The involvement of ultraviolet light in the induction of skin cancer has been established in both man and animals. Roffo (1) in 1934 demonstrated that prolonged exposure of albino rats to sunlight would result in a high incidence of tumors in the exposed areas. Many other investigators have shown that ultraviolet irradiation will result in a high incidence of skin tumors in animals. Findlay (2), Blum (3, 4), Rusch and associates (5), Kelner and Taft (6), Griffin and coworkers (7–10). The carcinogenicity of ultraviolet light has also been established by Rogers (11, 12) by the rapid in vitro induction of pulmonary adenomas in fetal mouse lung exposed to short wave length ultraviolet. The evidence is also overwhelming that a large percentage of skin cancer in man is caused by prolonged exposure to sunlight. Macdonald (13) has reported that skin cancer is more prevalent in areas of high sun intensity. The tumors appear on the exposed surfaces of the body and fair complexioned individuals are more susceptible than dark skinned peoples or than individuals who undergo normal pigmentation and tanning.

Considerable effort has been expended in many different laboratories in order to obtain a better understanding of ultraviolet carcinogenesis. Some of the factors or mechanisms involved will be included in the discussion of this presentation. Certainly, the presence of specific chemical drugs or metabolites may alter the response of the skin to exposure to light of ultraviolet or other wave bands (3). Of all known substances the psoralens are perhaps the most active in this respect. The application of these drugs in the treatment of vitiligo or in the enhancement of pigmentation has been most adequately covered in this symposium. It was the observations of Fitzpatrick and associates (14) of enhanced pigmentation resulting from the pre-administration of methoxsalen (8-MP or 8-MOP) and subsequent ultraviolet or sunlight exposure that prompted our own interest as to the effect these drugs may have in skin cancer induction.

**EXPERIMENTAL METHODS AND RESULTS**

The procedure for the induction of skin tumors in all of these studies was that described by Rusch and associates (5) with some modifications by our group (7–9). It has been established that approximately 100 daily exposures of 10–20 minutes to the total spectrum of the high voltage mercury arc lamps (G.E. Uviare-UA-3) results in the appearance of ear tumors in a high percentage of albino mice. Approximately 10 percent of these animals will also develop tumors of the eye. From these observations it may be calculated that $14–16 \times 10^4$ ergs per sq. cm. of total ultraviolet energy is required for tumor production. Rusch et al. (5) have reported that $6–8 \times 10^4$ ergs per sq. cm. of “mid-ultraviolet” radiation was adequate for ear tumor formation in Swiss mice.

Methoxsalen was given either orally or intraperitoneally to determine what effect these compounds may have upon ultraviolet cancer induction in albino mice exposed to the total spectrum of uviare lamps (8). The groups were as follows (40 female Swiss mice per group):

- **Group 1A—Controls, U.V. only.**
- **Group 1B—Methoxsalen, 0.4 mg/mouse/day, injected intraperitoneally one hour before U.V. exposure.**
- **Group 1C—Methoxsalen, 0.5 gm/kg in ground Purina Laboratory Chow.**

After approximately 100 days the psoralen and also the ultraviolet irradiation were discontinued and the animals were observed periodically for erythemal effects and ear tumor formation. The findings are shown in Figure 1. Intraperitoneal administration of methoxsalen resulted
Fig. 1. Effect of 8-methoxypsoralen on the incidence of ear tumors in mice exposed to ultraviolet light. Series I: U.V., 15 minutes/day. Group 1A: Controls, U.V. only. Group 1B: 8-MP, 0.4 mg/day, injected intraperitoneally. Group 1C: 8-MP in diet, 0.5 mg/kg diet.

in a severe erythema response to the ultraviolet. This was evident within a few weeks after the study was initiated. In addition to the burning with subsequent scar tissue formation about the ears, eyes and face, these animals all developed ear tumors. The erythema and carcinogenic response observed in this group was increased considerably over that observed in the non-drug controls in this series. In contrast the more gradual incorporation of the psoralen by the oral administration produced a different response to the ultraviolet exposure. These animals had even less erythema damage than the controls and a lower tumor incidence than either of the other two groups.

From the above findings it appears that the mode of administration and the dosage of methoxsalen had considerable influence upon the animal response to ultraviolet irradiation. When the same amount of the psoralen (0.4 mg) was injected I.P. 24 hours instead of 1 hour before U.V. exposure the marked erythema and carcinogenic response was considerably lessened. This would indicate that the drug was metabolized and excreted during this period. We have no immediate explanation for either the enhancement or the protection afforded by the drug in this particular study. Attempts to achieve a greater protection from ultraviolet exposure by dietary administration of methoxsalen have not been successful. Under the conditions established in our laboratory, the oral administration of methoxsalen (up to levels of 1.0 gm/kg of diet) has either protected or has had no effect on the final tumor incidence when compared to the non-psoralen controls of the same series.

Kelner and Taft (6) and Griffin et al. (9) have demonstrated that the lower portion of the ultraviolet spectrum will initiate carcinogenesis in albino mice. Accordingly, we wished to ascertain if methoxsalen had any effect upon cancer induction resulting from prolonged exposure to the germicidal type lamps with a principle emission at 2537 Å. A study was carried out similar to that just described above, employing this lower wavelength source of ultraviolet light (9, 10). The results of this investigation are shown in Table 1. It is evident that this exposure will initiate carcinogenesis in the ears and eyes of albino mice. However, this wavelength is considerably less effective than the longer ultraviolet spectrum. Methoxsalen, given intraperitoneally, did not accentuate carcinogenesis following exposure to the 2537 Å lamps. Approximately 10-20 per cent of these mice developed ear tumors which was very close to that observed in the control groups. The psoralen dietary group in this series exhibited a very mild erythema response and no tumors appeared. In this respect the findings are similar to those obtained in the earlier study involving the entire ultraviolet spectrum.

The next phase of this investigation was directed to the effects of methoxsalen following exposure of albino mice to the longer portion of the ultraviolet spectrum. The same experimental approach was followed as described in the preceding studies. Woods' type ultraviolet lamps (Blak-Ray Model XX15 Long Wave Ultraviolet) were used for the source of radiation. From past observations we have found that mice could withstand prolonged exposure to this form of light without any erythema effects or appearance of tumors. Administration of methoxsalen, especially by the intraperitoneal route and subsequently followed by the long ultraviolet irradiation, resulted in the most severe erythema response ever seen in our laboratories, Table 2 (9, 10). These findings clearly point out the photosensitizing capacity of the psoralen compounds in animals exposed to long wavelength ultraviolet. In group 7, control mice were exposed to a total of $13.4 \times 10^8$ ergs per sq. cm. of the long wave ultraviolet with no visible signs of erythema damage and no tumors were evi-
TABLE 1 (Ref. 9)
Erythemal and Carcinogenic Response Associated With Administration of Methoxsalen and Subsequent Exposure to Ultraviolet Light (2537 Å)

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methoxsalen administration</td>
<td>Ultraviolet irradiation</td>
</tr>
<tr>
<td>1</td>
<td>I.P. injection of 0.4 mg 1 hr. prior to U.V. exposure</td>
<td>20 minutes daily first 3 months, further increased to 60 min. exposure for next 7 mo. (total $8.3 \times 10^8$ ergs/cm²)</td>
</tr>
<tr>
<td>2</td>
<td>Mixed in diet, 0.5 gm per kg. of chow</td>
<td>Slight erythemal damage of ears and eyes</td>
</tr>
<tr>
<td>3</td>
<td>No medication</td>
<td>Mild erythemal damage of ears and eyes</td>
</tr>
<tr>
<td>4</td>
<td>I.P. injection of 0.4 mg daily</td>
<td>Controls, no irradiation</td>
</tr>
</tbody>
</table>

Methoxsalen administration. In contrast (group 11, Table 2), wherein methoxsalen was injected intraperitoneally before irradiation, a total exposure of less than five per cent of the above ($4.3 \times 10^8$ ergs per sq. cm.) resulted in severe burning of the ears, eyes and face, corneal opacity, cataracts, and ear tumors in all animals. Possible explanations for these marked effects will be considered in the discussion section.

A small percentage of the total emission from ordinary fluorescent light is in the ultraviolet range (15). Kline and Rusch (16), however, were unable to induce tumors in albino mice following prolonged exposure to fluorescent light. In view of our findings with psoralen activation of long wave length ultraviolet, we have irradiated control mice, mice given methoxsalen in the diet (0.5 gm per Kg.) and mice injected with the psoralen intraperitoneally (0.4 mg per mouse), for long periods of time with ordinary fluorescent lighting. In two separate studies we have demonstrated an erythemal response and actual ear tumor formation in the psoralen-injected groups. No changes were seen in the other two groups. It would appear that the emission from the fluorescent lamps is activated by certain concentrations of psoralen or psoralen metabolites in the skin resulting in a considerable erythema response.

An understanding of the metabolism of the psoralen compounds is essential in order to explain or predict the photodynamic properties of these compounds. Attempts to obtain C¹⁴ labeled psoralens have not been successful; however, tritium labeled methoxsalen has been made available*. This preparation has an activity of approximately 75,000 counts per mg. There are inherent difficulties in making accurate counts on tritium labeled samples with conven-

* Through the courtesy of Dr. F. S. Rowland, Department of Chemistry, University of Kansas, Lawrence, Kansas.
TABLE 2 (Ref. 9)

Erythemal and Carcinogenic Response Associated with Administration of Methoxsalen and Subsequent Exposure to Long-Wave Length Ultraviolet Light

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methoxsalen</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>5</td>
<td>0.4 mg I.P. one hour before UV exposure</td>
<td>2 hours daily for 3 months (total $13.4 \times 10^9$ ergs/cm²)</td>
</tr>
<tr>
<td>6</td>
<td>0.5 g/Kg diet</td>
<td>Same as above</td>
</tr>
<tr>
<td>7</td>
<td>Controls</td>
<td>Same as above</td>
</tr>
<tr>
<td>8</td>
<td>0.4 mg I.P. one hour before UV exposure</td>
<td>30 min. daily for 3 months ($2.6 \times 10^9$ ergs/cm²)</td>
</tr>
<tr>
<td>9</td>
<td>0.5 g/Kg diet</td>
<td>Same as above</td>
</tr>
<tr>
<td>10</td>
<td>Controls</td>
<td>Same as above</td>
</tr>
<tr>
<td>11</td>
<td>0.4 mg I.P. 1 hour before UV exposure</td>
<td>10 min. daily for 6 week period. ($4.3 \times 10^8$ erg/cm²)</td>
</tr>
<tr>
<td>12</td>
<td>0.5 g/Kg diet</td>
<td>Same as above</td>
</tr>
<tr>
<td>13</td>
<td>Controls</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

* In these groups it was difficult to arrive at an accurate tumor incidence. The scar tissue as well as other remaining erythemal damage was still considerable. From both gross and microscopic examination the final tumor incidence in these groups was high.

DISCUSSION

Throughout this study we have been concerned with the extensive changes seen in the eyes of the animals given psoralen and exposed to ultraviolet light. In past investigations we have noted that approximately ten per cent of ultraviolet light-irradiated mice have developed eye tumors. Kelner and Taft (6) made somewhat similar observations following exposure to the 2537 Å lamps. Following the intraperitoneal administration of methoxsalen and subsequent exposure to the total spectrum of the mercury arc very obvious and extensive lesions have appeared. These results, while obviously of a preliminary nature, would indicate a concentration of the drug or its metabolites in the skin.

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psoralen and ultraviolet irradiation. Since our initial efforts were more concerned with skin tumorigenesis, little direct attention was paid to the eye changes. Currently, we are making detailed gross and microscopic studies of these changes in the eye.

Little is known concerning the actual mechanism in malignant transformation of tissues exposed to ultraviolet light. In all instances there is extensive destruction of tissues and cellular proliferation subsequently occurs. During this process of new cell formation or during mitosis the ultraviolet radiation probably results in alterations in the nucleoproteins of the chromosomes. The mutagenicity of ultraviolet light is well established. Recently, Haas and Doudney (18) have reported that E. coli cultures synchronized in growth and division exhibit an increased mutation frequency when exposed to short term incubation in a medium supplemented with purines and pyrimidines just prior to ultraviolet irradiation. They found that this increase in mutation frequency was attained during periods of maximum nucleic acid synthesis.

Presence of the psoralen compounds may result in biosynthesis of altered nucleoproteins when cells are exposed to ultraviolet radiation. Altenburg (18) has reported that application of methoxsalen to the polar caps of Drosophila resulted in a greater number of mutations when the organisms were exposed to ultraviolet light.

Other than the points already mentioned, we cannot explain the effects of the psoralen upon ultraviolet carcinogenesis. A better understanding is required of the metabolism or distribution of these compounds within the body. In mammals with the capacity to pigment we would like to believe that methoxsalen would promote pigmentation and thereby protect exposed areas from subsequent ultraviolet irradiation. It is even more difficult to explain the protective effect observed when dietary psoralen was given to the irradiated albino mice. With the intraperitoneal administration of the psoralen we may assume that relatively large quantities of the drug or its metabolites are concentrated in the skin. During ultraviolet exposure the drug exhibits the property of fluorescence giving off ultraviolet light of other wave lengths. The light emitted is optimal for the initiation of erythema and the other untoward effects that have been observed. The psoralens do possess fluorescent properties.

The studies involving the combination of psoralen and long wave length ultraviolet light would further indicate that fluorescence must play a major role in the mechanism of action of these drugs. It is possible that the psoralens only initiate pigmentation and other erythemal effects by exposure to the longer portion of the ultraviolet spectrum. There is need for further studies with controlled wave band irradiation. It should also be pointed out that the visible spectrum may exert considerable influence upon the induction of cancer by ultraviolet irradiation.

Finally, it must be recognized that the anatomical and chemical composition of the skin may vary considerably from species to species or within species. The careful studies of Baumann and coworkers (19–22) with respect to the metabolism and composition of the skin sterols point out these differences and also give some indication of the changes which may occur during ultraviolet or chemical carcinogenesis. From the many reports presented during this symposium it is apparent that some progress has been made regarding the action of the psoralens and other photosensitizing compounds and also in man’s reaction or response to his light environment.

REFERENCES


