Photochemical Inactivation of DNA and RNA Viruses by Psoralen Derivatives

By CARL VEITH HANSON, JOHN L. RIGGS AND EDWIN H. LENNETTE

Viral and Rickettsial Disease Laboratory, California State Department of Health, 2151 Berkeley Way, Berkeley, California 94704, U.S.A.

(Accepted 1 March 1978)

SUMMARY

Western equine encephalitis virus, and RNA virus, and herpes simplex virus type 1, a DNA virus, were efficiently inactivated in less than 1 min by exposure to long-wave ultraviolet light (320 to 380 nm) in the presence of several psoralen derivatives. The psoralen photochemical reaction was chosen for study due to its known specificity for nucleic acids. Neither the light nor any of the drugs alone caused appreciable inactivation. The inactivation kinetics and dependence on light intensity and on different derivatives of psoralen were studied. The high solubility of a new aminomethyl psoralen derivative was found to be advantageous in the photochemical inactivation of the RNA virus, but was not in the case of the more easily inactivated DNA virus. Within its limited solubility range trimethylpsoralen was superior to its aminomethyl derivative on a molar basis for the inactivation of both types of viruses under most of the conditions studied.

INTRODUCTION

Virus inactivating agents may act by modifying virus coats, by damaging the virus nucleic acid, or simultaneously through both mechanisms. Formaldehyde and thermal inactivations have been theorized, for example, to result more from effects involving proteinaceous surface components than from the nucleic acid reactions which are also known to occur (Bachrach, 1966; v. St Whitelock, 1960). Other virucidal treatments which modify or react significantly with proteins despite their better known interactions with nucleic acid include ionizing and non-ionizing radiation (v. St Whitelock, 1960), treatment with beta-propiolactone (Colburn & Boutwell, 1968) and photodynamic inactivation with visible light-sensitive dyes (Spikes & Livingston, 1969).

Photochemical inactivation of DNA viruses by derivatives of psoralen (Fig. 1) was first demonstrated by Musajo et al. (1965), and the technique was recently extended to an RNA virus (vesicular stomatitis virus) by Hearst & Thiry (1977). By the use of a highly intense light source we have found that both DNA and RNA viruses may be efficiently and totally photochemically inactivated under near-physiological or other gentle conditions within seconds. The psoralen photoreaction involves only the virus nucleic acid (Pathak et al. 1974). Since surface antigens are therefore expected to remain unmodified, the method has potential for the development of killed virus vaccines with improved immunogenicity. Due to the transforming capabilities of virus DNA or even DNA fragments (Graham et al. 1974), the mere elimination of infectivity is not sufficient for the preparation of safe DNA
virus vaccines for human use. The verifiable effects of the psoralen photoreaction directly on the entire virus DNA molecule, including extensive action beyond loss of infectivity, could contribute to the eventual solution of this problem.

Psoralen derivatives have been best known historically for their ability to photosensitize mammalian skin (Musajo & Rodighiero, 1962; Pathak & Kramer, 1969; Scott et al. 1976) and more recently for their successful use in the photochemotherapy of psoriasis (Parrish et al. 1974) and other clinical dermatoses (Gilchrest et al. 1976). Their specific advantages for the inactivation of micro-organisms include the following. (i) Psorals [which are non-reactive in the absence of long-wave ultraviolet (u.v.) light] readily pass through cellular and nuclear membranes and virus or bacterial envelopes and reversibly bind to nucleic
Psoralen photoinactivation of viruses

Acids in situ within intact micro-organisms. (ii) Upon exposure to long-wave (near) u.v. light (320 to 380 nm) the drug molecule is covalently bonded to one or two strands of nucleic acid by cyclobutane-type linkages to pyrimidines (Cole, 1971; Dall’Acqua et al. 1971). (iii) Both psoralen mono-adducts (involving a single nucleic acid base) and di-adducts (which cross-link double-stranded nucleic acid) block nucleic acid replication and gene transcription (Baccichetti et al. 1975; Bordin et al. 1975). While enzymic mechanisms exist for the repair of very low levels of psoralen cross-links (Baden et al. 1971; Day et al. 1975; Bordin et al. 1976; Cole et al. 1976), the levels of photoreaction attained in this study exceed by several orders of magnitude those levels known to survive in any system by repair. (iv) The extent of adduct formation may be monitored by measuring the binding of radioactive psoralens (Isaacs et al. 1977), and by visualizing psoralen cross-links in DNA by electron microscopy (Hanson et al. 1976; Cech & Pardue, 1977). These techniques verify the psoralen-sensitivity of nucleic acid contained within both viruses and nucleoprotein complexes such as chromatin. Since proteins, carbohydrates and lipids neither interfere with nor participate in the highly specific psoralen photoreaction with nucleic acid, viruses may be psoralen-inactivated, in principle, while intracellular or in the presence of large amounts of cellular debris. (v) The photoreaction of psoralens with double-stranded DNA greatly exceeds that with (single-stranded) RNA under equivalent conditions. However, the early failure to inactivate RNA viruses (Musajo et al. 1965) is overcome by the use of more intense u.v. light or a new psoralen derivative (Isaacs et al. 1977) with improved solubility and nucleic acid binding properties.

METHODS

Viruses and cells. Western equine encephalitis virus (WEE), strain A42 (small plaque type), and herpes simplex virus type 1 (HSV-1), MacIntyre strain, were both grown in roller bottle cultures of a newborn hamster kidney continuous cell line developed in our laboratory and designated O-853. The cells were grown as an attached monolayer at 36 °C in closed vessels containing Eagle’s minimal essential medium (MEM) in Earle’s balanced salt solution, 10% heat-inactivated foetal bovine serum (FBS), 200 units of penicillin and 200 μg of streptomycin per ml. The yields of viruses after purification (as described below) were 10⁶ to 10⁸ plaque forming units (p.f.u.) of WEE and 10⁵ to 10⁶ p.f.u. of HSV-1 per ml of infected culture fluid.

Preparation of purified viruses. Approximately 48 h after infection with either WEE or HSV-1, the attached cells were shaken off into their culture medium and frozen (at −20 °C) and thawed once to release intracellular viruses. After centrifuging at 750 g for 30 min to remove cellular debris, the fluids were centrifuged at 78000 g for 2 h at 4 °C to concentrate the viruses. The pellets were resuspended in phosphate-buffered saline (PBS), pH 7.5, with 5% FBS, with the aid of a Potter-Elvehjem tissue grinder and either frozen at −70 °C or layered on to the tops of 23 ml pre-formed 35 to 60% sucrose gradients in PBS. Centrifugation of the gradients in a Beckman Spinco SW25.1 rotor at 42000 g overnight at 4 °C resulted in a turbid band in the lower half of each gradient. The recovered bands were diluted in PBS plus 5% FBS and pelleted at 105000 g for 1 h at 4 °C. These pellets were resuspended in Earle’s balanced salt solution (EBSS) without FBS and without phenol red. Finally, the suspension was homogenized in a tissue grinder and centrifugally clarified at 250 g for 10 min. All preparations were freshly made, starting with the sucrose gradient step, immediately before assay and use in the inactivation experiments.

Psoralen derivatives. Psoralen (PSOR, Fig. 1a) was the generous gift of Dr M.A.
Pathak, Harvard University Medical School. 8-Methoxypsoralen ('methoxsalen', 'xanthotoxin', MOP, Fig. 1b) was purchased from Sigma Chemical Co., St Louis, Missouri. 4, 5', 8-Trimethylpsoralen ('trioxsalen', TMP, Fig. 1c) was purchased from the Paul B. Elder Co., Brian, Ohio. 4'-Aminomethyl-4, 5', 8-trimethylpsoralen (AMT, Fig. 1d) was synthesized as previously described (Isaacs et al. 1977) and was the kind gift of Dr J. E. Hearst, University of California, Berkeley.

Shortly before use, a stock solution of each of the derivatives was made by dissolving 1 mg of drug per ml of 100% dimethyl sulphoxide (DMSO) at room temperature. Additional working dilutions in DMSO were made at 100 × the desired final concentration so as to give a concentration of 1% DMSO in the virus suspensions (unless otherwise noted below).

Long-wave u.v. light sources. A high intensity source of long-wave u.v. light was constructed by horizontally opposing two 400 W General Electric (H400RSP33-1) mercury reflector spot lamps on either side of a completely jacketed pyrex glass sample chamber. A refrigerated 40% (w/v) solution of Co(NO₃)₂ was circulated through the 1 cm jacket of the chamber to remove simultaneously heat and unwanted visible and short-wave u.v. light. The 1 cm-thick sample was continuously stirred by a magnetically driven stir-bar.

A lower intensity light source was constructed by mounting a bank of eight 20 W ITT F20T12/BLB fluorescent lamps ('black lights') horizontally above a polished aluminum reflector and below a transparent 0.25 inch Plexiglass shelf. Samples contained in Falcon brand cell culture plates or dishes were placed on the shelf for irradiation from below. Temperature was controlled by placing the apparatus in a walk-in room at 4 °C. Measurement of light intensity must be made through the culture dish due to the varying absorption properties of dishes from different manufacturers.

The intensity of light from both light sources was monitored with an IL500 radiometer coupled to a calibrated SEE-115W-UVA silicon detector, both from International Light Inc., Newburyport, Massachusetts. The spectral sensitivity of this detector corresponds to the known action spectrum for psoralen photoreactions (approx. range 320 to 380 nm). Narrow-band filters were also used to compare the fractions of light emitted at wavelengths of 350 ± 5 nm and 365 ± 5 nm. In the high intensity device the sum of the light intensities from both sides was 180 mW/cm² (corrected for an average absorption of 32% by 1 cm of the Co(NO₃)₂ solution). Sixty % of this light was at 365 ± 5 nm and 12% at 350 ± 5 nm. The intensity of the fluorescent light source was 1.9 mW/cm² (after an average absorption of 26% of the light by a Falcon-brand culture dish). In this latter device 40% of the light is at 365 ± 5 nm and 17% at 350 ± 5 nm.

Photochemical inactivation treatment. One % (v/v) of a 100 × stock solution of the appropriate psoralen (in DMSO) was added to the virus suspension and thoroughly mixed by vortex immediately before transfer to the irradiation chamber. Short exposures in both types of irradiation device were accomplished with the aid of manually operated shutters.

Determination of virus inactivation. Photochemically treated and control viruses were serially diluted in 1 log steps in cold EBSS + 5% FBS in tubes shortly after treatment. A sample (0.025 ml) of each dilution was added to each of 4 wells of a Falcon 96-well microtitre plate with flat-bottomed wells containing confluent 0-853 cells under maintenance medium (Eagle's MEM + 2% FBS + antibiotics as above). The plates were incubated at 36 °C in a CO₂ atmosphere and examined daily for c.p.e. The end-point dilution for c.p.e. was calculated after 5 days of observation using the Reed-Muench formula (Reed & Muench, 1938) and finally expressed as the fraction surviving relative to untreated controls.

Determination of the magnitude and completeness of virus inactivation, as in any such
Psoralen photoinactivation of viruses

Psoralen photoinactivation of viruses

Fig. 2. Photochemical inactivation of HSV-1 at low light intensity (1.9 mW/cm²) by several psoralen derivatives. The concentration of each derivative in μg/ml is shown in parentheses. The virus suspension was photoreacted with 0.1 μg/ml (0.34 μM) AMT (▼▼▼▼), 1.0 μg/ml (3.4 μM) AMT (▲▲▲▲), 1.0 μg/ml (34 μM) AMT (■■■■), 0.1 μg/ml (0.44 μM) TMP (××××), 1.0 μg/ml (4.4 μM) TMP (△△△△), 1.0 μg/ml (44 μM) TMP (□□□□), and 10.0 μg/ml (53.8 μM) PSOR (○○○○). Symbols with downward arrows (▼, ▲, △, etc.) signify that within the limits of the assay no remaining virus infectivity could be detected at these points (nor at longer time points not shown in the figure).

study, is limited by the starting infectivity titre and the sample volume employed in the assay. Samples referred to below as being 'totally' inactivated are those producing no c.p.e. in any wells, including those wells inoculated with a total combined volume of 0.10 ml of undiluted treated sample.

Photoreaction of HSV-1 with radioactive TMP. Tritium-labelled 4, 5', 8-trimethylpsoralen (3H-TMP) was radioactively labelled by Dr J. E. Hearst as previously described (Isaacs et al. 1977). 3H-TMP at a specific activity of 2.62 × 10⁶ cts/min/μg and dissolved in ethanol (rather than DMSO) was used for the inactivation of HSV-1 by the same procedures used for the non-radioactive drugs. The DNA of treated HSV-1 and of untreated controls was extracted and purified by methods previously described for the study of DNA from Drosophila melanogaster nuclei (Hanson et al. 1976). The number of covalently bound drug molecules per base pair of DNA was computed by quantifying the DNA by u.v. absorption and the radioactive drug by scintillation counting.

Electron microscopy of nucleic acid. DNA was isolated from psoralen-treated HSV-1 and observed electron microscopically by methods previously described for the study of psoralen-treated DNA from Drosophila melanogaster nuclei (Hanson et al. 1976).

RESULTS

In the case of the DNA virus HSV-1, it may be seen in Fig. 2 that on a molar basis TMP is approximately 10 times more effective than AMT and 100 times more effective than
Fig. 3. Photochemical inactivation of HSV-1 at high light intensity (180 mW/cm²) by several psoralen derivatives. The explanation of symbols is the same as in the legend of Fig. 2.

Fig. 4. Photochemical inactivation of WEE at low light intensity (1.9 mW/cm²) by several psoralen derivatives. Symbols are as in the legend of Fig. 2.
Psoralen photoinactivation of viruses

Fig. 5. Photochemical inactivation of WEE at high light intensity (180 mW/cm²) by several psoralen derivatives, all at 1 µg/ml. The virus suspension was photoreacted with 1.0 µg/ml (4.6 µM) MOP (●---●), 1.0 µg/ml (5.4 µM) PSOR (○---○), and 1.0 µg/ml AMT (▲---▲) and TMP (△---△).

PSOR for photochemical inactivation at low light intensity. The anomalous effect of TMP at 10 µg/ml was reproduced in several experiments and is attributed to TMP crystals which are present above its aqueous solubility limit, which is 0.8 µg/ml (Dall’Acqua et al. 1971). Equivalent inactivation of this same virus at high light intensity (Fig. 3) requires a greater total light energy, but may be accomplished within as little as 4 s with 1 µg/ml of TMP.

The response of the RNA virus WEE to the photochemical treatment is distinctively different from that of HSV-1. At low light intensity (Fig. 4) TMP and AMT are equally effective at 1 µg/ml, and at the latter concentration are equivalent to PSOR at 10 µg/ml (data for PSOR not shown). Complete elimination of detectable infectivity by a single drug dose, however, was accomplished only at 10 µg/ml of AMT. The superior solubility of AMT is required to effectively achieve this latter dose.

The photochemical inactivation of WEE at high light intensity by four psoralen derivatives, all at 1 µg/ml, is shown in Fig. 5. The relative effectiveness of TMP and AMT is approximately the same as in the case of HSV-1 under the same conditions. Greater amounts of irradiation are required in the case of the RNA virus, however. PSOR and MOP (the latter most commonly used for dermatological photochemotherapy) are less effective under these conditions.

The effect on WEE of the above four derivatives at 10 µg/ml under high light intensity is shown in Fig. 6. Just as at low light intensity, 10 µg/ml of AMT is the most effective, with complete inactivation occurring within 10 s. The behaviour of TMP at 10 µg/ml remains anomalous (i.e. less effective than at 1 µg/ml). The effect of PSOR is seen to be proportional to its concentration, while the action of MOP is increased only slightly by a 10-fold increase in its concentration.

While virus inactivation may be accomplished at high light intensity by dramatically short treatments of 10 s or less, the total amount of light energy required for a given inactivation is greater than with the low intensity light source. This is emphasized by Fig. 7, in which the effect of 1 µg/ml of AMT on WEE is plotted as a function of cumulative light energy rather than exposure time. This is attributed at least partly to the relatively
Fig. 6. Photochemical inactivation of WEE at high light intensity (180 mW/cm²) by several psoralen derivatives, all at 10 μg/ml. The virus suspension was photoreacted with 100 μg/ml (46 μM) MOP (●—●), 100 μg/ml (54 μM) PSOR (○—○), and 100 μg/ml AMT (■—■) and TMP (□—□).

Fig. 7. Photochemical inactivation of WEE by 1 μg/ml AMT with the high intensity (●—●) mercury light source (180 mW/cm²) and the low intensity (○—○) fluorescent light source (1.9 mW/cm²). The horizontal scale is the cumulative light energy incident on the samples in joules/cm².
greater photodestruction of the drugs by the high intensity light source. The different spectral outputs of the two types of light sources (see Methods, above) may, however, also play an important role.

The exposure of WEE and HSV-1 in the dark (or under ordinary fluorescent laboratory lighting) to all concentrations of all the psoralen derivatives used in the above experiments had no measurable effect on virus infectivity. Similarly, in the absence of psoralens, and with or without 1% DMSO or 1% ethanol, there was no reduction of infectivity by exposure to the low intensity u.v. light for up to 32 min. Exposure to the low intensity u.v. light for 60 min or to the high intensity u.v. light for 100 s, however, produced up to 1 log of inactivation.

The data in Fig. 2 to 6 demonstrate the great sensitivity of virus infectivity both to psoralen concentration and to the amount of u.v. light during photochemical inactivation. Preliminary experiments with diluted virus suspensions have shown that the photoinactivation is much less dependent upon the ratio of drug molecules to virus particles (data not shown). This is consistent with the idea that only a small fraction of the available drug photoreacts with the virus nucleic acid. At any given time the amount of drug associated with virus nucleic acid and available for photoreaction is a function of the overall concentration of dissolved drug with which the nucleic acid-associated drug is in equilibrium (Isaacs et al. 1977).

In the case of HSV-1, an attempt was made to correlate the photochemical inactivation of virus infectivity with effects on virus DNA as measured by physico-chemical methods. The uptake of covalently bound (non-dialysable) TMP by the DNA of HSV-1 during treatment of the intact virions is shown in Fig. 8. In this experiment 5-0 μg/ml of TMP
Fig. 9. Electron micrographs of DNA fragments isolated from psoralen-treated HSV-1 virions and visualized in the electron microscope under denaturing conditions to cause strand separation. Except in the untreated control (a), the two strands are seen to come together at the sites of cross-links. The virus was photoreacted with TMP as described in the text for the times indicated: (b) 1 min; (c) 5 min; (d) 30 min. The small closed circular molecules are single-stranded DNA from fd bacteriophage, included for purposes of length calibration (Hanson et al. 1976). All parts of the figure are at the same magnification, and a length calibration in nucleic acid bases is indicated by the bar.
Psoralen photoinactivation of viruses 355

(from a 1 mg/ml stock solution in ethanol, rather than in DMSO as in the above experiments) was added initially and an additional 2.5 µg/ml was added every 5 min thereafter (as indicated by the arrows in Fig. 8) during irradiation at high light intensity. Infectivity was reduced from a starting titre of 10^6 per ml to undetectable by 1 min of treatment. Fig. 8 thus illustrates, for the most part, photochemical effects on the DNA occurring beyond the point of biological inactivation. The plateau effect between 1 and 5 min is believed to result from photodestruction of the unbound drug and necessitates the successive drug additions used to obtain the results in Fig. 8.

In this same experiment the TMP adducts resulting in DNA cross-links were visualized in the electron microscope as shown in Fig. 9. As expected, the spacing of cross-links becomes closer with increasing treatment and by 15 min cannot be measured accurately due to numerous cross-links at spacings below the resolution of the electron microscopy technique. By this technique the locations of cross-links throughout the intact DNA of entire HSV-1 chromosomes have been observed (data not shown).

The possibility of partial gene expression (or transformation) by psoralen-inactivated (non-infectious) HSV-1 was explored using a primary culture of baby hamster kidney cells. After adsorption of the treated virus, the cells were cultured for various periods and then probed with fluorescent-labelled antibodies. Although no virus gene expression nor cell transformation was detected, similar experiments including the use of purified virus DNA should precede development of psoralen inactivated vaccines.

DISCUSSION

The factors expected to govern the photochemical inactivation of viruses by psoralen derivatives include: (A) Properties of the derivative: (i) solubility; (ii) affinity for nucleic acid (‘dark’ binding); (iii) photoreactivity with nucleic acid (quantum yield); (iv) competing photo-breakdown of unbound drug; (v) sensitivity of the above properties to temperature and ionic strength of the medium. (B) Properties of the virus nucleic acid: (i) genome size; (ii) type (ribo vs. deoxyribo); (iii) single or double strandedness; (iv) conformational state as affected by association with other nucleocapsid components and by ionic species in the medium. (C) Spectrum and intensity of irradiation.

While the permeability of the virus coat to psoralen would appear to be an additional factor, the derivatives tested to date are remarkable for readily entering not only viruses but also intact bacteria (Bordin et al. 1976; Cole et al. 1976), yeast (Averbeek & Moustacchi, 1975), animal cells (Ben-Hur & Elkind, 1973) and cell nuclei (Hansen et al. 1976).

The binding properties and photoreactivity of AMT and TMP with purified DNA, along with the rate of photo-breakdown of these derivatives, have recently been discovered to be sensitive functions of ionic strength (Hyde & Hearst, 1978). In the latter study, at physiological salt concentrations the net potency of TMP was found to be several-fold greater than AMT on a molar basis, in general agreement with the inactivation of HSV-1 as shown in Fig. 2 and 3. The relative resistance of TMP to photo-breakdown under these conditions (Hyde & Hearst, 1978) probably contributes significantly to its greater activity, especially at high light intensity.

The inactivation of WEE may result partly from psoralen cross-links in double-stranded ‘fold-back’ regions which could exist in its single-stranded RNA (Marciani et al. 1973; Shen & Hearst, 1976). It is known, however, that psoralen mono-adducts (involving only single nucleotides) are sufficient to block the biological functions of nucleic acids (Baccichetti et al. 1975; Bordin et al. 1975). In either case, the higher aqueous solubility of AMT
(10 mg/ml minimum) would appear to be essential for its superior inactivation of WEE in Fig. 4 and 6. The superiority of 1 µg/ml of TMP at high light intensity (Fig. 5), as in the case of HSV-1, may be accounted for by its relative resistance to photo-breakdown.

The importance of remaining within the solubility limit of the drug is illustrated by the anomalous behaviour of insoluble levels of TMP (Fig. 2, 4 and 6). The mechanism of this behaviour is unknown, but may involve the light-shielding of viruses by crystals which absorb or scatter light. (1 µg/ml TMP introduced into solution as described above may be slightly super-saturated, as a visible precipitate does not appear at concentrations below 1.2 µg/ml even at 0 °C.)

The greater sensitivity of HSV-1 to psoralen photoinactivation (relative to WEE) is consistent not only with the greater drug-binding capacity of the DNA double helix, but also with the larger genome size of HSV-1. There is preliminary evidence that even a single psoralen cross-link in a DNA virus cannot be repaired by host enzymes (Day et al. 1975). In this regard, the time required to accumulate at least one cross-link per virion would be inversely related to the size of the genome. Consistent with this is the observation that 15 min of the low intensity irradiation with only 0.02 µg/ml AMT is sufficient to totally inactivate eukaryotic cells (C. V. Hanson & P. Arnstein, unpublished data on cultured rat cells).

Finally, the psoralen sensitivity of virus nucleic acids may be affected by nucleic acid conformation or interaction with proteins, as has already been demonstrated in eukaryotic nuclei (Hanson et al. 1976; Cech & Pardue, 1977; Wiesehahn et al. 1977). Data such as those in Fig. 8 and 9 are capable of revealing such features of nucleic acid as they exist in situ within the virion. In principle, even the relative rates of inactivation of infectivity by the psoralen treatment could reveal differences among viruses with respect to nucleic acid secondary structures (such as RNA fold-backs) in situ. In the study of Hearst & Thiry (1977), vesicular stomatitis virus (VSV) was somewhat more resistant to specific psoralen treatments than was WEE in the current study. The possible correlation of this result with the ‘extended genome’ expected in the helically symmetric nucleocapsid of VSV deserves further study. In particular, the above electron microscopy technique could reveal base-paired regions (of sufficient length) existing in intact RNA virions during psoralen treatment.

For simplicity, the inactivations in this study were performed on purified virus suspensions with very low turbidity. In practice, however, longer exposures to compensate for light lost through scattering should make it possible to inactivate viruses regardless of the amount of extraneous protein present and thus in marked contrast to the cases of inactivation by formaldehyde or beta-propiolactone. The preferential interaction of psoralens with double-stranded nucleic acids is also the opposite of that of formaldehyde (Bachrach, 1966) and therefore of importance in the development of inactivated DNA virus vaccines.

Studies are in progress to confirm the expected lack of modification of virus surface components by psoralen photoinactivation. In addition to their potential application to inactivated vaccines, psoralens could be valuable for the photochemotherapy of accessible virus infections.

We are grateful to Dr J. E. Hearst for newly synthesized and radioactively labelled psoralen derivatives, for the use of the electron microscope, and for many valuable discussions during the initiation of this study. We thank Professor M. A. Pathak for his generous gift of psoralen and we especially thank K. Nakamura for his expert technical assistance. This work was supported in part by a Biological Research Support Grant (BRSG 05549) from the National Institutes of Health to the California State Department of Health and by a special grant (No. 864) from the California Division of the American Cancer Society.
Psoralen photoinactivation of viruses

REFERENCES


(Received 26 October 1977)