An Experimental and Clinical Investigation of Factors Influencing the Therapeutic Ratio of Cancer Photochemotherapy.

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DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institution of learning.

ABSTRACT

Some of the factors affecting the therapeutic ratio, the incidence of tumour to normal tissue damage, of photochemotherapy using Photofrin II and 630 nm light have been studied in patients with superficially recurrent cancer and in rats using a transplanted fibrosarcoma.

The normal growth of the rodent tumour and its response to cytotoxic chemotherapy and radiotherapy were comparable with data for other rodent tumours. In the rats, porphyrin levels in tumour were similar to those in adjacent skin and muscle after a standard dose of photosensitizer suggesting that any therapeutic advantage after photochemotherapy was not due to preferential tumour retention of photosensitizer. Porphyrin levels fell more slowly in skin than tumour, muscle or plasma.

Tumour growth delay in rats given a standard dose of photosensitizer increased with dose of light up to 200 J (interstitial) and up to 200 Jcm^{-2} (superficial), above which no further growth delay was obtained. There may be, therefore, a maximum single dose of light that can be delivered effectively through a cut optical fibre or using superficial light. This may be due to tumour resistance to photochemotherapy, inadequate doses photosensitizer or poor light distribution within the tumour. Response to interstitial treatment decreased for tumours greater than 10-12 mm in diameter. If multiple interstitial fibres are to be used to treat larger tumours, they should, therefore, probably be placed no more than 10-12 mm apart. Tumour response increased with field size suggesting that there may be a tumour bed effect associated with superficial photochemotherapy.

In patients treated with Photofrin II, the decrease in plasma porphyrin levels conformed to a two compartment pharmacokinetic model with a distribution half-life of 3.0 to 12.5 h and elimination half-life of 3 to 10 days. Tumour uptake of photosensitizer should be complete by 50 h which is consistent with the normal minimum interval between giving photosensitizer and light. Elimination of porphyrin was relatively slow and there may be a period of several days when levels in tumour are sufficient for effective light treatment. The duration of cutaneous photosensitivity may be greater than the 12 to 40 days predicted from the elimination half-life.

In patients treated with superficial photochemotherapy, complete tumour response and skin necrosis within the irradiated area increased with dose of photosensitizer and light suggesting a poor therapeutic ratio. Necrosis healed in all patients without scarring and it may be more appropriate to use the final cosmetic result of treatment for measuring therapeutic ratio. Tumour response decreased with increasing tumour depth and the data suggest that only tumours less than 1 cm thick can be treated effectively with superficial light. Interstitial light produced no complete tumour responses but tumour growth delay did increase with light dose. No skin necrosis was observed after interstitial treatment.

The photosensitivity of normal skin was measured using a reflectance meter to measure the erythema produced by white light from a solar simulator. The skin of the posterior chest remained sensitive to 20 Jcm^{-2} of light for <18 to >50 days after Photofrin II injection.

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CONTENTS

CHAPTER 1: **INTRODUCTION**

Photosensitizers activated by light may be used to treat cancer. During the last 15 years there has been increasing interest in this new modality, photochemotherapy, and several thousands of patients have been treated. The clinical work has been accompanied by extensive *in vitro* and *in vivo* studies.

The potential of chemicals that absorb light to damage biological systems has long been recognised (Raab, 1900) and the first patients received photochemotherapy in 1903, when topical eosin and white light were used to treat skin tumours (Tappeiner and Jesionek, 1903). Over the next 70 years, there was only one other report of a patient being treated with photochemotherapy (Lipson, Gray and Baldes, 1966: as quoted by Dougherty, 1986).

Tumours in mice given systemic haematoporphyrin fluoresce red under ultra-violet light (Auler and Banzer, 1942). Haematoporphyrin and other metaloporphyrins, also, accumulate in regenerating and lymphatic tissue (Figge, Weiland and Manganiello, 1948). The accumulation of haematoporphyrin in tumours and its characteristic red fluorescence when exposed to ultra-violet light led to it being used to delineate tumours (Figge, Weiland and Manganiello, 1948; Peck *et al.*, 1955; Rassmussen-Taxdal, Ward and Figge, 1955).

A derivative of haematoporphyrin, haematoporphyrin derivative (HPD), is a better tumour localizer and causes less cutaneous photosensitivity than haematoporphyrin (Lipson, Baldes and Olsen, 1961). During the 1960's, haematoporphyrin derivative was used for

tumour detection (Lipson, Olsen and Baldes, 1964; Lipson et al.,1964; Georgie, Horger and Ward, 1968) and activated by white light to treat a patient with recurrent breast cancer (Lipson, Gray and Baldes, 1966; as quoted by Dougherty, 1984a).

In 1972, Diamond et al. demonstrated that haematoporphyrin and white light reduced tumour growth in vitro and in vivo. In 1975, Dougherty et al. reported the eradication of a transplanted mammary tumour in mice after photochemotherapy using HPD activated by 600 to 700 nm light from a Xenon arc lamp and in 1976 Dougherty's group began clinical trials of photochemotherapy (Dougherty et al., 1978).

Since these early reports, the results of many studies have been published (see reviews by Dougherty, 1984b; Kessel, 1984; Dougherty, 1986). The main areas of investigation are:-

1. Photochemistry of sensitizers
2. Selection of photosensitizer
3. Photosensitizer levels in tissue
4. Mode of action of photochemotherapy in tumours
5. Light delivery
6. Clinical studies.

Photochemistry of Sensitizers

The cytotoxic species in photochemotherapy is believed to be singlet oxygen (Weishaupt, Gomer and Dougherty, 1976). The sequence of events when the photosensitizer is activated are:-

$$\text{Sens} + h\nu \longrightarrow {}^1\text{Sens}^*$$

$${}^1\text{Sens}^* \longrightarrow {}^3\text{Sens}^*$$

$${}^3\text{Sens}^* + {}^3O_2 \longrightarrow {}^1O_2 + \text{Sens}$$

$${}^1O_2 + \text{substrate} \longrightarrow \text{Oxidation}$$

where Sens is the sensitizer; ${}^1\text{Sens}^*$ is the excited singlet state of the sensitizer; ${}^3\text{Sens}^*$ is the triplet state of the sensitizer; ${}^3O_2$ is the ground state triplet oxygen and ${}^1O_2$ is excited singlet oxygen.

This process requires the presence of oxygen and hypoxia has been shown to decrease response to photochemotherapy (Bown et al., 1986; Moore, Keene and Land, 1986). Although biological damage from photochemotherapy is mainly due to singlet oxygen production, other mechanisms of cytotoxicity have not been excluded. Henderson and Miller (1986) reaffirmed the predominant role of singlet oxygen production in cell killing due to photochemotherapy but suggested that free radicles also have some involvement.

Selection of Photosensitizers

The characteristics of a photosensitizer that make it suitable for photochemotherapy are:-

1. The toxicity of the drug is acceptable at the doses required for treatment.
2. A higher concentration of drug occurs in the tumour than in the surrounding normal tissue, so that selective tumour damage is produced.
3. Light of a relatively long wavelength activates the drug, as penetration of light in tissue increases with wavelength (Wilson et

al., 1984).

4. The drug is photochemically efficient, that is the drug produces sufficient singlet oxygen to cause tumour damage.

The photosensitizers in clinical use are porphyrins. Diamond et al. (1972) and Tomio et al. (1982) reported that haematoporphyrin is an active photosensitizer but Dougherty (1983) suggested that it is the impurities present in most haematoporphyrin preparations that are photoactive.

Haematoporphyrin derivative is an active photosensitizer for photochemotherapy. It has only one major side-effect, cutaneous photosensitivity (Dougherty et al., 1978), accumulates in higher concentrations in the tumour than the normal surrounding tissue (Lipson, Baldes and Olsen, 1961; Gomer and Dougherty, 1979), is activated by 630 nm light (Dougherty et al., 1975) and is relatively photochemically efficient (Dougherty, 1984b). Haematoporphyrin derivative is a complex mixture of substances (Bonnett et al., 1981; Moan and Sommer, 1983). More recently, Photofrin II (Photofrin Medical Co. Inc., Raritan, New Jersey) has been produced which is thought to be the active component of HPD (Dougherty, 1983; Dougherty, 1984a). It is reported to be mainly dihaematoporphyrin ether (Dougherty, Potter and Weishaupt, 1984) but the drug still contains a mixture of substances.

Dougherty (1984a) suggested that Photofrin II produces less cutaneous photosensitivity and is more phototoxic to the tumour than HPD but other authors found little difference between the two (Gomer and Razum, 1984; Cowled and Forbes, 1985). Cutaneous photosensitivity,

with the possibility of a severe reaction on exposure to sunlight (Dougherty et al., 1979; Dahlman et al., 1983), persists for about a month after systemic injection of Photofrin II but it may last longer in some patients (Dougherty, 1984a). This photosensitivity is dose limiting. In animal studies, tumour response increases with increasing dose of photosensitizer (Cowled and Forbes, 1985), thus if the problems of cutaneous photosensitivity could be overcome, it might be possible to increase the tumour response rate in man.

Other newer photosensitizers have been examined but only one has been used clinically, tetraphenylporphine sulphonate (TPPS). Topically applied TPPS in an azone base gave good results when used to treat superficial skin tumours (Sacchini et al., 1987). The longest wavelength of light that activates TPPS is 630 nm, so the limitations to the depth of the lesion that can be treated are as for Photofrin II. Also, when the drug is given systemically to animals it causes neurotoxicity (Winkleman and Collins, 1985). The phthalocyanines are active photosensitizers in vitro and in vivo and are activated by 675 nm light (Spikes, 1986; Tralau et al., 1987), which may increase the range of tumours that can be treated.

Photosensitizer Levels in Tissue and Plasma

The difference in concentration of photosensitizer between tumour and its surrounding normal tissue is thought to be due to slower clearance of porphyrin from tumour than normal tissue (Bugelski, Porter and Dougherty, 1981). This preferential retention of photosensitizer in tumour compared to normal tissue forms the main basis for a possible therapeutic advantage of photochemotherapy over most conventional

treatment modalities. If the optimum therapeutic ratio for photochemotherapy is to be achieved and if light has equal effects on tumour and normal tissue, light should be given when the difference between concentrations of photosensitizer in the tumour and normal tissue is maximum.

In two mouse models after intraperitoneal injection of [^{3}H] and [^{14}C] HPD, the concentration of drug was greater in transplanted tumour tissue than in muscle and skin but less than in liver, spleen and kidney (Gomer and Dougherty, 1979). Twenty four hours after injection, the concentration of photosensitizer in tumour was about twice that in skin in both tumour models but the level of photosensitizer in tumour was 1.8 times greater in one model and 8.7 times greater in the other model than in muscle. Similar uptake of HPD has been demonstrated in another murine tumour (Evensen et al., 1984).

The concentration of photosensitizer in the tumour and normal tissues in animals can be measured directly but this is more difficult in humans as repeated biopsies would be required. By examining the pharmacokinetics of drugs in blood, it may be possible to infer how they are handled by tissues. In patients, the decrease in plasma levels of haematoporphyrin and HPD with time after intravenous injection conform to a two compartment pharmacokinetic model (Zalar et al., 1977; Dougherty et al., 1984b).

Mode of Action of Photochemotherapy in Tumours

A combination of cellular and vascular damage may occur during and after photochemotherapy.

The main sites of damage within malignant cells are the mitochondria (Copolla et al., 1980) and the cell membrane (Moan et al., 1982). In contrast with ionizing irradiation and cytotoxic chemotherapy, photochemotherapy does not produce significant chromosomal damage (Ben-Hur et al., 1987).

Henderson et al. (1985) suggested that a physiological factor, possibly induced anoxia due to vascular occlusion, is responsible for tumour response to photochemotherapy in vivo. Cells explanted from tumours immediately after treatment did not show any decrease in clonogenicity, whereas tumours left in situ after treatment showed decreasing clonogenicity with increasing interval between treatment and explantation.

Other studies also suggest that the main site of damage due to photochemotherapy in vivo is the tumour vasculature. Bugelski, Porter and Dougherty (1981) showed a five fold increase in the concentration of HPD around the tumour vasculature and Selman et al. (1984) and Star et al. (1986) demonstrated occlusion of blood vessels after photochemotherapy. Fingar and Henderson (1987) suggested that there is a tumour bed effect associated with superficial photochemotherapy, however the cause of this has not been determined. The tumour bed effect seen after a single dose of ionizing radiation is mainly due to

vascular damage (Saeki, Shimazaki and Urano, 1971; Clifton and Jirtle, 1975).

If there is a tumour bed effect associated with photochemotherapy, field size would be expected to affect tumour response to superficial treatment.

Light Delivery

For superficial photochemotherapy, tumour response increases with increasing dose of light (Dougherty et al., 1975; Cowled and Forbes, 1985)

In the early studies of photochemotherapy, white light was used but photosensitizers are only activated by absorption of light at specific wavelengths. The development of lasers, with their high output of monochromatic light, has allowed the use of specific wavelengths of light corresponding to the peaks in the absorption spectrum of the photosensitizer. Two types of laser are in common use in photochemotherapy, Argon ion/dye lasers and metal vapour lasers. The former emit continuous wave light, while latter emit pulsed light with a very short peak power duration. Tumour response is the same after photochemotherapy using either an Argon ion/dye laser or a Gold vapour laser (Cowled, Grace and Forbes, 1984).

Photofrin II has five absorption peaks at around 405, 510, 540, 580 and 630 nm (Kinsey et al., 1981). Although the 630 nm absorption peak is the smallest, this wavelength of light is now most commonly used in

clinical practice because penetration of light in tissue increases with increasing wavelength (Wilson et al., 1984). Despite the use of 630 nm light, it is still only possible to treat effectively tumours less than 1 to 1.5 cm deep if light is applied through the skin (Dougherty, 1984b). Limited penetration of light in tissue is still a major factor restricting the application of photochemotherapy (Dougherty et al., 1981).

As light intensity decreases exponentially in tissue (Wan et al., 1981) and the penetration depth of 630 nm light is only 1 to 2.6 mm (Driver et al., 1988), doubling the dose of light applied at the skin surface will only increase the effective treatment depth by 1 to 2.6 mm. One way of overcoming poor light penetration is to deliver light through implanted optical fibres. Interstitial light delivery may, also, improve the therapeutic ratio for treatment of subcutaneous tumours by allowing the maximum light dose to be delivered to the tumour rather than at the skin surface so decreasing the risk of skin damage.

The amount of tumour necrosis produced by interstitial photochemotherapy using a single optical fibre to deliver light increases with increasing light dose (Tralau et al., 1987) but histological evidence of complete tumour necrosis is not necessarily associated with the death of all clonogenic cells (Fingar, Potter and Henderson, 1987). To assess the effects of interstitial photochemotherapy on tumours, dose response curves need to be established using a clinical end-point, for example tumour growth delay.

Penetration of light limits the volume of tumour tissue that can be treated with a single optical fibre. If implantation of multiple optical fibres is to be used to overcome this limitation, the spacing of fibres requires careful planning so that all of the tumour receives an adequate dose of light. Experience of treating spontaneous tumours in cats and dogs with photochemotherapy, suggests that optical fibres should be 1.5 to 2 cm apart when using multiple fibre implants (Dougherty et al., 1981).

It is difficult to compare interstitial and superficial methods of light delivery because of problems with light dosimetry. Light is prescribed as an applied dose, not an absorbed dose. Superficial light is prescribed as Jcm^{-2} delivered to the skin surface and interstitial light is prescribed as J emitted from the tip of the fibre. Although the total dose of light given at the skin surface can be calculated in Joules, this does not relate directly to a dose of light given interstitially because:-

1. Up to 50% of the light delivered to the skin surface is reflected without being absorbed (Dougherty, 1984b).
2. The amount of light penetrating to a subcutaneous lesion depends on the pigmentation of the skin which may vary between sites and individuals.
3. The depth of the tumour below the skin surface affects the amount of light it receives.
4. When treating tumours with superficial light a margin of normal tissue around the tumour is usually treated and it is difficult to predict how much of the light scattered from the normal tissue is absorbed by the tumour.

Clinical Studies

The complete response rate of cutaneous or subcutaneous tumours in patients treated with superficial photochemotherapy is 50 to 80 % (Dougherty et al., 1984; Dougherty, 1986). It is suggested that the maximum depth of tumour that may be effectively treated is 1.5 cm (Dougherty, 1984b) and that a higher therapeutic ratio may be obtained by allowing three or more days to elapse between drug and light administration (Dougherty et al., 1979; Forbes et al., 1980) but the relationship between tumour and normal tissue response to photochemotherapy and how this is affected by changes in dose of photosensitizer or light is still not clear.

Some patients with recurrent superficial tumour have been treated with interstitial photochemotherapy but the results are usually presented with those of superficial photochemotherapy (Forbes et al., 1980; Schuh et al., 1987). Grossweiner, Hill and Lobracio (1987) did, however, report complete responses in 10 of 12 patients with head and neck cancer treated with interstitial photochemotherapy.

Superficial photochemotherapy has also been used to treat locally recurrent head and neck tumours with some patients showing complete tumour response lasting for more than a year (Wile et al., 1984) and others showing good palliation of symptoms (Schuller et al., 1985).

The only major side-effect of photochemotherapy is cutaneous photosensitivity (Dougherty et al., 1978). In the absence of photosensitizers, excessive sunlight exposure causes erythema within a few hours due mostly to the effects of ultraviolet B light (290 to 320

nm). Photofrin II has an absorption spectrum from 300 to 640 nm making patients sensitive to all visible and ultraviolet A (320 to 350 nm) and B light.

Photosensitivity may be assessed by measuring the erythema induced by known doses of light. Visual assessment of erythema is subject to error depending on the light in which observations are made, the colour of the surrounding skin and the experience and visual acuity of the observer (Chamberlin and Chamberlin, 1980). The colour of Caucasian skin is determined mainly by the quantity of blood in the dermis (Lewis, 1926) and the erythema produced is due mainly to dilatation of superficial blood vessels. Reflectance meters may be used to measure the amount of haemoglobin present in the skin giving an objective measurement of erythema (Dawson et al., 1980; Farr and Diffey, 1984).

Summary

Photochemotherapy has been shown to be effective in treating superficial tumours. The therapeutic ratio of superficial photochemotherapy is based on a relatively higher concentration of photosensitizer being present in the tumour than in the surrounding normal tissue. To achieve an optimum therapeutic ratio, this difference in concentration should be at its maximum when light is given. Measurement of the levels of photosensitizer in tissue and plasma pharmacokinetics may allow the time when this difference is maximum to be predicted.

The response of tumours to superficial photochemotherapy increases with increasing dose of light and the diameter of necrosis around an optical fibre after interstitial photochemotherapy in vivo increases with increasing light dose but the relationship between light dose and tumour growth restraint after interstitial treatment may be different.

Delivering light interstitially may improve the therapeutic ratio of photochemotherapy in subcutaneous tumours, as the maximum light dose is given to the tumour rather than at the skin surface. Interstitial light may also overcome the problems of poor light penetration in tissue. If interstitial light is used to treat larger tumours, multiple optical fibre implants are required. The positioning of these fibres must be determined so that the whole tumour receives an adequate light dose.

Tumour response to superficial photochemotherapy may not be dependent on light dose alone. It has been suggested that there is a tumour bed effect associated with superficial photochemotherapy, therefore treatment field size may influence tumour response.

The only major side-effect of photochemotherapy is prolonged cutaneous photosensitivity, with the possibility of severe reactions on exposure to sunlight. If this photosensitivity can be measured and its duration predicted, for example from pharmacokinetic parameters, morbidity due to sunlight exposure may be avoided.

Objectives of this study

The purpose of this work was to examine some of the biological, physical and clinical factors limiting the usefulness of photochemotherapy in patients and to indicate ways of overcoming these limitations to enable the treatment's clinical development. The therapeutic ratio of a cancer treatment may be expressed by:-

$$\text{Therapeutic Ratio} = \frac{\text{Damage to tumour}}{\text{Damage to normal tissue}}$$

For photochemotherapy, the therapeutic ratio depends on the relative uptake and clearance of photosensitizer in tumour and normal tissues and the distribution and dose of light throughout the tumour and its surrounding normal tissues.

The number of patients suitable for treatment with photochemotherapy was likely to be small, so the clinical studies were supported by a programme of in vivo experiments, using an isogenic fibrosarcoma ($LSBD_1$) in BD_9 rats. Extrapolation of the results of animal tumour studies to the clinical situation requires caution but in vivo studies may give qualitative information about tumour response when clinical studies are not possible.

The main areas of investigation undertaken were:-

CLINICAL STUDIES

1. Pharmacokinetics of photosensitizer

The plasma pharmacokinetics of photosensitizer were examined to try to determine the optimum interval between drug and light administration

and the relationship between plasma drug levels and cutaneous photosensitivity (Chapter 5 and 7).

The photosensitizer used was Photofrin II, as this was the only drug licensed for treating patients with photochemotherapy.

2. Response to photochemotherapy

a)The optimum treatment parameters for superficial photochemotherapy were examined by assessing the effect of drug and light dose on tumour and normal tissue response (Chapter 6).

b) The effect of light dose on the response of tumour and skin to interstitial photochemotherapy was examined to determine whether interstitial light delivery improves the therapeutic ratio of photochemotherapy (Chapter 6).

As penetration of light in tissue increases with wavelength, 630 nm light, the longest wavelength of light that will activate Photofrin II, was used. The light sources were an Argon ion/dye laser or a Copper vapour/dye laser tuned to emit light at 630 nm.

3. Normal skin photosensitivity

To assess the degree and duration of normal skin photosensitivity, photopatch testing with known doses of white light from a solar simulator and using a reflectance meter to measure erythema was performed. The relationship between duration of photosensitivity and plasma levels and elimination of photosensitizer were examined (Chapter 7).

ANIMAL STUDIES

1. Levels of photosensitizer in tissue

a) To determine the optimum interval between drug and light administration, by measuring the level of porphyrin in plasma, tumour and muscle and skin adjacent to the tumour after a standard dose of photosensitizer (Chapter 3). This, also, allowed the relationship between plasma and tissue levels of photosensitizer to be examined.

b) To determine the relationship between dose of photosensitizer and level of porphyrin in tumour. If there is a direct relationship between drug dose and tumour levels of porphyrin, it should be possible to increase tumour response by increasing the dose of drug used. The influence of tumour size on porphyrin uptake was also examined as this may affect the response of tumour to photochemotherapy (Chapter 3).

During the course of the study Photofrin II was withdrawn. In response to this, the Department of Biochemistry, University of Leeds, produced polyhaematoporphyrin (PHP), which has the same High Performance Liquid Chromatography Profile (HPLC) as Photofrin II (Appendix 3.1) and produces the same level of photosensitization as Photofrin II (Chapter 4). PHP was used in vivo to study uptake of photosensitizer and in some experiments examining response to photochemotherapy.

2. Response to photochemotherapy

a) To compare superficial and interstitial photochemotherapy by establishing the light dose response curves for tumours treated with superficial and interstitial light (Chapter 4).

b) To examine the effect of interstitial photochemotherapy on tumours of different sizes and, by determining the maximum diameter tumour that can be effectively treated using a single optical fibre, to give guidance about the spacing of multiple optical fibre implants (Chapter 4).

c) To examine the influence of treatment field size on response to superficial photochemotherapy If there is a tumour bed effect associated with photochemotherapy, tumour response would be expected to increase with field size (Chapter 4).

Dose response curves for $LSBD_1$ treated with cytotoxic chemotherapy and irradiation were established. This allowed the response of $LSBD_1$ to conventional cancer treatments to be compared with the response of other rodent tumours (Chapter 2) and comparison of the efficacy and mode of action of photochemotherapy with conventional treatment in $LSBD_1$ (Chapter 4).

CHAPTER 2: $LSBD_1$ TUMOUR: ITS GROWTH AND RESPONSE TO CYTOTOXIC CHEMOTHERAPY OR RADIOTHERAPY

$LSBD_1$, an isogenic fibrosarcoma, implanted as subcutaneous fragments in the flank of BD_9 rats was used in this study because:-

1. There was a good "in house" supply of BD_9 rats.
2. BD_9 rats tolerate anaesthetic well and all irradiation was performed under anaesthetic.
3. There is a high tumour take rate using fragment implants (>90%) and they grow as spherical tumours which are not fixed to the skin or underlying tissue.
4. The tumours are easily accessible for treatment and grow freely without any pressure effects from surrounding structures or distress to the animals.
5. $LSBD_1$ very rarely produces gross distant metastases in the first three months after implantation, so animals do not die of disseminated disease before local tumour response has been assessed.

Origin of $LSBD_1$

The tumour, a poorly differentiated fibrosarcoma, arose spontaneously in the dorsal/lateral left flank of a male BD_9 rat in 1979. At post mortem, there was no obvious site of origin and the tumour was easily dissected from adjacent skin and lateral chest wall.

The tumour was maintained by subcutaneous injection of minced tumour into the flank of BD_9 rats until the sixth transplant generation. The

"healthy" tumour from this generation was aseptically removed and cut into 3 to 4 mm pieces. Three or 4 tumour pieces were placed in 1.8 ml cryotubes (Gibco Ltd., Paisley, U.K.) and the volume in the tube made up to 1.8 ml with 10% dimethyl sulphoxide (DMSO) and 10% foetal calf serum in 199 culture medium (Gibco Ltd.). The capped cryotubes were kept at 4 °C for 30 min, transferred to the gas phase of a liquid nitrogen freezer for 24 hours and then stored in the liquid phase at -196 °C.

Preparation of Tumours for Experimental Use

All animal studies were performed within the current U.K. Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia (1988).

The tumours used throughout were implanted into inbred BD_9 male or virgin female rats, given free access to 'CMRX Rat and Mouse Diet' (Labsure, Manea, Cambridgeshire) and water. They were kept in single sex groups of 5 or 6 per cage, in a room where the temperature was maintained at 20 to 21 °C. The room was lit for 12 hours a day (06.00 to 18.00) with fluorescent day light tubes fitted with a diffusing cover which produced a light intensity of less than 0.3 $mWcm^{-2}$ in the animals' cages. The room was left in darkness the rest of the day (18.00 to 06.00).

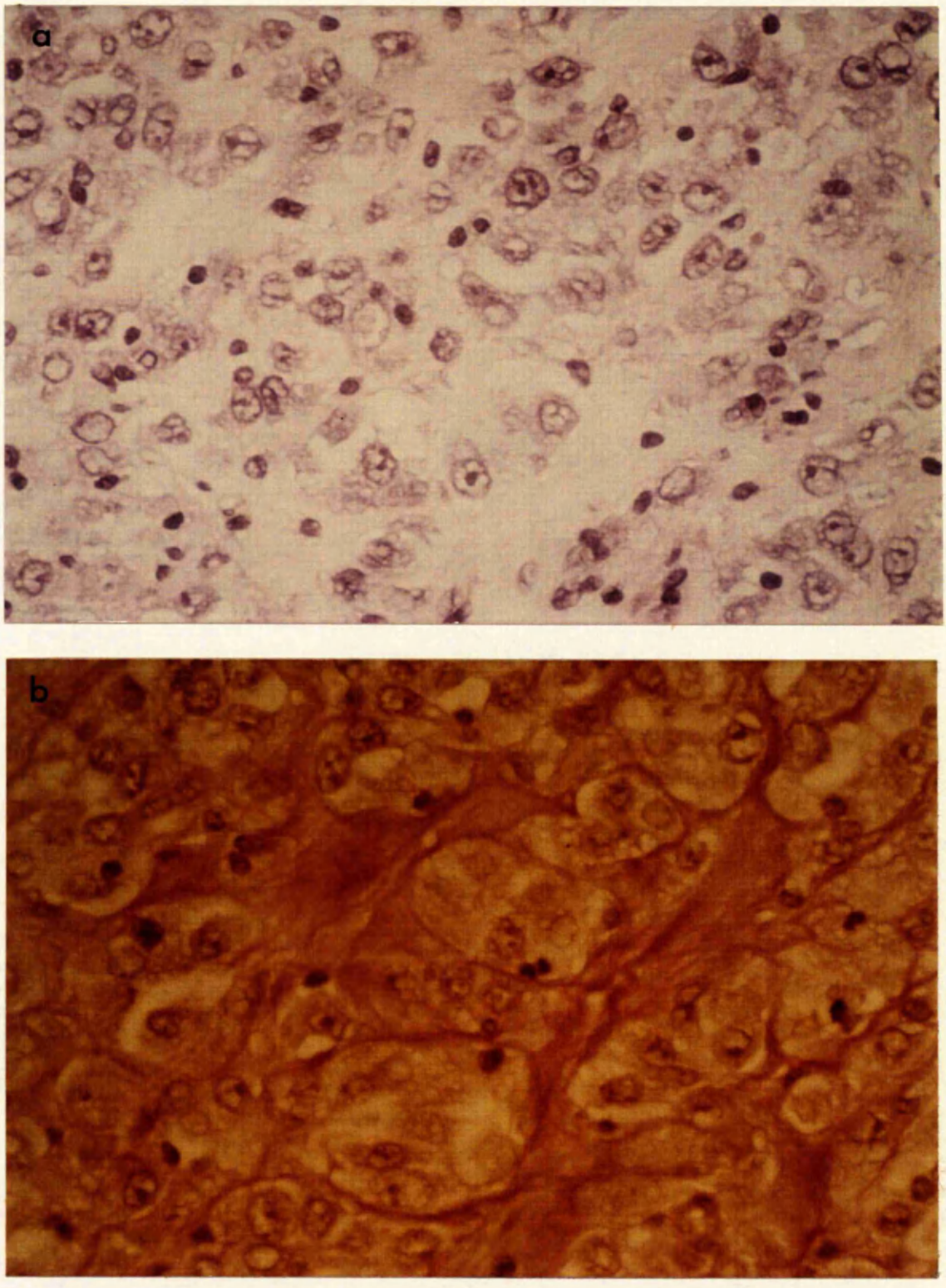
In March 1986, an ampoule containing sixth transplant generation tumour was taken from liquid nitrogen and thawed by immersion in warm water. The tumour fragments were removed and washed with normal

saline. The fragments were implanted subcutaneously into the left and right flanks of two male rats through a small skin incision which was closed with a single suture (3.0 silk, Davies & Gelk, Gosport, Hampshire). The seventh to tenth tumour generations were sequentially maintained by subcutaneous injection of minced tumour into the right abdominal flank of rats.

The eleventh to thirty first transplant generations were used sequentially for this study. Tumours were produced by aseptic implantation of a tumour fragment (about 3 mm in diameter) into the right flank of 200 to 400 g rats, through a small incision closed with a suture. The tumours grew as spheres unattached to the underlying muscle or overlying skin and very rarely ulcerated. The site of the tumour allowed it to grow freely without constraint, until it reached about 30 mm in mean diameter, and without detriment to the animals. Such tumours establish their blood supply from the vasculature of the underlying muscle wall and the overlying skin. The tumours rarely produced distant metastases but late local recurrence after tumour excision has been seen (personal observation).

Histology of the experimental tumours shows areas of round undifferentiated malignant cells with pleomorphic nuclei and prominent nucleoli and areas of more differentiated fibrous cells with elongated nuclei and banded chromatin (Figure 2.1). Connective tissue was abundant throughout the tumour and malignant cells were surrounded by collagen. The tumour contained numerous small blood vessels and there was little evidence of necrosis.

Figure 2.1



$LSBD_1$ fibrosarcoma.
a) Area of undifferentiated tumour (Haematoxylin and Eosin, x500)
b) Area of more differentiated tumour showing collagen formation (van Gieson, x500)

Assessment of Tumour Growth and Response to Treatment

Vernier calipers were used to measure, to the nearest millimetre, three mutually perpendicular tumour diameters, approximately five times a week. Measurements began when the mean tumour diameter was greater than 6 mm, usually 7 to 12 days after implantation. When the tumour reached a required diameter, designated T size, animals were randomized into control and treatment groups. Tumours differing by 3 mm or more in their three measured diameters on T day were excluded from the study.

Mean tumour diameter was plotted against time in days after T, to produce tumour growth curves. Tumour growth delay was used to assess dose response (Thomlinson and Craddock, 1967). This was calculated by subtracting the mean time taken for all untreated control tumours to grow from 8 to 10 mm to 25 mm diameter from the mean time taken for the treated tumours to grow from T to T+16 mm diameter. As mean tumour diameter increased directly with time for untreated tumours over the range of sizes studied, using these parameters allowed comparison of the effect of treatment on tumours of different sizes. Growth and dose response curves were fitted free-hand unless otherwise stated.

When tumour measurements were made, the animals were also weighed and any reaction to treatment, especially local skin damage, recorded. Weight loss greater than 10% of the animals pretreatment body weight was considered to be significant. Animals were killed by cervical dislocation when the end-point of the experiment was reached, either at a given tumour size or time after randomization. A full post mortem was performed on all animals and any gross changes recorded.

The Growth of the Untreated Tumour

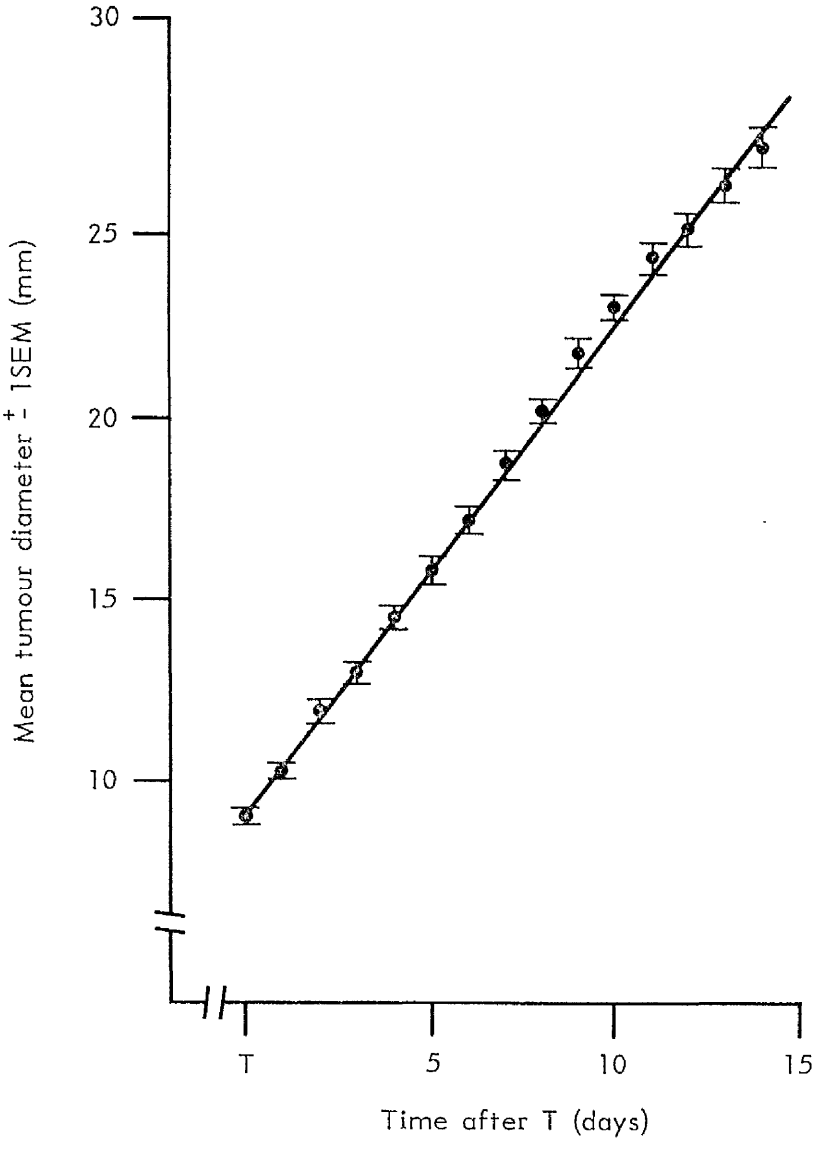
The growth curves of untreated tumours from the eleventh and twelfth (6 animals), twentieth and twenty first (10 animals) and twenty ninth and thirtieth (13 animals) transplant generations were compared from 8 to 10 mm (T) to 25 mm diameter (Appendix 2.1). The difference in growth rate between different transplant generations was not significant ($p>0.05$), except for the twentieth and twenty first transplant generations ($p<0.01$). These generations of tumour, however, responded to treatment in the same way as tumours from other transplant generations, therefore the data from all control animals was combined (Figure 2.2).

The control tumours took 10.8 $\pm$ 1.8 days (mean $\pm$ 1SE) to grow from 8 to 10 mm to 25 mm in diameter and the tumour volume doubling time was 2.4 days. The growth rate of $LSBD_1$ is comparable to that of other spontaneous transplanted rodent tumours (Denekamp, 1972; McNally, 1973; Moore and Dixon, 1978). In contrast, the volume doubling time of human mesenchymal sarcomas is 35 to 50 days (Charbit, Malaise, and Tubiana, 1973)

Response to Cytotoxic Chemotherapy

When mean tumour diameter was 8 to 10 mm (T), animals were randomized to receive no treatment, 25, 50, 100, 150, or 200 $mgkg^{-1}$ body weight of cyclophosphamide (Endoxana, WB Pharmaceuticals Ltd., Bracknall, U.K.) as a single intraperitoneal injection, remote from the tumour

Figure 2.2



Control growth curve: growth of all transplant generations of untreated $LSBD_1$ (T= 8-10 mm; n= 74). Curve fitted using linear regression analysis (r= 1.00).

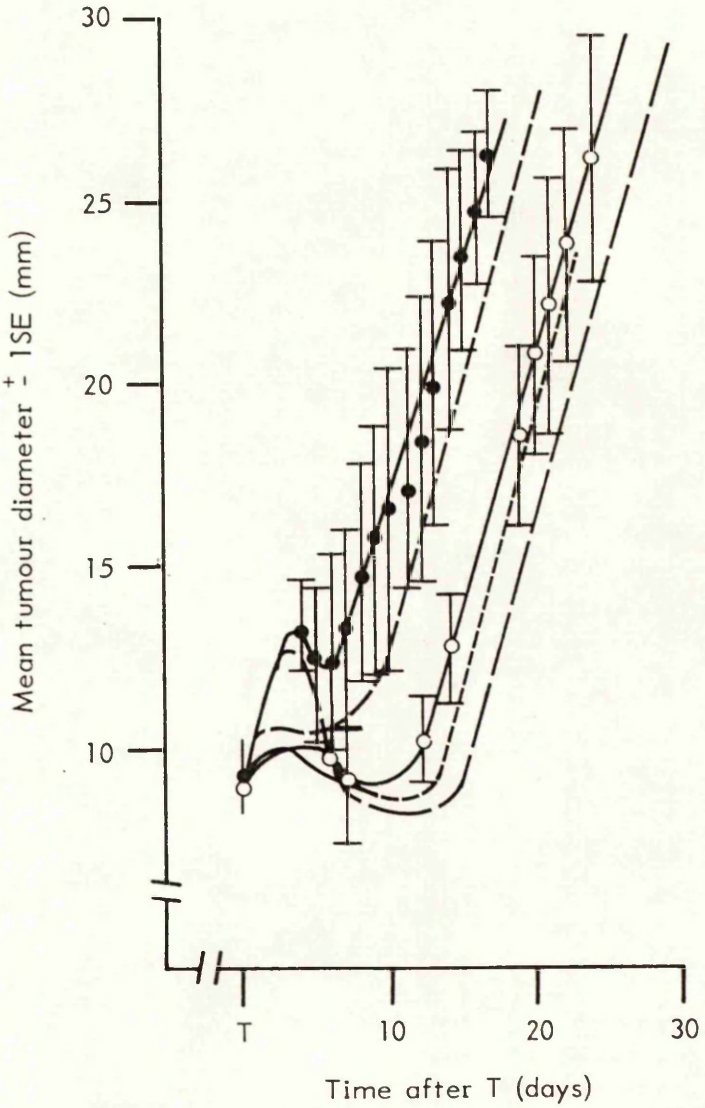
site. Tumours were measured until the mean diameter of the tumour reached 25 mm.

The tumour continued to grow for 2 to 3 days after treatment but then regressed (Figure 2.3; Appendix 2.2). Even after 200 $mgkg^{-1}$ of cyclophosphamide the tumour did not regress to less than its original size. Delay in regrowth of the tumour increased with dose but the rate of regrowth was not significantly altered by treatment ($p>0.10$) (Appendix 2.3). Thus, the dose response curve showed increasing tumour growth delay with increasing doses of cyclophosphamide, up to 150 $mgkg^{-1}$ (Figure 2.4; Appendix 2.3). Above this dose, there was no significant increase in tumour response, although the treatment related morbidity and mortality increased (Appendix 2.3). The treatment related deaths were due to gastro-intestinal haemorrhage (1 animal), probable septicaemia (1 animal) and haemorrhagic cystitis (1 animal).

The response of rodent tumours to chemotherapy is variable. Like $LSBD_1$, RIB_5 sarcoma in Johns' strain Wistar rats did not show regression of the tumour to less than its original volume (Peel and Cowen, 1972) but LMC_1 mammary carcinoma in the same strain did (Moore and Dixon, 1978). This is dependent on the rate of removal of dead cells as well as the number of cells killed by the treatment (Denekamp, 1972). Both the LMC_1 carcinoma and the RIB_5 fibrosarcoma show a dose response to cyclophosphamide similar to that observed for $LSBD_1$ (Peel and Cowen, 1972; Moore and Dixon, 1978).

The greater the number of clonogenic malignant cells killed by a cytotoxic agent the longer it takes for the tumour to regrow to its

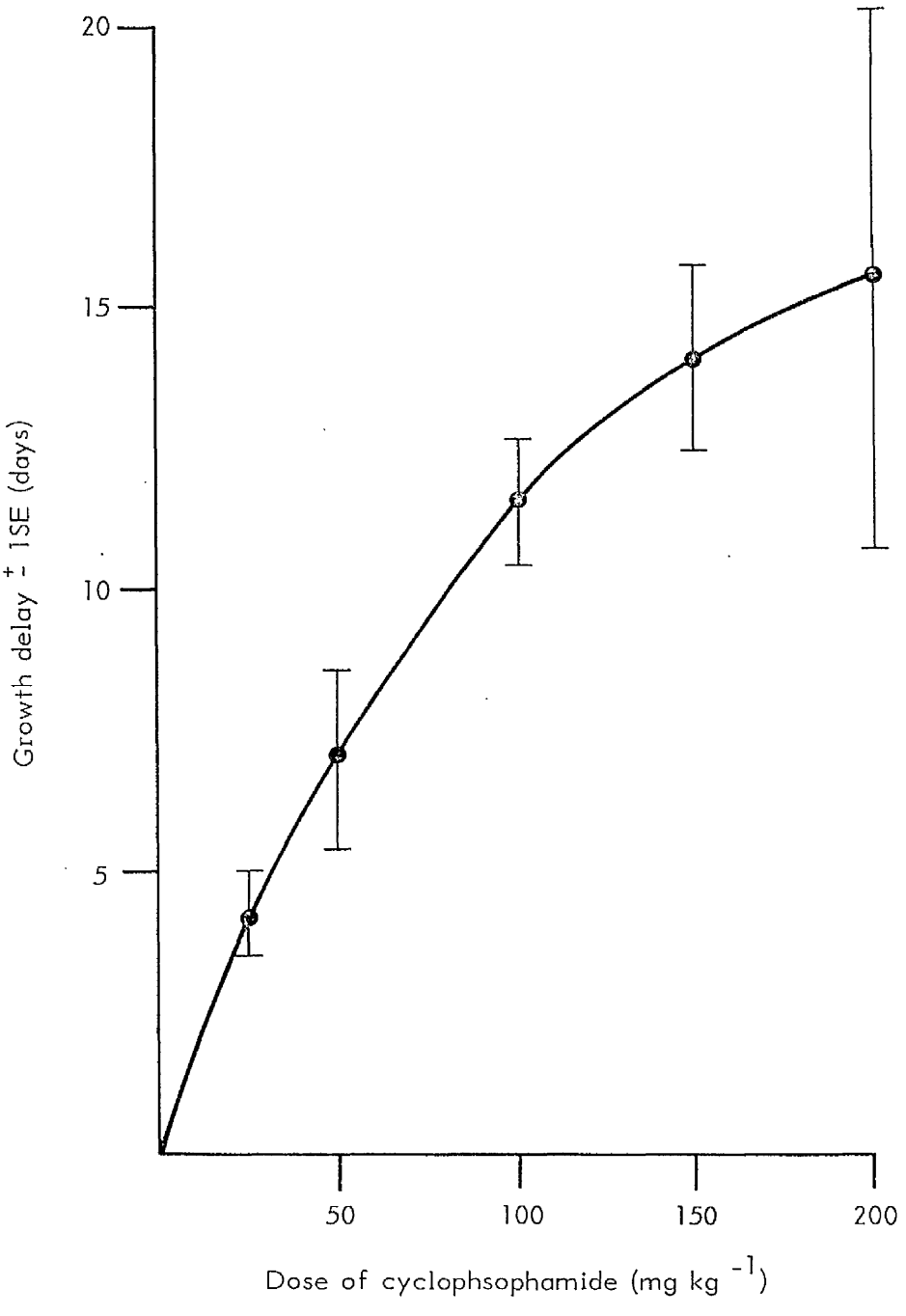
Figure 2.3



Regression and regrowth of $LSBD_1$ (T= 8-10 mm) after single doses of cyclophosphamide. (Some data points and error bars omitted for clarity, full data given in Appendix 2.2.)

- ● 25 $mgkg^{-1}$ cyclophosphamide
- — — — 50 $mgkg^{-1}$ cyclophosphamide
- ○ 100 $mgkg^{-1}$ cyclophosphamide
- — — — 150 $mgkg^{-1}$ cyclophosphamide
- ------ 200 $mgkg^{-1}$ cyclophosphamide

Figure 2.4



Dose response curve for $LSBD_1$ (T= 8-10 mm) treated with a single dose of cyclophosphamide

original size and therefore for $LSBD_1$ an increase in tumour growth delay with increasing dose of cyclophosphamide was to be expected. The possible plateau in the dose response curve at higher drug doses is presumed to be due to a resistant population, probably quiescent cells that retain clonogenic capacity (Hahn et al., 1973; Moore and Dixon, 1978).

Chemotherapy is a systemic treatment and cyclophosphamide may, also, affect normal tissue that is rapidly renewing, such as the gastro-intestinal tract and bone marrow. This may result in weight loss and bone marrow failure with resulting susceptibility to haemorrhage and infection which are known side-effects of cyclophosphamide. Haemorrhagic cystitis is, also, a recognized side-effect of cyclophosphamide. These effects are dose related and would, therefore, be expected to be to increase with dose of drug (Appendix 2.3).

Response to Radiotherapy

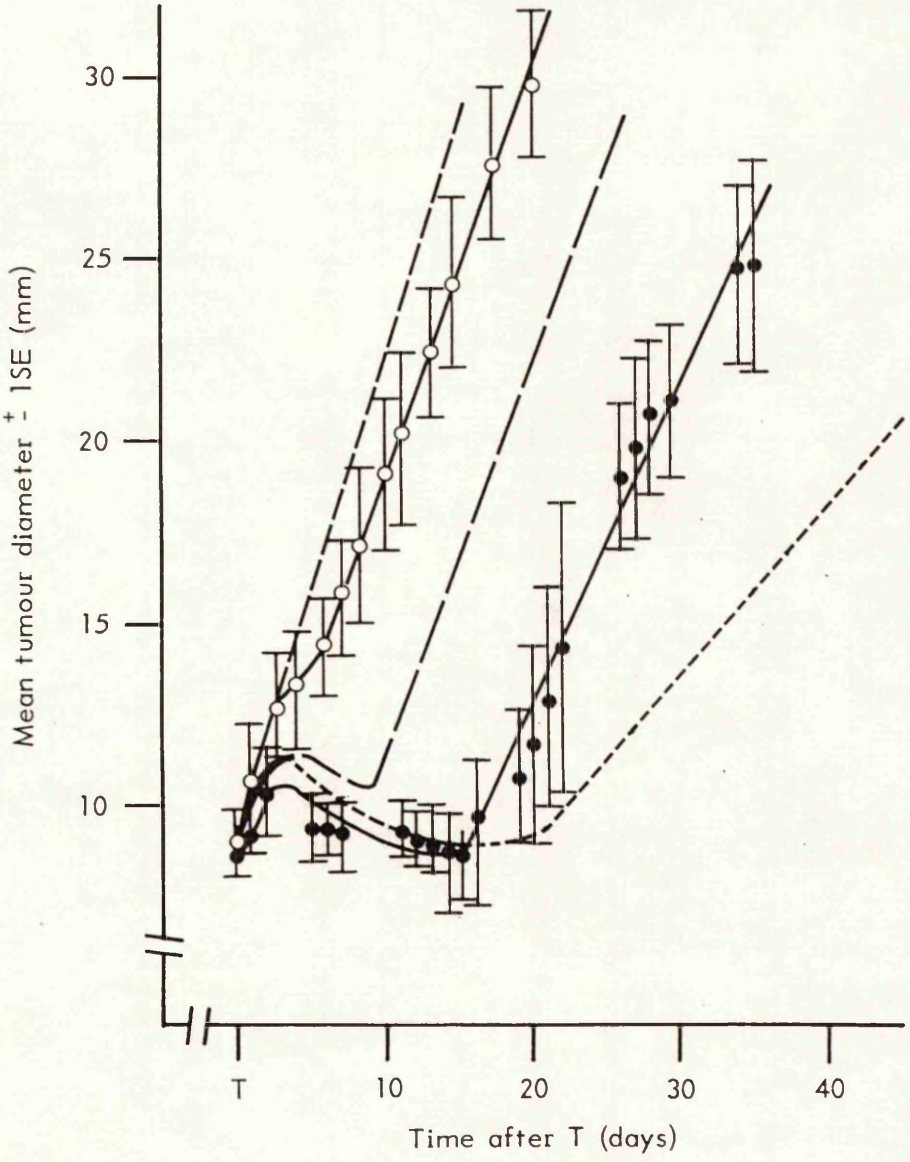
Radiotherapy was given using a single 2 cm diameter tangential field from a Cobalt 60 unit. The source to skin distance was 40 cm and a 1 cm "Perspex" filter provided full build up (Moore, 1976). When the tumours reached 8 to 10 mm diameter, the animals were randomized to receive no treatment or a single dose of 5, 10, 20 or 30 Gy, at a dose rate of 1.3 to 1.42 $Gymin^{-1}$. The animals were anaesthetized with Amylobarbitone Sodium (Sodium Amytal, Eli Lilly and Co. Ltd., Basingstoke, U.K.) 10 to 20 mg intraperitoneally, 5 min before treatment. The effects of the anaesthetic usually lasted for about 30 min.

Tumours continued to grow for 3 days after irradiation and then regressed but not to a smaller size than at the start of treatment (Figure 2.5; Appendix 2.4). As expected, the time taken for the tumour to start to grow again increased with increasing dose of irradiation (Appendix 2.5). In contrast with the response of $LSBD_1$ to cyclophosphamide, this was not the only factor causing tumour growth delay. The rate of tumour regrowth decreased with increasing dose of irradiation (Appendix 2.5). The dose response curve showed increasing tumour growth delay with increasing doses below 30 Gy (Figure 2.6).

After 30 Gy 2 of 6 animals showed significant weight loss (Appendix 2.5). Higher doses of irradiation were not used because of the likelihood of increasing treatment morbidity. Radiotherapy was given to small localized fields so systemic effects would not be expected. It was not possible to exclude all gut underlying the tumour from the treatment field and, therefore, the gut in this region may have been damaged. At post mortem, the animals showing significant weight loss were found to have peritoneal adhesions underlying the tumour site suggesting that weight loss was due to the local effects of irradiation on the gastro-intestinal tract.

Denekamp (1972) has suggested that the failure of rodent sarcomas to shrink within the first few days after irradiation is due to a relatively low cell loss factor. This may explain why $LSBD_1$ did not regress immediately after irradiation. As cell loss factor is a property of the tumour, delay in tumour regression after chemotherapy, as observed, may also be expected.

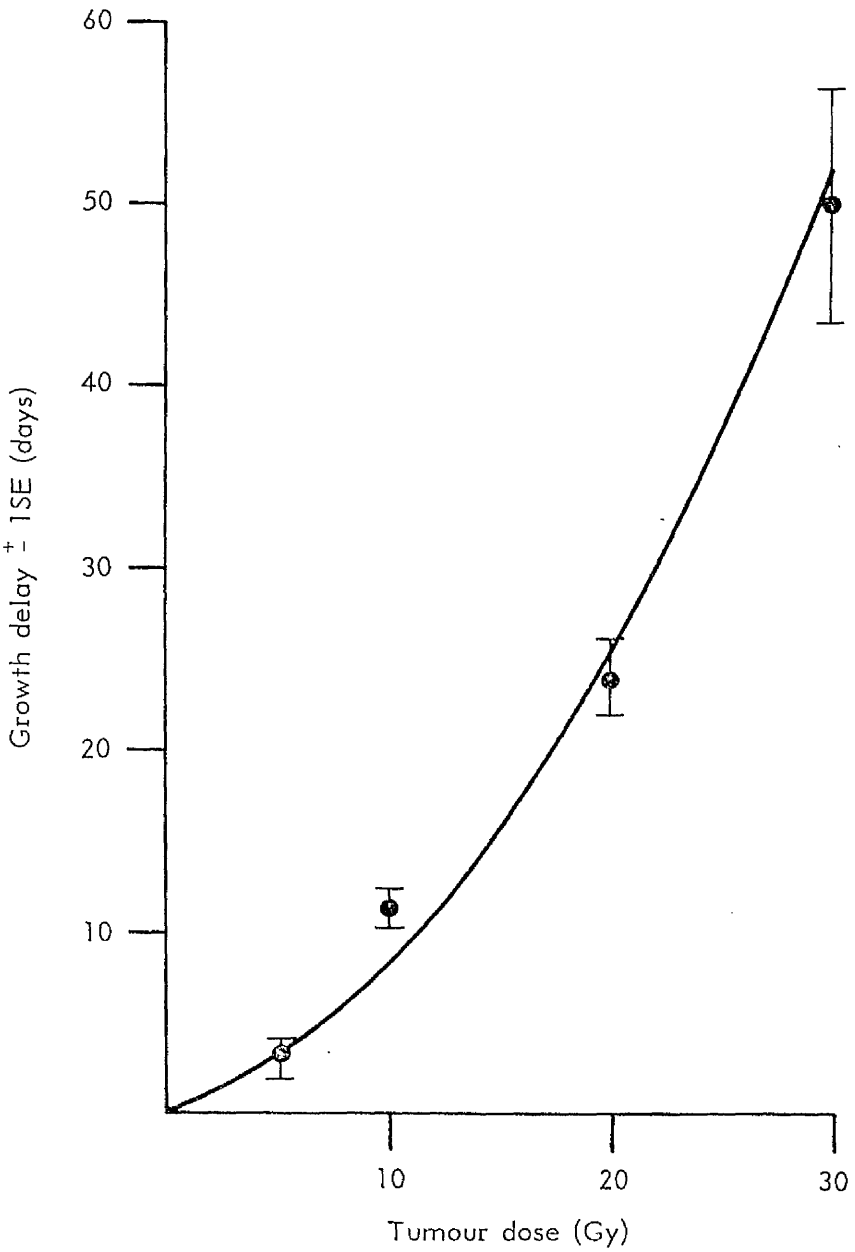
Figure 2.5



Regression and regrowth of $LSBD_1$ after single doses of ^{60}Co irradiation. (Some data points and error bars omitted for clarity, full data given in Appendix 2.4.)

——— Control, 0 Gy
o 5 Gy
— — — 10 Gy
● 20 Gy
------- 30 Gy

Figure 2.6



Dose response curve for $LSBD_1$ (T= 8-10 mm) treated with a single dose of gamma irradiation.

A decrease in the rate of tumour regrowth is common after single dose radiotherapy and usually results from "tumour bed effects" (Begg, 1980). A slow rate of clearance of dead cells within the tumour after radiotherapy may lead to slower regrowth of tumour initially. However, this is unlikely to be the cause of change of growth rate observed in $LSBD_1$, as the end point for regrowth delay was a tumour diameter 2.2 times greater than the treatment diameter (Begg, 1980).

Radiotherapy damages normal tissue in the treatment volume as well as the tumour and small blood vessels are particularly vulnerable to such damage. As the treated tumour regrows it must establish an increasing blood supply, but because of radiation damage this new blood supply is not able to keep pace with the tumour's requirements and regrowth is slowed in comparison to untreated tumours. This vascular damage is thought to be the major component responsible for the "tumour bed effect" (Saeki, Shimazaki and Urano, 1971; Clifton and Jirtle, 1975).

The irradiation dose response curves for 9 mm diameter RIB_5 sarcoma and LMC_1 carcinoma in air or hyperbaric oxygen breathing Johns' strain Wistar rats show a combination of two curves (Thomlinson and Craddock, 1967; Moore, 1976). The initial component is a curve attributable to the fully oxygenated cells and the second to the anoxic cells. A single component curve is seen if tumours are clamped to produce anoxia during irradiation (Thomlinson and Craddock, 1967; Moore, 1976), when only anoxic cells are present. The aerobic irradiation dose response curve for $LSBD_1$ did not have two components. This is most likely to be due to the doses of irradiation used being small, as the anoxic curves are only seen at higher doses. The break in the curve for the RIB_5 sarcoma is at about 15 Gy (Thomlinson and Craddock,

1967) and for the LMC_1 carcinoma at about 25 Gy. The response of $LSBD_1$ is unlikely to be due to the tumour being anoxic, as this would imply that the tumour is permanently anoxic which would render it incapable of survival, although general hypoxia cannot be excluded.

CONCLUSIONS

The response of $LSBD_1$ to chemotherapy and radiotherapy is similar to that observed in other isogenic rodent tumours. The response to chemotherapy suggests the presence of a resistant population of cells, probably clonogenic cells that are not cycling and, therefore, fail to respond to cyclophosphamide. These cells may, also, limit the effect of photochemotherapy as porphyrins are taken up by dividing cells (Figge et al., 1948).

Although tumour histology does not suggest the presence of major hypoxic damage, the absence of a biphasic radiation dose response curve does not exclude hypoxia. Also, the presence of non-cycling cells may be due to hypoxia. The presence of hypoxic cells in $LSBD_1$ may limit the effect of photochemotherapy, as hypoxia decreases response to photochemotherapy (Bown et al., 1986).

The response of $LSBD_1$ to radiotherapy suggests the presence of a "tumour bed effect" which occurs as a result of damage to the tumour vasculature. As it is suggested that photochemotherapy produces its main effect by causing vascular damage (Star et al., 1986), a tumour bed effect may occur after photochemotherapy.

CHAPTER 3: TUMOUR AND NORMAL TISSUE PORPHYRIN LEVELS IN BD_9 RATS

Since a literature search revealed no information about the uptake of Photofrin II in rodent tumours, the total porphyrin levels were measured in the tumour, surrounding normal tissue, that is underlying muscle and overlying skin, and plasma of $LSBD_1$ bearing BD_9 rats after a single intraperitoneal injection of PHP in order to:-

1. Determine the optimal interval between drug administration and light delivery in this tumour system.
2. Examine the relationship between dose of photosensitizer and tumour porphyrin concentration.
3. Measure the level of porphyrin present in tumours of different sizes at the time of light delivery.

MATERIALS and METHODS

Polyhaematoporphyrin Administration

Polyhaematoporphyrin was manufactured by the Department of Biochemistry, University of Leeds (Appendix 3.1).

Vials containing PHP (2.5 $mgml^{-1}$) were stored at -20 °C and when required were returned to room temperature. The drug needed for treatment was withdrawn and the remainder divided into 1.8 ml sterile cryotubes (Gibco Ltd.) and refrozen. Drug that was unused after being thawed twice was discarded.

The drug was given intraperitoneally remote from the tumour, at a dose of 0.5 to 40 $mgkg^{-1}$ body weight. The volume of injection was 0.4 to 5 ml. Before injection the skin overlying the tumour was shaved to remove all superficial hair.

Measurement of Porphyrin Levels

Animals were killed by cervical dislocation while under ether anaesthesia and 2 to 5 ml of blood taken immediately, by cardiac puncture. The blood was heparinised with 0.1ml of 1000 units ml^{-1} Sodium Mucous Heparin (Multiparin, CP Pharmaceuticals, Wrexham, Clwyd) per ml of blood, centrifuged (1000 r.p.m. for 30 min) and separated to obtain plasma samples. These were stored at -20 °C.

The whole tumour was excised and as much connective tissue as possible removed. A 1 cm square of full thickness muscle from immediately below and a 1 cm square of skin from immediately above the tumour were, also, taken. Animals killed at 168 h were shaved immediately before death to remove any new hair growth. Each specimen was placed separately in a 1.8 ml cryotube, "snap" frozen and stored in liquid nitrogen or a freezer (-20 °C) until assay.

Total porphyrin levels were measured by the Department of Biochemistry, University of Leeds, using spectrofluorimetry (Appendix 3.2).

Treatment Groups

Animals were randomized into 3 experiments:-

1. Variation in tissue porphyrin level with time after administration of drug.

Animals were all given 20 $mgkg^{-1}$ of PHP at a tumour size of 8 to 10 mm diameter. The animals were killed 15 min, 30 min, 1 h, 3 h, 6 h, 24 h, 72 h or 168 h after injection and plasma, tumour, muscle and skin samples taken for measurement of total porphyrin levels.

2. Effect of drug dose on porphyrin levels.

Animals were given no drug, 0.2, 2.0, 10.0, 20.0, or 40.0 $mgkg^{-1}$ of PHP when the tumour size was 8 to 10 mm diameter. Animals were killed 24 h later and the tumour excised for assay of total porphyrin levels.

3. Effect of tumour size on porphyrin levels.

Animals were given 20 $mgkg^{-1}$ PHP when tumour size was 8 to 10 mm, 12 to 14 mm, 14 to 16 mm diameter. Animals were killed 24 h later and the tumour taken for assay of total porphyrin levels.

RESULTS

The animals tolerated PHP injection well. At post mortem, those given 20 $mgkg^{-1}$ of PHP had free drug visible in the peritoneal cavity up to 6 h after injection but by 24 h only 1 out of 5 animals had free drug visible. Of those receiving 40 $mgkg^{-1}$ of PHP, 3 out of 5 had free drug in the peritoneal cavity, at 24 h.

After 20 $mgkg^{-1}$ of PHP, the highest porphyrin levels were in muscle with lower levels in tumour and skin (Figure 3.1; Appendix 3.3). Total porphyrin levels increased over 6 to 24 h and then decreased (Figure 3.1).

The porphyrin level in $LSBD_1$ was always less ($p<0.05$) than that in the underlying abdominal muscle wall (Figure 3.1) and the tumour to muscle porphyrin level ratio did not vary significantly ($p>0.05$) (Appendix 3.4). The total porphyrin level in tumour and skin were not significantly different ($p>0.05$) (Figure 3.1). The tumour to plasma porphyrin ratio did not vary significantly ($p>0.05$) before or after 24 h but the skin to plasma ratio was greater at 168 h than before 24 h ($p<0.05$) (Appendix 3.4).

The concentration of porphyrin in the tumour 24 h after injection was proportional to the dose of PHP given (Figure 3.2; Appendix 3.5). Tumour size did not significantly affect the total porphyrin level in $LSBD_1$ 24 h after injection of 20 $mgkg^{-1}$ of PHP (Appendix 3.6).

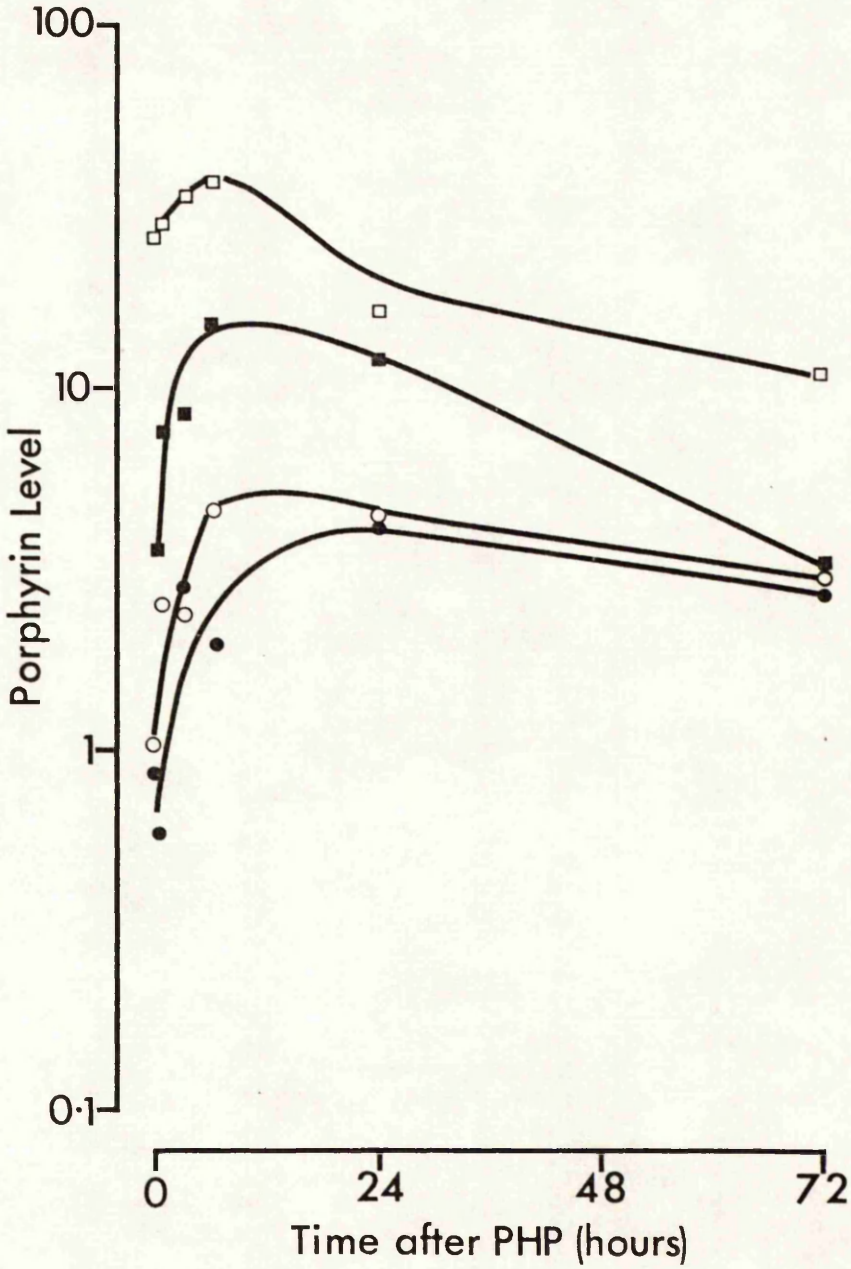
DISCUSSION

The variation in the total porphyrin levels measured in plasma, tumour and normal surrounding tissue may be due to:-

1. Variation in PHP absorption. Free porphyrin was visible in the peritoneal cavity of some animals 24 h after PHP injection suggesting that the rate of drug absorption varied.

2. Tumour position. The blood supply of tumour and surrounding normal tissue is dependent on position and variation in blood supply may

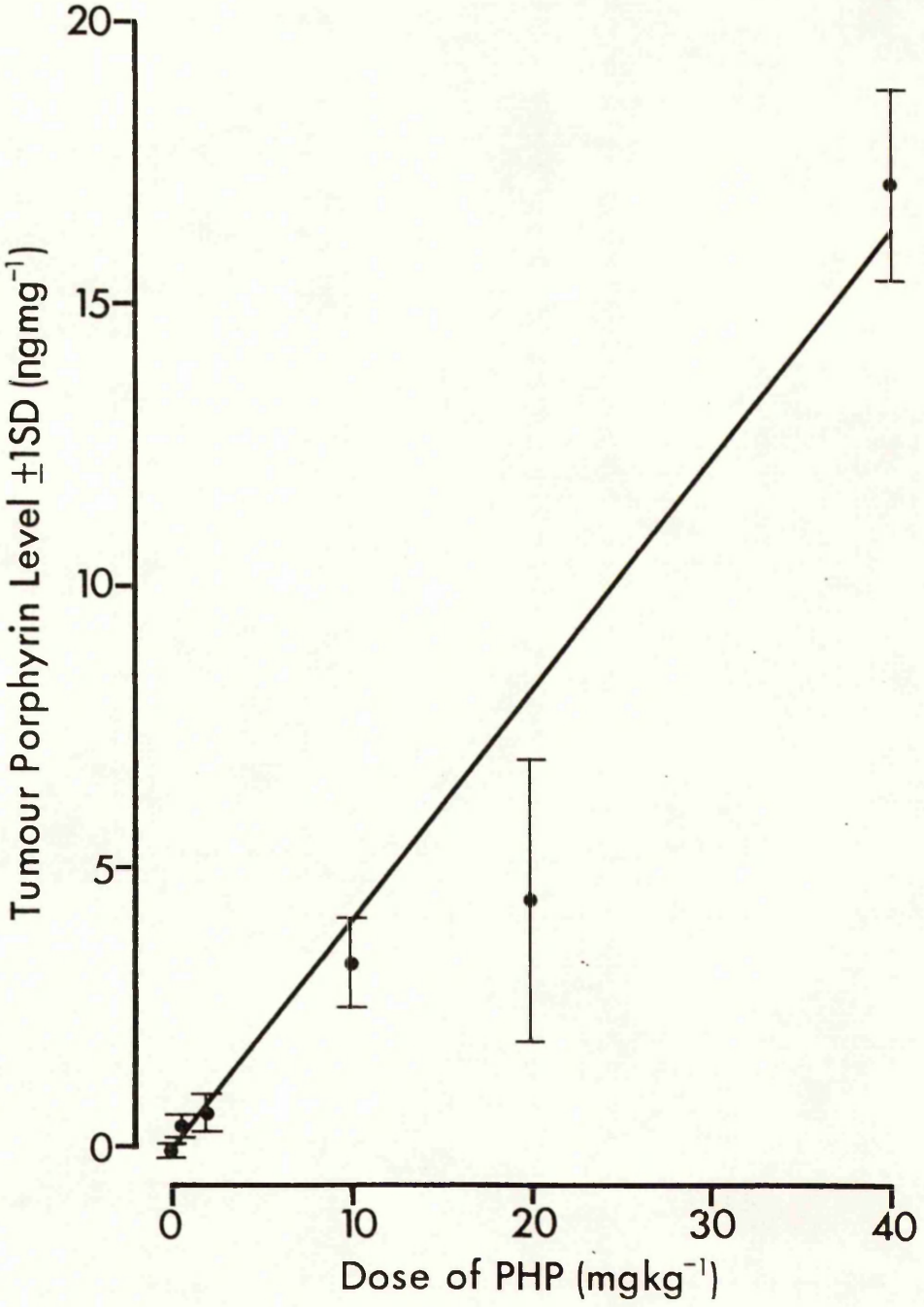
Figure 3.1



Total porphyrin concentration in $LSBD_1$ (T= 8-10 mm) bearing BD_9 rats after 20 $mgkg^{-1}$ of PHP. (Error bars omitted for clarity, full data given in Appendix 3.3.)

- ■ Plasma (μgml^{-1})
- ○ Tumour ($ngmg^{-1}$)
- □ Muscle ($ngmg^{-1}$)
- ● Skin ($ngmg^{-1}$)

Figure 3.2



Relationship between dose of PHP and total porphyrin concentration in $LSBD_1$ tumour (T= 8-10 mm) 24 hours after intraperitoneal injection (n= >5). Curve fitted using linear regression analysis (r= 0.96).

affect the availability of drug for uptake. This would not, however, explain the observed variation in plasma porphyrin levels.

3. Other host factors. The mechanism of uptake of porphyrin photosensitizer is not clearly understood (Girotti, 1983) but host factors should be minimized by using inbred animals.

4. The inclusion of subcutaneous tissue in specimens. Uptake of photosensitizer varies with tissue type (Gomer and Dougherty, 1979), therefore if all subcutaneous tissue was not removed from the tissue specimen variation in the total porphyrin level may occur. This would not explain the variation plasma or muscle porphyrin levels.

5. Sampling errors. Although the whole tissue sample was homogenized and stirred immediately before removal of the aliquots for assay, it is possible that the volume of homogenate removed was not representative of the whole sample. Again this would not explain the variation in plasma porphyrin levels.

Porphyrin levels in the blood, tumour and skin of mice after 10 $mgkg^{-1}$ of HPD were similar to those observed in this study after 20 $mgkg^{-1}$ of PHP (Gomer and Dougherty, 1979). Porphyrin levels in the muscle of mice after 10 $mgkg^{-1}$ of HPD (Gomer and Dougherty, 1979; Evensen *et al.*, 1984) were, however, lower than those measured after 20 $mgkg^{-1}$ of PHP. This may be related to where the muscle sample was taken from. This was not stated in the murine studies. In this study, the sample was taken from the BD_9's anterior abdominal wall immediately underlying the tumour because the porphyrin level there may influence response to photochemotherapy. The parietal peritoneum is adherent to the anterior abdominal wall. PHP was given by intraperitoneal injection and absorbed through the peritoneum, therefore the level of

porphyrin in muscle samples from the abdominal wall may be higher than in other muscles.

If the therapeutic ratio of photochemotherapy is dependent on the presence of a higher concentration of photosensitizer in tumour than surrounding normal tissue, the absence of such a difference in this study (Figure 3.1) suggests that the therapeutic ratio of photochemotherapy in the $LSBD_1/BD_9$ tumour system may be low. As the ratio of porphyrin levels in tumour and the surrounding normal tissue did not vary significantly (Appendix 3.4) and porphyrin levels in $LSBD_1$ were maximum between 6 and 24 h (Figure 3.1), laser irradiation was given 24 h after photosensitizer.

Porphyrin levels in $LSBD_1$ fell slowly (Figure 3.1), therefore there may be a relatively long interval when the level of photosensitizer in the tumour is sufficient for light to be applied effectively. The tumour to plasma porphyrin ratio did not change significantly with time but the variation between animals, indicated by the large standard deviations in the ratio, suggest that it may be difficult to predict tumour porphyrin levels for individual animals from plasma porphyrin levels.

If the porphyrin level in human skin declines slowly, as it did in the skin of BD_9 rats, prolonged cutaneous photosensitivity would be expected. The skin to plasma porphyrin ratio increased with time, suggesting that if porphyrin is cleared more slowly from skin than from plasma in BD_9 rats. If a similar relationship also occurs in humans and clearance of photosensitizer from plasma is used to

determine the duration of cutaneous photosensitivity, it may be underestimated.

The concentration of porphyrin in $LSBD_1$ increased with increasing dose of PHP (Figure 3.2). A similar relationship between tumour concentration and dose of Aluminium phthalocyanine has been demonstrated in another rodent fibrosarcoma (Tralau et al., 1987) but tumour response only increases with dose of Aluminium phthalocyanine over a certain dose range, above this dose tumour response decreases with increasing drug dose (Bown et al., 1986). This is due to the strong absorbance of light by Aluminium phthalocyanine at 670 nm, the wavelength of light used to activate the drug. Photofrin II and HPD are less likely to cause this diminution of tumour response with increasing high drug doses because of their weaker absorbance of light (Wilson, Patterson and Burns, 1986). Tumour response does increase with dose of HPD (Dougherty et al., 1975 and Cowled and Forbes, 1985). As concentration of porphyrin in $LSBD_1$ increased with increasing dose of PHP, tumour response might be expected to increase with dose of PHP. If the concentration of porphyrin in normal tissue, also, increases with the dose of photosensitizer, then normal tissue damage may increase in parallel with tumour response giving no therapeutic advantage.

Tumour size did not significantly affect the total porphyrin concentration in $LSBD_1$. The tumour levels were, however, an average of the level throughout the tumour and it is possible that some areas contained a relatively high concentration of photosensitizer while other areas contained a relatively low concentration of photosensitizer making them unresponsive to photochemotherapy.

CONCLUSIONS

1. The total porphyrin levels in $LSBD_1$ were the same or less than those in surrounding normal tissue.

2. Twenty four hours was a suitable interval between giving PHP and light when treating $LSBD_1$ tumour in BD_9 rats with photochemotherapy.

3. In BD_9 rats, the porphyrin levels in skin fell more slowly than those in plasma.

4. Porphyrin levels in $LSBD_1$ increased with dose of PHP, therefore tumour response to photochemotherapy may, also, increase with dose of photosensitizer but this may not improve the therapeutic ratio.

5. Total porphyrin level in $LSBD_1$ is not influenced by tumour size between 8 and 16 mm diameter.

CHAPTER 4: RESPONSE OF $LSBD_1$ TO PHOTOCHEMOTHERAPY

The objectives of the work described in this chapter were:-

1. To establish light dose response curves for $LSBD_1$ treated with superficial and interstitial photochemotherapy. This should allow superficial and interstitial light delivery to be compared.
2. To examine the effect of interstitial photochemotherapy on tumours of different sizes. By determining the maximum size of tumour that can be treated effectively with a single optical fibre, guidance may be given about the spacing of fibres in multiple fibre implants.
3. To examine the influence of field size on the response of $LSBD_1$ to superficial photochemotherapy. If there is a "tumour bed effect" associated with photochemotherapy, field size might be expected to influence the tumour's response to treatment

MATERIALS AND METHODS

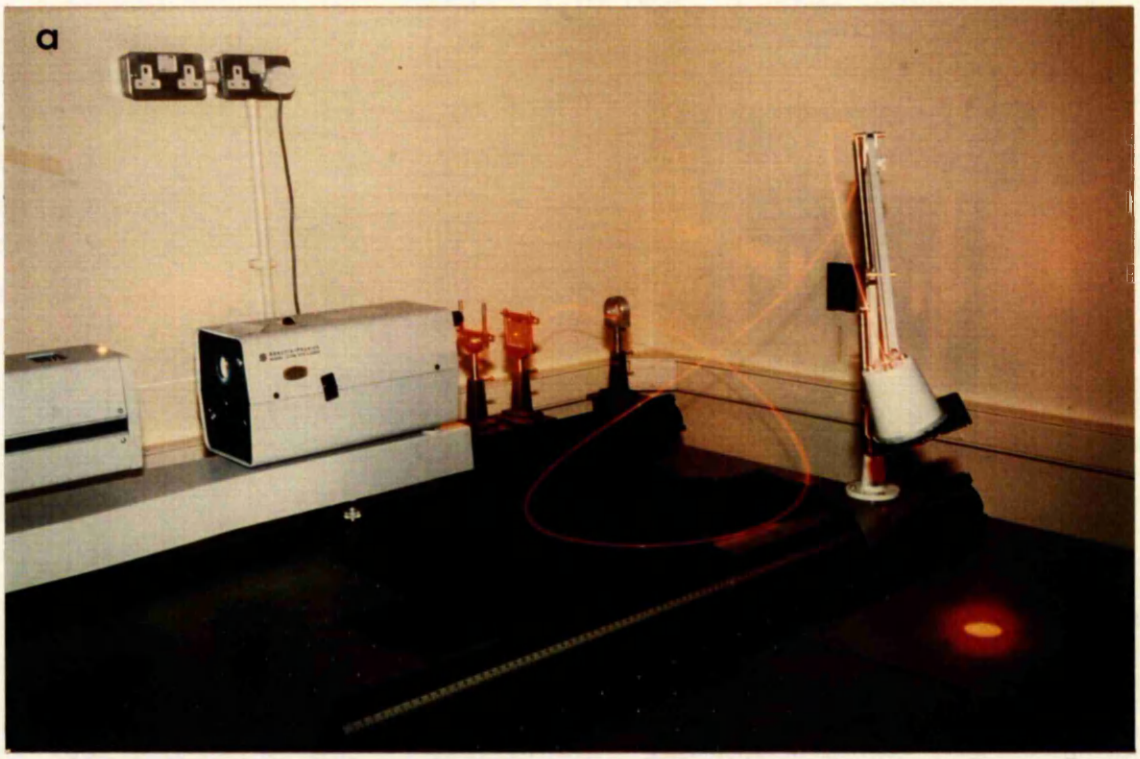
Light Delivery Systems

A 20 W Argon ion/dye laser (Spectra Physics Ltd., Hemel Hempstead, U.K.) (Figure 4.1a) or a 15 W Copper vapour/dye laser (Oxford Lasers Ltd., Oxford, U.K.) (Figure 4.1b) was used to produce 630 nm light for tumour irradiation.

Superficial light delivery

Light from the laser was focused into a 600 μm optical fibre fitted through a mode scrambler to produce even light distribution at its distal end. The light spread conically from the fibre tip which was

Figure 4.1



Lasers used for photochemotherapy.
a) Argon ion/dye laser
b) Copper vapour/dye laser

clamped vertically above the tumour to allow divergence of light to produce the required size of treatment field. This distance was measured for each laser and field size and was checked before each treatment to ensure that the set up was correct. The light emitted from the fibre was measured with a light meter (Photon Control Ltd., Cambridge; Figure 4.2) before and after each treatment.

The dose rate at the skin surface was calculated using the formula:-

$$\text{Surface Dose Rate} = \frac{\text{Light Emitted from Fibre in W}}{\pi r^2} \ (\text{Wcm}^{-2})$$

where r = the radius of the treatment field in cm.

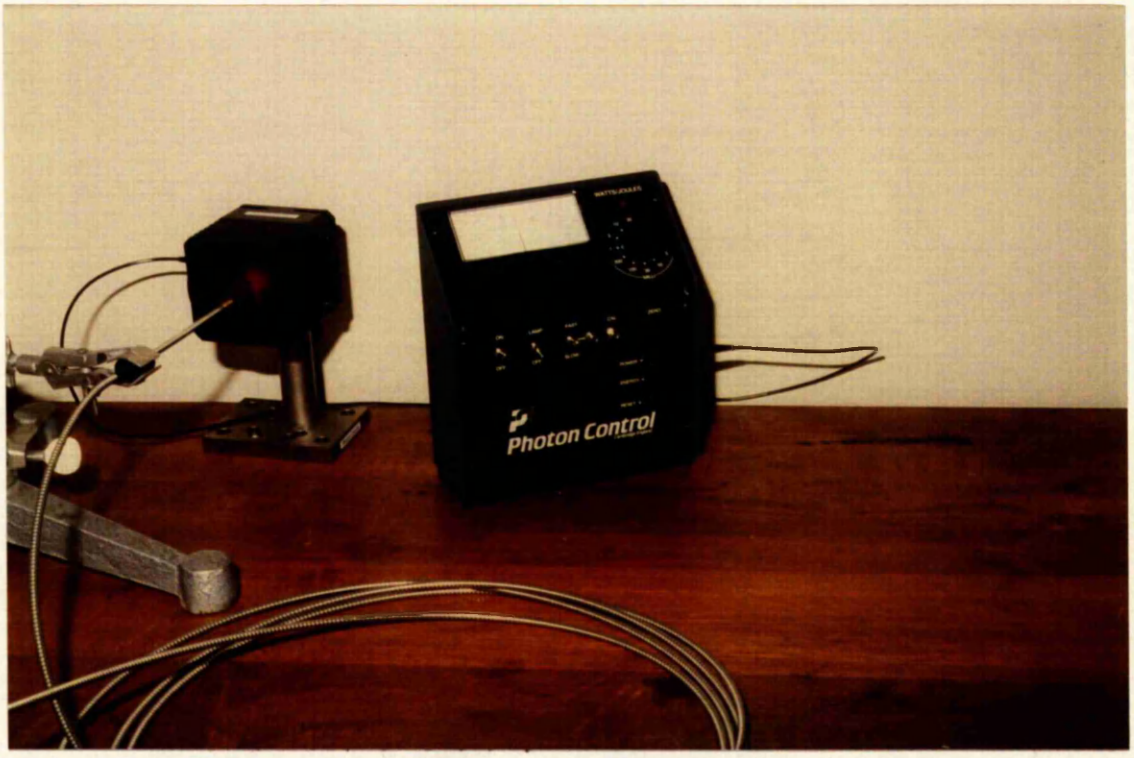
To minimize the risk of hyperthermia, the surface light dose rate was maintained at less than 150 mWcm^{-2} (Gomer and Razum, 1984) by reducing the output of the laser as necessary.

Interstitial Light Delivery

Light from the laser was focused into a 200 μm cut optical fibre with 1 to 2 mm of cladding removed from its distal end. The dose from the fibre tip was measured and the laser power was adjusted to produce the required dose rate (100 or 300 mW).

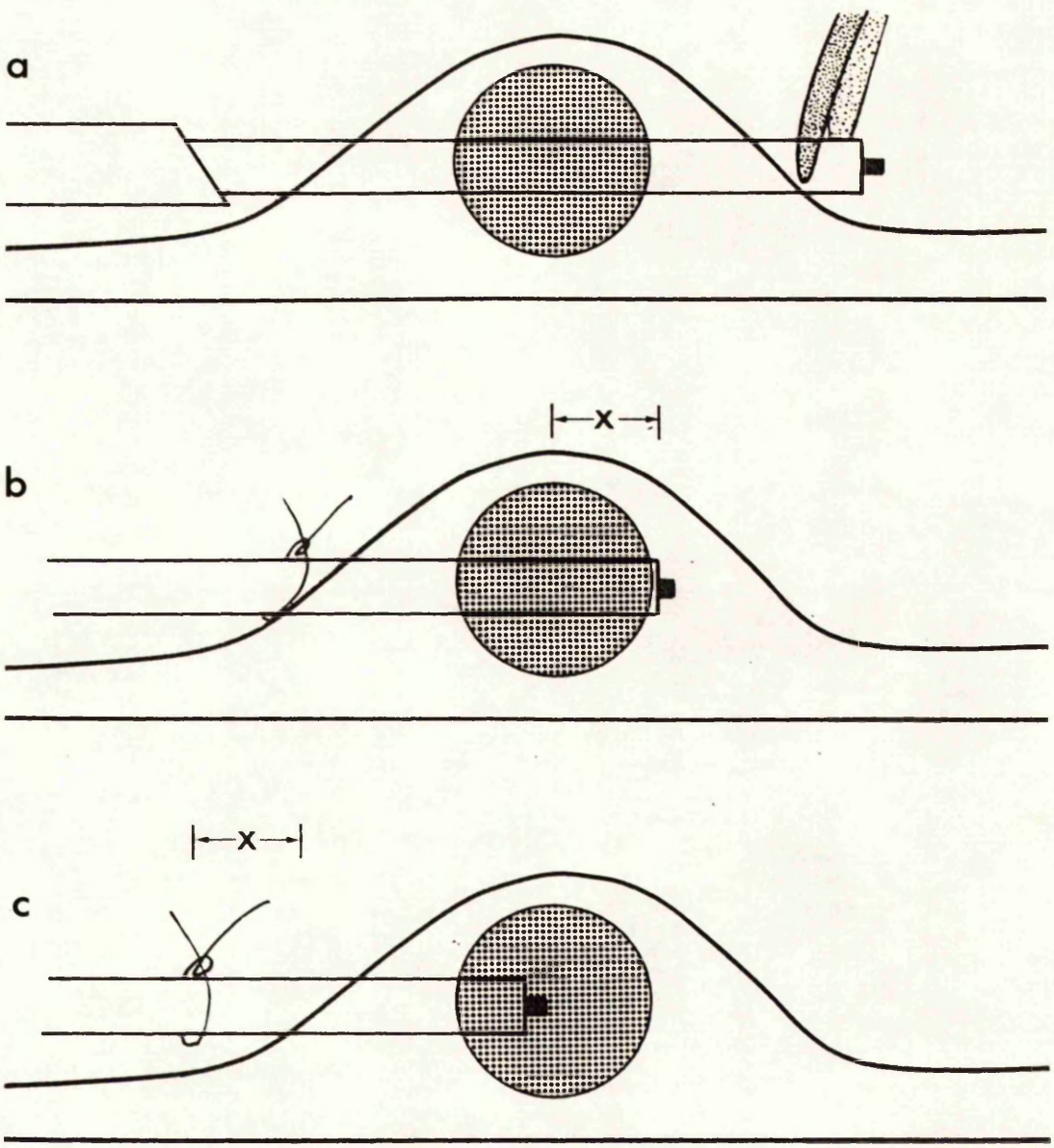
To introduce the optical fibre into the tumour, a sterile 17 G hypodermic needle was inserted through the skin of the anaesthetized rat 3 to 4 mm from the tumour, passed as near as possible through the centre of the tumour and then through the skin on the other side. The fibre was passed through the needle and held near the tip on the cladding while the needle was withdrawn (Figure 4.3a). For tumours greater than 11 mm diameter the fibre was withdrawn until its tip was

Figure 4.2



Light meter being used to measure the output from an optical fibre.

Figure 4.3



Method for insertion of an optical fibre for interstitial photochemotherapy.
a) Optical fibre held in place with forceps, while the needle used to introduce the fibre is withdrawn.
b) Fibre withdrawn so that its tip is just palpable at distal side of tumour, a tie marks the skin entry point of the fibre.
c) Fibre is withdrawn so that its tip is in the centre of the tumour.
(x = half the tumour diameter)

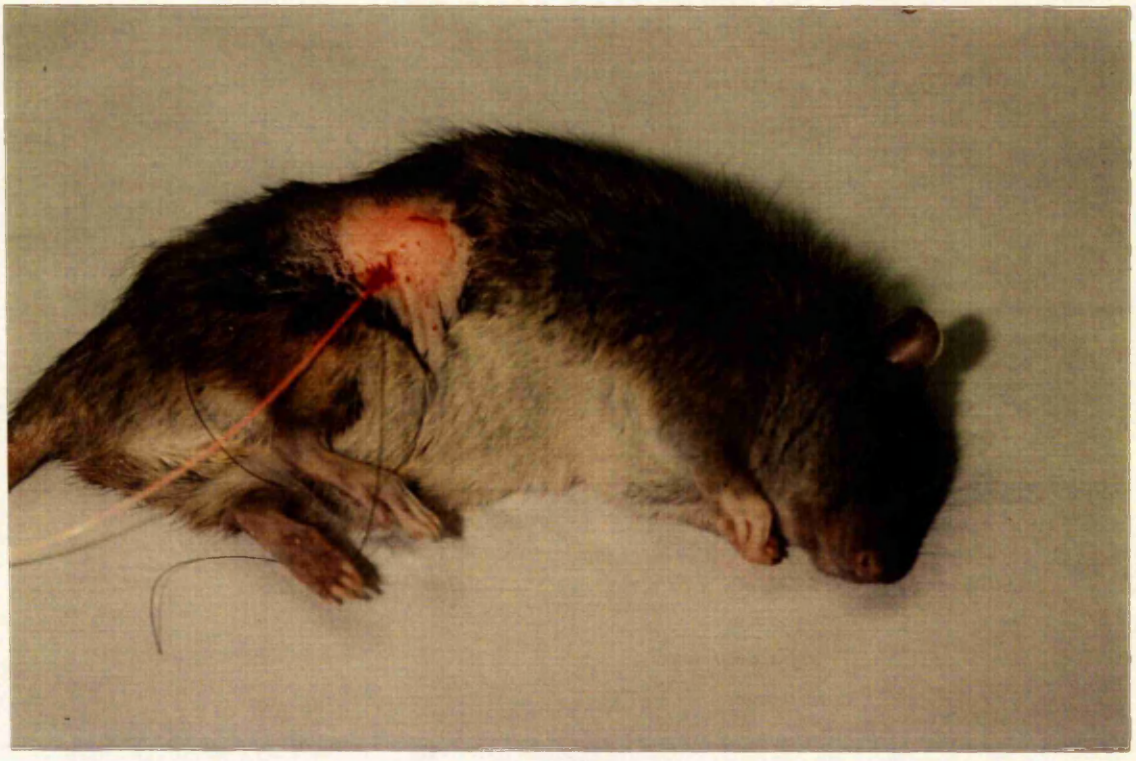
just palpable at the distal side of the tumour and thread tied round the fibre at its proximal skin entry point (Figure 4.3b). The diameter of the tumour along the axis of the fibre and the distance from the skin entry point to the distal edge of the tumour were measured. The fibre was then withdrawn to the centre of the tumour (Figure 4.3c). Thus the distance between the tie and the fibre entry point was equal to half the tumour diameter (x). After treatment, the distance from the tie to the fibre tip was checked. This should equal the distance from the fibre skin entry point to the distal edge of the tumour. Using this method it was possible to position the tip of the fibre so that it was always within 1 mm of the centre of the tumour. For tumours less than 11 mm in diameter the same procedure was followed, except that the position of the fibre for treatment was judged by eye so that the tumour was evenly illuminated (Figure 4.4).

The dose rate of light emitted from the fibre was measured immediately after removal of the fibre. The distal end of the fibre was then cut and a further 1 to 2 mm of plastic cladding removed before the next treatment. Before and after irradiation, the temperature of the skin overlying the tumour and 2 cm away from the tumour was also measured using a thermocouple (RS Components Ltd., Corby, Northants).

Treatment with photochemotherapy

When tumours reached 'T size' (p29, Chapter2), animals were anaesthetized with ether, given 20 $mgkg^{-1}$ of Photofrin II or PHP intraperitoneally at a site remote from the tumour and the skin overlying the tumour clipped and then shaved to remove all superficial

Figure 4.4



A BD_9 rat being treated with interstitial light from a single cut optical fibre.

hair. Twenty two to 26 h later, the animals were anaesthetized with Sodium Amylobarbitone, 10 to 20 mg given intraperitoneally, and their tumours exposed to 630 nm light.

The effects of light dose and field size on the response of $LSBD_1$ to superficial photochemotherapy were examined. Animals were randomized when their tumours reached 8 to 10 mm diameter (T size). They received no treatment, photosensitizer alone or 400 Jcm^{-2} light alone or photochemotherapy with 100 to 400 Jcm^{-2} light to 1.5 to 3.0 cm diameter fields. Each treatment group contained 6 animals, except if severe morbidity was observed when treatment was abandoned (see Results).

The response of $LSBD_1$ to interstitial photochemotherapy using a single optical fibre implanted in the centre of the tumour was investigated. Eight to 10, 10 to 12 mm, 12 to 14 and 14 to 16 mm diameter tumours were treated with interstitial photochemotherapy using 100 to 400 J of light at a dose rate of 300 or 100 mW. One group of animals (T = 8-10 mm) received 400 J of light alone and another group of animals (T = 10-12 mm) received 200 J of light alone. There were six animals in each treatment group.

Assessment of morbidity and tumour response was as described in Chapter2, p29.

RESULTS

Control Treatments

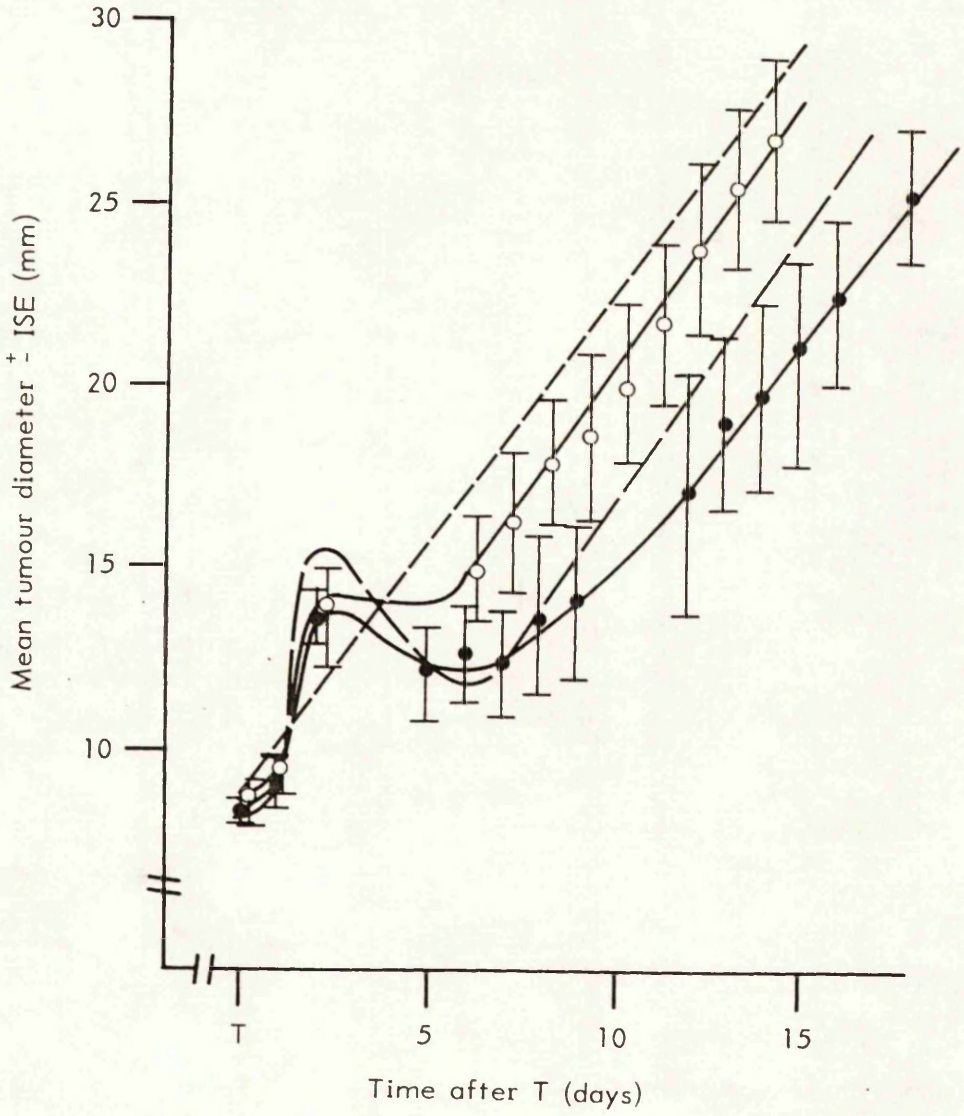
Photofrin II alone, 400 Jcm^{-2} of superficial light or 400 J of interstitial light alone did not have any significant effect on tumour growth (Appendix 4.1). Also, tumour response to superficial or interstitial photochemotherapy was the same when either Photofrin II and the Argon ion/dye laser or PHP and the Copper vapour laser was used (Appendix 4.2).

Superficial Photochemotherapy

Within 24 hours of superficial photochemotherapy there was a rapid increase in measured tumour diameter. This was due to oedema in the treated area which subsided during the next 2 to 4 days (Figure 4.5; Appendix 4.3). There was then a period of tumour growth delay followed by tumour regrowth. Tumours regrew at the same rate as untreated controls (Figure 4.5). For 1.5 cm diameter treatment fields, growth delay increased with increasing dose of light up to about 200 Jcm^{-2}, above this dose no further growth delay was obtained (Figure 4.6; Appendix 4.4).

For a standard light dose (150 Jcm^{-2}), tumour growth delay also increased with increasing diameter of the treatment field (Figure 4.7; Appendix 4.4). This was due to increasing tumour growth arrest. The rate of tumour regrowth was the same as the growth rate of untreated tumours (Appendix 4.5).

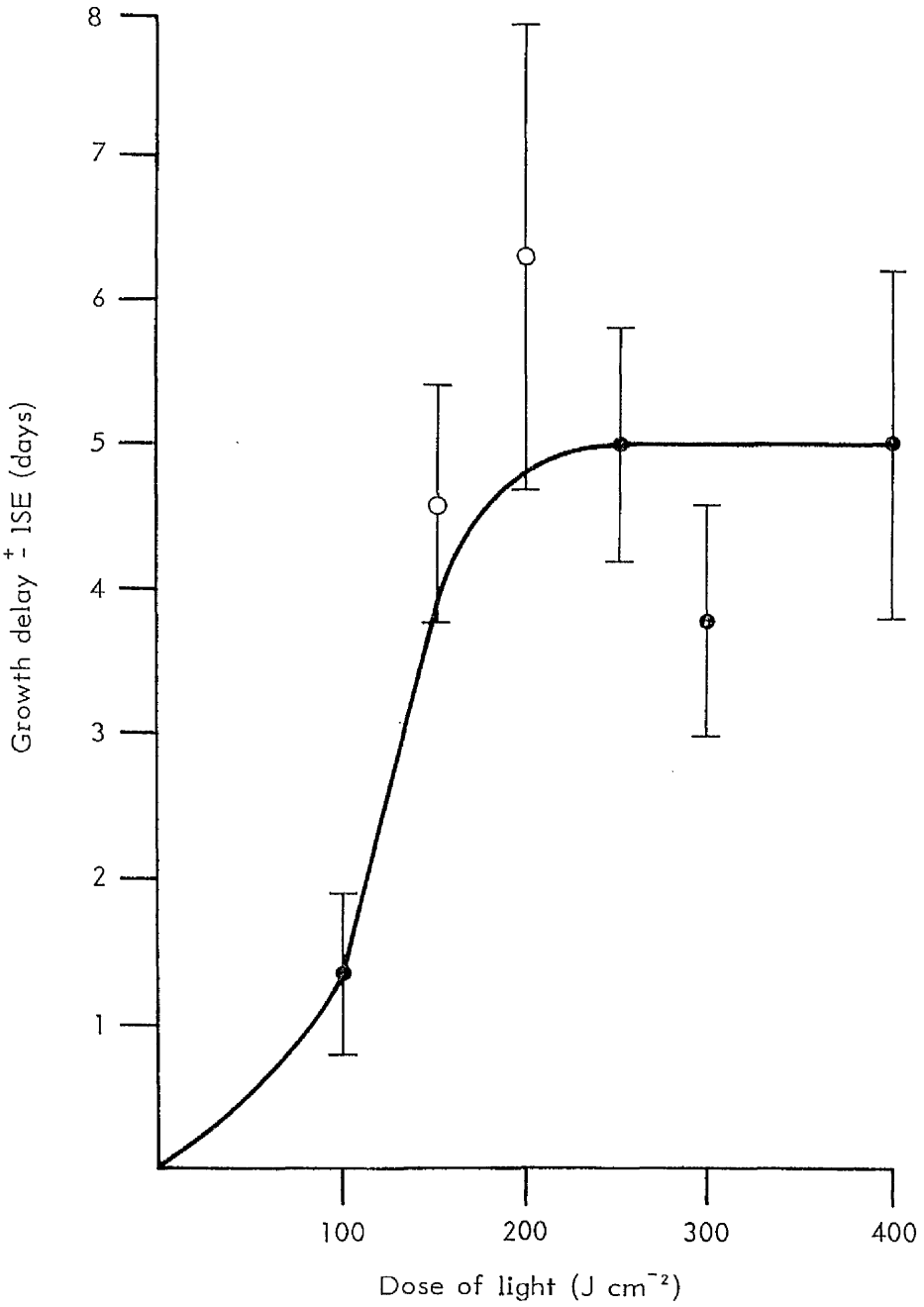
Figure 4.5



Growth of $LSBD_1$ (T= 8-10 mm) after superficial photochemotherapy using a 1.5 cm diameter treatment field. (Some error bars omitted for clarity, full data given in Appendix 4.3.)

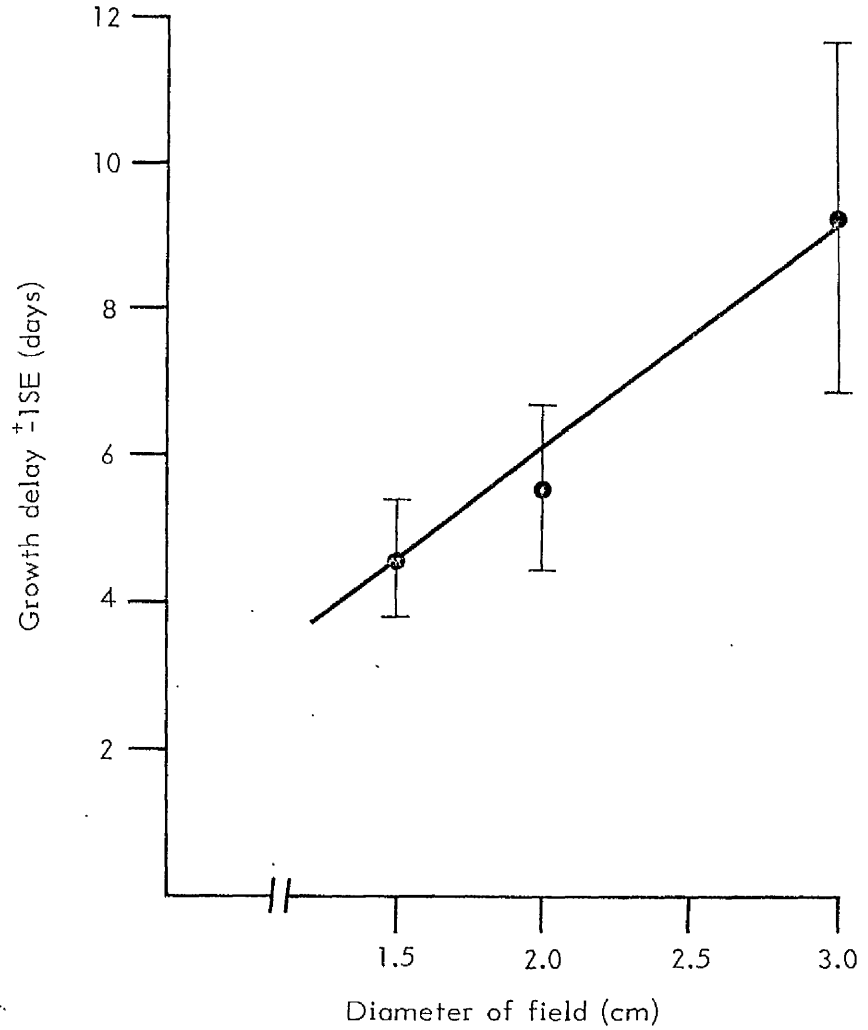
———— Control
o 20 $mgkg^{-1}$ PHP + 100 Jcm^{-2}
—— —— 20 $mgkg^{-1}$ Photofrin II + 150 Jcm^{-2}
● 20 $mgkg^{-1}$ PHP + 250 Jcm^{-2}

Figure 4.6



Light dose response curve for $LSBD_1$ (T= 8-10 mm) treated with superficial photochemotherapy using a 1.5 cm diameter treatment field.
○ Photofrin II 20 $mgkg^{-1}$
● PHP 20 $mgkg^{-1}$

Figure 4.7



Influence of field size on the response of $LSBD_1$ (T= 8-10 mm) to superficial photochemotherapy with 20 $mgkg^{-1}$ Photofrin II + 150 Jcm^{-2} of light. Curve fitted using linear regression analysis (r= 1.00)

The animals tolerated treatment with larger field sizes badly. Two hundred Jcm^{-2} of light given to 2 or 3 cm diameter fields was fatal to the 4 animals (2 in each group) treated. At post mortem, they had peritoneal adhesions causing gastrointestinal obstruction with or without perforation and fistula formation. No significant morbidity was seen with 1.5 cm diameter treatment fields even at 400 Jcm^{-2} (Appendix 4.4).

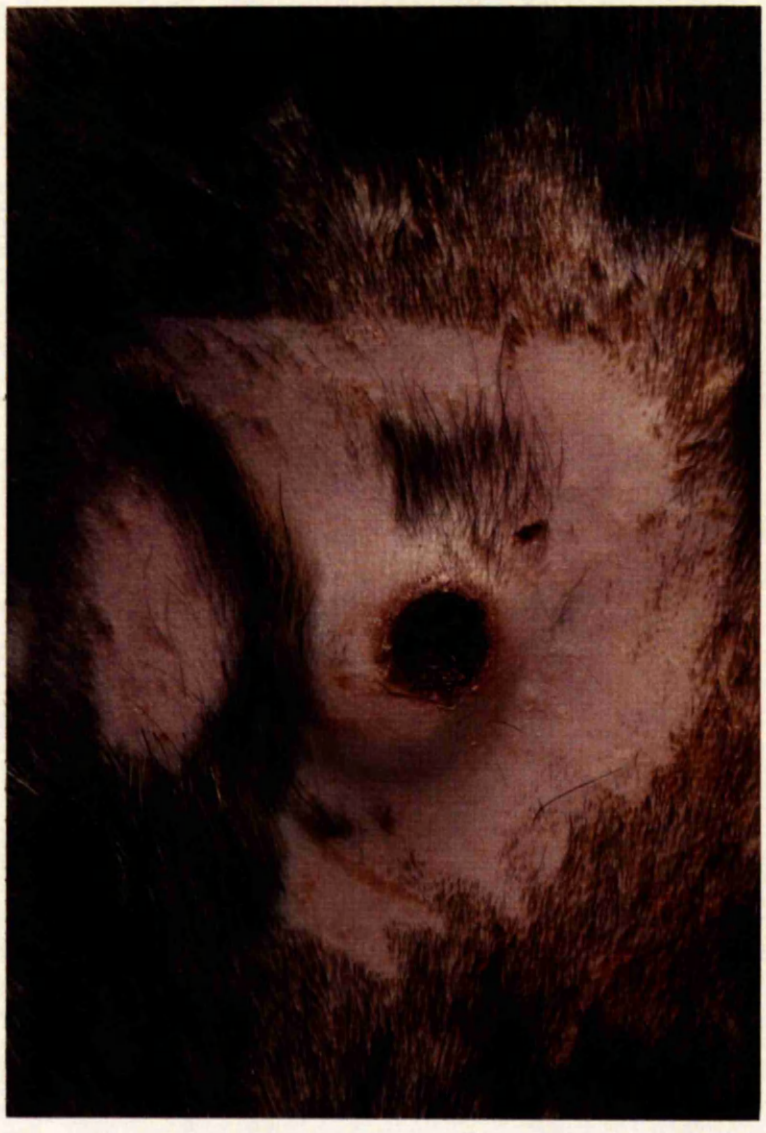
The only other side-effect of treatment observed was damage to the skin overlying the tumour. Skin necrosis occurred with formation of an eschar or scab about five days after treatment (Figure 4.8). These lesions were similar in appearence to those seen in the patients treated with superficial photochemotherapy (p100, Chapter 6). These healed from the periphery and the eschar which eventually separated during the next 2 weeks. This skin damage did not seem to distress the rats.

For 1.5 cm diameter treatment fields, the incidence of skin necrosis with formation of an eschar increased with light dose. This increase continued for doses greater than 200 Jcm^{-2} of light but tumour response did not (Appendix 4.4). Although tumour growth delay increased with field size, the incidence of skin necrosis did not (Appendix 4.4).

Interstitial Photochemotherapy

This, also, produced an increase in the measured tumour size and normal tissue oedema within 1 to 2 days of treatment which resolved

Figure 4.8



Typical appearance of skin necrosis in a BD_9 rat after photochemotherapy.

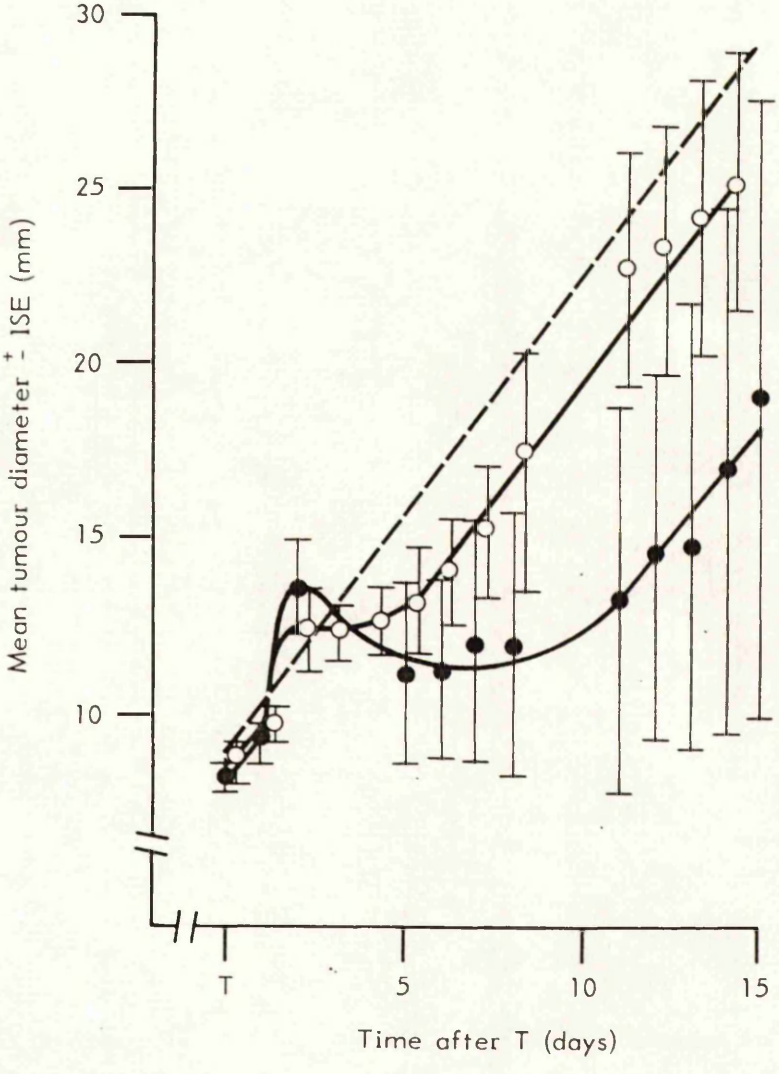
over the next 2 to 4 days. There was then a period of growth delay which increased with light dose up to 200 to 300 J and within experimental error the rate of tumour regrowth was the same as for controls (Figure 4.9, Appendix 4.6). The dose rate at which light was administered did not significantly affect tumour response (Appendices 4.7 and 4.8).

The dose response curve of 8 to 10 mm diameter tumours treated with interstitial photochemotherapy (Figure 4.10; Appendix 4.8) showed increasing growth delay with increasing light doses up to 300 J, above this there was no further growth delay. The large errors seen in the dose response curve for tumours treated with 300 J of light were due to a greater variability in response of individual animals to treatment in this particular group (Appendix 4.9).

The dose response curve for 10 to 12 mm diameter tumours treated with interstitial photochemotherapy was similar to that for 8 to 10 mm tumours (Figure 4.10). For 10 to 12 mm tumours increasing the light dose above 200 J produced no further increase in tumour response. The maximum mean growth delay for these tumours was 8 days, which was not significantly different from that for 8 to 10 mm tumours (10 days).

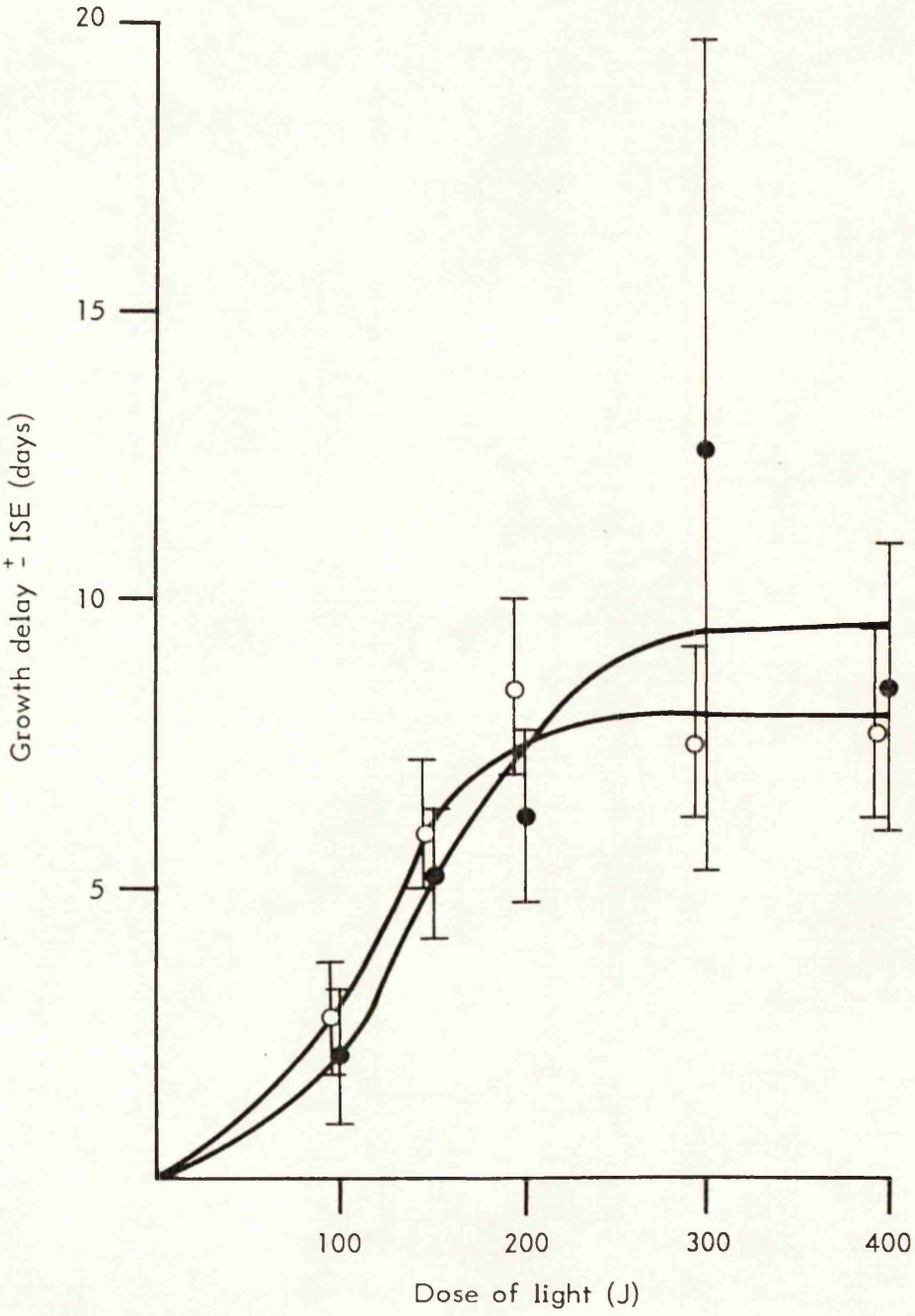
Tumour growth delay produced by interstitial photochemotherapy decreases with increasing initial tumour size (Appendix 4.10) and although there was an increase in measured tumour size and some oedema of the normal tissues for all sizes of tumour this was less marked with increasing tumour size.

Figure 4.9



Growth of $LSBD_1$ (T = 8-10 mm) after interstitial photochemotherapy.
——— Control
○ 20 $mgkg^{-1}$ polyhaematoporphyrin + 100 J light
● 20 $mgkg^{-1}$ polyhaematoporphyrin + 400 J of light

Figure 4.10



Light dose response curve for $LSBD_1$ treated with interstitial photochemotherapy.

● T = 8-10 mm, photosensitizer 20 $mgkg^{-1}$ PHP

○ T = 10-12 mm, photosensitizer 20 $mgkg^{-1}$ Photofrin II

The temperature of the skin overlying the tumour increased during interstitial photochemotherapy (Appendix 4.11). This increase was greater for animals receiving light at a dose rate of 300 mW than at 100 mW. Growth delay was not affected by the extent of the temperature rise and the increase in temperature was not related to the dose of light given (Appendix 4.11). Animals treated with light alone showed a smaller increase in skin temperature than those treated with porphyrin and light.

Interstitial photochemotherapy was well tolerated and there was no systemic morbidity. The incidence of skin necrosis increased with increasing dose of light and was similar for both 8 to 10 mm and 10 to 12 mm diameter tumours (Appendix 4.8).

DISCUSSION

There was considerable variation in the response of individual tumours to photochemotherapy (Figures 4.6 and 4.10; Appendix 4.9). The use of an isogenic tumour implanted in inbred rats should minimize biological variation but differences in tumour position may affect a its vascular supply which may alter the availability of photosensitizer (p44, Chapter 3) and so affect tumour response. As photochemotherapy is thought to exert its main effect *in vivo* by causing vascular damage (Star *et al.*, 1986 and Henderson *et al.*, 1985), variation in tumour vasculature due to its position may alter the tumour's response to treatment.

Differences in tumour position may, also, affect light penetration producing variation in tumour response. Skin pigmentation is patchy in BD_9 rats. For superficial photochemotherapy, tumours underlying pigmented areas would receive a lower light dose than those underlying non-pigmented skin. The thickness of the skin overlying the tumour may, also, vary with tumour position altering the dose of light reaching the tumour. These factors, however, would not explain the variation in tumour response to interstitial photochemotherapy. Insertion of optical fibres into tumours for interstitial light delivery caused bleeding which may have resulted in variation in light distribution, especially if the blood charred at the fibre tip during treatment. It is probable that a combination of these factors were in fact responsible for the variation in tumour response to treatment.

Tumour response after photochemotherapy, especially when high light dose rates are used, may be due, at least in part, to hyperthermia (Kinsey, Cortese and Neel, 1983). Such effects were unlikely in this study because :-

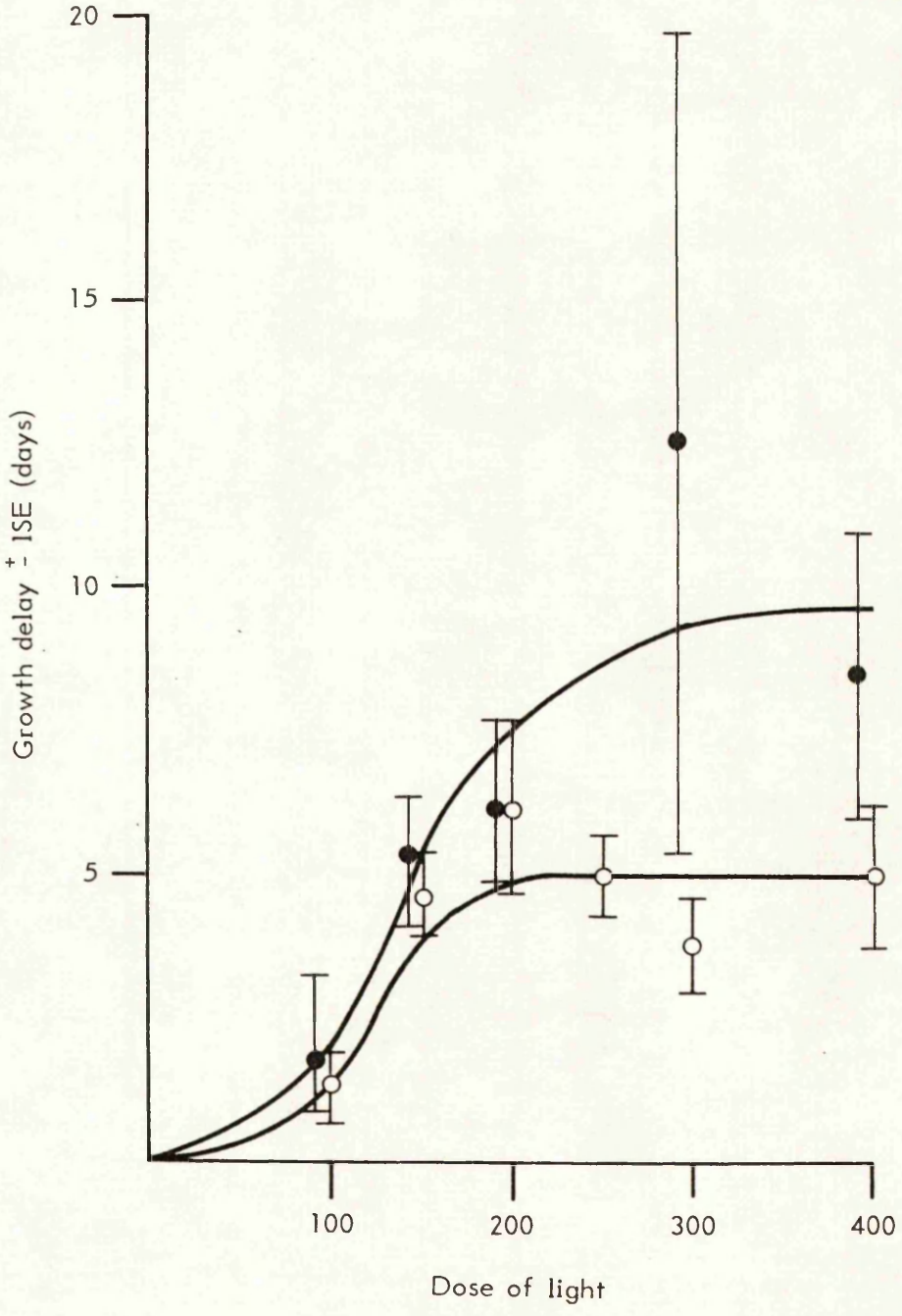
1. Light alone did not produce any tumour growth delay (Appendix 4.1).
2. Comparable tumour growth delay occurred with the the same dose of light given at a dose rate of 100 or 300 mW (Appendix 4.7), despite the latter producing a greater rise in temperature (Appendix 4.11).
3. In hyperthermia, the tumour effect is directly proportional to the duration of hyperthermia (Dewey et al., 1977). After photochemotherapy tumour response did not increase with doses of light above 200 to 300 J. If due to hyperthermia, tumour growth delay should have increased with increasing light dose because of the longer treatment times necessary.

Immediately after treatment there was a rapid increase in tumour size (Figure 4.5) associated with oedema of the surrounding normal tissue. This is a recognised phenomenon after photochemotherapy (Dougherty et al., 1978). The blood flow through $LSBD_1$ decreases within minutes of beginning illumination and does not return to normal within 24 hours after treatment (Feather et al., 1988a). Likewise, Benstead and Moore (1988) showed that blood flow in the mouse tail decreases rapidly and does not return to normal for about 5 days after photochemotherapy which is consistent with the period of time taken for oedema to subside in $LSBD_1$. Vascular stasis causes oedema and the increase in tumour size and oedema in surrounding normal tissues are probably due to the decrease in blood flow after photochemotherapy.

As after cytotoxic chemotherapy (p32, Chapter 2) and radiotherapy (p36, Chapter 2), tumour regression after photochemotherapy (Figure 4.5) took several days. Tumour regression after ionizing irradiation is related to the tumour's cell loss factor and its ability to remove dead tumour cells (Denekamp, 1972). These are properties of the tumour itself, therefore the rate of tumour regression may be expected to be similar after photochemotherapy and irradiation.

Dose response curves for superficial and interstitial photochemotherapy showed an increase in tumour response with increasing doses of light up to a certain dose and then no further increase in response (Figure 4.11). Fingar, Potter, and Henderson (1987) showed that there is a threshold dose of photosensitizer and light which must be exceeded to produce a cytotoxic effect. Above these doses, the effective therapeutic dose in photochemotherapy is a product of the doses of photosensitizer and light used (Cowled and

Figure 4.11



Light dose response curve for $LSBD_1$ (T= 8-10 mm) treated with superficial or interstitial photochemotherapy.
- ○ Superficial light (Jcm^{-2})
- ● Interstitial light (J)

Forbes, 1985; Fingar, Potter and Henderson, 1987). No clear threshold was observed in the dose response curve in $LSBD_1$. This may be due to 20 $mgkg^{-1}$ of Photofrin II, a relatively high dose of photosensitizer, being used so that relatively low doses of light were required to produce a tumour growth delay.

The plateau of the light dose response curve at high doses may have been due to:-

1. Insufficient concentration of photosensitizer in the tumour. Twenty $mgkg^{-1}$ of PHP, however, produced a tumour drug concentration similar to that found in other animal tumour systems used to study photochemotherapy (Gomer and Dougherty, 1979; Dougherty *et al.*, 1975). When photosensitizers are activated by light they are photodegraded (Evensen and Moan, 1988 and Mang *et al.*, 1987), so at higher light doses it is possible that all drug may be photodegraded so limiting the effect of photochemotherapy.

2. Insufficient light dose. The penetration depth of 630 nm light in $LSBD_1$ *in vivo* is 1.62 $\pm$ 0.18 mm (Driver *et al.*, 1988). Light intensity decreases exponentially with depth in tissue (Wan *et al.*, 1981), so doubling the applied light dose only increases the effective treatment depth of 630 nm light by 1.62 mm. An increase in tumour response might have been expected on doubling the light dose from 200 J to 400 J or 200 to 400 Jcm^{-2}, if limited penetration of light was the only factor responsible for producing the plateau in the dose response curves. To completely exclude this, however, the effects of larger doses of light may need to be examined.

3. The presence of a resistant population of cells. Haematoporphyrin is taken up by dividing tissues (Figge et al., 1948). If quiescent tumour cells do not take up Photofrin II, they would be resistant to photochemotherapy. Hypoxia may also produce resistance to photochemotherapy (Bown et al., 1986 and Gomer and Razum, 1984). Although $LSBD_1$'s response to ionizing irradiation does not suggest the presence of a large hypoxic fraction of cells, the level of hypoxia required to produce resistance to irradiation and photochemotherapy may not be the same.

4. Exhausting all of the vascular effect. Photochemotherapy in vivo is thought to exert its main effect by causing vascular damage (Henderson et al., 1985; Selman et al., 1984; Star et al., 1986). Once all blood vessels have been occluded, increasing doses of photosensitizer and light may not increase tumour response.

Vascular occlusion might be expected to limit the effects of photochemotherapy by producing tumour hypoxia (see above) but Henderson and Fingar (1987) showed that, although photochemotherapy produces hypoxia in vivo, this does not limit tumour response to photochemotherapy.

A combination of factors may explain the shape of the light dose response curves for $LSBD_1$ treated with photochemotherapy. Irrespective of the mechanism involved, the practical implication of these curves is that there seems to be a limit to the tumour response that can be produced by superficial photochemotherapy when only a small margin of normal tissue is included in the treatment field and, also, by interstitial photochemotherapy when using a single cut optical fibre.

Superficial photochemotherapy with 20 $mgkg^{-1}$ PHP and 200 Jcm^{-2} of light given to a 1.5 cm diameter treatment field produced a similar tumour growth delay to interstitial treatment using the same dose of photosensitizer and 150 J of light from a single cut optical fibre in 8 to 10 mm diameter $LSBD_1$ tumours. The total dose of light applied at the skin surface to give 200 Jcm^{-2} to a 1.5 cm diameter circle is 353 J. This was not however the amount of light absorbed by the tumour because the quantities of light reflected from the skin surface without absorption and absorbed are variable. Since tumour growth delay is related to tumour cell killing (McNally, 1973), it may be reasonable to assume that the doses of light absorbed by the tumour from superficial and interstitial photochemotherapy produced equivalent cell killing.

Tumour response increased with size of treatment field in superficial photochemotherapy (Figure 4.7). As the tumours treated were all the same size, the response of the tumour to photochemotherapy may be partly dependent on the response of normal tissue around the tumour, that is the tumour bed. Fingar and Henderson (1987) found that cure in a transplanted isogenic tumour in mice only occurred if the tumour bed was irradiated in addition to the tumour. If photochemotherapy causes vascular effects in the normal surrounding tissue as well as in the tumour and the tumour's regrowth depends on establishing a new blood supply which must grow in from the edges of the treatment field, then a larger treatment field would cause greater tumour growth delay because of the greater distance which new vessels must grow.

This tumour bed effect differs from that seen in radiotherapy as the rate of tumour regrowth after photochemotherapy was the same as the

growth rate of untreated tumours (Figure 4.5; Appendix 4.5), whereas after radiotherapy the rate of tumour regrowth decreases (Begg, 1980 and p36, Chapter 2). Irradiation has its main effect within the cell nucleus and, except at doses much greater than used in conventional radiotherapy, tissue injury is only expressed when the irradiated cells divide. When a tumour starts to regrow beyond its original size after irradiation, it needs to increase its vascular supply and endothelial cells are stimulated to divide. This causes the endothelial cell damage due to irradiation to be expressed which may retard vascular growth with a resulting decrease in the rate of tumour regrowth. In contrast photochemotherapy is thought to exert its main effect by causing interphase cell death (Nelson, Liaw and Berns, 1987 and Ben-Hur et al., 1987) which is expressed immediately after treatment without the need for cell division. Although photochemotherapy causes major vascular changes in vivo, the endothelial cells that survive may retain a normal clonogenic capacity, so that when tumour regrowth stimulates these cells to divide vascular growth can occur at the same rate as for untreated tumours. If so, tumour regrowth would not be restrained, as it is after irradiation.

Tumour response to interstitial photochemotherapy decreased with increasing tumour size (Appendix 4.10). This was expected as light intensity decreases exponentially with increasing depth in tissue (Wan et al., 1981). As tumours increase in size the centre of the tumour becomes necrotic with growth occurring mainly in the periphery of the tumour (Tannock, 1987). Using a single optical fibre implanted into the centre of the tumour to deliver light, may result in the highest dose of light being given to necrotic tissue and

a relatively low dose being given to more viable tumour at the periphery. This may add to the problems of poor light penetration and further reduce the effects of photochemotherapy in larger tumours. There is a minimum or threshold dose of light and photosensitizers required to produce a cytotoxic effect and if the light or drug dose falls below this level no cellular damage occurs (Fingar and Henderson, 1987). Below this threshold dose of light, there may be a rapid decrease in tumour response when a single implanted optical fibre is used to treat tumours of increasing size. In addition to the problems of light distribution, larger tumours contain greater numbers of clonogenic cells and, therefore, may be less responsive to treatment than smaller tumours.

The data (Appendix 4.10) suggests that the largest tumour that can be effectively treated with 200 J of light from a single cut optical fibre is 10 to 12 mm in diameter. The plateau observed in the dose response curves for 8 to 10 and 10 to 12 mm diameter tumours for interstitial photochemotherapy (Figure 4.10) suggest that increasing light dose will not overcome the problem of tumour size and that the maximum dose of light that can be effectively delivered from a single cut optical fibre is 200 to 250 J. If multiple optical fibres are to be used to deliver light, their spacing and the dose of light delivered through each fibre may be critical if the whole tumour is to be adequately and efficiently irradiated. These data suggest that for $LSBD_1$ fibres should be less than 12 mm apart (Appendix 4.10) and that not more than about 250 J of light (Figure 4.10) should be delivered from each fibre.

Photochemotherapy was generally well tolerated by the animals. All treatment related deaths were due to superficial photochemotherapy causing damage to the gastro-intestinal tract. The peritoneal cavity is only about 5 mm below the surface of the skin on the flank of a rat. The amount of light reaching the gut directly underlying the tumour was reduced by the tumour which was approximately 1 cm in diameter. For 1.5 cm diameter treatment fields there was only a 2.5 mm (an area of 1.00 cm^2) rim of gut that was not shielded from the direct light field but for a 3 cm diameter field a 10 mm (an area of 6.28 cm^2) rim was not shielded by tumour. In view of the difference in area of gut irradiated by different field sizes, gastro-intestinal toxicity may be expected to increase with increasing field size. While this was a major limiting factor to the animal studies of superficial photochemotherapy it is unlikely to be a problem in a clinical setting because human skin is thicker and unlike the rat most sites have some subcutaneous fat to protect vital structures. Gastro-intestinal toxicity would require consideration if direct intraperitoneal photochemotherapy was used, for example to treat peritoneal seeding of tumour cells.

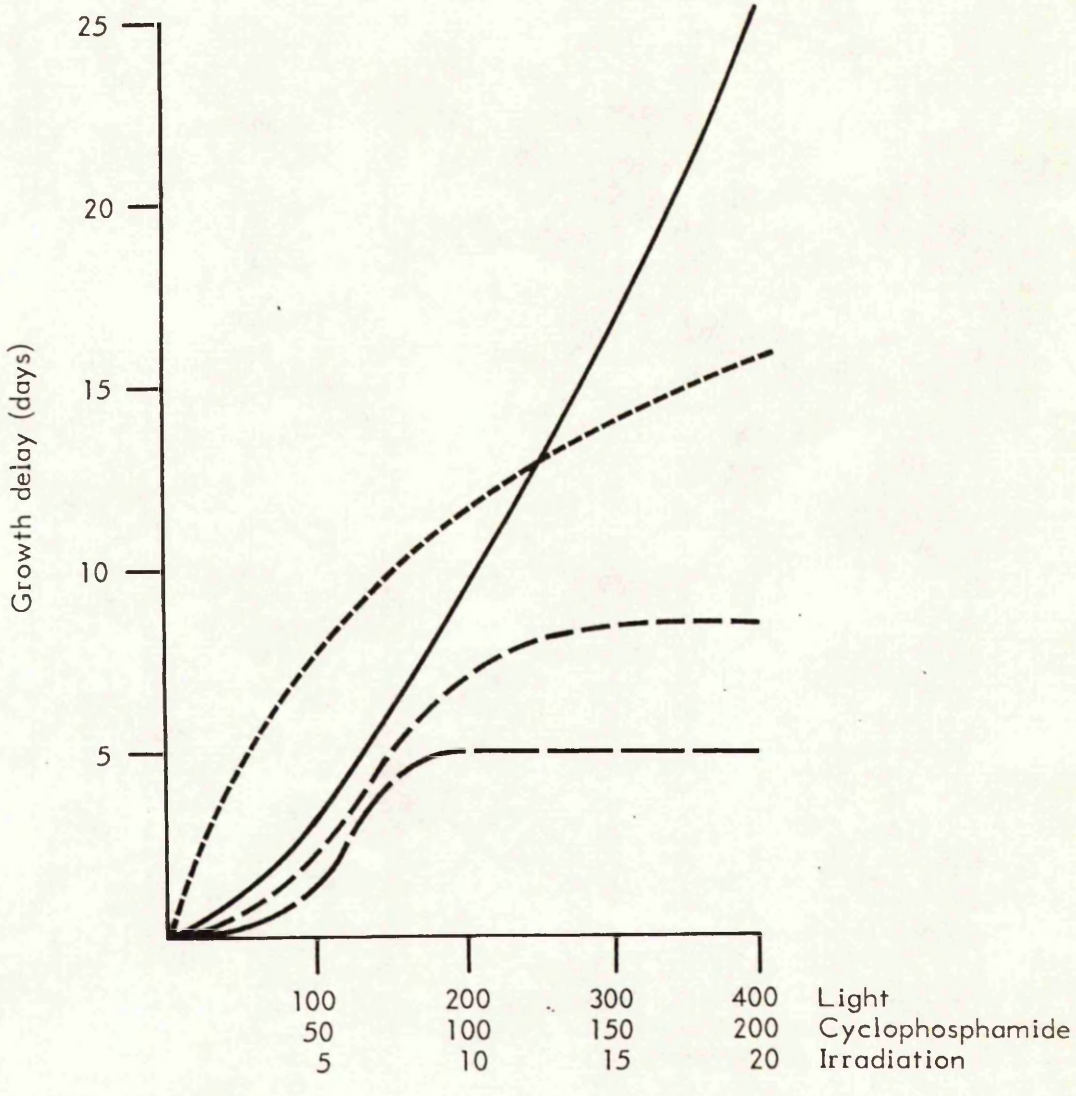
The incidence of skin necrosis increased with increasing dose of light for both superficial and interstitial photochemotherapy. A high incidence of skin necrosis may have been expected with superficial light delivery because the maximum light dose was delivered to the skin surface. For interstitial photochemotherapy, skin necrosis only occurred at higher light dose rates. Skin damage may have been due to hyperthermia. Skin temperature did not, however, increase with light alone (Appendix 4.11), suggesting that the temperature rise was related to treatment with photochemotherapy, perhaps due to a decrease

in blood flow decreasing skin cooling. If skin necrosis was due to hyperthermia, this contrasts with tumour response which was not affected by temperature increase (see above). Skin necrosis after interstitial photochemotherapy decreased with increasing tumour size (Appendix 4.8). This might be expected as light dose decreases exponentially with increasing depth in tissue (Wan et al., 1981) and the skin is at greater distance from the light source in larger tumours.

The response of $LSBD_1$ to cyclophosphamide or gamma irradiation was greater than its response to photochemotherapy (Figure 4.12). In previous comparisons of photochemotherapy and cytotoxic chemotherapy (Cowled, Mackenzie and Forbes, 1987) or radiotherapy (Grashew and Shapova, 1986; Pezzoni et al., 1984), tumour response to photochemotherapy was comparable to or better than response to conventional treatment. In these studies, the doses of cytotoxic drug or irradiation have, however, been relatively low and/or in a limited dose range.

Treatment with ^{60}Co gamma rays produced a physically homogeneous dose of ionizing radiation throughout the tumour. Treatment with superficial or single fibre interstitial photochemotherapy does not produce a homogeneous dose of light even throughout a 1 cm diameter tumour. It is, therefore, possible that despite using 400 J or 400 Jcm^{-2} of light, the maximum doses in this study, light distribution in addition to differences in biological mechanisms may have been responsible for the relatively poor tumour growth delay after photochemotherapy compared with after radiotherapy (Figure 4.12). It may be possible to improve light distribution within the tumour by

Figure 4.12



Dose response curves for $LSBD_1$ (T= 8-10 mm) treated with cyclophosphamide, ionizing radiation, interstitial and superficial photochemotherapy.

—— —— 20 $mgkg^{-1}$ PHP + superficial light (Jcm^{-2}), 1.5 cm diameter treatment field (ex Figure 4.6)

—— — —— 20 $mgkg^{-1}$ PHP + interstitial light (J) at 300 mW (ex Figure 4.10

------ Single dose of cyclophosphamide ($mgkg^{-1}$) (ex Figure 2.4)

——— Single dose of γ-irradiation (Gy) (ex Figure 2.6)

using multiple optical fibre implants and/or fibres with diffusing tips.

The blood supply of subcutaneously implanted tumours may be relatively poor, limiting the effects of treatments that require systemic administration of drugs. This may, also, explain why gamma irradiation produced greater tumour growth delay than photochemotherapy and make a comparison between cytotoxic chemotherapy and photochemotherapy more appropriate. At 100 $mgkg^{-1}$ cyclophosphamide, the dose of drug needed to produce greater tumour growth delay after chemotherapy than after photochemotherapy, significant morbidity occurred (Appendix 2.3). Although it may be possible to produce a greater tumour effect with cyclophosphamide than with photochemotherapy, the therapeutic ratio of interstitial photochemotherapy was better than the therapeutic ratio of cyclophosphamide.

CONCLUSIONS

These data suggest that:-

1. There is a limit to the tumour effect that can be produced by superficial photochemotherapy.
2. The tumour bed effect associated with photochemotherapy seems to be different from the tumour bed effect associated with radiotherapy.
3. There is a limit to the size of tumour that can effectively be treated by interstitial photochemotherapy using a single cut optical fibre to deliver light.
4. Optical fibres in a multiple fibre implant in $LSBD_1$ tumour should be about 10 mm apart.

5. To produce the optimum tumour response light must reach all relevant parts of the tumour and the surrounding normal tissue.

6. Photochemotherapy, as given in this study, is less effective than gamma irradiation in $LSBD_1$ tumour but that it may be as effective as cytotoxic chemotherapy with cyclophosphamide if therapeutic ratio is taken into consideration.

CHAPTER 5: PHARMACOKINETICS OF PHOTOFRIN II IN PATIENTS

The distribution and elimination of haematoporphyrin and HPD in humans conforms to a two compartment pharmacokinetic model (Zalar et al., 1977; Dougherty, 1984b). Since Photofrin II is the active component of HPD (Dougherty, 1983; Dougherty, 1984a), its pharmacokinetics may, also, be expected to conform to this model.

In a two compartment pharmacokinetic model, the distribution and elimination phases can be defined by half-lives. After four half-lives 93% of distribution or elimination of a drug has occurred and for practical purposes may be considered complete.

Full details of all patients treated with photochemotherapy in this study are given in Appendix 1 (pp 135-154). Plasma porphyrin levels were measured, during and after photochemotherapy, in 8 of these patients to determine the pharmacokinetics of Photofrin II.

PATIENTS AND MEASUREMENT OF PORPHYRIN LEVELS

All the 8 patients (Patients 1 to 6, 13 and 14, Appendix 1) had superficially recurrent malignant tumours, 6 of them with multiple sites of disease. They had normal blood counts, serum creatinine, urea and electrolytes levels. Patient 1 had slightly raised liver enzymes (GOT - 110 $i.u.l^{-1}$, normal <35 $i.u.l^{-1}$; Alkaline phosphatase - 35 KA units, normal range 3 to 12 KA units), although the serum bilirubin was normal. All other patients had normal liver function tests.

Vials containing Photofrin II (2.5 $mgml^{-1}$) were stored at -20 °C and thawed immediately before use. Patients were given 1.0, 1.5 or 2.0 $mgkg^{-1}$ Photofrin II intravenously over 5 min.

For each patient, 10 ml of venous blood was taken into a heparinized tube (Sarstedt, Numbrecht, W. Germany) immediately before drug administration, at 30 to 60 min and 3 to 6 h after injection, then daily for the next four days and at each follow up appointment (weekly at first then fortnightly) up to 67 days after treatment. The plasma total porphyrin levels were then measured (Appendix 3.2).

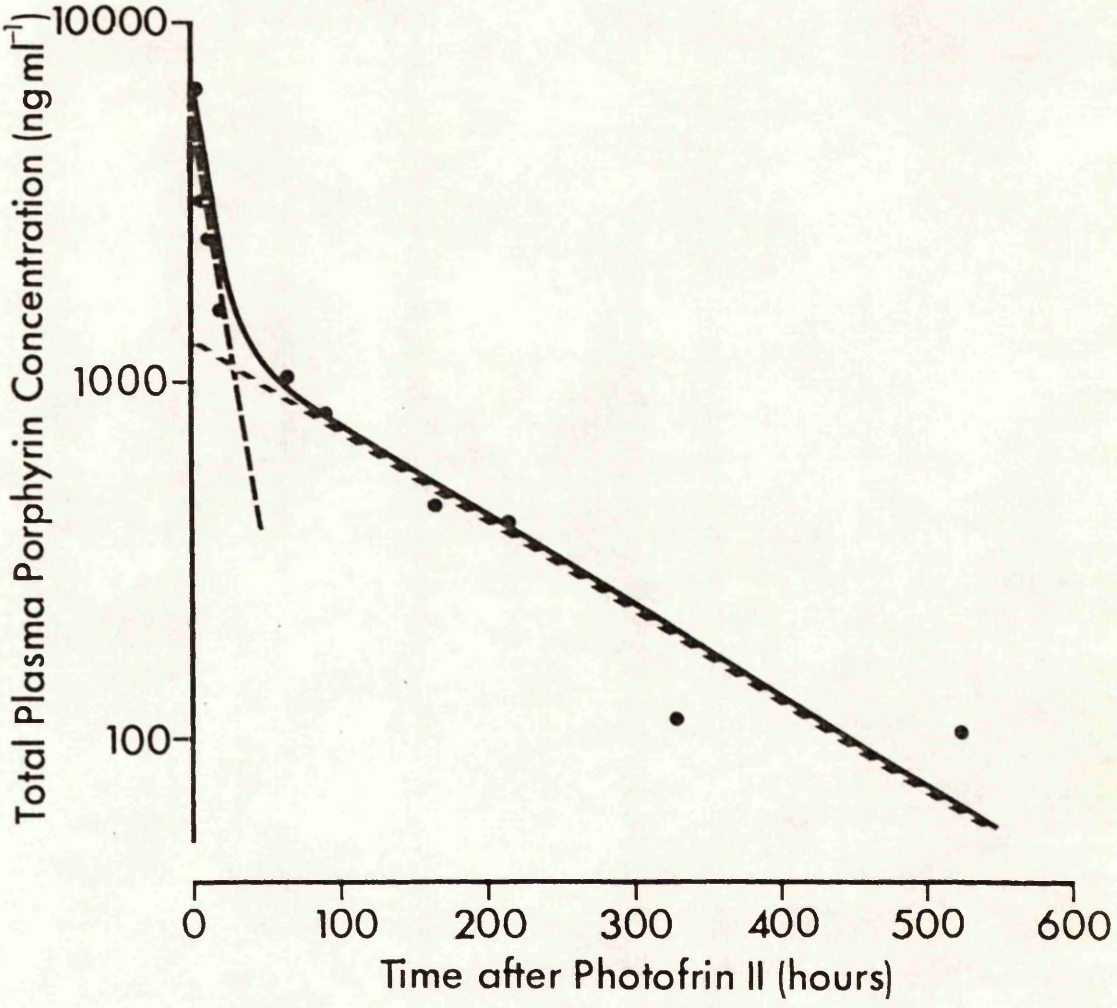
RESULTS

Plasma Porphyrin Levels

The mean porphyrin level in patients' plasma before Photofrin II administration was 10 ± 10 $ngml^{-1}$. For each patient, his or her pre-treatment porphyrin level was subtracted from the measured total porphyrin level before analysis of data.

The plasma porphyrin concentration decreased with time, initially rapidly and then more slowly (Appendix 5.1), indicating that the pharmacokinetics of Photofrin II conform to a two compartment model (Figure 5.1).

Figure 5.1



Plasma total porphyrin concentration plotted against Time on a log/linear scale (Patient 1) with calculated distribution, α, and elimination, β, curves.

α

β

Pharmacokinetic Analysis

The curve defining a two compartment pharmacokinetic model is described by the equation:-

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

Where C is the drug concentration at time t, A and B are constants and α and β are the slopes of the exponential functions. A and α refer to the initial or distribution phase, B and β to the second or elimination phase.

The half-life of Photofrin II in the first and second compartment were calculated by using:-

$$\text{half-life} = 0.693/\text{exponent } (\alpha \text{ or } \beta)$$

Values of A, B, α and β for a given set of experimental data may be estimated using Non Lin Program, (Research Biostatistics, Upjohn Co., Kalamazoo, Michagan, U.S.A.) which uses least squares estimation to determine these parameters. The program also provides the standard deviation and 95% confidence intervals for the calculated parameters.

A weighting of 1/y (where y is the plasma porphyrin measurement) was applied to the plasma porphyrin measurements, in order to correct for the unknown variance of the serum porphyrin measurements (Boxenbaum, Riegelman and Elashoff, 1973). The pharmacokinetic parameters calculated using this weighting had smaller standard deviations and if plotted graphically seemed to represent more closely the measured data points than other possible weightings (1 or $1/y^2$).

The exponent of the first or α curve were 0.070 to 0.213 h^{-1} and the exponent of the second or β curve 0.0035 to 0.0098 h^{-1} (Appendix 5.2). The α or distribution half-lives were 3.3 to 12.5 h, with a mean ($\pm$ 1 S.D.) of 8.5 $\pm$ 2.8 h, and the β or elimination half-lives were 70 to 242 h, with a mean ($\pm$ 1 S.D.) of 152 $\pm$ 58 h (Appendix 5.3) The elimination half-life was significantly longer than the distribution half-life ($p<0.001$). There was a 10 to 26 fold difference in duration of these two phases.

DISCUSSION

The pharmacokinetics of Photofrin II conformed to a two compartment model (Figure 5.1), with similar distribution and elimination half-lives to those reported for haematoporphyrin (Zalar et al., 1977) and HPD (Dougherty, 1984b). The standard deviations of the α and β exponents and hence the 95% confidence intervals for plasma half-lives may have been reduced by taking plasma samples more frequently but this would have placed unreasonable demands on the patients.

The α half-life for Photofrin II was 3.3 to 12.5 h (Appendix 5.3), thus by 50 h (4 half-lives) after giving the drug distribution of the photosensitizer from plasma to the tissues, including the tumour, should be complete. The β half-life was relatively long (3-10 days), suggesting that porphyrin levels in tissue fall slowly and that there is a period of several days when drug levels remain sufficient for light treatment to produce a cytotoxic effect. These data are consistent with the clinical practice of leaving at least 48 h between drug and light administration.

The slow elimination of drug is important if fractionated light delivery is to be used to try to improve the therapeutic ratio of photochemotherapy (Dougherty et al., 1979). More recently, it has been suggested that Photofrin II is degraded to an inactive form after light exposure (Mang et al. 1987), therefore drug elimination may not be the only factor controlling response to fractionated light delivery.

From the β half-life, elimination of porphyrin would be expected to be complete 12 to 40 days after Photofrin II administration. In a pharmacokinetic model, however, all of the peripheral or tissue compartment is considered together so an average elimination rate for all tissues is calculated. Some tissues may not clear photosensitizer at the rate suggested by the β half-life. In BD_9 rats, porphyrin levels in plasma and $LSBD_1$ fell at the same rate but they fell more quickly than in skin (Appendices 3.3 and 3.4). If this, also, occurs in patients, plasma porphyrin levels and pharmacokinetics may give a good estimate of porphyrin levels in tumour but they may underestimate the level of porphyrin in skin and the duration of cutaneous photosensitivity. The relationship between the pharmacokinetics of Photofrin II and the duration of cutaneous photosensitivity measured by photopatch testing are examined in Chapter 7.

CONCLUSIONS

1. The distribution and elimination of porphyrin in the plasma of patients treated with Photofrin II conformed to a two compartment pharmacokinetic model.

2. The distribution half-life of Photofrin II was 3.3 to 12.5 h, which is consistent with leaving at least 48 h between drug and light administration.

3. The elimination half-life of Photofrin II was 3 to 10 days, so there may be a period of several days when light may be effectively given.

CHAPTER 6: PHOTOCHEMOTHERAPY IN PATIENTS

The incidence of complete tumour control in recurrent cutaneous or subcutaneous tumours and of skin necrosis within the irradiated area were examined after superficial photochemotherapy, to assess the relationship between tumour and normal tissue response and how this is affected by changes in dose of photosensitizer or light. The response of three patients with recurrent oral cavity or oropharyngeal tumours to superficial photochemotherapy was, also, examined.

The tumour response and incidence of skin necrosis after interstitial photochemotherapy were examined using a standard dose of Photofrin II and varying doses of light delivered through cut optical fibres, to try to improve the therapeutic ratio of photochemotherapy.

PATIENTS AND METHODS

Superficial Photochemotherapy

Six patients (Patients 1 to 4, 7 and 8, Appendix 1) with a total of 34 assessable cutaneous or subcutaneous metastatic or locally recurrent tumours less than 1.5 cm in depth were treated. At five of the tumour sites the skin was already ulcerated. For each tumour four planar diameters were measured and the mean tumour diameter calculated.

Patients were given 1.0, 1.5 or 2.0 $mgkg^{-1}$ body-weight of Photofrin II intravenously, over 5 min. Forty eight to 72 h later the lesions were

treated with 630 nm light (Figure 6.1) from the Argon-dye laser, as described previously (p51, Chapter 4). The total doses of light given at the skin surface were 25, 50, 75 or 100 Jcm^{-2}, at a dose rate of 40 to 172 $mWcm^{-2}$. The tumours plus a 1 cm margin of normal tissue was irradiated. The treatment fields were 2.5 to 6 cm diameter circles. Light and drug doses were chosen so that different sized tumours were distributed evenly throughout the treatment groups.

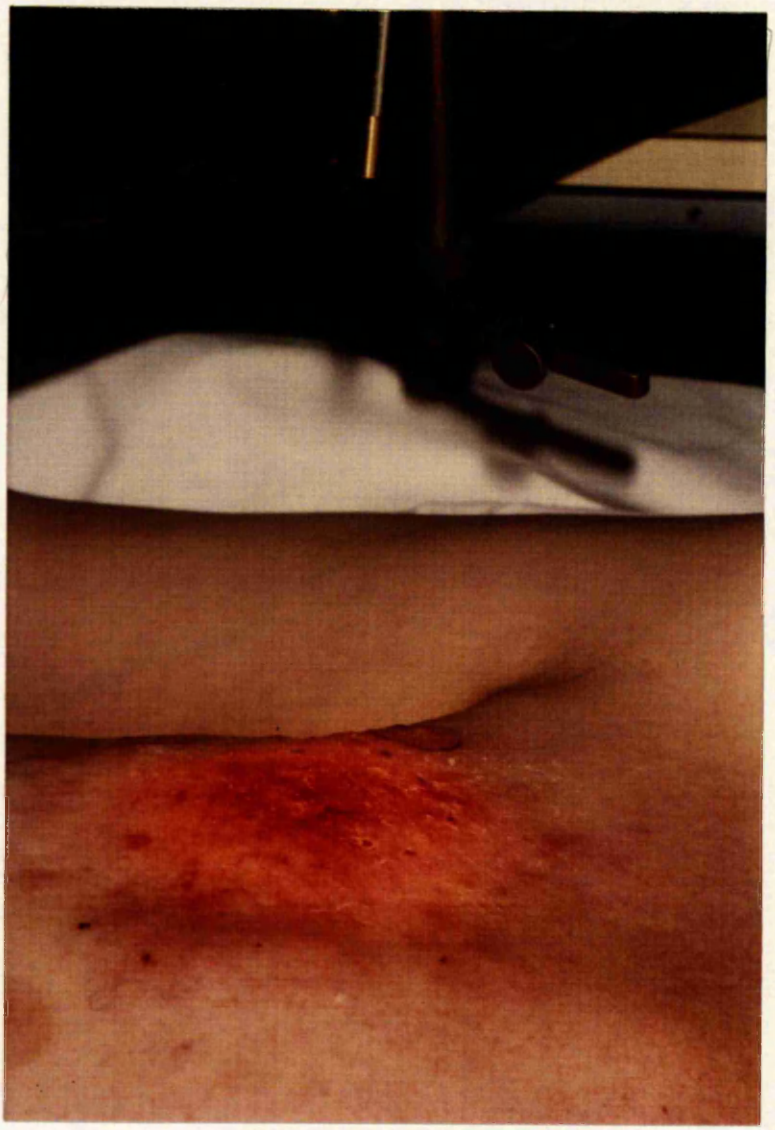
After treatment patients were reviewed weekly for 4 weeks and monthly thereafter, for 3 to 5 months. Complete clinical resolution of the lesion was used to assess tumour response and the incidence of damage to skin within the treated area was recorded using skin necrosis with formation of a black scab or eschar as the end-point.

Three patients with recurrent oral tumours were treated with superficial photochemotherapy (Patients 9 to 11, Appendix 1). None of the tumours showed evidence of boney invasion. Patient 10 had a lesion less than 5 mm thick (carcinoma in-situ) and in Patients 9 and 11 the lesions were estimated to be 5-10 mm thick. Patients were given 1.5 or 2.0 $mgkg^{-1}$ of Photofrin II and 40 or 50 Jcm^{-2} of light to a 2.5 or 3.0 cm diameter area. Disease free mucosa was shielded from the direct beam where possible.

Interstitial Photochemotherapy

Four patients (Patients 12 to 15, Appendix 1) with a total of 24 sites of cutaneous or subcutaneous recurrent tumour (volume 30 to 6000 mm^3) and without ulceration of skin were treated. Each patient also had

Figure 6.1



A patient being treated with superficial light (630 nm).

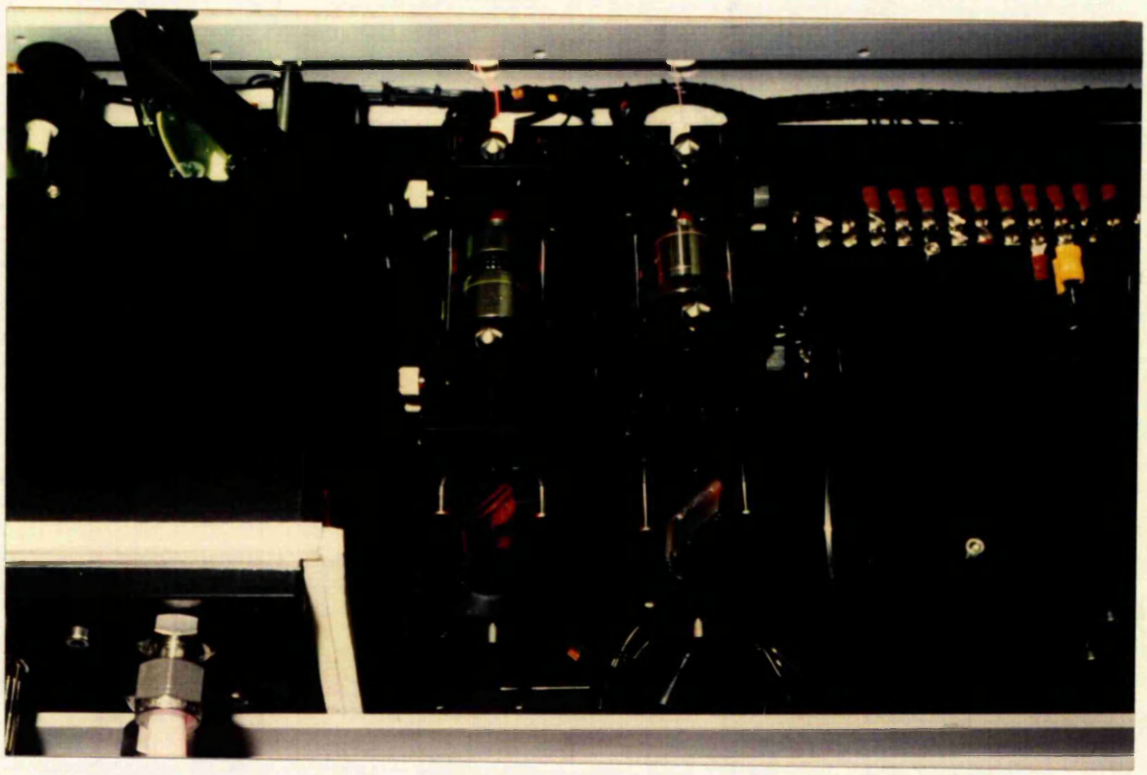
other superficial tumours that were not treated but were measured as controls.

The patients were given 1.5 $mgkg^{-1}$ of Photofrin II intravenously and 72 h later were treated with 50 to 300 J of 630 nm light from the Copper vapour/dye laser (p51, Chapter 4), at a dose rate of 100 to 300 mW. Light from the dye laser was divided by a beam splitter (Figure 6.2) so that two 200 μm optical fibres could be used simultaneously decreasing the total treatment time for each patient. Except at 2 sites (Patient 14), light was delivered to each tumour through a single cut optical fibre.

Optical fibres were inserted aseptically under a local anaesthetic (1% Lignocaine). A 17 G hypodermic needle was inserted through the centre of the tumour (Figure 6.3a), the fibre passed through the needle and held in place on the distal side of the tumour with forceps while the needle was withdrawn. The fibre was then partially withdrawn and its position adjusted to produce even illumination of the tumour (Figure 6.3b).

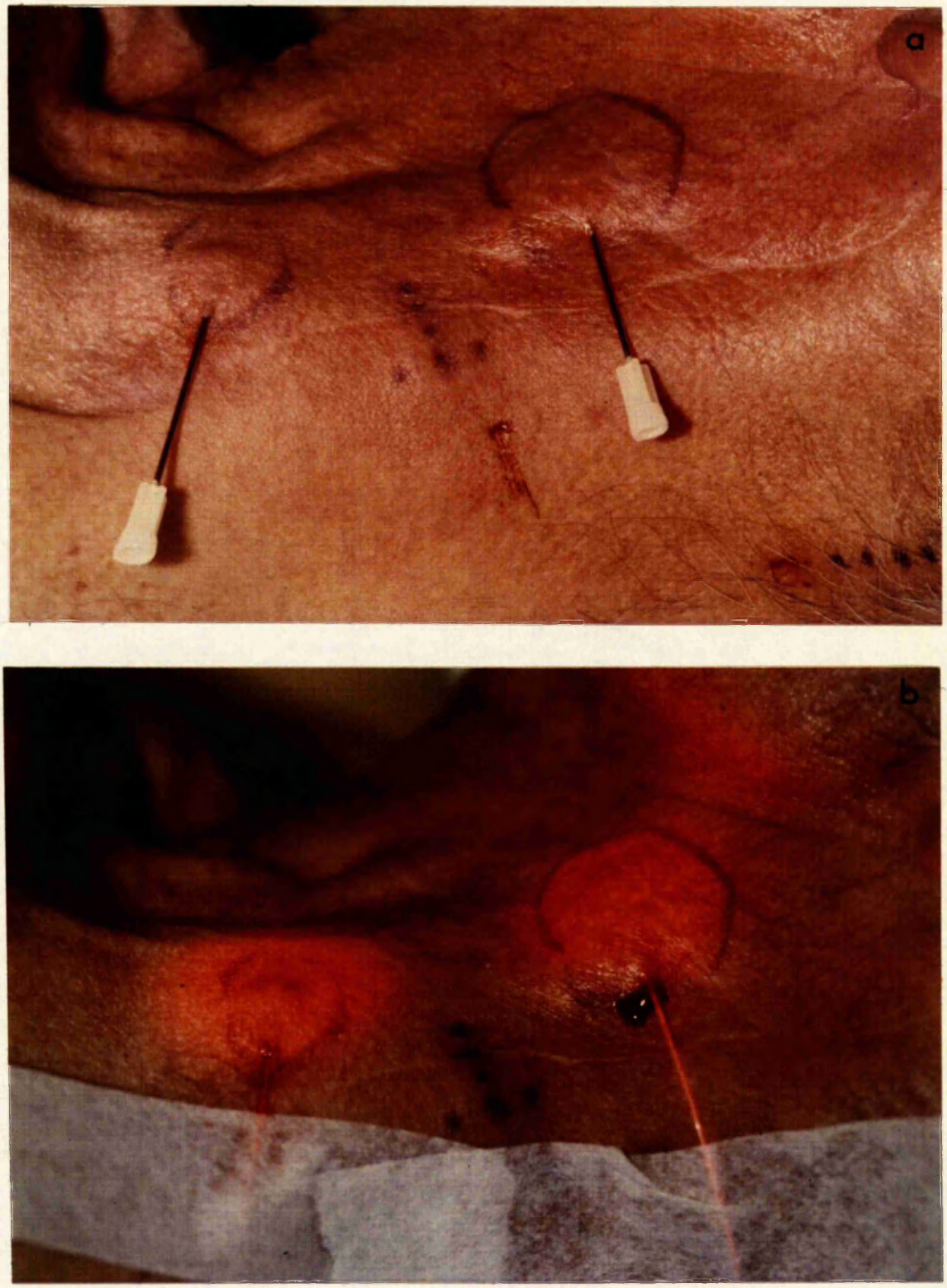
At the two sites where two optical fibres were used to deliver light, the fibres were inserted through the tumour so that they were parallel and 8 mm apart in tumour's central plane. The fibres were withdrawn a third of the length of their tracks through the tumour and a quarter of the total light dose delivered through each fibre at these points. The fibres were withdrawn the same distance again and the remaining light dose delivered.

Figure 6.2



Beam splitter used to focus light from the Copper vapour/dye laser into two 200 μm optical fibres for interstitial photochemotherapy.

Figure 6.3



Insertion of optical fibres for interstitial photochemotherapy.
a) 17 G hypodermic needle inserted through the tumour
b) Fibres positioned to give even illumination throughout the tumour.

The light output of the fibres was measured before and after each treatment with a light meter. The light dose rate from the fibre at the end of treatment was 78 ± 17% (mean ± S.D.) of the pre-treatment dose rate.

In patients 12 and 13, tumours of similar sizes were given different doses of light. Patient 15 had 6 tumours of different sizes (30 to 210 mm^3) each treated with 200 J of light. In patient 14, a single small lesion (60 mm^3) was given 75 J while the two larger lesions (6200 and 5600 mm^3) were treated with 2 optical fibres delivering 75 J to each of four points in the tumour.

All tumours were measured before photochemotherapy, 24 h after treatment, at weekly intervals for a month and then monthly for 2 to 5 months. For each tumour, four planar diameters were measured with Vernier calipers and a tumour volume calculated from each diameter, assuming that the tumours were spherical. The mean of these 4 volumes was used as the tumour volume.

RESULTS

Superficial Photochemotherapy

The overall complete tumour response rate was 47%. If only the 18 lesions treated with 1.5 or 2 $mgkg^{-1}$ of Photofrin II and 50 or 75 Jcm^{-2} of light are considered the complete response rate was 74%. Tumour control increased with dose of Photofrin II and light (Appendix

6.1). Complete tumour response occurred within three weeks of treatment and persisted during the period of follow-up.

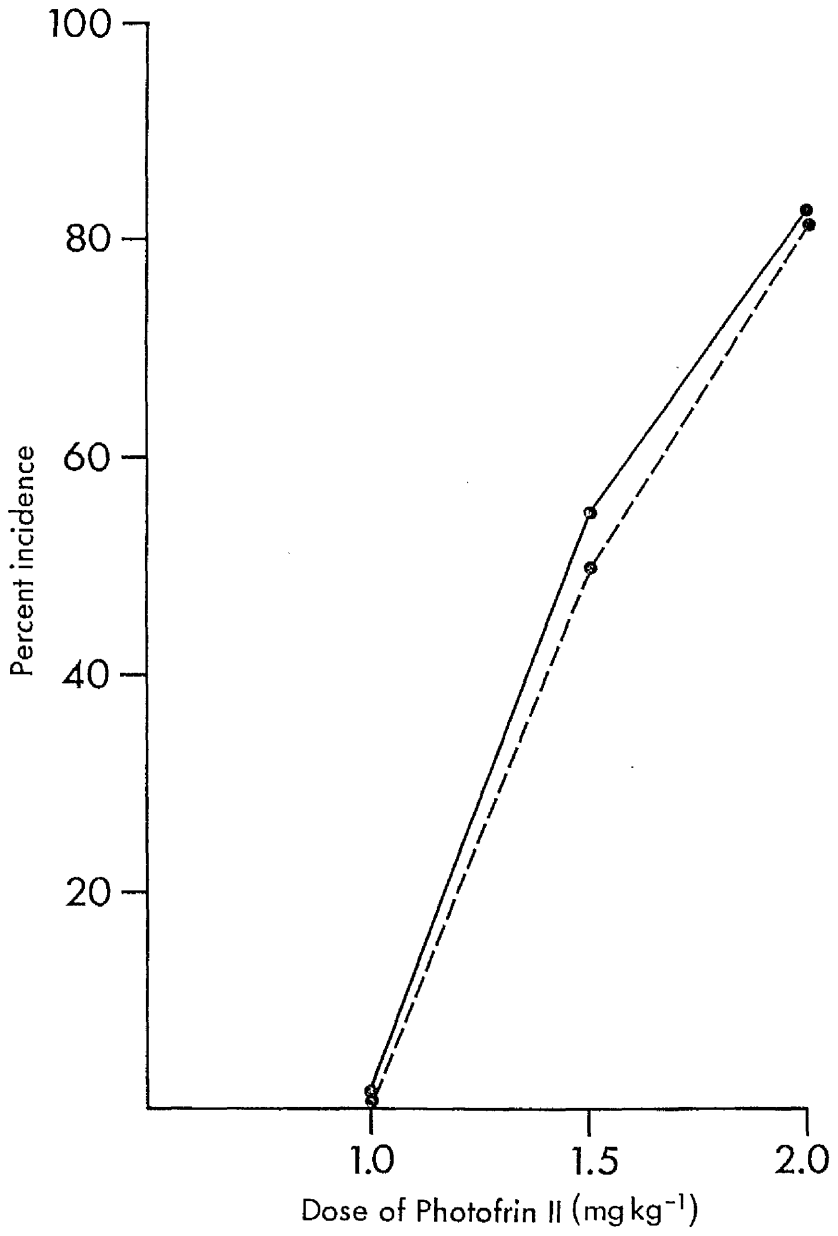
Ultrasound was not available to measure the thickness of the tumour below the skin surface. To assess the influence of 'tumour depth' on response to superficial photochemotherapy, the mean of the tumour's measured diameters was assumed, therefore, to be equal tumour depth for subcutaneous nodules with no skin infiltration. For tumour less than 10 mm thick complete tumour resolution occured more frequently than for larger tumours ($0.10>p>0.05$, Appendix 6.2).

The incidence of skin necrosis, also, increased with dose of Photofrin II and light (Appendix 6.3), and was closely associated with tumour response (Figures 6.4 and 6.5).

At those sites where skin necrosis occurred, there was blanching of the skin in the treated area surrounded by an annulus of erythema (Figure 6.6a) within hours of treatment. At one week there was intradermal haemorrhage in the centre of the treatment area (Figure 6.6b) and at two weeks, the skin had broken down and an eschar had formed (Figure 6.6c). Over the next 2 to 10 weeks the skin healed from the edges of the necrosed zone (Figures 6.6d and 6.6e). The mean diameter of the eschars produced was 51% $\pm$ 11% ($\pm$ 1 S.D.) of the diameter of the total area illuminated. There was a trend for the size of the eschar to increase with dose of drug and light (Figure 6.7).

The only abnormalities visible after healing were a small central scar and slight pigmentation which gradually faded (Figure 6.6e). The skin necrosis always healed completely, with no scarring or contraction,

Figure 6.4

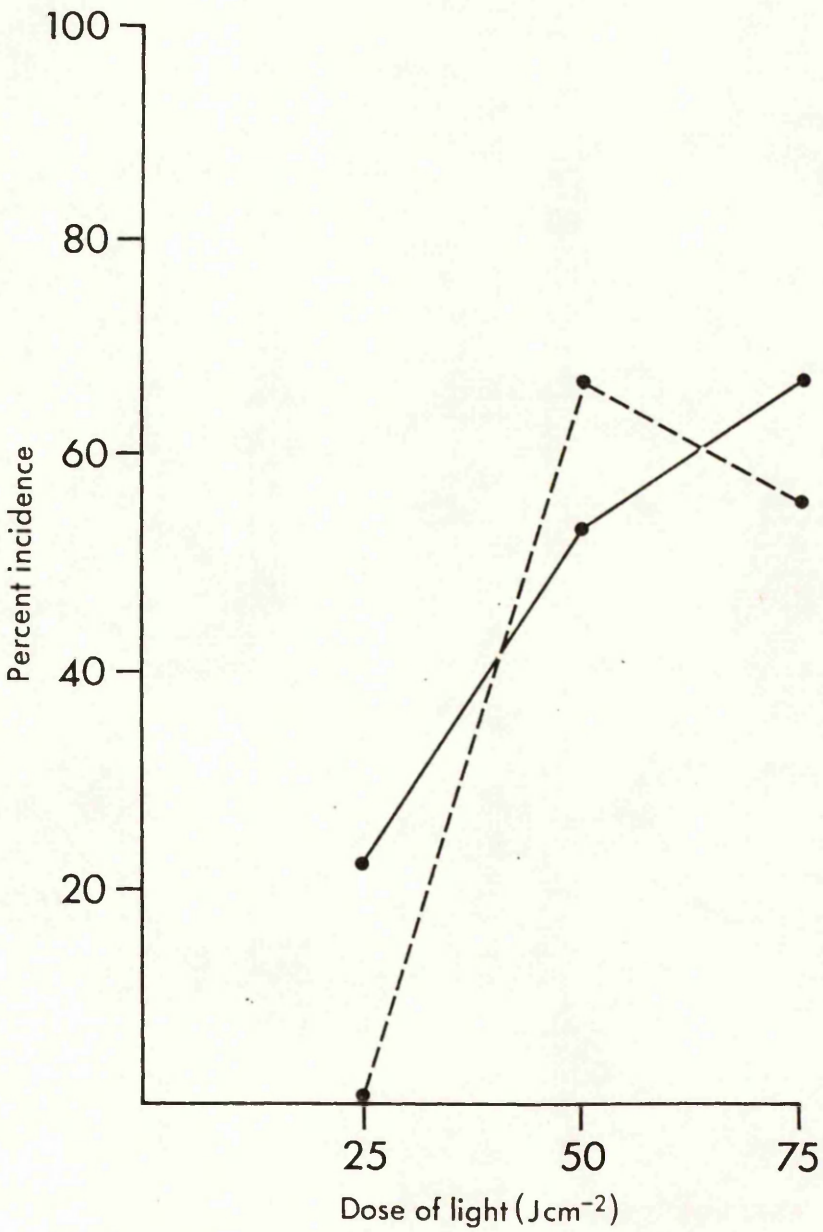


Relationship between the incidence of complete tumour response and skin necrosis and dose of Photofrin II.

——— Tumour response

— — — Skin necrosis

Figure 6.5

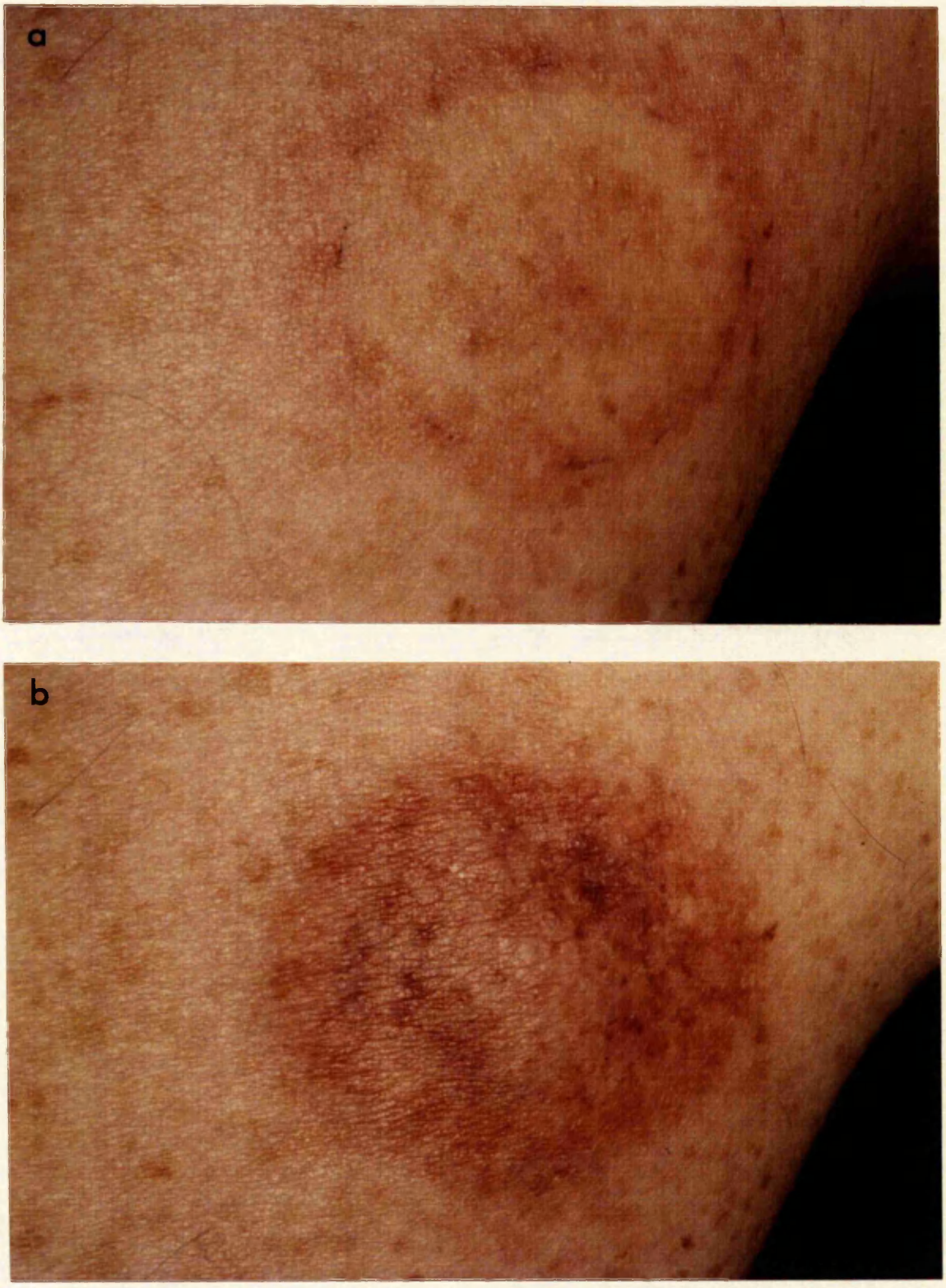


Relationship between the incidence of complete tumour response and skin necrosis and dose of light.

——— Tumour response

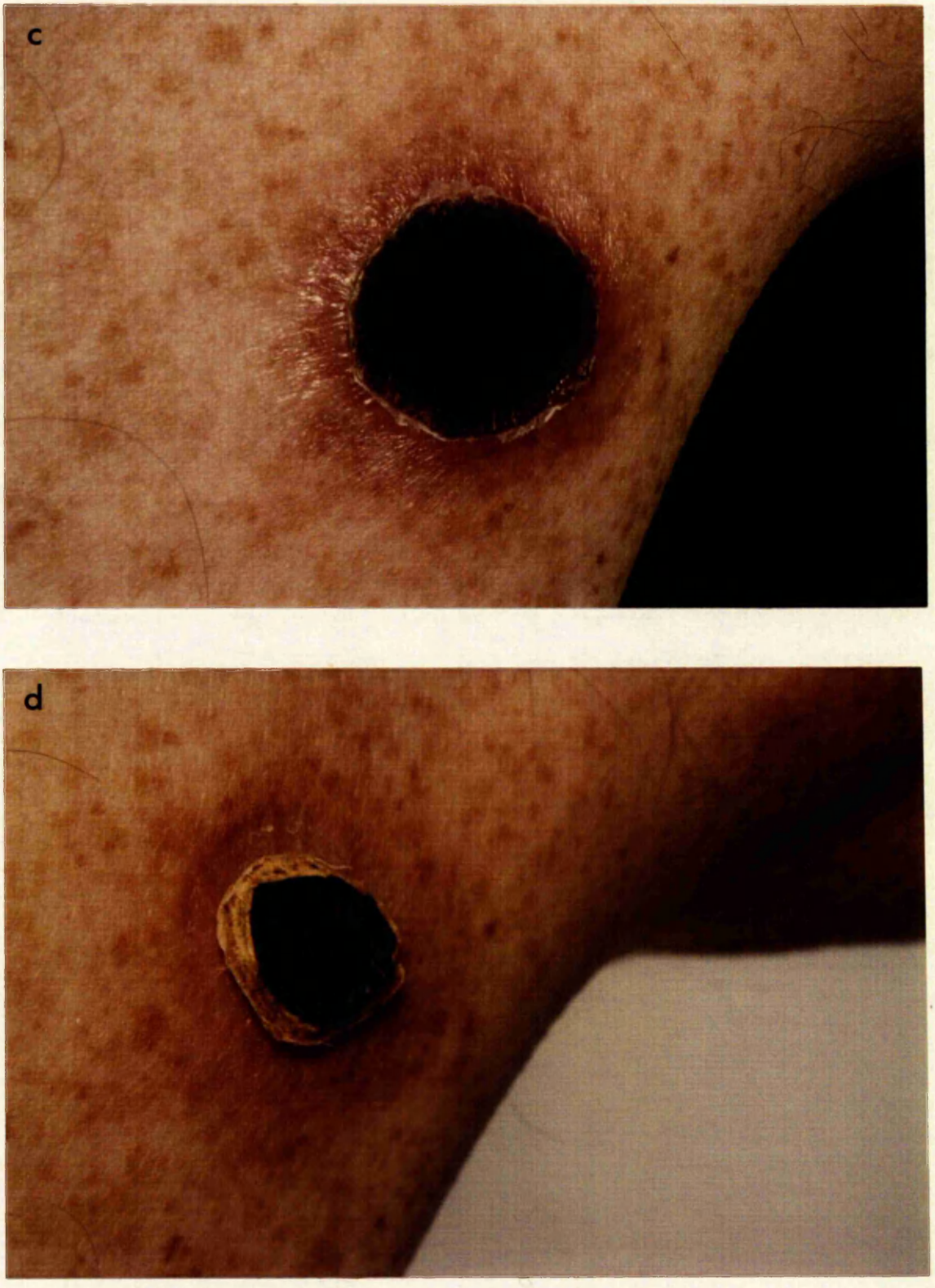
– – – – Skin necrosis

Figure 6.6



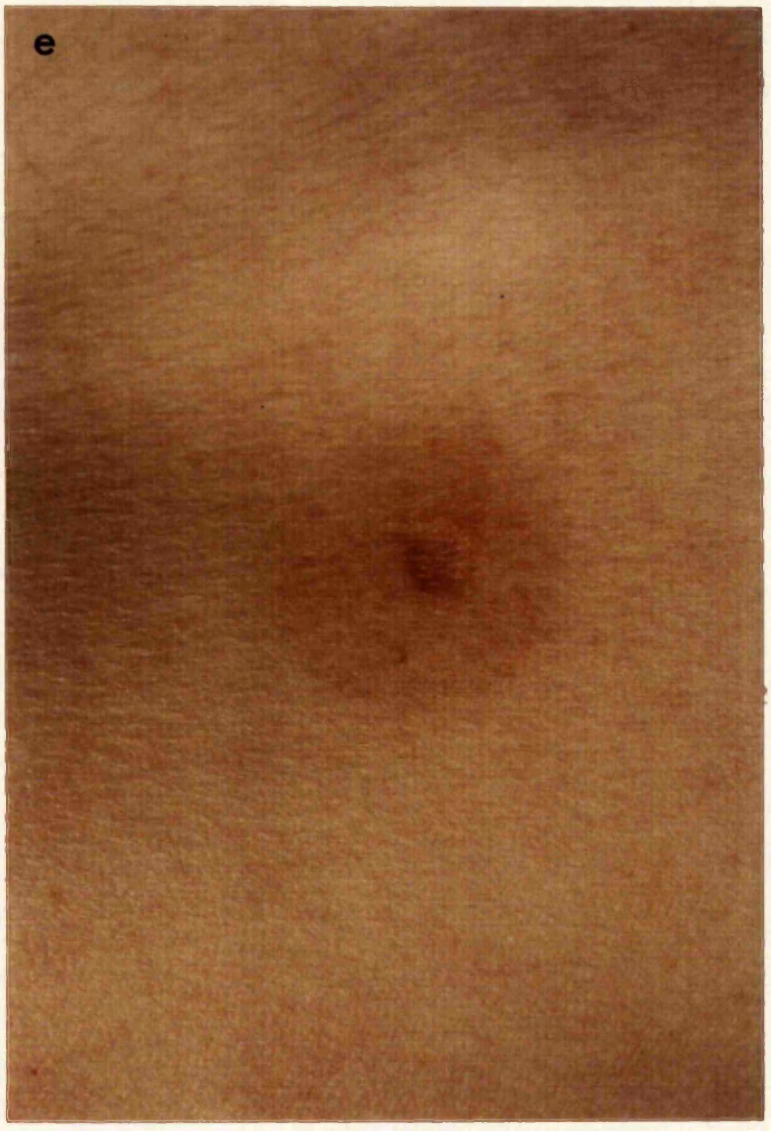
Skin changes after superficial photochemotherapy
a) 24 hours - blanching with an annulus of erythema
b) 1 week - intradermal haemorrhage

Figure 6.6 (continued)



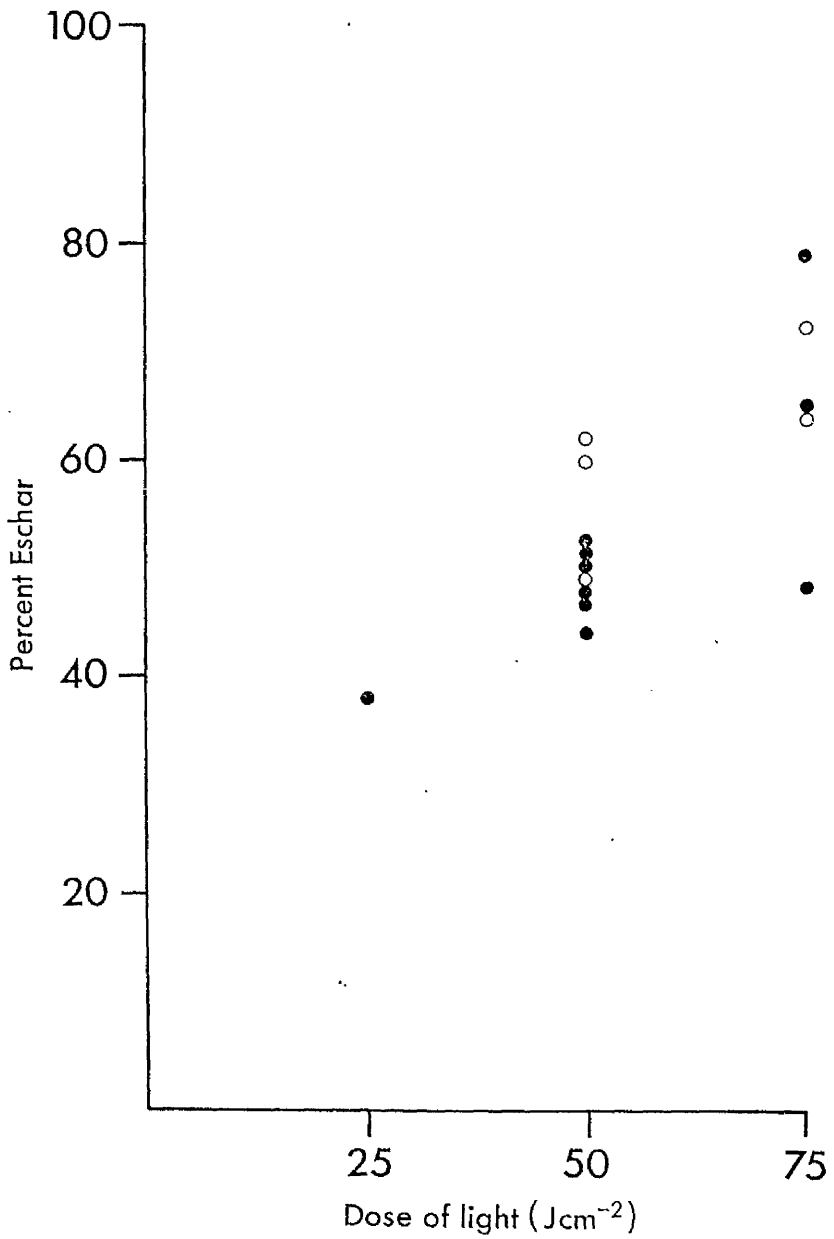
Skin changes after superficial photochemotherapy
c) 2 weeks - skin necrosis with formation of an eschar
d) 8 weeks - necrosis healing from the edges

Figure 6.6 (continued)



Skin changes after superficial photochemotherapy
e) Appearance of healed skin.

Figure 6.7



Relationship between eschar size (diameter of eschar expressed as a percentage of the diameter of the treated field) and dose of light.
- ● 1.5 mgkg^{-1} Photofrin II
- ○ 2.0 mgkg^{-1} Photofrin II

but at some sites this took 12 weeks. Skin necrosis was painless except at the largest treated area, where for 3 weeks after treatment the patient suffered discomfort which was relieved by co-proxamol.

Two of the patients with recurrent oral tumours showed complete regression of visible tumour with good relief of their symptoms. The third patient had a complete response and remained disease free in the treated area for a year but then required radical oral surgery for progressive disease elsewhere. These patients tolerated treatment well. Within a few hours of irradiation, there was blanching in the treated area and by 48 hours tumour necrosis occurred. Necrosis was confined to the tumour site and did not involve normal tissue included in the treatment field. This necrosis healed over the next two weeks.

Interstitial Photochemotherapy

None of the sites treated with interstitial photochemotherapy showed complete clinical resolution of the tumour and therefore tumour growth delay, the length of time taken for the tumour to regrow to its pre-treatment size, was used to assess tumour response (Yarnold, Bamber and Gibbs, 1986).

The volume of untreated tumours and tumours regrowing after treatment increased exponentially. Tumour volume plotted against time on a log/linear scale, therefore, gave a straight line which was calculated for individual tumours using linear regression analysis ($r = >0.80$). This line was used to calculate the time taken for treated tumours to regrow to their pre-treatment volume and for controls to grow to twice

their original volume (volume doubling time). For each patient, the tumour growth delay was divided by the volume doubling time of the untreated tumour, to allow comparison of tumour response between patients.

All except three tumour sites showed growth delay (Appendix 6.4). At 6 sites, although tumour was still palpable, tumour regrowth did not occur during follow up (11 to 21 weeks) (Appendix 6.4). Relative tumour response increased with the total light dose (Figure 6.8, Appendix 6.4) but this was not statistically significant ($0.10>p>0.05$). In Patient 15, who had six tumours of different sizes all treated with the same dose of light, tumour response decreased with increasing tumour volume (Figure 6.9, Appendix 6.4) ($p<0.05$).

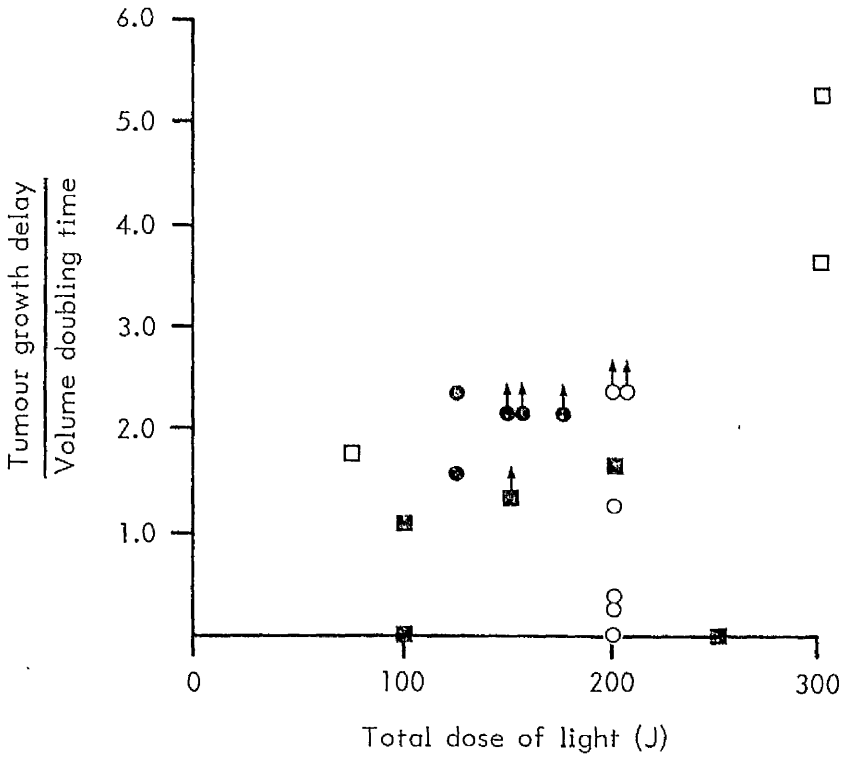
Patients tolerated treatment well and did not experience any pain after treatment. Skin necrosis did not occur after interstitial photochemotherapy.

DISCUSSION

Patients with multiple measurable metastases provide a good opportunity to assess treatment dose response curves, because the patient can act as his own control (Ash, Peckham and Steel, 1979; Urtasun et al., 1980).

In this study, the overall complete tumour response rate for superficial photochemotherapy was comparable with that observed by others (Dougherty, 1984a; Dougherty, 1986) and as reported by

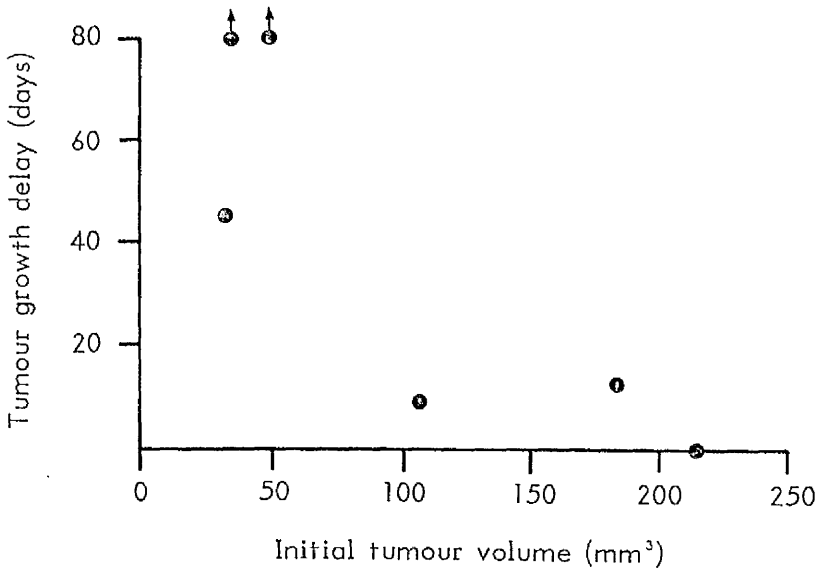
Figure 6.8



Tumour response to interstitial photochemotherapy
- ■ Patient 12
- ● Patient 13
- □ Patient 14
- ○ Patient 15

↑ indicates tumours that were not regrowing at time of last follow up.

Figure 6.9



Relationship between tumour volume and tumour response to 1.5 $mgkg^{-1}$ of Photofrin II and 200 J of interstitial light (Patient 12).

↑ indicates tumours that were not regrowing at time of last follow up

Dougherty et al (1978) tumour response increased with increasing dose of photosensitizer and light (Appendix 6.1). Tumour response, also, decreased with tumour diameter (Appendix 6.2).

These data suggest that superficial photochemotherapy may only be used effectively to treat cutaneous tumours less than 1 cm in depth (Appendix 6.2). The therapeutic ratio may be reduced by trying to treat tumours that are too large. Large tumours may contain more clonogenic cells than small tumours and therefore require more photosensitizer and/or light to control them. As the penetration depth of light in human tumours is 2.0 $\pm$ 0.5 mm (Driver et al., 1988), the light intensity at the base of 10 mm and 15 mm diameter tumours would be expected to be 0.67 and 0.02 % respectively of that absorbed at the skin surface. These factors may lead to insufficient treatment of the base of larger tumours. Since in this study, tumours of different sizes were evenly distributed throughout the treatment groups, tumour diameter perse should not have affected the trend for tumour response to increase with dose of photosensitizer and light.

The increase in complete tumour response was paralleled by an increase in the incidence of skin necrosis within the treated area (Figures 6.4 and 6.5), suggesting a relatively poor therapeutic ratio for superficial photochemotherapy. Skin necrosis, however, caused minimal discomfort to the patients and healed with a good cosmetic result in all patients. Early skin necrosis may, therefore, not be the most appropriate end-point for measuring the therapeutic ratio of superficial photochemotherapy. The cosmetic result after healing of skin necrosis may be more appropriate.

The mechanism of production and repair of skin damage is interesting because the initial damage appeared severe but caused minimal pain and always healed without scarring. The damage does not resemble a thermal burn, as in patients a full thickness burn would be expected to heal with fibrosis and a partial thickness burn, which may heal without scarring or contracture, is usually very painful (Bailey and Love, 1984). In rodent colonic mucosa thermal burns produced by lasers heal by fibrosis whilst damage due to photochemotherapy repairs leaving a relatively normal mucosa (Barr et al., 1987).

Necrosis produced by ionizing radiation, with the same appearance as that produced by photochemotherapy, would not be expected to heal. Ionizing radiation produces most of its acute effect by damaging clonogenic cells, for example stem cells in the skin. Such damage may limit the skin's ability to regenerate. Photochemotherapy, however, produces its acute effects indirectly via vascular damage, which may produce less damage to the stem cells and so have less effect on the skin's capacity for regeneration.

The light intensity in tissue decreases exponentially with increasing depth (Wan et al., 1981) therefore, receive a lower dose of light than the skin but the tumour showed persistent damage while skin damage was transient. This suggests that tumour is more sensitive to photochemotherapy than normal skin. This may be due to a greater concentration of Photofrin II in the tumour than the normal surrounding tissue or, as with radiotherapy, normal tissue may have a greater capacity for repair than tumour.

The parallel increase of tumour response and early skin damage with dose of Photofrin II suggests that increasing the dose of photosensitizer increases the porphyrin level in skin as well as tumour. As discussed previously (p49, Chapter 3), this may negate any therapeutic advantage produced by increasing the dose of photosensitizer.

The three patients with recurrent tumours in the mouth responded well to photochemotherapy. The oral mucosa is not pigmented and should allow relatively good light penetration making these tumours, which usually involve the surface, suitable for treatment with superficial light. The reaction produced by treatment in the mouth might be expected to heal more quickly than that in the skin because oral mucosa has a shorter regeneration time than skin (Hill, 1987). In view of the good response to treatment and absence of side-effects, the role of photochemotherapy in the management of oral tumours requires further investigation.

It was expected that interstitial light delivery would increase the dose of light delivered to the tumour and so improve tumour response to photochemotherapy. Although it did not produce any complete tumour responses, tumour response did increase with dose of light. It may be possible to improve tumour response and to produce complete regression by giving larger doses of light but the therapeutic ratio would only be improved if skin necrosis was still absent at higher light doses.

It is difficult to compare interstitial and superficial light doses (p17, Chapter 1). On the basis of the animal tumour studies (p74, Chapter 4,) and existing recommendations for comparing interstitial

and superficial light doses (McKenzie, 1985), the doses of interstitial light used in this study were thought to be equivalent to or greater than those used for superficial treatment. If the light doses used were comparable, these data suggest that interstitial photochemotherapy was less effective than superficial photochemotherapy. This may be due to:-

1. The tumours being too large to treat effectively with a single interstitial optical fibre. Tumour response decreased with increasing tumour size (Figure 6.9). This was expected because the intensity of light in tissue decreases exponentially with depth. Poor light penetration, however, seems unlikely to be the whole explanation because tumours of a similar size regressed completely after superficial treatment and to treat the whole tumour superficial light must penetrate through the skin plus the diameter of the tumour whereas interstitial light must only penetrate through the radius of the tumour.

2. The insertion of the optical fibre may alter the tumour's structure, perhaps by causing bleeding. Light can cause blood around the tip of an optical fibre to char (personal observation), decreasing the amount of light delivered to the tumour. The light output of the fibre measured after treatment may be an overestimate of the light dose rate in the tumour during treatment because removal of the fibre from the tumour may dislodge charred blood from its tip. It may be possible to decrease the risk of charring by using optical fibres with diffusing tips which decrease the light power density at the surface of the fibre.

Unlike human tumours, $LSBD_1$ showed no difference in tumour growth delay after superficial or interstitial photochemotherapy. This may be

due to differences in tumour vascularity. Subcutaneously implanted rodent tumours are usually less vascular than human tumours making bleeding around the optical fibre less likely in $LSBD_1$ than in human tumours.

Unlike the light dose response curves for $LSBD_1$ treated with photochemotherapy, the dose response curves in patients did not show a plateau. This may be due to:-

1. The doses of light in the clinical study being too low to demonstrate the plateau if it occurs.
2. The concentration of photosensitizer in human tumour being greater than in $LSBD_1$, as insufficient photosensitizer may limit the effect of photochemotherapy in $LSBD_1$ at higher light doses (p72, Chapter 4).
3. The factors limiting tumour response in humans may be different from those limiting the response of $LSBD_1$. For example, if the human tumours were more vascular than $LSBD_1$, they might have a smaller hypoxic fraction of cells than $LSBD_1$ or the dose of light that would produce complete vascular occlusion may be higher for human tumours (p73, Chapter 4).

CONCLUSIONS

1. Superficial photochemotherapy was well tolerated and an effective method of treating cutaneous and subcutaneous tumours less than 10 mm in diameter.
2. The incidence of early skin necrosis after superficial photochemotherapy was high. This healed in all cases with good cosmetic results and the use of early skin necrosis to assess the

therapeutic ratio of photochemotherapy may not be appropriate.

3. Tumour response increased with light dose for interstitial photochemotherapy and although it did not produce any complete tumour responses, neither did it cause skin necrosis. It may, therefore, be possible to produce complete tumour responses by increasing the dose of light without producing excessive normal tissue damage and maintaining a good therapeutic ratio.

4. Recurrent tumours of the oral mucosa responded well to superficial photochemotherapy.

CHAPTER 7: SKIN PHOTOSENSITIVITY TESTING

Cutaneous photosensitivity is the only major side-effect of photochemotherapy (Dougherty et al.). The erythema produced by known doses of white light in the skin of patients receiving photochemotherapy was measured to:-

a) allow patients to be advised about their skin photosensitivity

b) determine the relationship between photosensitivity and the pharmacokinetics of Photofrin II (Chapter 5).

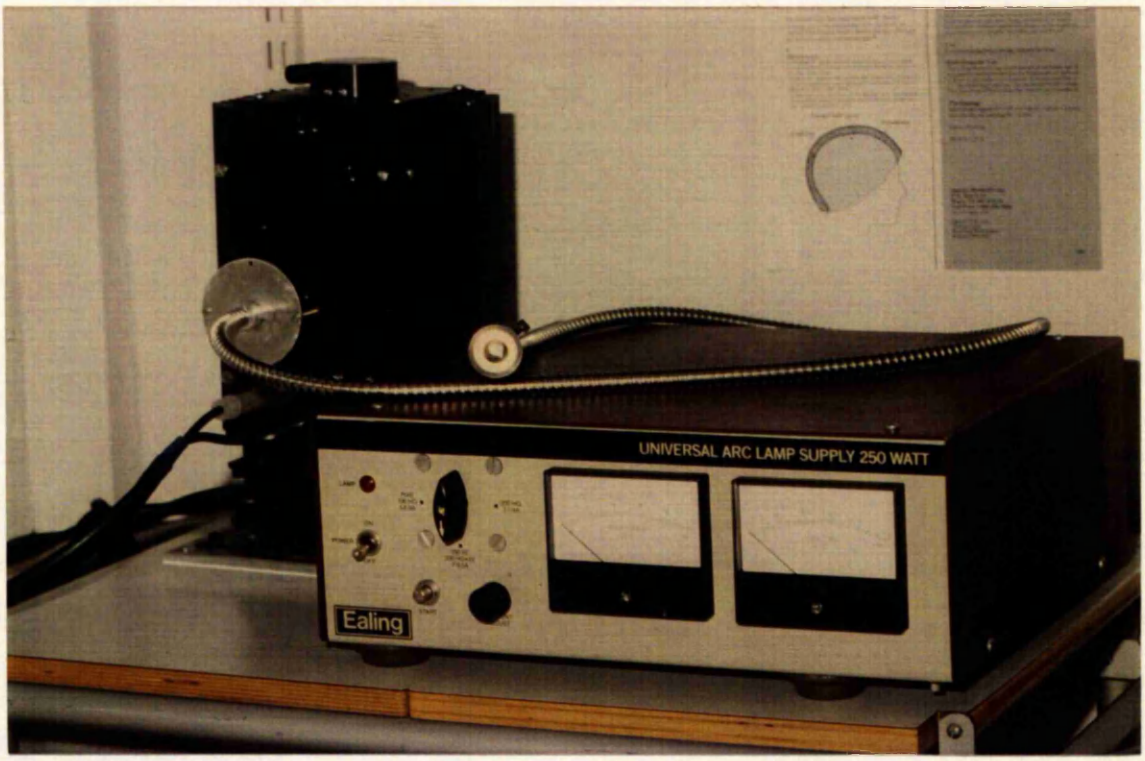
METHODS

Instrumentation

The solar simulator (Figure 7.1; Appendix 7.1) produced a 1.3 cm diameter circle of light with an irradiance of 164 $mWcm^{-2}$. This is about twice the light dose rate on a sunny Summer day in Leeds.

The haemelometer (Figure 7.2a) is a reflectance meter and was used to measure skin erythema. A full description and evaluation of the haemelometer is given by Feather, Ellis and Leslie (1988). A reflectance spectrophotometer (Figure 7.2b) was also used to measure skin erythema and is described in detail by Feather, et al. (1988). These reflectance meters quantify cutaneous haemoglobin, giving a Haemoglobin Index. This was used as a measure of cutaneous erythema (p19, Chapter 1).

Figure 7.1



The solar simulator used for photopatch testing.

Figure 7.2

Reflectance meters used to measure skin erythema.
a) the haemelometer
b) the reflectance spectrophotometer

Photopatch testing

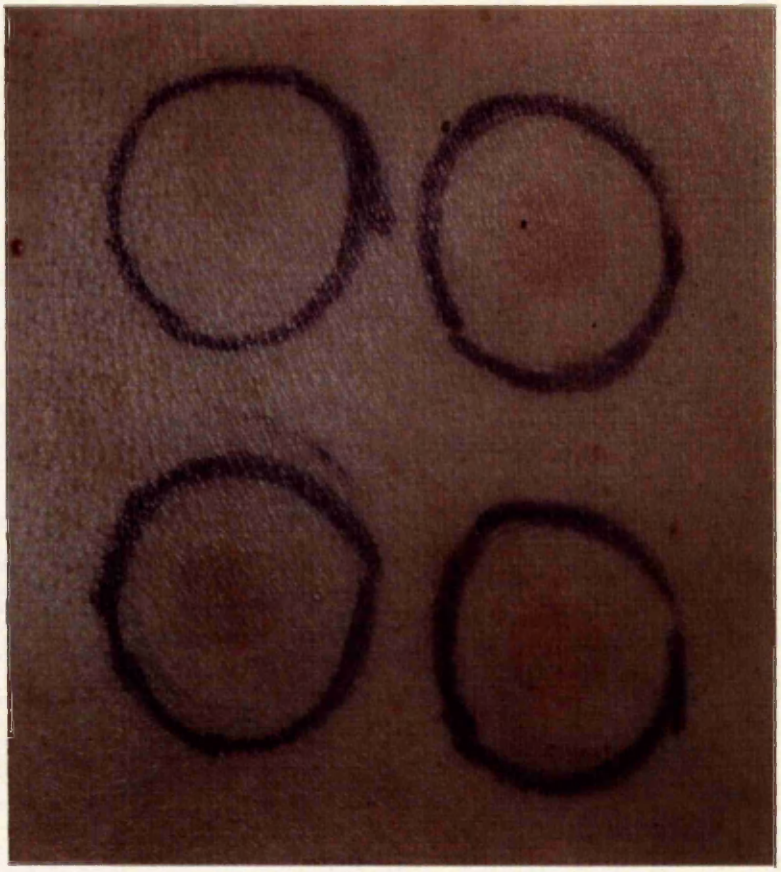
Eleven patients (Patients 1, 2, 4, 5, 6, 8, and 12 to 16, Appendix 1) were photopatch tested. They were given 1.0, 1.5 or 2.0 $mgkg^{-1}$ Photofrin II intravenously and their tumours irradiated 48 to 72 hours later with therapeutic doses of 630 nm light. None of the patients were taking any other photosensitizing drugs. Patients were advised to stay out of direct sunlight for one month after treatment and during the following month to avoid bright sunlight.

Photopatch testing was performed on the upper back, remote from sites of tumour, previous surgery or radiotherapy. Four (two rows of two) 1.3 cm diameter circles of skin about 2 cm apart were tested (Figure 7.3). They were exposed to 5, 10, 15 or 20 Jcm^{-2} of light, except in Patient 1 when the light doses used were 10, 20, 30 or 40 Jcm^{-2} or 20, 30, 40, or 50 Jcm^{-2}.

Two haemoglobin index readings were made at each test site and at a control site, the central area between the four test sites, using the haemelometer or reflectance spectrophotometer. The mean of these two readings was used to calculate the relative haemoglobin index. Measurements were made immediately before and two and a half to three hours after the test dose of light. In addition, the erythema produced by 20 Jcm^{-2} of light was measured at 20 minutes and 24 hours for Patients 1 and 6.

Patients 12, 13, 14 and 15 were photopatch tested immediately before drug administration and, when possible, all were tested 1, 4, 10, 30 and 45 days after Photofrin II injection. In patients 14 and 15

Figure 7.3



Erythema produced by photopatch testing.

testing with 20 Jcm^{-2} light was performed on the back of the hand. Patient 15, also, had a 3 cm diameter circle of skin covered with a sticking plaster before Photofrin II injection, to exclude light. The plaster was removed and the area tested 72 hours after injection.

The Relative Haemoglobin Index

The relative haemoglobin index was calculated using the formula:-

$$\text{Relative H.I.} = \frac{\text{H.I. control site at } t_0 \times \text{H.I. test site at } t_x}{\text{H.I. control site at } t_x \times \text{H.I. test site at } t_0}$$

where H.I. = Haemoglobin Index

t_0 = before test dose of light given

t_x = time after test dose of light given for which relative haemoglobin index is being calculated.

RESULTS

The patients did not develop any visible reaction to ambient light, even in the exposed skin of the face and hands, and they tolerated photopatch testing well. They did not experience discomfort or itching at the test sites. The patients tested before receiving Photofrin II did not show any significant reaction. Occasionally during the first four days after drug injection, patients developed erythema immediately after receiving the test dose of light. More commonly, however, erythema gradually developed over a few hours, subsiding again by 24 hours. These clinical observations were supported by the measurements made in Patients 1 and 6 (Appendix 7.2).

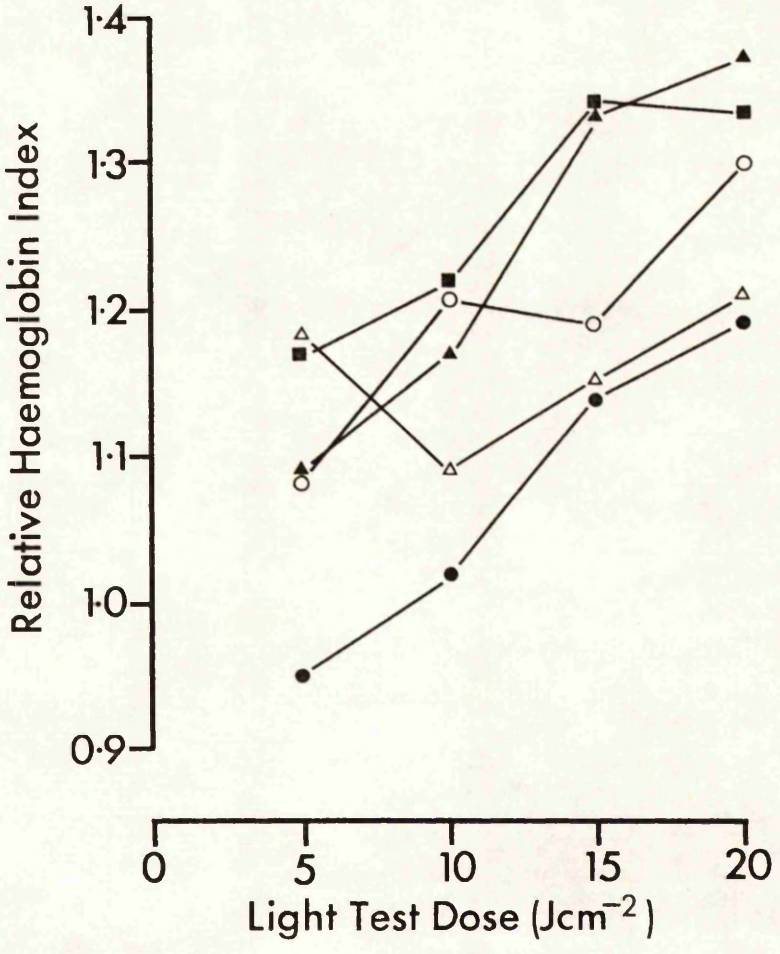
Generally the relative haemoglobin index increased with dose of light but about 4 days after Photofrin II injection, some patients showed a decrease in relative haemoglobin index with higher doses of light (Figure 7.4; Appendix 7.3).

The variation in relative haemoglobin index with time after Photofrin II was biphasic (Figure 7.5; Appendix 7.3). All except one of the nine patients photopatch tested over more than a month showed an increase in relative haemoglobin index 1 to 4 days after drug injection, followed by a fall in relative haemoglobin index a few days later. In six patients, this rise and fall occurred within four days. In the other two patients these changes occurred over eleven days. The patients then showed a second peak of erythema production, 11 to 50 days after receiving photosensitizer.

The duration of photosensitivity was difficult to assess because patients were followed up at increasing intervals and they were tested for a maximum of 6 weeks after receiving Photofrin II because patients had to remain at the hospital for three hours for testing to be performed. The results suggest that the skin of the back remains sensitive to photopatch testing for less than 3 to more than 6 weeks (Appendix 7.3).

Twenty Jcm^{-2} of light delivered to the back of the hand produced a smaller increase in relative haemoglobin index than the same dose of light given to the upper back (Appendix 7.4). This difference was greater at four days than at one day after drug administration (Appendix 7.4). In Patient 14, eleven days after drug, the relative haemoglobin index of the skin on the back of the hand had returned to

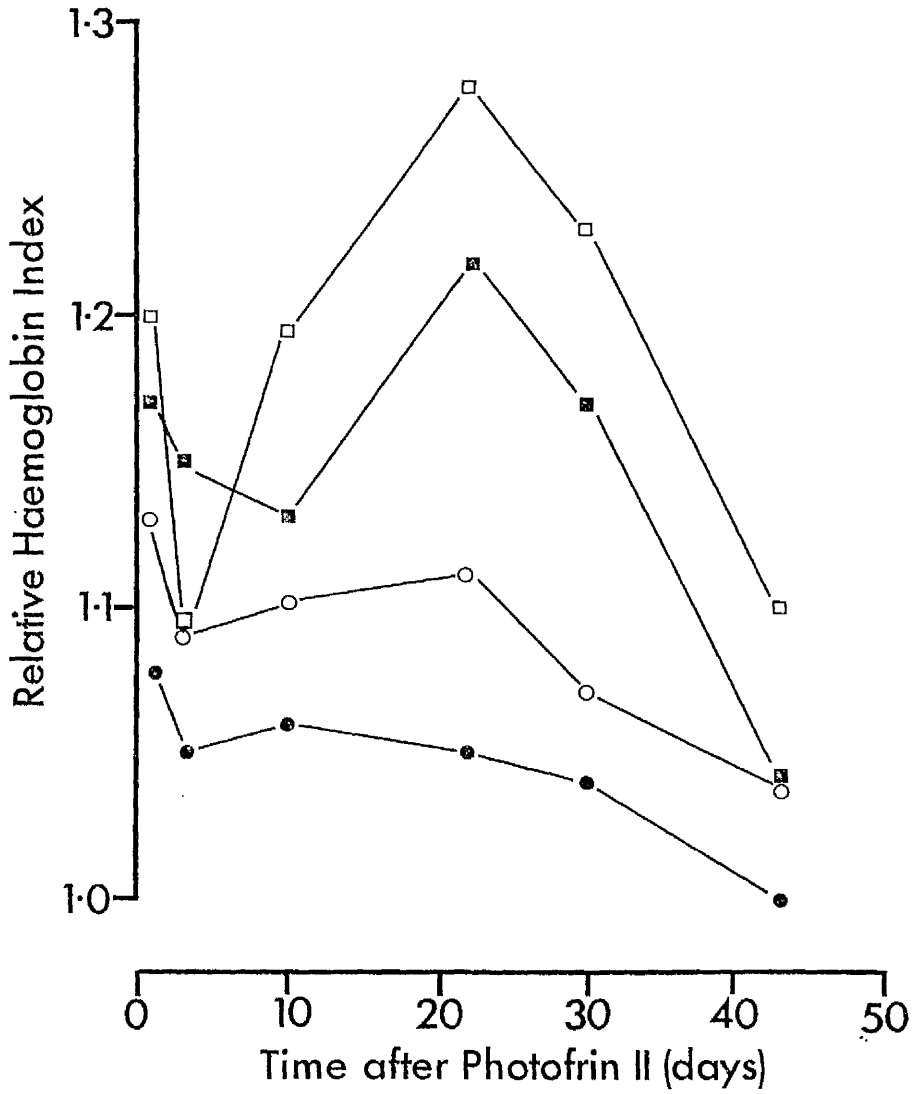
Figure 7.4



Relationship between relative haemoglobin index and test dose of light (Patient 16).

- ▲ 1 day after Photofrin II injection
- △ 4 days after Photofrin II injection
- ■ 17 days after Photofrin II injection
- ○ 32 days after Photofrin II injection
- ● 56 days after Photofrin II injection

Figure 7.5



Relationship between the relative haemoglobin index and time after Photofrin II injection (Patient 2).

- ● 5 Jcm^{-2}
- ○ 10 Jcm^{-2}
- ■ 15 Jcm^{-2}
- □ 20 Jcm^{-2}

normal despite that of the skin on the back increasing again. The area of skin which was covered with a plaster before Photofrin II administration (Patient 15) showed a greater response to photopatch testing four days after drug injection than adjacent skin that had not been covered (Appendix 7.4).

Correlation between serum pharmacokinetics of Photofrin II and cutaneous photosensitivity (Appendix 7.5)

The relative haemoglobin index measured on the skin of the back after 20 Jcm^{-2} of light did not correlate with plasma porphyrin levels, 10 or 11 days ($r = 0.02$) or 18 to 32 days (0.20) after photosensitizer. During the 6 weeks after Photofrin II injection, the erythema response of the skin on the back returned to normal in 1 patient (Patient 4). It was, therefore, not possible to correlate the duration of cutaneous photosensitivity, measured by photopatch testing, and the elimination half-life of Photofrin II. In all patients, except Patient 4, who had photopatch testing and pharmacokinetic studies performed (Patients 2, 5, 13 and 14), the duration of photosensitivity (Appendix 7.3) was greater than 4 times the elimination half-life of Photofrin II (Appendix 5.3). For practical purposes, elimination is usually considered to be complete by the time that four half-lives have elapsed.

DISCUSSION

Anything that alters cutaneous blood flow may affect skin colour (Lewis, 1926), for example changes in external temperature or posture.

The relative haemoglobin index compensates for such variations in normal skin colour by including measurements made before the test dose of light and at an adjacent control site. As the relative haemoglobin index is an objective measurement of erythema response, it was used to measure cutaneous photosensitivity.

It may have been desirable to leave more than two and a half to three hours between giving the test dose of light and measuring erythema, as the erythema response may have still have been developing at this time. This was, however, the maximum time interval which allowed photopatch testing to be performed on a regular out-patient basis.

The reflectance meters used give an objective measurement of erythema but there are some problems inherent in their use. Firstly, if the measuring head is applied to the test area with too much pressure, the blood supply to the skin may be impaired giving a falsely low reading. Secondly, the measuring probe must be applied so that no extraneous light can enter it because this may, also, give a falsely low reading. Thirdly, light emitted by the detection probe may produce erythema. The probe, however, gives the same dose of light to all sites measured, including the control, and this dose is small compared to the doses used for photopatch testing. Light from the probe is, therefore, unlikely to affect the relative haemoglobin index.

Photopatch testing using ultraviolet light may produce heating at the test site and so cause vasodilation and erythema. The light used in this study was unlikely to cause heating because:-

1. The energy from longer wavelengths of light is absorbed in a relatively larger volume of tissue than that of shorter wavelengths

because the penetration of light in tissue increases with increasing wavelength (Wilson et al., 1984). Longer wavelength of light are, therefore, likely to produce a smaller rise in temperature than the same dose of shorter wavelength light.

2. Skin response was measured two and a half to three hours after delivery of test doses of light when erythema due to heating should have subsided unless a thermal burn had been produced and patients did not experience pain which would have been expected with a burn.

3. Relatively small doses of light were used.

The site used for photopatch testing may affect the erythema response. The minimum erythema dose varies depending on which part of the back is being examined but the light dose response curve does not (Farr and Diffey, 1984). The data presented here was based on dose response curves.

Patients showed varying responses to photopatch testing (Appendix 7.3). The patients' natural photosensitivity may affect erythema response but each patient's response should be self consistent. As none of the patients who were photopatch tested before Photofrin II administration showed an erythema response, erythema may be assumed to be due to activation of photosensitizer in the skin and, therefore, dependant on the level of porphyrin in the skin. Uptake of the drug varies between patients and the effect of the drugs pharmacokinetics on photosensitivity will be discussed more fully later.

Generally, erythema response increased with applied light dose for each patient at a specific time after Photofrin II administration. In some patients, 3 to 4 days after receiving drug, the relative

haemoglobin index decreased with increasing doses of light (Figure 7.4; Appendix 7.3). It is common to see blanching after therapeutic doses of light (p99, Chapter 6). It is possible that at 3 or 4 days after drug administration, when drug distribution should be complete and a high concentration of drug may be expected in the skin, that the test doses of light were sufficient to cause blanching. The reflectance meters measure the amount of haemoglobin present in the skin and relative haemoglobin index can only be used as a measure of photosensitivity if the skin's response to light is erythema.

Erythema response decreased 4 to 11 days after Photofrin II injection and then increased again (Figure 7.5; Appendix 7.3). As discussed above, the trough may be due to test doses of light producing blanching and would reflect an increase rather than a decrease in photosensitivity. This suggests that photosensitivity increases to a maximum within a few days and then falls slowly over the next few weeks. If the decrease in erythema response at 4 to 11 days after giving photosensitizer is, however, due to a genuine decrease in cutaneous photosensitivity, this would be the optimum time for light delivery in superficial photochemotherapy to minimize skin damage.

Patients were found to be sensitive to small doses of light. Exposure to sunlight for one minute on a clear Summer day in Leeds or to artificial light for 7 h gives a light dose of about 5 Jcm^{-2}. Five Jcm^{-2} produced measurable erythema on the back of patients during photopatch testing but no erythema was seen in light exposed skin of the patients although their exposure to artificial light was not restricted. This lack of response to artificial light may be due to:-

1. Artificial light being of low intensity allowing the repair of

sublethal damage which may occur in photochemotherapy (Bellnier and Lin, 1985).

2. Exposed skin on the face and back of the hands being more pigmented and so more protected from light than the normally covered skin of the back.

3. Photofrin II degradation by light. This occurs in vivo (Mang et al., 1987) and drug present in the patients' exposed skin may be photodegraded producing a more rapid decrease in photosensitivity in these areas than in skin which is normally covered, for example the back. The possibility of photodegradation is supported by the greater photosensitivity of skin on the back of the hand when protected from ambient light by a plaster than adjacent skin that was exposed to ambient light (Appendix 7.4).

Skin that is normally exposed to environmental light was less photosensitive than skin that is normally covered. Photopatch testing of skin that is normally exposed to ambient light may, therefore, be a more appropriate basis for advising patients about the precautions necessary to avoid phototoxic reactions.

At present, the only way of preventing phototoxic reactions in patients treated with photochemotherapy is avoidance of sunlight. Commercially available sunscreens are designed to protect the skin against ultraviolet B light, for example Spectroban 15 (Stiefel Laboratories (U.K.) Limited, High Wycombe, Bucks.) protects against 280 to 315 nm light. The photosensitizers in current clinical use are activated by wavelengths of light up to 635 nm, therefore commercially available sunscreens do not protect patients against sunlight. Physical barrier creams, for example Titanium dioxide paste, will

block out all wavelengths of light but these are cosmetically unacceptable.

In the porphyrias, deposition of porphyrins in the skin causes increased photosensitivity. In some of these diseases, β-carotene protects patients from the harmful effects of light. β-carotene must be taken for a month before therapeutic levels are achieved. To protect patients having photochemotherapy, β-carotene would have to be started a month before treatment which may protect the tumour as well as the skin from the effects of light and it has, therefore, not been used.

If photodegradation of porphyrins in skin does occur, it may be possible to decrease cutaneous photosensitivity by exposure to controlled doses of light.

The correlation between photosensitivity and plasma total porphyrin level was examined about 10 days after Photofrin II injection. Before this time it was uncertain whether erythema response was a good measurement of photosensitivity (see above). From the porphyrin level measurements in skin and plasma in the animal study, a correlation between photosensitivity and plasma porphyrin level soon after drug administration but not later might have been expected but no correlation was seen (Appendix 7.5).

The relationship between the time of maximum skin photosensitivity and the distribution half life of Photofrin II was not examined because of the uncertainty about the cause of the decrease in erythema response between 4 and 11 days (Appendix 7.5). Photopatch testing before

photosensitizer administration did not produce an erythema response, it might, therefore, be expected that there would be no erythema response when the patients' photosensitivity returned to normal after Photofrin II injection, as was observed in one patient. The duration of photosensitivity was greater than would have been predicted from the elimination half-life of Photofrin II in 4 out of 5 patients (Appendices 5.3 and 7.3). This might be expected if porphyrins are cleared from the patients' plasma and skin at different rates, as in BD_9 rats (p44, Chapter 3).

In this study, it would not have been possible to advise patients about skin photosensitivity on the basis of plasma porphyrin levels or pharmacokinetic parameters of Photofrin II.

CONCLUSIONS

1. The erythema response of normal skin after Photofrin II injection was biphasic.
2. The photosensitivity of skin that was exposed to environmental light was less than that of skin that is usually covered. Photopatch testing of skin that is usually exposed to ambient light may, therefore, be a more appropriate basis for advising patients about the precautions necessary to avoid a phototoxic reaction.
3. There was no correlation between the results of photopatch testing and the plasma porphyrin measurements and the duration of photosensitivity was greater than would have been expected from the pharmacokinetics of Photofrin II. It was, therefore, not possible to predict the duration of cutaneous photosensitivity from blood testing.

CHAPTER 8: GENERAL SUMMARY AND CONCLUSIONS

The objective of this work was to examine some of the major factors presently limiting the clinical usefulness of photochemotherapy and possible ways of overcoming these limitations. Investigations were undertaken in patients with superficial recurrent cancer and an isogenic fibrosarcoma, $LSBD_1$, in rats. This was similar to other rodent tumours in its response to ionizing radiation and cytotoxic chemotherapy (Chapter 2). The photosensitizers used were Photofrin II or polyhaematoporphyrin and irradiation was performed with 630 nm light from an Argon ion/dye or Copper vapour/ dye laser. Photochemotherapy was tolerated well by both patients and rats.

The therapeutic ratio of photochemotherapy is believed to depend on a preferential retention of porphyrin in tumours producing a higher level of photosensitizer in the tumour than in the surrounding normal tissue. If this is so, the interval between drug and light administration may be critical in producing the optimum therapeutic ratio. Porphyrin levels in $LSBD_1$ were, however, the same or less than those in adjacent skin and muscle for 1 week after PHP injection (Chapter 3). This suggests that the therapeutic ratio of photochemotherapy in $LSBD_1$ bearing BD_9 rats is either low or not dependent on a difference in concentration of photosensitizer between tumour and surrounding normal tissue.

As repeated biopsies were not possible in patients, the total plasma porphyrin levels after Photofrin II were measured and pharmacokinetic analysis performed. The pharmacokinetics of Photofrin II in patients conformed to a two compartment model. The distribution half-life of

the drug was 3.3 to 12.5 h and, therefore, distribution of photosensitizer from plasma to the tissues, including the tumour, should be complete by 50 h. This is consistent with the minimum interval usually used between drug and light administration clinically. The elimination half-life of Photofrin II was relatively long (3 to 10 days) suggesting tissue porphyrin levels fall slowly and that there may be several days when photosensitizer levels are sufficient for effective light administration (Chapter 5).

Tumour response to treatment may be improved by using higher doses of photosensitizer. In $LSBD_1$, total porphyrin levels increased with dose of PHP (Chapter 3). If the porphyrin levels in normal tissue also increase with dose of drug, any increase in tumour response may be associated with an increase in normal tissue damage with no improvement in therapeutic ratio. In patients treated with superficial photochemotherapy, the increase in complete tumour response and skin necrosis with dose of Photofrin II (Chapter 6) suggested that increasing the dose of photosensitizer produced an increase in normal tissue as well as tumour porphyrin levels.

Tumour response may, also, be expected to increase with dose of light. The response of $LSBD_1$ increased with light dose initially but above 200 Jcm^{-2} of superficial or 200 J of interstitial light no further increase in tumour response was observed (Chapter 4). This plateau in the light dose response curve may be due to insufficient doses of photosensitizer or light being used, the presence of a resistant population of cells or, if tumour response is due to vascular damage, complete occlusion of the tumour's blood supply being produced by 200 J or J cm^{-2} of light.

In the clinical studies, tumour response increased with light dose (Chapter 6). The absence of a plateau in the light dose response curve may be due to the doses of light used being less than those required to produce a plateau or the limiting factor of tumour response in patients and $LSBD_1$ being different, for example human tumours are usually more vascular than implanted rodent tumours and may, therefore, have a higher concentration of photosensitizer, be less hypoxic than $LSBD_1$ or require a larger dose of light to produce complete vascular occlusion.

For superficial treatment, the increase in tumour response with light dose was accompanied by an increase in the incidence of skin necrosis suggesting a relatively low therapeutic ratio (Chapter 4 and 6). The use of skin necrosis to assess normal tissue damage after photochemotherapy in patients may not be appropriate because necrosis healed completely with good cosmetic results in all cases and only one patient suffered any symptoms. The final cosmetic result was good with little normal tissue scarring.

The response of $LSBD_1$ to superficial photochemotherapy increased with field size (Chapter 4) supporting the suggestion that there is a tumour bed effect associated with photochemotherapy. Although increasing the field size increased tumour response, it, also, increased the morbidity related to treatment offering no therapeutic advantage.

In the clinical studies, tumour response decreased with increasing tumour size (Chapter 6). This may have been due to larger tumours containing more clonogenic cells or more probably the limited

penetration of light in the tissue. The data suggest that tumours greater than 1 cm in depth cannot be effectively treated with superficial PCT (photochemotherapy).

Interstitial light delivery may improve the therapeutic ratio of photochemotherapy by delivering the maximum light dose to the tumour rather than the overlying skin. None of the patients treated interstitially had a complete tumour response but neither did any of them develop skin necrosis (Chapter 6). Tumour growth delay increased with light dose and possibly larger doses of light would produce complete tumour response without skin necrosis, so improving the therapeutic ratio of photochemotherapy. In BD_9 rats, however, skin necrosis increased with tumour response after interstitial photochemotherapy (Chapter 4).

Light delivery using multiple implanted optical fibres may overcome the problem of limited penetration of light in tissue. Tumour response decreased with increasing tumour diameter and the data from the animal tumour study suggests that fibres should be about 10 mm apart for multiple fibre implants (Chapter 4). The presence of a plateau in the light dose response curve, also, suggests that the maximum dose of light that can be effectively delivered through a single cut optical fibre is about 200 J.

Patients with locally recurrent oral tumours responded well to superficial photochemotherapy (Chapter 6). These tumours usually involve the mucosal surface and absence of pigment in the mouth make cancer in the oral cavity and oropharynx particularly suitable for superficial treatment.

The only major side-effect of photochemotherapy is cutaneous photosensitivity. This limits the dose of photosensitizer used in patients. Patients were advised to stay out of direct sunlight for a month after treatment and no phototoxic reactions occurred. For three to six weeks after treatment, the skin of the back was sensitive to 5 Jcm^{-2} of white light which is equivalent to spending 2 minutes in bright sunlight. Exposed skin on the back of the hand showed no response to 20 Jcm^{-2} after 10 days suggesting that duration of photosensitivity in skin that is usually exposed to light may be shorter than in skin that is usually covered. If photopatch testing is to be used to advise patients about photosensitivity, it may be more appropriate to test skin that is usually exposed to light. The problem of cutaneous photosensitivity may be overcome by using photosensitizers that are not retained in skin or using locally applied drugs to treat superficial lesions.

The limited penetration of light in tissue may be advantageous in treating superficial tumours, for example carcinoma in-situ. In most clinical situations, however, it remains a major limiting factor in PCT. The use of photosensitizer, such as phthalocyanines, that are activated by longer wavelengths of light may increase the depth of tumour that can be treated. Increasing the applied light dose may, also, increase the depth of tumour that can be treated with superficial PCT but this may produce little therapeutic gain because of the likelihood of increasing normal tissue morbidity (Chapter 6). It would, also, be relatively inefficient because of the exponential decrease of light intensity in tissue. This problem may be overcome by the use of interstitial light delivery. It seems probable that in the future superficial PCT using Photofrin II and 630 nm light will only

be used to treat tumours with surface infiltration and less than 1 cm in maximum depth and that other tumours will be treated with interstitial PCT. The use of diffusing optical fibres to deliver light will hopefully further improve the homogeneity of light distribution throughout the tumour.

Appendix 1: Contents

Summary of patients treated with photochemotherapy who form the basis of the results reported in Chapters 5, 6 and 7.

Appendix 1

Patient 1

Age: 49 Sex: Male

Diagnosis Small cell carcinoma of the bronchus

May 1985 - Presented with signs of superior vena caval obstruction and bilateral supraclavicular lymphadenopathy. Found to have locally extensive small cell bronchial carcinoma. Treated with cytotoxic chemotherapy and radiotherapy .

September 1985 - In complete remission clinically.

May 1986 - Developed several enlarging subcutaneous nodules which were treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.0 $mgkg^{-1}$ (Total 84 mg)

Light Delivery: Superficial (See next page)

Interval between Drug and Light: 2 days

Outcome The patient died at home in September 1986

Appendix 1

Patient 1- Light Delivery Details

Initial Tumour Diameter (mm)	Diameter of Treated Area (cm)	Total Light Dose (Jcm^{-2})	Light Dose Rate ($mWcm^{-2}$)
18	4.0	25	119
5	2.5	25	153
9	3.0	75	156
12	3.5	75	156
17	4.0	75	119
9	3.0	50	156
20	4.0	50	120
15	3.5	100	156

Appendix 1

Patient 2

Age: 49 Sex: Male

Diagnosis Malignant melanoma of the scalp

March 1985 - Presented with two lesions on the scalp. Treated by a wide excision and a skin graft

Over the next 18 months, the patient developed several more subcutaneous tumour that were treated with local excision.

September 1986 - Noticed several more superficial nodules. On examination, 17 site of recurrent disease were identified which were treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose - 1.5 $mgkg^{-1}$ (Total 90 mg)

Light Delivery - Superficial (See next page)

Interval between Drug and Light - 3 days

Outcome The patient died of brain metastases in April 1987.

Appendix 1

Patient 2: Light Delivery Details

Initial Tumour Diameter (mm)	Diameter of Treated Area (cm)	Total Light Dose (Jcm^{-2})	Light Dose Rate ($mWcm^{-2}$)
*	5.0	50	61
16	3.5	75	125
13	3.5	50	125
12	3.5	25	125
7	2.5	25	169
12	3.0	50	163
5	2.5	50	169
6	2.5	25	169
o	4.0	50	103
5	2.5	25	169
5	2.5	75	169
●	3.0	25	170
6	2.5	75	169
7	2.5	50	169
5	2.5	50	169
6	2.5	25	169
7	2.5	50	169

* 3 nodules (14, 16 and 10 mm diameter) treated within the area
o 3 nodules (each 4 mm diameter) treated within the area
● 2 nodules (5 and 6 mm diameter) treated within the area

Appendix 1

Patient 3

Age: 57 Sex: Female

Diagnosis Squamous carcinoma of the oral cavity

February 1986 - Presented with pain in the left lower alveolus. Found to have a T_2 N_1 tumour of the lower alveolus. Treated with radical radiotherapy.

October 1986 - Developed multiple cutaneous tumour recurrences on the anterior chest wall. Treated with cytotoxic chemotherapy but no tumour response.

December 1986 - Also, developed cervical lymphadenopathy. This and the cutaneous nodules on the chest wall were treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 2.0 $mgkg^{-1}$ (Total 137.5 mg)

Light Delivery :Superficial (See below)

Interval between Drug and Light: 3 days

Outcome In January 1987, weekly methotrexate was started because of progressive disease outside areas treated with photochemotherapy with some tumour regression initially. The patient died in May 1987.

Appendix 1

Patient 3: Light Delivery Details

Initial Tumour Diameter (mm)	Diameter of Treated Area (cm)	Total Light Dose (Jcm^{-2})	Light Dose Rate ($mWcm^{-2}$)
25	6.0	50	41
7	2.5	75	163
6	3.0	25	156
8	3.0	50	170
5	2.5	75	163
11	3.0	50	156

Appendix 1

Patient 4

Age: 64 Sex: Female

Diagnosis Adenocarcinoma of the left breast

September 1984 - Presented with an ulcerated tumour of the left breast and axillary lymphadenopathy. Treated with a left mastectomy and axillary dissection and post-operative radiotherapy.

January 1986 - Developed recurrent tumour in the mastectomy flap. Started on Tamoxifen.

July 1986 - Tumour not responding, Tamoxifen stopped and Megestrol Acetate introduced.

November 1986 - Tumour continuing to grow, so patient treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 105 mg)

Light Delivery: Superficial

Beam Size - 7 cm diameter circle

Treatment Field - 7 x 5 cm ellipse

Light Dose - 50 Jcm^{-2}

Light Dose Rate - 33 $mWcm^{-2}$

Interval between Drug and Light: 3 days

Outcome In January 1987, the patient developed a paraparesis due to spinal cord compression caused by spinal bone metastases and despite treatment died in February 1987.

Appendix 1

Patient 5

Age: 84 Sex: Male

Diagnosis Squamous carcinoma of the pinna

1982 - Partial amputation of the right pinna.

August 1986 - Developed a recurrence in the right parotid lymph node which was treated by excision and post-operative radiotherapy.

December 1986 - Developed a recurrence in the parotid region. Again treated with radiotherapy but without tumour response.

January 1987 - Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 87 mg)

Light Delivery: Superficial

Field Size - 5 cm diameter circle

Light Dose - 40 Jcm^{-2}

Light Dose Rate - 57 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient developed fixed mid-cervical lymphadenopathy and lung metastases and died in August 1987.

Appendix 1

Patient 6

Age: 72 Sex: Male

Diagnosis Large Cell Anaplastic Carcinoma of the Bronchus

April 1986 - Presented with a cough, weight loss and hoarseness. Found to have a tumour in the right upper lobe associated with hilar, mediastinal and right supraclavicular lymphadenopathy. Treated with cytotoxic chemotherapy but no tumour response.

May 1986 - Palliative radiotherapy to the mediastinum and right supraclavicular fossa.

July 1986 - Developed cutaneous tumour infiltration over the right anterior chest wall. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 72 mg)

Light Delivery: Superficial

Field Sizes - 2 cm diameter circle to 4 x 4 cm squares

Light Dose - 17 to 50 Jcm^{-2}

Light Dose Rate - 40 to 163 $mWcm^{-2}$

Number of Sites Treated - 23

Interval between Drug and Light: 2 and 4 days.

Outcome The patient developed a chest infection and died 24 days after treatment.

Appendix 1

Patient 7

Age: 79 Sex: Female

Diagnosis Mucoepidermoid Carcinoma of the Parotid Gland

October 1985 - Developed pain in the left side of the face due to a fixed ulcerated mucoepidermoid tumour of the parotid gland. Treated with radiotherapy producing complete tumour regression.

June 1986 - Tumour started to regrow.

September 1986 - Tumour threatening to ulcerate again. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 123 mg)

Light Delivery: Superficial

Field Size - 4 cm diameter circle

Light Dose - 75 Jcm^{-2}

Light Dose Rate - 150 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient died of a stroke in January 1987.

Appendix 1

Patient 8

Age: 65 Sex: Male

Diagnosis Pancoast Tumour.

May 1985 - Presented with numbness in the fingers of the right hand and impending superior vena caval obstruction. Treated with radiotherapy .

November 1986 - Developed a fungating tumour mass on the anterior chest wall. Treated with radiotherapy.

December 1986 - No tumour regression. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 93 mg)

Light Delivery: Superficial

Field Size - 5 cm diameter circle

Light Dose - 50 Jcm^{-2}

Light Dose Rate - 53.5 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient died at home in May 1987.

Appendix 1

Patient 9

Age: 43 Sex: Female

Diagnosis Squamous Carcinoma of the Tongue

June 1986 - Presented with pain and difficulty moving the tongue. Found to have a T_4 N_3 carcinoma of the tongue. Treated with neo-adjuvant chemotherapy and radical radiotherapy.

September 1986 - Developed lymphadenopathy in the left posterior triangle of the neck. Treated with radiotherapy.

March 1987 - Tumour recurred in the soft palate causing pain and dysphagia. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 2.0 $mgkg^{-1}$ (Total 109 mg)

Light Delivery: Superficial

Field Size - 2.5 cm diameter circle

Light Dose - 40 Jcm^{-2}

Light Dose Rate - 150 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient developed further cervical lymphadenopathy and recurrence in the tongue and died in October 1987.

Appendix 1

Patient 10

Age: 58 Sex: Male

Diagnosis Carcinoma in situ of the retromolar trigone

July 1985 - Presented with progressive leukoplakia in the right retromolar trigone and a biopsy showed carcinoma in situ. Treated with radical radiotherapy with complete regression of disease.

February 1987 - Recurrent tumour in the right retromolar trigone. Again biopsy showed carcinoma in situ which was treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 105 mg)

Light Delivery: Superficial

Field Size - 3.0 cm diameter circle

Light Dose - 40 Jcm^{-2}

Light Dose Rate - 85 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome Patient developed further leukoplakia and then invasive carcinoma in the floor of mouth treated by radical surgery in February 1988. Patient alive and well (November 1989).

Appendix 1

Patient 11

Age: 78 Sex: Female

Diagnosis Squamous Carcinoma of the Tonsil

November 1986 - Presented with dysphagia due to a tonsillar tumour eroding the soft palate. Treated with radical radiotherapy with complete tumour response.

November 1987 - Developed a local recurrence in the soft palate causing dysphagia and pain in the ear.

December 1987 - Recurrence treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$

Light Delivery: Superficial

Field Size - 3.0 cm diameter circle

Light Dose - 50 Jcm^{-2}

Light Dose Rate - 150 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient developed lung metastases and died in Summer 1988.

Appendix 1

Patient 12

Age: 58 Sex: Female

Diagnosis Adenocarcinoma of the breast

1971 - Presented with a locally advanced carcinoma of the right breast treated with radiotherapy.

1980 - Left mastectomy for intra-duct carcinoma.

1982 to 1987 - Local recurrences of tumour in right breast treated with hormone manipulation and then cytotoxic chemotherapy.

November 1987 - Chest wall recurrence progressing again. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 78 mg)

Light Delivery: Interstitial

Light Dose - 100 - 250 J

Light Dose Rate - 100 mW

Number of Sites Treated - 5

(Further Details - Appendix 6.4)

Interval between Drug and Light: 3 days.

Outcome Further chest wall recurrence treated with radiotherapy March 1988.

Appendix 1

Patient 13

Age: 62 Sex: Male

Diagnosis Squamous Carcinoma of the Ethmoid Sinus

October 1982 - Presented with a locally advanced tumour of the ethmoid sinus treated with radiotherapy and adjuvant chemotherapy.

1984 to 1987 - Had several cervical lymph node recurrences treated with surgery, radiotherapy and cytotoxic chemotherapy.

October 1987 - Further cervical lymph node recurrence. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 109 mg)

Light Delivery: Interstitial

Light Dose - 125 - 175 J

Light Dose Rate - 200 mW

Number of Sites Treated - 5

(Further Details - Appendix 6.4)

Interval between Drug and Light: 3 days.

Outcome The patient died of brain metastases October 1988.

Appendix 1

Patient 14

Age: 69 Sex: Female

Diagnosis Adenocarcinoma of the breast

September 1985 - Right simple mastectomy.

November 1977 - Developed a mass in the right axilla. Treated with radiotherapy.

1981 to 1987 - Locally recurrent disease controlled by hormone manipulation and then cytotoxic chemotherapy.

December 1987 - Disease progressing again. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 65 mg)

Light Delivery: Interstitial

Light Dose - 300 J (75 J at 4 points in the tumour)
or 75 J (at centre of tumour, single point)

Light Dose Rate - 200 or 300 mW

Number of Sites Treated - 3

(Further details - Appendix 6.4)

Interval between Drug and Light: 3 days.

Outcome Patient developed further local recurrence which was treated with cytotoxic chemotherapy. Patient died of a chest infection in January 1989.

Appendix 1

Patient 15

Age: 50 Sex: Female

Diagnosis Adenocarcinoma of the breast

February 1983 - Presented with a locally advanced carcinoma of the right breast. Treated with radical radiotherapy and Tamoxifen.

December 1986 - Developed a local recurrence. Treated with a mastectomy and Aminoglutethamide.

July 1987 - Chest wall recurrence treated with local excision.

January 1987 - Further chest wall recurrence. Treated with photochemotherapy.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 135 mg)

Light Delivery: Interstitial

Light Dose - 200 J

Light Dose Rate - 100 mW

Number of Sites Treated - 6

(Further Details - Appendix 6.4)

Interval between Drug and Light: 3 days.

Outcome - Developed further chest wall recurrence and then lung metastases. The patient was still receiving chemotherapy in January 1989.

Appendix 1

Patient 16

Age: 76 Sex: Female

Diagnosis Bowen's Disease of the perineum

November 1984 - Locally recurrent Bowen's disease, previously treated with local excision and skin grafting, treated with radiotherapy producing complete tumour response.

March 1985 - Further local recurrence treated with radiotherapy again.

August 1986 - Disease recurred locally and was treated with Fluorouracil cream which produced severe skin irritation.

December 1986 - Referred for photochemotherapy. Patient had extensive disease involving the perineal region and the skin of both buttocks. Only the right buttock was treated.

Photochemotherapy

Photofrin II Dose: 1.5 $mgkg^{-1}$ (Total 88 mg)

Light Delivery: Superficial

Beam Size - 8.0 cm circle

Treatment Field - 7.5 cm diameter semi-circle

Light Dose - 30 Jcm^{-2}

Light Dose Rate - 18 $mWcm^{-2}$

Interval between Drug and Light: 3 days.

Outcome The patient was alive but had persistent Bowen's disease disease in November 1989.

Appendix 2.1

Day	Mean Tumour Diameter ± 1 S.E. (mm)			Mean Diameter ± 1 S.E.M. (mm)
	Transplant Generations			
	11 & 12th	20th & 21st	29 & 30th	All Tumours
T	9.7 ± 0.9	9.0 ± 0.4	8.7 ± 0.6	9.0 ± 0.1
1	11.1 ± 1.2	10.4 ± 1.0	9.9 ± 1.1	10.3 ± 0.1
2	13.0 ± 2.4	11.8 ± 1.1	12.2 ± 1.3	12.0 ± 0.2
3			13.6 ± 1.4	13.0 ± 0.2
4		13.5 ± 1.9	15.9 ± 1.6	14.6 ± 0.2
5		14.9 ± 2.2		15.8 ± 0.7
6	17.5 ± 3.8	16.3 ± 2.0	18.8 ± 2.0	17.2 ± 0.3
7	19.1 ± 3.4	17.5 ± 2.3	20.4 ± 1.9	18.8 ± 0.3
8	20.6 ± 3.3	19.0 ± 1.9	22.3 ± 1.7	20.2 ± 0.3
9	22.0 ± 3.6	20.0 ± 1.4	24.0 ± 2.0	21.8 ± 0.3
10	23.3 ± 3.7	21.9 ± 1.6	25.6 ± 2.5	23.1 ± 0.3
11	24.8 ± 2.9	22.9 ± 1.4		24.4 ± 0.4
12		24.4 ± 1.5		25.2 ± 0.4
13				26.4 ± 0.4
14	27.4 ± 1.8			27.6 ± 0.5
No. of Animals	6	10	13	74

Growth of untreated $LSBD_1$ tumours (T= 8-10 mm) from eleventh and twelfth, twentieth and twenty first, twenty ninth and thirtieth and all transplant generations.

Appendix 2.2

Day	Mean Tumour Diameter ± 1 S.E. (mm)				
	Dose of Cyclophosphamide ($mgkg^{-1}$)				
	25	50	100	150	200
T	9.3 ± 1.0	9.9 ± 0.9	9.2 ± 1.0	9.2 ± 0.6	9.6 ± 0.8
1		10.4 ± 1.9		11.0 ± 1.3	
2				12.0 ± 2.0	
3				12.8 ± 2.5	
4	13.3 ± 1.3			12.1 ± 2.1	
5	12.4 ± 2.3				9.4 ± 1.9
6	12.6 ± 2.9	10.6 ± 2.7	9.7 ± 1.4	9.6 ± 0.7	9.4 ± 2.2
7	13.2 ± 2.7	10.9 ± 3.0	9.0 ± 1.6	8.9 ± 0.8	9.1 ± 2.3
8		11.6 ± 4.0		8.6 ± 0.5	
10	16.5 ± 4.0			8.8 ± 0.6	
11	17.1 ± 3.8			8.9 ± 1.1	
12	18.5 ± 3.8	14.6 ± 4.5	10.4 ± 1.2		8.6 ± 2.0
13	20.0 ± 3.9	15.9 ± 4.8		8.6 ± 1.4	
14	22.5 ± 3.5	17.4 ± 5.5	12.9 ± 1.5	8.5 ± 1.5	10.4 ± 1.6
15		19.5 ± 6.0		9.6 ± 1.6	11.6 ± 2.3
16		21.3 ± 6.1		10.5 ± 2.3	
17	26.3 ± 1.8			11.6 ± 3.0	
18	28.3 ± 2.1			13.1 ± 3.6	
19		26.5 ± 6.5	18.7 ± 2.4		17.8 ± 4.6
20		28.0 ± 5.6	20.9 ± 2.6	15.7 ± 4.8	19.8 ± 5.3
21			22.1 ± 3.5	18.4 ± 4.7	20.3 ± 4.7
22			23.9 ± 2.5	20.3 ± 5.6	22.3 ± 6.5
24			26.2 ± 3.3		
25			28.3 ± 4.0		
27				25.0 5.0	
28				26.6 4.7	
29				28.1 4.4	
30				29.2 4.1	
No of animals	6	6	6	5	4

Response of $LSBD_1$ (T= 8-10 mm) to a single intraperitoneal injection of cyclophosphamide.

Appendix 2.3

Drug Dose ($mgkg^{-1}$)	Growth Delay ± 1SE (days)	Regrowth Delay ± 1SE (days)	No. Animals with > 10% Weight Loss	Treatment Related Deaths
25	4.3 ± 0.7	11.0 ± 3.2	0/6	0/6
50	7.3 ± 1.6	11.2 ± 1.6	0/6	0/6
100	11.5 ± 1.1	11.3 ± 2.5	3/6	0/6
150	14.1 ± 1.6	10.4 ± 1.7	4/5	1/6
200	15.6 ± 4.8	15.0 ± 6.7	4/4	2/6

Response of $LSBD_1$ (T= 8-10 mm) to a single intraperitoneal dose of cyclophosphamide.

Appendix 2.4

Day	Mean Tumour Diameter ± 1 S.E. (mm)			
	Dose of Gamma Irradiation (Gy)			
	5	10	20	30
T	8.8 ± 0.9	8.9 ± 0.6	8.5 ± 0.50	9.2 ± 0.7
1	10.6 ± 1.7	10.0 ± 1.5	9.1 ± 0.50	11.1 ± 1.0
2			10.3 ± 1.16	
3	12.7 ± 1.5			11.2 ± 1.4
4	13.2 ± 1.6	10.9 ± 1.9		10.6 ± 2.0
5		11.2 ± 1.4	9.4 ± 0.88	
6	14.4 ± 1.3		9.3 ± 0.66	9.9 ± 1.5
7	15.7 ± 1.5	10.3 ± 1.6	9.3 ± 1.07	10.4 ± 1.3
8	17.1 ± 2.1	10.5 ± 1.6	10.2 ± 1.17	10.0 ± 1.4
9				9.5 ± 0.8
10	19.2 ± 2.2			9.9 ± 0.7
11	20.0 ± 2.4	12.3 ± 1.8	9.4 ± 0.8	10.0 ± 1.3
12		13.3 ± 1.7	9.1 ± 0.7	10.0 ± 2.2
13	22.3 ± 1.8	14.1 ± 1.6	9.1 ± 0.91	8.9 ± 0.8
14	24.4 ± 2.2		8.4 ± 1.3	8.5 ± 1.2
15			8.4 ± 0.9	8.9 ± 1.3
16		18.3 ± 3.5	9.3 ± 2.0	9.6 ± 1.9
17	26.6 ± 2.6			9.7 ± 1.5
18	27.7 ± 2.1	20.9 ± 2.4		
19	28.9 ± 2.0		10.8 ± 1.8	
20		22.8 ± 1.9	11.7 ± 2.7	9.5 ± 1.8
21		23.7 ± 1.9	13.0 ± 3.0	9.5 ± 2.0
22		24.3 ± 2.2	14.5 ± 4.0	10.0 ± 2.0
23				10.3 ± 2.2
24				10.7 ± 3.1
25		26.2 ± 2.6		11.8 ± 3.3
26		28.2 ± 2.7	19.1 ± 2.0	11.4 ± 3.9
27			19.9 ± 2.5	12.2 ± 4.0
28			20.8 ± 2.1	12.5 ± 5.0
29			21.1 ± 2.1	13.4 ± 4.8
30				13.6 ± 4.5
31				14.2 ± 4.6
33			23.9 ± 1.8	15.2 ± 4.6
34			24.7 ± 2.4	15.0 ± 5.6
35			24.9 ± 2.9	16.0 ± 4.8
36				16.4 ± 4.5
41				19.0 ± 4.5
42				19.6 ± 4.7
43				20.2 ± 0.5

Growth of $LSBD_1$ (T= 8-10 mm) after a single dose of gamma irradiation (n= 6).

Appendix 2.5

Dose of Radiation (Gy)	Growth Delay ± 1SE (days)	Regrowth Delay ± 1SE (days)	No. Animals with > 10% Weight Loss	Treatment Related Deaths
5	3.1 ± 0.8	12.0 ± 2.2	0/6	0/6
10	11.5 ± 1.2	13.6 ± 1.3	0/6	0/6
20	24.1 ± 2.1	19.2 ± 3.2	0/6	0/6
30	50.5 ± 6.7	35.7 ± 2.4	2/6	0/6

Response of $LSBD_1$ (T= 8-10 mm) to a single dose of gamma irradiation.

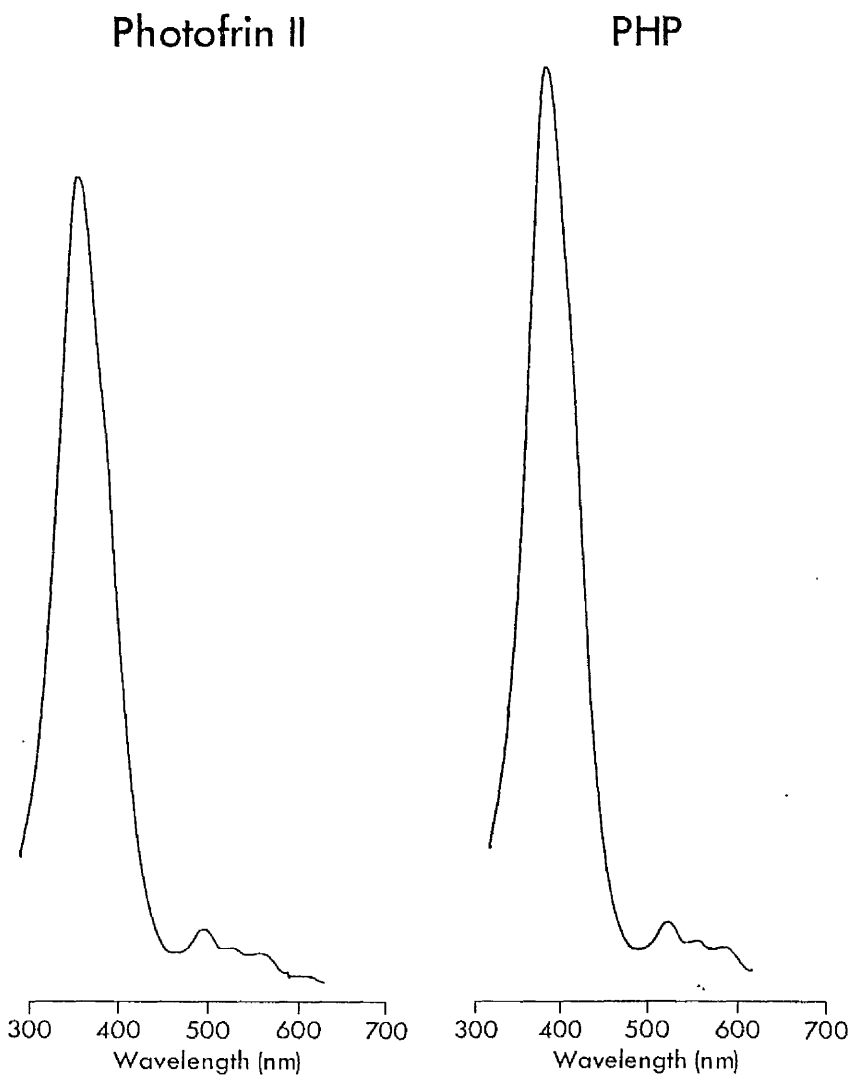
Appendix 3.1

Method used by the Department of Biochemistry, University of Leeds, to produce polyhaematoporphyrin

Haematoporphyrin derivative was manufactured as described by Lipson, Baldes and Olsen (1961). Diluted HPD was recirculated through a molecular sieve (Amicon Ltd, Gloucester.) with a molecular weight cut off of 10,000 to concentrate the solution and to remove any products with a molecular weight less than 10,000. The concentration of the residual solution was determined by spectrofluorimetry (Kontron SFM, Kontron, Watford; excitation set at 400 nm and emission at 640 nm) and then diluted with normal saline to produce a total porphyrin concentration of 2.5 $mgml^{-1}$.

The absorpton spectra of Photofrin II and PHP are the same (Appendix Figure 3.1).

Appendix Figure 3.1



Absorption spectra of Photofrin II and PHP.

Appendix 3.2

Method used by the Department of Biochemistry, University of Leeds, to measure total porphyrin levels

For plasma samples, a 200 µl aliquot was diluted to 1 ml with 50 mM Hepes pH 7.4 containing 10 mM cetyltrimethylammonium bromide (CTAB) buffer solution. For tumour, skin and muscle, each sample was weighed, homogenized in 5 ml of Hepes buffer solution and a 1 ml aliquot of the homogenate removed into a clean test tube.

The 1 ml diluted plasma or homogenized tissue samples was then shaken vigorously with a 5 ml mixture (4:1 v/v) of ethyl acetate and glacial acetic acid. The mixture was centrifuged for 5 min at 500 xG to improve separation of the two phases and to remove precipitated protein. The upper organic supernant was extracted with 4 ml of 0.5 N hydrochloric acid and the mixture centrifuged again to separate the two phases. The lower acid phase (approximately 6 ml) was heated for 90 min at 80 °C to hydrolyse the poorly fluorescing aggregates to the more fluorescent monomeric porphyrins, mainly haematoporphyrin and hydroxylethylvinyl deuteroporphyrin (HVD). The relative fluorescence was measured using a spectrofluorimeter (Kontron SFM) and compared to a standard haematoporphyrin solution (50 $ngml^{-1}$). The assay was performed in duplicate for each sample.

Appendix 3.3

Time (h)	Total Porphyrin Level ± 1 S.D.				n
	Plasma ($\mu g ml^{-1}$)	Tumour ($ng mg^{-1}$)	Muscle ($ng mg^{-1}$)	Skin ($ng mg^{-1}$)	
0.5	3.77±2.08	1.07±0.58	25.78±7.10	0.87±0.31	6
1	7.51±5.24	2.46±1.43	28.25±14.84	0.58±0.41	3
3	8.48±2.77	2.43±0.88	33.69±10.57	2.79±1.60	5
6	15.27±7.66	4.63±2.05	37.51±19.38	1.97±0.67	5
24	12.19±5.64	4.41±2.54	16.11±4.59	4.24±0.60	*
72	3.23±1.12	3.07±0.57	11.20±1.93	2.63±0.75	5
168	1.82±0.36	1.08±0.57	7.01±3.36	2.73±1.06	6

Variation in total porphyrin level with time after an intraperitoneal injection of 20 $mg kg^{-1}$ of PHP in $LSBD_1$ (T= 8-10 mm) bearing BD_9 rats. (n = number of animals)

* Plasma 9 animals
  Tumour 12 animals
  Skin and Muscle 5 animals

Appendix 3.4

Time (h)	Ratio of Total Porphyrin Levels ± 1 S.D.				No. of Animals
	Tumour: Muscle	Tumour: Skin	Tumour: Plasma	Skin: Plasma	
0.5	0.04±0.03	1.10±0.20	0.30±0.21	0.23±0.09	5
1	0.06±0.02	4.86±4.07	0.46±0.35	0.10±0.07	3
3	0.20±0.24	1.11±0.59	0.32±0.17	0.34±0.14	5
6	0.14±0.06	2.52±0.87	0.35±0.16	0.15±0.11	5
24	0.55±0.60	1.63±1.28	0.60±0.32	0.56±0.29	5
72	0.28±0.08	1.25±0.49	1.02±0.34	0.83±0.14	5
168	0.20±0.20	0.38±0.16	0.59±0.34	1.55±0.73	6

Variation in ratio of total porphyrin levels in tumour, plasma, muscle and skin with time after an intraperitoneal injection of 20 $mgkg^{-1}$ of PHP in $LSBD_1$ (T= 8-10 mm) bearing BD_9 rats.

(All tissue levels in $ngmg^{-1}$, plasma level in μgml^{-1})

Appendix 3.5

Dose of PHP ($mgkg^{-1}$)	Total Porphyrin Level $\pm$ 1 S.D. ($ngmg^{-1}$)	Number of Animals
0	0.10 $\pm$ 0.01	5
0.5	0.47 $\pm$ 0.24	5
2.0	0.67 $\pm$ 0.37	4
10.0	3.37 $\pm$ 0.70	5
20.0	4.41 $\pm$ 2.54	12
40.0	17.12 $\pm$ 1.69	4

Total porphyrin level in $LSBD_1$ (T= 8-10 mm) 24 h after an intraperitoneal injection of PHP.

Appendix 3.6

Tumour size (mm)	Total Porphyrin Level ± 1SD ($ngmg^{-1}$)	Number of Animals
8-10	4.41 ± 2.54	12
10-12	2.87 ± 1.07	5
12-14	3.94 ± 0.60	5
14-16	4.00 ± 1.92	5
Mean	3.96 ± 1.96	27

Total porphyrin level in $LSBD_1$ tumour 24 h after an intraperitoneal injection of 20 $mgkg^{-1}$ of PHP.

Appendix 4.1

Day	Mean Tumour Diameter ± 1 S.E. (mm)		
	Photofrin II only	Superficial Light Alone	Interstitial Light Alone
T	8.9 ± 0.4	8.3 ± 0.3	8.7 ± 0.4
1	10.0 ± 1.0	9.2 ± 0.6	10.0 ± 1.0
2	11.9 ± 1.3	10 4 ± 0 9	11.2 ± 1.4
3	13 1 ± 1.3	11.5 ± 0.6	12.5 ± 2.1
4	14.8 ± 1.6	13.5 ± 1.0	
5	16.2 ± 2.1	15.7 ± 1.9	16.1 ± 2.7
6	17.8 ± 2.3	16.9 ± 1.4	16.9 ± 2.8
7	19.7 ± 2.5	19.1 ± 0.6	19.2 ± 3.1
8	21.0 ± 2.2	19.8 ± 1.1	18.6 ± 3.5
9	22.2 ± 1.7	21.3 ± 1.6	19.3 ± 3.6
10	23.2 ± 2.5	22.6 ± 1.5	20.5 ± 4.1
11		23 9 ± 1.7	
12	25.7 ± 3.2	25.4 ± 2.1	22.9 ± 5.0
13	26.8 ± 3.6	26.8 ± 2.8	24.3 ± 4.8
14			25.6 ± 4.8
No. of animals	15	6	6

Effect of control treatments on $LSBD_1$ (T= 8-10 mm), 20 $mgkg^{-1}$ Photofrin II, 400 Jcm^{-2} superficial light or 400 J (at 300mW) interstitial light.

Appendix 4.2

Day	Mean Tumour Diameter ± 1 S.E.			
	P II + 150 Jcm^{-2}	PHP + 150 Jcm^{-2}	P II + 200 J	PHP + 200 J
T	8.6 ± 0.3	8.3 ± 0.3	10.5 ± 0.5	10.7 ± 0.4
1	9.1 ± 0.1	9.2 ± 0.6	11.3 ± 0.5	11.4 ± 0.8
2	14.3 ± 1.1		13.9 ± 1.5	
3		13.8 ± 1.3		13.8 ± 0.9
4			12.6 ± 1.6	
5		11.3 ± 1.5		12.2 ± 0.9
6	12.1 ± 1.2	12.2 ± 1.5		13.0 ± 1.6
7	12.4 ± 1.2	13.4 ± 2.5	12.4 ± 2.7	14.4 ± 2.1
8	13.0 ± 1.3	13.8 ± 2.8		15.5 ± 2.3
9			13.5 ± 3.5	15.9 ± 2.1
10		16.8 ± 2.7	14.5 ± 3.9	
11	16.5 ± 3.7	17.4 ± 3.6	15.6 ± 3.9	18.5 ± 2.5
12	17.9 ± 4.1	18.9 ± 4.4		19.4 ± 2.6
13	19.4 ± 3.5	19.0 ± 4.0		20.3 ± 2.9
14	21.1 ± 3.4	20.9 ± 3.3	19.1 ± 4.3	21.7 ± 3.1
15	22.0 ± 3.4	22.5 ± 3.5		23.1 ± 2.7
16		23.4 ± 3.2	21.5 ± 4.0	24.4 ± 3.0
17			23.2 ± 4.8	
18	25.6 ± 3.4			25.8 ± 2.8

Response of $LSBD_1$ to:-

Superficial photochemotherapy (T= 8-10 mm, 150 Jcm^{-2} light to a 2 cm diameter treatment field) -
Photofrin II (P II) + light from the Argon/dye laser
PHP + light from the Copper/vapour laser.

Interstitial photochemotherapy (T= 10-12 mm, 200 J light at 300 mW) -
Photofrin II + light from the Argon/dye laser
PHP + light from the Copper/vapour laser.

(Photosensitizer dose 20 $mgkg^{-1}$, n= 6)

Appendix 4.3

Day	Mean Tumour Diameter ± 1 S.E. (mm)		
	PHP + 100 Jcm^{-2}	P II + 150 Jcm^{-2}	PHP + 250 Jcm^{-2}
T	8.6 ± 0.5	8.2 ± 0.1	8.3 ± 0.5
1	9.3 ± 0.4	8.8 ± 0.3	9.2 ± 0.6
2	13.8 ± 1.6	15.5 ± 0.7	13.8 ± 0.8
3		15.1 ± 0.3	
4		13.6 ± 0.5	
5		12.4 ± 1.5	12.1 ± 0.6
6	14.9 ± 0.6	11.9 ± 1.6	12.7 ± 1.2
7	16.2 ± 1.9	12.4 ± 1.0	12.4 ± 1.4
8	17.9 ± 1.7		13.9 ± 2.0
9	18.6 ± 2.2	15.2 ± 0.9	14.2 ± 2.2
10	20.0 ± 2.1	17.3 ± 1.1	
11	21.7 ± 2.2	18.3 ± 0.8	
12	23.7 ± 2.3		17.1 ± 3.3
13	25.4 ± 2.2	20.8 ± 1.6	19.0 ± 2.4
14	26.7 ± 1.0	21.7 ± 1.7	19.7 ± 2.6
15		23.6 ± 0.7	21.1 ± 2.3
16		24.9 ± 1.3	22.3 ± 2.3
17		27.4 ± 2.2	
18			25.3 ± 1.9

Response of $LSBD_1$ (T= 8-10 mm) to superficial photochemotherapy with 20 $mgkg^{-1}$ PHP or Photofrin II (P II) and a 1.5 cm diameter treatment field. (n= 6)

Appendix 4.4

Light Dose (Jcm^{-2})	Field Size (cm)	Tumour Growth Delay ± 1SE (days)	Incidence of Eschar Formation	Incidence of Significant Weight Loss	Treatment Related Deaths
100°	1.5	1.3 ± 0.6	1/6	0/6	0/6
150°	1.5	4.6 ± 0.8	4/6	0/6	0/6
200°	1.5	6.3 ± 1.8	4/6	0/6	0/6
250°	1.5	5.0 ± 0.8	5/6	0/6	0/6
300°	1.5	3.8 ± 0.8	6/6	0/6	0/6
400°	1.5	5.0 ± 1.2	4/5	0/6	0/6
400*	1.5	0.5 ± 0.7	0/6	0/6	0/6
150●	2.0	5.6 ± 1.1	3/6	2/6	0/6
200●	2.0				2/2
100●	3.0	2.1 ± 1.2	0/8	0/8	0/8
150●	3.0	9.3 ± 2.4	4/6	0/6	0/6
200●	3.0				2/2

Response of $LSBD_1$ (T= 8-10 mm) bearing BD_9 rats to superficial photochemotherapy.

* Light only, no photosensitizer
° 20 $mgkg^{-1}$ PHP
● 20 $mgkg^{-1}$ Photofrin II

Appendix 4.5

Day	Mean Tumour Diameter ± 1 S.E. (mm)		
	Field Size Diameter (cm)		
	1.5	2.0	3.0
T	8.2 ± 0.2	8.6 ± 0.3	8.6 ± 0.4
1	8.8 ± 0.3	9.1 ± 0.1	9.2 ± 0.4
2	15.5 ± 0.7	14.3 ± 1.1	
3	15.1 ± 0.7	13.8 ± 1.3	
4	13.6 ± 1.2		
5	12.4 ± 1.5		11.1 ± 1.5
6	11.9 ± 1.6	12.1 ± 1.2	11.4 ± 1.7
7	12.4 ± 1.0	12.4 ± 1.2	11.7 ± 2.6
8		13.0 ± 1.3	
9	15.2 ± 0.9		
10	17.3 ± 1.1		12.4 ± 3.6
11	18.3 ± 0.8	16.5 ± 3.7	
12		17.9 ± 4.1	16.0 ± 5.7
13	20.8 ± 1.6	19.4 ± 3.5	
14	21.7 ± 1.7	21.1 ± 3.4	
15	23.6 ± 0.7	22.0 ± 3.4	18.4 ± 6.6
16	24.9 ± 1.3		19.6 ± 6.2
17	27.4 ± 2.2		20.4 ± 5.9
18		25.6 ± 3.4	21.7 ± 5.9
19			23.3 ± 5.6
20			24.4 ± 5.9
21			26.0 ± 6.5

Response of $LSBD_1$ (T= 8-10 mm) to superficial photochemotherapy with 20 $mgkg^{-1}$ of Photofrin II and 150 J cm^{-2} of light. (n= 6)

Appendix 4.6

Day	Mean Tumour Diameter ± 1 S.E. (mm)	
	PHP + 100J	PHP + 400J
T	8.6 ± 0.6	8.3 ± 0.3
1	9.7 ± 0.4	9.1 ± 0.5
2	12.5 ± 1.3	13.7 ± 1.3
3	12.3 ± 0.7	
4	12.7 ± 0.9	
5	13.2 ± 1.5	11.2 ± 2.6
6	14.1 ± 1.5	11.4 ± 2.6
7	15.2 ± 1.4	12.1 ± 3.4
8	17.4 ± 2.9	11.9 ± 3.7
11	22.8 ± 3.3	13.3 ± 5.2
12	23.3 ± 3.6	14.6 ± 5.2
13	24.2 ± 3.8	14.9 ± 5.8
14	25.2 ± 3.7	17.0 ± 7.4
15		19.1 ± 8.6
19		18.3 ± 5.8
20		21.5 ± 6.5

Response of $LSBD_1$ (T= 8-10 mm) to interstitial photochemotherapy with 20 $mgkg^{-1}$ of PHP and 100 or 400 J of light at 300 mW. (n= 6)

Appendix 4.7

Day	Mean Tumour Diameter ± 1 S.E. (mm)	
	Photofrin II + 200 J (300 mW)	Photofrin II + 200 J (100 mW)
T	10.5 ± 0.5	10.6 ± 0.4
1	11.3 ± 0.5	11.8 ± 0.3
2	13.9 ± 1.5	16.5 ± 0.7
3	13.8 ± 0.9	15.5 ± 0.4
4	12.6 ± 1.6	
5		13.5 ± 0.5
6		13.3 ± 0.9
7	12.4 ± 2.7	
9	13.5 ± 3.5	
10	14.5 ± 3.9	14.7 ± 2.7
11	15.6 ± 3.9	15.6 ± 2.6
12		16.1 ± 2.6
13		16.6 ± 2.9
14	19.1 ± 4.3	17.7 ± 3.1
16	21.5 ± 4.0	21.0 ± 3.6
17	23.2 ± 4.8	
19		24.9 ± 4.8
20		25.9 ± 4.5

Response of $LSBD_1$ (T= 10-12 mm) to interstitial photochemotherapy with 20 $mgkg^{-1}$ Photofrin II and 200J of light at a dose rate of 300 or 100 mW. (n= 6)

Appendix 4.8

T (mm)	Light Dose (J)	Tumour Growth Delay ± 1 S.E. (days)	Incidence of Eschar Formation
8-10^{o}	100	2.1 ± 1.2	2/6
8-10^{o}	150	5.3 ± 1.1	3/6
8-10^{o}	200	6.3 ± 1.5	2/6
8-10^{o}	200^{+}	4.5 ± 1.0	0/5
8-10^{o}	300	12.6 ± 7.2	3/6
8-10^{o}	400	8.5 ± 2.5	4/6
8-10*	400	1.6 ± 1.3	0/6
10-12$^{\bullet}$	100	5.9 ± 1.0	0/6
10-12$^{\bullet}$	150	6.1 ± 1.1	2/6
10-12$^{\bullet}$	200	8.5 ± 1.4	0/6
10-12$^{\bullet}$	200^{+}	9.3 ± 1.5	0/6
10-12$^{\bullet}$	300	7.7 ± 1.4	5/6
10-12$^{\bullet}$	400	4.8 ± 1.7	4/6
10-12*	200	2.6 ± 0.5	0/6
12-14^{o}	200	1.0 ± 0.5	0/6
15-16^{o}	200	2.0 ± 0.8	0/6

Response of $LSBD_1$ to interstitial photochemotherapy, light dose rate 300 mW or 100 mW (+). Photosensitizer:-

* None
- ● 20 $mgkg^{-1}$ Photofrin II
- o 20 $mgkg^{-1}$ PHP.

There were no treatment related deaths and none of the animals suffered significant weight loss.

Appendix 4.9

Day	Tumour Diameter for Individual Animals (mm)					
	1	2	3	4	5	6
T	9.0	8.0	8.0	8.7	9.3	9.0
1	9.7	8.6	9.3	10.0	10.3	9.3
2	12.0	12.0	13.6	13.0	11.7	12.3
3	13.0	9.3	13.6	13.0	11.7	12.3
4	11.0	9.0	11.0	11.0	13.3	12.0
5					14.0	12.3
6					15.6	10.7
7	12.0	9.0	12.3	15.5	15.8	13.0
8	14.3	8.0	13.0	19.7	16.0	12.8
9	15.6	8.0	14.0	18.0		
11					25.3	12.3
12					26.7	11.1
13	19.6	5.0	19.3	30.0	28.6	9.0
14	21.3	4.0	22.3	31.3	29.3	8.6
15	22.3	p	25.1	32.0	30.6	10.5
16	24.1	Nil	24.0			
17	25.5	Nil	25.7			
18	26.9	Nil	27.2			8.0
20		Nil				7.7
24		p				Nil
28		16.3				Nil
31		20.0				Nil
36		26.5				Nil
40						Nil
45						9.3
49						13.6
56						22.3

Response of individual $LSBD_1$ tumours (T= 8-10 mm) to 20 $mgkg^{-1}$ of PHP and 300 J of light (300 mW).

p = Palpable tumour 3 mm or less in diameter.

Appendix 4.10

Tumour Diameter (mm)	Tumour Volume (mm^3)	Tumour Growth Delay (days)
8-10	380	6.3 ± 1.5
10-12*	700	8.5 ± 1.4
12-14	1150	1.0 ± 0.5
14-16	1770	2.0 ± 0.8

Influence of tumour size (T) on the response of $LSBD_1$ to interstitial photochemotherapy with 20 $mgkg^{-1}$ PHP or Photofrin II (*) and 200 J of light at 300 mW. (n= 6)

Appendix 4.11

Treatment	Temperature at End of Treatment (°C)	Tumour Growth Delay (days)
200 J only (300 mW)	37.0	2.2
	37.5	2.2
	35.5	1.2
	39.5	4.2
	40.3	2.2
	36.0	3.2
P II + 200 J (300 mW)	41.0	2.2
	46.0	12.2
	43.5	11.2
	39.5	8.2
	40.0	6.2
	36.5	9.2
P II + 200 J (100 mW)	35.0	9.2
	32.5	11.2
	35.0	9.2
	34.0	12.2
	32.0	4.3
P II + 300 J (300 mW)	41.5	9.2
	39.0	4.2
	39.5	9.2
	40.0	6.2
	37.0	6.2
	42.0	11.2

Tumour growth delay and temperature of the skin overlying $LSBD_1$ tumours (T= 10-12 mm) in individual animals after light alone (200 J_1 at 300 mW) or after interstitial photochemotherapy using 20 $mgkg^{-1}$ Photofrin II (P II) and light (200 J at 300mW, 200 J at 100 mW or 300 J at 300 mW).

Appendix 5.1

Patient 1 (Dose* 1.0 $mgkg^{-1}$)										
Porphyrin level	6690	3218	2578	1602	1186	850	450	402	146	178
Time	1	18	24	42	66	90	165	217	361	525
Patient 2 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	7615	6062	1834	1006	489	440	91	50	24	
Time	0.5	3.3	22	44	68	175	255	538	730	
Patient 3 (Dose 2.0 $mgkg^{-1}$)										
Porphyrin level	5130	4004	1406	684	516	487	165	97	135	132
Time	0.8	3.25	24	50	78	119	167	288	436	504
Porphyrin level	47									
Time	792									
Patient 4 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	5923	4022	1854	553	398	287	350	217	107	169
Time	0.66	2.75	18	43	71	89	162	211	260	357
Porphyrin level	79	97	52	33						
Time	431	556	1252	1422						
Patient 5 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	6215	5379	1426	904	634	475	211	80	18	
Time	0.75	3.33	25	47	74	93	264	696	1608	
Patient 6 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	9605	4640	2253	1539	1053	920	666	536		
Time	0.5	5	21	28	45	76	99	170		
Patient 13 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	15407		9000	3240	2331	1190	579	501		
Time	0.83		5.67	26	49	96	237	453		
Patient 14 (Dose 1.5 $mgkg^{-1}$)										
Porphyrin level	17884		4327	3064	1478	1730	1298	151	144	
Time	0.33		23	49	82	115	211	787	1122	

Plasma total porphyrin levels ($ngml^{-1}$) after Photofrin II injection (Time in h).

* Dose = Dose of Photofrin II

Appendix 5.2

Patient	Weight (kg)	Photofrin II Dose ($mgkg^{-1}$)	$\alpha \pm 1$ S.D. (h^{-1})	$\beta \pm 1$ S.D. (h^{-1})
1	84	1.0	0.056 ± 0.007	0.0055 ± 0.0009
2	60	1.5	0.087 ± 0.015	0.0070 ± 0.0021
3	69	2.0	0.064 ± 0.013	0.0042 ± 0.0015
4	70	1.5	0.073 ± 0.011	0.0029 ± 0.0010
5	58	1.5	0.077 ± 0.009	0.0040 ± 0.0009
6	48	1.5	0.213 ± 0.051	0.0099 ± 0.0028
13	55	1.5	0.116 ± 0.036	0.0055 ± 0.0021
14	43	1.5	0 080 ± 0.014	0.0033 ± 0.0009

Calculated values of the exponents for the porphyrin distribution (α) and elimination (β) curves in patients treated with Photofrin II.

Appendix 5.3

Patient	Photofrin II Dose ($mgkg^{-1}$)	α Half-life (95% C.I.*) (h)	β Half-life (95% C.I.) (h)
1	1.0	12.5 (9.1-17.8)	126 (89-218)
2	1.5	8.4 (5.7-15.7)	99 (56-425)
3	2.0	10.9 (7.0-20.0)	165 (89-1219)
4	1.5	9.5 (7.1-14.0)	243 (135-1194)
5	1.5	9.0 (7.0-12.8)	174 (108-441)
6	1.5	3.3 (2.0-9.7)	70 (39-349)
13	1.5	6.0 (3.0-765.1)	127 (57--617)
14	1.5	8.7 (5.8-17.1)	211 (123-743)
Mean $\pm$ 1SD		8.5 $\pm$ 2.8	152 $\pm$ 58

Elimination (α) and distribution (β) half-lives of porphyrin in the plasma of patients treated with Photofrin II.

(*C.I. = Confidence Interval)

Appendix 6.1

Dose of Light (Jcm^{-2})	Dose of Photofrin II ($mgkg^{-1}$)		
	1.0	1.5	2.0
25	0/2	1/6	1/1
50	0/2	6/10	2/3
75	0/3	4/4	2/2
100	0/1		

Number of sites showing complete tumour response expressed as a fraction of the number of sites receiving the same dose of Photofrin II and superficial light.

Appendix 6.2

Patient	Tumour Depth (mm)	Light Dose (Jcm^{-2}) 25	50	75	100	Total
1	<5.0	0/1	-	-	-	0/1
	5.1-10.0	-	0/1	0/1	-	0/2
	10.1-15.0	-	-	0/1	0/1	0/2
	>15.1	0/1	0/1	0/1	-	0/3
2	<5.0	1/2	4/4	1/1	-	6/7
	5.1-10.0	0/4	3/4	1/1	-	4/9
	10.1-15.0	0/1	1/3	-	-	1/4
	>15.1	-	0/1	1/1	-	1/2
3	<5.0	-	-	1/1	-	1/1
	5.1-10.0	1/1	1/1	1/1	-	3/3
	10.1-15.0	-	1/1	-	-	1/1
	>15.1	-	0/1	-	-	0/1
1,2&3	<10					14/23
	>10					3/13

Tumour response rates after superficial photochemotherapy related to tumour 'depth'.

Patient 1 received 1.0 $mgkg^{-1}$ Photofrin II
Patient 2 " 1.5 " "
Patient 3 " 2.0 " "

Appendix 6.3

Dose of Light (Jcm^{-2})	Dose of Photofrin II ($mgkg^{-1}$)		
	1.0	1.5	2.0
25	0/2	0/6	0/1
50	0/2	7/10	3/3
75	0/3	3/4	2/2
100	0/1		

Number of sites showing eschar formation expressed as a fraction of the number of sites receiving the same dose of Photofrin II and superficial light.

Appendix 6.4

Patient/ Dose (J)	Initial Tumour Volume (mm^3)	VDT (days)	Growth Delay (days)	Growth Delay / VDT
12/100	130	40	43	1.1
150	490		56+	1.4+
200	530		68	1.7
250	400		0	0
100	250		0	0
13/125	1300	68	162	2.4
150	1300		148+	2.2+
175	850		148+	2.2+
125	150		109	1.6
150	220		148+	2.2+
14/ 75	60	16	29	1.8
300	5590		85	5.3
300	6200		52	3.7
15/200	210	34	0	0
200	30		45	1.3
200	30		80+	2.4+
200	190		13	0.4
200	110		9	0.3
200	50		80+	2.4+

Tumour response to interstitial photochemotherapy.

(+ tumours not regrowing at time of last measurement
VDT = Volume Doubling Time)

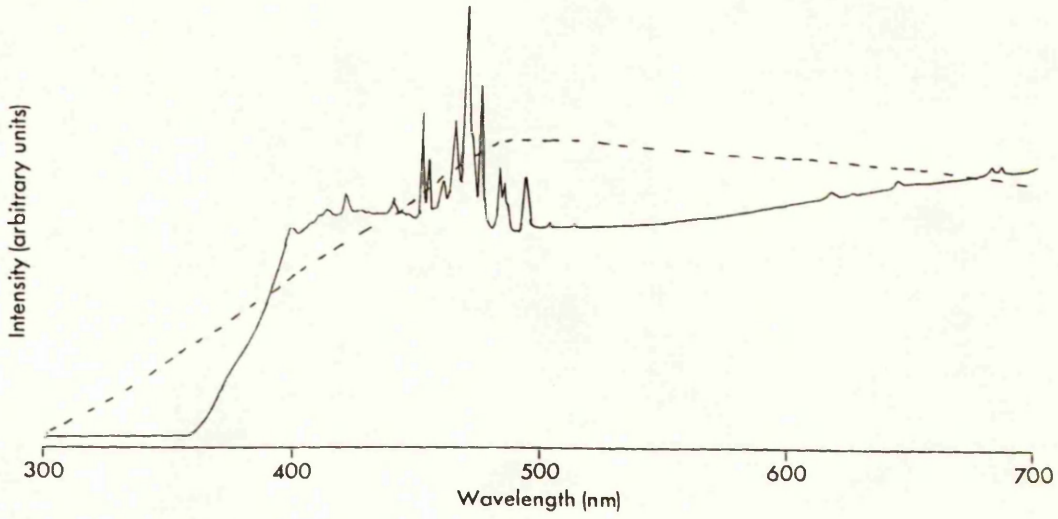
Appendix 7.1

The Solar Simulator

Light from a 150 W Xeron arc lamp was focused by a f/0.8 quartz condenser into a 1/4 inch diameter, 48 inch long glass fibre optic bundle (Ealing Electro-Optics, Watford, Herts) for delivery to the skin. The spectral distribution of the light used to irradiate the skin was measured with a monochromator(CF4)/photomultiplier (Hamamatsu R456, Hamamatsu Photonics, Enfield, Middlesex) system in the spectral region of interest, 300 to 700 nm. The spectral output, corrected for the spectral response of the detection system, was in good agreement with published spectra. Approximately 50% of the light emitted by Xenon arc lamps lay at wavelengths less than 700 nm. The ultraviolet component of the light was absorbed by the glass fibre bundle and no light was detected at wavelengths less than 350 nm. The total power exiting from the fibre bundle was 225 W, measured using a light meter (Photon Control Ltd.), giving an irradiance at the test site of 164 $mWcm^{-2}$.

Convolution of the Photofrin II absorbance curve with the Xenon arc and solar emission spectra confirms that these two sources are equivalent in terms of the total amount of light energy absorbed by the drug (Appendix Figure 7.1).

Appendix Figure 7.1



Spectra irradiance of the sun and the solar simulator.
- - - - - - - - Sun
———— Solar simulator

Appendix 7.2

Patient 1

Days after Photofrin II	Time after test (h)	Relative haemoglobin index				
		Test dose of light (Jcm^{-2})				
		10	20	30	40	50
4	0.25		1.02	0.99	1.03	1.03
	3		1.08	1.04	1.11	1.08
	24		1.05	1.01	1.07	1.05
10	0.25		1.01	1.00	0.99	1.06
	3		1.04	1.08	1.01	1.15
16	0.25	0.93	0.97	0.99	1.00	
	3	0.98	1.02	1.02	1.05	

Patient 6

Days after Photofrin II	Time after test (h)	Relative haemoglobin index			
		Test dose of light (Jcm^{-2})			
		5	10	15	20
2	0.33	0.99	1.04	1.03	1.15
	3	1.05	1.12	1.16	1.36
	21	0.96	1.03	1.04	1.08
7	0.25	1.10	1.04	1.07	1.08
	3	1.10	1.15	1.19	1.19

Photopatch testing results for Patients 1 and 6, erythema response measured with the haemelometer.

Appendix 7.3

Patient	Time after Photofrin II (Days)	Relative Haemoglobin Index			
		Test Dose of Light (Jcm^{-2})			
		5	10	15	20
2	1	1.08	1.13	1.17	1.20
	3	1.05	1.09	1.15	1.09
	10	1.06	1.10	1.13	1.19
	22	1.05	1.11	1.22	1.28
	30	1.04	1.07	1.17	1.23
	43	1.00	1.04	1.04	1.10
4	1	1.06	1.12	1.13	1.20
	4	1.04	0.99	1.01	1.03
	11	1.11	1.15	1.14	1.12
	18	1.04	1.07	1.00	1.00
5	1	1.08	1.12	1.20	1.21
	4	1.00	1.08	1.20	1.14
	10	1.11	1.08	1.11	1.15
	29	1.10	1.21	1.24	1.27
	42	1.17	1.29	1.25	1.22
8	1	1.08	1.19	1.32	1.22
	4	1.07	1.23	1.30	1.35
	11	1.02	1.09	1.31	1.31
	37	1.05	1.19	1.10	1.31
	51	1.08	1.12	1.15	1.21
12*	0	0.86	0.91	0.86	0.80
	2	0.67	1.11	1.24	1.49
	5	0.82	1.21	1.06	1.53
	12	1.32	1.50	1.92	1.50
	20	1.08	1.16	1.34	1.17
	31	1.19	1.64	1.63	1.80
13*	0	1.03	1.20	1.16	0.98
	1	1.12	1.37	1.56	1.65
	4	1.21	1.21	1.40	1.33
	10	1.66	1.49	1.85	1.35
	20	2.03	1.95	1.89	1.77
	31	1.11	1.22	1.47	1.06

Appendix 7.3 (continued)

Patient	Time after Photofrin II (Days)	Relative Haemoglobin Index			
		Test Dose of Light (Jcm^{-2})			
		5	10	15	20
14*	0	1.01	1.18	0.99	0.83
	1	1.84	2.80	2.02	1.85
	4	1.10	1.58	1.78	1.93
	12	1.52	1.81	2.71	2.00
	19	1.16	1.76	1.58	1.84
	33	1.15	1.16	1.37	1.44
	47	0.92	1.45	1.42	1.49
15*	0	1.14	0.97	0.86	0.83
	1	0.88	0.99	1.36	1.05^{o}
	4	1.17	0.98	1.36	1.22
16	1	1.09	1.17	1.33	1.37
	4	1.18	1.09	1.15	1.21
	17	1.17	1.22	1.34	1.33
	32	1.08	1.21	1.19	1.30
	56	0.95	1.02	1.14	1.19

Relative haemoglobin index 3 h after photopatch testing the skin of the back using the haemelometer or the reflectance spectrophotometer (*) to measure erythema.

(^{o} blanching visible within the test area)

Appendix 7.4

Patient	Time after Photofrin II (Days)	Relative haemoglobin index		
		Hand	Covered hand	Back
14	0	0.92		0.83
	1	1.23		1.85
	4	1.04		1.93
	12	0.91		2.00
15	0	0.89		0.83
	1	1.19		1.05^{o}
	4	1.14	1.50	1.22

Relative haemoglobin index 3 h after photopatch testing with 20 Jcm^{-2} of light on the skin of the back of the hand exposed to ambient light, of the back of the hand covered by a plaster and of the posterior chest wall.

(^{o} blanching visible within the test area)

Appendix 7.5

Patient	Time (Days)	Porphyrin level ($ngml^{-1}$)	Relative Haemoglobin Index
2	10	91	1.19
4	11	107	1.12
5	10	211	1.15
8	11	85	1.31
13	10	579	1.35
2	22	50	1.28
4	18	79	1.00
5	29	80	1.27
13	20	501	1.77
14	33	151	1.44
16	32	541	1.19

Plasma porphyrin level and erythema response (relative haemoglobin index 3 h after 20 Jcm^{-2} of light to the skin of of the back) 10 or 11 days and 18 to 33 days after Photofrin II injection.

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ADDENDUM

Therapeutic ratio of photodynamic therapy in the treatment of superficial tumours of skin and subcutaneous tissue in man.
D. Gilson, D. Ash, I. Driver, J.W. Feather & S.B. Brown, 1988.

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Therapeutic ratio of photodynamic therapy in the treatment of superficial tumours of skin and subcutaneous tissues in man

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Summary Six patients with a total of 34 assessible subcutaneous or cutaneous lesions were treated with photodynamic therapy using 1.0, 1.5 or 2.0 mg kg^{-1} of photofrin II and 25–100 J cm^{-2} of red light (630 nm). The incidence of complete tumour response and skin necrosis were used to try to assess the therapeutic ratio of photodynamic therapy. The tumour response rate was 47%. The rate of tumour control and necrosis increased in parallel with dose of photosensitizer and light used, implying a low therapeutic ratio. However, the use of necrosis with eschar formation as an end-point for severe normal tissue damage is questioned as the skin healed completely in all cases and with minimal discomfort to the patients.

Photodynamic therapy, the use of photosensitizers activated by light, has been used in man to treat superficial malignancy for some years (Dougherty *et al.*, 1978; Dougherty, 1984; Carruth & McKenzie, 1985). Selective retention of porphyrin in malignant tissue produces a relatively higher concentration of drug in the tumour than in the surrounding normal tissue (Gomer & Dougherty, 1979; Lipson *et al.*, 1961). This difference in concentration of porphyrin between normal and malignant tissue is the theoretical basis for the therapeutic ratio of photodynamic therapy. It is suggested that 3 days is left between giving the photosensitizer and irradiating the tumour to maximize the concentration difference (Dougherty *et al.*, 1979).

Previous studies have shown complete response rates of 50–80% (Dougherty, 1984) when photodynamic therapy is used to treat superficial tumours. This study examines how tumour response varies with dose of photofrin II (dihaematoporphyrin ether) and light (630 nm) and also tries to determine the doses of drug and light which will give maximum tumour response with minimum damage to normal skin within the irradiated area. This was done by examining the incidence of complete tumour regression and of skin necrosis within the irradiated area in cutaneous and subcutaneous tumours treated with photodynamic therapy.

Patients and methods

Between June and December 1986, six patients with a total of 34 assessible cutaneous or subcutaneous metastatic or locally recurrent tumours which were clinically <1.5 cm thick were treated with photodynamic therapy. At five of these sites the skin was already ulcerated.

Histology included squamous carcinoma (oral mucosa primary), small cell lung cancer, large cell anaplastic carcinoma, malignant melanoma, anaplastic parotid carcinoma and adenocarcinoma (breast primary).

Patients were given 1, 1.5 or 2 mg kg^{-1} body weight of photofrin II (Photofrin Medical Co. Inc., Raritan, New Jersey) intravenously. Forty-eight to seventy-two hours later the lesions were irradiated with red light (630 nm) from an argon-dye laser. The light from the laser was focused into a 600 μm optical fibre. The fibre passed through a 'mode scrambler' to flatten the light beam. The distal end of the fibre was positioned at an appropriate distance above the skin surface, so that divergence of the light beam gave the required size of treatment field.

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The tumours were treated with a 1 cm margin of surrounding normal skin and the diameter of the treated areas varied from 2.5 to 6 cm. The total doses of light given at the skin surface were 25, 50, 75 or 100 J cm^{-2}. Light and drug doses were chosen so that different sized tumours were spread evenly throughout the treatment groups. The light was delivered at a dose rate of 40–172 mW cm^{-2}, depending on the output of the laser and the size of treatment field.

After treatment patients were reviewed weekly for 4 weeks and monthly thereafter. Complete clinical resolution of the lesion was used to assess tumour response and the incidence of damage to skin within the irradiated area was recorded using skin necrosis and formation of a black eschar as the end-point.

Results

Within hours of treatment there was blanching within the irradiated area with an annulus of erythema around the treated zone. By one week there was intradermal haemorrhage in the centre of the treatment area. By two weeks (Figure 1), there was breakdown of the skin with a black scab or eschar overlying it. Over the next 4–12 weeks the skin healed from the edges of the necrosed zone. The only abnormalities visible after healing were a small central depressed scar and slight pigmentation which gradually faded (Figure 2).

The overall complete tumour response rate was 47%. If only the 19 lesions treated with 1.5 or 2 mg kg^{-1} of photofrin II and 50 or 75 J cm^{-2} of light are considered the complete response rate was 74%. Table I shows the increase of tumour control with increasing dose of photofrin II and light. Complete tumour response occurred within three weeks of treatment and persisted during the period of follow-up (3–5 months). Several sites showed partial regression of the lesion but tumour regrowth always began again within two months.

The incidence of skin necrosis also increased with dose of photofrin II (Table II), in a similar way to tumour response. The skin necrosis healed completely, with no scarring or contraction, in all cases but at some sites this took 12 weeks. Skin necrosis was painless except at one site which caused some discomfort which lasted for 3 weeks and was relieved by co-proxamol.

The size of the eschar was dependent on the size of the treatment field, the diameter of the eschar was $51 \pm 11\%$ (mean $\pm$ 1 s.d.) of the diameter of the total area illuminated. Although attempts were made to ensure a flat beam, differences between size of eschar and size of field

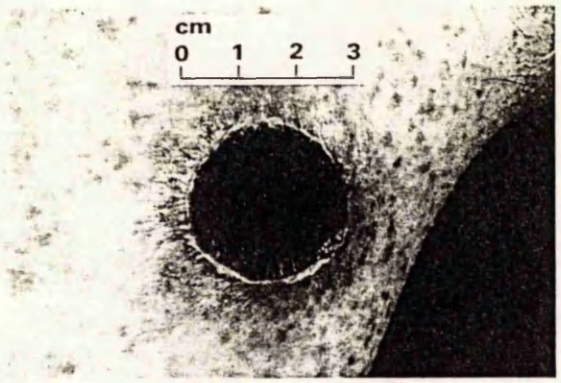

Figure 1 Typical appearance of skin necrosis, with formation of an eschar, two weeks after photodynamic therapy.

Figure 2 Appearance of the skin after healing of the skin necrosis showing pigmentation and a small central depressed scar.

illuminated could still have been due to a higher light flux in the centre of the beam. There was a trend for the size of the eschar to increase with increasing doses of light and drug (Figure 3). It was, also, our impression that the larger the eschar the longer the skin took to heal. Even the largest treated field which was a circle of 6 cm diameter healed within 12 weeks.

Discussion

The complete tumour response rate was comparable to that observed by other authors (Dougherty, 1984). Dougherty, also, commented that skin necrosis was common with higher doses of drug and light. The precise relationship between treatment parameters, tumour control and skin necrosis is difficult to discern from previous studies.

The data suggest a low therapeutic ratio for photodynamic therapy of superficial lesions when early damage to overlying skin is considered. The dose response curves produced for varying doses of photofrin II (Figure 4) and light (Figure 5) show that the incidence of tumour control is almost paralleled by that of skin necrosis. The use of skin necrosis and eschar formation as an end-point for skin damage within the irradiated area may not be appropriate, however, it produced minimal discomfort to the patients and in all cases the lesions healed completely and left a good cosmetic result. Also, skin damage was transient while tumour control persisted for the duration of follow-up.

The incidence of skin necrosis and probably the size of the eschar it produces are dependant on the doses of drug and light used. If the clinical impression that the larger the eschar the longer it takes to heal is correct, then increasing the doses of drug and light will produce not only higher chance of eschar formation but also these will take a longer time to heal.

Table I Number of sites showing complete tumour response expressed as a fraction of the number of sites receiving the same dose of photofrin II and light

Dose of light $J\,cm^{-2}$	*Dose of photofrin II*		
	1.0 mg kg^{-1}	*1.5 mg kg*$^{-1}$	*2.0 mg kg*$^{-1}$
25	0/2	1/6	1/1
50	0/2	6/10	2/3
75	0/3	4/4	2/2
100	0/1	–	–

Table II Number of sites showing eschar formation expressed as a fraction of the number of sites receiving the same dose of photofrin II and light

Dose of light $J\,cm^{-2}$	*Dose of photofrin II*		
	1.0 mg kg^{-1}	*1.5 mg kg*$^{-1}$	*2.0 mg kg*$^{-1}$
25	0/2	0/6	0/1
50	0/2	7/10	3/3
75	0/3	3/4	2/2
100	0/1	–	–

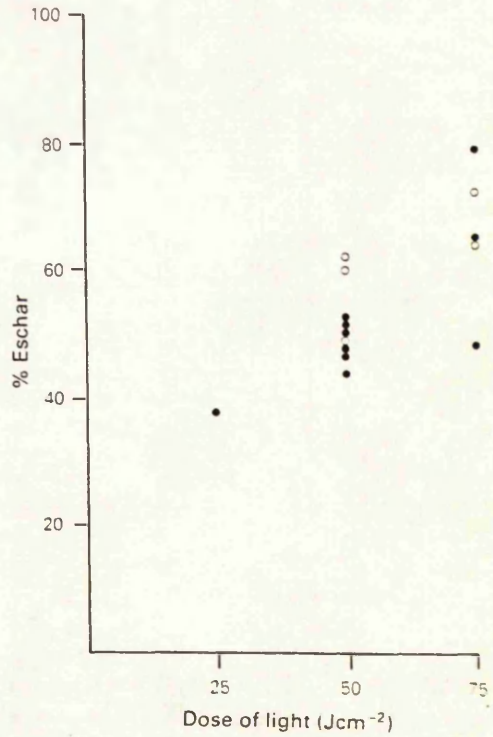

Figure 3 Variation in diameter of eschar expressed as a percentage of the diameter of the treatment field (percent eschar) with dose of light and photofrin II (● 1.5 mg kg^{-1} photofrin II, ○ 2.0 mg kg^{-1} photofrin II).

The mechanism of production and repair of this skin damage is interesting because the initial damage appears severe but it causes minimal pain and always heals without scarring. The damage does not resemble a thermal burn as one would expect a full thickness burn to heal with fibrosis but a partial thickness burn which may heal without scarring or contacture is usually very painful. Barr *et al.* (1987) have shown that in animal mucosa thermal burns produced by lasers heal by fibrosis whilst damage due to photodynamic therapy repairs leaving a relatively normal mucosa. The damage is also different from radiation necrosis as necrosis such as this usually fails to heal in the long term. Possibly, these differences are explained by the mode of action of photodynamic therapy which is postulated to be through causing vasoconstriction rather than by directly causing cell death (Star *et al.*, 1986; Henderson *et al.*, 1984).

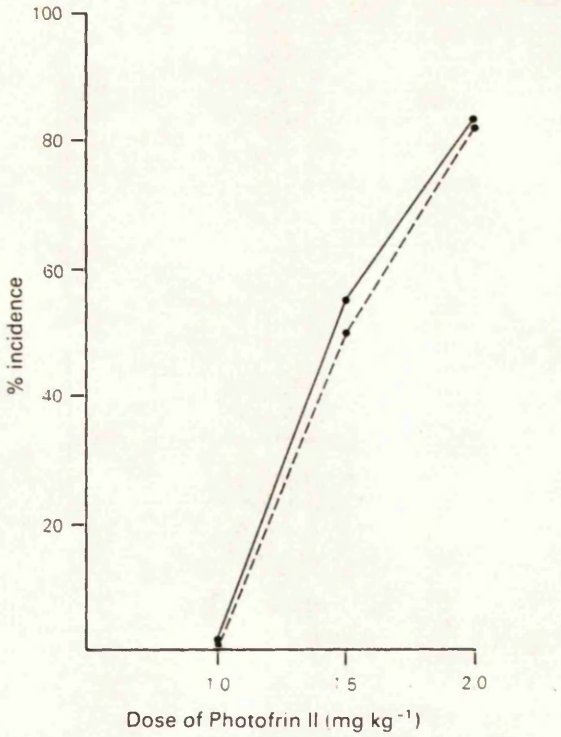

Figure 4 Relationship between incidence of complete tumour response and skin necrosis and dose of photofrin II (complete tumour response ———, skin necrosis – – –).

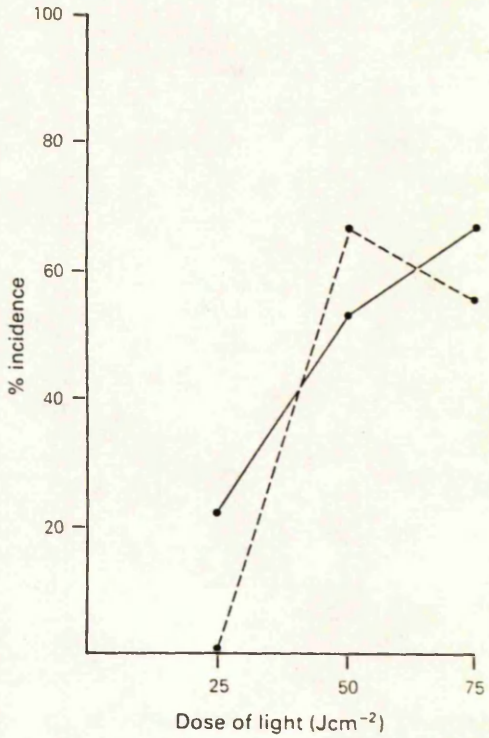

Figure 5 Relationship between incidence of complete tumour response and skin necrosis and dose of light (complete tumour response ———, skin necrosis – – –).

The dose of light decreases exponentially with increasing depth of tissue (Wan *et al.*, 1981). The tumour, in subcutaneous lesions lies below the skin and will therefore receive a lower dose of light than the skin but the tumour showed persistent damage whilst that to the skin was transient. This implies that the tumour is more sensitive to photodynamic therapy than normal skin, possibly due to the greater concentration of photofrin II in the tumour than the normal surrounding tissue. Whatever the mechanism of this difference, it is the basis for a relatively good therapeutic ratio, especially if early skin damage could be prevented.

One possible way of overcoming this is to use optical fibres implanted in the tumour to deliver light. This should increase the dose of light given to the tumour relative to the dose of light delivered to the normal surrounding tissue and it may also allow treatment of more deep seated tumours.

We conclude that photodynamic therapy is effective in treating superficial tumours and that refinement of light delivery systems may further reduce the side-effects of this relatively non-toxic treatment.

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