

at 37°C were taken at 30 frames/min using a Leicina super 8 camera and a Diavert microscope (Leitz, Wetzlar, W. Germany). Cell shapes and contact between neutrophils and substratum was analyzed by means of phase contrast pictures and reflexion contrast microscopy¹². Neutrophil-substratum adhesion was tested as follows: 10⁶ neutrophils suspended in 1 ml of the respective medium were incubated in tissue culture chamber/slides (Lab-tek, Naperville, Ill., USA) for 30 min at 37°C. The medium was poured off. Thereafter the slides were washed twice by addition of 1 ml of the respective medium and vigorous shaking on a Vortex-type mixer on position 30 for 10 sec (Eckli-Electronics, Zürich). Neutrophils per unit area were counted on ethanol-fixed, Giemsa-stained smears. The counts are expressed as percentage of the neutrophils applied. Viability had been tested by dye (nigrosin) exclusion¹³.

The results shown in table 1 demonstrate that 2% HSA (w/v) stimulated locomotion of neutrophils suspended in Gey's solution. The speed as well as the percentage of cells showing active locomotion was significantly higher if serum albumin was present in the medium, whereas viability as measured by dye exclusion was not changed. No preference for a particular direction of locomotion had been observed. We conclude that HSA has chemokinetic properties as determined by direct observation of moving cells and that neutrophils respond in the form of orthokinesis (for definitions, see Keller et al.⁷). The quantitative differences between the filter technique and direct observation are presumably of a technical nature. If the cells move slowly, displacement is measurable by direct observation but not necessarily by the filter technique used. Furthermore the substratum is somewhat different (glass vs mixed esters of cellulose).

Phase contrast pictures showed that neutrophils in Gey's solution alone have a more marked tendency to spread

than in Gey's solution containing 2% HSA. Examination by means of reflexion-contrast microscopy revealed that neutrophils in Gey's solution alone have larger and more intense dark areas, which are characteristic for close contact between cell and substratum. Functional studies on neutrophil adhesion substantiated these findings. The proportion of neutrophils remaining attached to the substratum was lower in presence of HSA (table 2). Taken together, the findings are interpreted to mean that HSA decreases attachment of neutrophils to the substratum. It appears to be reasonable that increased attachment correlates with decreased locomotion. The more firmly a cell is attached, the more force is presumably required for detachment which must also occur in the course of locomotion.

Chemokinesis seems to be a major regulatory process influencing neutrophil accumulation in inflamed tissues⁵. The expression of the intrinsic locomotor capacity of neutrophils in the form of random or directional locomotion in vitro depends on the presence of chemokinetic factors such as HSA. It is likely that serum albumin, as a major constituent of plasma, plays also a major role in promoting neutrophil locomotion in vivo.

Table 2. The influence of human serum albumin (HSA) on neutrophils adhesion

Culture medium	Neutrophils attached (%)
Gey's solution	51.9
2% HSA in Gey's solution	6.5

- 1 We thank Mrs R. Graf and Miss M. Schuster for technical assistance. This work was supported by the Swiss National Science Foundation.
- 2 H. U. Keller, *Immunology* 10, 225 (1966).
- 3 S. H. Zigmond and J. G. Hirsch, *J. expl Med.* 137, 387 (1973).
- 4 P. C. Wilkinson, *Expl Cell Res.* 103, 415 (1976).
- 5 H. U. Keller, J. Wissler, M. W. Hess and H. Cottier, in: Movement, metabolism and bactericidal mechanisms of phagocytes. Piccin, Padova (in press).
- 6 H. U. Keller, J. H. Wissler, M. W. Hess and H. Cottier, submitted for publication.
- 7 H. U. Keller, P. C. Wilkinson, M. Abercrombie, E. L. Becker, J. G. Hirsch, M. E. Miller, W. S. Ramsey and S. H. Zigmond, *Clin. expl Immun.* 27, 377 (1977).
- 8 M. H. Gail and Ch. W. Boone, *Expl Cell Res.* 70, 33 (1972).
- 9 L. Weiss and D. Graves, *J. Immun.* 115, 1362 (1975).
- 10 H. U. Keller, M. W. Hess and H. Cottier, Symposium on 'Clinical aspects of the complement system'. Thieme, Stuttgart 1977.
- 11 H. U. Keller, H. Gerber, M. W. Hess and H. Cottier, *Agents Actions* 6, 326 (1976).
- 12 J. S. Ploem, in: *Mononuclear Phagocytes*, p. 405. Ed. R. Van Furth. Blackwell Scientific Publ., Oxford 1975.
- 13 M. Claesson, *Scand. J. Haemat.* 6, 291 (1969).

Clinical and immunological studies on persons exposed to *Parthenium hysterophorus* L.¹

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Summary. Studies on 300 persons subjected by occupational hazard to the allergenic weed, *Parthenium hysterophorus* L. for periods ranging from 3 to 12 months revealed that 4% of them developed contact dermatitis of the exposed parts of the body, while 56% of them got sensitized to the weed without apparently exhibiting any dermatitis. None of them suffered from allergic manifestations like rhinitis or bronchial asthma during the period of study which extended for 2 years.

Although it is well established that *Parthenium hysterophorus* L. (Compositae), an alien weed growing wild in many parts of India, is a very potent contact allergen which is responsible for hundreds of cases of contact dermatitis⁵, it is not known what percentage of the population exposed to this weed become affected. Since the aggressive spread of *Parthenium* is posing both agricultural and health hazards, efforts are being made to eradicate the weed by various methods, including manual uprooting. In Bangalore (India), the city corporation has employed 300 manual labourers for removing the weed from

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- 5 A. Lonkar, J. C. Mitchell and C. D. Calnan, *Trans. a. Rep. St. John's Hosp. Derm. Soc., Lond.* 60, 43 (1974).

various parts of the city. We have made use of this rare opportunity, and carried out clinical and immunological studies on these persons subjected to occupational hazard due to *Parthenium hysterophorus* L., the results are presented in this communication.

Materials and methods. A small segment of a freshly collected leaf and a flower head of *Parthenium hysterophorus* L. as well as 5% (w/v) acetone extracts of the leaves and flowers were used to assess delayed hypersensitivity (type IV) by patch testing. Aliquots (50 μ l) of the acetone extracts of the leaf and flower head were spotted separately on 1 cm² Whatman No. 3 filter papers. On the back of each subject were placed separately each test material and covered with a 2 cm² polythene sheet fixed in the centre of a 5 cm² Johnson adhesive plaster. A filter paper spotted with acetone served as a control. The patches were removed at 48 h and the sites were evaluated 30 min later and again at 72 h. Sensitivity of the reaction was graded on a scale of 0 to 4 as follows: 0, negative; 1+, erythema; 2+, erythema and edema and/or beginning of papulation; 3+, fully developed vesiculation, papulation, edema and small bullae; 4+ large bulla, denudation, necrosis.

Results and discussion. Among the 300 workers engaged in uprooting *Parthenium* for variable periods of time stretching from 3 to 12 months, 12 persons developed contact dermatitis. All these patients presented typical clinical features which are consistent with known morphological picture of *Parthenium* dermatitis, recently described⁵. The lesions (erythematous papulovesicular or maculopapular eruptions) started first on the exposed areas, face, V of the neck, V of the chest and forearms. Apart from these areas, flexures, retroauricular areas and upper eyelids were conspicuously involved. Itching was intense. None of the patients gave any history of previous dermatitis of plant origin. All these patients exhibited typical delayed hypersensitivity (type IV) as denoted by a strong positive patch test response to acetone extracts as well as intact leaf and flower head of *Parthenium*.

The results of the patch test studies on *Parthenium* weed pullers are summarized in the table. Acetone extracts of

leaf and flower head gave consistently stronger as well as higher percentages of reactions than flower head and leaf as such, in all those cases where positive responses were elicited. Although only 4% of the weed pullers were actually suffering from clinical features of *Parthenium* dermatitis, 56% of them became sensitized on contact with the weed. It remains to be seen whether the remaining 52% of the persons who showed positive patch test response, would in due course develop allergic symptoms. In fact 1% increase in the incidence of dermatitis among the experimental group was noticed after a further contact for 12 months with the weed. Patch testing in a variety of other dermatological conditions (50 patients), used as controls showed consistently negative results. At the concentrations employed, extracts from the leaves as well as flowers did not exhibit primary irritant effects.

The above studies seem to suggest that the process of sensitization to *Parthenium* allergens need not necessarily bring about clinical manifestations of dermatitis. It is yet to be realized that mere mobilization of sensitized lymphocytes as denoted by the patch test results in some percentage of study group, does not necessarily lead to the clinical manifestation of *Parthenium* dermatitis, denoting there are factors as yet not understood which herald the actual clinical manifestations.

Sesquiterpene lactones commonly found in many Compositae plants are known to be potent allergens responsible for contact dermatitis⁶. Parthenin, the major sesquiterpene lactone thought to be responsible for allergic contact dermatitis to *Parthenium hysterophorus* L. is present in the leaves and flower heads of the weed, and particularly concentrated in the trichomes⁷. The persons suffering from contact dermatitis in the present study also gave positive reactions varying from 1+ to 3+ when patch tested with 1% parthenin in petrolatum.

None of the weed pullers constantly exposed to *Parthenium* developed immediate (type I) hypersensitive manifestations like allergic rhinitis, bronchial asthma or atopic dermatitis during the period of study which extended for 2 years.

Patch test studies on *Parthenium* weed pullers

a) Different patch test materials		
Antigen	No. of patients showing positive patch test*	%
Control	0	0
Leaf	31	10.3
Flower head	40	13.3
Acetone extract of leaves (5% w/v)	139	46.3
Acetone extract of flower heads (5% w/v)	134	44.7
b) Results of patch test		
Type of reaction	No. of patients*	%
Negative	132	44.0
+	98	32.6
++	38	12.7
+++	14	4.7
++++	18	6.0

* Total number of patients tested = 300.

6 J. C. Mitchell, Recent Adv. Phytochem. 9, 119 (1975).

7 E. Rodriguez, M. O. Dillon, T. J. Mabry, J. C. Mitchell and G. H. N. Towers, Experientia 32, 236 (1976).