Biochemical Pharmacology, Vol. 33, No. 8, pp. 1167–1180, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

COMMENTARY

PHOTOAFFINITY LABELS AS PHARMACOLOGICAL TOOLS*

JEFFREY S. FEDAN,†‡§ G. KURT HOGABOOM†‖ and JOHN P. O'DONNELL¶

† Physiology Section, NIOSH, Morgantown, WV 26505, U.S.A.; and ‡ Department of Pharmacology and Toxicology and ¶ School of Pharmacy, West Virginia University Medical Center, Morgantown, WV 26506, U.S.A.

Photoaffinity labels are ligands that have an inherent affinity for a binding site and possess biological activity but that also contain a photosensitive functional group which, when photoactivated with light, is capable of forming a covalent bond at or near the binding site. Unlike electrophilic affinity labels, their association with the recognition site will ordinarily be reversible until photolysis is initiated. The covalent linkage between photoprobes and binding sites has facilitated the biochemical and biophysical characterization and isolation of many cellular molecules. This is one important goal of the photoaffinity labeling technique. Short of this level of approach, there are several applications of photoaffinity labels, especially in pharmacology, related to the understanding of the functional interactions of agents with living cells. The purpose of this commentary is several-fold. First, there have been few reports on the use of photoaffinity labels in intact tissues. Our experience and that of others indicate that the technique is readily and directly applicable in intact cell and organ preparations. Therefore, we will advocate consideration of this particular application on a wider basis. Second, we wish to emphasize that in experimental designs some attention should be paid to the photochemical properties of these compounds. Third, guidance is provided on pitfalls which can be encountered and avoided; problem areas are discussed. Last, potentially useful applications not yet under study, which could evolve from studies on intact tissues, are suggested for future experimentation.

General methodological considerations

Several excellent and comprehensive reviews of the photoaffinity labeling technique are available [1–9]. These provide information on approaches to the synthesis of photoaffinity labels, photochemical mechanisms, advantages and disadvantages of photoaffinity labels, comparisons of different photoprobe moieties, and criteria and other theoretical considerations important to the design of valid experiments. Many of these reviews also contain extensive lists of compounds that have been employed in biochemical applications; little of this information will be repeated or updated here. The number of reports on photoaffinity labeling is increasing steadily as the advantages of this powerful technique become known. The focus of this discussion will be on the interaction of photoaffinity labels with receptors or with sites of drug action or of related pharmacological interest. Table 1 lists some current usages of photoaffinity labels from the pharmacological literature. This sampling provides representative examples of the kinds of investigations underway, and there are admittedly many omissions. For some labels the report cites only one of a series from that laboratory, and the one chosen was used to illustrate an applications aspect rather than an interpretive one. For some binding sites several different compounds have been studied; the merits and disadvantages of one compound compared to another are discussed sometimes in the individual reports.

Most of the receptor systems listed in Table 1 were well-characterized prior to the application of the photoaffinity labeling technique due to the availability of specific and reversibly-binding agonist and antagonist ligands with biological activity and high affinity. Experiments which establish the ability of the ligands to evoke or modify biological responses are often followed by biochemical assays of ligand–binding site interactions. In these assays, the interaction between the ligand and its binding site is "silent". Since reversibly-bound ligands of even the highest affinity will dissociate during chromatographic, electrophoretic or other procedures which could be used to isolate receptors, the ability to label covalently an otherwise silent site with a radiolabeled photoprobe has allowed significant progress to be made toward the characterization of receptors.

There are many agonists which are thought to induce responses via receptors, but for which a specific antagonist is or was not available. By itself, the fact that an agent elicits a response in a cell does not prove that a receptor exists. In many receptor systems for which specific antagonists are lacking, receptors and their subtypes have been characterized extensively with relative potency series for agonists. This alternative approach to the understanding of the interaction between endogenous agonists and their receptors cannot yield as much information as that provided by the effect of a specific antagonist

* Supported, in part, by NIGMS 5 T32 GM07039 and a grant from the American Heart Association-West Virginia Affiliate.

§ Address for correspondence: Jeffrey S. Fedan, Physiology Section, NIOSH, 944 Chestnut Ridge Road, Morgantown, WV 26505.

‖ Present address: Smith Kline French Laboratories, 620 Allendale Road, King of Prussia, PA 19406.

Table 1. Some applications of photoaffinity labels in pharmacology*

Binding site	Preparation	Parent ligand	Reference
	Membranes		
β-Adrenergic receptor	Turkey erythrocytes	Propranolol	10
β-Adrenergic receptor	Turkey erythrocytes	Iodocyanopindolol	11
β-Adrenergic receptor	Erythrocytes; several species	Iodopindolol	12, 13
β-Adrenergic receptor	Rat reticulocytes, liver	Acebutolol	14
β-Adrenergic receptor	Frog erythrocytes	Benzylcarazolol	15–17
α-Adrenergic receptor	Rat hepatic membranes	Prazosin	18
Insulin receptor	Several rat tissues	Insulin	19–22
Acetylcholinesterase	Human erythrocytes	Quarternary ammonium compounds	23
Nicotinic receptor	Torpedo membranes	Trimethisoquin	24, 25
Muscarinic receptor	Rat cerebral cortex	Tropine	26
Muscarinic receptor	Rat brain and heart membranes	3-Quinuclidinyl benzilate; *N*-methyl-4-piperidyl benzilate	27, 28
Angiotensin II receptor	Dog adrenal cortex	Angiotensin II	29
Opiate receptor	Bovine caudate nucleus	Enkephalins	30
N-Formyl peptide receptor	Neutrophil plasma membranes	Chemotactic peptide	31
Biogenic amine transporter	Bovine chromaffin granules	Serotonin	32
	Cell components, enzymes; Soluble systems		
Estrogen receptor	Rat uterus cytosol	Estrogens	33
α-Fetoprotein	Rat amniotic fluid	Estrogens	34
Na^+,K^+-ATPase	Purified enzyme	Digitoxin	35
Na^+,K^+-ATPase	Purified enzyme	Cymarin	36
Na^+,K^+-ATPase	Purified enzyme	ATP	37
Na^+,K^+-ATPase	Kidney microsomes, purified enzyme	Lipophilic probe	38
Na^+,K^+-ATPase	Purified enzyme	Ouabain, strophanthidin	39–41
Corticotropin receptor	Sheep pituitary gland	ACTH	42, 43
Neurophysins	Bovine posterior pituitary	Tripeptide (Met–Tyr–Phe)	44, 45
cAMP-dependent protein kinase	Soluble and membrane-bound enzyme; brain, heart, erythrocyte	cAMP	46–49
cAMP-dependent protein kinase	Erythrocyte membranes; holoenzyme	ATP	48, 50
cGMP-dependent protein kinase	Bovine arterial soluble enzyme	cIMP	51
Ribosomal protein S7	*Escherichia coli* ribosomes	Several antibiotics	52
DNA		Ethidium	53
Lysine-binding site	Human plasmin	Glycyl-L-lysine	54
S-Adenosylmethionine binding site	Porcine catechol *O*-methyltransferase	*S*-Adenosylmethionine	55
β-Subunit	Rabbit phosphorylase	ATP; ATP-2′-3′-dialdehyde	56
Ca^{2+}-ATPase	Sarcoplasmic reticulum	ATP	57
	Intact cells, Tissues		
Sodium channel	Isolated squid giant axon	Quinicrine	58
Sodium channel	Isolated frog skeletal muscle	Tetrodotoxin	59
Sodium channel	Neuroblastoma cells; rat brain synaptosomes	Scorpion toxin	60
Nicotinic receptor	Skeletal muscle	Acetylcholine	61
Nicotinic receptor	Isolated *Electrophorus* electroplaques	Di-, trimethisoquin	62
Nicotinic receptor	Isolated frog skeletal muscle	Quarternary ammonium compounds	23
Insulin receptor	Adipocytes	Insulin	63
Thyroid hormone nuclear receptor	Rat pituitary cells (GH_1)	3,5,3′-Triiodo-L-thyronine	64
Serotonin carrier protein	Guinea-pig brain synaptosomes, platelets	Imipramine	65
Adenosine receptor, adenosine deaminase	Human platelets	Adenosine	66
ADP receptor	Human platelets	ADP, AMP	67, 68
N-Formyl peptide receptor	Human polymorphonuclear leukocytes	Chemotactic peptide	69, 70
cAMP binding protein	Human sperm, *Dictyostelium discoideum*	Cyclic AMP	71, 72
Angiotensin II receptor	Isolated rabbit aorta, rat portal vein and uterus	Angiotensin II	73–75
Angiotensin II receptor	Isolated rat adrenal granulosa cells	Angiotensin II	29

Table 1 (*continued*)

Binding site	Preparation	Parent ligand	Reference
Histamine receptor	Isolated guinea-pig vas deferens, aorta; dog trachea	Histamine	76, 77
P_2-purinergic receptor	Isolated guinea-pig vas deferens, urinary bladder, Taenia coli; rabbit bladder	ATP	78–85
Calcitonin receptor	547D Breast cancer cells	Calcitonin	86†

* For simplicity, receptors are not classified by subtype; liberties have been taken to describe some preparations.
† An interesting approach to ligand derivatization by enzymatic means is presented in this report.

on physiological responses. To use an example from our own area of interest, an evaluation of the functional role of ATP in autonomic neurotransmission [87] has been difficult to establish directly, due to the unavailability of a specific ATP (P_2-purinergic) receptor antagonist [88], and evidence for a role of adenine nucleotides in neurotransmission was thus largely circumstantial [89]. Using the reasoning that agonist agents could gain antagonist properties by the addition of a photosensitive chemical moiety, an ATP photoaffinity label ($ANAPP_3$*) described by Jeng and Guillory [90] and Russell *et al.* [91] produced in intact smooth muscle preparations a specific antagonism of ATP-induced responses, thereby identifying the existence of the P_2-receptor [78] and allowing an appraisal of a neurotransmitter role for ATP [79, 81, 82, 84]. Thus, a photoaffinity antagonist provided information on the P_2-receptor which complimented the existing information on relative potencies of adenine nucleotides in smooth muscle [92, 93]. Hence, the value of a photoaffinity label in pharmacological studies is that it allows the characterization of a receptor and its interaction with endogenous substances with the same covalently-bound antagonist agent which can be used in subsequent biochemical analyses.

The application of photoaffinity labels in the pharmacological literature may be divided broadly (Table 1) into those involving broken cell preparations (isolated membranes, proteins, enzymes) and those in which cellular and functional integrity is maintained under reasonably physiological conditions. The latter preparations may be categorized according to whether they are dispersed in suspension (synaptosomes, adipocytes, platelets, etc.) or are used in an intact condition (muscles). The approach to photoaffinity labeling experiments will, to some degree, be influenced by the nature of the preparation, but the basic experimental design elements are similar. Briefly, the photoaffinity label should have an affinity for the binding site of the parent compound and bind reversibly to the site prior to photoactivation (i.e. under dark conditions). Following photolysis in the presence of the site, a covalent attachment of the photoaffinity label at or near the site should occur. The rate and extent of covalent insertion should be lessened if photoactivation is performed in the presence of a competing ligand. Prior photoincorporation of an unlabled photoprobe should diminish subsequently the amount of covalent insertion of a radiolabeled form of the compound. Transient exposure of the nonphotolyzed photoaffinity label under dark conditions with the binding site should not yield an irreversible effect like that observed following photolysis. A photoaffinity label will ordinarily be incapable of forming covalent attachments if it is adequately photolyzed beforehand in the absence of the binding site.

The light source used for photolysis must be suited to the absorption spectrum of the photoaffinity label. Most of the compounds in Table 1 are either azido or arylazido derivatives, and have absorption maxima at only ultraviolet or at both ultraviolet and visible wavelengths respectively [6]. Several kinds of apparatus are used. The simplest involves photolysis of preparations in quartz cuvettes with an ultraviolet lamp and is suitable for soluble enzymes, proteins, etc. and for membranes and cells which are kept suspended with mixing. Heating of the preparations during photolysis is not a problem with most ultraviolet light sources. Since ultraviolet light does not readily penetrate glass, of which most organ chambers for studies on isolated muscles are constructed, the design of apparatus for photoactivation of azido compounds in the presence of intact muscles may be cumbersome [74]. Arylazide analogs are perhaps better suited for studies on isolated preparations inasmuch as visible light sources and glass apparatus can be used. However, steps must be taken to avoid overheating of the preparations which can occur with high intensity visible light sources. One approach to this problem which is used very commonly is to photolyze chilled preparations. However, the practice of determining the kinetic properties of the nonphotolyzed photoprobe at one temperature but yet photolyzing at a lesser temperature does not consider entropic changes which may modify binding site structure and hence its affinity for the ligand [94]. An extreme case of temperature-dependent effects on receptors is represented by the proposal that a temperature-dependent qualitative change occurs in adrenergic receptors [95, 96]. In living cell preparations which maintain homeostasis with numerous active transport processes, lowering the temperature during photolysis may result in ionic imbalances. For example, reduced sodium–potassium pumping and the intracellular accumulation of calcium resulting from inhibition of sodium–calcium exchange will

* Abbreviations: $ANAPP_3$, arylazido aminopropionyl ATP (3′-*O*-{3-[*N*-(4-azido-2-nitrophenyl)amino]propionyl} ATP); $ANABP_3$, arylazido aminobutyryl ATP (3′-*O*-{4-[*N*-4-azido-2-nitrophenyl)amino]butyryl}ATP); and AAH, 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole.

affect the pharmacological behavior of cells. For all of the above reasons it would seem appropriate to photolyze preparations at a temperature which is relevant to ensuing experiments.

It is important to assess the possible effects of the irradiation procedure in control experiments [41]. Ideally, irradiation alone should have no effect. However, ultraviolet light damages the opiate receptor [29]. Preparations of functioning cells may be more sensitive to light effects than are purified systems. For example, 254 nm ultraviolet light (30 min; cf. Ref. 78) and >320 nm visible light (3 min; cf. Ref. 74) reduced contractivity of guinea-pig vas deferens and ileum and rabbit aorta, respectively, and ultraviolet and visible light evoke relaxation in several smooth muscles [75, 97–99]. The mechanisms of these light-induced effects are undefined, but could involve simple but important chemical alterations in cellular components [99]. For example, ultraviolet frequencies reduced sodium channel conductance [100] in nerve fibers as well as veratridine-stimulated, saxitoxin-sensitive ^{22}Na loss and saxitoxin binding in synaptosomes [101]. Information on the kinetics of photoactivation (*vide infra*) in combination with knowledge of the duration and type of irradiation that can be tolerated by a preparation should allow some balance to be struck so as to achieve desired levels of covalent insertion and, at the same time, reduce the toxic effects of light. For example, visible light was used to photoactivate an arylazido ATP analog ($ANAPP_3$) in the presence of the guinea-pig vas deferens after it was noted that a 30-min irradiation with ultraviolet light results in toxicity while visible light is innocuous [78]. More than an hour of irradiation with visible light is required to cause complete photodecomposition of the compound [83], while the process is completed within 5 min when a 254 nm source is used (unpublished observations). In hindsight, the greater photolytic efficiency of ultraviolet light compared to visible light might have allowed a much briefer irradiation period to be used, and the toxicity of shorter wavelengths might have been eliminated.

A large number of different experimental conditions and light sources of varying frequency and intensity have been used for photolysis. There is an understandable tendency for investigators who seek only qualitative effects to choose a suitable light source and other conditions which guarantee minimally that successful photoincorporation will be evident on polyacrylamide gels. Thus, while the properties of a nonphotolyzed compound are usually examined rigorously and the time-course of photoincorporation and effect is evaluated less frequently [15, 25–27, 30, 34, 37, 39, 47, 50, 56, 60, 68, 102–110], the kinetics of photoactivation, which is easily monitored spectrophotometrically [5, 18, 29, 34, 43, 55, 66, 68, 74, 76, 83, 108–115], is largely ignored. The latter two time-courses are closely linked. There are several ramifications of these considerations. The general lack of standardized procedures for given agents would make it difficult to obtain similar results in different laboratories. The rate at which binding sites become covalently labeled will be a function of the concentration of reactive intermediate (e.g. nitrene) produced by photolysis. Consumption of native compound will occur, and the concentration of native compound and its intermediate will decrease with time. Consequently, the rate of covalent insertion will decrease as well. Assuming that a given concentration of photoprobe and the binding site are at equilibrium prior to photolysis, the factors which would be expected to affect the rate of photoactivation, and hence the rate and extent of covalent insertion into binding sites, would include (1) the spectral characteristics and intensity of the light which reaches the compound in solution; this will be affected by the composition, geometry and reflectance of the vessel, the presence of filters, coolants, etc., (2) the distance of the light source from the preparation (light intensity declines with the inverse square of distance); in only a limited number of studies [32, 39] has the intensity of incident light been reported, and (3) the turbidity of the sample or the presence of other light-absorbing substances; for example, proteins, nucleotides, etc. absorb ultraviolet light in frequencies used for photolysis. Some additional considerations apply to studies on isolated intact tissues, e.g. muscles. Unlike membrane suspensions and soluble samples in which uniformity during photolysis can be maintained by mixing, only a small percentage of the photoprobe is in the biophase compared to the total amount present. Therefore, a bulk of the available compound will be photoactivated in free solution where there are no binding sites for insertion, resulting in substantial waste. Mixing of the bathing solution by bubbling oxygen and carbon dioxide, which often is needed to support tissue function and to maintain physiological pH, does not overcome this problem. (In an oxygen-rich environment, as Galardy and LaVorgna [75] have pointed out, photooxidation or free-radical reactions caused by hydrogen abstraction of products might occur. Purging of the physiological solutions with inert gases prior to photolysis [11, 12, 14, 25, 36, 45, 108, 109], while a practical solution to this particular problem in simpler systems, could compromise tissue viability, however.)

It may be possible to compensate for some sources of photoincorporation variability, namely, the light source and reaction vessels used, by performing the simple experiment of monitoring the time-course of spectral changes of the photoprobe in complete medium minus the sample and quantitating the kinetics of photodecomposition with, for example, apparent half-life. Several examples of this are found in the literature (*vide supra*). Adjustments in apparatus can then be made to obtain the reference apparent kinetics. When this is accomplished, the fractional absorbance change and the percentage of activation of native photoaffinity label, and hence the concentration of reactive intermediate at given time points and the rate and extent of photoincorporation, could approach uniformity in different laboratories. Earlier [83] we suggested that the extent of covalent insertion might be related only to the fractional amount of photoactivation regardless of the time required to reach the desired endpoint. Having reconsidered the fact that the rate of covalent insertion will be related to the instantaneous *concentration* of reactive intermediate, the photolysis period required to achieve a given frac-

tional photoactivation may be an important determinant as well. While it is assumed that a direct relationship exists between fractional changes in absorbance and the degree of photoconversion to reaction products, this information is not available but will be required to evaluate the validity of the above theoretical considerations.

Some additional concepts apply to achieving standardized conditions in isolated intact tissues. The intact tissues in which photoaffinity labels have been used successfully in functional studies (Table 1) are relatively thin, and, presumably, there was sufficient penetration of light into the opaque tissues to produce photoactivation at all depths. In support of this view is the finding that responses of smooth muscles to ATP added exogenously and to ATP released endogenously from nerves [79, 82, 84] were antagonized by $ANAPP_3$, following its photolysis in the presence of the tissues. However, since intact tissues will quench light, it is not known whether the photolabeling of receptors is uniform throughout the tissue; the blockade of receptors on tissue-surface cells would be expected to result in some pharmacological effect. Unlike studies on more isolated systems where the amount of binding sites (e.g. protein concentration) can be adjusted at will, the differences in tissue thickness resulting from biological or experimental variation are more difficult to control. This, in combination with the quenching properties of solid tissues, could affect the level of photoincorporation in individual experiments and affect quantitative measurements. The use of mirrored vessels and several light sources could be useful here. Autoradiographic analysis of the cross-sectional distribution of photolyzed isotopically-labeled photoprobes would help to define the limit of tissue thickness beyond which light of reasonable intensity would no longer be able to penetrate. In some large organs (e.g. heart, liver and kidney) dissection to achieve light-penetrability may not be desirable; some possible approaches to the use of photoaffinity labels in such systems will be mentioned later.

Qualitative pharmacological effects of photoaffinity labels on receptors

Information on the pharmacology of these compounds can be missed if their characterizations are restricted only to receptor-binding assays and no data are available on their abilities to induce, block or modify cellular responses. Studies in which physiological responses are measured are of value because it may be difficult using ligand binding assays to assess whether a photoaffinity label has agonist or antagonist properties before and after photolysis. For example, prior to photolysis diazo acetylcholine produced motor end-plate depolarization in isolated skeletal muscle; following photolysis, a prolonged depolarization remained [61], indicative of long-lived agonist effect. Similarly, Brandenburg *et al.* [63] observed that photolysis of an insulin photoaffinity label evoked a long-lasting lipogenesis in intact adipocytes; such activity would not have been predictable on the basis of electrophoretic studies [22] of the photoincorporation of such compounds into receptor proteins. Schmitt *et al.* [70] observed than an *N*-formyl peptide chemotactic factor derivative stimulated superoxide anion release in polymorphonuclear leukocytes which was blocked by a known receptor antagonist. Likewise, a calcitonin photoaffinity label was able to stimulate adenylate cyclase activity in a cancer cell line [86]. On the other hand, the retention of antagonist activity by photolyzed compounds which is similar qualitatively to that observed prior to photolysis is characteristic of a quarternary ammonium nicotinic receptor antagonist in isolated skeletal muscle [23], and of angiotensin II [73, 74] and H_1-histamine receptor [76, 77] photoaffinity analogs in isolated smooth muscles. The third possibility, that a photoaffinity analog with initial agonist activity is converted following photolysis into an antagonist, was seen with $ANAPP_3$ in several smooth muscle preparations [78, 81, 84] and with an angiotensin II analog in vascular smooth muscle [75].

In a large number of receptor systems, agonist affinity is modulated by GTP whereas antagonist affinity is not. A photoaffinity label which is an agonist prior to photolysis would be photoincorporated into GTP-regulated agonist-binding sites, while compounds which demonstrate antagonistic activity would label preferentially an antagonist site. It seems fundamental to suggest that photoaffinity analogs of agonists be evaluated in functional studies for their abilities following photolysis to continue to evoke or to block responses, to complement data on their abilities to interact with the binding of well-defined agonist and antagonist ligands in binding assays. Information on the nature of the incorporation site (i.e. agonist *vis à vis* antagonist affinity states) might thus be obtained.

Certain aspects of receptor theory require at present the use of functioning tissues or cells for their manifestation. The ability of an agent to evoke a response mediated via a receptor is a function of affinity of the receptor for the drug as well as of the ability of the drug to trigger a response once it occupies the receptor. This latter characteristic, which is structurally-related, is referred to as efficacy. Agonists possess both affinity and efficacy while antagonists have affinity but no efficacy. While theoretical details [115, 116] are beyond this discussion, the terms efficacy and intrinsic activity are not synonymous; the former applies to the ability of an agent to yield a response when "spare receptors" are present. The demonstration of the existence of spare receptors requires the use of a nonequilibrium competitive antagonist to cause the irreversible blockade of a fraction of the number of available receptors. As increasing numbers of receptors are blocked, dose–response curves for full agonists will be shifted progressively to the right, initially without a reduction in maximum response; extensive receptor blockade results eventually in a reduced maximum response. In studies on photoaffinity labels which have antagonist properties, the compounds behave prior to photolysis as equilibrium competitive antagonists in both ligand-binding assays [10, 14–16, 20, 26, 29, 30] and in functional studies [10, 29, 30, 73, 74, 76, 77]: there is competitive displacement of ligand in the former technique and a parallel shift of the dose–response curve to the right of control

without an effect on the maximum response in functional studies. Photolysis causes a transformation of the kinetic characteristics of the compound to that of a nonequilibrium competitive antagonist in functional studies [10, 14, 73, 74, 76, 77], as outlined above. A correlate to the concept of efficacy and spare receptors has not, to our knowledge, been observed in ligand-binding experiments. This is, perhaps, a result of the unavailability of nonequilibrium competitive antagonists for most receptors. However, the large number of photoaffinity labels available may facilitate a rigorous kinetic analysis of the effects of irreversible receptor blockade in ligand-binding assays. Conventional assays based only on equilibrium binding and competitive displacement utilize a number of mathematical relationships to obtain K_D (affinity of ligand), B_{max} (number of binding sites) and n (number of classes of binding sites), none of which include parameters for evaluating efficacy or spare receptors [117] in their derivation. It might be that B_{max} is reduced following photolysis as would be predicted by reducing the available number of binding sites. However, it has not been demonstrated whether receptor or post-receptor events are linked to the efficacy of a drug. There will be a need for substantial theoretical treatment of this problem, especially of the relationship between spare receptors, efficacy and B_{max}. An interesting beginning experiment could involve the influence of photoaffinity labels or nonequilibrium competitive antagonists on ligand-binding assays done in parallel with experiments on functioning cell or intact tissue preparations under two defined conditions: (1) where the antagonist produces only a shift of the dose–response curve of a full agonist to the right and (2) where both a rightward shift and a reduced maximum response for such an agonist are produced.

The restriction of photoaffinity labeling to only a single site will occur if the binding affinity of nonphotolyzed compound is very high, as is true for many compounds in Table 1, and little nonspecific incorporation of the highly reactive intermediates will result. In some systems in which biochemical methods are not available to obtain binding affinity data, and in which nonspecific incorporation and toxicity are possible, an alternative means of evaluating pharmacological specificity of photolabeling should be used. This can easily be performed with agonist or antagonist photoprobes which bind to receptors and exert an antagonist effect following photolysis by examining the effect of photolabeling on responses of the preparation to chemically-unrelated agonists. In a number of functional studies on intact muscle [73–85] and other [14] preparations, photoaffinity label analogs have been shown to exhibit a striking pharmacological specificity. It has been felt generally that photoaffinity analogs with only moderate affinity may not bind tightly enough to avoid nonspecific labeling effects. However, an arylazido histamine analog with a dissociation constant of only 10^{-5}–3×10^{-5} M produced after photolysis a specific antagonism of responses to histamine; responses mediated via other receptors were unaffected [76, 77]. This finding does not prove that only histamine receptors were photolabeled, but it does indicate that several receptors and mechanisms related to excitation–contraction coupling were not. An electrophoretic analysis using radiolabeled compound will be required to evaluate whether functionally "silent" sites were nonspecifically labeled.

Covalent bonding may occur following the photoactivation of a photoprobe which occupies the binding site (true photoaffinity labeling); alternatively, covalent bond formation may occur following the diffusion of reactive intermediate to the binding site of compound which is photoactivated in free solution (pseudophotoaffinity labeling) [6, 118]. While the qualitative pharmacological consequence of photoaffinity labeling by either mechanism should be the same, the photoactivation of excess nonbound ligand compound in free solution, especially if it has a low binding affinity, can result in substantial nonspecific incorporation. The mechanism of photoaffinity labeling may be deduced [6, 118] by photolyzing in the presence of a scavenger (albumin, para-aminobenzoic acid, sulfhydryl reagents) which provides alternative insertion sites for intermediate generated in free solution but which is not a preferred insertion site for intermediate generated at sites of high-affinity binding. Thus, scavengers will reduce the level of insertion if pseudophotoaffinity labeling has occurred [66, 118, 119]. The application of scavengers in functional cell or tissue preparations to elaborate photolabeling mechanisms and to reduce nonspecific labeling must take into account some factors which are of lesser importance in more purified preparations. For example, para-aminobenzoic acid, in concentrations used for scavenging, had pharmacological activity in the isolated guinea-pig vas deferens [119] which was, fortuitously, of benefit in interpreting whether a scavenging effect could have occurred at a receptor level. The use of sulfhydryl reagents such as dithiothreitol for scavenging carries the risk of modifying tissue behavior through the interaction of the agent with other cellular components, e.g. guanylate cyclase [120]. Some other considerations of more general significance include: (1) There should be no interaction between photoaffinity label and the scavenger prior to photolysis. For example, nonphotolyzed $ANAPP_3$ binds to albumin (unpublished observations). Photolysis of $ANAPP_3$ in the presence of albumin would lead to the erroneous conclusion that a reduced incorporation of $ANAPP_3$ into the P_2-receptor results from a scavenging effect on a pseudophotoaffinity labeling process. Additionally, sulfhydryl reagents can react chemically with and reduce arylazides of nonphotolyzed compounds to corresponding aryl amines [121], making the interpretation of a scavenging effect difficult. (2) Some evidence that a photochemical interaction between photoprobe and scavenger has occurred should be sought, especially where photoincorporation into the binding site is *not* reduced by the scavenger. The covalent insertion of photoprobe into the scavenger should yield the formation of a new reaction product. Rigorous chemical analyses of these kinds of products have not been performed; however, spectral changes, such as those which have been reported to occur following photolysis of $ANAPP_3$ in the presence of para-aminobenzoic acid [119], may be useful indicators of the generation of new products.

Photoaffinity labels may provide information on receptor conformation. The topology of most receptors and the configuration of the ligand-binding site complex, points of attachment of the ligand, etc., are unknown. The location of the photosensitive moiety within the receptor protein could be intimate to the recognition (endo) site for the parent compound or displaced from it (exo site). Following photolysis, access to the receptor will be prevented if a covalent bond is formed at the endo site, and also if the formation of the covalent bond at an exo site results in the occupation of the endo site by, perhaps, the parent moiety. Peptide mapping would be required to distinguish between these possibilities. The ability of a photoaffinity label to block receptors will be related to the ability of the receptor to accept the structural modification in the parent ligand as well as to the availability of sterically appropriate sites into which covalent insertion can take place. Structure–activity relationship information is important for completeness. These points can be illustrated with four examples. First, small and large deletions or additions to the N^1, N^6, and C^8 positions in adenine and in the 2′-hydroxyl of ATP produce substantial reductions or modifications in the activity of ATP analogs acting on the P_2-purinergic receptor of the guinea-pig vas deferens [93, 122]; however, 3′-hydroxyl deletions and additions are well tolerated. Thus, $ANAPP_3$, a 3′-hydroxyl derivative of ATP, is better suited for this system than 8-azido ATP [123] or adenine-derivatized analogs. Second, $ANABP_3$, a compound with a slightly larger 3′-hydroxyl-arylazide bridge, while its activity resembles that of ATP and $ANAPP_3$ before photolysis, is a more "potent" antagonist following photolysis than $ANAPP_3$ [83], and it has been suggested among other possibilities that the placement of the arylazide near the endo or exo insertion site is better than is the case with $ANAPP_3$. Third, nonphotolyzed AAH behaves as an equilibrium competitive antagonist of histamine-induced contractile responses in isolated guinea-pig aorta and dog trachealis; the dissociation constant for AAH in the two tissues is not different, implying that the H_1-histamine receptors are similar in the two tissues. In the guinea-pig aorta, photolysis of AAH results in a nonequilibrium competitive antagonism [77]; however, following photolysis the compound has no antagonistic effect in the dog trachealis, and it was suggested that the site available for covalent insertion of the compound at or near the H_1-receptor of the aorta is not present in the trachea. Lastly, differences in the abilities of benzodiazepine receptor ligands to photolabel central and peripheral receptors, despite their abilities to bind with high affinity to these sites, has been noted [124]. Thus, photoaffinity labels may allow the discrimination of subtle topological differences in receptors which, by other pharmacological criteria, appear identical.

Naturally photolabile ligands

Some ligands possess inherent photolability and can be activated with ultraviolet light to form covalent attachments with binding sites. Representative examples are given in Table 2. Undoubtedly there are other compounds already in use which possess this characteristic. It is both surprising and very interesting that such a complex molecule as α-bungarotoxin [131] should be photoactivatable. Since a large number of receptor ligands are already commercially available in isotopic form, it is, perhaps, more important at present that compounds be tested for photolability than the photochemistry involved be completely understood.

The main advantage to be gained using naturally photolabile ligands over photoaffinity analogs of ligands is that the bulky photosensitive moiety is not present. It can be reasonably expected that a covalent bond will be formed at the site of recognition rather than at exo sites. This should facilitate the understanding of the molecular location of groupings important in recognition of the ligand by the binding site. A second advantage is that the potency and

Table 2. Some pharmacological applications of naturally photolabile, underivatized ligands

Binding site	Preparation	Parent ligand	Reference
Benzodiazepine receptor	Rat brain membranes	Flunitrazepam	102–104, 124–126
Benzodiazepine receptor	Bovine cerebral cortex homogenates	Nitrazepam	125
Benzodiazepine receptor	Rat brain membranes	Clonazepam	125, 126
5-Hydroxytryptamine receptor	Bovine caudate membranes	Lysergic acid diethylamide	127
Dopamine receptors	Isolated *Mytilus* smooth muscle	Dopamine	128
Adenosine transporter	Rat forebrain membranes	Nitrobenzylthioinosine	129
Nicotinic receptor	*Torpedo* membranes	Triphenylmethylphosphonium	130
Nicotinic receptor	*Torpedo* membranes	α-Bungarotoxin	131
Nicotinic receptor	*Torpedo* membranes	Chlorpromazine, trimethisoquin, phencyclidine, perhydrohistrionicotoxin	132
Steroid binding proteins	Rat liver, rabbit uterus cytosol	α-β-Unsaturated steroids	105
Steroid receptor	*Xenopus* oocyte plasma membrane	Synthetic progestin (R5020)	106
Ecdysterone receptor	*Drosophila* salivary glands	Ecdysterone	133
Ribosomal protein S7	*Escherichia coli* ribosomes	Tetracycline	111
DNA		Anthracyline antibiotics	107
Opiate receptor	Isolated ileal smooth muscle	Morphine epoxide	134

efficacy of the compound will not have been changed as can occur when a parent ligand is derivatized with a photosensitive moiety.

The guidelines, criteria, pitfalls and other considerations mentioned here by us and reviewed in detail by others all apply to the use of naturally photolabile compounds. Since photoactivation of these compounds will require ultraviolet light (few ligands of pharmacological interest absorb light in visible frequencies), and in view of the potential effects of ultraviolet light discussed earlier, care will be needed to establish that covalent bonds do not occur as a result of light-induced molecular rearrangements in the binding site itself.

Heterobifunctional photoaffinity labeling

This is a fairly recent technique [112] which has been used to photolabel binding sites for peptide ligands (Table 3). It is similar in principle to affinity labeling but involves the use of heterobifunctional crosslinking reagents in which one end of the reagent containing a nucleophilic imidoester (which reacts spontaneously with primary amines, for example, in peptides) or other reactive grouping [136, 145] is connected via a bridge of varying length [135] to a photoactivatable moiety, usually an azido grouping. In a two-step procedure, the ligand is coupled first to the imidoester of a photoactivatable heterobifunctional crosslinking reagent, of which several are being used. Second, the coupled ligand product is incubated with the binding site and photolyzed, causing the formation of a covalent attachment of the derivatized ligand to the binding site, or a "cross-link". (The term "cross-link" is a carryover from the affinity labeling technique in which bifunctional cross-linking reagents are used. The term is less often used for photoaffinity labels which are not peptides.) Table 3 lists some representative recent applications of heterobifunctional photoaffinity derivatives in studies on both intact cell and broken cell preparations.

It is noteworthy that there are only a few reports [113, 137–139, 141, 142] on the biological activities of heterobifunctional photoaffinity labels or on the abilities of these compounds to initiate or modify responses mediated via the receptor sites for which they were designed. The emphasis of their use centers around the ability, when radiolabeled and used in conjunction with electrophoresis, to provide information on the molecular size of the binding site, under conditions in which the concentration of the *parent* ligand is known to interact with the binding site. In only a limited number of studies [108, 109, 112, 113, 136, 139, 146] have attempts been made to establish their photochemical kinetic characteristics and the kinetics and specificity of photoincorporation (i.e. a reasonably rigorous characterization of the compound with respect to the criteria for using the photoaffinity labeling technique).

One problem with the use of heterobifunctional photoaffinity label derivatives of macromolecular peptides is that there is some uncertainty in the location of the cross-linking reagent in the ligand. In ligands for which information on the number of primary amines has been determined from primary structure, the number of amines derivatized by the cross-linking reagent is in agreement with the theoretical number of equivalents per mole of ligand [139], while in other cases fewer amines are derivatized than are available [112, 137]. The tertiary structure of the proteins may restrict access of the cross-linking reagent to some amines. Nevertheless, when evidence is obtained that not all amines in the ligand have reacted with the cross-linker, the question may be raised as to whether the same amines have reacted in all the molecules so as to yield a homogeneous derivative or whether there are different extents and locations of derivatization, thereby yielding a heterogeneous population of molecules [112]. Insofar as the nature and size of substituents added to ligands are known to affect their affinity and efficacy, variability in the location of the cross-linking arms and of molecular topology could affect the binding of the compound prior to photolysis. If there is nonuniformity in the location of the cross-link arms, then the number and location of covalent insertions into the binding site could vary as well. It is not yet demonstrated whether endo or exo sites are labeled by these compounds.

This somewhat pessimistic appraisal of the pitfalls involved in the use of these compounds is entirely offset by the remarkable results obtained with them.

Table 3. Some applications of heterobifunctional photoaffinity labels

Binding site	Preparation	Parent ligand	Reference
Lectin receptor	Erythrocyte ghosts	Concanavalin A	112, 135
Lectin receptor	Pig spleen lymphocytes	Phytohemagglutinin	136
Insulin receptor	Adipocyte and liver plasma membranes	Insulin	19, 113
Lutropin receptor	Granulosa cells	Human choriogonadotropin	137, 138
Epidermal growth factor receptor	Mouse 3T3 cells	Epidermal growth factor	139
Glucagon receptor	Rat liver plasma membranes	Glucagon	140
Enzymes, binding proteins	Troponin I, myosin light chain kinase, Ca^{2+}-sensitive phosphodiesterase; adipocyte plasma membranes	Calmodulin	141, 142
Nicotinic receptor	*Torpedo californica* membranes	α-Bungarotoxin	143
β-Adrenergic receptor	Erythrocyte membranes	*p*-Aminobenzylcarazolol	144
N-Formyl peptide	Human polymorphonuclear leukocytes	Chemotactic peptide	29

The studies listed in Table 3 indicate that these compounds are very specific in their abilities to label discrete binding sites.

In some of the above studies [19, 112, 113, 135–139, 141] on heterobifunctional photoaffinity labeling, the ligands were derivatized with cross-linking reagent prior to their incubation with the binding site. An alternative methodology consists of derivatizating the ligand when it is bound to the binding site [140, 142, 147], using protocols similar to those used with nonphotoactivatable bifunctional cross-linking reagents (e.g. disuccinimidyl suberate; [145, 148]), and then photolyzing the preparation. An important difference between these approaches is that, in the latter one, amine groupings within the receptor (the ligand may dissociate and reassociate during exposure to cross-linker) or in moieties close to the recognition site would be expected to react with the cross-linking reagent as well as those contained in the ligand itself. Molecular perturbations in either location resulting from chemical additions could alter the binding between the ligand and the receptor, and result in an altered affinity of the site for the ligand prior to photolysis and/or in the formation of covalent bonds during photolysis to new regions. Should the latter occur then incorrect information on nearest neighbor relationships could evolve. It would seem that there is a need for a direct comparison between the results obtained by these two methods.

So as not to give the impression that photoactivatable heterobifunctional reactions are useful only for large ligands, it is worth mentioning two applications involving small ligands. 3′-Arylazido aminopropionyl-8-azido ATP contains two azido groupings and has been found to be of use in cross-linking both the α and β subunits of bacterial F_1-ATPase [108, 109]; this is of significance since 8-azido ATP preferentially labels the β subunit. Recently it has been demonstrated [144] that the high affinity β-adrenoceptor antagonist para-aminobenzylcarazolol binds covalently to the receptor following photolysis when bound ligand preparations have been treated with a photolabile heterobifunctional reagent. This finding has many ramifications for the reason that a large number of neurotransmitters are primary amines; irreversibly binding derivatives have not been described for many of them. Since, however, many of the neurotransmitters are already available in isotopic form, their covalent attachment to receptors might be easily accomplished. In addition, because the ligand itself is not derivatized neither its affinity nor its efficacy would be altered by the presence of an azido or arylazido grouping. For those amines in which the addition of a large adduct at the amine terminal is well-tolerated, the recent description [135] or relatively small, [^{125}I]-iodinated photoactivatable heterbifunctional reagents may allow a one-step synthesis of photoaffinity analogs with high specific activity.

Pitfalls and the need for controls

Currently, experiments are underway to characterize the interaction of [^{3}H]ANAPP$_3$ with the P_2-purinergic receptors of the smooth muscle of the guinea-pig vas deferens. This involves the photolysis in organ chambers of [^{3}H]ANAPP$_3$ (10^{-5} M) in the presence of the tissue (since ANAPP$_3$ interacts with cellular ATPases [90, 91], intact tissues must be used) and the subsequent polyacrylamide gel electrophoresis of samples, as is widely done. While the main results will be reported elsewhere, there are some features of this kind of approach which are of general applicability and which call for the use of appropriate controls. For example, in addition to the incorporation of [^{3}H]ANAPP$_3$ into high molecular weight proteins, ^{3}H is also observed in low molecular weight regions of the gels. This activity is observed only following photolysis. It appears in samples of the Krebs–Henseleit bathing medium taken following photolysis, and in samples of [^{3}H]ANAPP$_3$ photolyzed in water or in Krebs–Henseleit solution (tissues absent). The mobility of radioactivity in the latter two samples is somewhat different than in the tissue bath sample.

Several control experiments indicate that the activity reflects the formation of insertion products with undefined protein(s) which is released into the bathing medium from the tissue, the insertion of ANAPP$_3$ into a component of the Krebs–Henseleit physiological solution (as judged from samples photolyzed in Krebs–Henseleit solution lacking the tissue), or the co-insertion of ANAPP$_3$ into itself or its reaction products (as judged from samples of ANAPP$_3$ photolyzed in water). A misinterpretation regarding the characteristics of receptor-linked incorporation of [^{3}H]ANAPP$_3$ would have emerged had these controls not been performed. The co-insertion of high molecular weight photoprobes (e.g. peptides) will yield products of even higher molecular weight. We therefore advocate the use of controls (i.e. electrophoresis of samples of photolyzed complete medium minus binding sites, membranes, tissues, etc.) to rule out the possibility that regions of high specific incorporation are due to interligand insertions or the reaction of the photoprobe with the buffer or other ingredient.

Some other applications of photoaffinity labels

The current popularity of photoaffinity labels as tools for the isolation and characterization of cell components may diminish as alternative means, e.g. protein high performance liquid chromatography and affinity chromatography, are developed. However, their chemical properties make them amenable to other applications which have not been widely considered.

Macromolecular agonists and antagonists. It is often necessary to establish that a receptor has a cell-surface localization. This has been accomplished by eliciting responses with large molecular weight agonist-protein conjugates, as in the case of adenosine receptors [149–151], and with agonists attached to solid supports (agarose and glass beads; [152, 153]; these agents cannot be internalized by cells. Photo affinity labels can be used theoretically to prepare high molecular weight agonists. Ordinarily it is desirable (*vide supra*) that covalent bonds are formed only at the binding site, due to the selectivity and high affinity of the photoaffinity ligand for the site. However, the actual formation of the covalent bond

following photoactivation is chemically a relatively nonspecific event and occurs as a result of insertion into whatever moiety is near. Advantage can be taken of this chemical nonselectivity by using conditions which favor nonspecific covalent insertion [154] into proteins to create a high molecular weight conjugate. For example, photolysis of a high (10^{-3} M) concentration of $ANAPP_3$ in a solution containing bovine serum albumin resulted in a high molecular weight species (BSA-$ANAPP_3$) which, after dialysis and size-exclusion chromatography, caused contractile responses of the guinea-pig vas deferens which resembled qualitatively those evoked by ATP.* Presumably, the ATP adducts linked to the albumin through the 3′-arylazido aminopropionyl bridge had sufficient access to the cell-surface P_2-receptor [152] to trigger responses. The use of photoaffinity labels to produce high molecular weight conjugates may be more advantageous than the use of other chemical coupling agents (e.g. 1-ethyl-3,3′-dimethylisopropylcarbodiimide; [155]) insofar as uncertainty about where coupling to the ligand takes place is eliminated.

Anti-idiotypic antibodies. High molecular weight compounds such as BSA-$ANAPP_3$ should be immunogenic. Antisera to such agents can then be used to produce anti-idiotypic antibodies which can function as anti-receptor antibodies [156]. Such anti-receptor antibodies have proved useful in the characterization of beta-adrenergic [157] and insulin [156] receptors. The use of antibodies to characterize receptors has been reviewed recently [158]. Following labeling with fluorescein isocyanate, peroxidase or ^{125}I, the antibodies would permit via immunohistochemical and autoradiographic techniques identification of the cellular location of receptor sites as well as the distribution, localization and prevalence of such sites in complex organs, such as brain, where direct photoactivation of the photoaffinity label with light would be difficult. Anti-idiotypic antibodies could be considered as a class of specific pharmacological antagonists for both *in vivo* and *in vitro* experiments, and it is likely that, at least in the case of photoaffinity labels with relatively low affinities for the binding site, the affinity of the anti-receptor antibody for the receptor would exceed that of the native photoligand.

If cells contain multiple recognition sites for a photoaffinity label, as is the case with $ANAPP_3$ which photolabels ATPases [90, 91] as well as the P_2-receptor [78], affinity chromatographical methods, such as those involving agarose-ATP, might not discriminate between the enzymes and the receptor. In addition, a photolabeled receptor is no longer a native one, and only biophysical characterization would be possible after isolation of the labeled species. An immunoaffinity chromatography system utilizing an anti-idiotypic antibody derived from anti-BSA-$ANAPP_3$, if there should be little cross-reactivity with intracellular ATPases, might allow the facile separation of P_2-receptors in solubilized cell membrane fractions from otherwise similar binding sites or enzymes which recognize ATP. Analogies may be easily extrapolated to other systems.

* J. S. Fedan, R. J. Head, G. K. Hogaboom and J. P. O'Donnell, unpublished observations.

In vivo and therapeutic applications. The delivery of external light of sufficient intensity to internal organs to cause photoactivation is not possible with the light sources used ordinarily in *in vitro* experiments. This would seem to limit the usefulness of photoaffinity labels as *in vivo* probes in studies on deep tissues (e.g. brain), in which integrative physiological relationships may be of interest, and as therapeutic agents, where delivery of light via invasive techniques is often undesirable. There are a number of approaches which circumvent this problem. For example, several tissues (e.g. heart, hippocampus, cerebellum) have been transplanted successfully into the anterior eye chamber, where they become innervated, vascularized and retain near-normal characteristics as judged electrophysiologically [159–162]. The small size of the transplanted tissues, and the fact that they are externalized, could permit their exposure to photoaffinity labels and subsequent photolysis with conventional light sources.

The photoactivation of therapeutic agents to treat lung and other cancers in humans is now being evaluated by Dougherty and colleagues [163–167], Hayata *et al.* [168, 169], and others [170], and the results of their work are promising and exciting. A derivative of the photosensitive dye hematoporphyrin (HpD) is sequestered and retained by malignant tissues. Upon exposure to red light (>600 nm), which also penetrates tissues [164] to some degree, the compound in the presence of oxygen is thought to cause the formation of oxygen free radicals [171] which are cytotoxic. Following the i.v. administration of HpD to laboratory animals and humans, external irradiation with red light caused remission of superficial or relatively superficial tumors [163, 165]. In humans, malignant cutaneous and subcutaneous lesions have responded to photoradiation therapy of this type, and a red laser-equipped fiberoptic bronchoscope was used to irradiate loci of pulmonary squamous metaplasia in advanced cases and resulted in a remarkable regression of the lesions. So as to allow more general utility of the procedure, the problem of delivering adequate light to internal sites may be overcome in the future, as suggested by these workers [166, 168], by the implantation of fiberoptic devices into tumor tissue.

A somewhat older but interesting approach to the treatment of psoriasis and other dermatological diseases involves phototherapy and photochemotherapy, the latter in which photosensitizing agents (e.g. psoralens) are used in conjunction with ultraviolet light to enhance its local toxicity [172–174]. The ability of ultraviolet light to penetrate tissues is limited, and anyone who has experienced a severe sunburn will appreciate its toxicity. Since adjunctive therapeutic agents such as psoralens are already administered parenterally to patients, since the toxicity of ultraviolet light is wavelength-related [173], and since the formation of DNA-psoralen or other photoproducts [172] might be expected to occur in several different cell types, it would seem reasonable to suggest that this photochemotherapeutic approach could be extended, using the technique of delivering light locally [166, 168], to the management of superficial malignancies of internal organs.

Ultimately, the general application of light to acti-

vate or enhance the effect of therapeutic agents or adjuncts using noninvasive procedures is limited by the degree to which irradiation of reasonable energies penetrates tissues. While this is a real problem when dealing with nonionizing radiation (e.g. ultraviolet and visible light), ionizing radiation (e.g. X-rays) has no difficulty traversing soft tissues. In addition to its use in diagnostic imageing procedures, X-rays are used widely, with and without hypoxic cell radiosensitizing agents [175–177], for the radiotherapy of malignancies. Two points can be made here which introduce the next concept: the emphasis on the clinical use of ionizing and nonionizing radiation has been directed solely and understandably to cancer and other disorders of cell growth; and the penetrability of ionizing radiation allows it to reach areas of the body where it could interact with and modify the chemical characteristics of drugs. It is for the latter reason that we view as extremely important a recent preliminary report by Cashman *et al.* [178] that X-rays were able to activate a photoaffinity label (8-azido cyclic AMP) in *in vitro* experiments involving *Salmonella typhimurium*. No followup studies have appeared in the literature. 8-Azido cyclic AMP and all non-*aryl* azido photoaffinity labels ordinarily require ultraviolet light to cause photolysis, and the limitations, toxicities, etc. of short wavelength light in a number of experimental and clinical situations have been discussed (*vide supra*). Nitrophenyl arylazides do not absorb red light, of the kind used in HpD treatment; because living tissues are red, the blue light used for photolysis of these compounds would be readily quenched. To amplify on the authors' [178] speculation, it would now seem tenable that photoaffinity labels in general could be valuable research and therapeutic agents in *in vitro, in vivo* and clinical situations to produce, at will, a noninvasive "photoactivation" of the compounds with X-rays. While the ramifications of this finding by Cashman *et al.* may be obvious, it may be helpful to discuss its potential impact given that photoaffinity labels produce specific and long-lasting pharmacological and often antagonistic effects. In the neurosciences, the local infiltration or iontophoresis of compounds during X-radiation of discrete brain areas with focused beams of sufficient intensity to penetrate bone might allow an evaluation of the effect of receptor blockade, the possible identification of transmitter roles and neural circuits, and the labeling of cell components in brain tissue. The treatment of glaucoma with cholinergic and beta-adrenergic blocking photoaffinity labels could not be accomplished safely using ultraviolet or high intensity visible light for photoactivation because retinal damage would result; photoactivation by X-rays might be a suitable alternative to achieve a long-lived pharmacological effect. Numerous situations exist in therapeutics where systemic and/or side effects of a drug require that the dose administered be kept lower than would be desirable to produce a more profound effect at local sites. Two of many such examples would be the restriction of the pharmacological effect of certain calcium entry blockers (e.g. nifedipine), which cause hypotension, in life-threatening coronary vasopasm to the coronary vasculature, and the restriction of the actions of chemotherapeutic agents to the tumor. It seems possible, on a conceptual basis, that the infusion of high concentrations of such agents into the vasculature supplying the tissue of interest during X-radiation could raise local concentrations to the desired levels, minimize other systemic effects as the drug is diluted in the peripheral circulation, as well as produce a more permanent effect than would otherwise be possible. (The local infusion of HpD has also been suggested by Hayata *et al.* [168]). The therapeutic application of photoaffinity labels in conjunction with X-rays will undoubtedly require careful consideration of the toxicity of ionizing radiation on non-tumerous tissues as well as the design of X-ray apparatus capable of producing focused beams of small size. For photopharmacology to become a reality, it will be necessary to more fully understand X-ray activation of photoaffinity labels and the interactions and effects of these agents in intact tissues.

Acknowledgement—The authors are grateful to Terry Shave for expert secretarial assistance during preparation of the manuscript.

REFERENCES

1. R. Chowdry and F. H. Westheimer, *A. Rev. Biochem.* **48**, 293 (1979).
2. A. M. Tometsko and F. M. Richards (Eds.), Applications of Photochemistry in Probing Biological Targets, *Ann. N.Y. Acad. Sci.* **346**, 1 (1980).
3. J. Czarnecki, R. Geahlen and B. Haley, *Meth. Enzym.* **56**, 642 (1979).
4. W. S. Hanstein, *Meth. Enzym.* **56**, 653 (1979).
5. R. J. Guillory and S. J. Jeng, *Meth. Enzym.* **46**, 259 (1977).
6. H. Bayley and J. R. Knowles, *Meth. Enzym.* **46**, 69 (1977).
7. B. S. Cooperman, in *Ageing, Carcinogenicity and Radiation Biology* (Ed. C. Kendric) p. 315. Plenum Press, New York (1976).
8. J. A. Katzenellenbogen, in *Annual Report in Medicinal Chemistry* (Ed. R. V. Heinzelman), Vol. 9, p. 222. Academic Press, New York (1974).
9. R. J. Guillory, in *Current Topics in Bioenergetics* (Ed. G. R. Sanadi), Vol. 9, p. 268. Academic Press, New York (1979).
10. F. J. Darfler and G. V. Marinetti, *Biochem. biophys. Res. Commun.* **79**, 1 (1977).
11. W. Burgermeister, M. Hekman and E. J. M. Helmreich, *J. biol. Chem.* **257**, 5306 (1982).
12. A. Rashidbaigi and A. E. Ruoho, *Proc. natn. Acad. Sci. U.S.A.* **78**, 1609 (1981).
13. A. Rashidbaigi and A. E. Ruoho, *Biochem. biophys. Res. Commun.* **106**, 139 (1982).
14. S. M. Wrenn and C. J. Homcy, *Proc. natn. Acad. Sci. U.S.A.* 77, 4449 (1980).
15. T. N. Lavin, S. L. Heald, P. W. Jeffs, R. L. Shorr, R. J. Lefkowitz and M. G. Caron, *J. biol. Chem.* **256**, 11944 (1981).
16. T. N. Lavin, P. Nambi, S. L. Heald, P. W. Jeffs, R. J. Lefkowitz and M. G. Caron, *J. biol. Chem.* **257**, 12332 (1982).
17. R. G. L. Shorr, M. W. Strohsacker, T. N. Lavin, R. J. Lefkowitz and M. G. Caron, *J. biol. Chem.* **257**, 12341 (1982).
18. H-J. Hess, R. M. Graham and C. J. Homcy, *Proc. natn. Acad. Sci. U.S.A.* **80**, 2102 (1983).

19. C. C. Yip, C. W. T. Yeung and M. L. Moule, *J. biol. Chem.* **253**, 1743 (1978).
20. S. Jacobs, E. Hazum, Y. Shechter and P. Cuatrecasas, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4918 (1979).
21. C. C. Yip, M. L. Moule and C. W. T. Yeung, *Biochem. biophys. Res. Commun.* **96**, 1671 (1980).
22. M. H. Wisher, M. D. Baron, R. H. Jones and P. H. Sonksen, *Biochem. biophys. Res. Commun.* **92**, 492 (1980).
23. H. Kiefer, J. Lindstrom, E. S. Lennox and S. J. Singer, *Proc. natn. Acad. Sci. U.S.A.* **67**, 1688 (1970).
24. R. Oswald, A. Sobel, G. Waksman, B. Roques and J-P. Changeux, *Fedn. Eur. Biochem. Soc. Lett.* **111**, 29 (1980).
25. R. E. Oswald and J-P. Changeux, *Biochemistry* **20**, 7166 (1981).
26. J. A. Moreno-Yanes and H. R. Mahler, *Biochem. biophys. Res. commun.* **92**, 610 (1980).
27. G. Amitai, S. Avissar, D. Balderman and M. Sokolovsky, *Proc. natn. Acad. Sci. U.S.A.* **79**, 243 (1982).
28. S. Avissar, G. Amitai and M. Sokolovsky, *Proc. natn. Acad. Sci. U.S.A.* **80**, 156 (1983).
29. A. M. Capponi and K. J. Catt, *J. biol. Chem.* **255**, 12081 (1980).
30. M. Smolarsky and D. E. Koshland, *J. biol. Chem.* **255**, 7244 (1980).
31. J. Niedel, J. Davis and P. Cuatrecasas, *J. biol. Chem.* **255**, 7063 (1980).
32. R. Gabizon, T. Yetinson and S. Schuldiner, *J. biol. Chem.* **257**, 15145 (1982).
33. J. A. Katzenellenbogen, H. J. Johnson, H. N. Myers, K. E. Carlson and R. J. Kempton, *Bioorg. Chem.* **4**, 207 (1978).
34. D. W. Payne, J. A. Katzenellenbogen and K. E. Carlson, *J. biol. Chem.* **255**, 10359 (1980).
35. C. Hall and A. Ruoho, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4529 (1980).
36. A. Ruoho and J. Kyte, *Proc. natn. Acad. Sci. U.S.A.* **71**, 2352 (1974).
37. K. B. Munson, *J. biol. Chem.* **256**, 3223 (1981).
38. M. Girardet, K. Geering, B. C. Rossier, J. P. Kraehenbuhl and C. Bron, *Biochemistry* **22**, 2296 (1983).
39. B. Forbush, J. H. Kaplan and J. F. Hoffman, *Biochemistry* **17**, 3667 (1978).
40. T. B. Rogers and M. Lazdunski, *Fedn. Eur. Biochem. Soc.* **98**, 373 (1979).
41. T. B. Rogers and M. Lazdunski, *Biochemistry* **18**, 135 (1979).
42. E. Canova-Davis and J. Ramachandran, *Biochemistry* **19**, 3275 (1980).
43. K. Muramato and J. Ramachandran, *Biochemistry* **19**, 3280 (1980).
44. Y. S. Klousner, W. M. McCormick and I. M. Chaiken, *Int. J. Pept. Protein Res.* **11**, 82 (1978).
45. D. M. Abercrombie, W. M. McCormick and I. M. Chaiken, *J. biol. Chem.* **257**, 2274 (1982).
46. B. E. Haley, *Biochemistry* **14**, 3852 (1975).
47. A. H. Pomerantz, S. A. Rudolf, B. E. Haley and P. Greengard, *Biochemistry* **14**, 3858 (1975).
48. J. R. Owens and B. E. Haley, *J. supramolec. Struct.* **9**, 57 (1978).
49. A. R. Kerlavage and S. S. Taylor, *J. biol. Chem.* **255**, 8483 (1980).
50. J. Hoppe and W. Freist, *Eur. J. Biochem.* **93**, 141 (1979).
51. J. E. Casnellie, D. J. Schlichter, U. Walter and P. Greengard, *J. biol. Chem.* **253**, 4771 (1978).
52. B. S. Cooperman, *Ann. N.Y. Acad. Sci.* **346**, 302 (1980).
53. K. L. Yielding and L. W. Yielding, *Ann. N.Y. Acad. Sci.* **346**, 369 (1980).
54. T. J. Ryan, *Biochem. biophys. Res. Commun.* **98**, 1108 (1981).
55. I. I. Kaiser, D. M. Kladianos, E. A. Van Kirk and B. E. Haley, *J. biol. Chem.* **258**, 1747 (1983).
56. M. M. King, G. M. Carlson and B. E. Haley, *J. biol. Chem.* **257**, 14058 (1982).
57. F. N. Briggs, W. Al-Jumaily and B. E. Haley, *Cell Calcium* **1**, 205 (1980).
58. G. S. Oxford and R. A. Hudson, *Biochem. biophys. Res. Commun.* **104**, 1579 (1982).
59. R. J. Guillory, M. D. Rayner and J. S. D'Arrico, *Science* **196**, 883 (1977).
60. D. A. Beneski and W. A. Catterall, *Proc. natn. Acad. Sci. U.S.A.* **77**, 639 (1980).
61. P. G. Waser, A. Hofmann and W. Hopff, *Experientia* **26**, 1342 (1970).
62. G. Waksman, R. Oswald, J-P. Changeux and B. P. Roques, *Fedn. Eur. Biochem. Soc. Lett.* **111**, 23 (1980).
63. D. Brandenburg, C. Diaconescu, D. Saunders and P. Thamm. *Nature, Lond.* **286**, 832 (1980).
64. A. Pascual, J. Casanova and H. H. Samuels, *J. biol. Chem.* **257**, 9640 (1982).
65. A. Ratman and V. Pribluda, *Biochim. biophys. Acta* **714**, 173 (1982).
66. N. J. Cusack and G. V. R. Born, *Proc. R. Soc. B* **193**, 307 (1976).
67. N. J. Cusack and G. V. R. Born, *Proc. R. Soc. B* **197**, 515 (1977).
68. D. E. Macfarlane, D. C. B. Mills and P. C. Srivastava, *Biochemistry* **21**, 544 (1982).
69. R. G. Painter, M. Schmitt, A. J. Jesaitis, L. A. Sklar, K. Preissner and C. G. Cochrane, *J. cell. Biochem.* **20**, 203 (1982).
70. M. Schmitt, R. G. Painter, A. J. Jesaitis, K. Preissner, L. A. Sklar and C. G. Cochrane, *J. biol. Chem.* **258**, 649 (1983).
71. L. J. Wallace and W. A. Frazier, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4250 (1979).
72. P. K. Schoff, I. T. Forrester, B. E. Haley and R. W. Atherton, *J. cell. Biochem.* **19**, 1 (1982).
73. Y. C. Kwok and G. J. Moore, *Proc. west. Pharmac. Soc.* **23**, 345 (1980).
74. Y. C. Kwok and G. J. Moore, *Molec. Pharmac.* **18**, 210 (1980).
75. R. E. Galardy and K. A. LaVorgna, *J. med. Chem.* **24**, 362 (1981).
76. J. P. O'Donnell, G. K. Hogaboom and J. S. Fedan, *Eur. J. Pharmac.* **73**, 261 (1981).
77. J. S. Fedan, G. K. Hogaboom and J. P. O'Donnell, *Eur. J. Pharmac.* **81**, 393 (1982).
78. G. K. Hogaboom, J. P. O'Donnell and J. S. Fedan, *Science* **208**, 1273 (1980).
79. J. S. Fedan, G. K. Hogaboom, J. P. O'Donnell, J. Colby and D. P. Westfall, *Eur. J. Pharmac.* **69**, 41 (1981).
80. J. S. Fedan, G. K. Hogaboom, D. P. Westfall and J. P. O'Donnell, *Eur. J. Pharmac.* **85**, 277 (1982).
81. D. P. Westfall, G. K. Hogaboom, J. Colby, J. P. O'Donnell and J. S. Fedan, *Proc. natn. Acad. Sci. U.S.A.* **79**, 7041 (1982).
82. P. Sneddon, D. P. Westfall and J. S. Fedan, *Science* **218**, 693 (1982).
83. J. P. O'Donnell, G. K. Hogaboom and J. S. Fedan, *Eur. J. Pharmac.* **86**, 435 (1983).
84. D. P. Westfall, J. S. Fedan, J. Colby, G. K. Hogaboom and J. P. O'Donnell, *Eur. J. Pharmac.* **87**, 415 (1983).
85. R. Frew and P. M. Lundy, *Life Sci.* **30**, 259 (1982).
86. J. M. Mosely, D. M. Findlay, T. J. Martin and J. J. Gorman, *J. biol. Chem.* **257**, 5846 (1982).
87. G. Burnstock, *Pharmac. Rev.* **24**, 509 (1972).
88. G. Burnstock, in *Regulatory Functions of Adenosine*

(Eds. R. M. Berne, T. W. Rall and R. Rubio), p. 49. Martinus Nijhoff Publishers, Boston (1983).
89. G. Burnstock, in *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides* (Eds. H. P. Baer and G. I. Drummond), p. 3. Raven Press, New York (1979).
90. S. J. Jeng and R. J. Guillory, *J. supramolec. Struct.* **3**, 448 (1975).
91. J. Russell, S. J. Jeng and R. J. Guillory, *Biochem. biophys. Res. Commun.* **70**, 1225 (1976).
92. M. H. Maguire and D. G. Satchell, in *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides* (Eds. H. P. Baer and G. I. Drummond), p. 33. Raven Press, New York (1979).
93. J. S. Fedan, G. K. Hogaboom, D. P. Westfall and J. P. O'Donnell, *Eur. J. Pharmac.* **81**, 193 (1981).
94. K. M. M. Murphy and S. H. Snyder, *Molec. Pharmac.* **22**, 250 (1982).
95. G. Kunos and M. Nickerson, *J. Physiol., Lond.* **256**, 23 (1976).
96. G. Kunos and M. Nickerson, *Br. J. Pharmac.* **59**, 603 (1977).
97. G. Burnstock and H. Wong, *Br. J. Pharmac.* **62**, 293 (1978).
98. A. P. Somlyo and A. V. Somlyo, *Pharmac. Rev.* **22**, 249 (1970).
99. L. Augenstein and P. Riley, *Photochem. Photobiol.* **3**, 353 (1964).
100. J. M. Fox, *Pflügers Archs* **351**, 287 (1974).
101. J. B. Weigele and R. L. Barchi, *J. Neurochem.* **35**, 430 (1980).
102. M. K. Battersby, J. G. Richards and H. Mohler, *Eur. J. Pharmac.* **57**, 277 (1979).
103. H. Mohler, M. K. Battersby and J. G. Richards, *Proc. natn. Acad. Sci. U.S.A.* **77**, 1666 (1980).
104. J. W. Thomas and J. F. Tallman, *J. biol. Chem.* **256**, 9838 (1981).
105. H. M. Westphal, G. Fleishmann and M. Beato, *Eur. J. Biochem.* **119**, 101 (1981).
106. S. E. Sadler and J. L. Maller, *J. biol. Chem.* **257**, 355 (1982).
107. J. P. Daugherty, S. C. Hixon and K. L. Yielding, *Biochim. biophys. Acta* **565**; 13 (1979).
108. M. A. Raftery, V. Witzemann and S. G. Blanchard, *Ann. N.Y. Acad. Sci.* **346**, 458 (1980).
109. H-J. Schafer, J. Mainka, G. Rathgeber and D. Zimmer, *Biochem. biophys. Res. Commun.* **111**, 732 (1983).
110. J. Kyte, *J. biol. Chem.* **256**, 3231 (1981).
111. R. A. Goldman, T. Hasan, C. C. Hall, W. A. Strycharz and B. S. Cooperman, *Biochemistry* **22**, 359 (1983).
112. T. H. Ji, *J. biol. Chem.* **252**, 1566 (1977).
113. C. C. Yip, C. W. T. Yeung and M. L. Moule, *Biochemistry* **19**, 70 (1980).
114. A. M. Tometsko, J. D. Hare, C. R. Tometsko, P. Y. Lam and D. Lipman, *Ann. N.Y. Acad. Sci.* **346**, 419 (1980).
115. R. F. Furchgott, in *Catecholamines* (Eds. H. Blaschko and E. Muscholl), p. 283. Springer, Berlin (1972).
116. J. C. Besse and R. F. Furchgott, *J. Pharmac. exp. Ther.* **197**, 66 (1976).
117. R. R. Ruffolo, *J. auton. Pharmac.* **2**, 277 (1982).
118. A. E. Ruoho, H. Kiefer, P. E. Roeder and S. J. Singer, *Proc. natn. Acad. Sci. U.S.A.* **70**, 2567 (1973).
119. J. S. Fedan, G. K. Hogaboom and J. P. O'Donnell, *Life Sci.* **31**, 1921 (1982).
120. L. J. Ignarro, H. Lippton, J. C. Edwards, W. H. Baricos, A. L. Hyman, P. J. Kadowitz and C. A. Gruetter, *J. Pharmac. exp. Ther.* **218**, 739 (1981).
121. J. V. Staros, H. Bayley, D. N. Standring and J. R. Knowles, *Biochem. biophys. Res. Commun.* **80**, 568 (1978).
122. G. K. Hogaboom, J. P. O'Donnell and J. S. Fedan, *Pharmacologist* **24**, 223 (1982).
123. B. E. Haley and J. F. Hoffman, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3367 (1974).
124. W. Sieghart and M. Karabath, *Nature, Lond.* **286**, 285 (1980).
125. R. W. Johnson and H. I. Yamamura, *Life Sci.* **25**, 1613 (1979).
126. W. Siegart and H. Moehler, *Eur. J. Pharmac.* **81**, 171 (1982).
127. A. C. Mahon and P. R. Hartig, *Life Sci.* **30**, 1179 (1982).
128. Y. Iwayama and I. Takayanagi, *J. Pharm. Pharmac.* **34**, 729 (1982).
129. P. J. Marangos, R. Clark-Rosenberg and J. Patel, *Eur. J. Pharmac.* **85**, 359 (1982).
130. P. Muhn and E. Hucho, *Biochemistry* **22**, 421 (1983).
131. R. E. Oswald and J-P. Changeux, *Fedn. Eur. Biochem. Soc. Lett.* **139**, 225 (1982).
132. R. E. Oswald and J-P. Changeux, *Proc. natn. Acad. Sci. U.S.A.* **78**, 3925 (1981).
133. K. Schaltman and O. Pongs, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6 (1982).
134. I. Takayanagi, R. Shibata, N. Miyata and M. Hirobe, *J. pharmac. Meth.* **7**, 185 (1982).
135. T. H. Ji and I. Ji, *Analyt. Biochem.* **121**, 286 (1982).
136. G. Dupuis and F. Radwan, *Can. J. Biochem. Cell Biol.* **61**, 99 (1983).
137. I. Ji and T. H. Ji, *Proc. natn. Acad. Sci. U.S.A.* **77**, 7167 (1980).
138. I. Ji and T. H. Ji, *Proc. natn. Acad. Sci. U.S.A.* **78**, 5465 (1981).
139. M. Das, T. Mizakawa, C. F. Fox, R. M. Pruss, A. Aharonov and H. R. Herschman, *Proc. natn. Acad. Sci. U.S.A.* **74**, 2790 (1977).
140. D. L. Johnson, V. I. MacAndrew and P. F. Pilch, *Proc. natn. Acad. Sci. U.S.A.* **78**, 875 (1981).
141. T. J. Andreasen, C. H. Keller, D. C. LaPorte, A. M. Edelman and D. R. Storm, *Proc. natn. Acad. Sci. U.S.A.* **78**, 2782 (1981).
142. R. R. Goewert, M. Landt and J. M. McDonald, *Biochemistry* **21**, 5310 (1982).
143. V. Witzemann, D. Muchmore and M. A. Raftery, *Biochemistry* **18**, 5511 (1979).
144. R. G. L. Shorr, S. L. Heald, P. W. Jeffs, T. N. Lavin, M. W. Strohsacker, R. J. Lefkowitz and M. G. Caron, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2778 (1982).
145. S. Paglin and J. D. Jameson, *Proc. natn. Acad. Sci. U.S.A.* **79**, 3739 (1982).
146. M. C. DeTraglia, J. S. Brand and A. M. Tometsko, *Ann. N.Y. Acad. Sci.* **345**, 59 (1980).
147. R. B. Moreland, P. K. Smith, E. K. Fujimoto and M. E. Dockter, *Analyt. Biochem.* **121**, 321 (1982).
148. D. B. Donner, *J. biol. Chem.* **258**, 2736 (1983).
149. R. A. Olsson, C. J. Davis and E. M. Khouri, *Circulation Res.* **39**, 93 (1976).
150. R. A. Olsson, C. J. Davis and E. M. Khouri, *Life Sci.* **21**, 1343 (1978).
151. J. Schrader, S. Nees and E. Gerlach, *Pflügers Archs* **369**, 251 (1977).
152. R. J. Head, J. P. O'Donnell, G. K. Hogaboom and J. S. Fedan, *Biochem. Pharmac.* **32**, 563 (1983).
153. J. C. Venter, *Pharmac. Rev.* **34**, 153 (1982).
154. F. Tejedor and J. P. G. Bollesta, *Analyt. Biochem.* **128**, 115 (1983).
155. H. Cailla, C. LeBorgne De Kaouel, D. Roux, M. Delaage and J. Marti, *Proc. natn. Acad. Sci. U.S.A.* **79**, 4742 (1982).
156. K. Sege and P. A. Paterson, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2443 (1978).
157. C. J. Homcy, S. G. Rockson and E. Haber, *J. clin. Invest.* **69**, 1147 (1982).

158. J. C. Venter, *J. molec. cell. Cardiol.* **14**, 687 (1982).
159. D. Taylor, A. Seiger, R. Freedman, L. Olson and B. Hoffer, *Proc. natn. Acad. Sci. U.S.A.* **75**, 1009 (1978).
160. L. Olson, A. Seiger, B. Hoffer and D. Taylor, *Expl. Brain Res.* **35**, 47 (1979).
161. L. Olson, A. Seiger, D. Taylor, R. Freedman and B. J. Hoffer, *Expl Brain Res.* **39**, 277 (1980).
162. D. Taylor, R. Freedman, A. Seiger, L. Olson and B. J. Hoffer, *Expl Brain Res.* **39**, 289 (1980).
163. T. J. Dougherty, G. B. Grindley, R. Fiel, H. R. Weishaupt and D. G. Boyle, *J. natn. Cancer Inst.* **55**, 115 (1975).
164. T. J. Dougherty, J. E. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Boyle and A. Mittleman, *Cancer Res.* **38**, 2628 (1978).
165. T. J. Dougherty, G. Lawrence, J. Kaufman, D. G. Boyle, K. R. Weishaupt and A. Goldfarb, *J. natn. Cancer Inst.* **62**, 231 (1979).
166. T. J. Dougherty, R. E. Thomas, D. G. Boyle and K. R. Weishaupt, *Cancer Res.* **41**, 401 (1981).
167. T. J. Dougherty, *Chest* **81**, 266 (1982).
168. Y. Hayata, H. Kato, C. Konaka, J. Ono and N. Takizawa, *Chest* **81**, 269 (1982).
169. Y. Hayata, H. Kato, C. Kanaka, J. Ono, Y. Matsushima, K. Yoneyama and K. Nishimiya, *Chest* **82**, 10 (1982).
170. S. H. Tomson, E. A. Emmett and S. H. Fox, *Cancer Res.* **34**, 3124 (1974).
171. K. R. Weishaupt, C. J. Gomer and T. J. Dougherty, *Cancer Res.* **36**, 2326 (1976).
172. J. A. Parrish, *J. invest. Derm.* **77**, 167 (1981).
173. J. A. Parrish, *Pharmac. Ther.* **15**, 313 (1982).
174. J. A. Parrish, *Pharmac. Ther.* **15**, 439 (1982).
175. J. F. Fowler and J. Denekamp, *Pharmac. Ther.* **7**, 413 (1979).
176. J. E. Biaglow, *Pharmac. Ther.* **10**, 283 (1980).
177. H. Monney, J. Parrick and R. G. Wallace, *Pharmac. Ther.* **14**, 197 (1981).
178. D. P. Cashman, J. R. Cashman, J. J. Puma and S. R. Wellings, *Fedn. Proc.* **39**, 1912 (1980).