types were treated. It is evident that the combined effect of HPD plus light (over 600 nm) is highly effective in destroying malignant tissue of all types included in the study. Highly pigmented tumors (e.g., melanoma) and the larger s.c. tumors required more aggressive treatment (i.e., HPD, 5.0 mg/kg, rather than HPD, 2.5 mg/kg) than did the nonpigmented or superficial tumors. In the early stages of the study, extensive skin damage was found. This was markedly reduced and essentially eliminated by reducing light exposure to 20 min (100 milliwatts/sq cm) or its equivalent and by extending the time interval between injection and light exposure to at least 3 days for 2.5-mg/kg doses of HPD and to 4 days or more for 5.0-mg/kg doses. With this method complete tumor response was obtained without adversely affecting normal overlying or adjacent skin. Since in each case the skin received a higher light dose than did the underlying tumor, the differences in response apparently reflect differential uptake and/or clearance between malignant tissue and normal skin.

Tumors responded in spite of their resistance to conventional therapy. Previous radiation therapy did not appear to affect either the tumor response or normal skin response adversely. Thus, in each of the 5 patients with recurrent chest wall breast carcinoma, a full course of radiotherapy had been administered which precluded a second series with this modality. Superficial tumors of this type responded well following a 2.5-mg/kg dose.

Maximum acute tumor necrosis achieved was about 2 cm. Since HPD appears to be retained for very long periods of time in malignant tissue, it may be possible to increase effective depth by increasing HPD dose and increasing the time between injection and light exposure to allow skin clearance. Alternately, one could presumably increase the

light intensity since with the currently used intensities we have observed complete tumor response without toxicity to normal skin. Possibly, a more effective means to achieve the same result is by the use of fiber optics coupled to an appropriate laser source. Preliminary experiments in our laboratory indicate that a helium:neon laser emitting at 632.8 nm or a tunable dye laser emitting anywhere between 631 and 635 nm is an effective alternative to the arc lamp. The use of very fine fiber optics that can be efficiently coupled to the laser allows implantation of the light source to any desired depth within the tumor. The tissue acts as a very effective means of distributing the light throughout a large volume of the mass. In addition this system allows the light to be delivered through various types of endoscopes, reaching tumors in the cervix, bronchus, bladder, etc. Thus, the capability to treat deep-seated large tumors by photoradiation therapy is at hand.

Accurate determination of light flux at various depths in tissues (dosimetry) is difficult because of the large contribution due to backscatter. We have discussed this question previously and made calculations to indicate the importance of this factor. The present data confirm the necessity of using techniques that allow measurement of backscatter. Although the methods are rather crude and subject to several errors, the general trends appear clear. Using the actinometric data and assuming the rat tumor as a reasonable model, we would predict that 4 to 5% of the red light would penetrate 2 cm into tissue. Since about one-half of light at this wavelength is transmitted through human skin (6), the overall light flux would be 2 to 2.5% of the incident intensity. Thus at an effective incident light intensity of 25 milliwatts/sq cm (620 to 640 nm), the intensity at 2 cm would be approximately 0.5 milliwatt/sq cm (10^4 ergs/sec/sq cm). This is apparently the approximate intensity required to cause acute coagulation necrosis following 30 min of photoradiation (HPD, 5 mg/kg; Case 2).

While thermal effects due to the red light (all IR radiation is removed by filtration) may be contributory to some of the effects seen, that this is not of primary importance is apparent from the absence of effect of the light alone observed in animal systems (4) as well as by a diminished effect of the therapeutic light on tumors as they clear the HPD (e.g., Table 2, Lines 7 and 8). The actual clearance rates for HPD from human tissues need to be better understood. Some patients have retained obvious HPD fluorescence in tumors for several weeks after injection, while tumors in others lose fluorescence of the drug within a few days. As pointed out previously, however, fluorescence of the dye in the tissue is not a reliable indicator of HPD content since fluorescence is easily obscured by skin pigmentation, which prevents effective penetration of the blue light used to excite the fluorescence. We are attempting to

determine a reliable measure of HPD content in biopsy samples of human tumors.

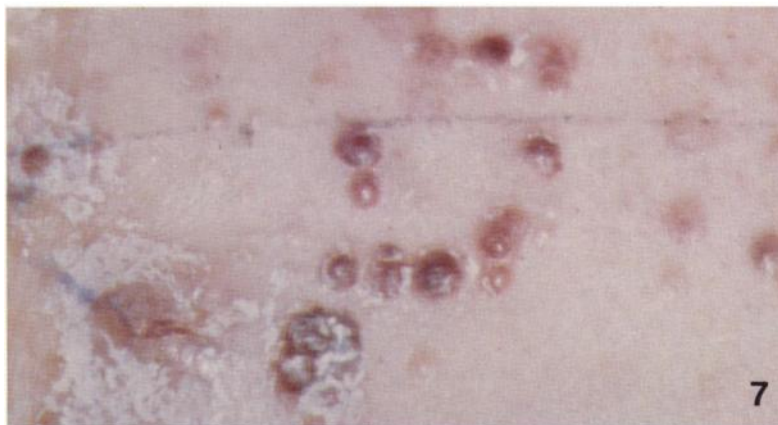
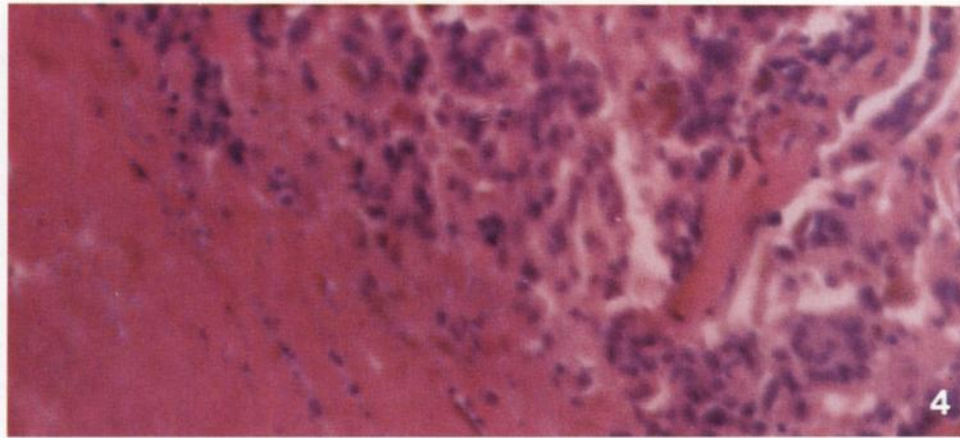
The response rate obtained in this group of refractory tumors following photoradiation is superior to previously reported treatments and should be considered in the armamentarium of cancer therapy. It is clear that the challenge is to develop the technology to allow treatment of less accessible, life-threatening tumors. In its present state photoradiation may be a useful method for treating recurrent chest wall breast carcinomas not controlled by chemotherapy, immunotherapy, or ionizing radiation.

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REFERENCES

1. Diamond, I., Granelli, S., McDonagh, A. F., Nielsen, S., Wilson, C. B., and Jaenicke, R. Photodynamic Therapy of Malignant Tumors. *Lancet*, 2: 1175-1177, 1972.
2. Dougherty, T. J. Photoradiation Therapy. Abstracts of the American Chemical Society Meeting, No. 014, Chicago, Ill., September 1973.
3. Dougherty, T. J. Activated Dyes as Anti-Tumor Agents. *J. Natl. Cancer Inst.*, 51: 1333-1336, 1974.
4. Dougherty, T. J., Gomer, C. J., and Weishaupt, K. R. Energetics and Efficiency of Photoinactivation of Murine Tumor Cells Containing Hematoporphyrin. *Cancer Res.*, 36: 2330-2333, 1976.
5. Dougherty, T. J., Grindey, G. B., Weishaupt, K. R., and Boyle D. G. Photoradiation Therapy II. *J. Natl. Cancer Inst.*, 55: 115-120, 1975.
6. Everett, M. A., Yeagers, E., Sayre, R., and Olson, R. Penetration of Epidermis by Ultraviolet Rays. *Photochem. Photobiol.*, 5: 533-542, 1966.
7. Gregorie, H. B., Horger, E. O., Ward, J. L., Green, J. F., Richards, T., Robertson, H. C., and Stevenson, T. B. Hematoporphyrin Derivative Fluorescence in Malignant Neoplasms. *Ann. Surg.*, 167: 820-827, 1968.
8. Hermens, A. F., and Barendsen, G. W. Changes in Cell Proliferation Characteristics in a Rat Rhabdomyosarcoma before and after X-irradiation. *European J. Cancer*, 5: 173-189, 1969.
9. Kelly, J. F., and Snell, M. E. Hematoporphyrin Derivative: A Possible Aid in the Diagnosis and Therapy of Carcinoma of the Bladder. *J. Urol.*, 115: 150-151, 1976.
10. Kelly, J. F., Snell, M. E., and Berenbaum, M. Photodynamic Destruction of Human Bladder Carcinomas. *Brit. J. Cancer*, 31: 237-244, 1975.
11. Lipson, R., Baldes, E., and Olsen, A. The Use of a Derivative of Hematoporphyrin in Tumor Detection. *J. Natl. Cancer Inst.*, 26: 1-8, 1961.
12. Rasmussen-Taxdall, D. S., Ward, G. E., and Figge, F. H. Fluorescence of Human Lymphatic and Cancer Tissue following High Doses of Intravenous Hematoporphyrin. *Cancer*, 8: 78-81, 1955.
13. Spikes, J. D. Porphyrins and Related Compounds as Photodynamic Sensitizers. *Ann. N. Y. Acad. Sci.*, 176: 496-508, 1975.
14. Tappener, H., and Jesionek, A. Therapeutische Versuche mit Fluoreszierenden Stoffe. *Munch. Med. Wochschr.*, 1: 2042-2044, 1903.
15. Tomson, S. H., Emmet, E. A., and Fox, S. H. Photodestruction of Mouse Epithelial Tumors after Oral Acridine Orange and Argon Laser. *Cancer Res.*, 34: 3124-3127, 1974.
16. Weishaupt, K. R., Gomer, C. J., and Dougherty, T. J. Identification of Singlet Oxygen as the Cytotoxic Agent in Photo-inactivation of a Murine Tumor. *Cancer Res.*, 36: 2326-2329, 1976.
17. Winkleman, J., and Rasmussen-Taxdall, R. S. Quantitative Determination of Porphyrin Uptake by Tumor Tissue following Parenteral Administration. *Bull. Johns Hopkins Hosp.*, 107: 228-233, 1960.



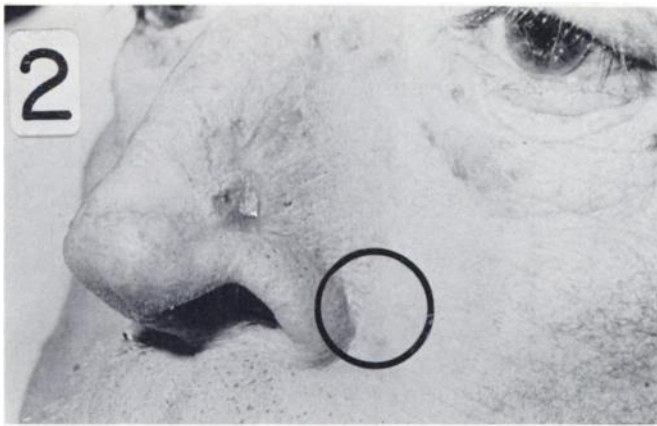


Fig. 2. Case 1, 7 months after treatment, indicating complete clearance of the treated tumor. Note continued growth of untreated lesion on side of nose.

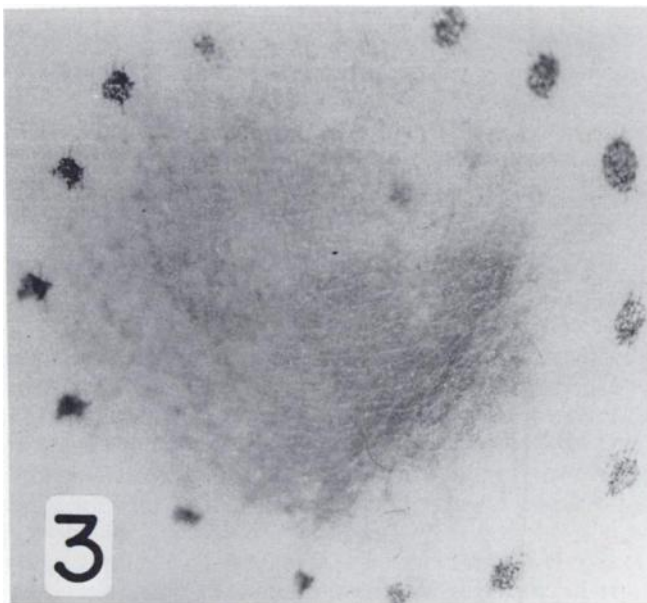


Fig. 3. Case 2. Treated tumor area 1 day after treatment, demonstrating erythema and edema in tumor area with relatively little effect in field outside of tumor area.

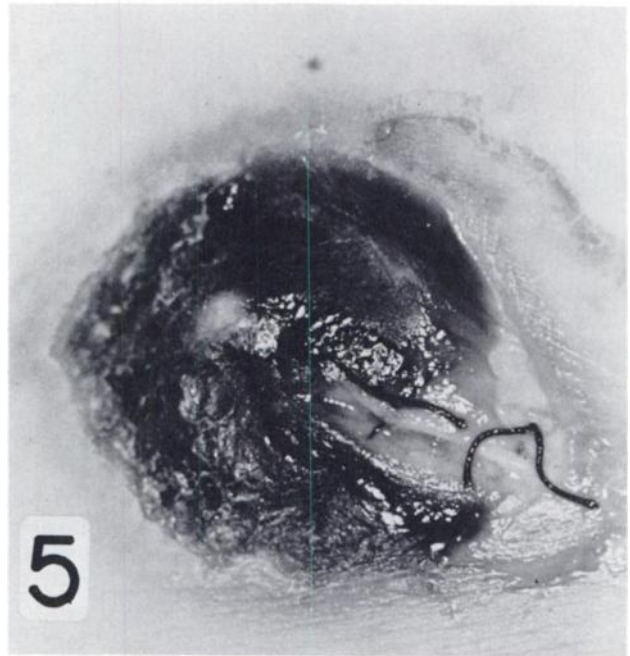


Fig. 5. Case 2, 17 days after treatment, indicating apparent complete tumor necrosis within treatment field. Lower right, biopsy scar.

Fig. 1. Case 1. Fluorescence of basal cell carcinoma lesion on the nose before treatment. Only the lesion adjacent to the tag was treated.

Fig. 4. Case 2. Biopsy section taken across treatment field and into adjacent nontreated area, 7 days after treatment. Complete coagulation necrosis is apparent within treatment field (approximately 2 cm deep), and apparent viable melanoma cells are outside field.

Fig. 6. Case 3. Metastatic breast carcinoma. Close-up of a selected field before treatment. 8 x 8 cm.

Fig. 7. Case 3. Field of Fig. 6, 1 day after treatment. Single 20-min treatment at 100 milliwatts/sq cm 5 days after HPD injection (Table 3, Line 4).