THE HISTOLOGY OF THE INTRACUTANEOUS TUBERCULIN REACTION IN HUMAN SKIN[1]

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The tuberculin test is becoming a diagnostic aid of increasing importance. The test is most useful among groups wherein the incidence of tuberculous infection is low. It is more useful as applied to younger individuals, to those who live in small communities, and to those who reside in areas wherein the positive reactors are few, such as parts of the Middle West.

Our knowledge of the tuberculin reaction is incomplete. The mechanism of its development is obscure and much remains to be learned about its immunologic and allergic significance. We have been unable to find a description in the literature of the histologic appearance of this important reaction at various phases of its development in human skin. Previous studies have been made on experimental and domestic animals. Undoubtedly, hundreds of thousands of tuberculin tests have been performed on human beings, apparently without knowledge of these fundamental facts on the part of those who performed them.

METHODS

It might be difficult to secure an adequate number of volunteers who would be willing to undergo the removal of specimens of skin for biopsy in order to permit the tuberculin reactions to be studied histologically. No possible benefit could be promised the individual and no obvious advancement of practical medical

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science could be foreseen. Such a study would obviously require the surgical excision of skin and subcutaneous tissue from a considerable number of individuals.

It occurred to us that material valuable for study could be obtained without discomfort, disfigurement or risk by the following simple procedure, which was carried out: intracutaneous tuberculin tests ("Mantoux tests") were made along the long axes of contemplated surgical incisions at varying times preceding operation. When the incision was made, the surgeon excised an elliptical portion of skin which contained the tuberculin reaction. The portions of maximal reaction were indicated by superficial scalpel marks which could be identified subsequently in sections. The specimens were fixed in a solution of 10 per cent neutral formalin, and were sectioned and stained. All specimens for biopsy were taken from the skin of the thorax, usually in the scapular region. All were obtained from volunteers who were patients at the Glen Lake Sanatorium, Oak Terrace, Minnesota. Forty-four specimens were obtained in this manner. These were obtained from one hour to fourteen days after injection of tuberculin.

All patients had active pulmonary tuberculosis requiring surgical collapse, usually thoracoplasty. All patients had been previously tested for tuberculin sensitivity, making it possible to adjust the dosage of the tuberculin solution to secure relatively uniform reactions of 1 plus or 2 plus severity without necrosis. Three types of tuberculin solution were employed: purified protein derivative ("P.P.D."); Dorset's old tuberculin ("O.T."); and "M.A. 100" (a protein fraction of tuberculin similar to P.P.D.). All injections were made intracutaneously.[4]

RESULTS

The following descriptions indicate the microscopic reactions noted in specimens biopsied at various time intervals after the injection of tuberculin:

One-hour reactions.—The early reaction may be partly attribut-

[4] The authors are grateful to Drs. Ernest S. Mariette, E. P. Fenger, T. J. Kinsella and G. K. Higgins, who generously co-operated in securing this material.

able to the trauma caused by the intracutaneous injection produced by the needle and also by the fluid which has been injected under considerable pressure. The latter action produces a separation of the connective tissue fibers, and hemorrhage into the region (fig. 1*a*). Careful search under the high power objective of the microscope discloses small collections of poly-

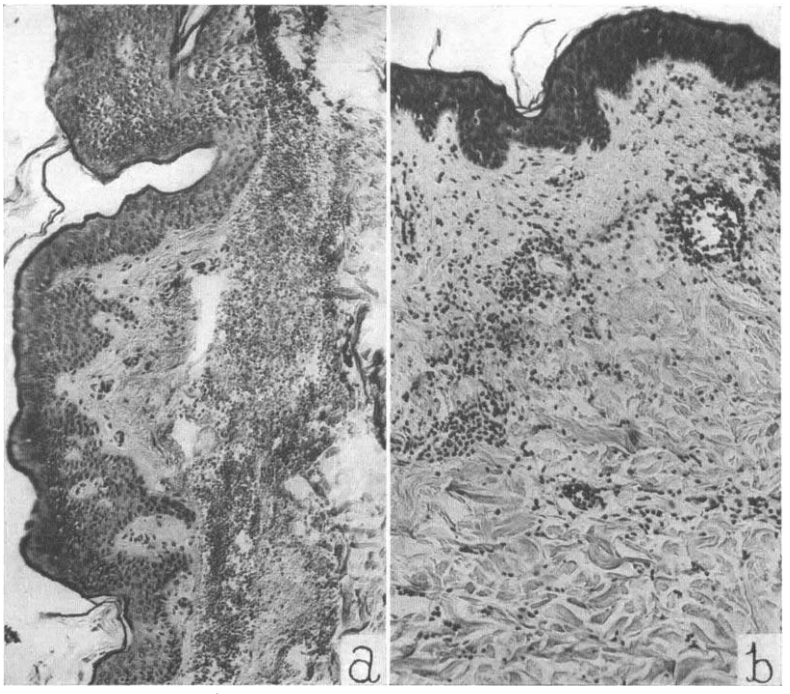

FIG. 1*a*. Traumatic hemorrhage produced by the intracutaneous injection of tuberculin; appearance of section at the end of one hour (hematoxylin and eosin × 100); *b*, two-hour tuberculin reaction, consisting of polymorphonuclear leukocytic infiltration (hematoxylin and eosin × 100).

morphonuclear leukocytes which have formed within one hour after injection. These collections are situated in the immediate vicinity of the hemorrhage. Usually, only a few leukocytes are seen in any one microscopic field. Small vascular channels nearby may contain an abnormal concentration of polymorphonuclear leukocytes within their lumens. In no instance is this collection of leukocytes impressive.

Two-hour reactions.—Within two hours, sections of skin obtained from near the site of injection demonstrate a very definite localized acute inflammatory response (fig. 1*b*). The infiltration consists of leukocytes which are predominantly of the polymorphonuclear type, with moderate numbers of eosinophilic granulocytes in unusual specimens.

Three-hour reactions.—Three hours after the injection of tuberculin, the inflammatory reaction is more severe and is more widely spread. In some specimens, the infiltration is rather diffuse throughout the papillary layer, with a tendency in some instances for the infiltrating cells to collect about the vascular tufts in the dermal papillae. Deeper in the reticular layer, a much greater proportion of infiltrating cells will be seen in the immediate vicinity of blood vascular channels (fig. 2*a*). Even at this early stage it is possible to detect a definite inflammatory reaction in the subcutaneous tissues. The infiltrating cells are predominantly polymorphonuclear leukocytes, and are seen both outside the vessels, as described previously, and within the lumens of smaller vessels. It is not difficult to find leukocytes within the wall of the vessel which apparently were fixed while undergoing diapedesis.

Six-hour reactions.—Gross inspection of a six-hour tuberculin reaction discloses little of diagnostic significance, but microscopically the reaction is well developed. The reaction is by no means as intense as that which will be seen a day or two later, but the types of cells and their localization are similar to those of a mature tuberculin reaction.

Prior to this time, the predominant cells will have been the polymorphonuclear leukocytes. However, the sections of positive tuberculin reactions biopsied after six hours show a great preponderance of mononuclear cells congregated in the tissue spaces. These cells are predominantly of the histiocytic type, with variable minority numbers of lymphocytes in different specimens. Polymorphonuclear leukocytes are difficult to find in the tissue spaces, even on careful search. Within small veins and capillaries, the polymorphonuclear cells have increased greatly, until many of these channels are densely packed with such cells.

Twelve-hour reactions.—Qualitatively, the changes at twelve hours are similar to the description in the preceding paragraph, but quantitatively, the reaction is much more intense than it was at six hours, with an increase in the number of cells which have collected in the tissues, and apparently, a widening zone of reaction (fig. 2*b*). In some specimens, the extravascular collections

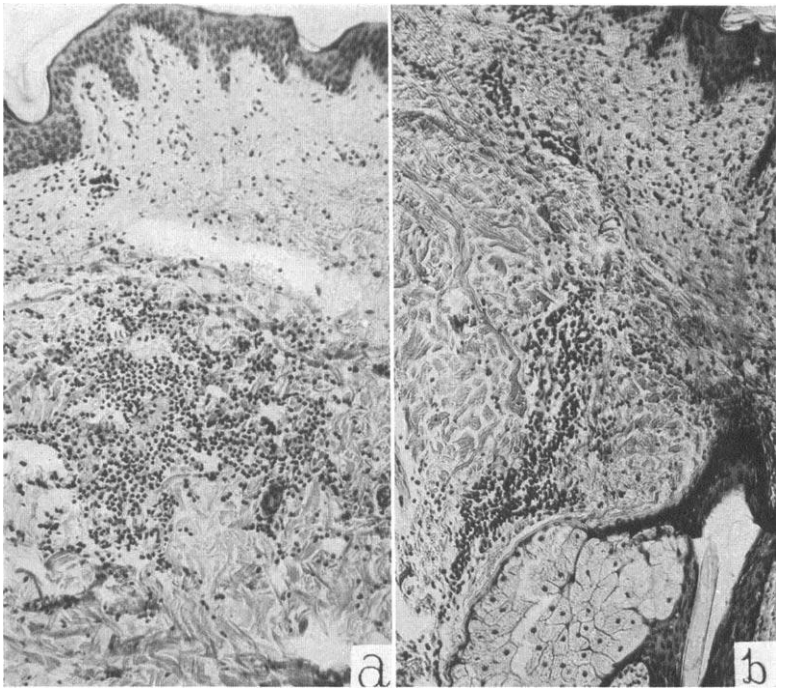

FIG. 2*a*. Three-hour reaction, showing increasing infiltration with polymorphonuclear leukocytes (hematoxylin and eosin × 100); *b*, twelve-hour tuberculin reaction, in which histiocytic cells predominate (hematoxylin and eosin × 100).

are almost entirely histiocytic cells; in others, a variable proportion of cells resembling lymphocytes are seen. In many cases the intravascular collections of polymorphonuclear leukocytes previously described are a striking feature. The papillary layer and upper reticular layers of the dermis seem to be involved most severely. Definite evidence of reaction is visible into the subcutaneous fat. The cells comprising the reactions are now seen

to congregate definitely about vascular channels and about the dermal appendages. During these early hours distention of the lymph spaces and engorgement of the capillaries can be detected.

Twenty-four hour reactions.—The intracutaneous tuberculin reaction is often sufficiently well developed to be recognized grossly by inspection and palpation within twenty-four hours.

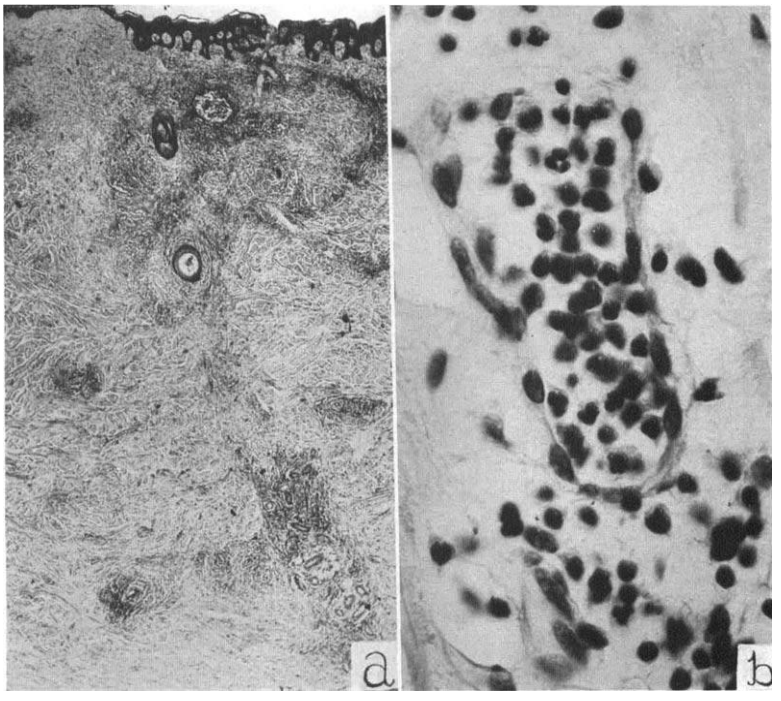

FIG. 3*a*. Twenty-four hour tuberculin reaction, demonstrating vigorous and extensive histiocytic infiltration (hematoxylin and eosin × 18); *b*, twenty-four hour reaction, showing the intravascular concentration of polymorphonuclear leukocytes (hematoxylin and eosin × 660).

Microscopically, the reaction by this time is vigorous and extensive (fig. 3*a*). It consists of a widespread focal mononuclear response. The majority of cells at this stage have the cytologic appearance of histiocytes, and in many regions these cells are packed together as closely as possible. The greatest concentration of reacting cells occurs around hair follicles, sebaceous glands,

sweat glands and blood vessels. These focal collections of cells are seen throughout the depth of the dermis, with limited but definite involvement of the subcutaneous fat in occasional portions. Figure 3*b* demonstrates how small vessels may have a dense concentration of polymorphonuclear leukocytes within their lumens, so that the contents of these vessels actually re-

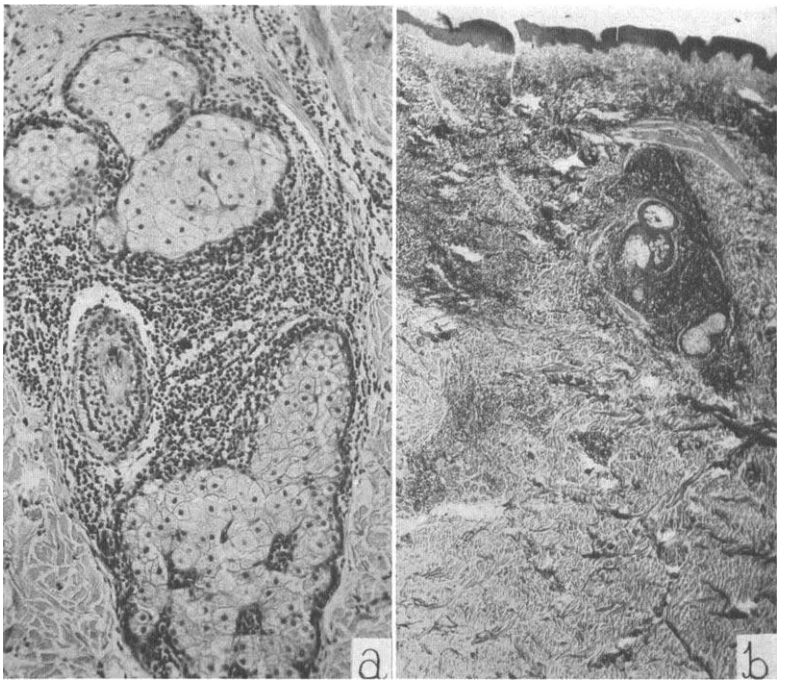

FIG. 4*a*. Fifty-two hour tuberculin reaction, illustrating the dense collection of histiocytes about the dermal appendages (hematoxylin and eosin × 100); *b*, seventy-two hour reaction, depicting the concentration of histiocytes about the dermal appendages (clear spaces are artefacts) (hematoxylin and eosin × 18).

semble pus from the microscopic viewpoint. It is difficult to believe that free circulation is taking place in these channels. Outside the vessels, polymorphonuclear leukocytes are rare at this stage. During our study, lymphocytic forms in the material we examined were usually not common after twenty-four hours. Exceptional specimens disclosed the presence of large numbers of

eosinophilic granulocytes, but usually these cells were few or absent.

Forty-eight to fifty-two hour reactions.—Microscopic examination of tuberculin reactions biopsied after forty-eight to fifty-two hours discloses a further increase in the vigorous response described for the twenty-four hour specimens (fig. 4*a*). No significant change can be detected at this stage in the appearance of the histiocytic cells comprising these dense collections, although their number has increased. In many regions they are as closely packed together as possible (fig. 4*a*), apparently distending the tissues and distorting the relationships of surrounding structures. In most specimens, only occasional cells of this type are found outside the rather sharply circumscribed collections. In other specimens, the reaction appears to be somewhat more diffuse. Rarely do these cells appear actually to invade glandular structures. It is difficult to demonstrate true edema or congestion, and it appears that the swelling and induration noted grossly can be accounted for, at least in part, by the great number of cells which distend the tissues.

Seventy-two hour reactions.—The extent and severity of the intradermal tuberculin reaction can be appreciated by inspecting the photomicrograph taken under the low power objective of the border of a seventy-two hour reaction (fig. 4*b*). This illustration also shows the maximal concentration of cells around glands, hair follicles and vascular channels. The cells are typical histiocytes (fig. 5*a*).

It is frequently possible to distinguish evidence of partial atrophy of the dermal appendages, especially of the sebaceous glands; and also degenerative changes, especially in the sweat glands. Early efforts at regeneration are sometimes visible in these dermal appendages.

One-week reactions.—The tuberculin reaction after one week is still of striking appearance microscopically (fig. 5*b*). Many of the histiocytic cells show evidence of differentiation as is described in detail in the following paragraph, dealing with the reaction after two weeks. Some of these histiocytic cells have nuclei resembling those of epithelioid cells, but no typical epithelioid

cells can be identified. In occasional specimens it is possible to find cells resembling Langhans' type of giant cell. No organized tubercles can be clearly recognized.

Two-week reactions.—The most impressive feature of these late reactions is the change in appearance of the majority of the histiocytic cells. Seen under the low power objective, the region

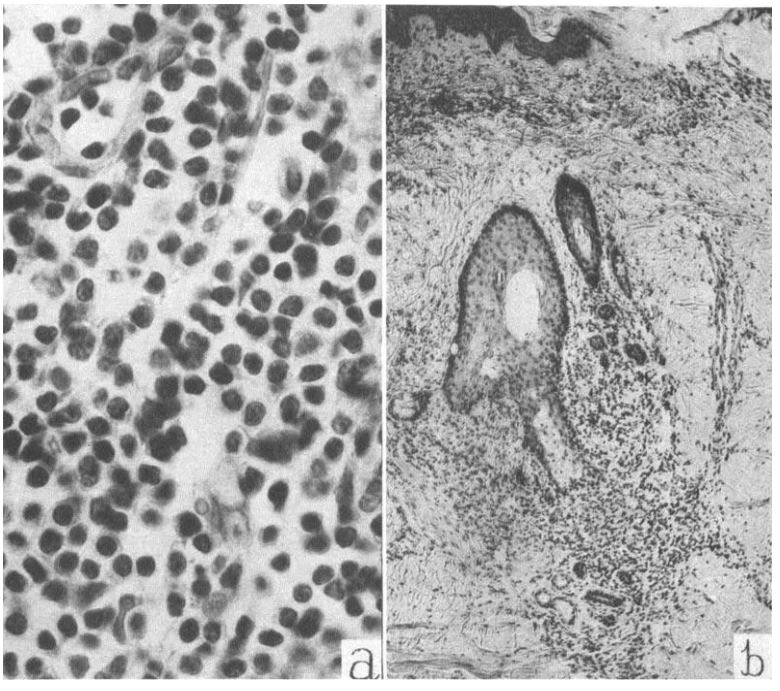

FIG. 5*a*. Seventy-two hour reaction, demonstrating the appearance of typical histiocytic cells (hematoxylin and eosin × 660); *b*, seven-day tuberculin reaction (hematoxylin and eosin × 60).

of reaction has the appearance of transparency (fig. 6*a*), contrasting with the dense appearance of earlier reactions (fig. 4*a*). These late reactions, viewed under the high power objective, demonstrate pronounced changes in both nuclei and cytoplasm of the histiocytic cells (fig. 6*b*). The nuclei of many such cells have become large, pale and vesicular. These nuclei are oval or occasionally spherical. The cytoplasm has undergone even more

striking transformation than that of histiocytic cells of the earlier reactions, being filled with moderate to large-sized vacuoles.

These unusual cells apparently are derived from the histiocytic forms, but the usual transitional stages have not been clearly followed. Their appearance is sometimes suggestive of epithelioid cells and others more closely resemble fibroblasts. They

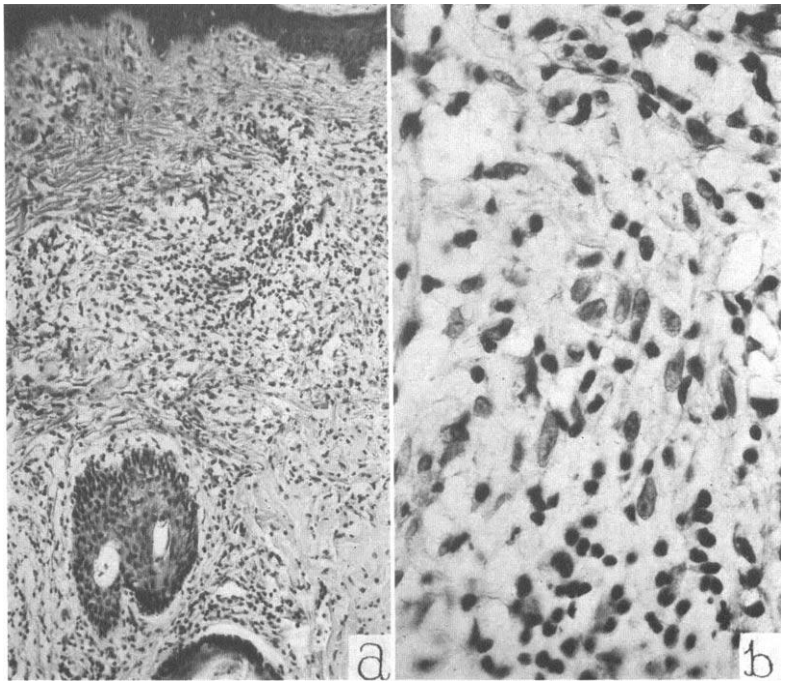

FIG. 6*a*. Fourteen-day tuberculin reaction (hematoxylin and eosin × 100); *b*, fourteen-day tuberculin reaction, showing nuclear details and the vacuolated cytoplasm of the differentiating cells (hematoxylin and eosin × 440).

are undergoing differentiation and appear destined to become ordinary connective tissue cells, losing their identity. However, we have not followed the reaction beyond the two-week period.

COMMENT

The reaction of human skin to the intracutaneous injection of tuberculin solution is one of surprising simplicity. Undoubtedly, larger doses of tuberculin would have produced a much more com-

plicated microscopic appearance than that which we viewed, due to the resulting necrosis, efforts at repair and possible secondary infection. It would be impossible clearly to separate these effects from the responses which tuberculin itself elicits.

The first phase of the reaction is one of polymorphonuclear infiltration. This phenomenon is of surprisingly short duration, being essentially completed within six hours. The factor of trauma produced by the needle and the disruption of tissue caused by the fluid injected under pressure must play some part in this phase of the reaction. However, Wartman has demonstrated that tuberculoprotein has the definite power of attracting polymorphonuclear leukocytes in vitro. In the study of the tuberculin reactions of cattle, Fitch and one of us (Feldman) (1) have shown that the polymorphonuclear response of these animals is much more prolonged than that which we have observed of humans, persisting into the second day. In human skin, the polymorphonuclear cells have almost disappeared within six hours. Their persistence within the lumens of small vessels is an interesting feature.

The remarkable obliterative endarteritis which Fitch and one of us (Feldman) observed in cattle was not detected in our present material. The differences between results of our tests and those of Fitch and one of us (Feldman) on cattle may have been caused by the difference in the dose of tuberculin administered. As stated previously, none of the reactions was sufficiently severe to produce significant necrosis.

Only a few of the specimens studied showed extensive infiltration with eosinophilic granulocytes. No relationship can be seen between the appearance of these cells and the type of tuberculin employed, the age of the reaction or any other factor. It seems probable that these cells appear in relation to some idiosyncrasy of the individual.

A few reactions exhibited multinucleated cells resembling Langhans' type of giant cell. There was no consistent appearance of such cells and in many reactions they were totally lacking. No true tubercle formation could be seen, but occasionally, foci of cells were seen which resembled epithelioid cells and gave the impression of abortive attempts in this direction.

No close correlation can be made between the appearance of the human tuberculin reaction and any form of cutaneous tuberculosis which we have seen described.

No differences could be noted between the reactions produced by the different tuberculin products employed (P.P.D., O.T. and M.A. 100).

Whether or not the histologic appearance of the tuberculin reaction is specific is problematic. To establish this point definitely it would be necessary to study the effects of other irritants when they are injected into human skin. The specificity of a positive reaction to a tuberculin test rests upon the fact that its gross manifestations develop only in sensitized individuals, in so far as we are aware.

SUMMARY

No comparable study of the tuberculin reaction in human skin has been found in the literature.

We have described the microscopic appearances of reactions appearing at from one hour to fourteen days after the intracutaneous injection of various tuberculin solutions.

Briefly, the changes may be summarized as follows: The first manifestation is a polymorphonuclear infiltration adjacent to the region of injection. This phase persists for less than six hours. After this time, mononuclear cells make their appearance. These cells are chiefly of the histiocytic type, with smaller numbers of lymphocytes. They are congregated about vascular channels and the dermal appendages. After several days, these cells undergo differentiative changes and apparently become cells of the type composing connective tissue.

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DISCUSSION

DR. HAMILTON MONTGOMERY, *The Mayo Clinic:* This work of Dr. Hinshaw's and Dr. Feldman's has required a lot of time and careful work in regard to controls. One is limited in the histologic types of reactions that occur in the skin in response to injections or to substances put on the skin. There is a difference between contact and atopic eczema histologically. I do not believe that the tuberculin reactions present a specific histologic picture. In fact, Dr. Hinshaw has shown me sections of the cutaneous reaction to the Shick test done with the same technique and which shows a similar type of reaction to the intracutaneous injection of tuberculin. The series, however, is not large enough to warrant final conclusions in this regard. I had the opportunity of studying the microscopic sections of Dr. Hinshaw's and Dr. Feldman's cases. In the sections taken two weeks after injection of tuberculin there were some cells which Dr. Feldman, I believe, would regard as fibroblasts but which to me were definite epithelioid cells often presenting a foamy cytoplasm such as we see in epithelioid cells in certain types of tuberculosis such as sarcoid or in the epithelioid cells seen in leprosy. If studies had been carried beyond the two-week period I would anticipate that a definite epithelioid tubercle might have been encountered in some cases. If these are epithelioid cells it is interesting that they should appear in response to injections chiefly of a purified protein derivative of tuberculin and which according to Sabin and Doan should call forth a hemorrhagic and lymphocytic type of reaction on the part of the tissue rather than a reaction of epithelioid cells; the epithelioid response being attributed to the phospholipid fraction of the tubercle bacillus.

DR. S. M. PECK, *New York:* For the last few years we have been interested in the histologic picture produced by the injections of various substances intradermally. These have included snake venom, trichophytin, and tuberculin. We noted that after the injection of snake venom a leucocytic reaction can be seen in less than one half hour. The examination of the tuberculin test showed that the later the histologic examination was made, the less the leucocytic reaction. If the examination was made late enough, a picture more like that of tuberculosis, that is, lymphocytic reaction and even necrosis became evident.

Our study was undertaken in an attempt to reproduce leucomids by the injection of trichophytin, tuberculin, and snake venom in patients with chronic and acute leucemia. We were rather surprised to see that the typical histologic picture for each injected substance as we had seen in controls was not changed very much even in those cases where we had profound changes in the blood pictures. In other words even the leucomids must have a definite mechanism for their development. This last must be clarified by the statement that the only characteristic picture of a true leucomid is that which is diagnosed as lymphadenosis cutis circumscripta.

DR. MARION B. SULZBERGER, *New York:* Doctors Hinshaw and Feldman are to be congratulated and thanked for engaging in this study. In view of the importance of the subject astonishingly few histologic studies have been made of

intracutaneous reactions to allergens such as tuberculin, trichophytin and oidiomycin.

Dr. Frances Pascher and I are now engaged in a comparative histologic study of tuberculin-, trichophytin- and oidiomycin-reactions.

The sort of response elicited seems to be largely dependent upon three factors: (1) the nature of the allergen, (2) the quantitative relationship between the time factors, the degree of sensitivity of the individual and the amount of extract injected; and (3) the quality or type of allergic sensitivity of the individual. For example, certain persons or sites with tuberculoid and sarcoid reactions have a greater than normal tendency to form epithelioid cells, regardless of the nature of the allergenic extract. Moreover, the form of response varies greatly with the time element. Usually polymorphonuclear cells are the first to be formed, then in the later biopsies more and more round cells are seen. But after several weeks to two months, one begins to see the tuberculoid structures. This chronologic sequence is seen more or less regardless of the allergenic extract injected, whether it be tuberculin or another bacterial or fungous extract. So that apparently tuberculoid structures are based upon immunologic tissue alterations, rather than being directly dependent on the chemical nature of the allergen.

DR. GEORGE S. WILLIAMSON, *Ottowa:* I am very much interested in this paper because it is a continuation of the study published in the Archives of Pathology by Feldman on the histology and activity of tuberculin in cattle. I think the reactions were quite similar to those shown today by Dr. Hinshaw on the screen, with the difference as he has indicated.

It is a matter of some importance to know whether there is a characteristic histologic response in the tuberculin type reaction. Within the last 2 years I have studied the histology of the tuberculin type reaction produced in neosalvarsan sensitized guinea pigs to neosalvarsan and to tuberculin. Microscopically these lesions are similar. It may be possible that the tuberculin type reaction is a characteristic response no matter what the exciting factor.

DR. H. C. HINSHAW, *Rochester, Minn.:* I appreciate very much the comments of the several men. I am particularly interested to learn that Doctor Sulzberger and his associates are making an effort to determine whether or not a specific type of histologic picture is produced by the intracutaneous injection of tuberculin. We have no evidence which would lead us to believe that tuberculin produces a specific histologic picture. Possibly the same reaction might occur from other chronic irritants. This does not mean that the tuberculin test is not specific in the clinical sense, because a positive reaction is evidence of sensitivity in the individual. Whether the picture is a histological entity remains to be seen. The P.P.D. tuberculin is pure protein derivative. It is produced by culturing tubercle bacilli on synthetic medium and precipitating the protein fraction.