HISTOPLASMIN AND TUBERCULIN SENSITIVITY IN RELATION TO PULMONARY CALCIFICATIONS AMONG UNIVERSITY OF WISCONSIN STUDENTS *

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In the last few years the problem of the marked variation in the frequency of pulmonary calcifications as observed in chest roentgenograms has been studied by several groups.[2, 3, 4, 5, 6, 7, 9] The highest incidence of pulmonary calcifications has been found in the central eastern half of the United States. These areas were not closely correlated with the areas of highest mortality from tuberculosis. Because a number of reports had demonstrated that a large number of these individuals had negative tuberculin reactions, a search for a non-tuberculous etiology was started. Smith [1] pointed out that the area of high incidence of pulmonary calcifications corresponded with the endemic area of histoplasmosis. Palmer [2] studied the histoplasmin and tuberculin reactions in approximately 3000 student nurses and found that the incidence of histoplasmin reactions was high in the same areas where the incidence of pulmonary calcifications was high. Zwerling and Palmer [5] reported that four times as many student nurses in Kansas City showed pulmonary calcifications as in Philadelphia, and that over four times as many of them were positive to histoplasmin. However, the tuberculin sensitivity was approximately the same in these two student groups. Furculow, High and Allan [6] reported from a study of over 17,000 persons in Kansas City that the frequency of pulmonary calcifications was over twice as high in the reactors to histoplasmin alone as to tuberculin alone.

Zwerling and Palmer [5] concluded that it was impossible to distinguish roentgenographically between the calcifications that occurred in the tuberculin positive and histoplasmin positive individuals. In a later study High et al.[8] reported that disseminated pulmonary calcification in 108 individuals was associated with a positive histoplasmin test in 104 instances and none of these individuals reacted only to tuberculin.

Our interest in the problem of pulmonary calcifications in the tuberculin negative individual was increased by the reports of Palmer and others [2, 3, 4] on the close correlation of histoplasmin sensitivity with pulmonary calcifications. The routine tuberculin testing and photofluorography of the students entering the University of Wisconsin allowed the isolation of a group of stu-

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dents who were tuberculin negative, but whose photofluorograms were interpreted as showing evidence of pulmonary calcifications or infiltrations. In the fall of 1945 each student was tested by the usual intradermal technic using the first and second strength P.P.D. The tests were read at 48 hours and a reaction of 5.0 mm. or greater of edema and redness was recorded as a positive reaction. From approximately 5000 students examined in this manner, a group of 160 was found to be tuberculin negative and to have pulmonary calcification or, much less frequently, infiltration roentgenologically. In April 1946, 116 of these students completed the retesting program which consisted of a tuberculin test using 1.0 mg. O.T. and 0.1 c.c. of a 1 : 1000 dilution of a histoplasmin solution. Both tests were given intradermally and read at 48 hours. A reaction of 5 by 10 mm. induration was required before the test was regarded as positive. The reactions to histoplasmin were clear cut and varied from 10 to 40 mm. in diameter. Of the 116 who completed the test, 67 were histoplasmin positive. None of the tuberculin tests was positive although the intervals between tuberculin tests varied from two to six months.

From these results it was apparent that we were dealing with an unusually high percentage of pulmonary calcifications unassociated with positive tuberculin or histoplasmin reactions. Palmer [2] reported 1.2 per cent calcification in 2141 student nurses who were negative to both antigens. Obviously, the first step was to check the accuracy of the roentgen interpretation of pulmonary calcifications. These 4 by 5 stereo photofluorograms had been interpreted routinely by one of us (E. C.). In routine reading of photofluorograms we believe the tendency to interpret vascular markings as calcification is greater than in the conventional 14 by 17 chest roentgenogram. Therefore, the photofluorograms of the 116 individuals were reviewed by the two of us independently. The few on which we differed were reëxamined and we agreed on the presence or absence of calcification. From this review of 116 photofluorograms we concluded that the diagnosis of calcification was not justified in 32. In 79 we agreed that calcification probably existed and that in five others there was pulmonary infiltration without calcification. These interpretations were made without the knowledge of the histoplasmin sensitivity.

In correlating these results with the histoplasmin reaction we found that 61 of the group who had the roentgen diagnosis of calcification were histoplasmin positive as were the five with non-calcified pulmonary infiltrates. The majority of these students showed extremely large calcified areas both in the hilum and in the parenchyma. Some of these areas were only partially calcified. In the 49 students who failed to react either to tuberculin or histoplasmin, there were 18 in whom we agreed calcification probably existed although the size, irregularity and density of the roentgen shadows were not such that we were certain of the diagnosis. To arrive at a more accurate classification of this group, we examined them fluoroscopically. Fourteen

of the 18 were available for study, as four were no longer in school. By fluoroscopy calcification was found in three; one with a relatively large left hilum calcification and two with peripheral calcifications 2.0 to 3.0 mm. in diameter. In the remaining 11 we concluded that no calcification existed although the large and prominent vascular markings thought to be probable calcified areas on the photofluorogram were usually discernible.

If we include the four not available for fluoroscopy in the group as showing calcification (and that seems improbable) we have a total of seven of 73 with calcification, or 9.6 per cent who failed to react either to tuberculin or to histoplasmin. This correlates very closely with Palmer's[2] study in which he found 25 of 294 or 8.5 per cent who had the roentgen diagnosis of calcification but were negative to histoplasmin and tuberculin. In our study coccidioidin skin testing was not done routinely as these students had no exposure in the areas where *Coccidioides immitis* is endemic. On 10 of the group who were retested at a later date with coccidioidin no positive reactions were noted.

All the students with positive histoplasmin tests were questioned as to their residence exclusive of their army experience. If a student had lived five-sixths of his life in one state, that state was tabulated as his residence. Wisconsin and Illinois led the represented states with 14 students each. Ohio was next with seven. Kentucky, Tennessee, New York, Mississippi, Maryland and Indiana were each assigned three students; Iowa, Oklahoma and the District of Columbia, two students each. One student was assigned to each of the states of Texas, Arkansas, Connecticut, Delaware, Nebraska. Comparison of the distribution of histoplasmin reactors according to residence with the number of students enrolled in the university from each of these states revealed an obvious disproportion in the incidence of histoplasmin sensitivity, the incidence among Wisconsin students being lower by actual percentage.

The second part of the study was done to determine the incidence of histoplasmin sensitivity in Wisconsin students. All the students entering in June 1946 were tuberculin tested as previously described and were also given 0.1 c.c. of 1 : 1000 histoplasmin solution intradermally. The readings were made as noted before. In this and in the succeeding group, 70 mm. photofluorograms were used instead of 4 by 5 stereo photofluorograms. Not considering army experience, we classified 381 students as lifetime residents of Wisconsin. Of these, 350 were men and 31 women. Forty-four positive histoplasmin tests were recorded, or 11.5 per cent. Tuberculin sensitivity was noted in 125 or 33 per cent. In 12 students both the histoplasmin and tuberculin test were positive. At this same time 184 students who were residents of the United States, but not Wisconsin residents, were tested. The number of foreign students tested was 12 which is too small to be significant. Only one of these 12 had a positive histoplasmin test and she had

spent her entire life in western Canada. Of the 184, 50 or 27.6 per cent were positive to histoplasmin. The tuberculin sensitivity was 41.3 per cent.

The states represented in the histoplasmin positive group were essentially the same as noted in the previous group with pulmonary calcifications; i.e., the central eastern states. The number was not large enough to be statistically significant if determined in percentages of the total students tested from the various states. Since it has been reported by Palmer [4] that states vary in histoplasmin sensitivity in different areas, we attempted to determine the situation among the reactors from Wisconsin. From this standpoint we divided the state into northern and southern parts. As Milwaukee is the only large city and is on the lake shore, we listed it separately. From northern Wisconsin there were eight histoplasmin reactors in 61 or 13.7 per cent. From Milwaukee eight of 66 were sensitive to histoplasmin or 11.6 per cent. In the rest of southern Wisconsin there were 28 positives from 252 individuals or 11.1 per cent. Therefore, we concluded that there was no significant variation in histoplasmin sensitivity in the various regions of Wisconsin, nor in the urban and rural populations.

Because the number of women in the previous group was small we tested the women who entered school in the fall of 1946 by the same method. One thousand eighteen students completed their skin testing. Eight hundred ninety-four of these were lifetime Wisconsin residents. Only 24 or 2.6 per cent were histoplasmin positive. Among the women from states other than Wisconsin 19 histoplasmin positive individuals were found in 124 or 15.3 per cent. Tuberculin sensitivity was found in 155 or 17.2 per cent of the Wisconsin women. In the non-resident group 27 of 124 or 21.7 per cent were positive to tuberculin. Again the out-of-state students who reacted to histoplasmin were scattered through the same group of states where histoplasmin sensitivity has been shown to be high.

In addition to studying the incidence of histoplasmin sensitivity in these students, we were interested in the incidence of calcification in the various groups as classified according to skin tests. For purposes of more accurate diagnosis of calcification than possible with 70 mm. photofluorograms, the majority of the patients with positive histoplasmin tests was fluoroscoped. Also, the majority of individuals with calcifications reported and who were negative to tuberculin and histoplasmin was checked by fluoroscopy; but in 12 individuals included in the group of 26 we were unable to obtain this study, hence they were listed under the roentgen diagnosis of "healed primary" which was the only method of reporting calcification in the forms used. Three students who showed minimal pulmonary infiltrates without obvious calcification were included as calcification in the charts that follow.

In addition to the students reported in these surveys we have observed several who have small pulmonary infiltrates which are indistinguishable roentgenologically from minimal pulmonary tuberculosis. These students have failed to react to 1.0 mg. O.T. but have reacted to histoplasmin. The majority gave histories of one or more previous negative tuberculin tests.

Discussion

From a routine survey of 5000 students by means of photofluorograms and tuberculin testing, 160 students were found to be tuberculin negative and to have pulmonary calcifications. On 116 of these students histoplasmin tests were done. Upon review of the photofluorograms of this group we excluded 32 in whom we deemed the original diagnosis of calcification unjustified. Of the remainder, 66 were histoplasmin positive. Only three individuals in whom calcifications were shown to exist by fluoroscopic examination were negative to both histoplasmin and tuberculin skin tests.

Although Wisconsin is an area of comparatively low incidence of histoplasmin sensitivity, the general pattern of the occurrence of pulmonary calcifications is the same as for areas of higher incidence; i.e., calcification is two to four times as frequent in the histoplasmin reactor as in the tuberculin reactor (tables 1 to 4). In the composite group the incidence of pulmonary calcifications was 38.3 per cent in the histoplasmin sensitive group and 9.2 per cent in the tuberculin positive group.

The fact that histoplasmin sensitivity does not necessarily mean that the infective agent is *Histoplasma capsulatum* is well recognized. However, the high degree of correlation between histoplasmin reactions and pulmonary calcification suggests that these calcifications are the result of previous infections with *Histoplasma capsulatum* or a very closely related fungus.

Table I

Wisconsin Residents, June 1946 (381 students)

	H+ T−	H+ T+	T+ H−	T− H−
Calcification	7	5	10	4
No calcification	21	7	100	202
No x-ray	4	0	3	18
Total	32	12	113	224
Per cent x-rayed showing calcification	25.0	41.7	9.1	1.9

Table II

Non-Resident, June 1946 (184 students)

	H+ T−	H+ T+	T+ H−	T− H−
Calcification	12	12	11	5
No calcification	12	14	36	66
No x-ray	1	0	4	11
Total	25	26	51	82
Per cent x-rayed showing calcification	50.0	46.1	23.4	7.0

TABLE III

Resident and Non-Resident Women, September 1946 (1018 students)

	H+ T−	H+ T+	T+ H−	T− H−
Calcification	12	4	7	17
No calcification	17	5	140	470
No x-ray	3	2	24	317*
Total	32	11	171	804
Per cent x-rayed showing calcification	41.4	44.4	4.8	3.4

* This large number was due to the film shortage at the time of the routine entrance examinations. In view of the negative skin tests these students were not required to report at a later date for roentgen study.

TABLE IV

All students studied

	H+ T−	T+ H+	T+ H−	T− H−
Calcification	31	20	28	26**
No calcification	50	26	276	738
No x-ray	8	2	31	346
Total	89	48	335	1110
Per cent x-rayed showing calcification	38.3	43.5	9.2	3.4

** 12 of this group had 70 mm. film only.

In a group of 381 lifetime residents of Wisconsin the histoplasmin sensitivity was 11.5 per cent. In this group there was no significant variation in the northern and southern portions of the state, nor in the one large urban group from Milwaukee. Among the resident female students the incidence of histoplasmin reactors was much lower, 2.6 per cent. The reason for this variation is not entirely clear. However, from previous studies [6] histoplasmin sensitivity shows substantial sex differences, especially after the age of 20, and the men were slightly older than the women in the groups which we studied.

CONCLUSION

1. Histoplasmin sensitivity among students at the University of Wisconsin reflects what is known of the geographic distribution of such sensitivity in the United States.

2. A significant proportion of hilum and pulmonary calcifications seen in chest roentgenograms is not associated with tuberculin sensitivity. In our series 66 of 73 such calcifications in tuberculin negative individuals were associated with histoplasmin sensitivity.

3. The incidence of pulmonary calcifications of the entire group surveyed in this study was 38.3 per cent in the students reacting only to histoplasmin and 9.2 per cent in those who reacted to tuberculin alone.

4. Small pulmonary infiltrates indistinguishable roentgenologically from tuberculous lesions may be associated with histoplasmin sensitivity and not tuberculin sensitivity.

5. From the roentgenologic finding of pulmonary calcifications or even infiltrations, we believe it erroneous to assume a diagnosis of tuberculous infection without further proof.

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