Today's Concept of the Tuberculin Test*

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The tuberculin test is the most effective means for detecting tuberculous infection, and hence is an essential tool in ultimate eradication of the disease. In clinical practice it has been generally accepted that a positive tuberculin skin reaction indicates the possibility of tuberculosis, whereas a negative reaction, with few exceptions, rules out a tuberculous infection. Since the advent of isoniazid chemotherapy, the tuberculin test has become essential for tuberculosis prevention by reason of its usefulness in revealing tuberculosis in the incipient stage, at which time administration of isoniazid may prevent the development of overt disease.

Until recently, all positive tuberculin skin reactions have been attributed to infection by Mycobacterium tuberculosis. This view has been challenged lately with the increasing knowledge of other mycobacterial infections causing tuberculin cross-sensitivity in man. This tuberculin cross reaction has posed a new problem in the interpretation of the tuberculin test. The purpose of this article is to bring up to date the interpretation of the tuberculin skin reaction in the light of present-day knowledge of tuberculosis and other mycobacterial infections based on studies in the Houston area, as well as on published works in current literature. The tuberculin patch test and the recently introduced Heaf and tine tests will be discussed in terms of their reliability and feasibility.

Materials and Methods

The tuberculin antigens. Old tuberculin (OT) was first prepared by Robert Koch, and is made by heat-sterilizing cultures of tubercle bacilli, filtering off the dead bacilli, and evaporating the filtrate to one-tenth of its original volume. It is a crude preparation containing many substances which may give rise to non-specific reactions. Its main drawback is that the actual content of active principle is not standardized and the potency of the preparation varies from batch to batch.¹

Purified protein derivative (PPD) consists of the active protein principle obtained from filtrates of autoclaved cultures of tubercle bacilli which have been grown on a synthetic medium and extracted either by trichloroacetic acid or by neutral ammonium sulfate precipitation. The latter method was used in the manufacture of one large batch by Seibert, which was adopted by the World Health Organization in 1952 as the International Standard Tuberculin, designated as PPD-S.² Commercially, PPD antigen prepared from Mycobacterium tuberculosis is available in three different strengths as follows:

<table>
<thead>
<tr>
<th>Strength</th>
<th>Mg. per dose†</th>
<th>Tuberculin Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>0.00002</td>
<td>1 TU</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.0001</td>
<td>5 TU</td>
</tr>
<tr>
<td>Second</td>
<td>0.005</td>
<td>250 TU</td>
</tr>
</tbody>
</table>

†mg. of protein per 0.1 ml. of solution.

Purified tuberculin RT 23 is a new batch of PPD prepared by the Statens Seruminstitut, Copenhagen, at the request of UNICEF for international use. It is estimated that the quantity prepared in this large batch will cover the global demand for purified tuberculin for human use for several years.³⁴

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Other PPD antigens for investigative use will be described under Tuberculin Cross Reactions.

Methods of tuberculin testing. The intradermal or Mantoux test (Fig. 1) is the standard method now in use. It is a quantitative test because a measured amount of antigen is inoculated. A dose of 0.0001 mg. (5 TU) is given intradermally in 0.1 ml. This dose will detect practically all tuberculous infections and is generally used for routine testing. When tuberculosis is strongly suspected, a lower dose of 0.00002 mg. (1 TU) is recommended to avoid a possible severe local reaction. The test is read 48 to 72 hours after injection by inspection of the skin reaction in a good light, and the extent of both induration and edema are carefully determined by gentle stroking and palpation of the area with the fingers. The margins of the palpable induration and edema should be marked with a pen and the transverse diameter measured to the nearest millimeter. A reaction of 6 mm. or more is considered positive. Large reactions are more significant than small ones.

Other methods of tuberculin testing currently in use are the Heaf test, the tine test, and the patch test. They are better suited than the intradermal test to large scale screening, but are not intended for diagnostic use because of lack of dosage control. The relative merit of the three tests will be discussed later.

The Sources of Tuberculin Sensitivity

Mycobacteria are widely distributed in nature; many species are saprophytic, and some parasitic in warm-blooded animals. The most important of the latter is M. tuberculosis, which is the most common cause of tuberculosis in man.

Among the vast number of mycobacteria other than M. tuberculosis several species have been found to be pathogenic for man. Not all of these mycobacteria have as yet been taxonomically classified.

Until recently, the tubercle bacillus, M. tuberculosis, was assumed to be the only source of tuberculin skin sensitivity. Recent studies have shown that much low-grade tuberculin skin sensitivity in healthy persons is probably caused by infection with mycobacteria other than M. tuberculosis. This phenomenon is known as the tuberculin cross reaction in contradistinction to the specific tuberculin reaction which is caused by M. tuberculosis. It is important to distinguish the two types of tuberculin skin sensitivity because of the great difference in their significance.

The Tuberculin Cross Reactions

Animal experiments. The problem of tuberculin cross reaction has been studied extensively in experimentally infected guinea pigs by the Tuberculosis Research Section of the United States Public Health Service. Also in recent years, carefully designed experimental observations have been made in guinea pigs at the Statens Seruminstitut in Denmark. As a result of these studies, important facts regarding tuberculin cross reaction have been revealed:

**Figure 1:** The intradermal tuberculin test.
1. The sources of tuberculin cross sensitivity have been traced to a number of strains of mycobacteria other than *M. tuberculosis*. Inoculation of these organisms into guinea pigs induces cutaneous hypersensitivity to the standard tuberculin (PPD-S) prepared from *M. tuberculosis*.

2. The antigenic properties of the different species of mycobacteria overlap. Thus, guinea pigs inoculated with one species may exhibit cutaneous hypersensitivity to antigens derived from several other species.

3. The PPD antigen derived from the homologous organism with which infection has been induced generally elicits a significantly stronger skin reaction than equivalent doses of antigens prepared from other mycobacteria. Hence, differential skin testing using a battery of antigens can be helpful in identifying the infecting mycobacteria.

*Studies in human population.* The United States Public Health Service has prepared PPD antigens from a variety of mycobacteria isolated from human sources for epidemiologic studies. Some of the antigens are as follows:

PPD-Y from *M. kansasii* (Runyon Group I, formerly called photochromogens).

PPD-BRIDGES from the Bridges strain (Runyon Group II or scotochromogens).

PPD-B from the Battey strain (Runyon Group III or non-chromogens).

PPD-S from *M. tuberculosis*.

The above PPD antigens were prepared in a manner similar to the preparation of PPD-S by the Seibert technique.11-18 For differential intradermal testing in man, solutions of each antigen are prepared to contain 0.0001 mg. protein in 0.1 ml.

Numerous skin test surveys in human populations have been carried out both in the United States and abroad. It has been noted that a tuberculin cross reaction is usually a low-grade reaction characterized by induration and edema of less than 10 mm. in response to a dose of 0.0001 mg. of PPD-S. Not infrequently there may be little or no skin reaction to this dose and higher dosage (0.002 mg. or 0.005 mg.) may be required in order to elicit a skin response. The following observations have shed new light on the nature of the tuberculin cross reaction.

1. The prevalence of low-grade tuberculin skin sensitivity in tuberculin surveys has been related not to the prevalence of tuberculosis, but to certain geographic areas, an observation which has strongly suggested an environmental rather than a human source of infection.418,19 A higher prevalence has been noted in rural than in urban areas; higher in the Southeastern United States than in the North and West;151 higher in the tropical belt of the world than in cooler climates.18,19

2. In the Southeastern United States, individuals showing a low-grade tuberculin skin reaction to PPD-S usually exhibit a stronger skin reaction to PPD-B. This skin reaction pattern indicates that the antigenic property of the infecting agent is more closely related to the Battey organism than to *M. tuberculosis*.47

3. Low-grade tuberculin skin sensitivity is infrequent in patients with bacteriologically proven disease caused by *M. tuberculosis*.18

In studying origins of tuberculin skin sensitivity in Philadelphia children, Mellman10 found that a number of positive tuberculin reactions could not be attributed to tuberculosis. Simultaneous intradermal testing with PPD-S and PPD-B revealed that tuberculin reactions of less than 10 mm. were usually associated with stronger reactions to PPD-B, suggesting a Battey type infection with a cross reaction to PPD-S. Such tuberculin cross reactions have been found in children with no tuberculosis contact and no radiographic or bacteriologic evidence of tuberculosis.

Studies on low-grade sensitivity in school children in Cardiff, Wales was recently reported by Griffith and associates.46 Comparative Heaf multiple puncture tests were carried out in 2,464 children; mammalian tuberculin was used on one arm and an antigen prepared from one of the unclassi-
fied mycobacteria on the other. The latter included a photochromogen, a group of scotochromogens, a locally isolated non-chromogen, the Battey bacillus and a fast growing non-chromogen. The authors concluded that a good deal of the low-grade skin sensitivity (Heaf Grade I or II) appeared to result from sensitization by bacilli of the Battey-Avian group, and that high-grade tuberculin skin sensitivity (Heaf Grades III or IV) usually resulted from infection with *M. tuberculosis*.

**Tuberculin cross reactions in healthy elementary school children.** Low-grade tuberculin skin sensitivity is common among elementary school children in the geographic area of Houston, Texas. In 1959 the pupils of Sunnyside Elementary School were tuberculin tested with 5 TU PPD. Among the 1,080 pupils participating in the survey only 1.6 per cent showed a positive reaction (6 mm. and more) at the 48 hour reading. The negative reactors were then tested with 250 TU PPD and 22.4 per cent gave a positive reaction at 48 hours. The prevalence of low-grade tuberculin skin reactors is shown in Fig. 2.

To study the source of the low-grade tuberculin sensitivity, a systemic skin test survey was made using multiple PPD antigens derived from a variety of mycobacteria prepared by USPHS. A number of elementary schools in the Houston area participated in the project. A prevalence of low-grade skin reactors to standard tuberculin (PPD-S) was again noted. The frequencies of positive skin reactions (6 mm. and more) to PPD-S and to the other PPD mycobacterial antigens in two suburban schools were as follows:

<table>
<thead>
<tr>
<th>School</th>
<th>PPD-S</th>
<th>PPD-Y</th>
<th>PPD-B</th>
</tr>
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<tbody>
<tr>
<td>Carver (1961)</td>
<td>8.2%</td>
<td>17.0%</td>
<td>39.6%</td>
</tr>
<tr>
<td>Bethune (1961)</td>
<td>9.0%</td>
<td>19.1%</td>
<td>45.1%</td>
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</table>

It was further noted that in these two schools the majority of the skin reactions to PPD-S were small in size. The association of low-grade tuberculin reactions with stronger reactions to PPD-B suggests infection by a "Battey" or antigenically related type of organisms rather than by *M. tuberculosis*.

Further study on the source of low-grade tuberculin sensitivity was carried out in 1962 in two elementary schools in Houston. PPD-Bridges (a scotochromogen) was administered intradermally to each pupil in addition to PPD-S, PPD-B, and PPD-Y. In St. Rose of Lima Elementary School 547 pupils were tested, and in Lantrip Elementary School 718 pupils participated. Frequencies of positive reactions to each antigen are shown in Fig. 3. It may be noted that skin sensitivity to the scotochromogen antigen is as common as to the Battey antigen.

Sensitization to Battey and to scotochromogen antigens begins early in life. The percentage of positive reactors at different age levels as observed in the four above-mentioned elementary schools, is shown in Fig. 4.

Low-grade tuberculin skin sensitivity in elementary school pupils appears to be related more to the Battey and scotochromogen types of infection. This point is illus-
trated in scatter-diagrams 5A, 5B, 5C of Fig. 5. Small tuberculin reactions of 12 mm. or less are associated with stronger reactions to PPD-B and PPD-Bridges (Figs. 5B, 5C). It is indicated, therefore, that the infecting agent is more closely related to the Battey and the scotochromogen organisms than to *M. tuberculosis*. The photochromogens appear to be a less frequent cause of low-grade tuberculin sensitivity in these children (Fig. 5A) than either the Battey organisms or the scotochromogens.

Clinical observations. Among the unclassified mycobacteria which were at one time considered to be saprophytes, certain strains have been found to be fully capable of infecting man and under certain conditions pathogenic and invasive. Patients with proved infection or disease caused by these mycobacteria provide an opportunity...
for studies of tuberculin cross reactions.

In the past three years, seven children with cervical lymphadenitis due to scotochromogens were seen at the Tuberculosis Division of Jefferson Davis Hospital. Tuberculin tests with 0.0001 mg. PPD-S showed doubtful reactions (erythema or edema with no palpable induration) in four cases, and positive reactions ranging from 13-19 mm. induration in three cases. However, in every case there was a stronger reaction to the scotochromogen antigen than to PPD-S. The skin reaction to PPD-S was interpreted as a cross reaction to a scotochromogen infection because scotochromogens were the only organism cultured from the diseased nodes, and there was no evidence of tuberculosis in these children.

In 1961, Davis and Comstock reviewed six cases of cervical adenitis in children due to chromogenic mycobacteria (yellow or...
orange colored colonies). Intradermal testing using 0.0001 mg. PPD-S showed low-grade reactions ranging from 3 mm. to 8 mm. in four cases and no reactions in two cases, while a stronger skin reaction was noted to the scotochromogen antigen.

In 1959, Chapman and Guy reported six cases of cervical lymphadenitis in children. Five cases were reported to be caused by photochromogens and one by scotochromogens. Three children had negative reactions to 1:1000 OT, but positive reactions to 1:100 OT; the fourth child had a small reaction of 5-6 mm. induration to 1:10,000 OT, and the remaining two had "positive" tuberculin reactions, with no mention of the sizes.

In 1959, Edwards and associates studied cutaneous reactions to PPD-B and PPD-S in patients with disease due to the "Battey" mycobacteria at the Battey Hospital in Rome, Georgia. Most of these patients had small reactions to the standard tuberculin, but stronger reactions to the homologous antigen, PPD-B.

Some mycobacteria species are so closely related to M. tuberculosis in their antigenic structure that differential skin testing may fail to distinguish them. Using OT antigens derived from M. tuberculosis and M. balnei respectively, Schafer in 1962 reported skin test results on 56 cases of infections due to M. balnei. Forty-eight of 56 cases showed positive reactions and the sizes of the reactions to the two antigens were so similar that the author found skin testing of little help in differentiating the balnei infection from the tuberculous infection. This work confirmed the earlier observations of Edwards and associates who found that M. balnei antigen gave reactions very similar in size from those elicited by RT tuberculin.

The Specific Tuberculin Reaction
Specific tuberculin skin sensitivity is caused by infection with M. tuberculosis, and is generally strongly positive. The size of the tuberculin skin reaction carries great significance when a standard dose of tuberculin is used. From extensive studies in human populations, Edwards and associates consider a reaction of 12 mm. or more to 0.0001 mg. PPD-S to be a specific response to infection with M. tuberculosis. Studies conducted in widely separated parts of the world also reveal that very few tuberculous patients have small tuberculin reactions, an observation which holds in persons who have either active or healed tuberculosis or have had close contact with tuberculous patients.

The Tuberculin Skin Testing Committee of the American Thoracic Society, after careful consideration, has chosen an arbitrary dividing line of 8-10 mm. of induration in response to the standard 5 TU (0.0001 mg.) dose of PPD-S. It is currently believed that this dividing line serves the practical purpose of separating most persons with tuberculous infections from those with tuberculin cross reactions due to infection with mycobacteria other than mammalian tubercle bacilli. Although it is understood that a few tuberculous infections may be missed, and that a few non-tuberculous infections may be called tuberculous, the 8-10 mm. dividing line appears feasible and practical. Clinical and epidemiologic studies conducted in the Houston area, have shown that a tuberculous infection generally gives reactions larger than 12 mm. to 0.0001 mg. PPD-S, when palpable edema, as well as central induration are included in the measurement of the tuberculin skin reaction.

Differential skin testing using multiple mycobacterial antigens is another approach to the identification of the specific tuberculin reaction. This concept is based on the fact that guinea pigs inoculated with one strain of mycobacteria will generally have stronger reactions to the antigen prepared from the same strain than to an equivalent dose of antigen derived from a different strain. By analogy it may be expected that persons infected with M. tuberculosis will react more strongly to the antigen prepared from M. tuberculosis (PPD-S) than to antigens prepared from other strains of mycobacteria.
In skin testing patients with disease due to *M. tuberculosis* in Rome, Georgia, Edwards and associates noted a stronger reaction to PPD-S than to PPD-B. At the Tuberculosis Division of Jefferson Davis Hospital in Houston, 100 patients with disease due to *M. tuberculosis* were skin tested with four mycobacterial antigens in 1962-63. The size of tuberculin (PPD-S) reaction as compared with that to PPD-Y, PPD-B and PPD-Br. are shown in scatter-diagrams 7A, 7B, and 7C in Fig. 7. It may be noted that with few exceptions, tuberculin reactions show more induration and edema than skin reactions to antigens from *M. kansasii* (PPD-Y), the Battey organisms (PPD-B), and scotochromogens (PPD-Br.). A typical example is shown in Fig. 6. This study confirms the earlier observation made at this hospital in 1960, when it was found that tuberculous patients showed stronger reactions to PPD-S than to PPD-B, PPD-Y as well as PPD-Aviun. This is known to us as the “tuberculin-predominating” pattern. In our experience this pattern is characteristic not only in patients with active disease due to *M. tuberculosis*, but is also the prevailing pattern of skin reaction in persons who have had contact with tuberculosis. The emergence of this type of reaction was seen to occur under our observation among tuberculous contacts in whom the tuberculin reaction converted from negative to positive.

**The Heaf Multiple Puncture Test**

While the intradermal skin test is the most accurate method for tuberculin testing, it suffers the disadvantage of being somewhat technically difficult. Children are apt to be frightened at the sight of a needle, and their alarm often increases the difficulty of performance and multiplies the error of the test. It is not surprising that many pediatricians limit the use of the intradermal test to a few cases presenting diagnostic problems. Large pediatric clinics are usually unable to provide the number of skilled personnel needed to make Mantoux testing a routine procedure. As a result, many children may go through health centers and clinics with tuberculous infection undetected. An easy and painless test for routine screening is therefore highly desirable.

The Heaf test is a multiple puncture tuberculin test developed by Heaf of Wales in 1951. It employs a “Heaf gun,” a spring-activated lancet which causes six prongs to be driven to a predetermined depth through a film of concentrated tuberculin solution on the skin. In the United States a modified Heaf gun with disposable lancet cartridges is known as the Sterneedle gun. The test is acceptable to children because there is no needle in sight and it is painless. Simplicity and ease of performance make it feasible for screening purposes. The antigen used is Weybridge PPD in a concentrated form containing 2 mg. of PPD in 1 ml. The degrees of reaction (Fig. 8) to the antigen have been described by Heaf and are as follows:

**Figure 6**: Differential skin testing using multiple PPD antigens.
FIGURE 8: Four grades of reactions to the Heaf test.

Grade I—4-6 discrete papules.
Grade II—Coalescence of papules forming an edematous ring.
Grade III—More intensive induration forming an elevated plaque.
Grade IV—Blister formation or sloughing.

The reliability and sensitivity of the Heaf test have been evaluated by a number of investigators. Henshaw, comparing the Heaf test and the Mantoux test (10 TU PPD) in 1,172 school children in Africa, reported in 1955 that there was a good correlation between the two and that the Heaf test was slightly more sensitive.

The British Tuberculosis Association reported in 1958 a cooperative study of six centers in England comparing the Heaf test and the Mantoux test (5 TU PPD) in 1,195 tuberculosis contacts. No important difference was found between the results of the two tests when read on the third day, and the Heaf test was regarded as being suitable for large-scale epidemiologic work.

However, further studies carried out in residential communities in Britain showed that the Heaf test was considerably more sensitive than the 5 TU Mantoux test. The greater number of Heaf reactors than Mantoux reactors was attributed to factors such as: the greater proportion of weakly positive reactors in the second test population; the late reading (7 day) of the Heaf test; and possibly other minor technical differences.

In New York, Robin and associates reported in 1960 a study comparing the Heaf test with the Mantoux test in 61,000 pupils entering New York high schools. It was noted that the Heaf test was more sensitive than the Mantoux test using 1:1000 dilution of Old Tuberculin or 5 TU PPD, and that part of the difference between the two tests could be eliminated if weak Grade I Heaf reactions were disregarded.

The authors and their associates have carried out a series of studies employing the Heaf test in the Houston area and com-
paring it with the 5 TU PPD Mantoux test. The results are summarized in the following paragraphs:

In the high tuberculin reactor group, the Heaf test and 5 TU Mantoux test gave very similar results. In 1960-61, 410 tuberculosis contacts were given both tests at the Tuberculosis Clinic. Reading at 48 hours showed that 238 had positive Mantoux reactions, 90 per cent of which were over 10 mm. in size, and that 254 had positive Heaf reactions, 90 per cent of which were Grade II or higher.

The Heaf test was found to be considerably more sensitive than the Mantoux test using 5 TU of PPD in children with essentially low-grade tuberculin skin sensitivity. To evaluate the Heaf test in persons with tuberculin cross reactions, the Heaf test was incorporated into the multiple mycobacterial skin test project which was carried out in Carver Elementary School in 1960. In this school each of 681 pupils was simultaneously given four skin tests, including the Heaf test and three Mantoux tests using 5 TU each of PPD-S, PPD-B, and PPD-Y. All tests were read at 48 hours. The analysis of the Heaf reactions against the reactions to PPD-S and PPD-B is shown as follows:

<table>
<thead>
<tr>
<th>Heaf Grade</th>
<th>Mantoux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade of Reaction</td>
</tr>
<tr>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>II, III, IV</td>
<td>I</td>
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</tbody>
</table>

Among the 74 children showing Grade I Heaf reactions, 61 had a negative Mantoux test, and 13 had a Battey-predominating type of Mantoux reaction (B>S) which were probably nontuberculous in origin. This observation is in harmony with the study recently reported by Griffith and associates in Cardiff. They noted that most of the children showing Grade I Heaf reactions had stronger reactions to the unclassified mycobacterial antigens than to the tuberculosis antigen and that the Battey-Avian group of mycobacteria were considered a likely source of such low-grade tuberculin sensitivity.

From the above-mentioned studies, it is recommended that when the Heaf test is used for tuberculosis screening, priority for follow-up examination should be given to the confluent reactors (ring, plaque, and blister) because among them true tuberculous infections are most likely to be found. The papule Heaf reaction (Grade I) must be classified as a doubtful reaction pending confirmation by the standard Mantoux test. This is particularly true in areas in which tuberculin cross sensitivity is prevalent. The Heaf test is not recommended for diagnostic use because of lack of dosage control.

The Tuberculin Tine Test

The tine test was developed by Rosenthal in 1961. Because of its recent introduction more time should be allowed for its evaluation. It is also a multiple puncture test, but differs from the Heaf test in that Old Tuberculin is used instead of PPD. The Old Tuberculin is dried onto specially constructed tines of a metal disk mounted on a small plastic holder. The test is read in 48-72 hours. An indurated papule of 2 mm. and over is considered a positive test.

In Chicago, Rosenthal compared the tine test with the Mantoux test using 1-10 TU Old Tuberculin. He reported that in the three groups of people studied, the tine test missed 7-13 per cent of the positive Mantoux reactors, and that false positive reactions occurred in 0-7.5 per cent of negative 10 TU Mantoux reactors.

In Boston, Badger and associates conducted a study comparing the tine and Mantoux tests using 5 TU PPD-S. Considering 2-3 mm. induration for a minimum positive tine, and 6 mm. induration for a minimum positive Mantoux, a good concordance of the two tests was observed. According to this study the optimum time for reading is 72 hours after the test.

In Houston, the authors and their associates made a preliminary study on the tine test in 1962 with an attempt to evaluate
the test in an area with a known high prevalence of low-grade tuberculin sensitivity. The study was carried out both in the high tuberculin reactor group and the low tuberculin reactor group.

For the high tuberculin reactors, 110 tuberculosis contacts were used. Each person was given simultaneously a tine test on one forearm and a Mantoux test, using 5 TU PPD-S, on the other. Both tests were read 48 hours later. Of the 24 persons showing a positive Mantoux reaction, 23 also reacted positively to the tine test. In most cases, both tests were strongly positive. Among the 86 persons having negative Mantoux reactions, 84 also had negative reactions to the tine test. Therefore, in a high tuberculin reaction group, the tine test compares well with the 5 TU Mantoux test.

For the low tuberculin reactor group, 95 sixth graders of St. Rose of Lima Elementary School were used. Each child was simultaneously given the tine test along with three Mantoux tests using 5 TU PPD-S, PPD-B, and PPD-Br., respectively. All tests were read at 48 hours. Considering 2-3 mm. induration for a minimum positive tine test, and 6 mm. induration for a minimum positive Mantoux reaction, the two tests were compared as follows: Only four children had a positive reaction to PPD-S. Three of the four were also positive to the tine test. Of 91 children showing a negative reaction to PPD-S, 21 had a positive reaction to the tine test with indurated discrete papules measuring 2-3 mm. It appears that the tine test would pick up a good deal of low-grade tuberculin sensitivity if 2 mm. and 3 mm. papules were to be considered as positive. The source of the low-grade sensitivity is probably the Battey-scotochromogen type of infection, because over 50 per cent of the 2-3 mm. reactors had a positive reaction to PPD-B and PPD-Br. of over 10 mm. induration.

Therefore, while the tine test compares favorably with the Mantoux test in Chicago and Boston, it is considerably more sensitive than the 5 TU Mantoux in Houston, where low-grade tuberculin sensitivity is comparatively prevalent. This preliminary study points to the need for further evaluation of the tine test, particularly in the low tuberculin sensitivity group.

The Patch Test

The tuberculin patch test is greatly inferior to the Mantoux test since it gives both false negative and false positive reactions. False negative reactions are more common in hot weather when excessive perspiration causes premature detachment of the patch. In office practice or on home calls when other tests are impractical, the patch test may be used, but only with a full realization of its limitations.

Discussion

There is no doubt that the problem of tuberculin cross reactions must be faced in many geographic areas. Cross reactions do not, however, make the tuberculin test less valid. Instead, an awareness and proper interpretation of tuberculin cross reactions only enhances the usefulness of the tuberculin skin test. In the past, many cross reactions were probably misinterpreted as indicating tuberculous infections with consequent erroneous diagnosis in clinical practice and undue inflation of the tuberculous infection rate in epidemiologic surveys.

The tuberculin available today is far from being pure. Even standard PPD contains protein fractions common to several types of mycobacteria. Taking into account the studies cited earlier, the authors have attempted to illustrate the present-day concept of the tuberculin reaction in Fig. 9 and Fig. 10.

Fig. 9 depicts the relative frequency of tuberculin cross reactions (crosshatch) at different levels of tuberculin sensitivity as measured by the size of the skin reaction to 5 TU of PPD-S. Cross reactions are very common when the sizes of reactions are less than 6 mm. (white zone), and they are less common when the reactions are 6-12 mm. in size (gray zone). Cross reactions are least common when the tuber-
culin reactions exceed 12 mm. in size (black zone).

The specific tuberculin reaction is indicated by the solid area in Fig. 9. Contrary to the cross reactions, they are predominately large reactions exceeding 12 mm. in size to 5 TU PPD-S, although some of the specific reactions may be smaller in size.

Fig. 9 also illustrates the frequency of tuberculin cross reactions at different geographic locations. Thus a physician practicing in area A, where cross reactions are very prevalent, can reasonably assume that a weak (6-12 mm.) reaction to 5 TU is likely to be nontuberculous in origin, whereas it would not be safe for a physician practicing in area B to make such an assumption, because of the infrequency of cross reactions in this area.

Fig. 10 shows the effect of the tuberculous infection on the perceptibility of the tuberculin cross reaction. In a population of low tuberculous infection rate (a), such as the elementary school children in Houston, the tuberculin cross reactions would be clearly evident\(^6\) and one could expect to find in such a group many weak tuberculin skin reactions. On the other hand, in a population of high tuberculous infection rate (b), such as tuberculosis contacts\(^6\), one would find the weak tuberculin cross reaction overshadowed by the strong spe-
specific tuberculin reaction in most instances. Consequently, weak tuberculin reactions would be few in the tuberculosis contact group. Finally, in the tuberculous patients (c) the weak cross reaction is seldom noticeable because it is almost completely overshadowed by the specific tuberculin reaction which is usually strongly positive.5,16,23

Interpretation of tuberculin test in healthy children. The previously mentioned concept of the tuberculin reaction has practical implications. When the tuberculin test is used for tuberculosis screening or for epidemiologic surveys, it is desirable to eliminate cross reactions insofar as possible in order to make tuberculosis detection more effective and to avoid undue inflation of the tuberculous infection rate in epidemiologic surveys. When 5 TU of PPD-S is used, an arbitrary dividing line at 8-10 mm. induration would serve to separate most of the cross reactions from the specific reactions except in localities where cross reactions are very prevalent. In the latter case it would be expedient to set the dividing line at 12 mm. or higher.

In the Houston area, where tuberculin cross reactions are very prevalent, an arbitrary dividing line at 12 mm. is preferred. In the elementary schools, practically all children showing a tuberculin reaction greater than 12 mm. had a “tuberculin-predominating” pattern of reaction when other mycobacterial antigens were used. This pattern is compatible with tuberculous infection, and a source of tuberculous infection frequently can be found. In the Houston area, however, the smaller tuberculin reactions were mostly “Battey-predominating,” and no source of tuberculosis was uncovered.

When multiple puncture tuberculin tests (Heaf and time) are used, specific tuberculin sensitivity is usually indicated by a high-grade skin reaction in which indurated papules coalesce to form a ring or plaque. In some cases, there may even be blister formation. On the other hand the low-grade reaction of discrete papules must be interpreted with caution. In areas in which the tuberculin cross reaction is prevalent, most of the discrete papules are probably indicative of infection with mycobacteria other than mammalian tubercle bacilli.

Tuberculin test for clinical diagnosis in children. In clinic practice and hospital work, the tuberculin reaction must be interpreted with greater reservation because the physician deals with sick children as individuals instead of with a group of predominantly healthy children. A skin reaction of 10 or 12 mm. induration to 0.0001 mg. PPD should be considered an indication of tuberculosis infection until proven otherwise. Smaller reactions cannot be dismissed as nontuberculous, because occasionally a tuberculous child may show a small reaction. When in doubt, further examinations should be made.

First, technical error should be ruled out by having the test repeated, preferably by the physician himself who will be sure that the antigen and equipment are adequate, the test properly administered, and the reaction carefully measured. Intradermal tuberculin testing, particularly in children, is an exacting procedure requiring experience and skill. Tests performed by inexperienced persons often give misleading results.

Further observation to indicate whether or not there has been a change in tuberculin sensitivity over a period of time often gives important clues. A small reaction in the incipient stage of a tuberculous infection could develop into a strongly positive reaction within a few weeks. On the other hand, a cross reaction will usually tend to wane in time or it may exhibit evanescence.

Investigation of contacts is helpful. The presence of a person with active tuberculosis in the child’s environment, or the finding of positive tuberculin reactions in the siblings are important pieces of circumstantial evidence.

It must be remembered that the tuberculin test is but one of many tests which can be used in identifying tuberculosis.
When tuberculosis is suspected, one should look for other evidences of this disease by physical examination, the chest roentgenogram, sputum or gastric smears and cultures and other types of examination.

The use of chemoprophylaxis for tuberculosis has made proper interpretation of tuberculin reaction more urgent than ever. It is now generally agreed that children who have an increased risk of developing clinical tuberculosis should be given isoniazid therapy for the prevention of manifest disease. In such cases, a positive tuberculin reaction is usually the only evidence of a tuberculous infection. If the tuberculin reaction proves doubtful, simultaneous skin test using multiple mycobacteria antigens can be very helpful. Unfortunately, the mycobacterial PPD antigens are in very limited supply at this time and probably will not be available for general use for some time to come. For young children, particularly infants in whom there is great risk of developing tuberculosis, it would be expedient to start chemoprophylaxis with isoniazid while other evidences of tuberculous infection or disease are being sought.

SUMMARY

(1) The tuberculin test is the most effective means of detecting tuberculosis in children. It is essential for early diagnosis and prevention of serious consequences of this disease.

(2) Recent studies on mycobacterial infections have provided better understanding of the tuberculin reaction. When a standard dose of 0.001 mg. PPD is used, the size of the reaction carries great significance. A large reaction usually indicates a tuberculous infection, whereas a small reaction may or may not be tuberculous in origin.

(3) The specific tuberculin reaction caused by the tubercle bacilli is usually strongly positive. In differential skin testing, the standard tuberculin (PPD-S) usually gives a stronger reaction than the other mycobacterial antigens.

(4) The tuberculin cross reaction may be caused by a variety of non-tuberculosis mycobacteria. It is usually weakly positive. In differential skin testing, the homologous mycobacteria antigen usually gives stronger reaction than the standard tuberculin.

(5) When the standard tuberculin (PPD-S) is used alone, it is not always possible to distinguish between the specific reaction and the cross reaction by its size. Generally speaking, the stronger the reaction, the greater the likelihood of its being a specific tuberculin reaction.

(6) The multiple puncture tuberculin tests are useful for routine screening. However, because of lack of dosage control they are not recommended for diagnostic use. In areas where low-grade tuberculin sensitivity is prevalent, the Grade I (discrete papules) reactions should be classified as doubtful instead of positive, because many of them probably represent a cross reaction from nontuberculous infections.

(7) The patch test is much inferior to the Mantoux test because it may give both false positive and false negative reactions. When other methods cannot be used, the patch test may be utilized as a preliminary measure pending confirmation by the standard intradermal test.

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Resumen

1. El medio mas efectivo para descubrir la tuberculosis en los niños es la tuberculíneracción. Es esencial para un diagnóstico temprano y
para prevenir las graves consecuencias de esta enfermedad.

2. Los estudios recientes sobre la micobacteria de la tuberculosis han proporcionado una mejor comprensión de la reacción tuberculínica.

Cuando se usa una dósis estandar de 0,0001 mg PPD, el tamaño de la reacción es de gran significación. Una reacción grande habitualmente indica una infección tuberculosa en tanto que una reacción pequeña puede ser o no, de origen tuberculoso.

3. La reacción tuberculínica específica causada por el bacilo de la tuberculosis es generalmente fuertemente positiva. En el estudio diferencial de las reacciones la tuberculina estandar (PPD-S) habitualmente da una reacción más intensa que los otros antígenos de micobacterias.

4. Pueden encontrarse reacciones tuberculínicas cruzadas con una variedad de micobacterias no tuberculosas. Generalmente en estas condiciones es debilmente positiva. En las pruebas diferenciales los antígenos de micobacterias homólogas suelen dar reacciones más intensas que la tuberculina estandar.

5. Cuando se usa la tuberculina es andar (PPD-S) solamente, no es posible siempre distinguir entre la reacción específica y la cruzada, por su tamaño. En general, a mayor reacción mayor posibilidad de que se trate de una reacción específica tuberculosa.

6. Las reacciones con la punción múltiple son útiles en la busqueda de rutina. Sin embargo, por la falta del control de la dosis, no son recomendables para uso diagnóstico. En las regiones donde hay una sensibilidad baja en la población el grado I (papulas discretas) debe ser clasificado como dudoso en lugar de positivo, porque muchas de estas reacciones son cruzadas de infecciones no tuberculosas.

7. La reacción del parche es muy inferior a Mantoux porque puede dar falsas positivas y falsas negativas. Cuando no se pueden usar otros métodos la reacción del parche puede usarse como preliminar pendiente de confirmación por el método estandar intradérmico.

**Resumé**

1. Le test tuberculínique est le moyen le plus efficace de détecter la tuberculose chez les enfants. Il est essentiel pour le diagnostic précoce et la prévention des conséquences graves de cette affection.

2. Des études récentes sur les infections mycobactériennes ont apporté une plus grande compréhension de la réaction à la tuberculine. Lorsqu'on utilise une dose standard de 0,0001 mg de tuberculine PPD, la taille de la réaction a une grande signification. Une réaction étendue indique habituellement une infection tuberculose, tandis qu'une petite réaction peut être d'origine tuberculose ou ne pas l'être.

3. La réaction spécifique à la tuberculine causée par le bacille tuberculique est habituellement fortement positive. Dans le test cutané différentiel, la tuberculine standard (PPD-S) donne habituellement une réaction plus importante que les autres antigènes mycobactériens.

4. Le test croisé à la tuberculine peut être provoqué par une variété de mycobacteries non tuberculeuses. Elle est habituellement faiblement positive. Dans le test cutané différentiel, l'antigène mycobactérien homologue donne habituellement une réaction plus importante que la tuberculine standard.

5. Lorsque la tuberculine standard (PPD-S) est utilisée seule, il n'est pas toujours possible de distinguer entre la réaction spécifique et la réaction croisée après la taille. En règle générale, plus la réaction est importante, plus il est vraisemblable qu'elle représente une réaction spécifique à la tuberculine.

6. Les tests tuberculiques avec scarifications multiples sont utiles pour l'examen rapide de pratique courante. Cependant, à cause du manque de dosage témoin, ils ne sont pas recommandés pour l'utilisation en vue du diagnostic. Dans les zones où une faible sensibilité à la tuberculine est prédominante, les réactions de type I (papules discrètes) devraient être classées comme douteuses au lieu de positives, parce que beaucoup d'entre elles représentent probablement une réaction croisée à partir d'infections non tuberculeuses.

7. Le test par tibre cutané est très inférieur au test de Mantoux parce qu'il peut donner à la fois des réactions faussement positives et faussement négatives. Quand les autres méthodes ne peuvent être utilisées, le tibre peut être employé comme mesure préliminaire attendant la confirmation par le test intradérmique standard.

**Zusammenfassung**

1. Die Tuberkulinprobe ist die wirksamste Methode zur Ermittlung der Tuberkulose bei Kindern. Sie ist wesentlich zur Frühdiagnose und zur Verhütung ernsthafter Krankheitsfolgen.


3. Die spezifische Tuberkulin-Reaktion, hervorderufen durch den Tuberkelbazillus, ist für gewöhnlich stark positiv. Bei der Differenzierung
von Hautproben ergibt das Standard-Tuberkulin (PPD-S) für gewöhnlich eine stärkere Reaktion als die anderen mykobakteriellen Antigene.


5. Wird das Standard-Tuberkulin (PPD-S) allein angewandt, so läßt sich nicht immer zwischen der spezifischen Reaktion und der Kreuzreaktion durch deren Größe eine Unterscheidung treffen. Allgemein gesagt: je lebhafter die Reaktion, desto größer die Wahrscheinlichkeit, daß es sich um eine spezifische Tuberkulin-Reaktion handelt.


7. Die Pfisterprobe ist der Mantoux-Probe erheblich unterlegen, da sie sowohl falsche positive als auch falsche negative Reaktionen ergibt. Können andere Methoden nicht angewandt werden, so kann die Pfisterprobe als eine vorläufige Methode benutzt werden bis zur Bestätigung durch die intrakutane Standard-Methode.

**References will appear in the reprints.**

For reprints, please write Dr. Hsu, 1801 Allen Parkway, Houston 77019.

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**TUMOR-SIMULATING INTRATHORACIC MARROW HETEROTOPIA IN THALASSEMAIA MAJOR**

The plain chest radiographs of the patients revealed multiple, soft, well-outlined, round or oval shadows located in the paraspinal region of the mediastinum. Tomography showed that the masses were located posteriorly in the costovertebral angle adjacent to the vertebral bodies and the proximal part of the ribs. The size ranged from 1 cm. to 6 cm. In the first case, the whole mediastinum was occupied, in the second, only the upper part, and in the third, the masses were located in the lower part just above the diaaphragm. In the two last cases, they located exclusively on the right side, whereas in the first the process was bilateral, although more prominent on the right. The superimposition of the masses often gives a lobulated appearance to the whole process, but sometimes a segmental arrangement is present.

The patients were all anemic young adult men suffering from Cooley’s disease. Radiographs of the bones showed changes typical of a marked degree of Cooley’s anemia, namely coarse trabeculation, expansion and extreme demineralization. Flattening of the vertebral bodies was also present. The results of isotopic studies with radioactive Cr⁵¹ and Fe⁵⁹ were consistent with the blood disease. Since masses of this sort in the posterior mediastinum are frequently neoplastic and these patients are subjected to surgical exploration, establishment of the correct diagnosis is important as unnecessary operations can then be avoided.


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**AN ELECTRONICALLY CONTROLLED HIGH PRESSURE INJECTOR FOR ANGIOCARDIOGRAPHY**

An electronically controlled, high-pressure injector for angiocardiotherapy has been described. The injector can deliver 50 ml. of contrast medium through a 9F angiography catheter in 750 milli-seconds, or with a smaller (30 ml.) syringe, 30 ml. can be delivered through an 8F catheter in 500 milliseconds. An electronic control unit enables injections to be made in a single diastole, in successive diastoles or according to other timing modes. Facilities have been developed to superimpose the time of injections on the electrocardiogram together with a display on cinematographic film.