

## EXPERIMENTAL INTRACUTANEOUS TUBERCULOSIS IN THE WHITE RAT

WILLIAM H. FELDMAN, D.V.M., M.S.,\* AND ALFRED G.  
KARLSON, D.V.M., M.S.†

(Received for publication November 6, 1939)

The uncertainty that exists concerning the true character of certain naturally occurring noncaseating tuberculosis-like lesions has been the inspiration for a limited number of investigations in which attempts have been made to produce comparable lesions experimentally. The work of W. Jadassohn is of particular interest. He reported the occurrence of sarcoid-like lesions in the skin of rats twenty to eighty days following injection with tubercle bacilli. Following the injection of the bacteria he observed the formation of small subcutaneous abscesses which disappeared rapidly and were replaced within the first two weeks by granulation tissue, leukocytes, lymphocytes and young connective-tissue cells. The histiocytes soon assumed an epithelioid character; the leukocytes disappeared and the lymphocytes in most cases appeared only in the peripheral zone. At the end of three weeks most of the lesions consisted essentially of nodules of epithelioid cells and peripherally situated lymphocytes. The character of the lesions remained unchanged from the end of the third week until the eightieth day.

Only occasionally were giant cells and necrosis observed. Typical acid-fast bacilli were not seen after the first week. There were observed, however, atypical, slender, beaded forms situated always within the cytoplasm of the epithelioid cells.

\* Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

† Fellow in Comparative Pathology, The Mayo Foundation, Rochester, Minnesota. Now affiliated with the University of Minnesota, Division of Veterinary Medicine, St. Paul, Minnesota.

Jadassohn considered these forms to be degenerating tubercle bacilli. Most of the attempts to demonstrate viable, virulent tubercle bacilli in the lesions by cultures and by the inoculation of guinea-pigs gave either negative or unsatisfactory results. Reinfection of rats with tubercle bacilli resulted in the same histologic response as primary infection. Jadassohn mentioned that a sensitivity to tuberculin did not develop in the infected rats and that their blood serum contained tuberculin-neutralizing properties or anticutins.

Jadassohn expressed the belief that his observations indicated an analogy between the experimental lesions in the skin of the rat and the sarcoid-like lesions in human beings; he mentioned four points of similarity: (1) a negative sensitivity to tuberculin in most cases of sarcoid and in all of the rats; (2) demonstration of procutins and of anticutins; (3) histologic similarity of the lesions, and (4) absence of demonstrable tubercle bacilli in the lesions of sarcoid and their occurrence only occasionally in the lesions of the rats. Pinner considered Jadassohn's observations "highly suggestive" and agreed with Jadassohn that the essential features of sarcoid lesions of man—namely absence of caseation and the presence of anergy and anticutins—had been produced experimentally in rats.

In order to determine whether or not lesions comparable to those described by Jadassohn might be induced in the skin of rats by agents other than human tubercle bacilli the following studies were done.

#### EXPERIMENT 1

*Methods.* Fifty-two adult white rats were divided into thirteen lots of four rats each and suspensions of each of the following bacteria were used to inject one lot of four rats intracutaneously.<sup>1</sup> The bacterial cultures were: *Mycobacterium tuberculosis bovis* 2904; *Mycobacterium tuberculosis avium* (recently isolated); acid-fast saprophyte from the tonsil of a hog; Hastings acid-fast;<sup>2</sup> BCG; Hagan soil acid-fast, rough; Hagan soil acid-fast, smooth; *Mycobacterium*

<sup>1</sup> The injections were made into the skin of the midportion of the back. The dose used was 0.01 mg. of bacteria suspended in physiologic sodium chloride solution.

<sup>2</sup> Isolated some years ago by Dr. E. G. Hastings, University of Wisconsin, from the mesenteric lymph node of a bovine animal. Apparently avirulent.

*smegmatis*; *Mycobacterium phlei*; *Mycobacterium chelonae*; *Mycobacterium tuberculosis hominis* 2080-27 (heat-killed); *Mycobacterium tuberculosis hominis* 2080-27 (viable) and *Escherichia coli*. Four additional rats were injected intracutaneously with 0.01 mg. of silicon dioxide.

Three weeks after the respective materials were injected two rats of each of the fourteen lots were killed for necropsy and portions of the following tissues preserved for subsequent study: skin at the site of inoculation, liver and spleen. In addition cultures were attempted from the spleens of all of the animals with the exception of the two that had been injected with silicon dioxide.

The remaining twenty-eight rats, with the exception of two that died after forty-five and sixty-six days respectively, were killed for necropsy seventy-two days after having been injected. One month before the animals were killed each was injected intracutaneously with mammalian and with avian tuberculins. At the time of necropsy the skin in the region where the respective inoculations had been introduced, and portions of the liver and spleen were preserved for subsequent study and cultures were attempted from the spleens of all of the rats that had been injected with bacteria.

*Results.* The attempts to secure cultures from the spleens of the respective rats were unsuccessful. Of the twenty-seven rats that were tested with tuberculins the reactions were negative in all except two. One of these had previously been injected with BCG and reacted comparably to both avian and mammalian tuberculins. The reactions after forty-eight hours consisted of moderately large, firm, erythematous areas of induration. The other rat that reacted to tuberculin had received *Mycobacterium phlei* intracutaneously. Both tuberculins elicited reactions which were of moderate severity.

Grossly, none of the tissues contained lesions of any kind. Microscopically, lesions were found in the tissues of only two of the rats and these were limited to the skin. One animal had been injected three weeks prior to necropsy with a recently isolated strain of *Mycobacterium tuberculosis avium*. The changes, which were confined largely to the lower zone of the hypoderm, were not typical of tuberculosis. They consisted essentially of a few intact foci of epithelioid cells surrounded by a dense mantle of histiocytes and a moderate number of lymphocytes (fig. 1). Eosinophilic granulocytes were not seen. There also occurred promiscuously throughout most of the reticular portion of the dermis small accumulations of histiocytes and occasionally a small epithelioid nodule was seen in this region. In sections

stained to reveal the presence of acid-fast bacteria, numerous acid-fast bacillary forms were readily seen. The majority of the acid-fast bacilli definitely occupied an intracellular position. Although single bacterial cells were readily demonstrable, the bacteria revealed a marked tendency to occur in clumps within the cytoplasm. The compressed elongated cells at the periphery of the larger foci were suggestive of fibroblasts. Giant cells were not seen.

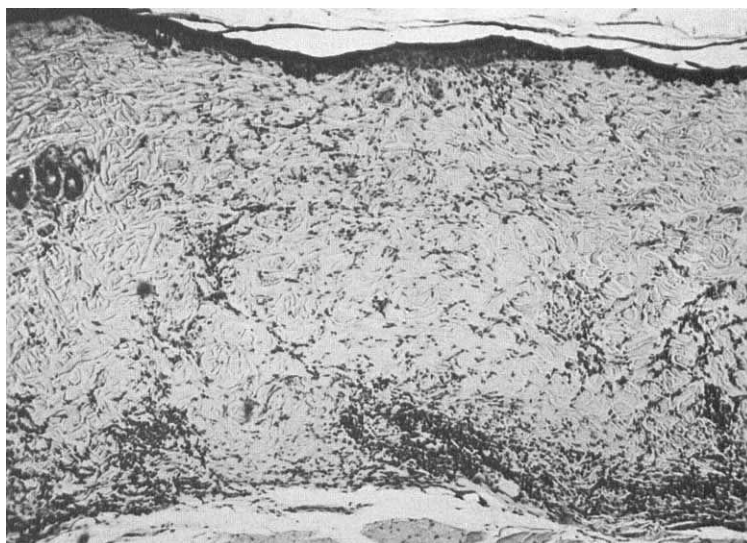


FIG. 1. Marked histiocytic and epithelioid reaction in lower portion of dermis of rat injected intracutaneously three weeks prior to necropsy with *Mycobacterium tuberculosis avium*. Experiment 1 ( $\times 60$ ).

The other rat in which lesions occurred in the skin had been injected seventy-two days prior to necropsy with the same strain of *Mycobacterium tuberculosis avium* used to inject the rat in which the previously described lesions were found. The lesions in the skin of both rats were similar in certain respects. The changes were most noticeable in the lower zone of the dermis and were characterized by the occurrence of a considerable number of diffusely deposited histiocytes and lymphocytes. A few small, atrophic epithelioid nodules that stained poorly were noted.

Giant cells, caseation or other necrobiotic changes were not seen. Acid-fast bacillary forms were present but these were not as numerous as those seen in the lesions of three weeks' duration. The bacteria were less general in their distribution, occurring in compact nests in intimate contact with the periphery of the nuclei of the histiocytic cells. Acid-fast bacilli were not seen in the epithelioid nodules.

In recapitulation it may be recounted briefly that of forty-eight white rats injected intracutaneously with a large number of strains of acid-fast bacilli, in none were the organisms isolated from the spleen. Demonstrable sensitivity to tuberculin developed in only two and lesions attributable to the inoculum occurred in the skin of only two animals, both of which had been injected with a virulent strain of *Mycobacterium tuberculosis avium*.

#### EXPERIMENT 2

*Methods.* The failure in experiment 1 to obtain lesions in a significant number of the rats made it desirable to extend the investigation using a larger number of recently isolated strains of *Mycobacterium tuberculosis hominis* than had been used previously. Accordingly thirty additional white rats of the same stock as those used in experiment 1 were secured. These were divided into five lots of six animals each. Each of five strains of *Mycobacterium tuberculosis hominis* that had been isolated from sputum one to two months previously and which were eventually proved to be typically pathogenic for guinea-pigs was used to inject six rats. The injections were made intracutaneously into the back, the dose for each rat being 0.01 mg. The six animals in each of the five groups were killed for necropsy as follows: two after one week, two after two weeks and two after four weeks. At necropsy the skin in the regions where the bacteria had previously been injected, was removed and preserved for microscopic study. Cultures were attempted from the spleens of all of the rats.

*Results.* No positive cultures were obtained from the spleens of the respective rats although incubation was continued for a period of twelve weeks. Gross evidence of cutaneous tuberculosis was not observed in any of the rats. Microscopically, definite lesions occurred in the skin of two of the ten rats killed one week after being injected. Among the ten rats killed after two weeks, lesions were found in the skin of eight. Lesions were not observed in the skin of the ten rats killed after four weeks. Of the five different strains of *Mycobacterium tuberculosis*

*hominis* used in infecting the animals, all produced lesions in one or more rats in each of the five groups. Microscopically, the changes in the skin of the two rats that showed lesions one week after inoculation were essentially similar. In the central portion of the dermis there occurred a large, irregularly outlined, poorly delimited region of epithelioid cells which extended to the subdermal connective tissues (fig. 2). An elongated, diffuse collection of histiocytes and epithelioid cells occurred for some distance

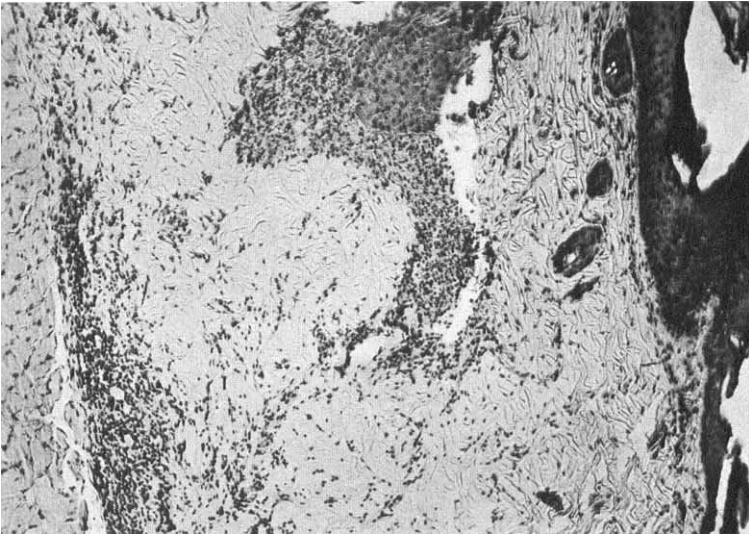


FIG. 2. Large irregular regions of epithelioid cells in central portion of dermis with diffuse collections of histiocytes and epithelioid cells in the depths of the dermis in a rat injected intracutaneously one week previously with *Mycobacterium tuberculosis hominis* ( $\times 60$ ).

along the lower border of the hypoderm. There were no giant cells of the Langhans type. Necrosis was not evident.

Although acid-fast bacilli were readily seen, the bacteria were not numerous. In so far as could be determined with certainty, the bacilli were for the most part within the cytoplasm of the epithelioid cells. Few bacteria were found in the regions that were predominantly histiocytic in character.

In the material obtained from the eight rats that had been injected two weeks prior to necropsy the severity of the lesions



varied. In most instances, however, there was a marked involvement of the lower third or lower half of the dermis characterized by the occurrence of diffuse collections of epithelioid cells and a lesser number of histiocytic and fibroblastic forms (figs. 3 and 4). A few lymphocytes were noted, but neither eosinophilic granulocytes nor giant cells of the Langhans type were seen. In some instances a tendency for the epithelioid cells to assume small, indefinite, nodular formations was noted

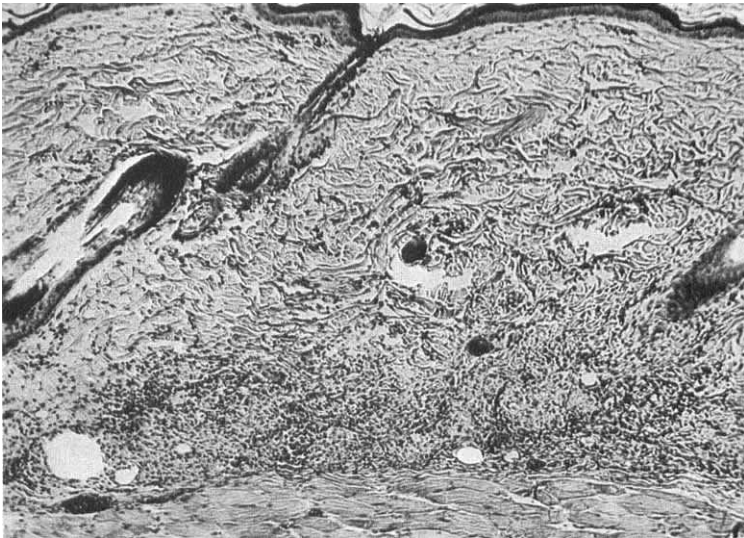


FIG. 3. Marked noncaseating epithelioid reaction in the lower third of the dermis of a rat injected intracutaneously two weeks previously with *Mycobacterium tuberculosis hominis* ( $\times 60$ ).

(fig. 5). Evidence of necrosis was absent. The changes extended to but did not violate the subcutaneous tissues.

In those instances in which the lesions were less pronounced the changes consisted of a promiscuous scattering of epithelioid cells extending from the epidermis through the entire hypoderm. In such regions epithelioid nodules were not prominent although when examined closely small nests of epithelioid cells were frequently discernible. Histiocytes were not numerous and the transition of epithelioid cells to fibroblastic forms was suggested.

In appropriately stained sections acid-fast bacillary forms,

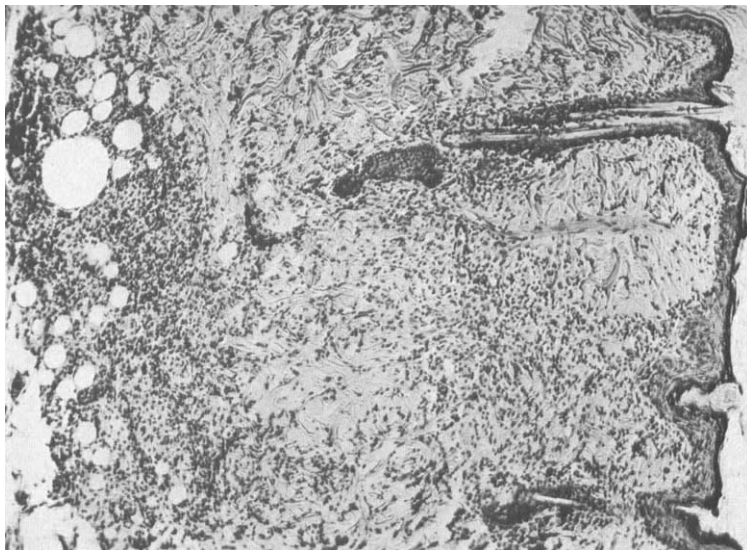


FIG. 4. Involvement of the entire width of the dermis in a rat injected intracutaneously two weeks previously with *Mycobacterium tuberculosis hominis*. The reaction is predominantly epithelioid in character ( $\times 60$ ).

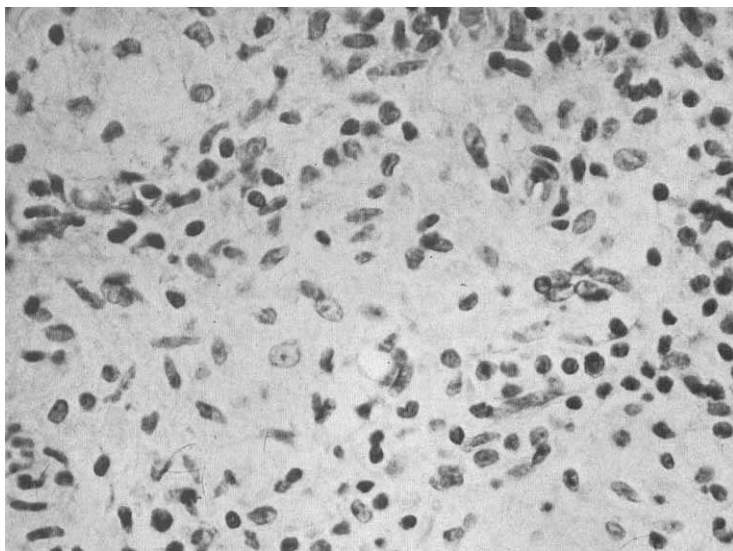


FIG. 5. Higher magnification of region of epithelioid reactions noted in the lower part of the dermis in figure 4. The epithelioid cells show a tendency to assume an indistinct nodular arrangement ( $\times 440$ ).



although not numerous, were easily demonstrated. With few exceptions the bacteria occupied an intracellular position.

To summarize experiment 2 briefly, it may be noted that five recently isolated strains of *Mycobacterium tuberculosis hominis* were each used to inject six rats intracutaneously. Ten rats were killed after one week, and lesions occurred in the skin of two. Of ten killed after two weeks lesions of variable severity were found in the skin of eight, while lesions were not found in the skin of the ten rats killed four weeks after injection. The lesions were essentially histiocytic or epithelioid in character. A tendency for the elements of the reactive process to maintain their structural integrity and to assume a more or less circumscribed form appeared characteristic.

#### COMMENT

These observations indicate, as the work of W. Jadassohn and of others has shown, that a localized, noncaseating, epithelioid, nodular type of tuberculosis may be induced in the skin of the white rat with virulent tubercle bacilli. That comparable lesions were not observed following the injection of presumably non-pathogenic forms of mycobacteria is perhaps significant. Our failure to recover acid-fast bacteria by cultural means from the spleens of any of the animals indicates that most of the bacteria were probably restrained and eventually destroyed by the elements of the local tissue reactions. The failure to find lesions in the ten rats injected with virulent *Mycobacterium tuberculosis hominis* and killed four weeks later is somewhat at variance with Jadassohn's observation since he noted lesions in the skin of rats that had been injected eighty days prior to necropsy. Each of the five strains used (in experiment 2) induced cutaneous lesions in one or more of the rats that were killed within the first two weeks and, although some rats were evidently more resistant to infection than others, it seems strange that lesions were not observed in the ten animals killed four weeks after injection. It would seem reasonable to believe that lesions had occurred in at least some of the ten rats and that the changes had undergone resolution before the time of death. This, however, should be

investigated further. The fact that lesions were found in one rat (experiment 1) that had been injected seventy-two days before death with a virulent strain of *Mycobacterium tuberculosis avium* indicates the variability of the susceptibility of the white rat to the tubercle bacillus.

Whether or not observations such as these are pertinent as regards the problem of the causation of sarcoid or so-called non-caseating tuberculosis of the human being is problematic. The lesions induced experimentally do have a general resemblance to those of sarcoid although there are also certain dissimilarities that may of course be due to differences inherent in the hosts' reaction to the infectious agent. Pinner in his admirable review has marshalled an impressive array of indirect evidence to substantiate the contention that the different clinical entities designated as noncaseating tuberculosis are the result of infection with tubercle bacilli. There remain, however, sufficient reasons to justify an attitude of skepticism and to await the results of further investigations before accepting the tuberculous character of these lesions as definitely established.

#### SUMMARY AND CONCLUSIONS

In an attempt to induce noncaseating epithelioid lesions in the skin of the white rat, two experiments were performed. In the first experiment each of twelve different cultures of mycobacteria, including a large number of saprophytic strains, and one strain of *Escherichia coli* were used to inject four rats intracutaneously. In addition four rats were similarly injected with silicon dioxide. Two rats of each group of four were killed after twenty-one days and the remaining two after seventy-two days. Lesions of a noncaseating epithelioid character occurred in only two of the animals, both of which had been injected with a virulent strain of *Mycobacterium tuberculosis avium*. In experiment 2 five strains of recently isolated *Mycobacterium tuberculosis hominis* were each used to inject six rats intracutaneously. Two rats of each lot of six were killed after one week, two after two weeks and two after four weeks. Non-caseating epithelioid lesions were found microscopically in the

skin of two of the ten rats killed after one week, and in eight of the ten rats killed after two weeks. Lesions were not seen in the skin of the ten rats that were killed four weeks after inoculation.

It is concluded that (1) tuberculosis-like lesions do not occur in the white rat following the intracutaneous injection of avirulent forms of mycobacteria, *Escherichia coli* or silicon dioxide; (2) localized noncaseating epithelioid lesions occur following the intracutaneous injection of *Mycobacterium tuberculosis hominis* and *Mycobacterium tuberculosis avium* of standard virulence, and (3) the infective bacteria appear to remain localized and when lesions occur the tissue changes show a tendency to undergo resolution.

#### REFERENCES

- (1) JADASSOHN, WERNER: Die Inokulationstuberkulose der Rattenhaut. (Ein Beitrag zur Frage der Hauttuberkulose mit positiver Anergie.) Arch. f. Dermat. u. Syph. **167**: 169-173, 1932-1933.
- (2) PINNER, MAX: Noncaseating tuberculosis; an analysis of the literature. Am. Rev. Tuberc. **37**: 690-728 (June) 1938.