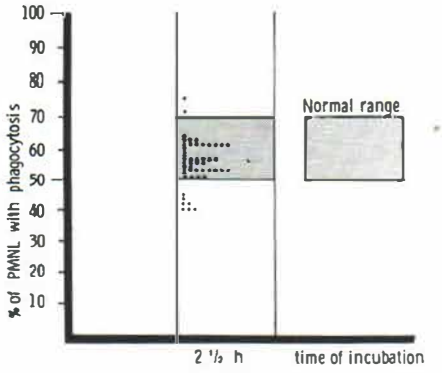

Fig. 2. Results of phagocytic activity of PMNL in RHS ($n=49$).

DISCUSSION

Recent investigations on T lymphocytes in patients suffering from RHS demonstrated some abnormalities of T cell function reflected by decreased numbers of T lymphocytes in peripheral blood and/or impaired lymphocyte transformation capacity (4, 6). A reduction in the number of positive skin reactions to various recall antigens, and a reduction of C_4 have also been observed. The state of humoral immunity seems to be less important, since even in patients with a lack of immunoglobulins, no predisposition to RHS was observed (10).

There is evidence from our results that in the majority of patients with RHS some additional alteration in PMNL function does exist, manifesting itself in a marked disturbance of chemotactic activity and reduced intracellular destruction of C.a. (Fig. 1). On the other hand, the phagocytic capacity of PMNL is close to normal and the NADH-dependent oxidase activity, on, which the intracellular production of H_2O_2 depends, is not impaired at all (Fig. 2).

Further data (unpublished so far) on peripheral blood lymphocytes in patients suffering from RHS demonstrate some derangement of the T-cell system. Disturbances in the PMNL system, together with abnormalities in various T cell, complement (C_4) and macrophage functions (3, 10), apparently point to a disordered balance in the complex organization of cellular defence mechanisms in many patients with protracted RHS.

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Accidental Induction of Photocontact Allergy to *Heracleum laciniatum*

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Abstract. Photocontact allergy to psoralens in *Heracleum laciniatum* occurred in two persons volunteering for investigations into phototoxicity of plant homogenates and purified psoralens. Photoallergy was noted following the fifth exposure in case 1, and the sixth in case 2. Testing with diluted solutions demonstrated allergy to sphondin, isobergapten and pimpinellin.

Key words: Photocontact allergy; *Heracleum laciniatum*, Sphondin; Isobergapten; Pimpinellin

Psoralens are the cause of the phototoxic reaction which will occur in everybody after exposure to sufficient light energy of the relevant wavelengths. This is a non-allergic phenomenon which can be induced at will in humans and animals (7). Photoallergic reactions in man are infrequent and are dependent on an acquired altered reactivity of the skin. As immunologic phenomena they require an induction period prior to sensitization (1).

The sensitizing capacity of *Heracleum laciniatum* and particularly of some naturally occurring psoralens present in the plant is reported following accidental induction of photocontact allergy.

MATERIALS AND METHODS

We are at present evaluating the phototoxic reactions of *Heracleum laciniatum*. Two of the authors served as test objects exposed to repeated exposures. Both are healthy middle-aged men with no history of skin diseases or phytophotodermatitis.

Case 1

He settled down in Tromsø one year before this study was initiated. He had never been in contact with *Heracleum laciniatum* before the experimental induction of photosensitivity to the plant. Photopatch tests were carried out in August 1981 on the lower thoracic back skin with homogenates of root, stem, leaves and flowers prepared by crushing in a mortar. The test procedure is described elsewhere (5). Three of the test sites reacted with erythema, infiltration, and vesicles covering an area with a diameter of approximately 1.3 cm. Dark controls were negative in all the tests.

We extracted and purified bergapten (5-methoxypsoralen), isobergapten (5-methoxyangelicin), pimpinellin (5.8-dimethoxypsoralen), isopimpinellin (5.6-dimethoxyisopsoralen) and sphondin (6-methoxyangelicin) (8). The purity of the compounds was confirmed by high pressure liquid chromatography. Each was dissolved in 96% ethanol and the following dilution series of all compounds was prepared: 0.1 %, 0.01 %, 0.001 % and 0.0001 %. The results of photo-epicutaneous testing with pure psoralens in September 1981 are set out in Table 1, showing erythema, infiltration, and vesicles, to 0.1% of bergapten and pimpinellin, erythema to 0.01% of bergapten and pimpinellin, and slight erythema to 0.001% of bergapten and 0.1% sphondin. Dark controls were negative in all the tests.

On December 20th an action spectrum study with a monochromator was made with leaves of *Heracleum laciniatum* crushed in a mortar, as described elsewhere (5). Altogether 27 positive reactions were seen, of which 19 were erythematous, 4 papular and 4 even vesicular.

Due to the insufficient response of some of the central wavelenghts with the light doses used, repeated exposure on 19 test spots with plant homogenates was made on December 30th. This time the photosensitivity reactions were much more pronounced (see Table 1). Of the 14 positive reactions, 2 were seen as intense erythema and 12 as intense erythema and infiltration.

Table 1. *Results of photopatch test with psoralens and plant homogenate, September 1981 and January 4th 1982, Case 1*

Plant homogenates were diluted with 96% ethanol
Grading of results: − negative; + erythema; ++ Erythema and oedema; +++ erythema, oedema, papules or vesicles

Psoralens and their concentrations		Reaction
September 1981		
Bergapten	0.1%	+++
	0.01%	+
	0.001%	+
	0.0001%	−
Pimpinellin	0.1%	+++
	0.01%	+
	0.001%	−
Sphondin	0.1%	+
	0.01%	−
Isobergapten	0.1%	−
	0.01%	−
Isopimpinellin	0.1%	−
	0.01%	−
Plant homogenate undiluted		+++
diluted 1:10		−
January 4th 1982		
Bergapten	0.001%	−
	0.0001%	−
Pimpinellin	0.01%	−
	0.001%	−
Sphondin	0.01%	++
	0.001%	+
Isobergapten	0.1%	++
	0.001%	+
Isopimpinellin	0.1%	−
	0.01%	−
Plant homogenate: diluted 1:100		++
diluted 1:1000		−

Now we suspected acquired photocontact allergy to psoralens in *Heracleum laciniatum*, which was why we repeated the photopatch tests on January 4 1982 with a dilution series of the psoralens, (Table 1). Only psoralen concentrations previously known not to be phototoxic were used. Plant homogenates diluted 1:100 and 1:1000 with 96% ethanol were also used for photopatch testing. After 2–4 days, erythematous and infiltrated lesions occurred in response to isobergapten 0.1% and 0.01% and sphondin 0.01% (Table 1).

Dark controls were always negative in all tests. On no occasion was a test placed on the same skin area as a previous test.

Case 2

He underwent experimental phototoxicity studies following essentially the same methods as described for case

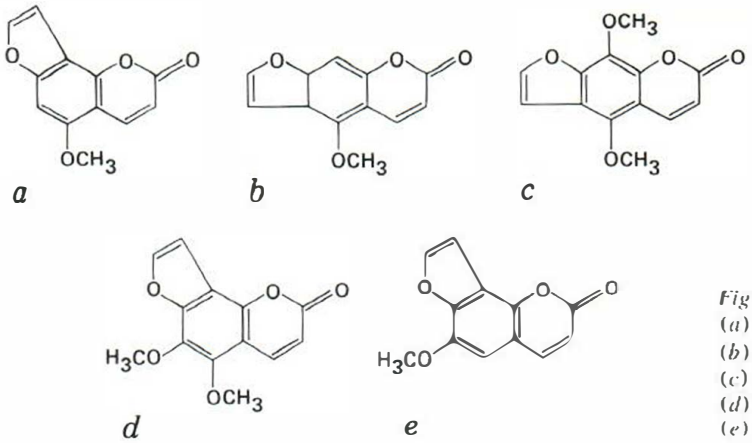

Fig. 1. The chemical structure of
(*a*) Isobergapten (5-methoxyangilicin)
(*b*) Bergapten (5-methoxypsoralen)
(*c*) Isopimpinellin (5.8-dimethoxypsoralen)
(*d*) Pimpinellin (5.6-dimethoxyisopsoralen)
(*e*) Sphondin (6-methoxyangelicin)

1, only using plant homogenates. During the course of 3 weeks starting in December 1981 experiments were carried out five times, giving normal phototoxic reactions causing little discomfort. In particular, no itching was noted in the positive reactions. The sixth experiment elicited unexpectedly intense reactions and an intense itching was noted. The results were similar to those described for case 1 (Table II). Prior to sensitization an UVA dose of 168 mJ/cm² from the bank of four fluorescent tubes gave a 1+ reaction (contact time for leaf homogenate was 1 hour). The seventh experiment gave, in contrast, a 2+ reaction with itching for leaf homogenate and also for 0.1% pimpinellin with a UVA dose of 54 mJ/cm². Subsequently dilutions of pimpinellin 0.001%, sphondin 0.01% and isobergapten 0.01% also produced positive reactions. Dark controls were all negative.

DISCUSSION

The cases presented demonstrate that not only phototoxic reactions, but also photoallergic reactions may be encountered after external contact with *Heracleum laciniatum*. The fact that neither subject had been in contact with the plant prior to the experiments nor had any history of other photocontact reactions, emphasizes the plant furocoumarins isobergapten, pimpinellin and sphondin as potentially powerful photoallergic contact sensitizers. Photocontact allergic reactions to psoralens seem to be rare, although a few reports have appeared. Ljunggren (6) reported a case of acquired photocontact allergy to furocoumarins in plants and found by photopatch testing with serial dilutions that 8-methoxypsoralen, 5-methoxypsoralen and imperatorin were photoallergens. Several authors have described photoallergy to 8-methoxypsoralen and imperatorin (3, 10, 11).

The assessment of photoallergenic potential can be made with the "photomaximization test" by repeated photopatch testing on the same area for a total of six times (4). Our cases demonstrate that it is not necessary to repeat the photopatch test on the same area. There is obviously no correlation between phototoxic potency and photoallergic sensitizing capacity. As described elsewhere (5), testing with a 0.25% solution of pimpinellin required a light dose of 1.2 J/cm² at 360 to give a one-plus reaction and 0.63 J/cm² at 335 nm. 0.25% sphondin gave no reactions in 5 persons tested at 335 nm with 1.2 J/cm², whereas in vitro testing confirmed both these relative phototoxic potencies and isobergapten to be non-phototoxic. The angular structure of psoralens (Fig. 1) seems to be of importance in eliciting photoallergy, whereas the linear psoralens such as bergapten are more potent photosensitizers.

Our cases further demonstrate that photoallergy to *Heracleum laciniatum* requires far less drug and

Table II. *Radiation output of the monochromator at some central wavelengths*

Half-bandwidth 5 nm. Skin reactions expressed as + = erythema and + + = erythema and oedema (Case 1)

	Exposure December 20th		Exposure December 30th	
Central wave length	Light dose (mJ/cm²)	Reaction	Light dose (mJ/cm²)	Reaction
315	46	+	12	++
330	52	+	26	++
355	124	+	78	++

radiation than does phototoxicity. These observations agree with earlier findings that, compared with phototoxic reactions, the eliciting of photocontact allergy requires far lower concentrations of the compounds once photosensitization has developed, and also that it requires less radiation energy (2, 7).

The photoallergic reactions to isobergapten, pimpinellin and sphondin may be explained on the basis of multiple sensitization, but photo-cross-reactions among these closely related agents are indeed likely. The only difference between isobergapten and sphondin is that the methoxy group is located in position 5 for isobergapten instead of position 6 for sphondin, while pimpinellin has two methoxy groups, one in position 5 and one in 6 (Fig. 1).

In conclusion, we have observed that plant products of *Heracleum laciniatum*, probably essentially psoralens, are potentially strong photocontact allergens. It is understandable that the photoallergy is easily overlooked, as the dermatitis is assumed to be phototoxic, which is the common reaction.

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^{65}Zinc Absorption in Untreated and D-Penicillamine-treated Patients with Generalized Scleroderma: Determination by Whole-body Counting Technique

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Abstract. ^{65}Zinc absorption in patients suffering from generalized scleroderma was studied by means of whole-body counting technique following a single dose of ^{65}Zn. In 4 untreated patients the mean ^{65}Zn absorption was calculated to 35% (range 20–59%). Five patients receiving oral D-penicillamine had a numerically higher mean absorption value of 55% (range 37–74%). The results corroborate earlier studies on the effect of D-penicillamine on ^{65}Zn absorption in rats.

Key words: Generalized scleroderma; D-penicillamine; ^{65}Zinc absorption; Whole-body counting

In 1957 Rukavina (8) suggested that the abnormal collagen metabolism in generalized scleroderma (GS) might be due to lack of or excess of certain trace elements or, alternatively, depend on changes in metallo-enzyme function following chelation. Details of such hypothetical mechanisms remain unclarified, but the concept has led to the introduction of various chelating drugs for treatment of GS (1).

D-penicillamine was introduced for GS in 1966 by Harris & Sjoerdsma (4). Later on, Blumenkrantz & Asboe-Hansen demonstrated D-penicillamine in vitro to be a potent inhibitor of collagen synthesis (2).

Recently, Weismann & Knudsen (10) found that oral D-penicillamine increases zinc absorption in rats. This led us to investigate whether therapeutical doses of D-penicillamine for GS exert the same effect in man.

MATERIALS AND METHODS

^{65}Zn absorption studies were performed on patients suffering from GS (i) before any medical treatment was initiated, and (ii) after at least 3 months' therapy with 750 mg D-penicillamine per day. The study was performed from July 1979 to the end of 1980. A total of nine absorption studies