*In this 2-year followup of a survey performed in an epidemic area of North American blastomycosis it was found that blastomycin skin sensitivity may be retained for at least 2 years in the absence of histoplasmin sensitivity or evidence of clinical blastomycosis.*

# Followup of Blastomycin Sensitivity in an Epidemic Area

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TO GAIN more information about sensitivity to the blastomycin skin test and its relation to reactivity to the complement fixation test, a second survey in the area of Grifton in Pitt County, N. C., was conducted on May 21, 1956. Covering a sample of the persons found positive to the blastomycin skin test or the blastomycosis complement fixation test in the first survey in April 1954, it provides data on conversions and reversions.

The survey in April 1954 was instituted because of an epidemic of North American blastomycosis affecting 11 patients during the winter of 1953–54 in Grifton (*1*). It included 70-mm. chest X-rays, tuberculin, histoplasmin, and blastomycin skin tests, and blastomycosis complement fixation tests. No cases of blastomycosis or histoplasmosis were discovered, although one case of active pulmonary tuberculosis was found.

Of the 1,648 persons surveyed in 1954, 2.9 percent were blastomycin sensitive and 6.4 percent were histoplasmin sensitive. There was no correlation of the blastomycosis complement fixation test with blastomycin skin sensitivity. Of the patients with positive blastomycin skin reactions, 4.7 percent had positive blastomycosis complement fixation tests, as opposed to 2.8 percent positive reactors in the total population tested, but 30.2 percent of the individuals with positive histoplasmin skin tests also had positive blastomycosis complement fixation tests, as opposed to only 6.4 percent in the total population.

These findings strongly suggested cross-reactions between the histoplasmin skin test and the blastomycosis complement fixation test. Unfortunately, histoplasmosis complement fixation tests were not done; it was therefore not possible to test the hypothesis that the correlation between the two might be explained by cross-reactions between the histoplasmosis and the blastomycosis complement fixation tests. On the basis of evidence from the 1954 survey it was believed that the sensitivity to both histoplasmin and blastomycin antigens in some

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individuals might be explained partly by a common mode of infection rather than a cross-reaction (*2*).

Since the 1954 survey, 5 more cases of blastomycosis have been diagnosed in the same county, and 4 of these patients lived within a 4-mile radius of Grifton at the time of onset of disease. (None of the 5 patients, however, had been included in the 1954 survey.) Of the 4 cases occurring in the Grifton area, 3 had onset of pulmonary disease during the fall and winter of 1954–55, and the fourth patient noted a nodular lesion on the left leg in mid-January 1956. The diagnosis of blastomycosis was established by smear and culture in all of these patients, and all were treated satisfactorily with 2-hydroxystilbamidine.

The occurrence of these cases, as well as the desire to learn more about blastomycin and histoplasmin skin test sensitivity, prompted the second survey.

## Method of Study

Because of limitations of time and personnel, as well as the subsidence of the community interest and hysteria engendered by the 1953–54 epidemic, it was decided that a maximum of information could be obtained by skin testing again all individuals who had had positive blastomycin skin tests or positive blastomycosis complement fixation tests in the 1954 survey. In the 1954 survey, 42 individuals given a blastomycin skin test had induration of 5 mm. or more at 48 hours, and 43 individuals, a complement fixation titer of 1:1 or more to *Blastomyces*. Since 2 individuals had both a positive skin test and complement fixation test, the total was 83. Letters were sent to this group requesting that they return for reexamination.

Of the 83 positive reactors in 1954, 43 reported for reexamination. These 43 constituted an excellent and presumably random sample of the 1954 group. Twenty-three of the 42 with positive blastomycin skin tests (54.7 percent) and 22 of the 43 with positive blastomycosis complement fixation tests, including the 2 individuals with both positive skin tests and positive complement fixation tests, composed the 1956 group. The 43 individuals ranged in age from 3 to 65 years, and 23 of them were under age 20.

One-tenth milliliter of each of the two antigens was injected intradermally. Blastomycin antigen was placed in the right forearm, and histoplasmin in the left forearm, both injections at approximately the same level. New syringes and needles were used for each antigen. In no instance was a syringe or needle re-used during this survey.

The histoplasmin (lot H–42) was diluted 1:100. The blastomycin was a *Blastomyces* vaccine prepared from 6-day yeast phase cultures grown on brain-heart infusion blood agar slants at 37° C. The yeast cells were suspended in saline and heat-killed at 56° C. for 2 hours; the vaccine was diluted 1:1,000 by volume in a Hopkins tube before use. The vaccine dilution, although not strictly an extract, is referred to as blastomycin in this paper. Both of these antigens were supplied by Dr. Norman F. Conant, of Duke University.

At the time the skin tests were read, the individuals surveyed were interrogated about the state of their health. All skin tests were read 48 hours after injection of the antigens by measuring the diameter of areas of erythema and induration with a millimeter ruler. The tests were considered positive when induration was 5 mm. or more and doubtful when erythema was 10 mm. or more.

All 43 participants contributed blood for blastomycosis and histoplasmosis complement fixation tests. These tests were performed at the Communicable Disease Center of the Public Health Service in Chamblee, Ga., through the cooperation of Dr. Kenneth W. Walls.

The *Histoplasma* antigen used for the complement fixation tests was histoplasmin prepared from single strains of mycelial *Histoplasma capsulatum* grown on C. E. Smith's culture medium for the preparation of coccidioidin, incubated at 25° C. for 6 months (*3*). The cultures were killed with merthiolate, Seitz filtered, and then pooled.

The blastomycosis antigen used for the complement fixation test was prepared from one isolate of *Blastomyces dermatitidis* grown in brain-heart infusion agar at 37° C. for 5 days. The cells from this yeast-phase growth were washed from the agar, killed with merthiolate,

centrifuged and washed. A 20 percent suspension of cells was ground with sand (50 percent weight/volume) and centrifuged, to remove the particulate matter. The supernatant fluid was used for the antigen. This was the same type antigen used in the complement fixation tests performed in the earlier study (*2*). The complement fixations were titered by the usual "box" titration.

## Results

None of the 43 persons surveyed in 1956 gave a history of pulmonary disease, and none of the histoplasmosis complement fixation tests were reactive. The reactivity of the group to the blastomycin and histoplasmin skin tests and to the blastomycosis complement fixation test is shown in table 1. The changes in reaction from 1954 to 1956 are summarized in table 2.

**Table 1. Reactions to blastomycin and histoplasmin skin tests and to blastomycosis complement fixation test, Grifton, N. C., 1954 and 1956**

| Reaction | 1954 | 1956 |
|---|---|---|
| Blastomycin skin test: | | |
| Positive | 23 | 15 |
| Negative | 20 | 28 |
| Histoplasmin skin test: | | |
| Positive | 9 | 15 |
| Negative | 29 | 28 |
| Not done | 5 | 0 |
| Blastomycosis CF test: | | |
| Positive | 22 | 4 |
| Negative | 17 | 39 |
| Not done | 4 | 0 |

**Table 2. Changes in blastomycin and histoplasmin skin tests and blastomycosis complement fixation test over 2-year period (1954–56), Grifton, N. C.**

| Items | Skin test | | CF test |
|---|---|---|---|
| | Blastomycin | Histoplasmin | |
| Maintenance of reactivity | 15 | 6 | 2 |
| Conversion to positive test | 0 | 7 | 2 |
| Reversion to negative test | 8 | 3 | 20 |
| No reactivity | 20 | 22 | 15 |
| Total tested in both surveys | 43 | 38 | 39 |

**Table 3. Correlation of blastomycin and histoplasmin skin tests with each other and each with blastomycosis complement fixation test in the same individual, Grifton, N. C., 1954 and 1956**

| Test correlations | 1954 | 1956 |
|---|---|---|
| Blastomycin and histoplasmin: | | |
| Both positive | 6 | 4 |
| Blastomycin only positive | 14 | 11 |
| Histoplasmin only positive | 3 | 11 |
| Both negative | 15 | 17 |
| Blastomycin with blastomycosis CF test: | | |
| Both positive | 2 | 1 |
| Blastomycin only positive | 17 | 14 |
| Blastomycosis CF only positive | 20 | 3 |
| Both negative | [1] 0 | 25 |
| Histoplasmin with blastomycosis CF test: | | |
| Both positive | 5 | 1 |
| Histoplasmin only positive | 3 | 14 |
| Blastomycosis CF only positive | 15 | 3 |
| Both negative | 11 | 25 |

[1] No individuals in this group because of selection of persons for 1956 survey.

NOTE: Only persons tested to both agents included.

Many of the persons with positive blastomycin (15 of 23, or 65.2 percent) and histoplasmin (6 of 10, or 60 percent) skin tests remained positive although reversions to negative occurred in both groups. Two of the three individuals whose histoplasmin skin tests reverted to negative had positive reactions to both histoplasmin and blastomycin in 1954. One of these two, a 3-year-old white girl, had an induration of 5 mm. to both antigens in 1954 and no induration to either in 1956. The other, a 9-year-old white boy, had 12-mm. induration with 40-mm. erythema to blastomycin and 15-mm. induration with 20-mm. erythema to histoplasmin in the first survey. Two years later the blastomycin reaction had 5-mm. induration with 11-mm. erythema, but the histoplasmin produced no erythema or induration. The third of the histoplasmin reverters was a 33-year-old woman. She had a negative blastomycin test in both surveys with 12-mm. induration to histoplasmin in 1954 and 4-mm. induration 2 years later.

Of the 8 persons whose blastomycin skin test reverted to negative, 2 maintained reactivity to histoplasmin, 3 showed no reactivity to histoplasmin, 1 was not tested with histoplasmin

at the time of the first survey and was nonreactive to both tests in the second survey, 1 developed histoplasmin reactivity, and 1 lost reactivity to both antigens as mentioned above. Of the 6 individuals with reactivity to both antigens, 2 were still reactive 2 years later.

Twenty of the 22 (91 percent) serums previously reactive to the blastomycosis complement fixation test were nonreactive in 1956. Seven conversions to a positive reaction to the histoplasmin skin test and two conversions to the blastomycosis complement fixation test were observed, but none to the blastomycin skin test. Three strengths of histoplasmin were used in 1954, and 5 of the 7 histoplasmin converters were in the group tested with the weakest histoplasmin (H–42, 1:1,000).

Table 3 shows the correlations of the two skin tests with each other and with the blastomycosis complement fixation test. All the possible combinations of positive and negative reactions to these three tests occurred.

## Discussion

There were no cross reactions between the histoplasmosis and blastomycosis complement fixation tests; however, only 4 individuals had reactive blastomycosis complement fixation tests in the second survey. In the absence of clinical blastomycosis, a positive blastomycin skin test was maintained for as long as 2 years. Reversion to negative occurred in a considerable number of individuals, 8 of 23, or 34.8 percent. Reactivity to the blastomycosis complement fixation test was maintained 2 years in 2 individuals, and conversions of the blastomycosis complement fixation from nonreactive to reactive were observed.

The conversions of the histoplasmin skin test from negative to positive were not unexpected in this endemic area (*4*). The reversion from positive to negative in 1 individual may be explained by cross-reaction to the blastomycin skin test; in the other 2, by loss of sensitivity. These reversions are not unique, such a change having been reported in the past (*5*, *6*). Reversion occurs also with the coccidioidin test in coccidioidomycosis (*3*) and the tuberculin test in tuberculosis (*7*).

A positive intradermal reaction to blastomycin with a negative reaction to histoplasmin can occur, and although cross-reactions undoubtedly exist, the possibility that positive reactions to both antigens indicate dual infection, apparent or subclinical, must be considered. This is suggested because over a period of 2 years, of the 6 persons with dual reactivity, 1 lost histoplasmin reactivity retaining blastomycin sensitivity, 2 lost blastomycin reactivity retaining histoplasmin sensitivity, 2 maintained reactivity to both antigens, and 1 lost sensitivity to both. Individuals who have positive intradermal reactions to both antigens and maintain this reactivity over long periods of time may represent examples of subclinical dual infection, whereas those who lose reactivity to one antigen only may represent cross-reactions. Cross-reactivity could also be invoked to explain these changes in sensitivity; however, the occurrence of dual infections in man (*8*) and dogs (*9*) would suggest that this may not always be the case. Changes in the size of the reaction to the two antigens with time also may prove to be useful in differentiating the cross-reaction from inapparent or apparent infection.

Correlation of the results of all the tests in each individual shows that all the possible combinations of positive and negative blastomycin skin tests with reactive and nonreactive complement fixation tests may occur in persons who do not have clinical blastomycosis but who live in a blastomycosis epidemic area.


## Summary

To examine further the reactivity of healthy individuals in a blastomycosis epidemic area to the blastomycin and histoplasmin skin tests, a second survey was conducted in the area of Grifton in Pitt County, N. C., in May 1956. It included 43 of the 83 persons who had had positive blastomycin skin tests or positive blastomycosis complement fixation tests in a survey in April 1954.

In the 2-year period, 34.8 percent of the previously positive blastomycin skin test reactors had reverted to negative, and 91 percent with previously positive complement fixation to blastomycosis were now negative. No conversions to positive blastomycin skin tests were found in the selected group included in this

survey, but two individuals previously negative to the blastomycosis complement fixation test were found to be positive.

Both skin sensitivity to blastomycin and blastomycosis complement-fixing antibodies tended to decrease in the 2-year period, but the complement-fixing antibodies disappeared in a higher percentage of the individuals tested.

Seven persons became reactive to the histoplasmin skin test and three lost their reactivity. The loss of reactivity in one instance might possibly represent cross-reactivity to the blastomycin skin test. None of the group surveyed had a positive histoplasmosis complement fixation test.

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## Training in the Care of Prematures

The tenth year of the institutes for physicians and nurses in the care of premature infants will begin in the fall of 1958. The institutes are sponsored by the New York State Department of Health and the U. S. Children's Bureau.

The training is planned to meet the needs of physicians and nurses in charge of hospital premature nurseries and premature centers, and medical and nursing directors and consultants in State and local programs.

Attendance at each institute is limited to six physician-nurse terms. For physicians, the program lasts 2 weeks, and for nurses, 4 weeks. There is no tuition fee, and stipends are provided to help cover expenses during attendance. The institutes are scheduled to begin September 22, 1958; November 3, 1958; January 12, 1959; February 23, 1959; and April 20, 1959.

For additional information, write to Box 143, Institute in the Care of Premature Infants, New York Hospital, 525 East 68th Street, New York 21, N. Y.