CLINICAL AND EXPERIMENTAL STUDIES WITH 8-METHOXYPSORALEN IN VITILIGO*

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Reports on the effective treatment of vitiligo have created great interest among physicians. El Mofty (1, 2) found that the use of crystalline constituents of the plant Ammi Majus Linn., together with ultraviolet light exposure, resulted in repigmentation of vitiliginous areas in over 75 per cent of patients with this disorder.

The crude plant has been used orally since ancient times in Egypt. The patients usually took 4 to 12 grams after meals for 15 days during which time vitiliginous areas were exposed to sunlight until inflammation and vesiculation occurred. After the acute reaction subsided, normal pigment was restored in the previously involved areas of leukoderma. Side effects of this crude preparation included severe vomiting, diarrhea, coma, abdominal pain, nephritis, hepatitis with cirrhosis, and exfoliative dermatitis. Despite the marked toxicity of this preparation, considerable interest was aroused because of its therapeutic effect. Concentrated alcoholic extracts of the plant were used orally and locally with good results. However, the extracts were only slightly less toxic than powder from the crude plant.

Recently Fahmy and Shady (3—5) reported the isolation of three crystalline principles from the Ammi majus Linn. fruits which were named Ammoidin, Majudin, and Ammidin. Schonberg, Sina, Fahmy and Shady (5, 6) found these compounds to be identical with the previously known chemicals xanthotoxin, bergapten, and imperatorin, respectively. The three compounds are psoralen (furo-coumarin) derivatives and are named chemically as shown in Fig. 1 corresponding to the above sequence.

El Mofty (1, 2) and others (7—10) used the three compounds separately and in various combinations. The drugs were given orally and applied locally to the involved areas. In doses which were effective therapeutically no significant toxic reactions occurred. Vitiliginous areas became repigmented in as many as 77 per cent of the patients.

The results of clinical and experimental studies with 8-methoxypsoralen† (xanthotoxin, ammoidin) are reported below.


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† 8-methoxypsoralen was obtained through the courtesy of the Memphis Chemical Company; Cairo, Egypt and the Paul B. Elder Company; Bryan, Ohio.
CLINICAL STUDIES

The clinical studies were divided into two parts: first, the effect and toxicity of 8-methoxypsoralen administered orally to patients with vitiligo; second, the effect and toxicity of this drug on the erythema response in three light sensitive individuals with albinism. All patients with vitiligo and albinism were given the drug orally. On occasions some 8-methoxypsoralen was applied topically. However, we wanted to determine the effect of a single agent administered in

![Chemical structures]

Fig. 1. 8-methoxypsoralen 5-methoxypsoralen 8-isoamyleneoxypsoralen

only one way. Throughout the experimental period clinical laboratory studies consisting of routine urinalyses, complete blood counts, tests for urinary porphyrins, prothrombin times* and liver function tests were made. The liver function tests included thymol turbidity, cephalin cholesterol flocculation, gamma globulin, free and total serum bilirubin, and serum bromsulphalein.

Case 1. C. C., a white female, age 13, with vitiligo. About 6 months before seeking medical advice, the patient first noted the spontaneous occurrence of depigmented spots over the upper mid back. These areas enlarged slightly, and other small areas of vitiligo appeared in the same vicinity. Gradually, during the next 6 months, lesions appeared about

* Prothrombin times were carried out to determine whether 8-methoxypsoralen, which is a coumarin derivative, has an anticoagulant action similar to dicoumarol.
the anterior neck and upper chest. No depigmented area had shown regression. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient took orally 30 mg. of 8-methoxypsoralen daily and used an R.S., G.E. sunlamp in erythema doses daily over the involved areas of the back for a period of 3 months. After 2 months of this treatment, the lesions on the back filled in from the edges with diffuse pigmentation. A small amount of perifollicular repigmentation was also noted in the vitiliginous areas. During the following months complete repigmentation occurred.

After 2 months of oral medication with 8-methoxypsoralen, the anterior neck and upper chest were exposed to the sunlamp for a period of 1 month. Pigmentation was also restored in these sites. She noted that exposure of normal skin to strong sunlight for 4 hours on a hot day did not result in erythema, whereas such exposure prior to therapy had resulted in marked erythema.

No toxicity followed the use of 8-methoxypsoralen. Laboratory studies showed normal blood counts, urinalyses, prothrombin times, liver function tests, except for a 1+ cephalin cholesterol at 48 hours, and no porphyrinuria following 3 months of 8-methoxypsoralen therapy.

Case 2. A. L., a white female, age 27, with vitiligo. Twenty-five years ago, an area of depigmentation developed spontaneously over the left knee and popliteal space. Ten years ago, areas of leukoderma appeared about the finger tips of both hands and over the interdigital web between the third and fourth digits of the right hand. Shortly thereafter, depigmentation occurred over the other finger webs, dorsal surfaces of the hands, and elbows. Eighteen months ago, a large patch of depigmentation gradually formed on the extensor surface of the left forearm; and a small spot of vitiligo appeared on the flexor surface of the middle of the left forearm. No depigmented areas had shown regression, and the lesions had been stable for 1 year prior to treatment. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient ingested 10 mg. of 8-methoxypsoralen daily for two months without change in any of the areas of vitiligo. After 1 month with no medication, the patient was given 50 mg. orally of 8-methoxypsoralen daily and she exposed the involved areas to sunlight frequently for a period of six months. After 4 months of therapy, and shortly after a moderate sunburn of the vitiliginous areas, perifollicular repigmentation was noted over the left elbow, the left thumb, and particularly over the extensor surface of the left forearm. The patient discontinued therapy for a period of 3 months during which time there was no change in the newly repigmented areas. She then took 30 to 50 mg. orally of 8-methoxypsoralen daily and used an R.S., G.E. Sunlamp daily in erythema doses over the involved areas for 4 months. During this time perifollicular repigmentation increased in all vitiliginous areas.

Fig. 3. The arm of case 2 after 4, 6, and 8 months of therapy
except over the knuckles. At this point three sites of vitiligo were treated locally with Thorium X in a concentration of 150 microcuries or 1000 e.s.u. per cc. of base. One site was treated with an alcoholic solution, another with an ointment, and the third with 0.1 per cent 8-methoxypsoralen added to the alcoholic solution. The size of the lesions was determined, and 1 cc. of the various preparations was used to cover 100 cm.² of involved area. On examination two weeks later, marked erythema and vesiculation were noted in the vitiliginous areas. Hyper-pigmentation was pronounced in the normal skin areas which came in contact with Thorium X. Thorium X did not increase repigmentation in any of the test areas although it did cause darkening of the perifollicular repigmentation which had returned previously. No toxicity to 8-methoxypsoralen occurred.

Laboratory data showed normal blood counts, urinalyses, prothrombin times, liver function tests, and no porphyrinuria during and following 20 months of intermittent therapy with 8-methoxypsoralen.

Case 3. M. F., a Negro female, age 15 months, with vitiligo. Six months prior to examination the patient's parents noted over a period of 1 month the spontaneous occurrence of depigmentation about the medial portion of the right orbit, right upper lip, and right temporal region. The parents were greatly concerned about the patient's appearance. The depigmented areas had shown no regression, but had remained stable in size, and no new sites had developed during the five months preceding examination. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient ingested 10 mg. of 8-methoxypsoralen each day and exposed the involved areas to erythema doses of sunlight whenever possible for a 12 month period beginning in May 1951. Three months after initiating treatment, normal pigmentation began to fill in the areas of vitiligo from the edges. In five months there was considerable return of pigmentation along the right upper lip and right lower eyelid. Pigmentation also increased along the medial portion of the right upper eyelid and in the spot over the right temporal region. By the eleventh month, the area about the mouth had entirely filled in with normal pigment. One month later the area along the medial margin of the orbit began to expand by gradually becoming depigmented at all margins.

No toxicity to the use of 8-methoxypsoralen was noted. The child gained 10 pounds over the period of treatment, and growth and development were normal.

Laboratory data showed normal blood counts, urinalyses, and no porphyrinuria during and following the twelve months of 8-methoxypsoralen therapy.
Case 4. F. S., a white male, age 33, with vitiligo. Seventeen years ago, the patient first noted the spontaneous occurrence of depigmentation over the upper back and shoulders. During the succeeding years, other leukodermic areas appeared over the anterior and posterior trunk, neck, face, and upper extremities. No depigmented areas had shown regression. The lesions had remained stable for the last few years, and no new sites had developed. The family history was of interest in that the patient's father and brother also had vitiligo. Except for the vitiliginous areas, the physical findings were within normal limits.

The patient took 10 mg. orally of 8-methoxypsoralen each day and exposed the involved areas to erythema doses of sunlight whenever possible for a period of 4 months beginning in February, 1951. The patient was next seen in September, 1951, at which time he stated that he had not become sunburned in the involved areas during the summer despite a more than usual amount of exposure to sunlight. On examination, no repigmentation was noted in any of the vitiliginous areas. The patient then took 10 to 30 mg. orally of 8-methoxypsoralen each day and used an R.S., G.E. Sunlamp daily on the involved areas in erythema doses over a 9 month period. He also exposed these areas to sunlight whenever possible. At the end of this time the patient stated that there was less reaction in the involved areas on exposure to sunlight and that a small area of vitiligo beneath his chin had become repigmented. Upon examination under the Wood’s lamp, pigmentation in the vitiliginous patches could be seen. The patient was a farmer who for the 15 years preceding treatment could not work outside on a sunny day unless all vitiliginous areas were covered. Since receiving treatment, he has been able to work in the fields without taking such precautions.

At times the patient noted vague feelings of nausea and increased nervous tension while taking 20 mg. of the drug daily. These side effects did not necessitate discontinuing the medication.

Laboratory data showed normal blood counts, urinalyses, liver function tests, prothrombin times, and no prophyrinuria during and following 1 year's treatment with 8-methoxypsoralen.

Case 5. G. S., a white male, age 49, with vitiligo. This patient, a brother of F. S., first noted the spontaneous occurrence of depigmentation of the chin 18 months prior to examination. Other small areas of leukoderma appeared over the face, neck, upper extremities, and scrotum during the following 9 months. No depigmented areas had shown regression. The lesions had remained stable for 9 months, and no new sites had developed during the period of observation. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient ingested 10 to 30 mg. of 8-methoxypsoralen daily and used an R.S., G.E. Sunlamp in erythema doses daily over the involved areas for a period of 9 months. He also exposed these areas to sunlight whenever possible. In 6 months slight diffuse brown pigmentation appeared in a few of the vitiliginous areas over the patient's face. Gray hair in these areas grew out black. In 8 months the patient reported that he was able to tolerate sunlight on the depigmented areas much better than before treatment. At times he complained of vague feelings of nausea, epigastric distress, and increased nervous tension while taking 20 to 30 mg. of the drug daily. These side effects did not necessitate discontinuing the medication.

Laboratory tests showed normal blood counts, urinalyses, liver function tests, except for a 1+ cephalin cholesterol at 48 hours, prothrombin times, and no porphyrinuria during and following treatment with 8-methoxypsoralen for 1 year.

Case 6. H. L., a white female, age 36, with vitiligo. Three years ago, the patient first noted the spontaneous occurrence of depigmentation over the dorsal surfaces of the hands. During the last three years the patient developed areas of vitiligo over the forearms. No depigmented areas had shown regression. The patient complained of the unsightly appearance of the lesions and of sunburning in the involved regions. With the exception of the vitiliginous areas, physical findings were within normal limits.

The patient took 20 mg. orally of 8-methoxypsoralen each day, and for a period of 6 weeks was given daily increasing doses of ultraviolet light with a Hanovia ultraviolet lamp
on the involved areas of the hands and forearms. She reached a final level, of 8 minimal erythema doses. No definite return of pigmentation was noted following treatment. Five months afterwards, the patient's ultraviolet light sensitivity time, tested in the previously treated vitiliginous areas, was 2 minutes, or 4 minimal erythema doses, suggesting a decrease in ultraviolet light sensitivity despite the lack of apparent repigmentation. She again ingested 20 mg. of 8-methoxypsoralen each day and exposed the involved areas to sunlight whenever possible for a period of 2 months. No return of pigment was noted. However, the areas had not sunburned as they did prior to therapy. The patient noted diarrhea if she took more than 20 mg. of 8-methoxypsoralen daily.

Laboratory data showed normal blood counts, urinalyses, liver function tests, prothrombin times, and no porphyrinuria following the last 2 months of 8-methoxypsoralen therapy.

Case 7. F. W., a white female, age 31, with vitiligo and congenital abnormalities of the fingers and toes. Nineteen years ago, the patient first noted the spontaneous occurrence of depigmentation about the mouth. Since then she had developed extensive areas of vitiligo over the body surface, particularly in the covered areas. Recently new areas of vitiligo were noted on the flexor surfaces of the forearm. It was possible that part of the vitiliginous area had become repigmented spontaneously since the onset of the disease. The family history was of interest in that the patient's grandfather, her father's cousin, a nephew, and a sister all had vitiligo.

On physical examination shortening and radial deviation of the fifth digit of each hand were noted. In addition there was ulnar deviation of the third finger of each hand. Webbing was present between the second and third toes of each foot. With the exception of the vitiliginous areas, the remaining physical findings were within normal limits.

The patient took 30 mg. orally of 8-methoxypsoralen each day and used an R.S., G.E. Sunlamp daily in erythema doses to the involved areas for a period of 1 month. In addition three sites of vitiligo were treated locally with Thorium X in a concentration of 150 microcuries per cc. of base. One site was treated with an alcoholic solution, another with an ointment, and the third with 0.1 per cent 8-methoxypsoralen added to the alcoholic solution. The size of the lesions was determined, and 1 cc. of the various preparations was used to cover 100 cm.² of involved surface.

All areas treated with Thorium X became very erythematous and slightly edematous within 48 hours. In 4 days hyperpigmentation occurred in the area of normal skin in contact with Thorium X. Twenty days after the applications the vitiliginous areas were still erythematous and mild desquamation was present in all treated sites. Hyperpigmentation of normal skin areas was very marked and showed no evidence of fading. No areas of vitiligo showed any repigmentation. The patient developed an eruption of typical pityriasis rosea about 1 week following the use of Thorium X. Although there were lesions over the entire trunk, including the vitiliginous areas, none developed in the sites treated with Thorium X. No toxicity to the use of 8-methoxypsoralen occurred.

Laboratory data showed normal blood counts, urinalyses, prothrombin times, liver function tests, except for a 3+ cephalin-cholesterol flocculation test at 48 hours, and no porphyrinuria after 4 weeks of treatment with 8-methoxypsoralen.

Case 8. D. E., a Negro female, age 15, with vitiligo. Eight years ago the patient first noted the spontaneous occurrence of depigmentation over the left upper chest followed by areas of depigmentation over the left upper arm and forearm and left fourth and fifth digits. Four years ago areas of leukoderma appeared over the left upper back. The patient felt that a few of the vitiliginous areas had filled in slightly with normal pigmentation during the last 2 years. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient took 30 mg. orally of 8-methoxypsoralen daily and used an R.S., G.E. Sunlamp in erythema doses daily on the involved areas for a period of 7 months. In the last months three sites of vitiligo were treated locally with Thorium X in a concentration of 150 microcuries per cc. of base. One site was treated with an alcoholic solution, another with
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an ointment, and the third with 0.1 per cent 8-methoxypsoralen added to the alcoholic solution. The size of the lesions was determined, and 1 cc. of the various preparations was used to cover 100 cm.² of involved surface.

All the Thorium X treated areas became very erythematosus and slightly edematous within 48 hours. In about 8 days hyperpigmentation was noted in the normal skin areas in contact with Thorium X. Twenty days after the applications the depigmented areas were still erythematous, hyperpigmentation of the normal skin areas remained, and moderate desquamation was evident. No areas of vitiligo had shown any repigmentation. No toxicity was noted to the use of 8-methoxypsoralen.

Laboratory data showed normal blood counts, urinalyses, liver function tests, prothrombin times, and no porphyrinuria during and following treatment for 7 months with 8-methoxypsoralen.

Case 9. D. D., a white female, age 23, with vitiligo. Twelve years ago the patient first noted the spontaneous occurrence of depigmentation over the knees and volar aspects of the wrists. During the last four years, the patient developed successive areas of vitiligo of the ankles, finger tips, axillae, trunk, genitalia, and anal and perioral regions. No depigmented areas had shown regression. The lesions had remained stable for the last year, and no new sites of vitiligo had developed during the period of observation. With the exception of the vitiliginous areas, the physical findings were within normal limits.

The patient took 30 mg. orally of 8-methoxypsoralen each day and used an R.S., G.E. Sunlamp in erythema doses daily on the involved areas for a period of 2 months. In the last month three sites of vitiligo were treated locally with Thorium X in a concentration of 150 microcuries per cc. of base. One site was treated with an alcoholic solution, another with an ointment, and a third with 0.1 per cent 8-methoxypsoralen added to the alcoholic solution. The size of the lesions was determined, and 1 cc. of the various preparations was used to cover 100 cm.² of involved surface.

All the Thorium X treated areas became very erythematous and slightly edematous within 48 hours. In 4 days hyperpigmentation was noted in the normal skin areas in contact with Thorium X. The vitiliginous areas were still erythematous. Twenty days after the applications, the depigmented areas were no longer erythematous; however, the hyperpigmentation of the normal skin areas had not decreased. No areas of vitiligo had shown any repigmentation. No toxicity resulted from the use of 8-methoxypsoralen.

Laboratory data showed normal blood counts, urinalyses, liver function studies except for a 2+ cephalin cholesterol at 48 hours, and no porphyrinuria after 2 months of therapy with 8-methoxypsoralen.

Of the nine patients with vitiligo treated with 8-methoxypsoralen, seven were females and two males. Three patients showed striking improvement, two showed less but definite improvement, and four showed no change.

The patients ranged in age from 15 months to 49 years and were treated for a period of 1 to 20 months. The daily dose of the drug varied from 10 to 50 mg. orally given in divided doses supplied in 10 mg. capsules. Six of the patients showed no reaction to the drug, and three had mild symptoms not requiring discontinuation of the medication. One patient had nausea and increased nervous tension, the second, nausea and epigastric distress, and the third, diarrhea if the daily dose was greater than 20 mg. All laboratory studies, except for two, 1+, one 2+, and one 3+ cephalin-cholesterol flocculation tests after 48 hours, were negative. Since all 24 hour tests were negative, no significance was attached to the one, two, and three plus results.

During the above studies it was observed that three of the nine patients developed marked increase in tolerance to ultraviolet light. It was not known
whether this inhibition of the erythema reaction to sunlight was due to previous exposure to ultraviolet light (11) or to the drug. To study this problem further, 8-methoxypsoralen was given to three individuals with albinism; and the minimal erythema dose responses were followed. All tests for light sensitivity were performed at new sites each time.

Case 10. K. W., a white male, age 24, with albinism. The patient had very fair skin, white hair, blue-grey eyes, lateral nystagmus, and photophobia. Except for a few black beard hairs and one minute pigmented macule on the mid-back, no evidence of normal melanin pigmentation was present. The patient did not become tanned on exposure to sun.

The patient ingested 30 mg. of 8-methoxypsoralen daily for 3 months. His ultraviolet light erythema time prior to and 3 weeks after beginning treatment was 15 seconds. At the end of 2 months of treatment, the patient's ultraviolet light erythema time was 30 seconds, indicating a definite decrease in sensitivity. The patient noticed no decrease in photophobia, but he believed that his skin was less photosensitive on exposure to sunlight following treatment. No toxicity resulted from the use of 8-methoxypsoralen.

Laboratory data showed normal blood counts, urinalyses, prothrombin times, liver function studies except for a 1+ cephalin cholesterol at 24 and 48 hours, and no porphyrinuria following 3 months of treatment with 8-methoxypsoralen orally.

Case 11. J. H., a white male, age 32, with albinism. The patient had very fair skin, white hair, pink eyes, lateral nystagmus, and photophobia. There was no evidence of normal melanin pigmentation.

The patient took 30 mg. orally of 8-methoxypsoralen daily for 3 months. His ultraviolet light erythema time prior to therapy as well as 4 weeks afterwards was 5 seconds. At the end of 2 months of treatment, the ultraviolet light erythema time was 30 seconds, indicating a definite decrease in sensitivity. After 3 months therapy, the erythema time was 15 seconds. The patient noticed no decrease in photophobia, but he believed that his skin had become less photosensitive on exposure to sunlight. No toxicity resulted from the use of 8-methoxypsoralen.

Laboratory data showed normal blood counts, urinalyses, liver function studies, except for a 1+ cephalin cholesterol at 48 hours, prothrombin times and no porphyrinuria during and following 3 months of treatment with 8-methoxypsoralen orally.

Case 12. D. W., a white female, age 19, with albinism. The patient had very fair skin, white hair, blue eyes, lateral nystagmus, and photophobia. There was no evidence of normal melanin pigmentation present.

The patient took 30 mg. orally of 8-methoxypsoralen daily for 2 months. Prior to therapy her ultraviolet light erythema time was 10 seconds. After treatment for 1 month it was 15 seconds, and after 2 months it was 10 seconds, suggesting a possible decrease in sensitivity. She was able to swim outdoors on a hot sunny day for 1 and ½ hours without showing any erythema when examined 15 hours after exposure. The patient stated that such exposure prior to therapy would have produced a marked reaction. No toxicity resulted from the use of 8-methoxypsoralen.

Laboratory data showed normal blood counts, urinalyses, prothrombin times, liver function tests, except for a 2+ cephalin cholesterol at 24 hours and a 3+ reaction at 48 hours, and no porphyrinuria during and following 2 months of treatment with 8-methoxypsoralen orally.

Two of the albinos were treated for 3 months and the third for 2 months. All showed decreased sensitivity to ultraviolet light. Two stated that they no longer wore hats when out in the sun. There was no effect on the photophobia or lateral nystagmus.

None of the albinos developed any symptoms from the drug. All laboratory
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studies other than the cephalin cholesterol flocculation test were negative. One patient showed a 1+ test at 24 and 48 hours after 3 months of therapy, a second showed a 1+ test in 48 hours after 3 months and a third showed a 2+ at 24 hours and a 3+ at 48 hours after 2 months.

TOXICITY STUDIES

Recent reports showed that for guinea pigs the minimal lethal dose was 400 mg. per kg. for 8-methoxypsoralen and 800 mg. per kg. for 8-isoamyleneoxypsoralen (12). Death from 8-methoxypsoralen was attributed to adrenal hemorrhage and from 8-isoamyleneoxypsoralen to acute necrosis of the liver. Guinea pigs given sublethal doses of 200, 250, 300 and 350 mg. per kg. orally and sacrificed after 30 hours showed cloudy swelling, fatty degeneration, and acute hemorrhagic necrosis of the liver. The kidneys were severely congested. Hematuria was present. At doses of 300 mg. per kg. there was congestion and lipid depletion of the adrenals. Similar doses of 8-isoamyleneoxypsoralen produced only some cloudy swelling of the liver. Repeated oral administration of 8-methoxypsoralen in doses of 1 and 2 mg. per kg. for 5 months had no effect on growth but resulted in liver necrosis in some of the animals.

For mice the minimal lethal dose was 310 mg. per kg. for 8-methoxypsoralen, and 370 mg. per kg. for 8-isoamyleneoxypsoralen (2). In our experiments, adult male mice were divided into 5 groups of 8 animals and given 8-methoxypsoralen by intraperitoneal injection in 5% acacia (½ ml. volume) in doses of 100, 200, 300, 400, and 600 mg. per kg. At the end of 48 hours, deaths occurred as follows: 1 in the 200 mg. per kg. group, 2 in the 300 mg. per kg. group, 3 in the 400 mg. per kg. group, and 4 in the 600 mg. per kg. group.

In the course of studies to determine whether these drugs could alter the effect of large amounts of x-ray on adult female rats, it was observed that when 8 control rats were given 200 mg. per kg. of 8-methoxypsoralen by intraperitoneal injection in 5% acacia, 3 died in 24 hours and the rest lived (13). Of eight rats treated similarly but given 500 r, 2 died in 24 hours and the rest lived. Of eight rats treated as above but given 900 r, 2 died in 24 hours and the rest in the following two weeks. The early deaths probably resulted from toxicity to 8-methoxypsoralen and the late deaths from radiation. These studies showed that of 24 rats given 200 mg. per kg. 7 died within 24 hours and the rest lived. Of 8 rats given 400 mg. per kg. and 900 r, 7 died in 24 hours.

El Mofty gave three patients 150 mg. of 8-methoxypsoralen in divided doses 3 times daily (2). Severe nausea, vomiting, giddiness, sense of fatigue, and headache were experienced by all. One patient developed diarrhea. Urinalyses were negative. Three patients given 100 mg. in divided doses showed less severe symptoms than those seen in patients receiving 150 mg. per kg. It was claimed that a dose of 50 mg. produced no symptoms. Doses of 8-isoamyleneoxypsoralen of 1 mg. per kg. produced no symptoms. The drugs had no effect on three patients with bronchial asthma and two patients with hyperthyroidism. A patient with a papular necrotic tuberculid could tolerate only half of the expected dose. A patient with mild diabetes mellitus who did not require insulin became worse.

Our studies showed that a child of 15 months could take 10 mg. daily for months without deleterious effect on growth or weight. For adults, doses ranging from 20 to 50 mg. were given for long periods of time. There was great variation in individual tolerance. Whereas one person took 50 mg. daily without difficulty, another did well on 20 mg. daily but developed symptoms when given 30 mg. The symptoms observed were nausea, epigastric distress, increased
nervous tension, and diarrhea. One of our patients developed pityriasis rosea while on the drug. It was our feeling that this eruption was not related to the drug. Extensive laboratory studies were negative except for the cephalin cholesterol flocculation test which was carried out on 11 of the 12 cases. Of the 11 patients, 7 had questionable changes in the cephalin cholesterol flocculation test after receiving the drug for 1 to 3 months. In 5 cases only the 48 hour tests were positive; in the other two, one was 1+ and the other 2+ at 24 hours.

![Figure 5](image)

**FIG. 5.** Molecular extinction coefficient of 8-methoxypsoralen

The above data indicate that up to 50 mg. of 8-methoxypsoralen daily in divided doses is safe for adults. It appears worthwhile to do cephalin cholesterol flocculation tests monthly for 3 months and then at less frequent intervals to check liver function.

**IN VITRO STUDIES**

All *in vitro* experiments were carried out with 8-methoxypsoralen before and after irradiation of the compound with ultraviolet light. This procedure was followed because in the treatment of vitiligo, the drug is inactive alone, ultraviolet light being required for a therapeutic effect. It is possible that 8-methoxypsoralen is converted to a new compound which in turn may be the active agent
stimulating the melanocyte* to produce pigment. Another possibility is that the drug and ultraviolet light potentiate one another’s effect on the pigment producing cell.

Both 8-methoxypsoralen and 8-isoamyleneoxypsoralen absorb greatly throughout the ultraviolet light spectra. The molecular extinction coefficient curves are shown in Fig. 5. If a dilute solution of 8-methoxypsoralen is irradiated with ultraviolet light for 3 and 6 hour periods of time, a decrease in optical density is obtained as shown in Fig. 6. Thus it appears that irradiation of 8-methoxypsoralen with ultraviolet light destroys the compound. Ascorbic acid and ferrous ions have no apparent effect on this change in absorption. When a relatively

concentrated solution is irradiated, no change is observed in the ultraviolet absorption spectrum; but a yellow colored solution is produced. The absorption of this yellow solution is shown in Fig. 7. The solution prior to irradiation does not give any absorption in this region. Exposure of these solutions to light from an ordinary incandescent bulb did not bring about the above changes.

One half ml. portions of the dilute and 0.4 ml. of the concentrated solutions before and after irradiation were added to 0.2 ml. mammalian tyrosinase (approximately 3 units) (14), 0.5 ml. substrate (0.5 mg. tyrosine or dopa in buffer)

* In accordance with terminology adopted at the Pigment Cell Conference (N. Y., 1951), the term melanocyte will be used to designate the mature pigment forming cell, thus replacing the older term melanoblast.
and sufficient 0.1 M phosphate buffer at pH 6.8 to make a total volume of 3 ml. Oxygen uptake measurements at 38°C on the tyrosine-, dopa-, and glutathione inhibited-tyrosinase reactions were negative. No effect on pigment formation was observed. The preparations did not affect the tyrosine or dopa histochemical tests in human skin slices.

![Graph](image)

**Fig. 7.** The effect of ultraviolet light on the optical density of 8-methoxypsoralen 666.5 mg. per liter in a solution of 1 part ethanol, 0.4 parts 10 per cent Tween 20 and 4.6 parts water. Ultraviolet irradiation was obtained from an R.S., G.E. Sunlamp 2 inches above the solution. The volume of the solution was kept constant by adding fresh solvent as rapidly as the solution evaporated.

![Chemical Structure](image)

**PSORALEN**

**Fig. 8.** Psoralen

These solutions were also tested to determine whether they could act as the melanocyte stimulating hormone (melanophore hormone). Tests with isolated frog skin were negative.

**TERMINOLOGY**

In 1933, Jois, Manjunath and Venkatia Rao (15) isolated a substance from the seeds of *Psoralea corylifolia* Linn. to which they assigned the name *psoralen*. 
This chemical consisting of a coumarin and furo group is the nucleus for the compounds discussed in this paper. The nucleus and the method of numbering these compounds is shown in Fig. 8. In 1911, Priess (16) and Thoms (17) isolated 8-methoxypsoralen from *Peganum xanthoxyloides* and gave it the name xanthotoxin. In 1947, Fahmy and Shady (3) used the term ammoidin. Originally, 8-isoamyleneoxypsoralen, isolated from *Imperatoria ostruthium* was called imperatorin (18) and later ammidin (3). Since 1891, 5-methoxypsoralen, isolated from oil of bergomot, had been known as bergapten (19) and later as majudin (4). We believe it is best to retain the correct chemical names as shown in Fig. 1. For general use, the term psoralen derivative is suggested.

**SOURCE OF PSORALEN COMPOUNDS**

The psoralen derivatives discussed in this paper have been isolated from a variety of natural sources including, expressed citrus peel oils, bergomot oil, and the plants, *Ammi majus* Linn., *Angelica archangelica*, *Angelica glabra*, *Seseli indicum*, *Scirrha lauroela* Hook, *Imperatoria ostruthium*, *Aegle marmelos*, *Ruta graveolens*, *Luvanga scandens* and others. In addition, these compounds have been made synthetically. The psoralens present in natural oil, such as bergomot, stimulate the melanocytes and produce the hyperpigmentation seen in Berloque dermatitis.

**DISCUSSION**

Many substances have been used to treat vitiligo, including vitamins of the B complex (especially PABA), pituitary extracts, heavy metals and psoralen derivatives. With the exception of the recently purified psoralen compounds therapy has always had a variable effectiveness. Our clinical and animal toxicity studies parallel those of El Mofty (2) and Elwi (11).

The Egyptian workers used mixtures of 8-methoxy- and 8-isoamyleneoxy-psoralen both locally and orally, followed by exposure of the vitiliginous areas to ultraviolet light, and reported results better than ours. We used 8-methoxypsoralen orally only and exposed the involved areas to ultraviolet light. It is planned to try mixtures of the psoralen derivatives in future studies. Unfortunately, no simple system is available by which compounds can be tested. Clinical evaluation is obviously difficult because many patients must be studied for long periods of time before definite conclusions can be reached.

When properly used the psoralen derivatives are not toxic. The LD$_{50}$ for rats and mice is about 400 to 600 mg. per kg., while the therapeutic dose for human beings is less than 1 mg. per kg. When symptoms of nausea and gastrointestinal distress occur, the dosage should be decreased. The only clinical laboratory study considered important is the cephalin cholesterol flocculation test. This determination should be carried out once a month for 3 months and then at less frequent intervals.

Individuals handling the drug should not let it come in contact with the skin. One of our laboratory workers' arm came in contact with an alcoholic solution of 8-methoxypsoralen. Shortly thereafter, following exposure to ultraviolet light, he developed a hyperpigmented spot on the arm. Fig. 9.
Until now there have been no reports on the use of radiant energy, other than ultraviolet light, given in conjunction with oral and local therapy. Thorium X was used locally on 3 of our patients because this substance is known to be excellent for stimulating pigmentation. No improvement was noted following a single application of Thorium X. However, this procedure merits further testing.

It is of interest to speculate on the mechanism of action of these drugs. It is not known whether ultraviolet brings about conversion of the psoralen derivatives to more active substances or whether the two factors act synergistically to produce an increase in melanin formation. A combination of both mechanisms might be at work.

The psoralen compounds before and after exposure to ultraviolet light have no effect on tyrosine- or dopa-tyrosinase reactions or on the glutathione inhibited reaction. Also, the substances do not act as does the melanocyte stimulating hormone. Coumarins are known to inhibit sulfhydryl groups. While such inhibition was not shown from our studies with psoralens and glutathione inhibited tyrosinase, the possibility of inactivation of SH groups by psoralens should be tested. An agent, at the proper location in the skin, that could react with SH groups, plus ultraviolet light—which is known to inactivate SH groups—might stimulate melanin formation. In addition, the chemical structures of the psoralen derivatives partly resemble that of alloxan. Alloxan, which is known to react with sulfhydryl groups, may produce diabetes mellitus in animals through such a reaction. (20). A patient with diabetes mellitus, who did not require insulin before receiving psoralen derivatives, lost weight; and insulin
was necessary for diabetic control afterwards (2). These findings are consistent with inhibition of sulfhydryl groups by the psoralens.

The apparent increase in tolerance to ultraviolet light after ingestion of the drug is in marked contrast to the increased sensitivity to ultraviolet light following local application. Since these substances absorb strongly throughout the ultraviolet region and have absorption peaks at 3000 and 2500 Å, it is possible that they absorb erythema producing rays in much the same way as natural melanin, which protects the skin by absorbing visible and ultraviolet light. These drugs should be tried on patients with various light sensitivity diseases such as lupus erythematosus, xeroderma pigmentosum, hydroa aestivale, etc. They should also be used with ultraviolet light in those disorders in which ultraviolet light acts therapeutically, such as psoriasis.

Although it is not known why local use of the drug results in increased sensitivity to ultraviolet light, it may be of significance that the locally applied preparations supply a very high concentration of the drug to the skin, whereas oral therapy allows only a very low concentration to reach the skin.

**SUMMARY**

1. Oral administration of 8-methoxypsoralen (xanthotoxin, ammoidin), together with exposure of areas of vitiligo to ultraviolet light, is effective treatment for some cases of vitiligo.

2. Oral use of this drug decreases the erythema response of patients to ultraviolet light, whereas local application increases this response.

3. In appropriate doses 8-methoxypsoralen is well tolerated by patients. Complete blood studies, urinalyses, examinations for urinary porphyrins, prothrombin times, and liver function tests, except the cephalin cholesterol flocculation test, were within normal limits. Some patients had weakly positive cephalin cholesterol flocculation tests after 2 months therapy. It is suggested that this test be done monthly for 3 months, and then at less frequent intervals, on patients receiving the drug.

4. The LD₅₀ for adult mice and rats is approximately 400 to 600 mg. per kg.

5. The molecular extinction coefficient for 8-methoxypsoralen and 8-isoamyl-enepsoralen is given.

6. Solutions of 8-methoxypsoralen before and after exposure to ultraviolet light have no effect on the tyrosine-, dopa-, or glutathione inhibited-tyrosinase systems, or on the histochemical skin tests for tyrosinase activity. The solutions also did not affect the melanocytes of isolated frog skin.

7. The effect of ultraviolet light on the optical density of solutions of 8-methoxypsoralen is shown.

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