

North American Blastomycosis in an Epidemic Area

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AFTER 10 patients with North American blastomycosis, all from the same area in Pitt County, N. C., were admitted within a month and a half to Duke Hospital, we thought a survey for blastomycosis would be desirable.

Since the epidemic included a child as young as 7 years and a man aged 77 (1), plans were made to examine all persons within a 4-mile radius from the center of Grifton in Pitt County. About 750 were expected. Knowledge about the epidemic became so widespread and community interest so intense, however, that 1,648 people appeared. Many came from nearby areas. The survey was conducted during the week of April 12-16, 1954.

Methods of Study

The survey included skin tests with *Blastomyces* vaccine, histoplasmin, and old tuberculin; complement fixation (CF) tests of blood specimens; and 70-mm. chest X-rays. Children

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under 6 years were not X-rayed because of the technical difficulty in taking satisfactory pictures, but children in that age group were skin tested. When feasible, their blood samples were obtained also.

The *Blastomyces* vaccine and histoplasmin tests were placed on the right forearm at widely separated points. The tuberculin tests were made on the left forearm.

A 0.1 ml. portion of each of the 3 antigens was injected intracutaneously. In no instance were different antigens used in the same syringe. All syringes were new and were marked with a diamond point to indicate which antigen was to be used. After initial use each syringe was resterilized with other syringes used for the same antigen. The syringe was autoclaved before it was refilled.

The skin tests were read by measuring the diameter of areas of erythema and induration with a millimeter ruler. Skin tests were considered positive when induration was 5 mm. or more, or doubtful when erythema was 10 mm. or more and induration less than 5 mm. All other results were considered negative.

Because of the unanticipated response to the survey, it was necessary to use 3 lots of histoplasmin. Patients (group 1) numbered 1 to 650 were tested with lot HKC-5 diluted 1:500. Patients (group 2) numbered 651 to 1,200 were tested with lot H-42 diluted 1:100, and patients (group 3) numbered 1,201 to 1,648 were tested with lot H-42 also, but the histoplasmin was diluted 1:1,000.

Lot HKC-5 and lot H-42 of histoplasmin

Table 1. Blastomycin and histoplasmin reactions in entire study population

| Blastomycin (mm. induration) | Histoplasmin (mm. induration) | | | | | | | Total |
|------------------------------|-------------------------------|-----|-----|-------|-------|-----|---------|-------|
| | 0 | 1-4 | 5-9 | 10-14 | 15-19 | 20+ | Unknown | |
| 0 | 809 | 65 | 35 | 10 | | 1 | 65 | 985 |
| 1-4 | 224 | 167 | 10 | 14 | 6 | 1 | 16 | 438 |
| 5-9 | 18 | 3 | 5 | 3 | 2 | | 4 | 35 |
| 10-14 | 4 | 1 | | | 1 | | | 6 |
| 15-19 | | | | | | | | |
| 20+ | | 1 | | | | | | 1 |
| Unknown | 3 | | | | | | 180 | 183 |
| Total | 1,058 | 237 | 50 | 27 | 9 | 2 | 265 | 1,648 |

were supplied by Dr. Michael L. Furcolow, chief of the Kansas City Field Station of the Communicable Disease Center, Public Health Service.

Undiluted old tuberculin (Wyeth, Inc.) was supplied by Dr. William M. Peck of the North Carolina Sanatorium for Tuberculosis at McCain and was diluted 1:1,000 before use.

The *Blastomyces* vaccine was prepared in Dr. Conant's laboratory from 6-day yeast phase cultures grown in brain-heart infusion on blood-agar slants at 37° C. The yeast cells were then killed by suspension for 2 hours in a saline solution heated to a temperature of 56° C. The vaccine dilution, though not strictly an extract, will be referred to hereafter as blastomycin.

CF tests with *Blastomyces* antigen were performed by Dr. Joseph Schubert at the laboratories of the Communicable Disease Center, Public Health Service, Atlanta.

Results of Tests

Logistic difficulties made it impossible to test everyone with each antigen; therefore 1,465 persons were tested with blastomycin, 1,383 with histoplasmin, and 1,325 with tuberculin. Forty-two had positive reactions to blastomycin (2.9 percent), 88 to histoplasmin (6.4 percent), and 340 to tuberculin (25.6 percent.)

The fact that acquired histoplasmin sensitivity in some persons will give a cross reaction to blastomycin is well known (2, 3). On the other hand, the converse situation of a positive blastomycin skin test with a negative histoplasmin skin test has been observed not in-

frequently at Duke Hospital in patients with clinical blastomycosis though similar instances have been reported rarely in the literature (4, 5). Of the 1,380 persons tested with both histoplasmin and blastomycin in the survey, 11 had positive tests with both antigens and 27 had a blastomycin skin test of 5 mm. or more of induration and a histoplasmin test showing 4 mm. or less of induration (table 1). Despite the insignificant reactions to histoplasmin, in 6 of the 27 induration from the blastomycin was larger than 10 mm. Conversely, 77 individuals reacted to histoplasmin but not to blastomycin. Thirty-two of these had more than 10 mm. of induration with histoplasmin. The degree of cross sensitivity must, therefore, be small.

As previously noted, 3 histoplasmin antigens were used in the survey: HKC-5 diluted 1:500, H-42 diluted 1:100, and H-42 diluted 1:1,000 (tables 2, 3, and 4). Since H-42 1:100 and HKC-5 1:500 were considered to give analogous reactions, no comparison of the relative size of induration was made (6). The difference in size of reaction to H-42 1:100 and H-42 1:1,000 might be of considerable magnitude, however.

To test that possibility, 13 persons, most of whom were known to react positively to histoplasmin, were tested with 1:1,000 and 1:100 dilutions of lot H-42. The more concentrated solution was injected into the right forearm of 7 individuals and into the left forearm of 6. Both tests were negative in 3 of the 13 persons, but in 3 others induration measured less than 5 mm. with the 1:1,000 dilution and more than 5 mm. with the 1:100 dilution. The remaining

7 had positive reactions to both antigens, but in general the induration was several millimeters larger at the site of the 1:100 dilution.

Dependence of reactivity on the concentration of the dose was also evident in the 3 test groups. Eighty-eight, or 6.36 percent, of all 1,383 individuals tested with histoplasmin were positive. The percentage of positive reactions in group 1 was 6.69 (41÷613, table 2). The percentage in group 2 was 7.57 (29÷383, table 3) whereas in group 3 it was 4.65 (18÷387, table 4). The lower percentage of positive reaction in group 3 may have resulted from the more diluted antigen of histoplasmin.

Nevertheless, the variable of a more dilute antigen in the 387 tests in group 3 does not influence the preceding conclusions concerning cross reactions since only 8 of the 27 individuals with positive blastomycin and negative histoplasmin reactions were tested with the weaker strength of histoplasmin. Seven of the eight

had no reaction to the histoplasmin. Since all controls having a positive reaction to the more concentrated dose of histoplasmin also had some induration from the weaker material, it is doubtful that the 7 would have shown positive reactions to the 1:100 dilution. The individual who showed less than 4 millimeters of induration from the 1:1,000 dilution might have been definitely positive to histoplasmin if he had been injected with the 1:100 dilution. However, that possibility would not invalidate the general conclusion that individuals can be highly sensitive to blastomycin without demonstrating histoplasmin sensitivity. The frequency of the converse situation (histoplasmin sensitivity and blastomycin negativity) is obvious from the tables.

On the other hand, some degree of association between histoplasmin and blastomycin sensitivity in a population can be shown statistically from probability tables. For example, from

Table 2. Blastomycin and histoplasmin reactions in group 1: histoplasmin lot HKC-5 diluted 1:500

| Blastomycin (mm. induration) | Histoplasmin (mm. induration) | | | | | | | Total |
|------------------------------|-------------------------------|-------|-------|-------|-------|-------|---------|-------|
| | 0 | 1-4 | 5-9 | 10-14 | 15-19 | 20+ | Unknown | |
| 0..... | 272 | 39 | 11 | 6 | ----- | 1 | ----- | 329 |
| 1-4..... | 114 | 137 | 4 | 7 | 6 | 1 | ----- | 269 |
| 5-9..... | 6 | 2 | 3 | 1 | ----- | ----- | ----- | 12 |
| 10-14..... | ----- | 1 | ----- | ----- | 1 | ----- | ----- | 2 |
| 15-19..... | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 20+..... | ----- | 1 | ----- | ----- | ----- | ----- | ----- | 1 |
| Unknown..... | ----- | ----- | ----- | ----- | ----- | ----- | 32 | 32 |
| Total..... | 392 | 180 | 18 | 14 | 7 | 2 | 32 | 645 |

Table 3. Blastomycin and histoplasmin reactions in group 2: histoplasmin lot H-42 diluted 1:100

| Blastomycin (mm. induration) | Histoplasmin (mm. induration) | | | | | | | Total |
|------------------------------|-------------------------------|-------|-------|-------|-------|-------|---------|-------|
| | 0 | 1-4 | 5-9 | 10-14 | 15-19 | 20+ | Unknown | |
| 0..... | 248 | 21 | 15 | 3 | ----- | ----- | 15 | 302 |
| 1-4..... | 53 | 23 | 4 | 4 | ----- | ----- | 2 | 86 |
| 5-9..... | 6 | ----- | ----- | 1 | 2 | ----- | 1 | 10 |
| 10-14..... | 3 | ----- | ----- | ----- | ----- | ----- | ----- | 3 |
| 15-19..... | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| 20+..... | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| Unknown..... | ----- | ----- | ----- | ----- | ----- | ----- | 42 | 42 |
| Total..... | 310 | 44 | 19 | 8 | 2 | ----- | 60 | 443 |

table 5 it can be seen that considerably more persons reacted positively to both antigens than can be explained on a random basis from the number of reactors to each antigen separately. Eleven individuals reacted to both antigens whereas only 2.4 would have been expected in this group. The difference is highly significant statistically (chi square equals 30.0).

This association may be caused not only by a general hypersensitivity of the skin in these individuals or by a similarity in the antigenic components in the two test materials, but also by a common mode of spread of the diseases so that there would be a statistically significant increased probability of exposure to both diseases over the random chance of acquiring the two on independent bases. Of the 11 individuals who reacted to both blastomycin and histoplasmin, 7 had negative tuberculin tests, 3 had positive tuberculin tests, and 1 was not tested for tuberculosis. These figures are similar to those of the general population and do not indicate general hypersensitivity of the skin in these indi-

viduals. The failure of the blastomycin and histoplasmin tests to show cross reactivity in 104 individuals out of the 115 that were positive for either test (table 5) speaks against the presence of strong common antigens while recent reports of simultaneous infections with blastomycosis and histoplasmosis in humans and dogs is strong evidence for the last postulate of a common mode of transmission (7, 8).

The age variation in the percentage of reactors in the study of tuberculin, histoplasmin, and blastomycin is shown in the figure. The high prevalence and rapid increase of tuberculin sensitivity with age is well known for Pitt County. The graph shows a gradual and poorly defined increase in both histoplasmin and blastomycin reactivity with age. The poorly defined histoplasmin reactivity is in marked contrast to the conditions pertaining in the Mississippi Valley areas (9). The lack of correlation between the reactions of tuberculin and of histoplasmin or blastomycin sensitivity is well demonstrated by the chart.

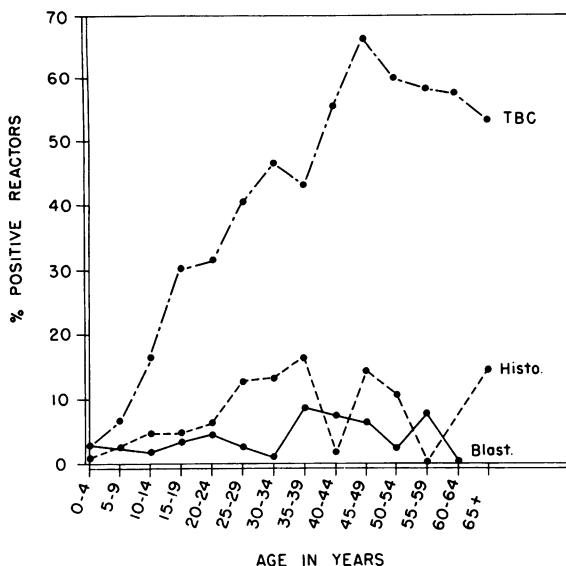
Table 4. Blastomycin and histoplasmin reactions in group 3: histoplasmin lot H-42 diluted 1:1,000

| Blastomycin (mm. induration) | Histoplasmin (mm. induration) | | | | | | | Total |
|------------------------------|-------------------------------|-----|-----|-------|-------|-----|---------|-------|
| | 0 | 1-4 | 5-9 | 10-14 | 15-19 | 20+ | Unknown | |
| 0..... | 289 | 5 | 9 | 1 | | | 50 | 354 |
| 1-4..... | 57 | 7 | 2 | 3 | | | 14 | 83 |
| 5-9..... | 6 | 1 | 2 | 1 | | | 3 | 13 |
| 10-14..... | 1 | | | | | | | 1 |
| 15-19..... | | | | | | | | |
| 20+..... | | | | | | | | |
| Unknown..... | | | | | | | 106 | 109 |
| Total..... | 356 | 13 | 13 | 5 | | | 173 | 560 |

Table 5. Correlation between prevalence of blastomycin and histoplasmin sensitivity

| Blastomycin | Histoplasmin | | | | Total |
|---------------|--------------|----------|----------|----------|-------|
| | Positive | | Negative | | |
| | Observed | Expected | Observed | Expected | |
| Positive..... | 11 | 2.4 | 27 | 35.6 | 38 |
| Negative..... | 77 | 85.6 | 1,265 | 1,256.4 | 1,342 |
| Total..... | 88 | 88.0 | 1,292 | 1,292.0 | 1,380 |

Variation with age in prevalence of positive skin reactions to tuberculin (TBC), histoplasmin (Histo.) and blastomycin (Blast.).



Blastomycosis CF tests were performed on 1,275 serums of the 1,648 individuals in the survey. Twelve serums were anticomplementary, 1,220 were negative, and 43 were positive to the CF tests. The titers ranged from undiluted to 32-fold dilutions. The number of positive skin tests in each group showing different degrees of titer are presented in table 6. Complement fixation showed no obvious correlation with blastomycin or tuberculin skin sensitivity. However, although the percentage of positive tuber-

culin tests and of positive blastomycin tests in the group showing complement fixation is similar to the percentage in the general study population, the percentage of histoplasmin reactors is much greater in the former group (30.2 percent) than in the latter (6.4 percent). Further, the individuals having positive CF titers failed to show a much greater prevalence of positive blastomycin skin sensitivity than did the general population (table 6). The significance and meaning of these observations await clarification.

The survey included 1,465 (70 mm.) fluorophotographs; 1,393 (95.1 percent) were negative, 4 were unsatisfactory, and 68 showed some evidence of pathology involving either the heart or lungs. Forty-six large roentgenograms were taken of suspicious lung lesions. However, although many small scars were noted, only one individual was found to have active pulmonary tuberculosis. This result was most surprising in view of the high percentage of tuberculin reactors (25.6 percent) in the study population. Only 17 of the 72 individuals with suspicious fluorophotographs had positive tuberculin tests, 6 had positive histoplasmin tests, and 6 were positive to blastomycin. No instances of pulmonary blastomycosis or histoplasmosis were uncovered although a 31-year-old male who moved to Grifton one month (March 1954) before the survey developed cough and chest pain shortly thereafter. He did not attend the

Table 6. Relationship between skin sensitivity and Blastomyces complement fixation test

| Complement fixation titer | Persons with positive reactions | | | Persons with negative skin tests | Persons without skin tests | Total |
|---|---------------------------------|--------------|-------------|----------------------------------|----------------------------|-----------|
| | Tuberculin | Histoplasmin | Blastomycin | | | |
| Undiluted..... | 2 | 3 | ----- | 2 | ----- | 7 |
| 1:2..... | 6 | 2 | ----- | 6 | ----- | 14 |
| 1:4..... | ----- | 2 | ----- | 6 | 1 | 9 |
| 1:8..... | 1 | 4 | 1 | 0 | 2 | 8 |
| 1:16..... | 1 | 1 | 1 | 0 | 1 | 4 |
| 1:32..... | ----- | 1 | ----- | 0 | ----- | 1 |
| Total..... | 10 | 13 | 2 | 14 | 4 | 43 |
| Percent of total showing positive skin tests ¹ | 23.3 | 30.2 | 4.7 | ----- | ----- | ----- |
| Percent of total population showing positive skin tests..... | 25.6 | 6.4 | 2.9 | ----- | ----- | ----- |

¹ Percent of total showing positive blastomycin complement fixation who showed a positive skin sensitivity test.

survey because of his recent arrival. In July 1954 he was diagnosed at Duke Hospital as having blastomycosis.

Summary

In an epidemic area in Pitt County, N. C., 1,648 individuals were surveyed for North American blastomycosis by Duke University School of Medicine. Blastomycin skin tests and complement fixation tests were made, and 70-mm. fluorophotographs were taken. Sensitivity to histoplasmin and tuberculin was determined simultaneously.

Of the population, 2.9 percent were sensitive to blastomycin, 6.4 percent were sensitive to histoplasmin, and 25.6 percent had positive reactions to tuberculin. The *Blastomyces* CF test did not correlate with skin sensitivity. No individuals with cutaneous or pulmonary blastomycosis were discovered, and only one individual with active pulmonary tuberculosis was found.

The correlation between blastomycin and histoplasmin sensitivity was carefully studied, but there was little evidence in this survey that antigens common to blastomycin and histoplasmin would explain completely the unexpectedly greater number (11) of individuals who were sensitive to both histoplasmin and blastomycin. The evidence may implicate a common mode of contact with the agents causing sensitivity to these two materials. There was no correlation

between sensitivity to tuberculin and sensitivity to either blastomycin or histoplasmin.

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Dr. Charles-Edward Amory Winslow

February 4, 1877-January 8, 1957

Dr. C.-E. A. Winslow has made an immeasurable contribution; his genius and untiring efforts have placed an indelible mark on the entire world of public health. Many thousands of health workers, like myself, are forever indebted to his teaching, his guidance, and his wise counsel. To the generations that come after us, his wisdom will have an undying influence and stimulus. We have lost a great man whom we have loved, honored, and revered, and whose memory we will always cherish.

—Surgeon General Leroy E. Burney