

FUNGICIDAL AGENTS IN THE TREATMENT OF ALLERGY TO MOLDS

LEO H. CRIEP, M.D., ROMAYNE A. TEUFEL, B.S.,* AND CRAIG S. MILLER, B.S.**
PITTSBURGH, PA.

THE present study deals with a determination of the efficacy of various agents reputed to be fungicidal. It was undertaken in the hope of finding a suitable, inexpensive, nontoxic, and nonstaining agent which could effectively reduce the mold content in homes of patients allergic to molds.

LITERATURE

The role of nonpathogenic fungi in the etiology of allergy of the respiratory tract has been well established.¹⁻⁹ For the most part, they occur seasonally.¹⁰ Their growth and distribution vary with geographic and climatic conditions. Thus, wind,¹¹ location,¹² temperature, and humidity¹³ determine the abundance of outdoor fungi in a given area. The extent and severity of allergic manifestations produced as a result of exposure to air-borne fungus spores depend, as is the case in pollen allergy, on the extent of sensitivity to these spores as well as on their quantity in the inspired air. Since fungi are ubiquitous, it follows that the air content of outdoor spores is reflected in their distribution in the indoor house dust and air.^{14, 15} Richards¹⁶ compared the occurrence, type, and abundance of molds indoors and outdoors. He finds the colony count obtained as a result of outdoor exposure higher than that obtained indoors; the same molds are distributed in the same relative proportion in both areas. He also indicates that there is a higher concentration in damp houses. He identifies the following indoor molds: *Alternaria tenuis*, *Aspergillus fumigatus*, *Cephalosporium acremonium*, *Cladosporium herbarium*, *Penicillium* (several types), and *Pullularia*. Saito²³ and Rostrup²⁴ report practically the same indoor molds as indicated above, while others¹⁷ find, in similar areas, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Pullularia*. On indoor sampling, Merksamer²⁶ and others²¹ indicate, in addition to the above fungi, *Mucor*, *Rhizopus*, *Phoma*, yeast, *Penicillium* and *Aspergillus*. Prince and associates¹⁷ and others²² state that even under normal conditions the spore and colony count may be higher indoors than outdoors. This is especially true in barns, greenhouses, haylofts,²³ and in old houses that are dark, dusty, musty, and damp. In such areas one may find patches of molds growing on walls, leather (shoes, luggage), fabrics (woolens, cotton), wood, pillows, mattresses, upholstery, furniture, and other articles.^{16, 23} These growths are

From the Allergy Research Laboratory, Veterans Administration Hospital, Pittsburgh, Pennsylvania, and the Section on Allergy, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

Received for publication Feb. 3, 1958.

*Biochemist, Veterans Administration Hospital, Allergy Research Laboratory, Pittsburgh, Pennsylvania.

**Grantee of the American Foundation for the Study of Allergic Diseases.

erroneously referred to by the layman as "mildew." They are an added source of mold spores in the house. Nilsby^{19, 20} has shown that the number of mold colonies is greater in damp "nonhygienic" houses than in clean dry ones, and Rennerfelt²¹ and also Swaebly and Christensen²² found fewer colonies in clean homes than were found outdoors. Spores are easily distributed indoors by air currents as shown by Christensen,²⁸ who recovered them on the fourth floor of a house within five minutes after liberating them on the first floor. These spores easily reached another room located fifteen feet away from the source, even though the door was open only two inches. This permeation, infiltration, and contamination of the air from one part of a house to another take place easily through the aid of natural air currents. It would seem that occasionally such air dissemination varies to some extent with the type of mold studied.²⁹ Suitable culture techniques³⁰ are employed in the identification of these molds.

CASE REPORTS

This study was stimulated by our recent experience with three cases of respiratory allergy—two of severe perennial nasal allergy and a third of bronchial asthma and nasal allergy. The condition of these patients improved only partially with treatment. It was then realized that they were highly allergic to molds. A brief summary of our clinical findings in each case follows.

CASE 1.—The patient, a 15-year-old atopic girl, has had severe perennial and seasonal nasal allergy for the past five years. She was found to be sensitive to many inhalants (including molds) and some foods. In spite of good cooperation and adequate specific therapy, she continued to be uncomfortable, especially during the summer and fall. Positive direct skin test reactions were obtained to *Penicillium*, *Aspergillus*, *Alternaria*, and *Hormodendrum* with a positive passive transfer to the first three molds. It was then discovered that the patient lived in an old damp home. A visit to her house showed a dusty, musty attic and a basement room full of "mildew." Fungicidal agents were then used with good therapeutic results.

CASE 2.—A housewife, 35 years of age, presented clinical evidence of persistent, severe, perennial nasal allergy with polyps. This condition was present over a period of seven years. She had a positive family history of allergy; she had atopic dermatitis earlier in life and showed clinical sensitivity to house dust, peanuts, and chocolate. She gave positive skin test reaction to some inhalants and foods and a marked direct positive reaction to *Alternaria*, *Hormodendrum*, and *Penicillium*—reactions which transferred passively. In spite of complete and adequate therapy, the patient continued to be uncomfortable, especially when she was at home. The house was located in a river valley and, therefore, was in a damp, foggy area. Exposure of plates yielded a high mold colony count. The patient experienced marked relief following the use of fungicidal agents in her house.

CASE 3.—A 16-year-old girl gave a history of bronchial asthma over a period of ten years. This condition became worse during the past four or five years, after she moved to a new location. Investigation reveals that five years ago a storage room was added to the basement of the house. This room was damp, poorly ventilated, and received little sunlight. There were two storage cabinets in this room, and these were used to store seasonal clothes. A visit to the house revealed "moldy, mildewed" patches on many articles in this enclosure. There was "mildew" on a leather golf bag, on clothes stored in the cupboards, and on the walls. The molds were freely distributed throughout the house by air currents. During the

winter, this circulation of molds was further aided by forced air heating. This patient subsequently volunteered the information that she would invariably develop a paroxysm of asthma and nasal symptoms when visiting the basement. There was marked improvement in her condition following adequate treatment of the house with effective fungicidal agents.

The realization that the three patients just described were highly allergic to molds and that the mold content of the air in their homes was high led to an investigation as to how the element of such exposure may best be eliminated.

PAST EXPERIENCE WITH FUNGICIDAL AGENTS

Various procedures have been employed in the past to reduce indoor mold spores. These usually involve an attempt to increase the ventilation in the patient's house (particularly in the basement and attic), to reduce and avoid house dust, to dehumidify the air, and to increase exposure to the sun. In many instances, these procedures either are not possible under existing conditions or are only partially effective. Some time ago, we³¹ presented evidence that electrostatic air cleaners filter out of the air all particulate matter, including pollen, spores, and dust, and also that mechanical filters, although less effective, are helpful. Centrally installed electrostatic cleaners are part of the equipment of only the newer homes, however, and therefore are not generally available. Rooks and colleagues³² find that mechanical filters and air conditioners are not very efficient in removing mold spores from indoor air. Furthermore, such procedures simply filter out the spores and do not affect the growth of molds. A great deal of work has been done with reference to the applicability and mechanism of action of fungicides employed to impregnate materials and fabrics in order to reduce their contamination with molds. Thus, Baskin and Kaplan³³ determined that plasticized vinyl-coated fabrics become variably resistant to fungi following treatment with solubilized copper-8-quinolate and phenyl mercuric acetate. Marsh and associates,³⁴ Butler and Marsh,³⁵ and Corey and Shirk³⁶ report that various bisphenol compounds are effective fungicidal agents to be used locally as "mildew" preventives on cotton fabrics. Others found certain B-nitrostyrenes and some cyclohexane derivatives to be good fungicides.³⁷ Volini and associates³⁸ determined that three different derivatives of monothiouonium prevent the growth of *Hormodendrum*, *Helminthosporium*, and *Candida albicans*. Nitrostyrenes³⁹ and triacetin (glycerol triacetate)⁴⁰ are effective against pathogenic fungi as well as against *Alternaria*, *Penicillium*, and *Aspergillus*. Plants and leaves of woodland trees have naturally occurring fungicidal and fungistatic substances.^{41, 42} It would appear that in some instances at least the mechanism of the toxic action of these fungicidal compounds is the inhibition of fungus enzymes⁴³ or the coagulation of protoplasm. All of these procedures, however, have limited practical clinical application, since they are not readily available for use in large areas or under any other than experimental conditions. The literature makes only casual reference to the possible use of general fumigation of houses with formaldehyde vapor,⁴⁴ a procedure which has obvious practical drawbacks.

FUNGICIDAL AGENTS EMPLOYED IN THIS STUDY

Three agents reputed to have fungicidal properties were employed in this investigation. Following is a description of each of these agents:

*Hyagen** is a 10 per cent solution of alkyl (C9 to C15) tolyl methyltrimethyl ammonium chloride containing 90 per cent of inert ingredients. This product is noninflammable, nonirritating, nonvolatile, noncorrosive, and odorless. It does not attack fabrics or other materials, but it should not be permitted to contaminate food. It is recommended that it be used as a spray.

Roccal† (10 per cent solution) is a nonmetallic organic compound, a mixture of high-molecular alkyl dimethylbenzyl ammonium chloride. This product is freely soluble in water, is colorless, and has a slight aromatic odor. It is available in a more purified form as Zephiran chloride, a 12.5 per cent solution. This product is used in operating rooms. In an aqueous dilution of 1-10,000, it inhibits the growth of *Trichophyton interdigitale* after exposure for four to six minutes.⁴⁵ Zephiran chloride has a definitely high bactericidal and bacteriostatic effect as demonstrated by its phenol coefficient for various pathogenic organisms, including pathogenic fungi.⁴⁶⁻⁴⁹

Trioxymethylene‡ (crystalline paraformaldehyde) is a white amorphous powder of high molecular weight. Depolymerization occurs at a temperature of 180 to 200 degrees; hence, it is a convenient source of anhydrous, gaseous formaldehyde. This product has a melting point of 120 to 130 degrees. The commercial product contains 95 per cent formaldehyde. When heated, it is converted to formaldehyde.

TECHNIQUE

This study deals with an evaluation of the fungicidal activity of the three agents just described. The study was done on the basis of the ability of these agents to interfere with mold growth as determined on exposed Petri dishes. This investigation was carried out under six different sets of conditions to determine: (1) the effect of dipping plates into or spraying them with the agents under consideration, (2) the effect of incorporating the agents into the medium, (3) the effect of spraying agents in experimental hospital rooms, (4) the effect of subliming trioxymethylene powder in such rooms, (5) the mold content of dust in the patient's home, and (6) the effect of exposing trioxymethylene in the patient's home.

Petri dishes, 180 mm. in diameter, were employed. These plates contained sterile Mehrlich's medium with sodium chloride, 75 grams per liter. Following exposure for ten minutes, the Petri dish was closed and sealed with cellophane tape. It was not moved or disturbed until ten days to two weeks later, when all of the colonies present were counted. An attempt was made to identify the more prevalent fungi. The effectiveness of the agents employed was determined by the reduction in the number of mold colonies (Figs. 1 and 2).

*Marketed by the Miami Products and Chemical Company, Dayton, Ohio.

†Marketed by Winthrop Laboratories, Inc., 1450 Broadway, New York, N. Y.

‡Available commercially.

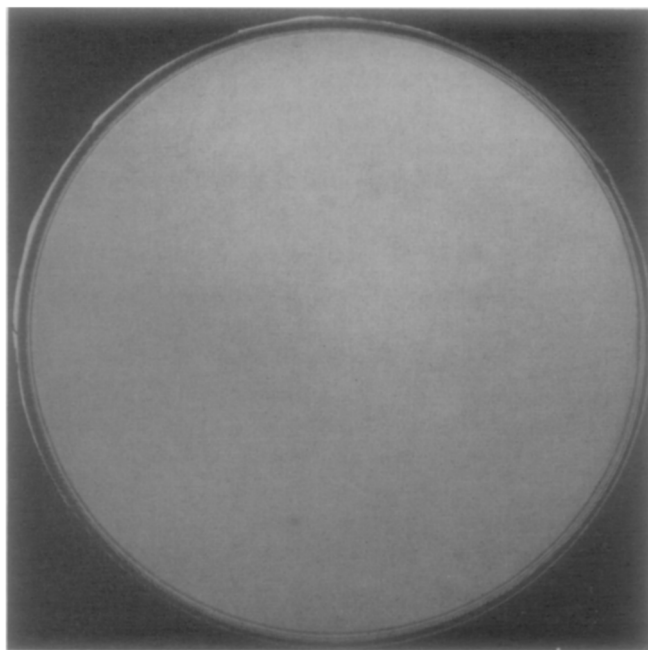


Fig. 1.—Trioxymethylene incorporated into medium prevents growth of molds regardless of method by which plate is exposed.

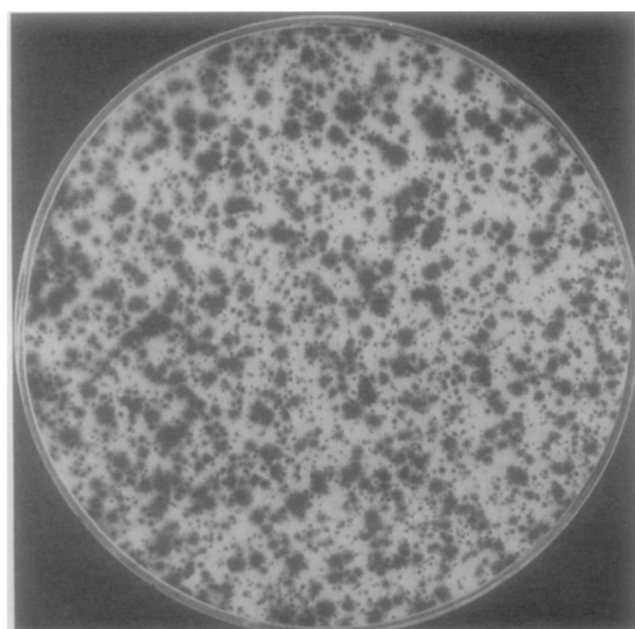


Fig. 2.—In instances where agent is not effective, the colonies are too numerous to count.

TABLE I. EXPERIMENT 1.
EFFECT ON COLONY GROWTH WHEN PLATE IS SPRAYED WITH OR DIPPED INTO AGENT
(EIGHTY-TWO PLATES)

AGENT	DILUTION	NUMBER OF COLONIES WHEN PLATE SPRAYED WITH AGENT*	NUMBER OF COLONIES WHEN PLATE DIPPED INTO AGENT*
Tap water		Too many	Too many
Sterile tap water		Too many	Too many
Normal saline		Too many	Too many
Sterile normal saline		Too many	Too many
Plain media		Too many	Too many
Hyagen	1-150	Too many	Too many
	1-300	Too many	Too many
	1-600	Too many	Too many
Zephiran chloride	1-100	0	No growth
	1-500	Only around edges	84
	1-1,000	Too many	Too many
Roccal	1-100	18	0
	1-500	218	52
	1-1,000	Too many	118
Trioxymethylene	1-10	56	16
	1-100	Too many	Too many
	1-1,000	Too many	Too many
	1-2,000	Too many	Too many

*Too many indicates that the plate was covered with such a luxuriant growth that the colonies were confluent and therefore too numerous to count.

Experiment 1 (Table I, Fig. 3). Effect of Spraying Plates With or Dipping Them Into the Agent.—This experiment was carried out in several hospital rooms. These were approximately the same size—15 feet 3 inches by 10 feet 1 inch by 9 feet 4 inches, or 1,435 cubic feet. Each room has one door and two

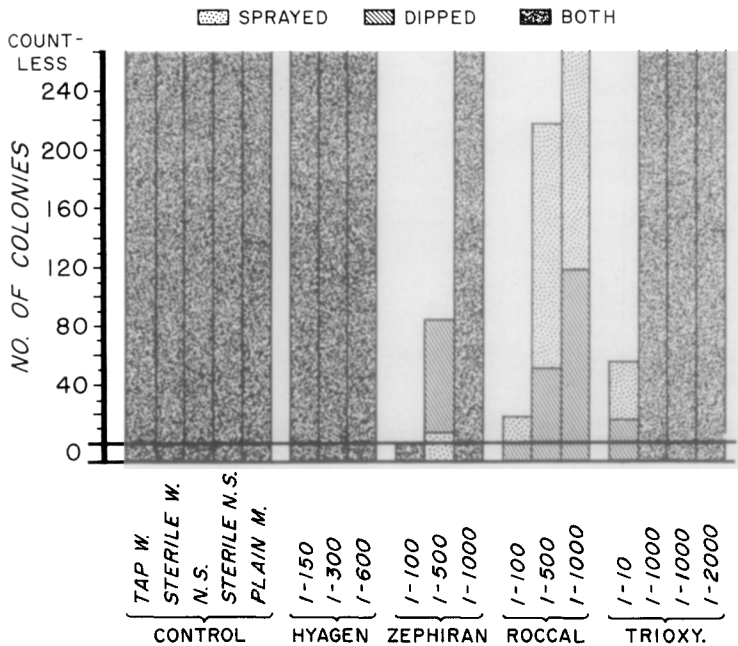


Fig. 3.—Effect on colony growth when plate is sprayed or dipped into agent. (Eighty-two plates.)

windows, and one cupboard of about 87 cubic feet projects into it. The door to the closet has a ventilating louvre, 12 by 18 inches, allowing for ventilation. A high concentration of mold spores was obtained in each of these rooms by operating an electric fan in front of several Petri dishes containing a luxuriant growth of fungi. Various dilutions were made of Hyagen, Roccal, Zephiran, and trioxymethylene. These dilutions were obtained from the concentration of the product as available from commercial sources. Trioxymethylene goes into solution with great difficulty; hence, the dilutions indicated are not always accurate. Some Petri dishes containing the sterile medium were dipped and others were sprayed with these various dilutions of the agent. The treated plates were then exposed for ten minutes in these rooms. Controls using tap water and saline, both sterile and nonsterile, as well as plain Mehrlich's medium were made. A total of eighty plates was used. The number of colonies indicated represents an average of several plates read under each set of conditions. The results are shown in Table I and Fig. 1. In the case of all controls, the growth was so abundant that there were too many colonies to permit an accurate count. Hyagen concentration as high as 1-150 had no inhibiting effect. Zephiran began to show an appreciable effect in a 1-500 dilution and permitted no growth in a 1-100 dilution. Roccal showed little effect in a dilution of 1-1,000, was moderately effective in 1-500, and was quite satisfactory in a dilution of 1-100. Trioxymethylene markedly reduced the growth when employed in a 1-10 dilution.

TABLE II. EXPERIMENT 2.
EFFECT ON COLONY GROWTH WHEN AGENT IS INCORPORATED INTO MEDIUM (130 PLATES)

AGENT	DILUTION	EXPOSED TO ROOM AIR (NO. OF COLONIES AFTER TWO WEEKS)	PLATES STREAKED WITH SPORE WASHINGS (NO. OF COLONIES AFTER TWO WEEKS)	ADDITION OF 1 C.C. OF SPORE SUSPEN- SION
Tap water		Too many	Too many	Too many
Sterile water		Too many	Too many	Too many
Nonsterile water		Too many	Too many	Too many
Sterile water		Too many	Too many	Too many
Plain media		Too many	Too many	Too many
Hyagen	1-3	0	3	0
	1-15	2	20	1
	1-30	16	52	15
	1-60	2	63	17
	1-100	Too many	Too many	Too many
Zephiran chloride	1-100	2	0	0
	1-500	2	11	0
	1-1,000	3	14	2
	1-2,000	9	60	80
	1-4,000	41	Too many	Too many
Roccal	1-100	0	6	0
	1-500	2	51	0
	1-1,000	8	58	1
	1-2,500	24	58	Too many
	1-5,000	85	Too many	Too many
Trioxymethylene	1-10	5	0	0
	1-20	0	0	0
	1-100	0	0	0
	1-500	0	0	0
	1-1,000	0	22	0

Experiment 2 (Table II, Fig. 4). Effect on Mold Colony Growth When Agent Is Incorporated Into the Medium.—Plates were exposed by three methods: One series of plates was exposed for ten minutes in a hospital room in which a high concentration of mold spores was liberated; another series of plates was streaked with loops containing washings from heavy fungus cultures; to the last group of plates was added 1 c.c. of a suspension of molds containing approximately 50,000 spores. A total of 130 plates was thus used. The results are seen in Table II and Fig. 2. Mold growth is completely uninhibited in the controls. In the dilutions employed, trioxymethylene is most effective. Generally speaking, the more concentrated the fungicidal agent, the greater the inhibition of growth.

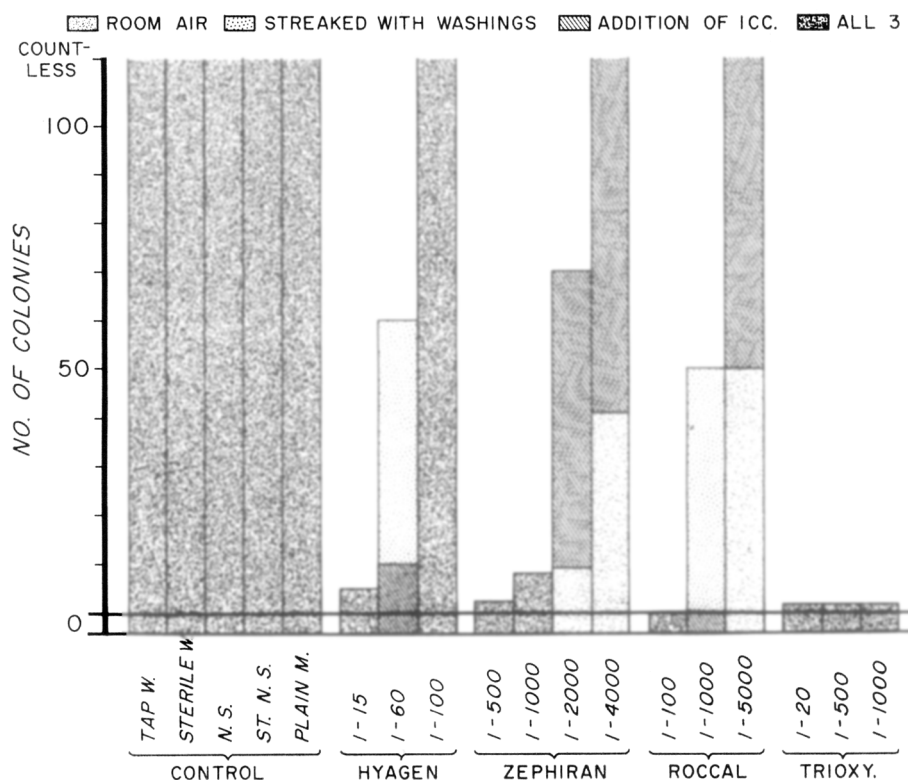


Fig. 4.—Effect on colony growth when agent is incorporated into medium. (One hundred thirty plates.)

Experiment 3 (Table III, Fig. 5). Effect on Mold Colony Growth When Agents Are Sprayed Into Hospital Rooms.—The doors and windows of these rooms remained closed. All plates were exposed for variable periods of time, as indicated in Table III. Hyagen had no effect. Roccal and trioxymethylene were about equally effective in interfering with mold growth. The count was definitely lower in instances of prolonged plate exposure, since there was thus allowed a longer period of time for fungicidal action. Zephiran was not

TABLE III. EXPERIMENT 3.
EFFECT ON COLONY GROWTH WHEN AGENTS SPRAYED INTO ROOM (28 PLATES)

AGENT	DILUTION	PLATE EXPOSURE	GROWTH IN TWO WEEKS
Control		10 minutes	Too many
Hyagen	1-15	10 minutes	Too many
		3 hours	Too many
		24 hours	Too many
		24 hours	Too many
Roccal	1-500	10 minutes	25
		3 hours	34
		24 hours	1
		24 hours (1 day late)*	1
Trioxymethylene	1-500	10 minutes	35
		3 hours	44
		24 hours	8
		24 hours (1 day late)*	2

*This plate was exposed for twenty-four hours one day after the room was sprayed.

employed, since its cost was prohibitive for experiment. The odor of formaldehyde was noticeable only at the time the room was sprayed. A total of twenty-eight plates were exposed in this experiment.

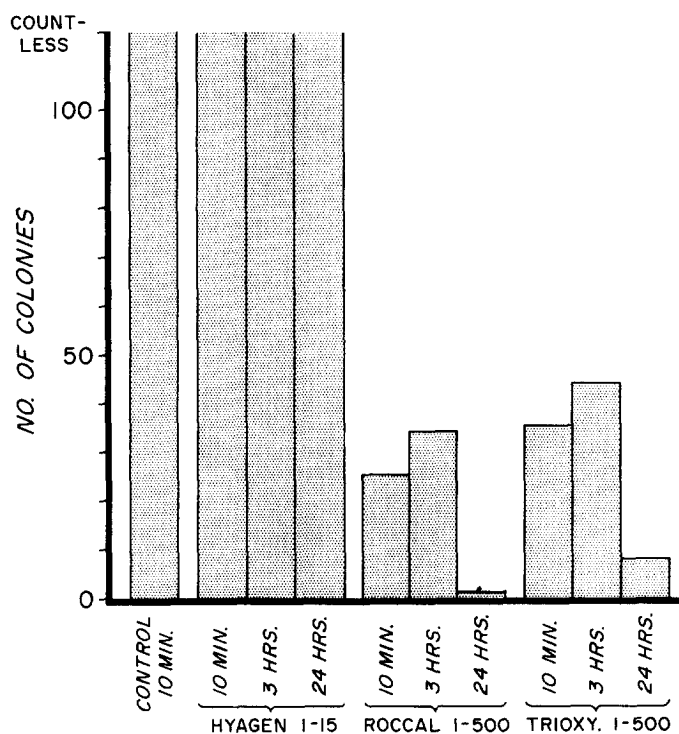


Fig. 5.—Effect on colony growth when agents are sprayed into room. (Twenty-eight plates.)

Experiment 4 (Table IV). Effect of Trioxymethylene on Mold Colony Growth When Agent Is Employed in Hospital Rooms.—Control plates were exposed as usual before trioxymethylene was used. At this point, an open jar containing trioxymethylene was placed on the floor of the closet in a hospital

room and the door was closed. The contained air space was continuous with the room air space by a louvre in the door measuring twelve inches by eighteen inches. Windows and doors were kept closed and a period of forty-eight hours was allowed for equilibrium to become established. Trioxymethylene was thus volatilized into room air. The odor of formaldehyde was detectable in the cupboard but not in the room proper with the doors and windows closed. Plates were exposed at the end of forty-eight hours when 2 of the 10 grams of trioxymethylene had sublimed. The experiment was then repeated with the doors and windows open. No attempt was made in this experiment to introduce molds into the room as was done in previous experiments. Hence, the total count is lower.

TABLE IV. EXPERIMENT 4.
EFFECT OF TRIOXYMETHYLENE POWDER ON MOLD COLONY GROWTH WHEN AGENT IS EXPOSED IN HOSPITAL ROOM (36 PLATES)

	NUMBER OF COLONIES	
	CUPBOARD	ROOM
Control windows closed	28	15
after Trioxymethylene	4	2
Control windows open	46	11
after Trioxymethylene	30	6

However, it is the relative count obtained before and after the use of trioxymethylene that is significant. The results are indicated in Table IV. Note the drop in colony count, both in the cupboard and in the rooms, with doors and windows both open and closed following the use of this agent. In the two days in which 2 grams of trioxymethylene had volatilized, the agent was eighty per cent effective in reducing the mold colony count. In this concentration, no irritating formaldehyde odor was present. No attempt was made to determine its concentration in the room air, although such techniques are available.

TABLE V. MOLD COLONIES FOUND TEN DAYS AFTER PLATING DILUTIONS OF HOUSE DUST (26 PLATES)

DILUTION	HOUSE 1	HOUSE 2
1-100	Too many to count	Too many to count
1-200	43	37
1-500	37	48
1-1,000	45	30
1-10,000	8	2

Experiment 5 (Table V, Fig. 6). Mold Content of House Dust.—This experiment was designed to indicate the mold content of the house dust in the homes of two patients (Cases 1 and 2). This house dust was diluted with sterile distilled water. As might be expected, small quantities of house dust thus obtained show a fairly high mold content indicating the necessity of reduction and avoidance of house dust. We plated 0.1 c.c. of each dilution.

Experiment 6 (Table VI, Fig. 7). The Effect of Trioxymethylene on Mold Colony Growth in Patients' Homes.—In each of these homes there was presented ample evidence of a high mold content. Hyagen was not employed in this

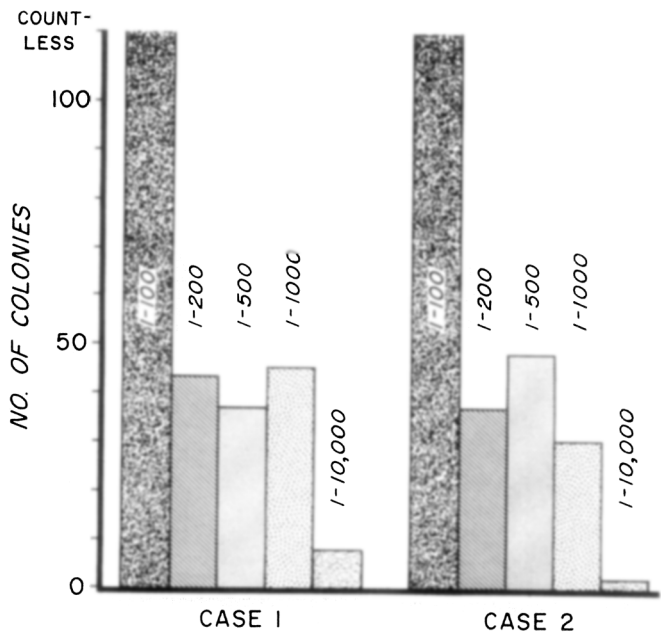


Fig. 6.—Mold colonies found ten days after plating dilutions of house dust. (Twenty-six plates.)

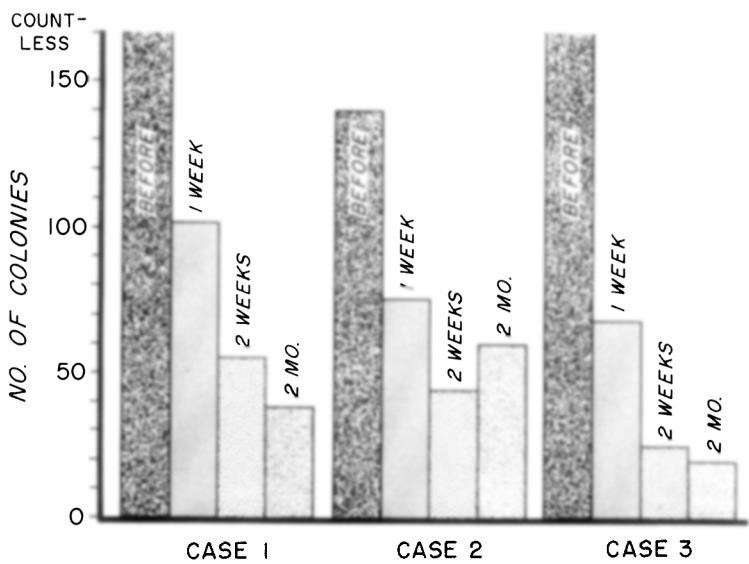


Fig. 7.—Effect of trioxymethylene on mold colony growth in basement of patient's home. (Thirty-five plates.)

experiment because its fungicidal effectiveness was doubtful in light of the results obtained in our previous experiments. Roccal, while inexpensive, has the disadvantage that its use involves spraying of home furnishings and possible food contamination. On the other hand, trioxymethylene is not toxic, is easily employed, and circulates throughout the house by natural air currents. Approximately 10 grams of the powder was placed in an open jar and located in closed storage cabinets, basement rooms, attics, and in at least one other room in the house. Multiple Petri dishes were exposed both before and at various intervals after the introduction of trioxymethylene into the house. The results are indicated in Table VI and Fig. 6. A total of thirty-five Petri dishes was employed. The molds identified as growing in homes under investigation were, in order of frequency, as follows: *Aspergillus* and *Penicillium* in the "mildew," *Alternaria*, *Hormodendrum*, *Cladosporium*, *Helminthosporium*, and *Chaetomium*.

TABLE VI. EFFECT OF TRIOXYMETHYLENE ON MOLD COLONY GROWTH IN PATIENT'S HOME (35 PLATES)

TRIOXYMETHYLENE USED	HOUSE (CASE 1)			HOUSE (CASE 2)			HOUSE (CASE 3)		
	BASE- MENT	UP- STAIRS	CLOSET	BASE- MENT	UP- STAIRS	CLOSET	BASE- MENT	UP- STAIRS	CLOSET
Before	Too many	128	134	140	139	100+	Too many	92	Too many
1 week after (Sept.)	102	60	22	76	90	44	68	34	32
2 weeks after (Sept.)	55	24	10	44	32	16	24	28	40
2 months after (Dec.)	38	28	18	60	38	14	20	30	12

In the basement and in basement closets in Homes 1 and 3, before the use of fungicidal agents, there were too many mold colonies to count. There was a considerable "mildew" evident in both of these locations. After one week of exposure to trioxymethylene, there was a tremendous drop in the count in all areas. By that time about one-half of the powder placed in open jars had sublimed. There was a faint odor of formaldehyde in the basement room as well as a strong odor in the closed closets. Weeks and again two months later the count was still very low and there was no "mildew" present. The count in a dry, clean home of similar size but located higher and away from the river was considerably lower without the use of any fungicidal agents. The outdoor count in the same areas as the location of the three homes under discussion was too high to count. As stated elsewhere, following a reduction in mold content in these homes, there occurred a definite clinical improvement in the condition of the patients. One of the patients (Case 3) could not walk into the basement room because the odor of formaldehyde, while barely perceptible, was too irritating to the patient's nasal mucous membrane.

SUMMARY AND CONCLUSIONS

What are the conclusions that can be drawn from this study? The clinical course of respiratory allergy, bronchial asthma, and perennial allergic rhinitis in mold-sensitive patients is affected, as one might expect, by exposure to fungus

spores. The concentration of these spores is high in old, damp homes. A good fungicidal agent is one which must meet several requirements: it must be easily applied to living quarters; it must not be toxic; it should not be a severe respiratory irritant; it must be relatively inexpensive; and, finally, it must be demonstrated that the product is effective in abolishing "mildew" and in reducing the mold colonies obtained by proper Petri dish exposure, thus bringing about clinical improvement.

A total of 337 plates was exposed under the different conditions related in this article. Of the three fungicidal agents employed, Roccal and trioxymethylene are found to be satisfactory. Both are relatively inexpensive and commercially available. Roccal, however, must be introduced by spraying. This is easy enough in a basement room, but it offers the possibility of food contamination and may affect house furnishings. Trioxymethylene is more easily employed but, if used in large enough quantities, may produce an irritating odor. Under the conditions of our experiments, it proves quite effective in reducing the mold content of the home and is a contributory factor in the clinical improvement of mold-sensitive patients.

REFERENCES

1. Bernston, H. S.: Asthma Due to Mold—*Aspergillus Fumigatus*, *J. A. M. A.* **95**: 189, 1930.
2. Hopkins, J. G., Benham, R. W., and Kesten, B. M.: Asthma Due to Fungus—*Alternaria*, *J. A. M. A.* **94**: 6, 1930.
3. Morrow, M. B.: Mold Fungi in the Etiology of Respiratory Allergic Diseases. VII. Further Survey Studies, *Ann. Allergy* **5**: 442, 1947.
4. Prince, H. E., Arbesman, C. E., Sellers, E. D., Petit, P. T., Brown, E. A., and Morrow, M. B.: Mold Fungi in the Etiology of Respiratory Allergic Diseases. XII. Further Studies With Mold Extracts, *Ann. Allergy* **7**: 597, 1949.
5. Prince, H. E., Epstein, S., Figley, K. D., Wittich, F. W., Dell, H. L., and Morrow, W. B.: Mold Fungi in the Etiology of Respiratory Allergic Diseases. IX. Further Studies With Mold Extracts, *Ann. Allergy* **7**: 301, 1949.
6. Credill, B. A.: Report of a Case of Bronchial Asthma Due to Molds, *J. Michigan M. Soc.* **32**: 167, 1933.
7. Morrow, M. B., and Wheeler, E. C.: Mold Fungi in the Etiology of Respiratory Allergic Diseases. XIV. Fungi in the Aerobiological Populations; The Fungus Flora of *Tillandsia* Species (Ball and Spanish Moss), *Ann. Allergy* **8**: 761, 1950.
8. Prince, H. E., and Morrow, M. B.: Mold Fungi in the Etiology of Respiratory Allergic Diseases. XV. Selection of Molds for Therapy, *Ann. Allergy* **12**: 253-261, 1954.
9. Hyde, H. A., Richards, M., and Williams, D. A.: Allergy to Mold Spores in Britain, *Brit. M. J.* **1**: 886-890, 1956.
10. Bernstein, T. B., and Feinberg, S. M.: Air-Borne Fungus Spores: A Five-Year Survey of Daily Mold Spore Content of Chicago Air, *J. ALLERGY* **13**: 231, 1942.
11. Alvarez, G. C., and Castro, G. F.: Quantitative Studies of Air-Borne Fungi of Havana in Each of the Twenty-four Hours of the Day, *J. ALLERGY* **23**: 259, 1952.
12. Harsh, G. F., and Allen, S. E.: A Study of Fungus Contaminants of the Air of San Diego and Vicinity, *J. ALLERGY* **16**: 125, 1945.
13. Kaplan, L.: Survey of Air-Borne Fungus Spores of the Boston Area in Relation to Inhalant Allergens, *Ann. Allergy* **10**: 109, 1952.
14. Schaffer, N., Seidmon, E., and Bruskin, S.: The Clinical Evaluation of Air-Borne and House Dust Fungi in New Jersey, *J. ALLERGY* **24**: 348-354, 1953.
15. Wallace, M. E., Weaver, R. H., and Scherago, M.: A Weekly Mold Survey of Air and Dust in Lexington, Kentucky, *Ann. Allergy* **8**: 202-211, 1950.
16. Richards, M.: Atmospheric Mold Spores In and Out of Doors, *J. ALLERGY* **25**: 429-439, 1954.
17. Prince, H. E., and Morrow, M. B.: Molds in the Etiology of Asthma and Hay Fever With Special Reference to the Coastal Area of Texas, South. M. J. **30**: 754, 1937.
18. Maunsell, K.: Respiratory Allergy to Fungus Spores, in *Progress in Allergy*, Vol. 4, Basel, 1954, S. Karger, pp. 457-527.

19. Nilsby, I.: Allergi framkallad av mögesporer, Svenska läk.-tidning **44**: 941, 1947.
20. Nilsby, I.: Allergy to Molds in Sweden; A Botanical and Clinical Study, *Acta allergol. Kbh.* **2**: 57, 1949.
21. Rennerfelt, E.: Nagra Undersokningar over Luftens Halt av Svampsporer, Svenska Bot. Tidskr. **41**: 283, 1947.
22. Swaebly, M. A., and Christensen, C. M.: Molds in House Dust, Furniture Stuffing, and in the Air Within Homes, *J. ALLERGY* **23**: 370, 1952.
23. Saito, K.: Untersuchungen über die atmosphärischen Pilzkeime. I. Mittheilung, *J. Coll. Sci. Tokyo* **18**: 58, 1904.
24. Rostrup, O.: Nogle Undersogelser over Luftens af Svampekim, Bot. Tidskr. **29**: 32, 1908.
25. Flensburg, E. W., and Samsøe-Jensen, T.: Studies in Mold Allergy; Mold Spore Counts in Copenhagen, *Acta allergol. Kbh.* **3**: 49, 1950.
26. Merksamer, D.: Mold Allergy, *New York J. Med.* **57**: 115, 1957.
27. Brown, G. T.: Sensitization to Fungi, *J. ALLERGY* **3**: 522, 1932.
28. Christensen, C. M.: Intramural Dissemination of Spores of Hormodendrum, *J. ALLERGY* **21**: 409, 1950.
29. Morrow, H. B., and Thiers, H. D.: Mold Fungi in the Etiology of Respiratory Allergic Disease. XI. Phytopathogenic Fungi in Aerobiological Populations, *Ann. Allergy* **7**: 592, 1949.
30. Prince, H. E.: Miscellaneous Inhalants and Molds, *Ann. Allergy* **9**: 575-584, 1951.
31. Criepp, L. H., and Green, M. A.: Air Cleaning as an Aid in the Treatment of Hay Fever and Bronchial Asthma, *J. ALLERGY* **7**: 120, 1935.
32. Rooks, R., Caplan, R. M., and Shapiro, R. S.: Air-Borne Fungus Spore Removal From Indoor Air by Mechanical Filters and Air Conditioners of Sampling Tests, *J. ALLERGY* **27**: 32, 1956.
33. Baskin, A. D., and Kaplan, A. M.: Mildew Resistance of Vinyl Coated Fabrics Determined by Mixed Spore Culture and Soil Burial Method, *Appl. Microb.* **4**: 288-293, 1956.
34. Marsh, P. B., Butler, M. L., and Clark, B. S.: Fungicidal Activity of Bisphenol, *Indust. & Engin. Chem.* **41**: 2176-2184, 1949.
35. Butler, M. L., and Marsh, B.: Fungicidal Activity of Bisphenols as Mildew Preventives on Cotton Fabric, *Indust. & Engin. Chem.* **38**: 701-705, 1946.
36. Corey, R. R., and Shirk, H. G.: The Influence of Chemical Structure on Fungal Activity. IV. The Effect of Bisphenolic-Type Compounds, *Arch. Biochem.* **56**: 196-203, 1955.
37. Huitric, A. C., Pratt, R., Okano, Y., and Kumler, W. D.: Antifungal Activity of B-Nitrostyrenes and Some Cyclohexane Derivatives, *Antibiotics & Chemother.* **6**: 290-293, 1956.
38. Volini, M., Stubbs, R. K., and Ereoli, N.: Trypanocidal Antibacterial and Antifungal Effectiveness of Monothiuronium Derivatives, *Antibiotics & Chemother.* **6**: 603-606, 1956.
39. Bocobo, F. C., Curtis, A. C., Block, W. D., and Harrell, E. R.: In Vitro Study of Antifungal Activity of Nitrostyrenes, *Proc. Soc. Exper. Biol. & Med.* **85**: 220-222, 1954.
40. Knight, S. G.: The In Vitro Antifungal Activity of Triacetin, *Antibiotics & Chemother.* **7**: 172-174, 1957.
41. Topps, J. H., and Wain, R. L.: Fungistatic Properties of Leaf Exudates, *Nature, London* **179**: 652-653, 1957.
42. Spencer, D. M., Topps, J. H., and Wain, R. L.: Fungistatic Properties of Plant Tissues, *Nature, London* **179**: 651-652, 1957.
43. Byrde, R. J. W., Martin, J. T., and Nicholas, D. J. D.: Effect of Fungicides on Fungus Enzymes, *Nature, London* **178**: 638-639, 1956.
44. Kalb, C. H.: Molds as Inhalant Allergens, *Ann. Allergy* **8**: 695-697, 1950.
45. Wallace, G.: Report on Second Annual Swimming Pool Conference, June 4-5, 1940, State Department of Public Health, Division of Sanitary Engineering, University of Illinois.
46. Dunn, C. G.: Mixture of High Molecular Alkyl-Dimethyl-Benzyl Ammonium Chlorides as Antiseptic, *Proc. Soc. Exper. Biol. & Med.* **35**: 427-429, 1936.
47. Dunn, C. G.: Antiseptic and Germicidal Properties of Mixture of High Molecular Alkyl-Dimethyl-Benzylammonium Chlorides, *Am. J. Hyg.* **26**: 46-52, 1937.
48. Dunn, C. G.: Fungicidal Properties of Alkyl-Dimethyl-Benzyl Ammonium Chlorides, *Proc. Soc. Exper. Biol. & Med.* **37**: 661-663, 1938.
49. Dunn, C. G.: A Comparative Study of Some Antiseptic and Germicides With Special Reference to Alkyl-Dimethyl-Benzyl Ammonium Chlorides, *Am. J. Surg.* **41**: 268-271, 1938.