

## BOOSTER EFFECT OF HISTOPLASMIN SKIN TESTING IN AN ELDERLY POPULATION<sup>1</sup>

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Ganley, J. P. (Office of Biometry and Epidemiology, National Eye Institute, NIH, Bethesda, Md. 20014), R. E. Smith, D. B. Thomas, G. W. Comstock and P. E. Sartwell. Booster effect of histoplasmin skin testing in an elderly population. *Am J Epidemiol* 95: 104-110, 1972.—A second histoplasmin skin test was administered to 97 individuals aged 50 years or older who had less than 7 mm of induration on a test given 3 weeks earlier. Of 76 people with 0 to 1 mm of induration on the first test, 35.5% had reactions of 2 to 13 mm on the second test and would be classified as converters. Of 21 people with 2 to 6 mm of induration on the first test, 19.0% had less than 2 mm of induration on the second test and would be classified as reverters. Among those with 2 mm of induration or more on the second test, those with initial reactions of 0 to 1 mm had a mean increase in induration of 7.2 mm, and those with initial reactions of 2 to 6 mm had a mean increase of 6.5 mm. The net conversion rate of 23.7% was attributed to the booster effect of the first test on the cellular immune system. Among this study group, all of whom were over 50 years of age, the booster effect showed no change with sex or age. The estimated age-specific prevalence of histoplasmin reactors, using the combined results of the first and second tests, was higher than that estimated from the results of the first test alone; both estimates declined sharply with age. In an elderly population, the booster effect must be taken into account, both as a cause of skin test conversion, and as a means of obtaining a more complete estimate of past sensitivity to the test antigen.

chorioretinitis; hypersensitivity, delayed; histoplasmosis; skin tests

### INTRODUCTION

Induration, as a manifestation of delayed type hypersensitivity in response to tuber-

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culin skin testing, tends to decrease, and in fact may disappear with passage of time after infection with *Mycobacterium tuberculosis* in experimental animal models (1, 2), in Calmette-Guérin bacillus vaccination (3), and in human cohort studies (4). This same waning of sensitivity is presumed to occur with other delayed skin reactions, such as reactions to histoplasmin, brucellergen and coccidioidin.

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Skin testing with tuberculin (3, 5-7) and histoplasmin (8) can produce a booster effect in some people who have been previously sensitized, either by vaccination or exposure to the organism. Histoplasmin-sensitized individuals who do not react to the first test may thus convert to positive on the second test without an intervening infection with *Histoplasma capsulatum*; when initially positive, their induration may be larger, appear sooner, and disappear more quickly on the second test (8). In sensitized individuals, the histoplasmin skin test may also stimulate the production of collodion-agglutinating (9) and complement-fixing antibodies (10-12). However, in nonsensitized individuals, repeated intracutaneous injections of diluted histoplasmin antigen do not induce delayed type skin reactions, either in animal or human subjects (9, 13).

A study of the association of chorioretinal scars and histoplasmosis (14) made it important to ascertain as completely as possible all individuals who had ever been infected with *H. capsulatum*. For this purpose, a single histoplasmin skin test was not sufficient because it might not cause a reaction among persons whose sensitivity had waned. Consequently, the test was repeated to identify those previously infected individuals who reacted poorly to the first test, but in whom the anamnestic effect of the first test might produce a positive response on the repeat test.

The results of retesting an elderly population who had little or no reaction to an initial histoplasmin skin test form the subject of this report.

#### MATERIALS AND METHODS

In the spring of 1970, a community survey was conducted in Walkersville, a small community in Frederick County, Maryland, to determine the prevalence of chorioretinal scars and their association with histoplasmosis. Details of the study are given elsewhere (14). Members of the community

who were 13 years of age and older were skin tested on the volar surface of the left forearm with 0.1 ml of histoplasmin, lot H-42, diluted 1:100 one week prior to testing.<sup>8</sup> The skin test results were read 48 hours later by two of us (D. B. T. and G. W. C.) who had previously established the comparability of our reading technique. Using a rule with a hidden scale<sup>4</sup> to minimize observer bias, the broadest transverse diameter of induration was measured and recorded in millimeters.

Persons 50 years of age and older who had less than 7 mm of induration from the initial histoplasmin test were asked to return three weeks later for a second skin test in the opposite forearm. The dose of antigen and the techniques of testing and reading were the same as those employed for the first test. The results of the first skin test were not known at the time of the second reading.

The interval between tests was set at three weeks because it was believed that this period would permit maximum anamnestic response of the cellular immune system to the first skin test in those individuals who had been previously sensitized. Furthermore, it was desirable to select as brief an interval between tests as possible to minimize the probability of reinfection. It seems reasonable, in this population with no known exposure to environmental conditions capable of massive infection with *H. capsulatum*, to assume that so few reinfections could have occurred in this brief period that they may safely be ignored.

#### RESULTS

The distribution of tested individuals by the size of their reactions to histoplasmin is shown in figure 1. The distribution is obviously bimodal. It is clear that the best

<sup>8</sup>Supplied through the courtesy of Dr. Lydia B. Edwards, Tuberculosis Program, Center for Disease Control, U.S. Public Health Service, Rockville, Maryland.

<sup>4</sup>Developed by Randolph B. Turnbull, Memphis, Tenn.

separation between those who reacted to the test dose of histoplasmin and those who did not can be achieved by dividing the population into those with less than 2 mm and those with 2 mm or more of induration. For the purposes of this paper, these two groups will be called "negative" and "positive", respectively (15).

The prevalence in the community of individuals who reacted positively to the initial skin test is shown separately for each sex in figure 2. At all ages, there were fewer positive reactors among women than among men. After a sharp rise prior to age 25, there was only a slight further increase in prevalence until about age 50. The frequency of positive reactions started to decline in the sixth decade of life, and fell

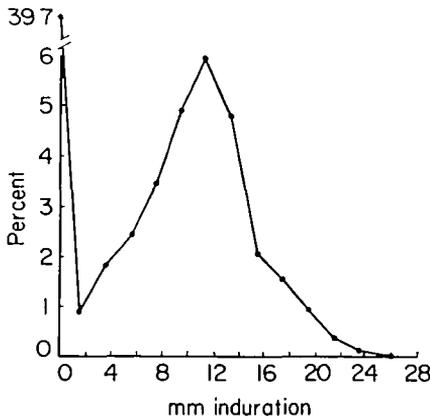


FIGURE 1. Distribution of individuals by size of reaction to histoplasmin.

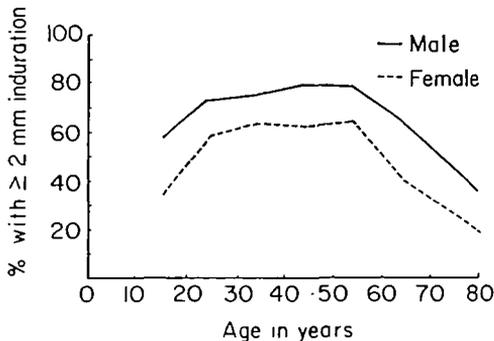


FIGURE 2. Age-specific prevalence of reactors to histoplasmin on first test by sex ( $\geq 2$  mm induration).

TABLE 1

Number and percentage of people from Walkersville community histoplasmin retested, by age and sex

Sex	Age (years)	First skin test			Second skin test		
		No. in community	No. examined	% examined	No. eligible*	No. retested	% retested
Male	50-59	62	42	67.7	16	13	81.2
	60-69	51	28	54.9	13	10	76.9
	70+	39	17	43.5	12	8	66.6
Total		152	87	57.2	41	31	75.6
Female	50-59	60	42	70.0	28	19	67.8
	60-69	67	44	65.6	32	27	84.3
	70+	62	37	59.6	34	20	58.8
Total		189	123	65.0	94	66	70.2

\* Persons eligible for the second skin test were those aged 50 years or older with reactions 6 mm or less in diameter on the first test.

rapidly after the age of 60. This decline in histoplasmin sensitivity among older persons has been observed in other surveys (16, 17).

If it is assumed that all persons in the tested population were exposed to similar risks of becoming infected with *H. capsulatum* throughout their early life, then it appears that loss of histoplasmin sensitivity exceeded its acquisition at about age 50. The group of persons older than 50 should thus contain a large proportion of previously infected individuals who failed to react to the initial test. Some of these nonreactors should be identifiable as previously sensitized subjects by the booster effect of the initial test.

Of the 341 persons in the community 50 years of age and older, 210 or 61.6 per cent were tested initially. Nearly two-thirds of those tested (135 persons) reacted with less than 7 mm of induration, and 97 (71.8 per cent) of them came to be retested three weeks later. As is shown in table 1, the differences in participation by sex were slight. Persons 70 years of age and older did not participate in either test to the same extent as younger individuals.

TABLE 2

Distribution of persons by induration to histoplasmin on first and second tests among those with less than 7 mm induration on first test\*

Milli-meters of induration on first test	No. of persons with indicated millimeters of induration on second test										Total no. of persons
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	
0-1	49	5	2	9	3	4	4				76
2-3	2			2	1				1	1	7
4-5	2				1	3	3	1			10
6				1		2	1				4
Total	53	5	2	12	5	9	8	1	1	1	97

\* Numbers in italics indicate no change between the two tests

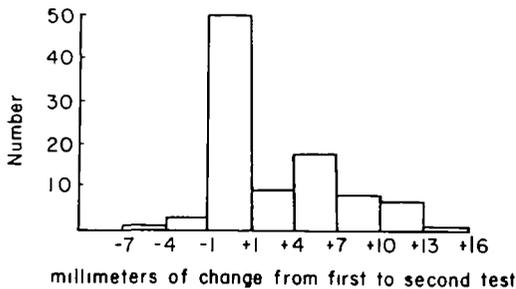


FIGURE 3. Frequency distribution of differences in induration between first and second histoplasmin skin test measurements on 97 individuals.

The results of the second test according to the degree of induration to the first test are shown in table 2. Slightly more than half of the tested persons, 50 in number, had essentially no change from one test to the other; all but three had no detectable induration on either occasion. Four others with small amounts of induration on the first test had no induration on the second. The remaining 43 showed increases of 2 to 16 mm.

The frequency distribution of differences in induration between the first and second histoplasmin skin tests is shown in figure 3. There appear to be two parts to this distribution. The first, centering at 0 mm, is largely composed of persons with no induration to either test. The other part of the distribution, with a mode at +5 to +7 mm of induration, represents the anamnestic response evoked by the first skin test.

Among persons whose skin test reactions increased by 2 mm or more, there was little indication of correlation between the size of reaction to the first and second skin tests. The 27 individuals with 0 to 1 mm of induration on the first test whose second tests were 2 to 13 mm larger had a mean reaction size to the second test of 7.2 mm. A somewhat smaller mean increase, 6.5 mm, was observed among the 16 persons with 2 to 6 mm initially and an increase of 2 to 16 mm on the second test, resulting in a mean induration on the second test of 10.9 mm. Differences between males and females in the size of the booster effect were insignificant.

Using the definition of a positive histoplasmin reactor as a person with at least 2 mm of induration, the conversion, reversion and net conversion rates are shown in table 3 by sex and by age. Among the total group,

TABLE 3

Changes in histoplasmin sensitivity on a second test given 3 weeks later, by age and sex

	Conversion*			Reversion†			% net conversion‡
	No. tested	No. converting	% converting	No. tested	No. reverting	% reverting	
Sex							
Male	23	10	43.4	8	3	37.5	22.6
Female	53	17	32.0	13	1	7.0	24.2
	$\chi^2 = .87, d f = 1, .3 < p < 5$			Fisher exact test, $p = .13$			
Age (years)							
50-59	20	11	55.0	12	3	25.0	25.0
60-69	31	10	32.2	6	1	16.6	24.0
70+	25	6	24.0	3	0	0	24.0
	$\chi^2$ for linear regression = .02 < $p$ < .05, d.f. = 1			Fisher exact test, $p = .33$			
Total	76	27	35.5	21	4	19.0	23.7

\* Conversion defined as those with 0-1 mm of induration on the first histoplasmin skin test who developed  $\geq 2$  mm of induration on the second test.

† Reversion defined as those with 2-6 mm of induration on the first histoplasmin skin test who developed  $< 1$  mm of induration on the second test.

‡ Percentage net conversion = (converters - reverters) / total No. tested  $\times 100$ .

35.5 per cent of the 76 individuals with reactions of 0 to 1 mm on the first test had larger reactions on the second test, while 19.0 per cent of the 21 with initial reactions of 2 mm or more had negative reactions on the second test. The net conversion rate was 23.7 per cent for the total group. Both conversion and reversion rates were lower among females than among males, and both rates decreased with increasing age. The net conversion rates, however, were essentially the same for both sexes and for each age group.

By applying the observed conversion and reversion rates in table 3 to the total Walkersville population over the age of 50 who had participated in the initial survey, new age-specific prevalence ratios of histoplasmin sensitivity were calculated. The highest prevalence ratio, 81.2 per cent, occurred in the 50 to 59 year age group. This fell with increasing age to 51.8 per cent among persons over 70 years of age. The rate of decline in prevalence by age was similar to that based on the results of the initial skin test survey, even though the prevalence of positive histoplasmin reactors was higher at

all ages when estimated from the results of both skin testing programs (figure 4).

#### DISCUSSION

Several experimental and observational factors can influence the variability of measurements of delayed type skin sensitivity, and must be considered in analyzing these findings. Nissen Meyer et al. in the administration and reading of tuberculin skin tests, found a small experimental error with a standard deviation of 2 mm in the diameter of induration; the reading error alone accounted for 1.3 mm (18).

However, if these errors are random, the number of individuals whose second tests were larger than their first tests should equal those with second tests smaller than their first tests. A frequency distribution of such changes between tests should have its mode at 0. A slight systematic error superimposed on these random errors would cause the distribution of changes from one test to the other to be skewed. A larger systematic effect would cause a bimodal distribution, such as that shown in figure 3.

It is possible that the part of this observed bimodal distribution which is centered away from 0 resulted from some systematic error or bias. However, efforts to achieve standardized and accurate injection technique, measurement of reactions with gauges designed to keep the reading hidden until the pointers had been positioned at each end of the transverse diameter of induration, and the fact that the second reading was made with no knowledge of the first test except that it had caused less than 7 mm of induration all make major systematic errors unlikely. New infections are not a likely cause of the increased sensitivity observed at the second testing because the period between tests was only three weeks. The most likely cause is the booster effect resulting from the stimulation of the cellular immune system by the first test.

The booster effect did not change with age in this population. As a consequence, the revised estimate of past infection based

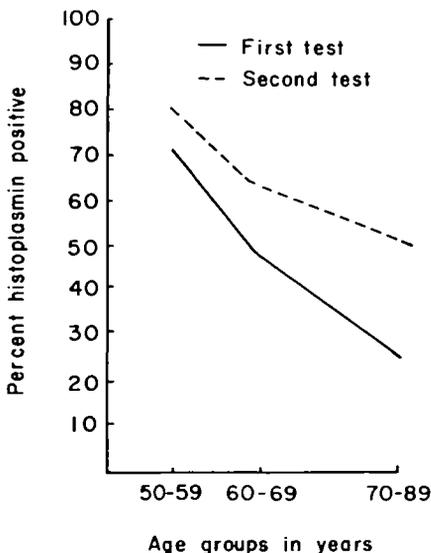


FIGURE 4. Age-specific prevalence rates to histoplasmin on first test compared with estimated rates for community computed from second test three weeks later.

on the results of both tests still showed a marked decrease with age after 50 years. Although the true frequency of past infection cannot be known, it seems unlikely that the probability of having been infected with *H. capsulatum* at some time in the past really decreased with age. A more likely cause of the decreasing frequency of positive reactors after the age of 50 is that some older persons may have lost sensitivity to such an extent that the ordinary diagnostic test is not a sufficient stimulus to cause skin sensitivity which can be detected by the same dose given subsequently.

Hence it is possible that a repeat test, while improving the estimate of past infections, may still fail to reveal them all. This appears to be an unavoidable error in studies designed to investigate the association of previous infections with pathologic conditions that may have developed soon after initial infection with an organism, such as atrophic scars of presumed ocular histoplasmosis or pulmonary calcifications.

The booster effect has not previously been taken into consideration in community surveys to determine converters to histoplasmin (16, 17). The anamnestic response may have been a component of the 38 per cent conversion rate that Doto et al. (17) found in their first 6-month retest period. Experience with tuberculin testing suggests that virtually all of the booster effect is elicited by the initial test, and that this effect may persist for at least a year (15). If a similar effect were to apply to repeated histoplasmin testing, this would suggest that the 17 to 23 per cent conversion rate observed by Doto et al. (17) in subsequent 6-month test intervals was closer to the true conversion rate, and that the excess conversion in the first period was due to the booster effect. Similar considerations could apply equally well to studies of tuberculin conversion rates, results of sequential testing with weak and strong doses of tuberculin, and conversions attributed to Calmette-Guérin bacillus (BCG) vaccination, especially among older persons.

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