

DIAGNOSTIC VALUE
OF CULTURE PROCEDURES AND
PROVOCATION TESTS
IN SUSPECTED MOLD ALLERGIES

By

A. LIEBESKIND
Haifa, Israel.

It has been established, beyond doubt, that mold spores in the air may play an important role in the etiology of allergic diseases, especially naso-bronchial allergy and a great deal of publications in the recent allergological literature is concerned with the systematic study of aeromycology in various areas of the world and new methods (1) and apparatuses like *Andersen's* (11, 12) are described from time to time. But on a parallel line we witness an immense development of mycology itself in taxonomic studies, medical, industrial and agriculture mycology and many other fields linked to the commonly known molds (2, 8, 13, 15, 16, 20). The laboratory procedures, the culturing and identification methods are recently enriched through an extensive literature (4, 5, 6, 7).

Apart from the airborne molds („sensu stricto“) caught by our investigation method there are many sources of molds in our environment in industry, agriculture, food processing, timber work etc. which may evoke an allergic reaction in suitable individuals.

Allergy against molds material develops not seldom in patients suffering from allergic naso-bronchial disorders, whose diagnostic and therapeutic results are so far unsatisfactory although, based on a very thoroughly collected anamnesis and,

various skin and provocation tests because the proper allergenic factor has not yet been revealed. Thus in such situation, both the practising allergologist and his unhappy patient face the problem of reconsideration of the whole treatment and have to look for the real offending allergen, which has been overlooked so far (14). It is by no means an easy task and requires an attitude of mind of the practising allergologist, an adequate knowledge of mycological problems, an experience in cultivation procedures and identification methods and sometimes a very painstaking and time-consuming work.

The present work was undertaken with two aims. Firstly to try to decide if a proper mold allergen is present in the suspected and incriminated material which can be responsible for the allergic response of the patient and if so, to try to eliminate the errors in diagnosis and therapy.

MATERIAL

The study includes 11 allergic patients, who present the above mentioned problem. Part of them had not undergone any specific treatment, others were treated in our allergy clinic with specific hyposensitisation, based on the results of various skin-tests with routine allergenic extracts and provocation tests and in addition with antiallergic drugs, but without success. We were therefore forced to reconsider our etiologic diagnosis, looking for the offending allergen in materials suspected of containing molds with which the patient could have been in contact.

Case 1. R. P. female 34, worker in a citrus grove, complained of sneezing, running of the nose, coughing with wheezing, while eating grapefruit and other citrusfruit. She was „labelled“ as allergic to citrusfruit and had been advised to avoid eating them. Thoroughly inquired, she admitted that sometimes she could tolerate the forbidden citrusfruit. A sample of a grapefruit, brought to us for examination with fragrant odour, and a soft, rotten appearance showed blue-green patches which finally, after being mycologically examined turned out to be *Penicillium italicum*. It appeared later, that she could eat all citrusfruit, except those infested with this species of molds.

Case 2. J. A. male 29, fruitshop-owner with a history of rhinitis allergica and

No.	Name, Sex, age	Occupation	Clinical diagnosis	Former treatment
1	R. P. 34 ♀	Orangegrove worker	Rhinitis Coughing	Symptomatic treatment; drugs
2	J. A. 29 ♂	Fruitshop- owner	Rhinitis Asthma bronchial.	Hyposensitisation Symptomat. drugs
3	B. J. 52 ♂	Teacher	Rhinitis allergica	Symptomatic drugs drops
4	D. M. 29 ♀	Agriculture worker	Rhinitis Asthma bronchiale	Hyposensitisation symptomat. drugs
5	D. A. 9 ♂	Schoolboy	Rhinitis Asthmoid bronchit.	None
6	S. M. 32 ♂	School- teacher	Rhinitis Asthmoid bronchit.	None
7	R. J. 42 ♂	Building contractor	Urticaria recidivans	Dermatologic, symptomatic treatment
8	A. S. 38 ♂	Worker in dye factory	Rhinitis Cough	Symptomatic drugs nasal drops
9	O. D. 46 ♂	Agriculture worker	Rhinitis Asthmoid bronchitis	None
10	E. A. 26 ♂	Citrusgrove worker	Rhinitis allergica Asthma bronchial.	Hyposensitisation, symptomatic drugs Steroids.
11	N. S. 50 ♀	Housewife	Rhinitis Cough	Hyposensitisation, drugs

Material mold infestated	Mold species	Provocation tests	Skin tests intra-dermal
Grapefruit	<i>Penicillium italic.</i>	Positive	Positive marked immediate
Apples	<i>Penicillium expansum</i>	Positive	Positive marked immediate
Wall-paper	<i>Aureobasidium pullulans</i>	Positive	Not done
Potatoes	<i>Phytophthora infestans</i>	Positive	Not done
Potatoes	<i>Phytophthora infestans</i>	Positive	Not done
Potatoes	<i>Phytophthora infestans</i>	Positive	Not done
Scrapings from hyperkeratotic plaques of heels	<i>Trychophyton rubrum</i>	Positive	Positive marked immediate
Scrappings from his apron and trousers	<i>Chaetomium</i> sp.	Positive	Positive marked immediate
Leaves and tomatoes growing in glass houses (plastic)	<i>Cladosporium fulvum</i>	Positive	Positive moderate-marked immediate
Oranges Grapefruit	<i>Penicillium digitatum</i>	Positive	Positive marked immediate
Wall-painting	<i>Phoma violaceum</i>	Positive	Positive marked immediate

asthma bronchiale. Despite hyposensitisation with housedust extracts, bacterial vaccines and mixed mold extract allergens finally forced to use nebulizers and corticosteroids. On the shelves in his fruit shop there was a heap of mixed old and fresh apples with very distinct brown rot, identified by mycological examination as *Penicillium expansum*.

Case 3. B. J. male 52, teacher, complained of running of the nose, sneezing and post nasal drip while sitting in his study, especially during the evening hours. Treatment with nasal drops and antihistaminics was so far unsuccessful. His study was covered with wall-paper and in some places beneath the wall-paper sheets we found dirty greyish and black masses, which by mycological investigation turned out to be colonies of *Aureobasidium pullulans*. After removal of the wall-paper from his study and after appropriate treatment, he recovered completely.

Case 4, 5, 6. 3 villagers from Western Gallilea suffering from rhinitis and asthmoid bronchitis. D. M. female 29, received during a period of one year hyposensitisation treatment against molds and housedust, as indicated by the skin-test results. Following this treatment her condition improved, but after a long time she appeared again in my clinic in a miserable condition. Upon questioning her about any changes in her environment she replied that she had slept in a room, where she stored potatoes. Her child, a school boy D. A. 9, and also her neighbour, S. M. male 32, schoolteacher, had started to suffer from very frequent and troublesome attacks of rhinitis and asthmoid bronchitis. Examination of the spoiled potatoes revealed an infestation of the potatoes with *Phytophthora infestans* (9).

In all these three cases a provocation nasal test confirmed our diagnosis. After removing the spoiled potatoes from both houses remarkable relief for the suffering patients ensued.

Case 7. R. J. male 42, building contractor, complained of urticaria recidivans, since two years with intervals of some weeks; he received symptomatic treatment with antihistaminics and corticosteroids with negative results. Chronic hyperkeratotic skin changes on both soles, heels and sides of the feet (so called „moccasin distribution“) called attention. This was diagnosed in England and in the Netherlands years before as chrome dermatitis, induced by the leather of the shoes; However, after changing his leather shoes for linen shoes and using various ointments and other therapeutic dermatological procedures he had not obtained any improvement and had left the affection untreated for many years.

He did not suppose that this could be the cause of his present illness, urticaria recidivans, which sometimes proceeded as urticaria generalisata. A direct microscopic examination of the scraped material from the hyperkeratotic lesions revealed some mycelial elements, micro- and macroconidia, and only by using Sabouraud nutrient medium we were able to grow a characteristic culture of *Trychophyton rubrum* with fluffy red appearance on the reverse of the Petri-dishes.

Case 8. A. S. male 38, worker in a dye-plant, complained of sneezing, running of the nose and molesting, unproductive cough, especially when wearing his work-apron. His previous examination including skin-test as well patch test with several chemical substances used in the plant, did not reveal any positive results and his symptomatic treatment consisted only of nasal drops and antihistaminics. On his work-apron, although very often changed, we found dark greyish spots and plaques, which in the Petri-dishes and subsequently on subcultures with Czapek medium presented themselves as *Chaetomium* sp. with their black perithecia and long, dark coloured, stiff hair. The growth of these spots and plaques, even on very frequently changed, clean aprons, indicated a steady source of mold production in the environment of the patient.

Case 9. O. D. male 46, agriculture worker suffered from rhinitis and asthmoid bronchitis; he believed that his work, growing tomatoes in greenhouses (plastic), was the cause of his illness. From the leaves of tomatoes we succeeded to grow in Petri-dishes and subsequently in appropriate subcultures, *Cladosporium fulvum* (Fulvia), and we could confirm our diagnosis in positive provocation nasal-tests.

Case 10. E. A. male 26, a citrus grove worker, suffered from rhinitis allergica and asthma bronchiale. Hyposensitisation treatment, antihistaminic drugs, inhalation therapy and corticosteroids were without any effect. At the citrus grove we found many shrunken oranges and grapefruits, on which the infested places were olive or yellowish green cultures and subcultures from these appeared in Petri-dishes as typical colonies of *Penicillium digitatum*. A provocation nasal-test with these molds confirmed our diagnosis.

Case 11. N. S. female 50, housewife, complained of stuffy and running nose, headache, and irritation of her throat leading to unproductive cough. Previous treatment including hyposensitisation, antihistaminics and antibiotics was without positive result. In her apartment we found on the dark red painted walls pink purplish spots in clusters, and in mycological cultures we were able to disclose typical *Phoma* pycnidia, probably belonging to the described *Phoma violaceum*. A provocation nasal-test confirmed our suspicion.

Recently (10) we described three cases of rhinitis allergica and asthma bronchiale due to Basidiomycetes: *Agaricus bisporus*, *Merulius* (*Serpula lacrimans*) and *Lycoperdon perlatum*.

METHODS

Cultures for morphological study of molds are usually grown on Petri-dishes, in plugged test tubes, or in both. Several different culture media used for the propagation and study of the molds are listed in (Table 2) according to our experience.

The method used to isolate a particular mold from material substratum like in our material and to obtain a pure culture depends somewhat on circumstances. On the whole in such cases direct cultures can seldom be made without introduc-

TABLE 2

Nutrient media used in Petri dishes and subcultures.

Kind of Nutrient medium:	Formula from:
Potatoes-glucose-agar	Centr. v. Schimmel Cult. Baarn
Corn-meal agar	Centr. v. Schimmel Cult. Baarn
Malt extract-agar	Modification by G. Smith
Czapek's agar	Centr. v. Schimmel Cult. Baarn
Sabouraud-glucose-agar	Modification by Alkiewicz (3.)
a. milieu d'épreuve	
b. milieu de conservation	

ing gross contamination with other microorganisms. The best way of isolating the dominant mold from material of this type is to prepare series of dilution cultures, commonly called „plating out“ (13). Fortunately, in our mycological examination it was not necessary to make dilution cultures because of the prominence and clearness of the dominant species of mold, grown already on the Petri-dishes. But it was our routine in most cases to „plate out“ suspected material on two or three different media, as an important mold may grow so well on a rich medium, e.g. P.D.A. potato dextrose agar or malt extract agar, that it swamps a slow growing species, or, on the other hand, an important species may grow very slowly or not at all on a syntetic medium like Czapek agar and be completely missed, unless a more suitable medium is used. Table 3 shows the „plating out“ technique.

For preventing the growth of bacteria we added to our nutrient media 0.35 g per liter of Rose Bengal which has been found to be very satisfactory in combating a gross bacterial contamination (13). The first stage is the inoculation of the Petri-dishes containing appropriate nutrient media with the suspected material; these Petri-dishes were incubated at 20° C approximately for 3-5 days, depending on the growth of the colonies. The colonies developed were counted, identified, and isolated subculturing them on tube slants containing alternatively different nutrient media. These subcultures as a second stage of our investigations were used as a source for the mass culture of various molds and for the preparation of antigenic extracts, used in the subsequent skin and provocation tests.

The preparation of allergenic extracts for skin-tests is a rather complicated procedure, and we were able to prepare them in only 5 out of 11 cases, according to the method described by *Van der Werff* (20). The greatest difficulty in preparation of the allergenic extracts was the lack of possibility for correct standardisation through measuring the total nitrogen content.

We performed in all cases nasal provocation tests according to *E. Fuchs* (19), because our patients suffered particularly from upper respiratory distress especially from the nose. The major difficulties in nasal provocation testing

TABLE 3
Plating out.

Source of molds	Nutrient media in Petri dishes	Nutrient media in subcultures
Grapefruit	Malt extract-agar	1. Czapek's-agar 2. Corn meal-agar
Apples	Malt extract-agar	1. Czapek's-agar 2. Potato glucose-agar
Wall paper sheets	Czapek's-agar	1. Czapek's-agar 2. Malt extract-agar 3. Corn meal-agar
Hyperkeratotic skin scrapings	Sabouraud glucose-agar	1. Sabouraud-glucose 2. Sabouraud-(milieu de conservation)
Spots and plaques on apron	Czapek's-agar	1. Malt extract-agar 2. Corn meal-agar
Grapefruit	Malt extract-agar	1. Corn meal-agar 2. Potato glucose-agar 3. Czapek's-agar
Wall painting	Czapek's-agar	1. Malt extract-agar 2. Corn meal-agar 3. Potato glucose-agar
Potatoes	Potato glucose-agar	—
Tomato leaves	Corn meal-agar	1. Malt extract-agar 2. Czapek's-agar 3. Potato glucose-agar

are the standarisisation of antigen challenge and the measurement as stated by *G. Taylor* (17). But we did without this somehow complicated method proposed by *Taylor*, relying only on the macroscopic appearance of the exposed nasal mucosa, compared with the mucose of the unexposed nares of the same individual under the same conditions. In each case we performed (even several times) a control provocation test with 0.9 per cent NaCl to prove the specificity of the introduced allergen. The eosinophils in the nasal secretions were often found in clusters and were counted. Only when found in a great number (sometimes 100 in the field) it was valued as a positive result of the provocation test.

RESULTS

Our clinical material comprises 11 patients, 10 with upper respiratory allergies and one with urticaria recidivans. In all cases we succeeded in finding the offending allergen as a mold in the environment of the patient, using mycological culture procedures. We could confirm our diagnosis in all cases by provocation nasal-tests and in some cases by skin tests.

DISCUSSION

All these patients were previously treated. Four of them had hyposensitisation injections for a relatively long time in accordance with the results of skin tests and received symptomatic treatment with antiasthmatics and corticosteroids for a short period. The results of this extant treatment were unsatisfactory. We attempted to consider the former etiological diagnosis by checking the history very thoroughly, looking for other sources of the offending allergen. This task was not easy, but the growing experience in culturemaking procedures, which we have acquired during the last few years enabled us to undertake this aim.

Case 1 (Table 1) proved that the patient P. R. is not allergic to citrusfruit. She has no food allergy, but is allergic to molds infestating the citrusfruit in her environment. Cases 2 and 10 (Table 1) proved to be hypersensitive to other species of penicillium, as demonstrated above. In case 3 (Table 1) we could reveal the offending mold allergen growing in colonies beneath the sheets of wallpaper. Cases 4, 5, 6, (9.) required a very thorough study of the natural history of *Phytophthora infestans* before we could start culturing the Oomycetes, and it was very interesting to apply the appropriate temperature during the examination of the samples as advised by *Alexopoulos* (2). Case 7 initially presented a great problem, because of the undoubted diagnosis of shoe dermatitis; we could not link it with the present illness, urticaria recidivans, although such cases were described some years ago

(18). This kind of urticaria appeared as a dermatophytid and the patient, after getting treatment with griseofulvin and hypensensitisation with *Trichophyton rubrum* extract, has now become free of urticarial symptoms, through three years of observation. In case 8 it was very difficult to think about the infestation with *Chaetomium*; our first attention was turned to the dye and cellulose material he got in touch with during his work, but with totally negative results with chemical and industrial products, we finally took into the consideration the presence of *Chaetomium* in spot colonies on his work-apron. Case 11 (Table 1) was at the very beginning hopeless and only the calling at her apartment and thorough search for the offending allergen lead to the correct etiological diagnosis and enabled us to confirm it.

SUMMARY AND CONCLUSION

In many cases of allergic disorders we are forced to reconsider the diagnosis, especially in cases with unsuccessful results. The task is not easy, especially when the practising allergist gets involved in mycological work, a field which is in itself immense, requiring special experience and substantial knowledge of mold problems. But, overcoming these obvious difficulties, the practising allergologist, if he is „moldminded“, can perform the mycological investigation with very modest tools and equipment in his daily practice.

ACKNOWLEDGEMENT

I am very grateful to Dr. G. De Vries and his assistants, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, for their generous help in improving my technical knowledge during my stays there.

REFERENCES

1. Agarwal, M. K., Shrivastava, D. N. & Mukerji, K. G.: Studies on the allergenic fungal spores in Delhi, metropolitan area, India. *J. Allergy* 44, 193, 1969.
2. Alexopoulos, C. J.: Einführung in die Mykologie. G. Fischer, Stuttgart, 1966.
3. Alkiewicz, J.: Mikologia Lekarska. P. Z. W. L. Warszawa, Poland, 1966.

4. *Barnett, H. L.*: Illustrated Genera of Imperfecti Fungi. Second Edition. Burgess Publ. Company, Minneapolis, 1967.
5. *Funder, S.*: Practical Mycology, Oslo, Norway, Broggers Boktrykkeri, 1935.
6. *Hazen, E. L., Gordon, M. A. & Reed, F. C.*: Laboratory Identification of Pathogenic Fungi Simplified. 2 Ed, C. C. Thomas Publ. Springfield Ill. 1968.
7. Laboratory Procedures in Clinical Mycology, T. M. 8, 227-8, Headquarters Dept. of Army, 1964.
8. *Müller, E. & Loeffler, W.*: Mykologie. G. Thieme Verlag. Stuttgart 1968.
9. *Liebeskind, A.*: False-Mildew (Phytophthora Infestans) As An Allergenic Factor. Acta Allergol. 24, 362, 1969.
10. *Liebeskind, A.*: Mykologische Probleme bei Allergischen Berufskrankheiten. Allergie u. Asthma 16, 875, 1970.
11. *Salomon, W. R.*: Simplified Application of The Andersen Sampler. To the Study Of The Airborne Fungus Particle. J. Allergy 45, 1, 1970.
12. *Sayer, W. J., Shean, D. B. & Ghossein, J.*: Estimation Of Airborne Fungal Flora By Andersen Sampler Versus The Gravity Settling Culture Plate. J. Allergy 44, 214, 1969.
13. *Smith, G.*: An Introduction to Industrial Mycology. Sixth Ed. Edw. Arnold Publ. London, 1969.
14. The Editor's Page: Overlooking the Obvious. Rev. Allergy 23, 737, 1969.
15. *Thom, C. & Raper, K. B.*: Manual of the Aspergilli. Baillière, Tindall & Cox. London 1945.
16. *Thom, G.*: The Penicillia. The Williams & Wilkins Co. Baltimore, 1930.
- 16 a. *Raper, K. & Thom, C.*: Manual of Penicillia. Baillière, Tindall & Cox. London 1949.
17. *Taylor, G.*: Nasal Provocation Testing (Abstr.) Meeting of The British All. Soc. London 7 Jan. 1969.
18. *Weiner, A. L. & Goldman, L.*: Localized Urticarial Reactions Associated with Trichophyton Rubrum Infections of The Skin. Cutis 3, 59, 1967.
19. *Werner, M. & Ruppert, V.*: Praktische Allergie-Diagnostik. G. Thieme Verlag. Stuttgart, 1968.
20. *Van der Werff, P. J.*: Mould Fungi and Bronchi Asthma. H. E. Stenfert Kroese N. V. Leiden, 1958.
21. Van Wetenschappen Koninklijke Nederlandsè Akademie. Centraalbureau Voor Schimmelcultures. Baarn, The Netherlands Twenty seventh Edition List of Cultures, 1968.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.