ANNOTATIONS

This section of the Journal *is devoted to rapidly published one page research notes*

(See Instructions to Authors in January–February and July–August issues.)

Effect of Solvents on Cutaneous Penetration of Acridine Orange

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The interaction between triheterocyclic dyes (neutral red, proflavine, acridine orange, toluidine blue) and deoxyribonucleic acid is well known (BLAKE and PEACOCK, *Biopolymers* 6: 1225-1253, 1968; GERSH and JORDAN, *J Mol Biol* 13: 138-156, 1965; LERMAN, *Proc Natl Acad Sci* 49: 94-102, 1963). In the presence of fluorescent or incandescent light, these dyes will consistently inactivate herpes virus under the parameters established in the laboratory (HIATT, *Trans NY Acad Sci* 23: 66-78, 1970; WALLIS, TRULOCK, and MELNICK, *J Gen Virol* 5: 53-61, 1969). Although clinical application of this phenomenon of photodynamic inactivation for treatment of recurrent herpes simplex infections has been moderately successful (FELBER ET AL, *JAMA* 216: 835, 1971; FRIEDRICK, *Obstet Gynecol* 41: 74-77, 1973), clinical factors have not been determined for maximum effectiveness. One of the limiting factors is the ability of the dye to reach the virus. Partial resolution of this problem has been achieved by the unroofing of viral vesicles before dye application. Theoretically, solvents should help carry the dye through epithelium to the virus and would be of great value when herpetic vesicles are not yet clinically evident. The purpose of this study is to test the effects of water, ethanol, acetone, and dimethylsulfoxide (DMSO) on the cutaneous penetration of acridine orange (AO), a fluorescent triheterocyclic dye with potential clinical use for viral photodynamic inactivation.

The backs of six rats were shaved and marked off into quadrants. Quadrants on each of three anesthetized rats were swabbed for one minute with solutions of 1% AO in either water, 50% ethanol, 50% acetone, or 50% DMSO. Quadrants of the other three rats were first scrubbed with one of these solvents for one minute and then swabbed for one minute with 1% AO in water. Skin biopsy specimens were immediately frozen and sectioned in a cryostat for fluorescent microscopy. Prescrubbing the skin with ethanol or acetone resulted in total epithelial fluorescence whereas all others produced only patchy superficial staining (Table).

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Since an ethanol prescrub appeared to best enhance AO penetration, the lower lips of 12 humans were subjected to this procedure. One half of the lip was scrubbed with 95% ethanol for one minute and then swabbed with 1% AO in water for one minute. The other half was swabbed with AO only. After air drying, transparent tape was used to strip off epithelial cells (BAKER and KLIGMAN, *J Invest Dermatol* 45: 273-274, 1967). Twelve tapes were taken from each patient. The tape strips were then attached to a slide, viewed through a fluorescent microscope, and cells were counted. Almost all cells were fluorescent on the first few tape strips. By the tenth strip, 50 to 75% of the epithelial cells exhibited fluorescence from the half pretreated with ethanol whereas less than 25% of the cells from the other half showed AO fluorescence. Fluorescence of the epithelial cells when present appeared evenly distributed throughout the cell with occasional nuclei staining more intensely than cytoplasm.

It is apparent that prescrubbing with ethanol promotes cutaneous penetration of AO and may be of clinical value in the photodynamic treatment of recurrent herpes simplex infections. This alteration in treatment regimen, as well as other possibilities (for example, use of monochromatic light, different time-dose relationships, use of other untested triheterocyclic dyes), merit clinical trial to achieve the maximum success rate possible for this treatment modality.

TABLE

FLUORESCENCE OF RAT SKIN BIOPSY SPECIMENS (Graded on a Scale of 1+ – 3+*)

	1% AO Solvent	1% AO in Water
Water	1+	2+
Ethanol	1+	3+
Acetone	1+	3+
DMSO	1+	1+

* 1+ indicates patchy fluorescence limited to keratinized layer; 2+, patchy fluorescence into prickle cell layer; and 3+, complete epithelial fluorescence to basement membrane.

† Skin was prescrubbed with solvent.