ALTERATION OF TUBERCULIN REACTION PATTERNS
AT THE SITE OF PREVIOUS REPEATED TESTS

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The pattern of tuberculin reaction is altered at the site of previous tests. At a previously used site, reaction appears earlier and fades sooner than at a new site. Von Pirquet reported this phenomenon as early as 1909. He made cutaneous tuberculin tests on his own thigh and found that reactions were accelerated at the sites of previous tests. Reaction appeared after four hours, attained its maximum after 12 hours and began to fade after 24 hours. He considered this phenomenon as the result of local accumulation of antibody. Weiss confirmed Pirquet’s finding in 1926. But thereafter this phenomenon had been ignored until Yanagisawa found it again in 1942.

In Japan each individual citizen is required by law to undergo the tuberculin test once a year up to 30 years of age. Since the law provides that the test be given on the flexor surface of the left forearm, many persons are injected with tuberculin repeatedly at the same site. In 1942 Yanagisawa noticed the acceleration of tuberculin reaction at the repeatedly used site, comparing reactions at the used and new sites 24 and 48 hours after injection. Since this finding was considered to be of great importance in the practice of tuberculin test, many Japanese investigators, Suzuki (1948, 1951), Koike (1948, 1951), Gotô (1950), K. Maeda (1954), Shiota (1955, 1956), M. Ikegami (1956), Terada (1956), Nobechi (1958), H. Ikegami (1958), Satomi (1958), Ogura (1958), Okada (1958, 1959), Murai (1960), H. Kobayashi (1960), Y. Maeda (1960), Inaba (1960), Y. Kobayashi (1962) et al. studied this phenomenon. Edwards and Magnus (1955) also observed this phenomenon independently.

None of these investigators, except Pirquet, observed the whole course of the reaction from its beginning. In 1956 the present authors observed the course of tuberculin reaction at the used site from immediately after injection and found a new type of reaction, appearing 3 to 4 hours after injection differing from either the immediate type reaction or delayed type reaction. A report of this new reaction, early reaction, was published in 1957 (Matusima et al. 1957). Recently Duboczy (1961) also made a report about this reaction. In the present paper further detailed investigation results about this early reaction and altered reaction patterns at the used site are reported.
MATERIALS AND METHODS

Tuberculin: Tuberculin used in the present study was a 1/2,000 old tuberculin solution approved by the National Institute of Health, Japan. Injection dose was 0.1 ml.

Injection sites: Tuberculin was injected at two sites, namely, the used site and the new site. As the used site the skin area on the flexor surface of arm or on the back, in which tuberculin had previously been injected, was employed and as the new site the corresponding area, in which tuberculin had never been injected.

Observation methods: Means of the longitudinal and transverse diameters of erythemas and those of definite indurations were used as their sizes, respectively. When induration was hardly perceptible, its diameter was calculated as zero.

Subjects: Students, nurses, student nurses and school children were subjected to the present observations. Many of them had previously been vaccinated with BCG. But in this study the vaccinée were not separated from non-vaccinée.

EXPERIMENTS

1. Pattern of Tuberculin Reaction at the Used Site

In June of 1956, 24 student nurses were injected with tuberculin in two sites, namely, the upper one third point of the left forearm where tuberculin had been previously injected 9 to 28 times (the used site) and the corresponding point of the right forearm where tuberculin had not been injected as far back as they remembered (the new site). Reactions were observed 1/2, 1, 2, 3, 4, 6, 8, 12, 24 and 48 hours after injection.

In 18 out of 24 student nurses the reaction at the used site differed from that at the new site (Fig. 1). In both sites local swelling due to the injected solution disappeared within two hours. At the used site remarkable erythema and induration with an edematous appearance and a clear-cut boundary were observed three to four hours after injection. (In the present paper this reaction is tentatively referred to as early reaction.) The intensity of reaction attained its maximum after 12 to 24 hours and showed a tendency to decrease thereafter. After 48 hours induration was hardly perceptible in most of the

Fig. 1. Patterns of tuberculin reactions at the repeatedly used site (left) and a new site (right). Upper line; average size of erythemas. Lower line; that of definite indurations. Average for 18 cases.
cases and in some cases only a vague pigmentation remained in the area where the reaction had been observed.

At the new site no remarkable erythema nor induration was observed after three to four hours. Erythema appeared after eight to 12 hours and definite induration after 24 hours. The intensity of reaction attained its maximum after 48 hours.

In the remaining six student nurses early reaction was observed on both forearms. Reaction was reexamined at the lower third point of the right forearm, and at this skin area early reaction was not observed in four of them.

In summary, in the site where tuberculin tests had repeatedly been given, an early reaction and an accelerated delayed reaction were observed. Early reaction with remarkable erythema and induration appeared after three to four hours. Reaction attained its maximum after 12 to 24 hours and declined markedly after 48 hours.

2. Influence of the Repetition of Injection on the Pattern of Tuberculin Reaction

In September of 1956, 17 patients in a sanatorium for school children were given tuberculin injection in the right forearm where tuberculin had not been injected previously. Thereafter, every 8 to 10 weeks, tuberculin of the same lot was injected at the same site. This was repeated four times. Reactions were observed 4, 8, 12, 24 and 48 hours after each injection (Fig. 2).

In the first test no early reaction was observed and the intensity of reaction increased as time elapsed.

In the second test, remarkable erythema and induration appeared after four hours. The reaction attained its maximum intensity after 12 to 24 hours. Bulae were observed in six out of 17 children. As a whole, the reaction was more intensive than in the first test.

In the third, fourth and fifth tests, while the early reaction was nearly the same as in the second test, the intensity of the delayed reaction after 24 to 48 hours decreased in accordance with the repetition of the injection. In the fifth test, induration was hardly perceptible in almost all cases after 48 hours.

In summary, the pattern of tuberculin reaction at the used site changed with the

![Fig. 2. Alteration of tuberculin reaction patterns by repetition of tests at the same site. From left to right; reactions at the first, the second, the third, the fourth and the fifth tests. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for 17 cases.](image)
number of repeated tests. *Early reaction* was observed from the second test on. Delayed reaction was accelerated and intensified in the second test, while thereafter it became weaker, as the test was repeated.

3. **Extent of the Area Influenced by the Preceding Tuberculin Reaction**

In January of 1957, 89 school children were given tuberculin injections in the prescribed site of the right forearm where tuberculin had never been injected. To mark the injection site the distance from the tip of the middle finger was recorded. The longitudinal diameters of erythemas were measured 48 hours after injection. One month later the children were divided into four groups and each group was given a second injection in a different site.

In group A of 10 children the distance between the first and second injection sites was made equal to the radius of the erythema of the first reaction, so that the areas of the two reactions might partially overlap.

In group B of 21 children the distance between the two sites was made equal to the diameter of the first reaction, so that the areas of the two reactions might come in contact with each other, but not overlap.

In group C of 44 children the distance was made greater than the diameter of the first reaction, so that the two reaction areas might not touch each other.

In group D of 14 children with strong reactions the erythema was composed of two parts, a central erythema with definite induration and a surrounding pallid erythema (double erythema). The distance between the two injection sites was so chosen that the central erythema of the second reaction might overlap only the area of the surrounding pallid erythema of the first reaction, but not the central one.

As a control, tuberculin was also injected in a corresponding new site on the left forearm. These two reactions at experimental and control sites were observed 4, 8, 24 and 48 hours after injection (Fig. 3).

In group A, in almost all children the reaction patterns at experimental and control sites was different from each other. At the experimental site *early reaction* was observed in all children, but at the control site it was found only in one child. Therefore, the influence of the preceding reaction was evident when a part of the second reaction overlapped the area of the preceding one.

In group B, 14 out of 21 children showed *early reaction* at the experimental site, while they showed no *early reaction* at the control site. Therefore, influence was recognized in about two thirds of children in whom the second injection site was so chosen that the areas of the first and second reactions might touch each other.

In group C, only four out of 44 children showed *early reaction* at the experimental site, and in the remaining 40 children the reaction pattern was almost the same at the experimental and control sites. Thus, little influence was noticed when the second injection site was chosen apart from the first one with a distance greater than the diameter of the first reaction.

In group D with double erythema the reaction pattern at the experimental site was almost identical with that at the control site in all children. Therefore, no influence was recognized when the central erythema of the second reaction overlapped only the area of the surrounding pallid erythema of the first reaction.

In summary, the extent of the area influenced by the preceding tuberculin reaction...
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Fig. 3. Extent of the area influenced by the preceding tuberculin reaction. A; erythemas of the first and the second reactions partially overlapped. Left; reactions at the experimental site. Right; reactions at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for 10 cases. B; erythemas of the first and the second reactions came in contact with each other but did not overlap. Average for 21 cases. C; the two reaction areas did not touch each other. Average for 44 cases. D; strong reactions with double erythemas, the central erythemas of the second reactions overlapped only the surrounding pallid erythemas of the first reactions but did not come in contact with the central ones. Average for 14 cases.

was limited to the area of the erythema of that reaction. But, in a strong reaction with double erythema the surrounding pallid erythema had no influence on the subsequent reaction.

4. Duration of the Influence of the Preceding Tuberculin Reaction on the Subsequent one at the Same Site

In April and July of 1956, 266 school children received tuberculin injection in a new site on the right forearm. After one year (50 children), two years (46 children), three years (55 children), four years (50 children), five year (32 children) and six years (33 children), tuberculin was injected again in the previously injected site and in a new site as a control. Reactions were observed 4, (8, after one year), 24 and 48 hours after injection (Fig. 4).

In all six groups, reaction patterns at the used and new sites were different from each other. At the used site the early reaction was observed in all children after one to five years. After six years it was not found in four out of the 33 children.

The course of the delayed reaction in the used site was highly accelerated in many
Fig. 4. Duration of the influence of the preceding tuberculin reaction on the subsequent one at the same site. From upper left to lower right: reactions one year, two years, three years, four years, five years and six years after the first test. Average for 50, 46, 55, 50, 32 and 33 cases respectively. Left; reactions at the site of previous tests. Right; reactions at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations.

cases of the first two groups (after one and two years). After 48 hours a definite induration was observed only in 26% and 23% of cases respectively at the used site, while it was observed in 76% and 75% at the new site. Thereafter this acceleration was observed in fewer cases. After four to six years a definite induration was observed after 48 hours in 64%, 61% and 58% of cases respectively at the used site, in contrast to 84%, 80% and 82% at the new site.

In summary, the influence of the preceding tuberculin reaction was evident after one and two years. Thereafter, it became less remarkable but was still manifest even after six years.

5. Influence of Tuberculin Injection on the Subsequent Tuberculin Reaction at the Same Site in Tuberculin Non-sensitive Persons

Five infants, who had never received BCG vaccination and in whom tuberculin reaction was negative, were injected with tuberculin in the new site of the right forearm and with a control solution (diluted Sauton's culture medium) in the corresponding new site of the left forearm. Every two weeks these two materials were injected in the same sites five times, respectively. In one infant a pallid erythema with slight induration was observed after 8 to 12 hours at the fourth and fifth injection, but no typical early reaction was observed in any of the infants.

Thereafter, 0.04 mg of BCG was inoculated in these infants. After one month tuberculin reaction converted to positive in all infants. Then tuberculin was injected in three sites on the forearms, namely, the two sites where tuberculin or the control solution had previously been injected and a new site as a control. Reactions were observed 4, 8, 12, 24 and 48 hours after injection (Fig. 5).

The reaction patterns were almost the same at these three sites. No early reaction
In summary, tuberculin injection had no influence on subsequent tuberculin reaction at the same site when the injected subject was not sensitive to tuberculin.

6. Influence of Tuberculin Injection on the Subsequent Tuberculin Reaction at the Same Site in the Subjects Who were Sensitive to Tuberculin in the Past but Did Not React to it at the Time of the Present Experiment

a. A 1/2,000 tuberculin solution was injected in a new site on the right forearm in 23 school children. They had previously received BCG inoculation and had once showed positive tuberculin reactions. But at the present experiment their sensitivity to tuberculin had decreased and the reaction to the used tuberculin solution was negative after 48 hours. After one month tuberculin was injected again at the same site and at a new site as a control. Reactions were observed 4, 8, 24 and 48 hours after injection (Fig. 6). At the used site early reactions and accelerated, intensified delayed reactions were observed in 17 out of 23 children in contrast to the new site.

b. A 1/50,000 tuberculin solution was injected at a new site on the right upper arm in 11 adults, who had showed positive reaction to a 1/2,000 tuberculin solution. At the present experiment they all showed negative reaction to the used tuberculin solution after 48 hours. After one month 1/2,000 tuberculin solution was injected at the same site and at a corresponding new site on the left upper arm. Reactions were observed 4, 8, 24 and 48 hours after injection (Fig. 7). At the used site early reactions were observed in 8 out of 11 cases in contrast to the new site.

In summary, if the examined subject was suspected to be sensitized to tuberculin by BCG inoculation or natural infection, tuberculin injection could influence the subsequent tuberculin reaction at the same site, even when the first reaction was negative because
Fig. 6. Influence of tuberculin injection on the subsequent tuberculin reaction at the same site in previously BCG vaccinated subjects. Left; reactions at the site where tuberculin had previously been injected and no reaction had been observed. Right; reactions at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for 23 cases.

Fig. 7. Influence of injection with highly diluted tuberculin solution on the subsequent tuberculin reaction at the same site. Left; reactions at the site where 1:50,000 tuberculin had previously been injected and no reaction had been observed. Right; reactions at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for 11 cases.

of the low tuberculin sensitivity of the examined subject (in the former experiment) or because of the high dilution of the used tuberculin solution (in the latter experiment).

7. Influence of Other Materials Injected Intradermally on the Subsequent Tuberculin Reaction at the Same Site

0.06 mcg of PPDs, 10 u of Agglutinogen of *H. pertussis* and 15 u of "Communin" (a filtrate of culture medium of *E. coli*) were injected at three new sites, respectively, on the back of eight students. Every two weeks each of these three materials was injected twice or three times at the same site. One month after the last injection PPDs was injected at all three sites and at a new site as a control. Reactions were observed 4, 8, 24 and 48 hours after injection (Fig. 8).

At the site where PPDs was repeatedly injected remarkable early reaction and typical
acceleration of the delayed reaction were observed in all cases. At the sites where Agglutinogen or "Communin" had previously been injected, the typical alteration of reaction pattern with early reaction and accelerated delayed reaction were not noticed except in one case, though the reaction was intensified in three other cases. In the remaining four cases the reaction pattern was the same as at the new site.

In summary, other materials injected intradermally could not influence the subsequent tuberculin reaction in the same manner as tuberculin itself.

Fig. 8. Patterns of tuberculin reactions elicited by PPDs injected in the sites where other materials had previously been injected. PPDs→PPDs; reactions at the site where PPDs had previously been injected. Aggl.→PPDs; reactions at the site where agglutinogen of H. pertussis had previously been injected. "Com."→PPDs; reactions at the site where filtrate of culture medium of E. coli ("Communin") had previously been injected. PPDs; reactions at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for eight cases.

8. Influence of the Injection of Tuberculin Fractions on the Subsequent Tuberculin Reaction at the Same Site

a. 0.06 mcg of PPD¹ was injected in two new sites on the backs of seven students, and 0.06 mcg of CF¹ (polysaccharide fraction of old tuberculin) was also injected at two other sites. After one month PPD was injected at two used sites, where PPD or CF had previously been injected respectively, and at a new site as a control. CF was also injected at the remaining two used sites and at a new site. Reactions were observed 4, 8, 24 and 48 hours after injection (Fig. 9).

Early reaction was observed only at the site where PPD was injected twice. At the site, where at first CF and secondly PPD were injected, reaction patterns were almost the same as at the new site. At sites where CF was injected secondly neither early reaction nor delayed reaction was noticed.

b. Old tuberculin was injected at two new sites on the back of four students. After one month old tuberculin and "digested tuberculin"² were injected at one of these two used sites respectively, and at two new sites as controls. Reactions were observed 4, 8,

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¹ These fractions were provided by Department of Tuberculosis, the National Institute of Health, Japan.
² Old tuberculin was incubated with an equal amount of 100,000 u/ml trypsin solution at 27°C for 4 hours, heated to 100°C, centrifuged at 3,000 rpm for 15 minutes. The supernatant was dialyzed for 48 hours and diluted to 1/2,000 of the original material. It was shown to have no potency to elicit positive reactions in tuberculin sensitive subjects.
Fig. 9. Interaction between the reactions elicited by tuberculin fractions, PPD and CF (polysaccharide fraction of old tuberculin). PPD→PPD; reactions elicited by PPD at the site of previous PPD injection. CF→PPD; those at the site of previous CF injection. PPD; those at a new site. PPD→CF; reactions elicited by CF at the site of previous PPD injection. CF→CF; those at the site of previous CF injection. CF; those at a new site. Average for seven cases.

24 and 48 hours after injection (Fig. 10).

At the site where tuberculin was injected twice early reaction was observed in all four cases in contrast to the new site. At the site where at first tuberculin and secondly "digested tuberculin" were injected, early reaction was not observed and the reaction pattern was almost the same as at the new site.

In summary, the results of these experiments suggested that the tuberculin fraction responsible for the early reaction might be a protein fraction and not a polysaccharide fraction.

9. Influence of the Injection of the Tuberculin Active Polypeptide (TAP Yamamura) on the Subsequent Tuberculin Reaction at the Same Site

Old tuberculin and 0.25 mcg of tuberculin active peptide (TAP Yamamura), which was reported to have no sensitizing capacity by Someya, were injected, respectively, at two sites on the back of five students. After one month old tuberculin and TAP were injected at four used sites and at two new sites after the manner of the preceding experiment with PPD and CF. Reactions were observed 4, 8, 24 and 48 hours after injection (Fig. 11).

At all four sites where injection was repeated, early reaction and accelerated delayed reactions were observed in contrast to the new sites, and all the reaction patterns were almost the same.

In summary, the injection of TAP also influenced the subsequent tuberculin reaction at the same site. Between old tuberculin and TAP no significant difference was observed in the effect of modifying the subsequent tuberculin reactions.
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Fig. 10. Reactions elicited by digested tuberculin at the site of previous tuberculin tests. OT–OT; reactions elicited by old tuberculin at the site of previous old tuberculin injection. OT; those at a new site. OT–Dig. OT; reactions elicited by digested tuberculin at the site of previous old tuberculin injection. Dig. OT; those at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for four cases.

10. Histological Study of the Tuberculin Reaction at the Used Site

Tuberculin was injected at three sites (A, B and C) along the prospective incision line on the back of 35 tuberculosis patients, who were determined to undergo thoracoplasty or pulmonary resection. The site A was used as a control site. After one month tuberculin was again injected at the site B. After three to six months, just before the operation, tuberculin was injected at the two used sites (B and C) and at a new site (D) as a control. It was injected 2, 4, 12, 18, 24, 48 and 72 hours before operation in every five patients, respectively. The skin including these four sites (A, B, C and D) was removed at operation and subjected to histological study. Sections were stained with hematoxylin and eosin, Van Gieson stain, the Weigert’s elastic tissue stain and silver impregnation for reticulin. Invading cells were calculated and classified into 1. neutrophilic leucocytes and 2. lymphocytes and large mononuclear cells (monocytes and histiocytes).

The principal histological findings observed in the skin of the reaction sites were dilatation and congestion of capillaries, stasis, cell infiltration and thickening of collagen fibers, etc.

The differences between the findings in the used and new sites were already noticed
Fig. 11. Interaction between reactions elicited by old tuberculin and tuberculin active peptide (Yamamura) (TAP). OT–OT; reactions elicited by old tuberculin at the site of previous old tuberculin injection. TAP–OT; those at the site of previous TAP injection. OT; those at a new site. OT–TAP; reactions elicited by TAP at the site of previous old tuberculin injection. TAP–TAP; those at the site of previous TAP injection. TAP; those at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for five cases.

after two hours. After four hours these differences became significant. In the used sites remarkable edema and diffuse, dense cell infiltration were observed in the stratum papillare and subpalillare, while in the new site only slight edema and sporadic cell infiltration were noticed. But the invading cells were almost the same in the used and new sites and composed of about 45 to 50% neutrophilic leucocytes and about 45% lymphocytes and large mononuclear cells (Fig. 12 and 16).

After 12 hours stasis was found in all three sites. In the used sites, cell infiltration was more intensified in stratum papillare and subpapillare and spread into the epidermis, stratum reticulare and subcutis. Formation of vesicles was observed in the epidermis. In the new site edema became remarkable but cell infiltration was yet localized and clustered. The kinds of invading cells were almost the same as those after 4 hours but in the new site the percentage of neutrophilic leucocytes diminished slightly (Fig. 13 and 16).

After 18 hours, the percentage of neutrophilic leucocytes showed a marked decrease in all the three sites.

After 24 hours the difference between the used and new sites decreased and diffuse cell infiltration was also observed in the new site. Invading cells were composed of about 20 to 25% neutrophilic leucocytes and about 70% lymphocytes and large mononuclear cells in all the three sites (Fig. 14 and 16).

After 48 hours the intensity of cell infiltration decreased in the used sites (more markedly in site B, where tuberculin was injected three times, than in site C, where it
Fig. 12. Histological findings. 4 hours after injection. (a) Used site. Diffuse cell infiltration with predominance of neutrophilic leucocytes. (b) New site. Sporadic cell infiltration, also with predominance of neutrophils.

Fig. 13. Histological findings. 12 hours after injection. (a) Used site. Intense cell infiltration in dermis, spreading into epidermis and subcutis. Predominance of neutrophilic leucocytes. (b) New site. Clustered cell infiltration, also with predominance of neutrophils.

Fig. 14. Histological findings. 24 hours after injection. (a) Used site. (b) New site. Diffuse cell infiltration with predominance of mononuclear cells in both sites.
Fig. 15. Histological findings, 48 hours after injection. (a) Used site. Cell infiltration separated in masses by thickened collagen fibers. Predominance of mononuclear cells. (b) New site. Diffuse, dense cell infiltration, also with predominance of mononuclear cells.

Fig. 16. Invading cells at the used and new sites. Average for five cases at each observation time.
was injected twice. Collagen fibers became thickened separating the invading cells in masses. In contrast to this, cell infiltration in the new site attained its maximum intensity and became more remarkable than in the used sites. Invading cells were composed of nearly the same cells as after 24 hours, namely about 20% neutrophils and about 75 to 80% mononuclear cells (Fig. 15 and 16). After 72 hours the reactions in the three sites weakened and became similar to each other.

In summary, in the used sites the early inflammatory reaction with neutrophilic leucocytes, which was also observed to a slight degree in the new site, was significantly intensified and the delayed reaction with mononuclear cells declined earlier than in the new site.

11. Alteration of Reaction Patterns in the Other Delayed Type Skin Reactions Repeated at the Same Site

a. 0.1 ml of 1/20 diphtheria toxoid solution was injected intradermally on the flexor surfaces of the right upper arms of five nurses and student nurses. After 1 month, diphtheria toxoid was again injected at the same site and at a corresponding new site of the left upper arm. Reactions were observed 4, 8, 24 and 48 hours after injection.

At the used site early reaction was observed in all five cases and remarkable acceleration of delayed reaction was noticed in two cases, while at the new site no early reaction was observed (Fig. 17).

b. 10 u of H. pertussis Agglutinogen was injected intradermally on the back of three students. After 1 month Agglutinogen was again injected at the same site and at a corresponding new site. The observation times were the same as in the above experiment.

At the used site early reaction and acceleration of delayed reaction were observed in all three cases in contrast to the new site (Fig. 18).

In summary, in other delayed type skin reactions such as Moloney reaction or H. pertussis Agglutinogen reaction almost the same alteration of reaction patterns as in the tuberculin reaction was observed.
12. Alteration of Reaction Patterns in the Tuberculin Patch Test Repeated at the Same Site

A tuberculin patch test and an intradermal test were given at two new sites on the flexor surfaces of the left upper arms of 13 subjects, respectively. In the patch test a hydrophilic ointment with 33% old tuberculin was applied for 24 hours. After one month the following two experiments were carried out.

a. In nine of the 13 subjects tuberculin patch tests were again undertaken at the two used sites and at a corresponding new site on the right upper arm. Reaction was observed 24, 48 and 72 hours after the application of the tuberculin ointment and classified into six grades after Murohashi's classification (Murohashi et al. 1962).
At both used sites reaction was already remarkable after 24 hours in seven out of nine cases and declined after 72 hours in four cases. In contrast, at the new site it was hardly perceptible after 24 hours in all cases except two, appearing thereafter and attaining its maximum after 48 to 72 hours (Fig. 19).

b. In the remaining four subjects tuberculin was injected intradermally at the two used sites and at a corresponding new site of the right upper arm. Reaction was observed 4, 8, 24 and 48 hours after the injection.

At the site where a tuberculin patch test had previously been given almost the same alteration of reaction pattern was observed as at the site where tuberculin was injected twice intradermally (Fig. 20).

**Fig. 20.** Influence of preceding percutaneous tuberculin reactions on the subsequent intradermal reactions. Used site (Intradermal): intradermal tuberculin reactions at the site of previous intradermal tests. Used site (Patch): those at the site of previous patch tests. New site; those at a new site. Upper line; average size of erythemas. Lower line; that of definite indurations. Average for four cases.

In summary, alteration of reaction pattern was also observed in tuberculin patch tests repeated at the same site. Moreover, interaction between the tuberculin intradermal and patch tests were proved.

**DISCUSSION**

In the skin area at which a tuberculin test has previously been given the reaction pattern is altered. Namely, *early reaction* is elicited and the course of delayed reaction is accelerated. Reaction appears as early as three to four hours after injection, attains its maximum after 12 to 24 hours and thereafter usually fades away.

It is highly probable that these two phenomena, the appearance of *early reaction* and the acceleration of delayed reaction, are related with each other. Without *early reaction*, the delayed reaction would not be accelerated. But they do not always run parallel to each other. In the experiment 2, in which tuberculin was repeatedly injected at the same skin area, *early reaction* appeared at the second injection, but the acceleration of delayed reaction with marked decline after 48 hours was not manifest until more injections had been repeated. At the second injection the delayed reaction was intensified
in many cases.

Edwards (1955) noticed the unexpectedly high frequency of accelerated, intense, bullous reactions at the second test, but if she had repeated more tests at the same site, she would have found a progressive decrease of the intensity of reactions. Terada (1956) found nearly the same alteration of reaction pattern in 1955, one year before the present study, repeating tuberculin test every ten days at the same site. But in his experiment the interval between the two tests seems to be somewhat too short, to exclude the direct effect of the local inflammation. Inaba (1960) also confirmed the above-mentioned results.

This alteration of reaction pattern is not systemic but local, as many authors have pointed out. Once tuberculin test is given in an area of skin, the mode of reaction of that skin area to subsequent tuberculin injection is modified. Therefore, tuberculin should always be injected in new sites, according to Nobechi’s proposal, whenever tuberculin reactions are compared with each other simultaneously or successively. For this purpose, where should the injection site be chosen? The experiment 3, investigating the extent of the modified skin reactivity, proved its localization into the area of the erythema of the preceding reaction. Consequently, in order to avoid the influence of the preceding reaction, the distance between the present and foregoing injection sites must be greater than the diameter of the preceding erythema. Very large erythemas over 4 cm in diameter, are usually divided into two areas, the central erythema with definite induration and the surrounding pallid one (double erythema), and the latter is proved to have no influence upon the mode of reaction of the corresponding skin area. Murai (1960) also confirmed this fact. Accordingly, if tuberculin is injected in the site more than 5 cm away from the foregoing injection site, the reaction would avoid the influence of the preceding one. In practice, 6 points can be chosen as adequate injection sites, namely, the upper and lower third points of both forearms and the lower third point of both upper arms.

Then how long may this modified reactivity of the injected skin area last? In experiment 4 this problem was pursued for six years after the first test, but the full length of the duration could not be determined. Here again the appearance of early reaction and the acceleration of delayed reaction did not necessarily run parallel to each other. Namely, early reaction was always observed in almost all cases from one to six years, but as to the acceleration of delayed reaction, the longer the interval between the first and second tests, the less manifest was this phenomenon. It seems probable that this marked acceleration of delayed reaction will disappear earlier than the early reaction. Duboczy (1961) demonstrated that in the site of previous tuberculin tests given 23 years ago even early reaction was not observed.

In experiments 1 to 4 all subjects were tuberculin sensitive and showed positive reactions at the first tests. When tuberculin is injected in a person who has no sensitivity to it, would the reactivity of the injected skin area also be modified? Experiment 5 answered this question with “No”. As the subjects were infants in this experiment, it seems possible that their poor capacity for antibody production might be the reason of these negative results. But Kobayashi (1962), making a similar experiment in school children, achieved almost the same result, and Maeda (1960) also confirmed this fact using guinea pigs.

In contrast to this, the results of experiment 6 show that in the subjects suspected to be sensitized to tuberculin, tuberculin injection can modify the reactivity of the skin area, even if it elicits no positive reaction. Consequently, it seems possible that the
alteration of the reactivity of skin area by tuberculin injection needs the systemic tuberculin sensitivity as a postulate, but does not always need positive local reaction.

Duboczy (1962) reported early reaction observed in tuberculin negative persons at the site of previous tests. In his case 1 a superintendent nurse who had never had a positive tuberculin reaction, showed an early reaction, which began within 3 hours, continued 24 hours and disappeared within 48 hours (at the site where she had previously had negative tests). This finding appears different from the present authors’ observations. However, since the subject was a nurse in a tuberculosis hospital, it seems possible that she had already been systemically sensitized to tuberculin, by natural infection, as the school children in experiment 6 a, who had been vaccinated with BCG but showed negative reactions. Moreover, her reaction might be considered as a combination of early and delayed reactions, as it continued for 24 hours. Recently the present authors observed a nearly pure early reaction at the used site of a sarcoidosis patient, which appeared within 4 hours, attained its maximum in 12 hours and dissapeared almost completely within 24 hours.

The next problem is whether the change of reactivity of the tested skin area is the result of local sensitization by injected tuberculin, or is it merely the nonspecific consequence of local inflammation. Experiment 6 proved that, without perceptible local inflammation, injection of tuberculin in a tuberculin sensitive person can change the reactivity of the skin area. Experiment 7, moreover, demonstrated that local inflammations elicited by other materials can not produce the typical modification of reactivity to tuberculin. These experimental results appear to support the conception of specific local sensitization. In experiment 7 the injection of other materials intensified a subsequent tuberculin reaction in a few cases. Maeda (1960) observed similar intensification of reaction in guinea pigs, using staphylococcal vaccine and phenol.

Tuberculin reaction is a representative delayed type reaction, caused by the protein fraction of tuberculin. Is the early reaction at the used site, appearing as early as three to four hours after injection, also caused by the protein fraction or by the polysaccharide fraction? Experiment 8 with tuberculin fractions and digested tuberculin proved that it is caused by the protein fraction, not by the polysaccharide fraction.

In experiment 9 the tuberculin active peptide (TAP Yamamura) was used. This polypeptide was prepared by Yamamura (1960) from cells of M. tuberculosis and proved by Someya (1962) to have no sensitizing effect, when injected into guinea pigs with adjuvant. In the present experiment TAP was also proved to have the potency to modify the reactivity of the injected skin area and to elicit early reaction and the acceleration of delayed reaction. Ókushi (1961) reported a similar observation using TAP but he noticed a slight difference between the effects of TAP and old tuberculin in contrast to the present observation.

As the conclusion of the above experiments the present authors would like to say that the appearance of early reaction and the acceleration of the course of delayed reaction at the used site are the results of the local sensitization brought about by the injection of tuberculin protein into a tuberculin sensitive person.

Hioki (1957) made a histological study of tuberculin reaction in the human akin. He infected tuberculin on the back of tuberculosis patients undergoing thoracoplasty or pulmonary resection. Injections were given at different lengths of time before operation and the injected skin areas were removed at operation and studied histologically. Similar histological study of tuberculin reaction at the repeatedly used sites was attempted after his method.
Despite the edematous appearance of the early reaction, its principal histological finding is not edema, but diffuse cell infiltration with predominance of neutrophilic leucocytes. Similar but slight cell infiltration is simultaneously found in the new site. After 24 hours histological changes characteristic of delayed type reaction with mononuclear cell infiltration are found in both sites and after 48 hours this cell infiltration declines in the used site in contrast to the new site. The shift from neutrophilic leucocytic infiltration to mononuclear cell infiltration occurs about 18 hours after injection in both sites. Consequently, from the viewpoint of invading cells, the course of histological changes in the used site is not accelerated. It seems possible to conclude, that in the used site a neutrophilic leucocytic infiltration, observed to a slight degree in the early stage of the ordinary tuberculin reaction, is markedly intensified, or a new reaction with neutrophilic leucocytic infiltration (early reaction) is added to the ordinary course of the tuberculin reaction.

Maeda (1960) attained nearly the same results using guinea pigs.

In the above-mentioned histological study, the sections at different times were derived from different subjects. In another experiment skin areas showing reactions 4, 12, 24 and 48 hours after injection were removed from the same individuals. The course of the histological changes in the used site was the same as in the above experiments.

Experiment 11 shows that this alteration of reaction patterns is also found in other delayed type reactions e. g., Moloney reaction and H. pertussis Agglutinogen reaction. Iidaka (1959) observed the same alteration of reaction patterns using lepromin in leprosy patients and found a cross reaction between lepromin and tuberculin, which seem to be closely related to each other in antigenicity.

Moreover, a similar alteration of reaction patterns is also found with the patch test. Therefore, it seems necessary that every delayed type intradermal or patch test be given at a new site where that test has never been undertaken.

As for the early reaction itself, this reaction is also observed in some cases even at an unquestionably new site, for example, on the flexor surface of the upper arm or on the back (Takahashi 1960). In these cases its appearance seems to be related to the systemic tuberculin sensitivity, and not to the local one. Concerning this problem, further studies are required.

**SUMMARY**

The pattern of tuberculin reaction is altered at the site of a previous test. Reaction appears earlier and fades sooner. This phenomenon was previously noticed by v. Pirquet in 1909. Since Yanagisawa reported the acceleration of the course of tuberculin reaction at the repeatedly used site, intensive studies were made by many Japanese investigators. In the present paper further observations on this phenomenon are reported.

The course of tuberculin reaction at the repeatedly used site was observed from immediately after injection. Remarkable erythema and induration with edematous appearance (early reaction) appeared as early as three to four hours. Reaction attained its maximum after 12 to 24 hours, and declined markedly thereafter.

This alteration of reaction pattern was observed from the second test on. Reaction was intensified in the second test, but thereafter it became weaker as the tests were repeated.

This phenomenon is not systemic, but local. The extent of the area influenced by the preceding tuberculin reaction was limited to the area of its erythema. But the surrounding pallid erythemas observed in strong reactions had no influence on the subse-
The duration of this influence was pursued for a period of six years. It became less remarkable as time elapsed, but was still manifest after six years.

As to the influence of negative reaction, no modification of the local skin reactivity was observed in tuberculin nonsensitive subjects. But in subjects suspected to have been sensitized to tuberculin, injection of tuberculin could modify the local skin reactivity without positive reaction.

Other materials injected intradermally could not modify the local skin reactivity to tuberculin in the characteristic manner.

*Early reaction* was proved to be elicited by the protein fraction of tuberculin, not by the polysaccharide fraction. Also tuberculin active peptide (Yamamura), reported to have no sensitizing effect, modified the local skin reactivity and elicited *early reaction*.

Histological study of the tuberculin reaction in the used site of human skin was performed. The principal histological finding of the *early reaction* was proved to be diffuse cell infiltration with predominance of neutrophilic leucocytes. As a whole, in the used site the early inflammatory reaction with neutrophilic leucocytes, which was also observed to a slight degree in the new site, was intensified, and the delayed reaction with mononuclear cells declined earlier than in the new site.

From the above results it seems probable that the alteration of reaction pattern at the used site, namely, the appearance of *early reaction* and the acceleration of delayed reaction, is the result of the local sensitization brought about by the injection of tuberculin protein into a tuberculin sensitive subject.

It was proved that such an alteration of reaction pattern at the used site also occurs in other delayed type intradermal reactions and in patch tests.

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