Associations Between Kveim Test Results, Previous B.C.G. Vaccination, and Tuberculin Sensitivity in Healthy Young Adults

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A trial of tuberculosis vaccines in more than 54,000 adolescents, nearly all aged 14 to 15½ years and attending school in urban or suburban areas, was started in England in 1950-2 (Medical Research Council, 1956, 1959, 1963). Those found to be tuberculin-negative on entry to the trial—that is, with no induration, or one of less than 5 mm., both to 3 and to 100 tuberculin units (T.U.) of Old Tuberculin—were vaccinated with B.C.G. or vole-bacillus vaccine or left unvaccinated, according to a method of random allocation. All the participants, including those who were tuberculin-positive to 3 or to 100 T.U. initially, were followed up intensively, with annual postal inquiries, home visits, radiographic examinations, and tuberculin tests, until September 1960 (which represented an average period of 8.8 years).

Of the tuberculin-negative participants, 13,598 were allocated to the B.C.G.-vaccinated group. All had had a clear chest radiograph immediately before vaccination, and just over half, not selected in any special way, had further tuberculin tests within a year after vaccination (nearly all within six months); of these, 17 (0.23%) were still negative to 100 T.U.; the remainder were positive either to 3 T.U. (85.3%) or to 100 T.U. (14.4%).

It was expected that some of the small number who were still tuberculin-negative would represent failures of the vaccination (or of the tuberculin tests) for technical reasons, but it seemed likely that others had a temporary or lasting tuberculin anergy, a condition recognized by previous workers—for example, Ustvedt (1951), Borrie (1952), Stewart and Beckwith (1960), and Sparrow and Carré (1960). We considered such individuals to be of particular immunological interest, not merely as regards their subsequent susceptibility to tuberculosis, but also because they might include some occult cases of sarcoidosis, in which disease a depression of tuberculin sensitivity and little or no enhancement thereof after B.C.G. vaccination are common (see Israel and Sones, 1961).

During the follow-up no case of sarcoidosis (nor any case of tuberculosis) was discovered either among the above 17 participants, who were known to have been negative to 100 T.U. soon after B.C.G. vaccination, or among any of the 35 additional participants in whom evidence that they had not become positive to 100 T.U. following B.C.G. vaccination was based on tests only made later (the possibility that some of these 35 had become positive and then reverted to negativity can therefore not be excluded). (In the total of 54,239 participants in the vaccines trial, 38 cases of definite or possible sarcoidosis and 504 cases of tuberculosis are known to have developed during the intensive follow-up; as just implied, none of these cases conformed to the criteria of the above 52 participants.) Despite these negative findings for sarcoidosis in the vaccines trial records for the 52, it was decided in 1960 to make a special

investigation of as many of them as were available and still tuberculin-negative. These volunteers, by then young adults, were to be given a second B.C.G. vaccination, and, if still tuberculin-negative subsequently, a special clinical examination for signs of sarcoidosis. A blood sample would be taken and a Kveim test (a test which is often positive in clinical sarcoidosis, and is regarded by many authorities as specific for this disease) would also be made. In addition, as explained fully below, Kveim tests were to be made on those who had become tuberculin-positive after the second vaccination, and also on other volunteers, all drawn from the participants in the vaccines trial, but with different histories of tuberculin sensitivity and of B.C.G. vaccination, in the hope of elucidating further the relation between tuberculin sensitivity, the Kveim test, and sarcoidosis.

There were particular advantages in basing the present study entirely on the participants in the vaccines trial, since this ensured that all the volunteers came from one broad community sample, and were of similar age; moreover, they had had serial tuberculin tests and chest radiographs extending over a period of 8 to 10 years, so that their tuberculin status could be closely defined. Although the vaccines trial has therefore been a source of suitable subjects, and of past information about them, the present study is entirely separate.

Fortunately, Kveim test material shown to have a substantial degree of specificity for sarcoidosis (Siltzbach, 1961b; Hirsch *et al.*, 1961; Anderson *et al.*, 1963) was made available to us for the whole inquiry, through the kindness of Dr. L. E. Siltzbach, of New York.

In the initial stages of the study it became clear that relatively few of the 52 participants who had not become tuberculin-positive after B.C.G. vaccination in 1950-2 would be available for a second B.C.G. vaccination; some were geographically inaccessible or for various reasons unavailable, and by 1960-2 many were no longer tuberculin-negative. However, a number of the latter had never shown more than an occasional weak tuberculin-positive reaction (5-9 mm. induration to 100 T.U.), and thus could also provisionally be regarded as having tuberculin anergy. It was therefore decided to adopt a definition for non-conversion to tuberculin sensitivity after vaccination (see below) so that such participants, as well as those consistently with 0-4 mm. induration to 100 T.U., would be eligible for the second B.C.G. vaccination; but even so, only 13 of the 52 (including 6 of the 17 referred to above) were available for this vaccination. In addition, however, this definition for non-conversion permitted the inclusion of 6 additional vaccines trial participants, not in the 52, whose first post-vaccination tuberculin test with 100 T.U. showed 5-9 mm. induration and who had never shown larger reactions subsequently.

Thus, in all, 19 originally non-converted subjects were available for the second B.C.G. vaccination and the subsequent

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Investigations (groups 1 and 2 below). The other subjects, with different histories of tuberculin sensitivity and of B.C.G. vaccination, who were also drawn from the vaccines trial for comparative Kveim testing, totalled 48 (groups 3 to 8 below).

Plan and Conduct of the Study

Definitions

To permit a clear description of this study and its findings it is desirable to assign special meanings to certain terms not used in connexion with the tuberculosis vaccines trial, and to avoid using others adopted in that trial. In particular, the arbitrary concepts of tuberculin-negative and positive have been replaced by those of non-reactors and reactors, and the (also arbitrary) definition of non-conversion to tuberculin sensitivity following vaccination is slightly wider than that used in the vaccines trial. (It may be noted, in justification for this widening, that the results for the additional subjects were similar to those for subjects who conformed to the trial definition—see below. Moreover, even this widened definition is probably more stringent than in studies not using 100 T.U. tests.) The terms used here are as follows:

Reactor: An individual showing any detectable induration to a specified dose of tuberculin.

Non-reactor: An individual showing no induration at all to a specified dose of tuberculin.

Converted: Refers to an individual (with 0–4 mm. induration to 100 T.U. before vaccination) showing induration 5 mm. or more in diameter to 3 T.U. (or 10 mm. or more to 100 T.U.) following B.C.G. vaccination.

Non-converted: Refers to an individual (with 0–4 mm. induration to 100 T.U. before vaccination) showing induration 0–4 mm. in diameter to 3 T.U. and 0–9 mm. to 100 T.U. after B.C.G. vaccination. (This definition has thus been widened to include a post-vaccination induration 5–9 mm. in diameter to 100 T.U., since few individuals consistently showed induration 0–4 mm. in diameter to 100 T.U. after B.C.G. vaccination.)

Original: At the time of entry to the vaccines trial—namely, in 1950–2.

Recent: In connexion with the present investigation—namely, in 1960–3.

Selection of Subjects for Kveim Tests

Kveim tests were made on 19 originally non-converted subjects (groups 1 and 2 below) as well as on 48 subjects, in six other groups, also drawn from the vaccines trial participants.

1. *Non-converted by original and recent B.C.G. vaccinations* (12 subjects).—0–4 mm. induration to 100 T.U. at original test (in fact, no induration at all in all 12); given B.C.G. vaccine in 1950–2, typical scar noted (in 10 subjects), 0–9 mm. induration to 100 T.U. at subsequent tests; given B.C.G. vaccine in 1960–2, typical scar noted (in 11 subjects), 0–9 mm. induration to 100 T.U. at subsequent tests. (Four of the 12 subjects in this group had 0–4 mm. induration to 100 T.U. at every test following both vaccinations; three of these (referred to later as subjects A, B, and C) had no induration at all to 100 T.U. at any of these tests; subject A showed no scar following the first, and subject B no scar following either, vaccination.)

2. *Non-converted by original but converted by recent B.C.G. vaccination* (7 subjects).—0–4 mm. induration to 100 T.U. at original test (in fact, no induration at all in all 7); given B.C.G. vaccine in 1950–2, typical scar noted, 0–9 mm. induration to 100 T.U. at subsequent tests; given B.C.G. vaccine in 1960–2, typical scar noted, 5 mm. or more induration to 3 T.U., or 10 mm. or more to 100 T.U., at subsequent tests.

3. *Converted by original B.C.G. vaccination (originally non-reactors)* (10 subjects).—No induration at all to 100 T.U. at original test; given B.C.G. vaccine in 1950–2, typical scar noted, 5 mm. or more induration to 3 T.U. at subsequent tests; no subsequent vaccination.

4. *Converted by recent B.C.G. vaccination (previously non-reactors)* (10 subjects).—No induration at all to 100 T.U. at original test; left unvaccinated in 1950–2, no induration at all to 100 T.U. at subsequent tests; given B.C.G. vaccine in 1960–2, typical scar noted, 5 mm. or more induration to 3 T.U. at subsequent tests.

5. *Converted by recent B.C.G. vaccination (previously weak reactors)* (5 subjects).—0–4 mm. induration to 100 T.U. at original test; left unvaccinated in 1950–2, 0–9 mm. induration to 100 T.U. at subsequent tests (and at some time 5–9 mm.); given B.C.G. vaccine in 1960–2, typical scar noted, 5 mm. or more induration to 3 T.U. at subsequent tests.

6. *Natural strong reactors to tuberculin* (5 subjects).—10 mm. or more induration to 3 T.U. at original test; not vaccinated in 1950–2 (or later), 5 mm. or more induration to 3 T.U. at subsequent tests (in fact, nearly always 10 mm. or more).

7. *Natural weak reactors to tuberculin* (10 subjects).—0–9 mm. induration to 100 T.U. at original test; not vaccinated in 1950–2 (or later), 0–9 mm. induration to 100 T.U. at subsequent tests (and at some time 5–9 mm.).

8. *Non-reactors to tuberculin* (8 subjects).—No induration at all to 100 T.U. at original test; left unvaccinated in 1950–2 (and later), no induration at all to 100 T.U. at subsequent tests.

The general procedure for recruiting subjects to these groups was first to select individual participants in the vaccines trial who were potentially eligible on the basis of their vaccination and tuberculin test history. Their eligibility was checked with a further tuberculin test, followed, if indicated, by B.C.G. vaccination and a tuberculin test three to five months after the vaccination. The Kveim test was given after the eligibility of the subject for the appropriate group had been confirmed. In group 1 the interval between confirmation of eligibility (by tuberculin test) and the Kveim test ranged from one week to six months. In group 2 there was a delay of about two years before the Kveim tests could be given, and these tests were therefore immediately preceded by a second confirmatory tuberculin test (in five of the seven subjects). In the other groups the interval was at most one month.

This procedure involved screening much larger numbers of individuals than could finally be included in the groups. For groups 1 and 2, as already explained, every potentially eligible trial participant had to be covered by the screening process (including the known cases of sarcoidosis and tuberculosis, although none of these conformed). For the other groups sufficient numbers were obtained from an alphabetical register of the trial participants, excluding those geographically inaccessible, presumptively non-cooperative, or with current major illness; known cases of tuberculosis were excluded, and by chance no known case of sarcoidosis was selected. Although the selection of suitable volunteers from among those eligible for the various groups cannot therefore be regarded as random, there is no reason to suppose that the selection procedure has introduced any salient differences between the groups, apart from the essential contrast in their vaccination and tuberculin test histories. The proportions of eligible trial participants who were finally included in each group certainly varied from category to category (being, for example, very much higher for group 1 than for group 3 or group 8), but these proportions cannot be determined since the screening procedure involved several stages, which differed according to the category. In retrospect, it would have been more satisfactory if groups 3 to 8 had included more subjects; but it was important at the time of selection to preserve a balance between the sizes of the individual groups, and, moreover, the amount of Kveim test material available from the current lot was very limited.

Between them the eight categories covered a wide range of different vaccination and tuberculin-test histories, and permitted a number of hypotheses to be examined. The investigation was started with subjects in some of these categories only, but the early results were so surprising that it became necessary to extend the comparisons by including new categories and expanding others. It is, however, simpler to present the findings

in a different order from that in which the information became available.

Kveim Test

The Kveim test consisted of an injection of test material, followed by biopsy of the site at least four weeks later. Histological assessments were made, the macroscopic appearances before biopsy having been recorded separately.

The Kveim test material was all derived from the spleen of a patient ("J") aged 24 years, suffering from sarcoidosis. She had no history of tuberculosis or of B.C.G. vaccination, and was negative to 250 T.U. of P.P.D. in July 1954. The spleen had been removed surgically in May 1954 for hypersplenism, and has since been stored in the frozen state. (Histologically, the spleen was permeated by non-caseating epithelioid cell follicles, an appearance compatible with splenic sarcoidosis.) The test material was prepared by Dr. M. W. Chase and Dr. Siltzbach (see Chase, 1961; Siltzbach, 1961a) and supplied from a single lot (lot 7, type 1, 2.4 mg./ml., a dose of 0.2 ml. containing 480 µg. of alcohol-precipitable material). It was sent by air to London in four separate deliveries, and was stored at 4° C. until used. (The results were similar with all four deliveries, and have therefore been pooled.)

The tests were made by one of us (D.N.M.) according to the following procedure, based on that recommended by Siltzbach and Ehrlich (1954). The phial of Kveim-test material was shaken well and 0.2 ml. was injected intracutaneously, from a tuberculin syringe, on the flexor aspect of the forearm. The site of injection was recorded for subsequent identification by a careful standard measuring technique. The diameter of any induration at the test site was recorded by the tester two and four weeks after making the test, and the site was also examined on other occasions, and finally just before the biopsy. Care was taken on each occasion, by stretching the skin over the site after cleaning with ether, not to overlook a tiny or deep-seated nodule.

All 67 volunteers had agreed specifically to the Kveim test and biopsy (following a detailed explanation), but three (two from group 1, one from group 8) having been tested, refused the biopsy. Immediately before the biopsy, which was performed (in all but two instances) by Mr. S. C. F. Stephenson, an area enclosing the test site and any visible (or palpable) nodule was ringed with a skin pencil. If there was no detectable nodule the test site was indicated by intersecting pencil lines. The full thickness of skin down to the subcutaneous tissue, enclosing the nodule or test site, was excised under procaine anaesthesia. Thus the biopsy was made even if no nodule was detected. Of the 64 biopsies, 58 were made between four and seven weeks after the test, and six between 7 and 17 weeks (including four which were delayed because there was no visible nodule). Sections were prepared and stained with haematoxylin and eosin (a few with other stains) in the Department of Histology, General Hospital, Birmingham (Dr. D. B. Brewer).

The same slides were submitted separately to two independent assessors, Dr. Siltzbach and Professor J. G. Scadding. Each batch of slides (there were three separate batches for Assessor 1 and two for Assessor 2) contained individuals from several but not all of the eight groups of subjects, presented in a random order. The set of slides for each individual was identified only by a code number. The assessors were unaware of the vaccination or tuberculin-test history of any of the individuals, and of the clinical and radiographic findings.

The result of each Kveim test was classified by the assessors as positive, equivocal, or negative on histological grounds,¹ without any knowledge of the naked-eye appearances of the test site. (Each assessor originally graded his positive readings, but these gradings were later agreed to be insufficiently meaningful to be used.) The term negative includes a report of

"normal skin" (which could, however, mean that a reaction had been missed by the sections), as well as "non-specific reactions."

Because the time-table of the investigation made it impracticable for the assessors to read the slides in a single randomized series, an extra precaution was taken. Five of the early sets of slides, selected to represent the various assessments, were renumbered and resubmitted to each assessor, without his knowledge, as part of the final batch. Each assessor reproduced his former verdict (and with the same grading of the positive reactions) on the second reading.

In addition to the histological assessments, the size of any nodule at the test site (diameter of induration at four weeks) was used to express the result of the test quantitatively. In subjects with positive Kveim test results this measure is referred to as the "specific nodule size" (Siltzbach, personal communication).

Tuberculin Tests

The tuberculin tests, both during the vaccines trial and in connexion with the present investigation, were all made according to a standard procedure: (a) An intracutaneous (Mantoux) test was made on the forearm with 3 T.U., using 0.1 ml. of 1/3,000 Old Tuberculin (international standard strength; a single batch of heat-concentrated synthetic medium human-type tuberculin, prepared in June 1950 by the Ministry of Agriculture, Fisheries and Food, Veterinary Laboratory, Weybridge, Surrey; tests made in 1960 and 1962 have shown that this batch is still of international standard strength); the greatest diameter of palpable infiltration (induration) at 72 hours was recorded in millimetres. (b) If there was no induration, or if the greatest diameter of induration was less than 5 mm., another intracutaneous test was made on the same forearm with 100 T.U., using 0.1 ml. of 1/100 Old Tuberculin; the greatest diameter of induration at 72 hours was again recorded.

The number of tuberculin tests per subject, during the vaccines trial and up to the time of the Kveim test, ranged from 3 to 11 (from 4 to 9 for the subjects in group 1).

B.C.G. Vaccines

For all the original vaccinations (groups 1, 2, and 3), and for the recent vaccinations in groups 1 and 2, liquid B.C.G. vaccine was used. The other recent vaccinations (groups 4 and 5) were all made with freeze-dried vaccine, the liquid product being no longer available. (The results indicate that this difference in type of vaccine was irrelevant.) The batches of liquid vaccine were routine issues, freshly prepared by the State Serum Institute, Copenhagen. Each batch was stored at 4° C. and used within eight days of its receipt and within 14 days of the harvesting of the cultures. The batches of freeze-dried vaccine (derived from the State Serum Institute's strain) were routine issues prepared by Glaxo Laboratories Ltd., Middlesex, and were also stored at 4° C. until used. The dose of each vaccine was 0.1 ml. injected intracutaneously, in male subjects in the left deltoid region, and in female subjects in the upper and outer part of the left thigh (original vaccination) and either in the left thigh or in the left deltoid region (recent vaccination). Each subject who had a recent vaccination was

¹ "A positive Kveim reaction consists of a collection of epithelioid cell follicles with intermixture of occasional Langhans-type giant cells and non-specific inflammatory cells. Small areas of fibrinoid change in the granulomas are not uncommon. The entire appearance simulates that seen in spontaneous cutaneous sarcoids. In an equivocal Kveim reaction, epithelioid cells may be few and are not the predominant cell type in the test granuloma. The follicles are poorly circumscribed and may contain large areas of necrosis, inordinate numbers of giant cells, and birefringent material observed under polarized light" (Siltzbach, personal communication; see also Siltzbach and Ehrlich, 1954; Siltzbach, 1961a, 1961b, 1961c).

examined on several occasions during the first few weeks after the vaccination, to note the course of the local reaction at the vaccination site.

The fact that more than 99% of the trial participants given B.C.G. vaccine had become tuberculin-positive (to 100 T.U.) within a year after the original vaccination (see above) indicates that the batches used in 1950–2 were technically satisfactory. Information has been obtained on tuberculin conversion following the routine use (in the national B.C.G.-vaccination scheme for schoolchildren in Britain) of nearly all the batches used for the recent vaccinations in the present investigation; these batches also appear to have been technically satisfactory.

Clinical Examinations

At the time when the Kveim test was performed each of the 12 subjects in group 1 (non-converted by original and recent B.C.G. vaccinations) was examined by one of us (D. N.M.) for possible lesions (notably of the eye and skin) which might indicate sarcoidosis; particular attention was paid to possible changes in any scars (including the two B.C.G. scars). A personal and a family history of definite or possible tuberculous manifestations were taken. (The medical records of the vaccines trial were, of course, also available for each subject.) A further intracutaneous test was made with 100 T.U. mixed with cortisone, to see if the latter affected the result (Pyke and Scadding, 1952); and a blood sample was examined for its content of serum calcium, total protein, and protein fractions (Dr. R. Gaddie). At a later date seven of the subjects in group 1 were given a special ophthalmological examination, including the use of a slit-lamp (Mr. S. J. Crews).

In the other groups no detailed clinical examinations were made at the time of the Kveim test (but again the medical records of the vaccines trial were available).

Radiographic Examinations

Several chest radiographs (nearly all 35 mm.) were available for all 67 subjects, extending back to 1950–2. These series were supplemented with recent full-size chest radiographs, taken within six months before or after the Kveim test, and submitted for assessment to Professor J. G. Scadding. His readings were made after he had assessed the Kveim test results, and the radiographic series were submitted in a different order from that of the slides.

To permit an up-to-date assessment of the subjects in groups 1 and 2, additional full-size chest radiographs were taken shortly before the completion of this report, and were submitted separately to the assessor.

Skin Tests with Agents other than Tuberculin

Ten of the subjects in group 1 (non-converted by original and recent B.C.G. vaccinations) were tested to see whether delayed-type skin hypersensitivity to agents other than tuberculin was also depressed, as occurs with some antigens in clinical sarcoidosis. They were given skin tests with materials prepared from *Candida albicans* by Dr. R. W. Riddell (see Citron, 1957) and from streptococci—namely, “varidase” (streptokinase-streptodornase), this being a material to which a large proportion of normal individuals would be expected to react (see Lawrence, 1952).

The *C. albicans* suspension contained 0.5% organisms; a dose of 0.1 ml. of a 1/2 dilution in physiological saline was given intracutaneously and the test read at 48 hours. The dose of varidase was 5 units in 0.1 ml. intracutaneously, and the test was read at 48 hours. For comparison, the seven subjects in group 2 (non-converted by original, but converted by recent,

vaccination), as well as a total of 44 vaccine trial participants with tuberculin sensitivities conforming to category 3, 6, 7, or 8, were given the same skin tests (but only 12 of these 44 subjects had had a Kveim test in the present investigation).

Results

Examination of those not Converted by Original nor by Recent B.C.G. Vaccination (Group 1)

Apart from the Kveim tests (see below), the results of the special examinations of those not converted by the original nor by the recent B.C.G. vaccination (group 1) were virtually negative. All 12 subjects were apparently healthy and leading normal lives. There was no evidence of present or past tuberculosis. No current lesions resembling sarcoidosis were detected, nor was there any suggestion of this disease in the past records; in particular, there was no history or record suggesting erythema nodosum, and no evidence of recent or past ocular sarcoidosis, despite the special ophthalmological examination which was made on the seven subjects in this group who showed positive Kveim reactions with large specific nodules. The radiographic series (3 to 10 films per individual during a 10-year period, most of them having 6 to 8 films) were all passed as normal by the assessor. The only family history of tuberculosis was of a minimal lesion (sputum consistently negative on culture) in a sister of subject A, detected 15 months after his second vaccination.

Serum calcium and proteins were estimated in 10 of the 12 subjects. The serum calcium ranged from 9.1 to 10.6 mg./100 ml. (range by same method for 17 healthy adults outside the present series, 9.2 to 10.4 mg.). The values (obtained by paper electrophoresis) for total protein and protein fractions were also in the normal range (judged from the same 17 healthy adults), with the exception of low albumin concentrations in three subjects. The serum albumin ranged from 2.7 to 4.9 g./100 ml. (compared with 4.0–4.7 g.), the serum globulin from 2.7 to 3.8 g./100 ml. (compared with 2.7–3.8 g.), and the gamma-globulin from 1.1 to 1.7 g./100 ml. (compared with 0.9–2.0 g.).

In this group the mean induration to 100 T.U. plus cortisone was 6.8 mm., compared with 4.8 mm. to the tuberculin alone. In the four subjects with 0–4 mm. induration to 100 T.U. without cortisone at the time of these tests, the mean increase with cortisone was 4.2 mm.; in the eight subjects with 5–9 mm. induration, however, the mean increase was only 0.9 mm.

It may be added that 10 of the 12 subjects had shown an accelerated (Koch) local reaction at the site of their recent vaccination (before the Kveim test), the reaction appearing one to five days after the vaccination in nine and at seven days in one; of the other two subjects, one (subject B), showed no local reaction and one had a reaction appearing at 20 days. Hence, in spite of the persistent low level of their sensitivity to tuberculin, most of this group showed evidence of having been sensitized by their first vaccination.

Examination of the Other Subjects

The other 55 subjects (groups 2 to 8) were also apparently healthy and leading normal lives. There was no suggestion of sarcoidosis in their past records (known cases of tuberculosis had not been considered for inclusion—see above). The assessor reported the radiographic series (2 to 13 films per individual over a 10-year period, most of them having 7 or 8 films) as normal for all the subjects, with three exceptions.

One participant (group 2) had appearances suggestive of chronic bronchitis and emphysema. One participant in group 4 (converted by recent B.C.G. vaccination—previously non-reactor) showed shadowing in the right hilar region in

1955 (not noted as abnormal in the routine of the vaccines trial), followed by calcification in 1963; these appearances were assessed as compatible with a primary tuberculous infection. The recent B.C.G. vaccination was in 1962, and produced conversion to 3 T.U. three months later. The Kveim test given at this time was assessed as positive (Assessor 1) and equivocal (Assessor 2), with a 2-mm. nodule. The third participant was in group 8 (non-reactor to tuberculin); she had normal films until 1959, when a 35-mm. film showed indefinite shadowing in the upper part of the right lung (no full-size radiograph was obtained). The Kveim test, given in 1961, was negative (both assessors).

Of the seven subjects in group 2 (not converted by original, but converted by recent, vaccination), six had shown an accelerated (Koch) local reaction at the site of their recent vaccination (before the Kveim test), the reaction appearing three to seven days after the vaccination; in the other subject the reaction appeared at 22 days. These accelerated reactions to the recent vaccination, and the occurrence of typical scars in all seven following the original vaccination (see above), have led us to conclude that the original vaccination had established an infection and was thus valid, and that the non-conversion by it was not a technical fault.

Kveim Tests

Of the 64 biopsies, Assessor 1 judged 16 to be Kveim-positive and 5 to be equivocal. Assessor 2 judged 8 to be Kveim-positive and 5 equivocal. The readings of the two assessors on the same subject are compared in Table I; the differences between the two sets of assessments do not appear to indicate a basic disagreement between the assessors, but result from a general tendency for Assessor 2 to read at a lower level than Assessor 1. Thus all five equivocal readings of Assessor 1 were read as negative by Assessor 2, whereas all five equivocal readings of Assessor 2 were read as positive by Assessor 1. In only three instances was one reader's assessment "positive" and the other's "negative." The readings of the two assessors have been kept separate in presenting the results of the Kveim tests.

TABLE I.—Comparison of Kveim Test Readings by the Two Assessors

Assessor 2		Assessor 1			
		Positive	Equivocal	Negative	Total
	Positive	8	0	0	8
	Equivocal	5	0	0	5
	Negative	3	5	43	51
	Total	16	5	43	64

In Table II the size of the nodule at the test site four weeks after the injection of the Kveim material is compared with the histological assessments of the test. There is an association between the two sets of findings. Thus, of 10 sites with a nodule 5 mm. or more in diameter, eight were found Kveim-positive by Assessor 1 and five by Assessor 2. At the other extreme, of 22 sites with no visible (or palpable) nodule, two were found positive by Assessor 1 and one by Assessor 2. It is of interest that the great majority of the small nodules

TABLE II.—Comparison of Histological Assessments of Kveim Test With Size of Nodule at Test Site

Assessor	Kveim Test	Total Biopsies	Diameter of Nodule Four Weeks After the Injection (mm.)				
			8-7	6-5	4-3	2-1	0
1	Positive	16	2	6	4	2	2
	Equivocal	5	1	0	1	2	1
	Negative	43	1	0	9	14	19
2	Positive	8	1	4	2	0	1
	Equivocal	5	1	2	0	1	1
	Negative	51	2	0	12	17	20
	Total	64	4	6	14	18	22

(1-4 mm.) were negative (23 out of 32—Assessor 1—and 29 out of 32—Assessor 2).

Table III shows the sizes of the nodules four weeks after the injection in each of the eight categories, without regard to the histological findings. In group 1, 10 of the 12 tested had nodules, and these were all large; in group 2, on the other hand, only three of the seven had nodules and these were all small. It is also of interest that none of the eight non-reactors to tuberculin (group 8) had detectable nodules, in sharp contrast to the small nodules in 12 of the 15 natural strong and natural weak reactors (groups 6 and 7), and in even sharper contrast to the large nodules in 10 of the 12 persistently non-converted subjects (group 1).

TABLE III.—Size of Nodules in the Various Vaccination and Tuberculin Test Categories

Vaccination and Tuberculin Test Category	Total Tested	Diameter of Nodule at Four Weeks (mm.)				
		8-7	6-5	4-3	2-1	0
1. Non-converted by original and recent B.C.G. vaccinations	12	5	5	0	0	2
2. Non-converted by original, but converted by recent, B.C.G. vaccination	7	0	0	2	1	4
3. Converted by original B.C.G. vaccination (originally non-reactors)	10	0	0	7	2	1
4. Converted by recent B.C.G. vaccination (previously non-reactors)	10	0	0	2	4	4
5. Converted by recent B.C.G. vaccination (previously weak reactors)	5	0	1	0	2	2
6. Natural strong reactors to tuberculin	5	0	0	2	2	1
7. Natural weak reactors to tuberculin	10	0	0	1	7	2
8. Non-reactors to tuberculin	8	0	0	0	0	8
Total	67	5	6	14	18	24

Because so many of the nodules, particularly the small ones, were not Kveim-positive, the nodule sizes without histological assessments, given in Table III, may prove misleading. From this point, therefore, only the sizes of the nodules for the subjects found to be Kveim-positive—that is, the specific nodule sizes—are used.

The histological assessments of the Kveim tests in each of the eight categories, together with the specific nodule sizes, are set out in Table IV. It is evident that those not converted by the original nor by the recent B.C.G. vaccination (group 1) are in a special position. Despite the absence of any clinical evidence of current or past sarcoidosis, 7 of the 10 had positive (and one equivocal) Kveim reactions (Assessor 1), and four had positive (and three equivocal) reactions (Assessor 2); a notable finding was that the specific nodules were all large. (Of the three subjects with no induration at all to 100 T.U. after either vaccination, subject A had a positive reaction according to both assessors and a 5-mm. nodule, and the other two refused biopsy and do not appear in the table; subject B had a 7-mm. nodule and subject C no nodule.)

The findings among those not converted by the original, but converted by the recent, B.C.G. vaccination (group 2) are also remarkable. Of the seven, three had positive (and one equivocal) Kveim reactions (Assessor 1), and two had positive (and none equivocal) reactions (Assessor 2); here the specific nodules were small (in one instance not present). Thus positive Kveim reactions occurred in group 2, but rather less often than in group 1; the specific nodule sizes were substantially less.

In each of groups 3, 4, and 5 (which had a single B.C.G. vaccination, either original or recent, followed by a normal tuberculin conversion), one to three positive Kveim reactions were found by one or other assessor, but the proportions are less than in groups 1 and 2; there are no obvious differences in specific nodule size between groups 3, 4, and 5 and group 2 (two vaccinations, converted only after the second). In the remaining groups (6 to 8, none of which had B.C.G. vaccination), only one subject (in group 7) had a positive Kveim reaction (Assessor 1 only), with a small specific nodule.

Arising from the findings in Table IV, the possibility that Kveim positivity and also specific nodule size are associated with non-conversion, previous B.C.G. vaccination, or both, has been examined more closely. Table V summarizes the histological assessments, as well as the sizes of the specific nodules, according to tuberculin status and previous B.C.G. vaccination.

Considering non-conversion first, evidence on its relevance is provided by the comparison of groups 1 and 2, which have in common two vaccinations and non-conversion after the first, but which differ in that the subjects in group 1 were not converted, whereas those in group 2 were converted, after the second vaccination. In terms of frequency of positive Kveim reactions alone, the difference between these two groups does not reach statistical significance; but if specific nodule size is taken into account as well, the difference is significant at the 1% level for Assessor 1 and borders on significance at the 5% level for Assessor 2. The substantially larger specific nodules, accompanied by the rather higher prevalence of Kveim positivity, in group 1 thus appear to be related to the *persistence* of the non-conversion in this group through two vaccinations. Because there was no group of non-converted subjects who were given Kveim tests *before* their second vaccination, it is not possible to assess the relevance of non-conversion following the first vaccination.

Turning to previous B.C.G. vaccination, direct evidence on its relevance (uncomplicated by non-conversion) is provided by the comparison of groups 3, 4, and 5 (vaccinated once, followed by normal conversion) with groups 6, 7, and 8 (not vaccinated). Among the 25 vaccinated subjects there were positive Kveim reactions in five, with one large and three small specific nodules (Assessor 1), and in two, with one large and one small specific nodule (Assessor 2). In contrast, among the 22 unvaccinated subjects there was one positive Kveim reaction with a small

specific nodule (Assessor 1 only). This contrast suggests an association of Kveim positivity with previous vaccination, but the differences do not attain statistical significance. Further evidence for such an association is provided by the seven subjects of group 2 (vaccinated twice and converted after the second), of whom three (Assessor 1) and two (Assessor 2) had positive Kveim reactions, again in contrast with the unvaccinated subjects. However, since these subjects had not been converted by their first vaccination 10 years previously, non-conversion, as well as vaccination, could have been a factor in their Kveim positivity. (The findings in group 1—vaccinated twice and never converted—have not been used as evidence of the relevance of vaccination because of the further complication, in this group, of non-conversion following their second, recent, vaccination and the association, shown above, between this persistence and their Kveim test results.)

In summary, when specific nodule size is considered together with the frequency of positive Kveim reactions, there is an association of the Kveim test results with persistent non-conversion to tuberculin sensitivity by B.C.G. vaccination. In addition, the available data give indications of an association of Kveim positivity with previous B.C.G. vaccination.

From Table IV it is also possible to examine whether there is an association between Kveim positivity and the interval since a (single) B.C.G. vaccination (group 3 compared with groups 4 and 5 combined). In 10 subjects vaccinated in 1950–2 and tested in 1961–2 there was one positive Kveim reaction (both assessors), and in 15 subjects tested three to six months after vaccination in 1961–2 there were four positive reactions (Assessor 1) and one (Assessor 2). From these small numbers there is a slight suggestion that Kveim positivity may decrease in frequency with a large increase in the interval since vaccination; there is no obvious difference in specific nodule size.

TABLE IV.—*Histological Assessments of Kveim Test, and Sizes of Specific Nodules,* in the Various Vaccination and Tuberculin Test Categories*

Vaccination and Tuberculin Test Category	Total Biopsies	Assessor 1							Assessor 2								
		Diameter of Specific Nodule at Four Weeks (mm.)					Kveim-positive	Kveim-equivocal	Kveim-negative	Diameter of Specific Nodule at Four Weeks (mm.)					Kveim-positive	Kveim-equivocal	Kveim-negative
		8-7	6-5	4-3	2-1	0				8-7	6-5	4-3	2-1	0			
1. Non-converted by original and recent B.C.G. vaccinations ..	10	2	5	0	0	0	7	1	2	1	3	0	0	0	4	3	3
2. Non-converted by original, but converted by recent, B.C.G. vaccination	7	0	0	2	0	1	3	1	3	0	0	1	0	1	2	0	5
3. Converted by original B.C.G. vaccination (originally non-reactors)	10	0	0	1	0	0	1	1	8	0	0	1	0	0	1	0	9
4. Converted by recent B.C.G. vaccination (previously non-reactors)	10	0	0	1	1	1	3	0	7	0	0	0	0	0	0	2	8
5. Converted by recent B.C.G. vaccination (previously weak reactors)	5	0	1	0	0	0	1	1	3	0	1	0	0	0	1	0	4
6. Natural strong reactors to tuberculin	5	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	5
7. Natural weak reactors to tuberculin	10	0	0	0	1	0	1	1	8	0	0	0	0	0	0	0	10
8. Non-reactors to tuberculin ..	7	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7
Total	64	2	6	4	2	2	16	5	43	1	4	2	0	1	8	5	51

* For definition, see text.

TABLE V.—*Histological Assessments of Kveim Test, and Sizes of Specific Nodules, According to Tuberculin Status and Previous B.C.G. Vaccination*

Group	Tuberculin Status and Previous B.C.G. Vaccination	Total Biopsies	Assessor 1						Assessor 2					
			Diameter of Specific Nodule at Four Weeks (mm.)			Kveim-positive	Kveim-equivocal	Kveim-negative	Diameter of Specific Nodule at Four Weeks (mm.)			Kveim-positive	Kveim-equivocal	Kveim-negative
			8-5 (Large)	4-1 (Small)	0				8-5 (Large)	4-1 (Small)	0			
1	Vaccinated twice and never converted . . .	10	7	0	0	7	1	2	4	0	0	4	3	3
2	Vaccinated twice, and converted after the second . . .	7	0	2	1	3	1	3	0	1	1	2	0	5
3, 4, and 5	Vaccinated once, followed by normal conversion . . .	25	1	3	1	5	2	18	1	1	0	2	2	21
6, 7, and 8	Not vaccinated . . .	22	0	1	0	1	1	20	0	0	0	0	0	22

Because of the time-table of the investigation in those vaccinated twice, the interval between the second vaccination and the Kveim test for those who had been converted by this vaccination (group 2)—namely, about two years—was longer than that for those who had not been converted by it (group 1)—namely, about six months. The absence of any clear association between frequency of Kveim positivity and the interval since a single vaccination, noted above, suggests that this relatively small difference in interval is unlikely to have seriously affected the comparison of Kveim positivity between groups 1 and 2.

In contrast to B.C.G. vaccination, natural mycobacterial infection (as judged by the observed tuberculin sensitivity) does not appear to have an appreciable association with Kveim positivity. There were no positive Kveim reactions among five strong reactors to tuberculin (group 6) and only one (Assessor 1 only) among 10 weak reactors (group 7).

The data also provide a little information concerning the relation between Kveim positivity and the degree of non-conversion after the first vaccination (not tabulated). Of the 10 subjects biopsied in group 1, six had shown only 0–4 mm. induration to 100 T.U. at the tests following the original vaccination, whereas four had shown 5–9 mm. on at least one occasion; of the six subjects whose tuberculin response was the more refractory, three (Assessor 1) and two (Assessor 2) were Kveim-positive, with large specific nodules; of the four subjects with the slightly less refractory response, four (Assessor 1) and two (Assessor 2) were positive, with large specific nodules. In group 2 three subjects had the more refractory tuberculin response after the original vaccination, and one (Assessor 1 only) was Kveim-positive, with a small specific nodule; four subjects had the slightly less refractory response and two (both assessors) were positive, with small or absent specific nodules. Within this narrow range there is no indication of a falling off in Kveim positivity in either group 1 or group 2 with diminishing refractoriness of tuberculin response following B.C.G. vaccination. These findings provide a retrospective justification for the decision to widen the definition of non-conversion in this study from that used in the vaccines trial (see above).

Skin Tests with Agents other than Tuberculin

The results of the skin tests with the *C. albicans* and varidase materials are summarized in Table VI. It is of interest that the mean induration of delayed-type skin reactions to *C. albicans* for those non-converted by the original and recent B.C.G. vaccinations (a large proportion of whom were Kveim-positive, with large specific nodules) was 2.1 mm., significantly less (at the 1% level) than the mean indurations of 8.4 mm. among those non-converted by the original, but converted by the recent, vaccination, 8.1 mm. among those converted by the original vaccination, 7.5 mm. among natural strong reactors to tuberculin, 6.1 mm. among natural weak reactors to tuberculin, and 7.5 mm. among non-reactors to tuberculin.

TABLE VI.—Results of Skin Tests with *Candida albicans* and Varidase Test Materials

Vaccination and Tuberculin Test Category	Total Tested	Mean Induration To:		No. also Given Kveim Test in Present Investigation
		<i>Candida albicans</i> (mm.)	Varidase (mm.)	
1. Non-converted by original and recent B.C.G. vaccinations	10	2.1	10.5	10
2. Non-converted by original, but converted by recent, B.C.G. vaccination	7	8.4	Not tested	7
3. Converted by original B.C.G. vaccination (originally non-reactors)	9	8.1	10.2	3
6. Natural strong reactors to tuberculin	4	7.5	Not tested	4
7. Natural weak reactors to tuberculin	10	6.1	9.0	5
8. Non-reactors to tuberculin	21	7.5	9.0	0

The reduction in induration to *C. albicans* appeared to characterize each of the 10 subjects tested from group 1. Thus only one reaction in this group was as large as 5 mm., whereas five of the seven reactions in group 2, and 38 of the 44 reactions in the other four groups combined, were 5 mm. or more. There was no apparent relation between the size of the *C. albicans* reaction within group 1 and either the occurrence of Kveim positivity or the specific nodule size, with either assessor.

The mean indurations to varidase streptococcal material were similar (between 9.0 and 10.5 mm.) in all four groups tested.

Discussion

This work was conceived as a study of the clinical and immunological characteristics of young adults who had failed to develop tuberculin sensitivity after an otherwise apparently satisfactory B.C.G. vaccination—that is, one giving a visible local reaction at the vaccination site, followed by a typical scar—and of the possible relation of this tuberculin anergy to sarcoidosis.

Special attention was paid to the 12 subjects whose failure to be “converted” to tuberculin sensitivity had persisted through two vaccinations—the original one on entry to the Medical Research Council’s tuberculosis vaccines trial at the age of 14 to 15½ years, and the recent one in the present investigation about 10 years later; tuberculin sensitivity, which had been absent before the first vaccination, was either absent or only very weak at every subsequent test.

No clear indication has been obtained of the cause or mechanism of this persistent non-conversion. The 12 subjects were apparently healthy, and, although specially examined (including slit-lamp examination of seven), showed no clinical or radiographic evidence of past or present sarcoidosis. The serum calcium and protein fractions were not abnormally high. On the other hand, among the various skin tests given, cortisone, which can elicit or enhance weak tuberculin sensitivity in sarcoidosis (Pyke and Scadding, 1952; Citron and Scadding, 1957), also did so in these subjects; but since the same may occur in tuberculous patients desensitized with tuberculin (Citron and Scadding, 1957), our findings may merely be a consequence of the very low level of tuberculin sensitivity in our subjects. There was a depression of delayed-type skin sensitivity to *Candida* antigen, as has also been reported in sarcoidosis (Sones and Israel, 1954; Citron, 1957; Buck and Sartwell, 1961).

Despite these negative findings from periodic observations extending over 10 years, it is still possible, in view of their tuberculin anergy, either that these subjects had had clinical sarcoidosis in the past or that the disease was occult at the time of the present investigation. But if so, it is surprising that no clinical or radiographic evidence of sarcoidosis should have been detected in any of them at any time (the evidence—including a further chest radiograph—in the two years since the present investigation has also been negative). Moreover, in the whole of the vaccines trial, none of the known cases of clinical sarcoidosis has occurred in anyone who failed to be converted to tuberculin sensitivity by their original vaccination.

The Kveim test is often positive in clinical sarcoidosis. Because these persistently non-converted subjects had been found to be clinically normal, the large proportion showing positive Kveim reactions (assessed histologically) was unexpected, raising the possibility that some factor other than sarcoidosis might be responsible. The emphasis of the study was therefore shifted to a comparison between the prevalence of Kveim positivity in this group and that in the various other groups, with well-defined contrasting histories of tuberculin sensitivity and B.C.G. vaccination, which were assembled for investigation.

The 55 subjects in these other groups were also apparently healthy and without clinical or radiographic evidence of

sarcoidosis. None of these groups contained persistently non-converted subjects. Yet positive Kveim reactions were unexpectedly found in certain of them too.

Although all the groups in this study were small—and in the case of the “non-converted” this was dictated by the numbers available—the subjects were of similar age and were derived from the same community sample—namely, the participants in the vaccines trial, all of whom entered that trial in 1950–2. Moreover, the assessments of Kveim positivity were made by two experienced assessors and were based entirely on the microscopical appearances, the assessors not knowing if there was a visible nodule and not being informed of any clinical or radiographic findings. The size of the nodule was recorded separately, and for those with positive Kveim reactions has been used (as the “specific nodule size”) to express the result of the test quantitatively.

Two Associations with Kveim Positivity

From consideration of the results of the Kveim tests in all eight groups, two associations have emerged. The first is between the Kveim test results and *persistent* non-conversion to tuberculin sensitivity by B.C.G. vaccination; positive Kveim reactions were rather more frequent and the specific nodules much larger in the twice-vaccinated but persistently non-converted (group 1) than in the twice-vaccinated and finally converted (group 2).

The second association is of Kveim positivity with previous B.C.G. vaccination. The main evidence for this association is the more frequent positive Kveim reactions in those vaccinated once, followed by normal conversion, than in those not vaccinated. While this difference does not itself attain statistical significance, evidence supporting such an association is provided by the more frequent positive Kveim reactions in those vaccinated twice and converted after the second vaccination than in those not vaccinated—although non-conversion to the first vaccination, 10 years previously, cannot be excluded as a factor in the Kveim positivity of this vaccinated group. From the available evidence there are thus indications of an association of B.C.G. vaccination with subsequent Kveim positivity. It would clearly be valuable to know if this conclusion was confirmed on larger numbers of vaccinated and unvaccinated subjects; as already explained, it was not practicable to increase the numbers in these groups in the present investigation. However, the conclusion receives some further support from studies of Kveim positivity in other groups of subjects without clinical sarcoidosis or tuberculosis (see Siltzbach, 1961a, 1961b). No B.C.G.-vaccinated group has been included specifically in these surveys. The reported prevalences of Kveim positivity have been extremely low, as in our unvaccinated, but unlike our B.C.G.-vaccinated, subjects.

Although an association has been shown between Kveim test results and non-conversion to tuberculin sensitivity persisting through two vaccinations, it has not been possible to assess the relevance of non-conversion after a *single* vaccination, because all those not converted by their first vaccination were given a second before their Kveim test. This is a shortcoming of the design of the present study, which is inherent in the way it developed.

We are unaware of any publication describing Kveim tests in apparently healthy persons who had received one or more B.C.G. vaccinations, whether they were converted to tuberculin sensitivity or not. There are, however, a number of reports suggesting that there may be some association between B.C.G. vaccination and the subsequent appearance of clinical sarcoidosis or of radiographic features of this disease. Most of these reports present details of occasional instances where the disease followed the vaccination. In some instances the vaccination had resulted in tuberculin conversion and in others not; some of the subjects had received one and others two vaccinations; the interval from

vaccination to the onset of sarcoidal manifestations varied from a few days to several years (Jackson, 1957; Birkhäuser, 1957; Ellman and Andrews, 1959; Scadding, 1960; Hoffbrand, 1963; and others). It is impossible from reports of individual cases to gauge the frequency with which vaccination is followed by the disease, and coincidence may be responsible for some or even all of the reported instances (especially since the B.C.G. may have been given selectively because the subject was found to be tuberculin-negative, this negativity being, however, due to incipient sarcoidosis). If there is a causal relationship, it would indicate a parallel with our evidence for an association between positive Kveim reactions and previous B.C.G. vaccination.

In this connexion some remarkable findings in the mass campaign carried out in 1950–2 by the Danish Tuberculosis Index need to be mentioned (Groth-Petersen, Knudsen, and Wilbek, 1959). Of nearly 750,000 adults examined from the Danish population, 213,000 had never been vaccinated and were tuberculin-negative; 120,000 had been vaccinated previously and were tuberculin-positive, and 30,000 had been vaccinated in the past but were found to be tuberculin-negative (to 10 T.U.). “Suspicious” pulmonary (including hilar) shadows were found in 0.46%, 0.24%, and 0.57% of these three groups respectively; and of these shadows the percentages reported as indicating enlarged hilar glands without pulmonary disease were about 20%, 25%, and 50% respectively. The authors considered that B.C.G. vaccination itself must have had something to do with the relatively large figures for the vaccinated but tuberculin-negative group, and discuss the possibility that some of the shadows may have represented sarcoidosis; but they were unable to come to a definite conclusion from the data.

The present results raise the question whether Kveim positivity is associated with other mycobacterial infections besides B.C.G. Our groups of natural strong and weak reactors to tuberculin showed a positive result in only 1 out of 15 subjects; hence if there is any association between Kveim positivity and natural mycobacterial infection it is not appreciable in this series. This is in keeping with reports that Kveim tests give negative, doubtful, or only rarely positive reactions in patients with tuberculosis (James and Thomson, 1955; Nelson and Schwimmer, 1957; Hirsch *et al.*, 1961; Siltzbach, 1961a; Steigleder *et al.*, 1961; Kenney and Stone, 1963). On the other hand, there are contrary reports that positive Kveim reactions occur in a high proportion of tuberculous patients (Israel and Soneş, 1955; Daniel and Schneider, 1962), though with different test material Israel *et al.* (1958) failed to repeat their earlier findings. This conflict of evidence in the literature might be due to variations in the test material, to differences in interpretation, or to differences in the proportions of active disease between the reported series. With regard to the possible relevance of activity of mycobacterial infections, there is a slight suggestion in the present investigation that those given Kveim tests within six months of B.C.G. vaccination (groups 4 and 5) showed positive reactions more often than those tested about 10 years after vaccination (group 3).

There are a number of possible explanations for an association of Kveim positivity with previous B.C.G. vaccination in apparently healthy subjects, and for the strikingly frequent positive Kveim reactions, with large specific nodules, in those showing persistent failure to be converted to tuberculin sensitivity by vaccination.

One explanation (akin to that newly proposed by Siltzbach—personal communication) is that, despite the lack of clinical evidence, the positive Kveim reactions are due to naturally occurring occult (or past) sarcoidosis which has been uncovered by means of B.C.G. vaccination. According to this conception, the vaccination has identified the anergic individuals, and has in some way increased the capacity of certain other individuals (also with occult disease, or with a predisposition to this) to give a positive Kveim reaction; the occurrence of large specific nodules in the persistently anergic individuals, and of small

specific nodules in the others, could be accounted for by differences in the activity or in some other feature of their occult sarcoidosis. This explanation implies that a surprisingly large proportion of apparently healthy young adults have occult sarcoidosis.

An entirely different possibility is that healthy young adults rarely have occult sarcoidosis, but that B.C.G. vaccination can give them this disease in an occult form; if this were so, one might have expected many more cases of *clinical* sarcoidosis to have been reported in association with previous B.C.G. vaccination, having regard to the vast numbers vaccinated throughout the world.

A third possibility is that in these clinically normal subjects the positive Kveim reactions are not due to occult or clinical sarcoidosis, but are the result of some novel immunological change brought about by the B.C.G. vaccination itself; the immunological change would then seem to be related in some way to tuberculin status, being especially prominent in subjects with persistent tuberculin anergy. We favour this last explanation, mainly because we have failed to detect sarcoidosis, occult or clinical, in our subjects.

It is hoped that the present findings may lead to a fuller understanding of the nature of the Kveim test and possibly also of sarcoidosis.

Summary

Positive reactions to the Kveim test were found in a large proportion of clinically normal young adults who had failed to be "converted" to tuberculin sensitivity following two B.C.G. vaccinations. This unexpected finding was the prelude to a wider inquiry, centred on the Kveim test. In all, Kveim tests were made in 1960-3 on 67 former participants in the Medical Research Council's tuberculosis vaccines trial. The subjects were carefully selected to form eight contrasted groups, differentiated on the basis of their history of an "original" B.C.G. vaccination—that is, in 1950-2, on entry to the vaccines trial at the age of 14 to 15½ years; their tuberculin state during an eight- to ten-year period of follow-up; the giving of a "recent" B.C.G. vaccination—that is, in 1960-2, in connexion with the present investigation; and the results of recent tuberculin tests.

The short titles of the groups were as follows: (1) non-converted by original and recent B.C.G. vaccinations (12 subjects); (2) non-converted by original, but converted by recent, B.C.G. vaccination (7 subjects); (3) converted by original B.C.G. vaccination (originally non-reactors) (10 subjects); (4) converted by recent B.C.G. vaccination (previously non-reactors) (10 subjects); (5) converted by recent B.C.G. vaccination (previously weak reactors) (5 subjects); (6) natural strong reactors to tuberculin (5 subjects); (7) natural weak reactors to tuberculin (10 subjects); (8) non-reactors to tuberculin (8 subjects).

Various examinations were made on the 67 subjects during the present investigation, including a chest radiograph, and, in group 1, special blood and ophthalmological examinations. In addition, the medical records of the vaccines trial, including a series of chest radiographs, were available.

The Kveim tests were all made according to a recommended procedure with a single lot of material from a sarcoid spleen, supplied by Dr. L. E. Siltzbach, New York. The results of the tests were classified as positive, equivocal, or negative on histological grounds, from biopsies of the test site (almost all at four to seven weeks), by two independent assessors (one being Dr. Siltzbach), neither of whom was aware of the clinical or radiographic findings, of the vaccination and tuberculin-test history of any of the subjects, or of the naked-eye appearances of the test site. One assessor's histological readings of the 64 biopsies were at a lower level (8 Kveim-positive and 5 equivocal) than the other's (16 positive and 5 equivocal), but despite

this tendency their readings did not conflict. The size of any nodule at the test site in Kveim-positive subjects—that is, "specific nodule size"—was used to express the result of the test quantitatively.

All the subjects were apparently healthy and leading normal lives. There was no evidence of sarcoidosis, past or present (apart from a depression of skin sensitivity to *Candida albicans* antigen in association with persistent tuberculin anergy—group 1), or of definite tuberculosis. Yet a large proportion of group 1 (vaccinated twice but never converted) had positive Kveim reactions, with large specific nodules; in group 2 (vaccinated twice and finally converted) the proportion with positive reactions was also substantial, but rather less than in group 1, and the specific nodules were small or absent. In groups 3, 4, and 5 (vaccinated once and converted normally) positive Kveim reactions were also found, but in smaller proportions than in groups 1 and 2. Positive reactions were almost absent from groups 6, 7, and 8 (not vaccinated).

The Kveim test results were related to persistent non-conversion to tuberculin sensitivity, in that positive Kveim reactions were rather more frequent and the specific nodules much larger in those never converted (group 1) than in those finally converted (group 2) after two vaccinations. Further, detailed consideration of the findings gave indications that B.C.G. vaccination itself was a factor in subsequent Kveim positivity, but that natural mycobacterial infection was not. Various possible explanations for these associations are presented. We consider it less likely that the positive Kveim reactions in these clinically normal subjects indicate occult sarcoidosis than that the vaccination has brought about some novel immunological change which has manifested itself in positive reactions to the Kveim test, this change being especially prominent in those with persistent tuberculin anergy.

The present results may lead to a fuller understanding of the Kveim test and of the aetiology of sarcoidosis.

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Studies on the Secretion of Human-pituitary-growth Hormone

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The development of a sensitive and specific radio-immunoassay for human growth hormone in plasma (Hunter and Greenwood, 1962, 1964) has allowed studies of the levels of human growth hormone in the varying physiological states represented by childhood, adolescence (Greenwood, Hunter, and Marrian, 1964), and pregnancy and lactation (Greenwood, Hunter, and Klopfer, 1964).

Acute changes in growth-hormone secretion have been induced in normal adult subjects by Roth, Glick, Yalow, and Berson (1963a, 1963b). These authors have shown that the secretion of growth hormone as measured by plasma-growth-hormone levels is suppressed by the administration of glucose. Secretion was stimulated by hypoglycaemia, inhibition of glucose utilization, prolonged fasting, and muscular exercise. Results presented here confirm their findings and also suggest that growth-hormone secretion is reduced but not suppressed by protein administration. Results obtained from a series of 11 subjects during hypoglycaemia induced by insulin or tolbutamide suggest that the response in growth-hormone secretion to this stimulus could be of value in the investigation of patients with suspected pituitary disorders.

Material and Methods

With the exception of the hospital patients noted in Table II and the Chart, subjects were healthy adult volunteers with no history of endocrine disease, aged from 24 to 48.

The diagnoses, insulin or tolbutamide doses, and blood-sugar values recorded for the hospital patients shown in Table II and the Chart were kindly supplied by clinical colleagues at the hospitals mentioned in the acknowledgments. Different methods for the analysis of blood sugar were used for these patients and for the normal subjects. Values recorded are comparable only for each patient.

Human growth hormone was estimated by a radio-immuno-electrophoretic technique (Hunter and Greenwood, 1962). The estimations were carried out with heparinized plasma (0.05–0.4 ml. of a 1/4 dilution) in assays using 0.2 μ mg. 131 I-labelled growth hormone. The standards in the assay were from a laboratory reference preparation of growth hormone (I.C.R.F. Batch 3) prepared by the method of Raben (1959). This has an immunological potency of 124% of the Medical Research Council human growth hormone R.5. The levels of the first standard were 0.1 or 0.2 μ mg.; sensitivities of growth hormone

in plasma were >1 or >2 μ mg./ml. plasma respectively. The precision of the measurements in plasma over the ranges 1–5, 5–10, and 10–40 μ mg./ml. were ± 0.51 , ± 0.95 , and ± 2.22 μ mg. respectively. These values are estimates of the standard deviations of results from their means (Hunter and Greenwood, 1964).

Effect of intravenous glucose on plasma-growth-hormone levels. Plasma-growth-hormone (—●—●—●) and blood-sugar (---△---△---) levels were measured before and after intravenous glucose (25 g.) in a patient with a suspected islet-cell tumour. A blood sample was taken and glucose injected at the point indicated by the arrow. This study was carried out by Dr. V. Marks.

Results

Overnight Fasting Levels of Growth Hormone

Table I shows the levels of growth hormone found in healthy adults after an overnight fast (14 hours). The previous dietary habits were not recorded. Samples of plasma from 18 adults two to three hours after a meal have previously been reported (Greenwood, Hunter, and Marrian, 1964) and a mean value of 0.55 μ mg. ± 0.68 (standard deviation) was obtained. In nine of these subjects the level of growth hormone was indistinguishable from zero, in common with patients after hypophysectomy for metastatic breast cancer. In contrast detectable growth hormone was present in the normal subjects shown in Table I, and the mean level with subject G excluded was 2.92 μ mg. ± 2.95 (standard deviation). One subject (E, Table I) was tested on three occasions at mid-morning and no growth hor-

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