

Sensitivity of skin and bronchial mucosa to green algae

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*Six green algal strains were examined for their possible allergenicity to humans. Of 79 atopic patients tested with algal extracts, 47 gave positive skin tests. Non-atopic individuals showed no skin reactivity. Skin-sensitizing antibodies were passively transferred from 6 of 8 patients tested with *Chlorella vulgaris*. In the same group of patients, precipitating antibodies were not observed. Bronchial mucosal tests, evaluated by changes in the peak expiratory flow rate and by the appearance of clinical wheezing, were performed on 8 patients in whom cutaneous reactivity had been pronounced. Positive responses to aerosolized extracts of *Chlorella vulgaris* were obtained in 5 of these patients. These results suggest that positive skin tests to green algae are immunologically significant and that these organisms may be etiological agents in human respiratory allergies.*

Although algae are not commonly considered to be of clinical significance, they have occasionally been responsible for human disorders. Among the reported disturbances are cases of hypersensitization resulting from exposure to algae-infested waters. Since such manifestations have been documented only sporadically, the potential role of the algal cell as a human allergen remains unexplored. Of the cases recorded, almost all have been associated with members of the Myxophyceae.¹

Heise² demonstrated the allergenicity of the Myxophyceae in 1949 while examining the cause of hay fever symptoms related to lake swimming. Employing extracts prepared directly from the blue-green algal bloom, he was able to elicit intracutaneous reactions and a passive transfer of the sensitizing factor in one patient. Moreover, in the same patient, desensitization treatment with these extracts seemed to result in effective relief of the allergic symptoms. In 1953, Cohen and Reif³ observed persistent dermatitis in a patient exposed to the

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blue-green alga, *Anabaena*. Their subsequent examination of this patient led to the documentation of delayed cutaneous reactivity to the products of the organism.

Still to be investigated as a source of respiratory allergens are the airborne algae. Although little is known about their aerial distribution, substantial concentrations have occasionally been reported. In 1955, the blue-green alga *Gloeocapsa* was recorded at an average concentration of 800 cells per cubic meter of air for a period of at least three weeks.⁴ More recently, Brown, Larson, and Bold⁵ obtained counts of 228 and 102 impactions per cubic meter, while, in dust samples, algal densities of more than 3,000 cells per cubic meter were reported. Of the algae recovered, the Chlorophyceae and Myxophyceae were most common. Schlichting,⁶ on the other hand, cited only the Chlorophyceae, specifically members of the order Chlorococcales. Recently, McElhenney and co-workers⁷ reported that extracts from four Chlorophyceae elicited positive skin reactions in patients with histories of inhalant allergies. Of the 120 atopic patients tested, 82 per cent reacted to one or more of these extracts. Nonallergic individuals gave a negative reaction to all four algal extracts. In view of these findings, the present experiment was initiated to substantiate the allergenicity of several Chlorophyceae strains and to determine whether such strains could provoke asthmatic symptoms in atopic patients.

MATERIALS AND METHODS

Cultures

The six algal strains used in this study were bacteria-free cultures of *Chlorella vulgaris*, IU 263*; *Chlorella pyrenoidosa*, SEC 121; *Chlorococcum botryoides*, SEC 91; *Chlorococcum macrostigmatum*, IU 109; *Scenedesmus basilensis*, SEC 83; and *Ankistrodesmus falcatus* var. *acicularis*, SEC 28. All were selected from the order Chlorococcales on the basis of reports that members of this order have been commonly encountered in the course of sampling for air-, soil-, and waterborne algae.^{5, 6, 8-10}

Culture methods

The algae were inoculated into 250 ml. Erlenmeyer flasks containing 100 ml. of the following Chu medium: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 23.2×10^{-2} Gm.; K_2HPO_4 , 1×10^{-2} Gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5×10^{-2} Gm.; Na_2CO_3 , 2×10^{-2} Gm.; $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$, 4.4×10^{-2} Gm.; ferric citrate, 3.5×10^{-3} Gm.; citric acid, 3.5×10^{-3} Gm.; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1×10^{-7} Gm.; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 2×10^{-5} Gm.; ZnCl_2 , 4×10^{-4} Gm.; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.4×10^{-3} Gm.; H_3BO_3 , 2.4×10^{-3} Gm.; Tris(hydroxymethyl)-aminomethane, 1.0 Gm.; and distilled water, 1,000 ml. The flasks were then incubated under static conditions for 7 to 10 days at 20° C. with "cool white" fluorescent tubes providing illumination of 140 to 160 foot candles. The above procedures were employed in the maintenance of stock cultures with the exception of the incubation period which was extended to 30 days. To insure that the algae remained bacteria free during propagation, the cultures were tested

*Culture numbers refer to the algal culture collections at (IU) Indiana University, Bloomington, Ind., and at (SEC) Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

prior to harvesting in nutrient broth, thioglycollate broth, Chu medium plus 0.1 per cent sodium glutamate, and Chu medium plus 0.1 per cent Difco proteose peptone No. 3.

Preparation of algal extract

Cultures were harvested in a continuous-flow centrifuge, washed with distilled water, and air dried. The dried cells were suspended in Coca's solution* at a 1:40 or 1:50 dilution (grams per milliliter) and treated for 30 minutes in a 20 kilocycle ultrasonic disintegrator (Biosonic). Particulate matter was then removed from the extract by centrifugation at 10,000 g for 20 minutes. As a precautionary measure, the above procedures were carried out at temperatures ranging from 4 to 10° C. The resulting supernatant was passed through a sterile ultrafine sintered-glass filter and stored at 4° C. Prior to use, the extracts were tested for sterility.

Selection of test subjects

Seventy-nine patients under treatment at the Allergy Clinic of the Cincinnati General Hospital were selected for this study. All patients gave well-documented clinical histories of extrinsic allergic disease (chiefly, allergic rhinitis and asthma), and all displayed marked skin reactions for various inhalant allergies. Only asthmatic patients were selected for the bronchial provocation tests. Border-line cases, whose seasonal histories and symptoms were not clearly defined, were excluded from the study.

The control group consisted of 26 subjects who had negative family and personal histories of allergic diathesis. Prior to skin testing with algal extracts, each individual was pretested intracutaneously with pollen and dust allergens in order to exclude nonspecific skin reactors.

Clinical techniques

Direct intracutaneous tests were performed with 0.02 ml. injections of 1:1,000 dilutions of the above algal extracts. After 10 minutes, the test sites were examined and results recorded. For control purposes, each patient was also tested with saline, aqueous ragweed extract, and Endo house dust antigen.

Sera from 8 patients demonstrating marked cutaneous reactions (3 plus or greater) to *Chlorella vulgaris* were tested for skin-sensitizing antibodies by the passive transfer technique. The tests were performed by intracutaneously injecting 0.05 ml. of various dilutions of sera into the backs of nonsensitive volunteers. Twenty-four hours later, the multiple sites of injection were challenged with a 1:100 dilution of the algal antigen, and the intensity of the reactions determined 10 minutes after introduction of the antigen.

A micro-Ouchterlony slide was used for the agar gel diffusion studies. The test was performed with undiluted sera and cell extracts of *Chlorella vulgaris* at dilutions of 1:100, 1:1,000, and 1:10,000.

A *Chlorella vulgaris* extract was used exclusively for bronchial provocation

*Coca's solution: NaCl, 5.0 Gm.; NaHCO₃, 2.5 Gm.; phenol, 4.0 Gm.; and distilled water, 1,000 ml.

tests. Various dilutions were aerosolized in 1.0 ml. aliquots by means of a No. 40 DeVilbiss nebulizer attached to a pressure source by means of a Y-tube connection. Each dilution was inhaled for a period of 5 minutes, during which time the rate of respiration was controlled at 20 breaths per minute with the aid of a metronome. Positive responses, evaluated on the basis of at least a 20 per cent decrease in the predicted peak expiratory flow rate, were determined within 30 minutes after introduction of the antigen. Prior to challenging the subject, an aerosolized mixture of buffered saline was administered as a means of excluding nonspecific reactors.

RESULTS

Of the 79 atopic patients tested, 47 exhibited positive skin reactions of 2 plus or greater to one or more of the six algal antigens studied (Table I). No reactions were observed in any of the individuals who were selected as nonallergic control subjects.

It is evident from the data presented in Table II that each antigen evoked pronounced skin reactions (3 plus or greater) in a substantial number of patients. Of these 64 reactions, 51 per cent were elicited by *Chlorella vulgaris* and *Scenedesmus basileensis*. Multiple reactions of 2 plus or greater were observed in 68 per cent of the patients, who exhibited moderate to pronounced skin reactions. However, as seen in Fig. 1, the percentage of positive responses to successively greater numbers of antigens generally showed a marked decrease in these patients. Thus, reactivity to all six antigens was noted in only 5 per cent of the subjects. Patients were also examined for cross-reactivity to *Chlorella* and *Chlorococcum* antigens. The results of these comparisons failed to indicate

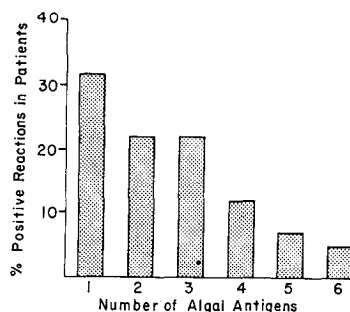
Table I. Peak skin reactivity obtained in persons tested with one or more algal extracts

Subjects	Incidence at different skin test grades*				
	4+	3+	2+	1+	0
Atopic	21	18	8	6	26
Nonatopic	0	0	0	0	26

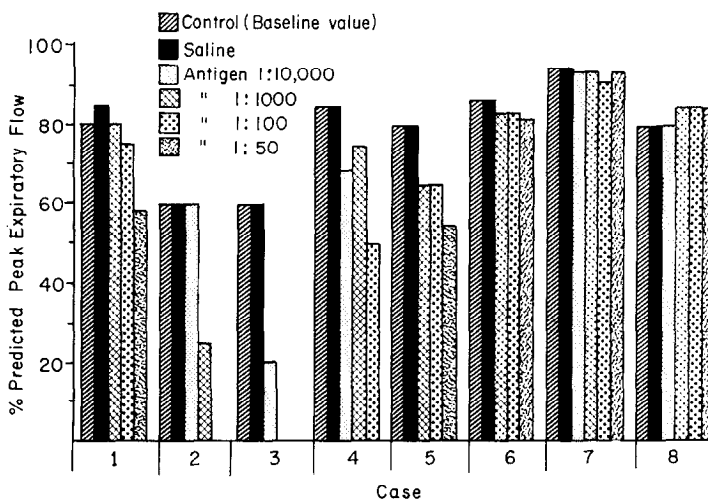
*0, no wheal or erythema; 1+, erythema alone; 2+, erythema and circular wheal measuring 0.5 cm. in diameter; 3+, erythema and circular wheal measuring 1.0 cm. in diameter; 4+, marked erythema and wheal formation with pseudopods.

Table II. Skin test results in atopic patients tested with 6 algal extracts

Algae	Test dilution (Gm./ml.)	Test dose (PNU/ 0.02 ml.)	Incidence at different skin test grades				
			4+	3+	2+	1+	0
<i>Chlorella pyrenoidosa</i>	1:1,000	1,300	2	6	7	4	48
<i>Scenedesmus basileensis</i>	1:1,000	2,500	5	10	3	10	39
<i>Chlorococcum botryoides</i>	1:1,000	3,500	6	2	12	6	41
<i>Ankistrodesmus falcatus</i>	1:1,000	1,130	1	5	6	6	49
<i>Chlorella vulgaris</i>	1:1,000	3,530	7	11	7	6	36
<i>Chlorococcum macrostigmatum</i>	1:1,000	1,000	2	7	5	6	47

**Fig. 1**

Analysis of single and multiple skin reactions, 2 plus or greater.

**Fig. 2**

Bronchial provocative tests in 8 patients.

any significant differences or similarities in their response to either the same or different genera.

Skin-sensitizing antibodies were passively transferred from 6 of the 8 patients studied (Table III). The undiluted sera of 5 of these patients yielded passive reactivity of 2 plus or greater. When test sites injected with diluted sera (1:10) were challenged with antigen, 5 of the sera continued to give positive reactions. One serum diluted 1:160 evoked a positive response to the antigen. No precipitation bands could be detected when sera from these same patients were tested against cellular extracts of *Chlorella vulgaris*.

A summary of the direct bronchial mucosal tests is presented in Fig. 2. Positive responses were obtained in 5 of 8 asthmatic patients upon whom this technique was performed. Included in this group were 7 patients who were tested for transferable antibodies. The remaining patient (Case 5) gave a 4

Table III. Passive transfer results in eight patients with marked cutaneous reactions to *Chlorella vulgaris* extract

Serum dilution	Skin test grades								Control sites	
	Sera test sites* challenged with <i>Chlorella vulgaris</i> (1:100)								Serum No. 1 challenged with saline	Ragweed reaginic serum challenged with WSR† (1:100)
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8		
Undiluted	4+	2+	1+	2+	3+	0	0	3+	0	4+
1:10	4+	1+	1+	1+	2+	0	0	0	0	4+
1:20	3+	0	0	0	1+	0	0	0	0	3+
1:40	3+	0	0	0	0	0	0	0	0	3+
1:80	2+	0	0	0	0	0	0	0	0	2+
1:160	1+	0	0	0	0	0	0	0	0	1+

*Each serum was placed on two recipients and the graded results were averaged.

†WSR, water-soluble ragweed extract.

plus direct cutaneous test for *Chlorella vulgaris*, but was not tested for P-K antibodies. In addition to decreases in peak expiratory flow rate, all of the 5 patients with positive responses developed auscultatory evidence of wheezing at the conclusion of their respective provocative episodes. Marked changes in peak expiratory flow rate and severe clinical wheezing were observed in 2 of these patients who were challenged with dilute solutions of the antigen. Both required epinephrine for relief of symptoms. One patient responded to a moderate concentration (1:100) of aerosolized antigen, whereas the remaining 2 patients reacted only to the strongest concentration (1:50). Concentrated material did not provoke measurable ventilatory changes in Cases 6, 7, and 8. Serum from Case 8 also failed to demonstrate the presence of P-K antibodies.

Provocative tests to concentrated (1:50) extracts of *Chlorella vulgaris* were performed on 6 asthmatic patients with negative skin tests to this organism and in 5 nonatopic patients, including one man who had sustained symptoms of chronic bronchitis for several years. None of these patients showed positive responses either immediately after challenge or two hours later.

DISCUSSION

Skin test results corroborated a previous report that cutaneous reactions to green algal extracts occur commonly in atopic persons.⁷ From our passive transfer studies, it would appear that this skin reactivity may be equated with reaginic-type antibodies. Significant bronchial provocative responses were also obtained, which suggests that reaginic antibodies to algal antigens may localize in bronchial mucosa and that the respiratory tract could be a preferred site for sensitization. Moreover, direct provocation of some atopic patients with *Chlorella vulgaris* resulted in severe clinical wheezing. Whether algal concentrations in the natural environment are sufficient to cause such clinical symptoms remains to be determined.

It is evident from the data presented in Fig. 1 that a substantial number

of patients reacted to only one of the six antigens tested. Analysis of these single reactions shows that they were not restricted to a particular algal species or genus. This would suggest that, within the limits of the study, algal sensitization of some patients was specific. The fact that multiple reactions to five or more algal antigens were uncommon tends to affirm the importance of individual reactions. Among the patients exhibiting multiple reactions, no cross-sensitivity could be established in closely related algal strains.

Our observations strongly suggest the possible role of green algae in human respiratory allergies. However, establishment of a definite causal relationship must await the results of epidemiological and immunological investigations. Of the probable routes of algal sensitization, inhalation of the organism deserves primary consideration. Schlichting⁶ postulated that the dissemination of algae into the atmosphere resulted primarily from wind currents and thermal updrafts. He also noted that fluctuations of airborne algal populations occurred with season and locality. Therefore, algal sensitization might be expected to vary accordingly.

Brief mention should be made of ingestion as an alternate avenue of human exposure to members of the Chlorophyceae. The epidemiological importance of this source should not be overlooked, in view of the likelihood of more frequent exposures to algae-infested waters and the artificial cultivation of green algae for human consumption.^{9, 11} In controlled feeding experiments with a mixture of *Chlorella* and *Scenedesmus* strains, consumption of 100 Gm. of algae daily for three days gave no evidence of serious side effects.¹² However, the question of possible algal sensitization was not considered in these preliminary trials. In view of our results, we recommend that the allergenic potential of these materials be closely scrutinized in future algal feeding studies.

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REFERENCES

1. Schwimmer, D., and Schwimmer, M.: Algae and Medicine, in Jackson, D. F., editor: *Algae and Man*, New York, 1964, Plenum Press, Inc., p. 368.
2. Heise, H. A.: Symptoms of Hay Fever Caused by Algae, *J. ALLERGY* **20**: 383, 1949.
3. Cohen, S. G., and Reif, C. B.: Cutaneous Sensitization to Blue-Green Algae, *J. ALLERGY* **24**: 452, 1953.
4. Gregory, P. H., Hamilton, E. D., and Sreeramulu, T.: Occurrence of the Alga *Gloeocapsa* in the Air, *Nature* **176**: 1270, 1955.
5. Brown, R. M., Jr., Larson, D. A., and Bold, H. C.: Airborne Algae: Their Abundance and Heterogeneity, *Science* **143**: 583, 1964.
6. Schlichting, H. E., Jr.: Meteorological Conditions Affecting the Dispersal of Airborne Algae and Protozoa, *Lloydia* **27**: 64, 1964.
7. McElhenney, T. R., Bold, H. C., Brown, R. M., Jr., and McGovern, J. P.: Algae: A Cause of Inhalant Allergy in Children, *Ann. Allergy* **20**: 739, 1962.
8. Smith, F. B.: The Occurrence and Distribution of Algae in Soils, *Proc. Florida Acad. Sc.* **7**: 44, 1944.
9. Palmer, C. M.: Algae in Water Supplies of the United States, in Jackson, D. F., editor: *Algae and Man*, New York, 1964, Plenum Press, Inc., p. 239.

10. Williams, L. G.: Plankton Population Dynamics, U. S. Public Health Service, Publ. 663, Suppl. 2, 1962.
11. Krauss, R. W.: Mass Culture of Algae for Food and Other Organic Compounds, Am. J. Botany 49: 425, 1962.
12. Powell, R. C., Nevels, E. M., and McDowell, M. E.: Algae Feeding in Humans, J. Nutrition 75: 7, 1961.