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ANTIGENS OF *PARIETARIA OFF.* POLLEN
(URTICACEAE), SEPARATED BY DISCONTINUOUS
ELECTROPHORESIS IN POLYACRYLAMIDE GEL.

By

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There is no doubt (Serafini (14, 15) 1950, 1957) that in Italy, the pollens of Urticaceae (*Parietaria off.*, *Urtica dioica*) represent the second major source (Graminaceae come first) of naso-ocular and/or bronchial allergic manifestations. It seems that the forms caused by *Urtica dioica* (Clerici & Sambataro (3) 1953) prevail in Northern Italy, and that, also according to our direct observations (Crifò (4) 1968), those caused by *Parietaria off.* are considerably more frequent in Southern Italy, Sicily and Sardinia. On the other hand, Italy lies in the very middle of the area where this plant grows (Gola (9) 1950); and, moreover, it chiefly occurs all along the coasts of the Mediterranean Sea (Alemany Vall 1967).

According to Mameli-Calvino (12) (1951), the frequent occurrence of *Parietaria off.*-sensitive cases, besides being closely related to the wide diffusion and almost perennial blossoming of this plant in our climate, might also be ascribed to the small size of its pollen (18-22 μ), to its hydrophilous properties and to the possible irritation caused by the impurities in it (hair, thorns, etc.), as well as to the potassium nitrate it seemingly contains in fairly relevant amounts.

Recently we were able to demonstrate (Crifò 1968) that the pollen of *Parietaria off.* possesses a permeating factor (or histamine-releasing factor) probably similar to that singled out by Kind *et al.* (10, 11) (1964, 1967) for the

pollen of Graminaceae. This factor is strongly inhibited by a non-enzymatic proteic constituent of the "normal," nasal secretion of man (Crifò (4) 1968), which, according to hitherto unpublished observations, would be of the gamma-globulin type.

In striking contrast with the remarkable progress in the knowledge of the antigenic characteristics of the respiratory-atopic excitants, we know almost nothing about those of the pollen of *Parietaria* in spite of its unquestionable importance in clinical practice.

The only data presently available, come from Di Nardo's and Navazio (8) attempts (1965) to isolate by paper chromatography allergy-inducing substances from crude pollen extracts. By crude extracts carried out in patients with a specific hypersensitivity, the most active compounds, did separate in the area of aminoacids and sugars.

In this article, we report the results of a preliminary study on the allergenic power of protein fractions of *Parietaria off.* pollen separated by means of discontinuous electrophoresis in polyacrylamide gel, from highly concentrate, crude extracts obtained by a procedure recently suggested by one of us (Crifò (5) 1969).

MATERIALS AND METHODS

Non-defated, commercially available *Parietaria off.* pollen was semipurified by a sieve for resins (Test Sieve n. 400, $37\ \mu$). A fraction A ($< 37\ \mu$) very rich in pollen, and residual fraction B ($> 37\ \mu$) poor in pollen and with abundant impurities (residues of leaves, thorns, etc.) were obtained.

An exactly weighed amount of each fraction was combined (w/v) in a buffer solution of glycine-tris (0.38 M in glycine, 0.04 M in tris, pH 9.5). The suspension was brought down to -70°C and was then subjected to eight compression by means of a bacterial press AB-Bios (Nacka, Sweden*). As reported elsewhere (Crifò (5) 1969), the use of bacterial press allows a high degree of pollen crushing, and gives crude extracts that are very rich in proteins and other constituents. After thawing, the crude extract was centrifuged for 1 hour at 16,000 rpm and at $+4^\circ\text{C}$, and the supernatant was used as such in the

* We are most grateful to the laboratories of Biological Chemistry of the Higher Health Institute for having allowed us to utilize their bacterial press.

subsequent tests. Such tests were also carried out with samples of crude extract, either dialyzed for 5 days at the temperature of $+4^{\circ}\text{C}$ against a glycine tris buffer, or depigmented by means of activated chochoal (Merk). Separation of soluble proteins was achieved by discontinuous gel electrophoresis in polyacrylamide, according to *Ornstein's* (13) (1964) and *Davis'* (7) (1964) technique. Solutions for the preparation of gels and buffers and experimental conditions under which electrophoresis was performed are the same as described by *Silano et al.* (16) (1967). Volumes of crude extract A, B or of their dialyzed or chochoal-depigmented samples, found to be experimentally suitable, were charged on gel in saccharose at final concentration of 12 per cent. Recordings of humid gels were made by means of a Chromoscan microdensitometer (Joyce, Loeb & Co., Cateshead, England).

The study of the allergenic power—if any—of protein fractions was carried out by isolating the areas corresponding to either to single protein fractions, or to very close fractions, by means of transversal cuts of non stained gels. Identification of the areas corresponding to these fractions was obtained by estimating the *rf* of the single fractions, calculated by dividing the distance of the band from the upper edge, by the overall gel length, and by making use of the indications supplied by a stained gel. The fractions thus isolated were eluted with 1 ml of a 0.9 per cent NaCl solution. After centrifugation at 16,000 rpm and at $+4^{\circ}\text{C}$, the supernatants were used as such or after suitable dilution, in skin reaction tests, repeated also after some time, in two patients suffering from nasal pollinosis ascribable only to the pollen of *Parietaria off.* Controls used in these tests were: the elution of a "control" gel not charged with a protein sample and treated as above; the eluting solution; commercial solutions of *Urticaceae* (I.S.M., Milano) and of *Parietaria off.* (Iofarma, Milano).

RESULTS AND COMMENTS

Fig. 1 shows the result of two gels which were charged with an equal volume of a crude extract, respectively of semi-purified pollen ($<37\mu$) and rich in impurities, to such an extent as to indicate at least some bands in gel charged with extracts from the fraction rich in impurities ($>37\mu$). In these experimental conditions, it clearly appears that the semi-purified pollen extract is very rich in material colourable as protein, so that any other vegetable impurities present in the pollen, should be considered of minor relevance as a source of such substances. It seems also likely that the substances colourable as proteins and present in the extract rich in impurities, also come from a percentage of pollen passed

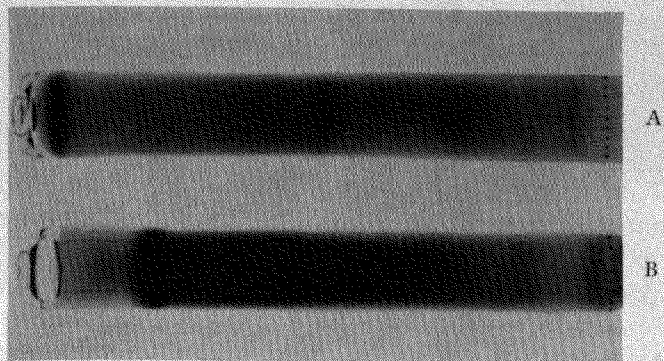


Fig. 1.

Parallely run gels: *A*, charged with 0.12 ml of crude extract obtained from the fraction retained by the sieve ($> 37 \mu$); *B*, charged with 0.12 ml of crude extract obtained from the fraction of semipurified *Parietaria off.* pollen. It is evident that, for this volume of sample, only some fractions are evident in the *A* gel, while an excess of proteins is clearly detectable in the *B* gel.

through the sieve and easily identifiable by means of fluorescent microscopy. Fig. 2 shows two gels which were charged with an equal volume of crude extract of semipurified pollen and of the same extract depigmented by prolonged dialysis.

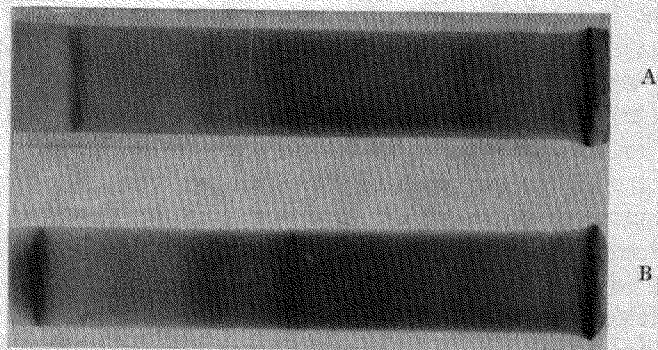


Fig. 2.

Parallely run gels, charged with 0.06 ml of sample, *A*, of crude extract from the semipurified fraction, depigmented by means of a prolonged dialysis; *B*, of the same fraction but non-depigmented. Depigmentation improves the view of protein bands.

As demonstrated also by the densimetric analysis of gels, such a process affords a better view and a better densimetric reading of the protein fractions, by eliminating some pigment bands and the background colouring. Superimposable results were obtained by depigmentation with activated charcoal: by this method also it is possible to obtain a slightly coloured extract, probably from protein-bound pigments and devoid of the free pigmentary component, which is of no allergological relevance as widely recognized (*Wodehouse & Coca* (17) 1946; *Augustin* (2) 1955) and as we were able to demonstrate as the superficial liposoluble fraction (*Crifò & coll.* (6) 1968) is concerned, and through recent observations concerning the hydrosoluble fraction of *Parietaria* pollen.

Fig. 3 shows a gel obtained with a crude extract of the pollen of *Parietaria off.*, depigmented by prolonged dialysis, according to the method described in the foregoing technical section. In the figure also the corresponding densimetric picture and the diagram of these protein fractions, with their *rf*, are shown. By the technique adopted by us, it was possible to demonstrate twelve protein fractions, the first of which, located almost in correspondence to the gel's upper edge, is marked in the diagram with an asterisk. The demonstration of all the fractions is only related to the protein content of the sample, *i.e.*, to the use of an adequate volume to be found experimentally because it varies according to the different extracts. By comparing two gels which were made to run parallel and charged, the first one with a suitable volume of semipurified and depigmented crude pollen extract, and the other with normal human serum, it was possible to ascertain that fractions VII and VIII of the pollen show an gel electrophoretic behaviour, that is roughly similar to that of albumin, whereas fractions IX, X, XI undoubtedly have a lower molecular weight since they migrate towards the prealbumin area. We could also observe that the mobility of fraction IV is hardly lower than that of the transferrin; and, lastly, that the fraction marked with

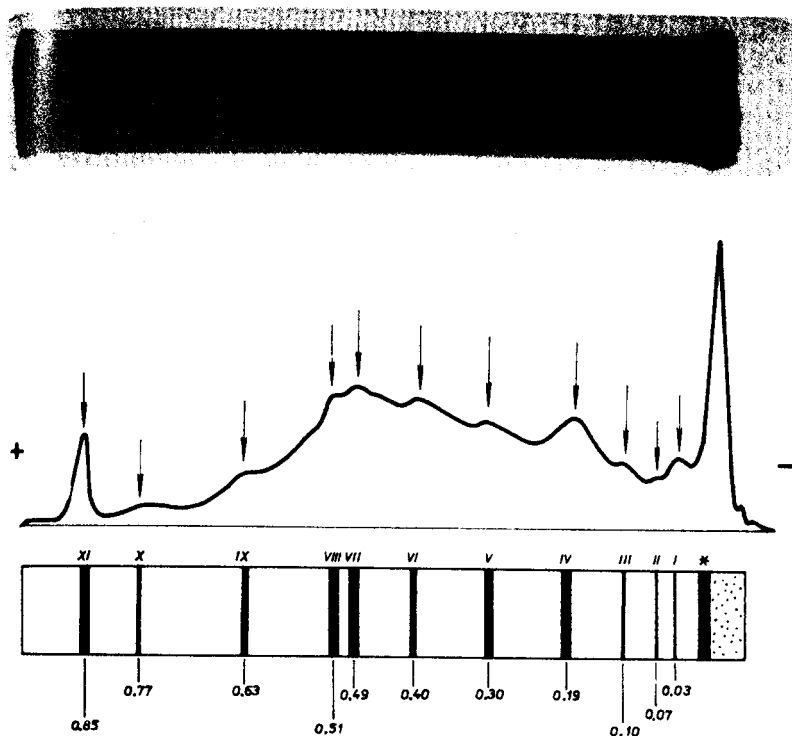


Fig. 3.

Gel charged with 0.06 ml of crude extract obtained by means of a bacterial press, from the semipurified fraction of *Parietaria off.* pollen. The corresponding densitometric picture and the pattern of the protein fractions with their relative r_f , are indicated. The fraction f very close to the edge, is marked with an asterisk.

an asterisk in the diagram of Fig. 4, shows the same mobility as the gamma globulins. This latter fraction is the only one which assumes a specific colouration in gels stained according to the technique suggested by Davis (7) (1964) for polysaccharides.

The diagram appearing in Fig. 4 summarises the preliminary findings of the study on the allergenic power of protein fractions isolated from the crude extract of the *Parie-*

(GEL. SUP.)		1	A	B
*		2	++++	++
I		3	+	—
II				
III		4	++++	++++
IV		5	—	—
V		6	++++	—
VI		7	—	++++
VII		8	++++	++++
VIII				
IX		9	—	++++
X		10	+	++++
XI		11	—	—
(PIGMENTO)		12	—	—

Fig. 4.

Results of the skin tests carried out in two patients (A, B) suffering from nasal atopy caused only by *Parietaria off.* pollen, using the elutions corresponding to the upper gel, to a band of pigment, to the isolable fractions and, in two cases, to couples of non-isolable fractions.

taria off. pollen. During these tests (carried out with skin tests at three different periods of the year, in two patients suffering from nasal pollinosis only ascribable to the pollen of *Parietaria off.*), also the upper gel elution and that of a pigment isolated in pure form from pigmented extracts could be tested. In all the tests performed, both the elution of the upper gel and that of pigment, were found to be inactive. Also negative were the tests made with the elution of fra-

tions IV and XI, whilst all the other tests carried out with the other fractions proved positive, even though some of them were positive in one patient and some in the other patient, and with different intensity.

We must stress, however, that as to the fractions I-II and VII-VIII, we possess but one global indication as it was impossible for us to isolate the single fractions.

In the first patient, who had been suffering for over twenty years from nasal manifestations of allergic nature caused by *Parietaria*, and in a form hardly reactive to hyposensitizing therapy, the allergometric picture has appeared to be chiefly supported by the fractions with a higher molecular weight. In the other patient, whose clinical manifestations date back for only three years, sensitization was mainly to fractions having a lower molecular weight. Furthermore, by carrying out the skin tests in different periods of the year, we noticed in both patients a considerable fluctuation in the intensity of the skin response, only with the fractions with a lower molecular weight.

SUMMARY

By using discontinuous gel electrophoresis in polyacrylamide, it was possible to demonstrate, in concentrated extracts of *Parietaria off.* pollen obtained by means of an original method, twelve protein fractions. Ten of these fractions, gave positive skin tests in patients suffering from nasal atopy caused by *Parietaria off.* pollen. It would appear from preliminary observations, that the fractions with a lower molecular weight, besides showing at different times of the year a fluctuating intensity in the skin tests reactions, probably may be regarded as the excitants of the onset of the disease.

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