Effect of Photosensitizer Concentration in Tissue on the Penetration Depth of Photoactivating Light

BRIAN C. WILSON, MICHAEL S. PATTERSON, DIANE M. BURNS[a]

Department of Medical Physics, Ontario Cancer Treatment and Research Foundation, and Departments of Radiology and Physics, McMaster University, and [a]Department of Radiology, McMaster University, 711 Concession Street, Hamilton, Ontario, Canada L8V 1C3

Correspondence to Brian C. Wilson

Abstract. The additional optical absorption in tissue resulting from the uptake of exogenous photosensitizers increases the effective attenuation of photoactivating light. This may be significant for the irradiation of solid tumours in photodynamic therapy, since it reduces the depth or volume of tissue treated. The effect has been studied in vitro by using dihaematoporphyrin ether (DHE) and 630 nm light in tissues representing a wide range of absorption and scattering conditions. While the attenuation may be markedly changed by small concentrations of DHE in pure scattering media, tissues with significant inherent light absorption are little affected by the additional absorption of DHE at concentrations relevant to clinical photodynamic therapy. However, it is shown that for other potential photosensitizers such as the phthalocyanines, which have substantially greater absorption at the treatment wavelength than DHE, the penetration of light in tissues may be significantly reduced.

INTRODUCTION

In clinical photodynamic therapy (PDT), as currently practised by using haematoporphyrin derivative (HPD) and 630 nm light, several factors limit the applications to relatively small, solid tumours (1, 2). These factors are: (a) the limited optical penetration of 630 nm light in tissue; (b) the relatively low uptake of HPD in tumour tissue, which cannot be increased by increasing the administered dose because of the need to minimize systemic skin photosensitivity; and (c) the low light absorption of HPD at 630 nm and the consequent low photoactivation. These factors are interrelated. For example, although the absorption and photoactivation of HPD is much higher at shorter wavelengths, this improvement is more than offset (3, 4) by the rapid decrease in optical penetration of light in tissues below 600 nm owing to haemoglobin absorption (5). There is current interest, therefore, in developing alternative photosensitizers which have much greater absorption in the long wavelength range, say above 600 nm, together with high tumour uptake. A potential problem in this approach is that, if its optical absorption and concentration in tissue are high enough, the photosensitizer itself may reduce the penetration of light in the target tissue.

There is both indirect and direct evidence for this effect. It is known, for example from the 'poisoned moderator' technique in neutron physics (6), that the attenuation of radiation in highly scattering materials may be markedly increased by the addition of small amounts of absorber. Qualitatively, this is because the path length of radiation in such a scattering medium is large, which increases the probability of absorption in propagation through a given thickness of material. The effect is expected also for the propagation of light in tissues since, at least above about 600 nm, lightly pigmented tissues are typically highly scattering (2, 7–13, and S.T. Flock et al, unpublished observations). Experimental evidence in tissue was provided by Profio and Sarnaik (12) who showed that the optical attenuation of brain tissue in vitro at 633 nm could be increased by the addition of small concentrations of Tryptan red dye, and that the changes were consistent with the predictions of diffusion theory. We shall use the same basic method as Profio and Sarnaik, but extend it both theoretically and experimentally.

Paper received 7 October 1986

Recently, Powers and Brown (14) measured the light transmission in normal rat brain in vivo at 633 nm in control animals and in animals injected intravenously 24 h previously with 10 mg/kg HPD. They observed a reduction in transmission in the latter group and attributed this to light absorption by the photosensitizer.

Further evidence has been reported by Bown et al (15), who measured the depth of necrosis produced in normal rat liver by using the photosensitizer aluminium chlorosulphonated phthalocyanine (AlSPc) and 675 nm light. For a fixed light dose the depth of necrosis first increased with increasing AlSPc concentration and then decreased. It was postulated that initially a higher photosensitizer concentration reduces the light irradiance required to produce any given degree of tissue damage, and hence increases the depth at which the combination of space irradiance and concentration exceeds some 'threshold' level. The subsequent reduction in depth of necrosis at higher concentrations is then due to a reduction in optical penetration caused by absorption of light by the AlSPc. This hypothesis was not tested quantitatively.

The final, indirect evidence comes from a study by Hisazumi et al (16) who measured the penetration of 630 nm light in normal rabbit auricle containing HPD, for both pulsed and continuous wave (CW) irradiation. The pulsed light was found to be more penetrating than the CW radiation, particularly at high-power density. This was attributed to transient bleaching of the HPD by the high-power pulsed light, and the resultant reduction in optical absorption. Improved control in treating transplanted tumours in mice with pulsed compared with CW irradiation was also interpreted as due to deeper penetration of the pulsed light. However, no differences in tumour regression between pulsed and CW laser for PDT irradiation were observed by Cowled et al (17). We shall examine quantitatively whether transient HPD bleaching by pulsed light could produce significant changes in optical penetration, and hence in tumour response.

The experiments reported here comprise measurements of the effective penetration depth of 630 nm light in various tissues in vitro and in a highly scattering medium of well determined optical properties, as a function of added absorber in the form of dihaematoporphyrin ether (DHE) or ink. The tissues were chosen to represent a wide range of inherent absorption and scattering coefficients. The data have been compared with diffusion theory calculations and, on the basis of the excellent agreement between theory and experiment, we have developed a generalized description which may be applied to any tissue and any photosensitizer of known optical absorption. This model will then be used to analyse in detail the data of Bown et al (15) for the depth of necrosis in phthalocyanine-sensitized liver. Conclusions will be drawn from this which have general implications for the future development of PDT.

THEORY

For a plane-collimated light beam incident normally on the surface, where depth $x = 0$, of an optically homogeneous, semi-infinite medium, the distribution of space irradiance (radiant energy fluence rate), ϕ, with depth, x, is exponential, except close to the surface and, applying the formalism of diffusion theory (2, 4, 9, 10, 12, 18), may be expressed as

$$\phi(x) = \phi_0 e^{-\Sigma}\,\mathrm{eff}^x \quad (1a)$$

$$= I_0(1-\gamma)\Sigma_{\mathrm{eff}}\Sigma_a^{-1}e^{-\Sigma}\,\mathrm{eff}^x \quad (1b)$$

where Σ_{eff} is the effective attenuation coefficient, Σ_a is the absorption coefficient, and γ is the total reflection coefficient at the surface. The 'coupling constant' $(1-\gamma)\,\Sigma_{\mathrm{eff}}\Sigma_a^{-1}$ relating the space irradiance near the surface, ϕ_0, and the incident irradiance, I_0, is approximate (9, 18). Further, for infinite beam diameter,

$$\Sigma_{\mathrm{eff}}^2 = 1/d_{\mathrm{eff}}^2 = \Sigma_a/\xi = 3\Sigma_a(\Sigma_a + \Sigma_s(1-g)) \quad (2)$$

where d_{eff} is the effective penetration depth (depth at which the irradiance falls to 1/e, or 37%), Σ_s is the scattering coefficient of the medium and g is the mean cosine of the scattering phase function. We note that the diffusion coefficient, ξ, and Σ_{eff} depend not on the value of the scattering coefficient Σ_s as such, but on the 'reduced' scattering coefficient $\Sigma_s' = \Sigma_s(1-g)$. For a finite beam diameter, equation 2 no longer holds exactly; the effective attenuation increases as the diameter decreases, and the diffusion equation has to be solved numerically (2), or techniques such as Monte Carlo computer simulation (19) have to be used to determine Σ_{eff}, given Σ_a, Σ_s and g. However, measurements by Marynissen and Star (10) for various tissues in vitro show that the penetration depth of 630 nm light is within 10% of the infinite beam diameter value for diameters above 3 cm. Thus, the effect of beam size is important only for very small irradiation fields. We also note

that equation 2 is accurate only for high 'reduced albedo' that is, for $\Sigma_s' \gg \Sigma_a$; otherwise the effective penetration depth is underestimated.

Diffusion theory has also been used to derive simple forms for the spatial irradiance distributions that correspond to interstitial optical fibre irradiation. These involve the same effective penetration depth as for the surface irradiation case (equation 2), so that the effects observed for the latter may be applied directly for the interstitial geometry. For a point source of illumination within an infinite, homogeneous block, the radial distribution of space irradiance, analogous to equation 1, is

$$\phi(r) = P\Sigma_{eff}^2(4\pi\Sigma_a r)^{-1}e^{-\Sigma_{eff}r} \quad (3)$$

where P is the input power. Again, the coupling constant relating the absolute values of P and ϕ is an approximation.

For either irradiation geometry, with the addition of a photosensitizer with absorption coefficient Σ_{ap} to tissue, the total absorption coefficient becomes

$$\Sigma_a = \Sigma_{at} + \Sigma_{ap} \quad (4)$$

where Σ_{at} is the inherent absorption coefficient of the tissue, and $\Sigma_{ap} = K.\epsilon_p$ where K is the concentration of the added absorber with extinction coefficient ϵ_p at the wavelength of interest. This makes the reasonable assumption that the photosensitizer does not alter the light-scattering properties of the tissue ($\Sigma_s' = \Sigma_{st}'$). The irradiance distribution is then correspondingly changed, by inserting Σ_a as given by equation 4 into equation 2, and then into equations 1b or 3.

MATERIALS AND METHODS

Fig. 1 shows the experimental set-up used. A 4-cm diameter collimated circular light beam from a 4-mW Helium–Neon laser (632.8 nm) was used to irradiate the phantom, which comprised an 8-cm perspex cube, the sides of which were painted matt black, and the base of which was transparent. This was filled with the various tissue-simulating materials. A 400 μm quartz optical fibre (numerical aperture in air = 0.4), connected to a single photon counter (5) was mounted in a biopsy needle on a stereotaxic frame co-axially with the light beam. It could be moved along the beam axis with a precision of $\pm$ 0.2 mm. The detected light flux, F, was recorded as a function of distance, x, of the fibre tip from the surface. Although the response of such a detector is directionally dependent, in the diffusion region $F(x)$ may be assumed (10)

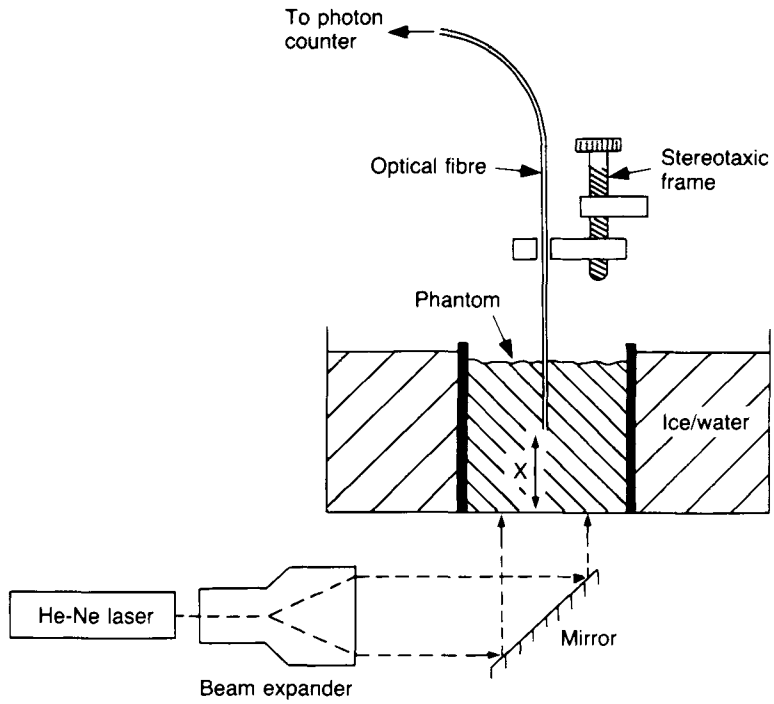

Fig. 1. Diagram of apparatus, showing the detector fibre mounted co-axially with the expanded He–Ne laser beam, which irradiates the surface of the tissue-simulating phantom.

proportional to $\phi(x)$. From the slope of F versus x in the diffusion region, the value of d_{eff} was determined.

Five different phantom materials were studied: a 1% aqueous solution of Nutralipid (Pharmacia Inc., Quebec), and four freshly ground tissues; white chicken muscle, pig brain, and bovine muscle and liver. Nutralipid is a fat emulsion which is highly scattering with very low light absorption (20). As will be discussed later, the reduced scattering coefficient, Σ'_{st}, is of the same order of magnitude for all four tissues, and for this concentration of Nutralipid, namely about $1\,\text{mm}^{-1}$. The absorption coefficients, however, cover a wide range; Nutralipid about 10^{-3}, chicken muscle and brain about 10^{-2}, bovine muscle and liver about $10^{-1}\,\text{mm}^{-1}$.

For Nutralipid, brain and chicken d_{eff} was determined using added DHE concentrations up to 40 μg per g tissue. For both bovine muscle and liver, however, this range does not produce significant change in d_{eff}, so that additional experiments were done using India ink as the added absorber, since this could be obtained at much higher initial concentrations than DHE.

The use of both the liquid Nutralipid and the ground tissues allows thorough mixing of absorber into the phantoms. The DHE used was the commercial material Photofrin II (Photomedica Inc., New Jersey). This was diluted to 0.84 mg/ml by using 10% fetal calf serum (FCS) in phosphate buffered saline (PBS), and kept at 0 °C in the dark for 30 min, to allow binding to protein. The tissues were ground and 5 ml of 10% FCS in PBS was added to each 200 g of tissue to facilitate uniform mixing of the small volume of DHE solution, which was added immediately thereafter. For the Nutralipid phantom, 5 ml of 10% FCS in PBS was added to 200 ml of 1% Nutralipid solution prior to adding the DHE. The preparation of the phantoms with ink as the absorber was essentially the same as for DHE, except that FCS was not added. The measurements of F versus x were carried out with the phantom placed in an ice/water bath. This minimized the changes in the optical properties that were observed as a function of time in tissues held at room temperature. The extinction coefficient, ϵ_{p}, of DHE in 10% FCS/PBS was measured in a

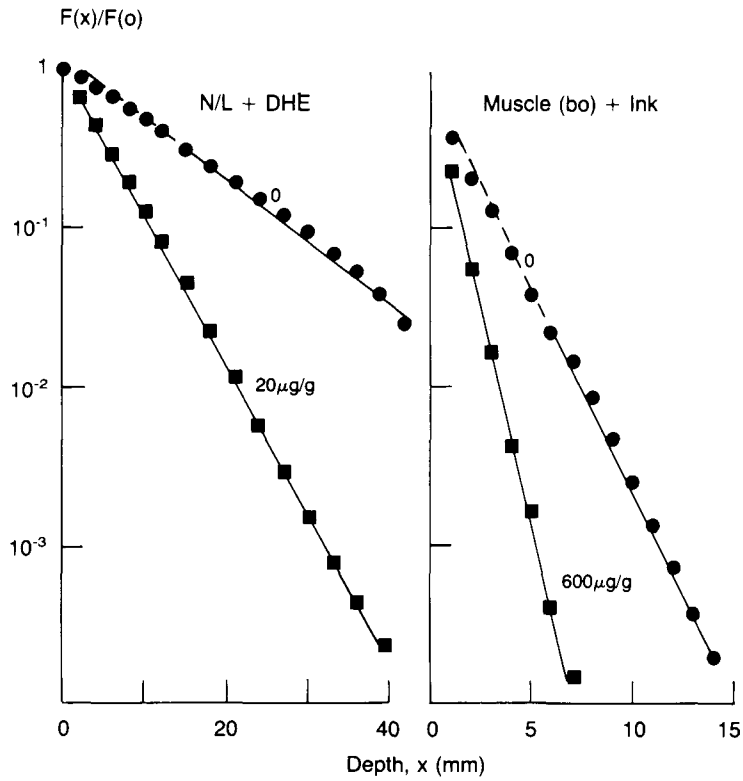

Fig. 2. Examples of the variation in detected light flux, $F(x)$, with depth in the phantom, normalized to the value at the surface (4-cm beam diameter). The straight lines are least-squares fits of $\ln F$ versus x to the final exponential part of each curve: the effective penetration depth, d_{eff}, is given by the negative slope of the fitted line. The statistical error on each point due to photon counts and variation in the laser output is comparable to or smaller than the symbol size. N/L, Nutralipid; bo, bovine muscle. The figures above each line indicate the concentration of added absorber (dihaematoporphyrin ether (DHE) or Ink).

spectrophotometer at 633 nm as $(4.9 \pm 0.2).10^{-4}\,mm^{-1}$ per $\mu g/g$. For ink, the corresponding value was $(4.8 \pm 0.2).10^{-4}\,mm^{-1}$ per $\mu g/g$.

RESULTS

Fig. 2 shows examples of the measured attenuation curves $\ln F$ versus x. For the highly scattering Nutralipid phantom with no added absorber, the final exponential decrease with depth is not established until a depth of about 10 mm is reached, compared with < 5 mm in muscle and brain and < 1 mm in liver. The effective penetration depth, d_{eff}, for each curve was obtained by a least-squares fit to $\ln F$ versus x in the exponential region of each curve. Fig. 3 shows the derived d_{eff} values as a function of the added absorber for each phantom material. The continuous curves in Fig. 3 were derived by fitting equations 2 and 4 to these data, with Σ_{at} and Σ'_{st} as the free parameters. The results are summarized in Table 1, together with comparable values derived from other published data.

It should be noted that for each tissue and concentration of added absorber the curve of $F(x)$ versus x was measured once only. Thus, it is not possible to place meaningful errors on the derived d_{eff} values, taking into account such possible sources of variation as non-uniform mixing of phantom and absorber, and differences in the tissue optical properties from sample to sample from the same animal, or from animal to animal. Such considerations may be addressed in future if the added-absorber method is used for the specific purpose of measuring accurately the optical properties of tissues. In the case of liver in Fig. 3, the large fluctuations in $F(x)$ are believed indeed to be

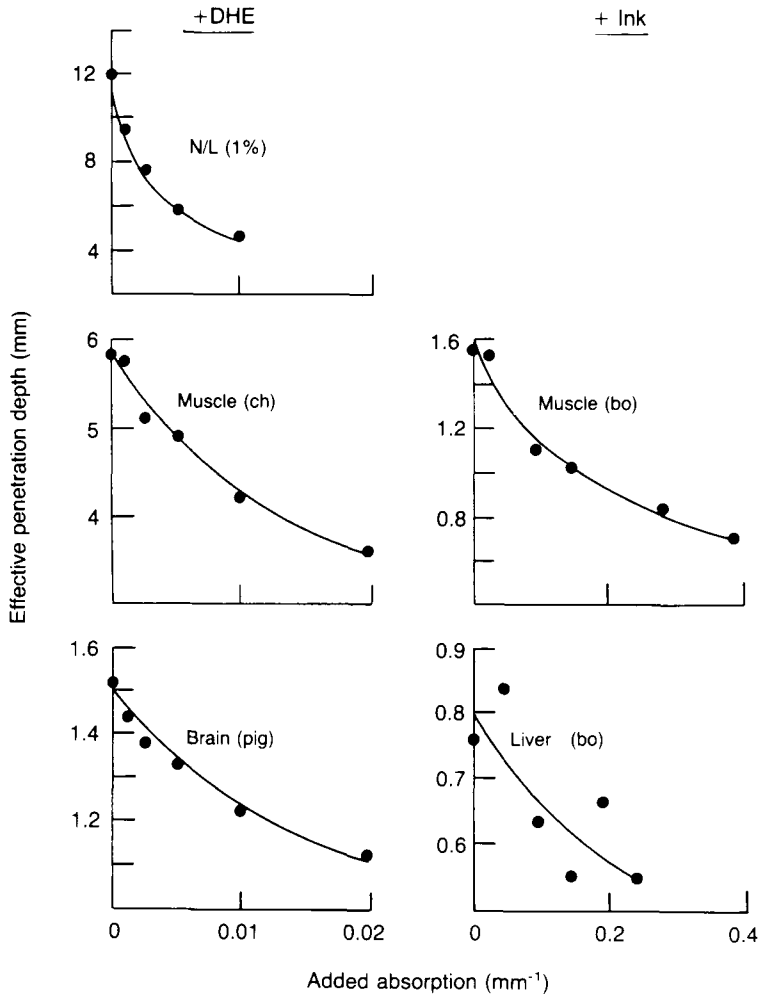

Fig. 3. Plots of the effective penetration depth, d_{eff} versus added absorption at 632.8 nm for the five different phantom materials, with either Photofrin II (DHE) or India ink as the absorber. The curves show the best least-squares fit to these data. N/L, Nutralipid; ch, chicken; bo, bovine.

Table 1. Derived values of the optical properties of tissues and of Nutralipid

Tissue	Absorption coefficient Σ_{at} (mm^{-1})	Reduced scattering coefficient Σ_{st} (mm^{-1})	d_{eff} (mm), with no added absorber
Liver (bovine)	0.27	1.7	0.8 (1.2)[c]
Muscle (bovine)	0.15 (0.04)[a]	0.7 (0.56)[a]	1.6 (1.4–4.2)[d,a,c]
Brain (pig)	0.026 (0.064)[b]	5.7 (5.2)[b]	1.5 (1.1[a]; 1.8–2.3)[e]
Muscle (chicken)	0.012 (0.03)[a]	0.8 (0.4)[a]	5.8 (5.9)[a]
Nutralipid (1%)	0.0016	1.5	11.8

[a]Other data, to be found in reference (10); [b]reference (12); [c]reference (21); [d]reference (4); [e]reference (5). See (2) for a summary of these other data.

due to the difficulty of homogenizing this tissue, and hence in obtaining uniform mixing of the ink. The optical properties for each tissue given in Table 1 must be considered as single-sample values.

DISCUSSION

The excellent fits of equations 2 and 4 to the variation of the measured d_{eff} values as a function of added absorption for a wide range of tissue types confirms the adequacy of the diffusion model to describe the effective penetration of 630 nm light in homogeneous tissues. The added-absorber method also allowed the fundamental tissue parameters Σ_{at} and Σ'_{st} to be determined as summarized in Table 1. In comparing the results with other published values for Σ_{at} and Σ'_{st}, one should realize that the latter have generally been obtained by methods that are substantially different from those used here. The one exception is the added-absorber experiment in pig brain by Profio and Sarnaik (12). (Note that the data from that paper have been reanalysed: A.E. Profio, private communication, to give the values in Table 1.) In general, there is good agreement with the published data for the reduced scattering coefficient and the effective penetration depth. For Σ_{at} the data agree to within a factor of 4 for muscle and brain. This degree of variation between experiments might be expected, however, (a) since the absorption coefficient of tissue is very markedly altered by physiological factors such as blood content and oxygenation status, and (b) because $\Sigma_{at} \ll \Sigma'_{st}$ makes measurement of Σ_{at} very sensitive to systematic biases in the different measurement techniques. Nevertheless, even this level of consistency between the data is encouraging at this early stage in the development of methods to measure the fundamental optical properties of tissues.

The main purpose of the present study was not, however, to measure Σ_{at} and Σ'_{st} values *per se* but, rather, to determine experimentally the relationship between the effective penetration depth and the concentration of added absorber, to demonstrate that this could be accurately described by diffusion theory, and hence to examine the implications of this for the penetration of photoactivating light in photodynamic therapy in vivo. These aspects will now be discussed in some detail.

Fig. 4 shows the predictions from equations 2 and 4 for the percentage decrease in d_{eff} as a function of added absorption, for a range of inherent absorption and reduced scattering coefficients. The Σ_{at} values for the Nutralipid and the four tissues measured above are also indicated. It can be seen that the percentage change in penetration depth for a given concentration of added absorber is strongly dependent on the inherent tissue absorption, being greatest where this is small. The change is much less sensitive to the reduced scattering coefficient of the tissue.

The significance of a given decrease in effective penetration depth depends on the depth to which one is trying to achieve a tumoricidal space irradiance and, in particular, on how large is this depth compared with the inherent penetration depth in the tissue. Thus, for surface irradiation, if the treatment depth $d_t = n.d_{eff_0}$, then the irradiance at d_t in the absence of photosensitizer is proportional to e^{-d_t}/d_{eff_0}, and with photosensitizer present is proportional to e^{-d_t}/d_{eff}, where d_{eff} is given by equations 2 and 4. The ratio of these space irradiances is plotted in Fig. 5 as a function of the percentage decrease in d_{eff}, for different values of the constant n. (The change in the coupling

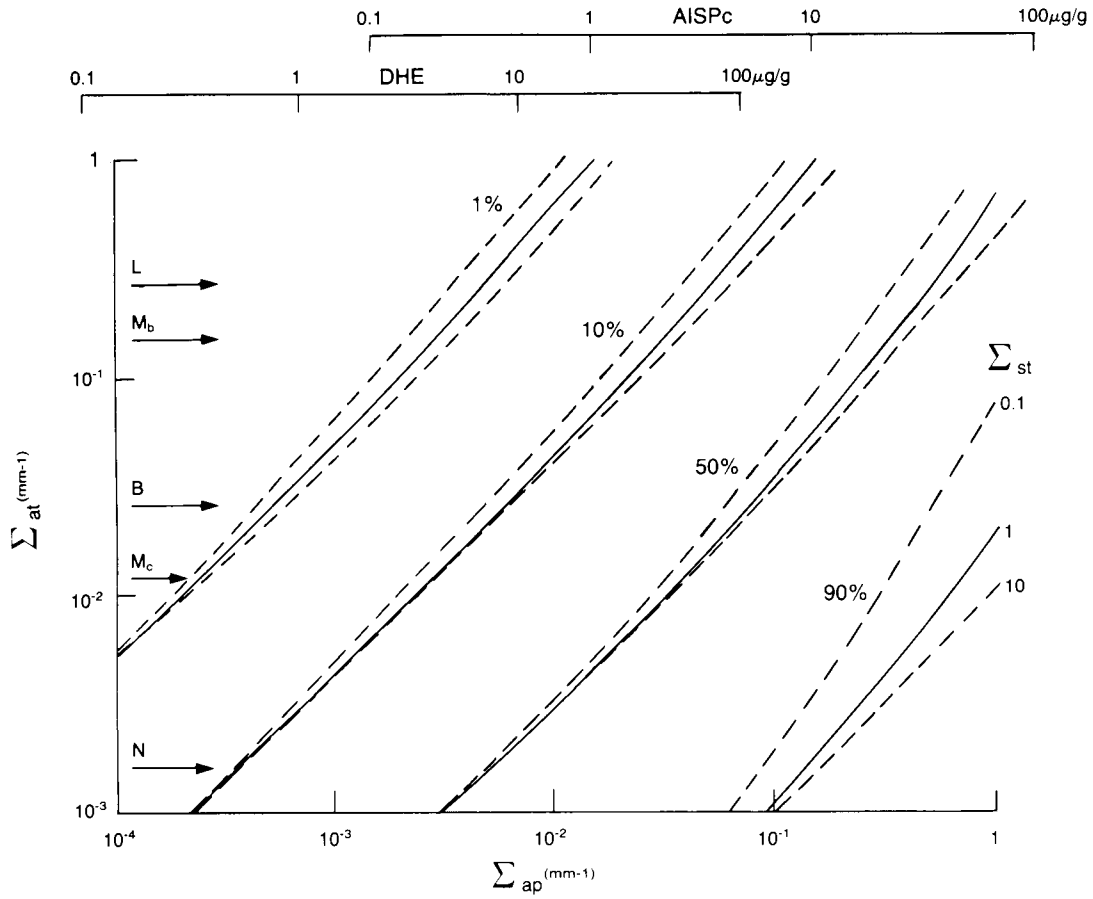

Fig. 4. Computer prediction of lines of equal percentage decrease in effective penetration depth, plotted as a function of tissue absorption Σ_{at} and added photosensitizer absorption Σ_{ap}. Each line corresponds to a particular value of tissue-reduced scattering coefficient Σ'_{st}. (Values of 0.1, 1 and 10 were used, as indicated on 90% lines.) The scales at the top give the concentrations of DHE at 630 nm and of aluminium chlorosulphonated phthalocyanine (AlSPc) at 675 nm having the equivalent Σ_{ap} value ($\epsilon_{DHE} = 7 \times 10^{-4}\,mm^{-1}/(\mu g/g)$; AlSPc = $1.5 \times 10^{-2}\,mm^{-1}/(\mu g/g)$). The arrows on the left-hand side indicate the Σ_{at} values of the various tissues (see Table 1). L, liver; M_b, bovine muscle; B, brain; M_c, chicken muscle; N, Nutralipid.

constants—see equations 1b and 3—with change in d_{eff} has not been included here. It would somewhat reduce the magnitude of the effect, depending on the value of Σ_{at}.) For example, in treating to five times the effective penetration depth of tissue ($n = 5$), a 10% reduction in d_{eff} due to the photosensitizer would require that the incident light exposure be increased by 74% to maintain the same space irradiance at this depth. Thus, except for treatment of very superficial tumours (when n is small), any change in effective penetration of the light due to photosensitizer absorption is greatly magnified in terms of the irradiance at depth. We note that similar considerations apply if the concentration of blood, or the

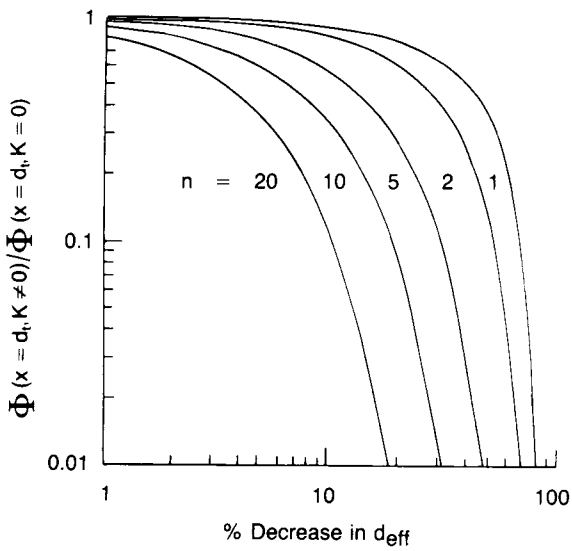

Fig. 5. Computer prediction of ratio of irradiances at treatment depth or radius, d_t, after and before the addition of photosensitizer, plotted as a function of the percentage decrease in effective penetration depth resulting from the photosensitizer absorption. K, concentration of photosensitizer; n, ratio of d_t to the effective penetration depth in the absence of added photosensitizer, d_{eff_0}.

haemoglobin oxygenation status, changes during irradiation (5).

In the tumour response studies of Hisazumi et al (16), using HPD and pulsed or CW red light to treat transplanted human bladder carcinoma tumours in nude mice, injected doses of 10 mg/kg HPD were used, and the tumours were 10 mm thick (d_t = 10 mm). Assuming for tumour tissue that Σ_{at} is approximately $10^{-1}\,mm^{-1}$, d_{eff_0} is approximately 2 mm, and that the HPD concentration was 20 μg/g in the tumour at the time of treatment, complete bleaching of the HPD by the pulsed light would increase the irradiance at 10 mm depth by around 70%, compared with CW irradiation. It is conceivable that this could alter the response rate. In the studies by Hizasumi et al, of light penetration through the normal rabbit ear, where the penetration depth ('extinction length') for high-power pulsed light was found to be 3.4 times that for CW light, an added absorption of around $0.1\,mm^{-1}$ would be required to account for the difference, even assuming Σ_{at} to be as low as $10^{-2}\,mm^{-1}$. For HPD this corresponds to 140 μg/g. Unfortunately Hisazumi et al do not state the dose of HPD administered in this experiment but it seems unlikely that the dose would have been in this very high range.

As regards the findings of Powers and Brown (14) that a 10 mg/kg injection of HPD in rats caused a marked change in the transmission of 630 nm light through normal brain, we will assume that Σ_{at} is approximately $10^{-2}\,mm^{-1}$ (Table 1). Applying equation 3 to the $F(r)$ versus r data of Powers and Brown, the effective penetration depths in control rats and in HPD-injected rats are approximately 1.6 and 1.3 mm, respectively. This change implies (Fig. 4) a tissue concentration of HPD greater than 10 μg/g, which is higher by 1–2 orders of magnitude than expected for normal brain according to other HPD uptake studies (22, 23). We note that in these experiments the light source was an interstitial 400-μm fibre carrying an optical power of 50 mW, and we suggest that the changes in light transmission observed may have been the result of the photodynamic response of the brain tissue, rather than being due to the added-absorber effect.

With HPD as used clinically, at injected doses of typically 2 mg HPD/kg body weight, the concentration, K, of the photosensitizer in the tumour tissue is probably less than 5 μg/g i.e. $\Sigma_{ap} = K.\epsilon_p < 3.5 \times 10^{-3}\,mm^{-1}$ (for $\epsilon_p = 7.10^{-4}\,mm^{-1}/(\mu g/g)$ at the 630 nm absorption peak). Even for tissues with very low inherent absorption of the order of $10^{-2}\,mm^{-1}$, a concentration of 5 μg/g would change d_{eff} by only about 5% and in treating larger tumours (say, n = 5), the decrease in space irradiance at depth would be only about 25%. For more typical tissues (Σ_{at} of the order of $10^{-1}\,mm^{-1}$), the corresponding changes in d_{eff} and irradiance at depth are about 1% and 5%, respectively. One may conclude, therefore, that the effect can normally be ignored for photodynamic therapy using clinically relevant HPD (Photofrin I or II) doses and 630 nm light. Only if the HPD dose is very high, so that $K > 10\,\mu g/g$ of the treatment depth is large ($n > 5$), or the tumour is very lightly pigmented ($\Sigma_{at} < 10^{-2}\,mm^{-1}$), will there be a substantial change in space irradiance at depth.

A significantly different situation holds, however, with respect to other photosensitizers. Thus (15), the phthalocyanine AlSPc has an extinction coefficient at 675 nm approximately 22 times that of HPD at the 630 nm peak, i.e. $0.015\,mm^{-1}/(\mu g/g)$. A 20%, or 50% decrease in d_{eff} would result from a concentration of only 1 μg/g in tissue with Σ_{at} of the order of 10^{-2} or $10^{-1}\,mm^{-1}$ respectively. Likewise a concentration of 10 μg/g would reduce the penetration depths by about 80% or 50%.

As stated in the introduction, for this particular sensitizer, Bown et al (15) have recently published experimental values for the radius of necrosis in normal rat liver at a fixed 675 nm light exposure (50 J at 100 mW) delivered via an interstitial fibre, using different injected doses of AlSPc. These data are shown in Fig. 6, with the injected amounts (in mg AlSPc/kg body weight) converted to concentration in liver (μg/g) from the fluorescence uptake measurements of Bown et al. The solid curve in this figure was obtained as follows. As has been observed in other studies of tissue response to PDT in vivo (1, 25, 26), a sharply-defined radius of necrosis, R, was produced in these experiments. It is, therefore, reasonable to postulate the existence of a 'threshold photodynamic dose', T, which must be exceeded for necrosis to occur. We then assume, for simplicity, that

$$T = \Sigma_{ap}\,\phi_R t \qquad (5)$$

where ϕ_R is the space irradiance at the effective treatment radius R, and t is the treatment time. ϕ_R is a function of the optical properties of the tissue and so depends on both Σ_{at} and Σ_{ap}. For the interstitial fibre irradiation technique we obtain from equation 3

$$T = Pt\Sigma_{ap}\Sigma_{eff^2}(4\pi\Sigma_a R)^{-1}\,e^{-\Sigma_{eff}R} \qquad (6)$$

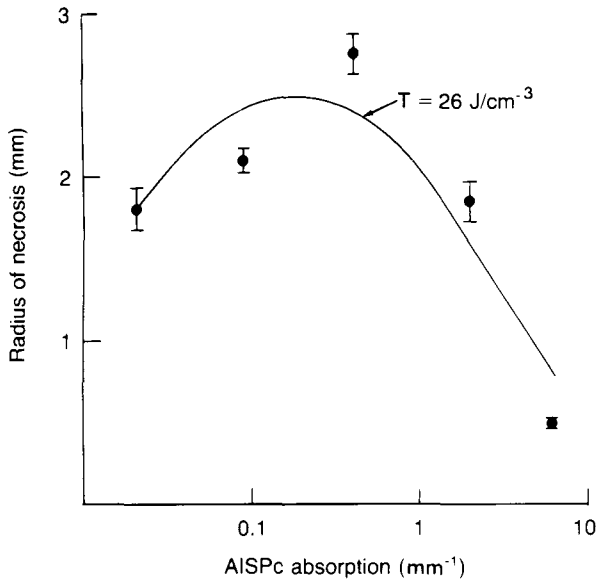

Fig. 6. Depth of necrosis in rat liver, irradiated by using an interstitial fibre, versus added absorption of the photosensitizer AlSPc at 675 nm. The measured values are from Bown et al (15), while the curve shows the best least-squares fit of equation 6 to these data (Σ_{at} and Σ'_{st} fixed at 0.27 and 1.7 mm^{-1}, respectively).

A similar form can be obtained from equation 1b for surface irradiation. Note that T then represents the total energy which must be absorbed by the photosensitizer per unit volume of tissue to produce necrosis.

The curve shown in Fig. 6 is the best least-squares fit to the data of Bown et al with Σ_{at} and Σ'_{st} for liver as from Table 1, and T as the single free parameter. The main discrepancy between the fitted curve and the measured data is at the highest photosensitizer concentration for which the irradiance falls off more rapidly than predicted by equation 6. However, equation 3, and hence equation 6, assumes an isotropic point source of light in the tissue. This produces a larger irradiance laterally than that from a real optical fibre of limited numerical aperture, so that, quantitatively, this discrepancy between theory and experiment would be expected.

CONCLUSIONS

The phantom studies reported here have served to validate the use of diffusion theory, and to investigate the effect of the optical absorption of photosensitizers on the effective penetration of photoactivating light. The effect ranges from being probably negligible in the case of HPD used clinically to potentially very important for sensitizers with high extinction coefficient and tissue concentration, as demonstrated by the example of AlSPc. The general model predictions given here may be used to determine whether or not the effect must be taken into account for any other photosensitizer of known absorption coefficient. The added-absorber method may also be valuable for determination of the absorption and reduced scattering coefficients of intact tissues, and thus complement and supplement other direct and indirect measurements of such optical properties.

The comparison of the model calculations with the data of Bown et al (15) further confirms the diffusion theory modelling and supports the concept of a threshold photodynamic dose. The analysis of in vivo data for radius or depth of damage from PDT as a function of light irradiance and photosensitizer concentration may be a useful means of comparing quantitatively the photodynamic effectiveness of different photosensitizers in different tissues in vivo, by determining the equivalent T-values. In such, more detailed analyses of additional effects such as photodestruction of the photosensitizer as recently suggested by Potter (27) may have to be taken into account.

ACKNOWLEDGEMENTS

This work was supported by The National Cancer Institute of Canada. The authors wish to thank Catherine Edwards for technical assistance and Donna Laking for preparing the manuscript.

REFERENCES

1 Dougherty TJ, Weishaupt KR, Boyle DG. Photodynamic sensitizers. In: DeVita VT et al (eds) *Cancer: Principles of Oncology*. Philadelphia: Lippincott, 1985, **2**:2272–9
2 Wilson BC, Patterson MS. The physics of photodynamic therapy. *Phys Med Biol* 1986, **31**:327–60
3 van Gemert JC, Berenbaum MC, and Gijsberg GHM. Wavelength and light-dose dependence in tumour phototherapy with haematoporphyrin derivative. *Br J Cancer* 1985, **52**:43–9
4 Doiron DR, Svaasand CO, Profio AE. Light dosimetry in tissue: application to photoradiation therapy. In: Kessel D, Dougherty TJ (eds) *Porphyrin photosensitization*, New York: Plenum, 1983:63–76
5 Wilson BC, Jeeves WP, Lowe DM. In vivo and postmor-

tem measurements of the attenuation spectra of light in mammalian tissues. *Photochem Photobiol* 1985, **42**:153–62
6 Profio AE. *Radiation shielding and dosimetry*, New York: Wiley, 1979
7 van Gemert JC, Hulsbergen Henning JP. A model approach to laser coagulation of dermal vascular lesions. *Arch Dermatol Res* 1981, **270**:429–39
8 Wan S, Anderson RR, Parrish JA. Analytic modeling for the optical properties of the skin with in vitro and in vivo applications. *Photochem Photobiol* 1981, **34**:493–9
9 Svaasand LO, Ellingsen R. Optical penetration in human intracranial tumors. *Photochem Photobiol* 1983, **38**:283–99
10 Marynissen JPA, Star WM. Phantom measurements for light dosimetry using isotropic and small aperture detectors. In: Doiron DR, Gomer CJ (eds) *Porphyrin photosensitization and treatment of tumors*, New York: A.R. Liss, 1984:133–48
11 Wilksh PA, Jacka F, Blake AJ. Studies of light propagation through tissue. In: Doiron DR, Gomer CJ (eds) *Porphyrin photosensitization and treatment of tumors*, New York: A.R. Liss, 1984:149–61
12 Profio AE, Sarnaik J. Fluorescence of HPD for tumor detection and dosimetry in photoradiation therapy. In: Doiron DR, Gomer CJ (eds) *Porphyrin photosensitization and treatment of tumors*, New York: A.R. Liss, 1984:163–75
13 Flock ST, Patterson MS, Wilson BC, Burns DM. Optical properties of tissues at 632.8 nanometers. *Photochem Photobiol* 1986, **43**:15S (abstr)
14 Powers SK, Brown JT. Light dosimetry in brain tissue: an in vivo model applicable to photodynamic therapy. *Lasers Surg Med* 1986, **6**:318–22
15 Bown SG, Tralau CJ, Coleridge Smith PD, Akdemir D, Wieman TJ. Photodynamic therapy with porphyrin and phthalocyanine sensitization—quantitative studies in normal rat liver. *Br J Cancer* 1986, **54**:43–52
16 Hisazumi H, Naito K, Misaki T, Koshida K, Yamamoto H. An experimental study of photodynamic therapy using a pulsed gold vapor laser. In: Jori G, Perria C (eds) *Photodynamic therapy of tumors and other diseases*, Padova: Liberian Progetto Editore, 1985:251–4
17 Cowled PA, Grace JR, Forbes IJ. Comparison of the efficacy of pulsed and continuous-wave red laser light in induction of photo-cytotoxicity by hematoporphyrin derivative. *Photochem Photobiol* 1984, **39**:115–17
18 McKenzie AL. How may external and interstitial illumination be compared in laser photodynamic therapy? *Phys Med Biol* 1985, **5**:455–60
19 Wilson BC, Adam G. A monte carlo model for the absorption and flux distributions of light in tissue. *Med Phys* 1983, **10**:824–30
20 Wilson BC, Muller PJ, Yanch JC. Instrumentation and light dosimetry for intra-operative photodynamic therapy (PDT) of malignant brain tumors. *Phys Med Biol* 1986, **31**:125–33
21 Preuss LE, Bolen FP, Cain BW. A comment on spectral transmittance in mammalian skeletal muscle. *Photochem Photobiol* 1983, **37**:113–16
22 Gomer CJ, Rucker N, Mark C, Benedict WF, Murphree AL. ^{3}H-hematoporphyrin derivative in athymic nude mice heterotransplanted with human retinoblastoma. *Invest Ophthalmol & Visual Sci* 1982, **22**:118–20
23 Jeeves WP, Wilson BC, Firnau G, Brown K. Studies of HPD and radiolabelled HPD in vivo and in vitro. In: Kessel D (ed) *Methods in porphyrin photosensitization*, New York: Plenum, 1986:51–67
24 Gomer CJ, Dougherty TJ. Determination of ^{3}H- and ^{14}C-hematoporphyrin derivative distribution in malignant and normal tissue. *Cancer Res* 1979, **39**:146–51
25 Berenbaum MC, Bonnett R, Scourides PA. In vivo biological activity of the components of hematoporphyrin derivative. *Br J Cancer* 1982, **45**:571–81
26 Pimstone NR, Gandhi SN. Optimal photodynamic band of red light on hematoporphyrin derivative (HPD) photoradiation. In: Doiron DR, Gomer CJ (eds) *Porphyrin photosensitization and treatment of tumors*, New York: Liss, 1984:673–8
27 Potter WR. The theory of photodynamic dosimetry: consequences of photodestruction of sensitizer. *SPIE Lasers Med* 1986, **712**: in press

Key words: Dihaematoporphyrin ether; Aluminium chlorosulphonated phthalocyanine; Penetration depth; Diffusion theory; Photodynamic therapy