

Studies on the Development of Inflammatory Lesions

II. Influence of Cortisone and Methylandrostendiol on the Tissue Reactions Caused by Dead Tubercle Bacilli.

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Introduction

In the first report¹⁾ of these serial studies, the author described the influence of steroid hormones such as cortisone and methylandrostendiol (MAD) on experimental tuberculosis in guinea pigs and it was proved that a modification of the pattern in the tuberculous lesions would occur following the administration of these hormones ; following the administration of cortisone, there were marked inhibition of production of fibroblasts and of new formation of collagenous fibers, disappearance of lymphocytes in the lesions and an extension of the area of the lesions. The administration of MAD caused an augmentation of the fibroblastic response, proliferation of epithelioid cells, and thus an increased tendency towards the productive type of lesion was observed. In this paper, the author wished to report on the influence of the steroid hormones on tissue reactions following the insertion of dead tubercle bacilli as a further investigation. Many investigators have already studied in detail the pathogenicity of dead tubercle bacilli and their chemical fractions. (Sabin, Roulet²⁾⁻¹¹⁾ etc.¹²⁾¹³⁾

More recently, with the advance of endocrinology, many studies of the internal factors in tissue reactions especially of certain hormones as well as the external factors in various diseases have been reported.

In order to understand the complex pattern in naturally occurring lesions, it is useful to analyse results of relatively simple experiments.

The author chose to study the modification of tissue reaction, especially fibroblastic reactions to dead tubercle bacilli under cortisone and MAD administration.

As was indicated in the first report of this series, steroid hormones such as cortisone and MAD influence inflammatory processes via the hormonal environment but do not affect tubercle bacilli directly. Therefore, the present

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experiments should be of interest.

Materials and Methods

Animals : Thirty male albino rats of the Wister strain (weight 110~130g.) were used for the experiment.

Human type tubercle bacilli (H37Rv) were cultured in Kirchner's medium, then collected, heated and dried at 37°C, in the usual manner, and then, 10 mg of the dead bacilli were inserted aseptically into the dorsal subcutis of each rat with a small trocar. The experimental animals were divided into 3 groups.

1) The first group was given cortisone (4 mg per kg body weight) by injection every day.

2) The second group was given MAD (2 mg per kg body weight) by injection every day.

3) The third group received no hormones and served as controls. The method of administration of the hormones was described in the first report.

The animals were sacrificed 3 days, 1 week, and 3 weeks after the beginning of the experiment (Table 1) and small pieces of the subcutis of the inserted areas were removed and fixed in a 10% formol solution. Tissue sections were prepared in the routine ways and stained with hematoxylin eosin and Van Gieson stain.

Table 1

	mg/daily	Number of animals (Experimental period)		
		3 days	1 week	3 weeks
Control group		4	3	3
Cortisone treated group	4.0mg/kg	4	3	3
MAD treated group	2.0mg/kg	4	3	3

Results Obtained

A. General condition.

There were no specific changes in the general condition of either the experimental or control groups. No animal died during the experiment. The average body weight of the cortisone administered group had decreased 7--10mg by the end of the experiment. There was no weight loss in the control and the MAD administered group.

B. Histopathological findings.

1) Control group.*a. Three days after the insertion.*

Numerous polymorphonuclear leucocytes and their degenerative forms were seen in the area. The tendency of degeneration or destruction seemed so intense that the central area of the lesion consisted chiefly of masses of dead bacilli, cellular fragments and fibrin.

The surrounding connective tissue was edematous and the blood vessels were dilated. Perivascular cellular infiltration was also seen.

In general, most of the cells were neutrophiles, and next most numerous were macrophages. Thus, although the exudative and degenerative processes of acute inflammation were so severe, there was no specific or fibroblastic reactions in this stage.

b. One week after the insertion.

Many neutrophiles and their destroyed forms remained in the area although they were fewer than in the early stage. On the other hand mononuclear cells were increased in the area and the shape of the cells became more inflated and the nuclei pale-staining, and some of the cells were identical to epithelioid cells. The production of connective fibers in the surrounding tissues was observed with a tendency to encapsulation. In some cases, the lesion formed a granuloma in the center of which masses of dead bacilli were divided in many pieces like islets and were surrounded by newly produced collagenous fibers. New formations of blood capillaries were also observed.

Epithelioid cells and Langhans' type of giant cells were observed in the granuloma. Collagenous fibers in an annular formation around the masses were demonstrated by Van Gieson's stain, but the fiber formation was not extensive,

c. Three weeks after the insertion.

In the masses of dead bacilli in the center of the lesion peculiar spaces accompanied with some neutrophiles were observed. The degenerative or destructive tendency of leucocytes was not so marked as it was in the earlier stages. In addition to neutrophiles, many mononuclear cells were observed in the granulation tissues around the masses, in which many collagenous fibers and blood capillaries were formed. There were many mononuclear cells besides fibroblasts and neutrophile leucocytes in this granuloma and some of the mononuclear cells had the typical shape of the epithelioid cells. There were also small numbers of plasma cells and lymphocytes. Epithelioid cells looked more swollen than those in human tuberculosis. Langhans' giant cells were also observed.

Thus, as the stage advanced, acute inflammatory processes were diminished and granulomas were formed, and the specific type of cells in tuberculosis such as epithelioid cells and Langhans' giant cells appeared. No caseous alteration occurred.

2) The cortisone administered group.

a. Three days after the insertion.

No specific finding was seen compared with the control group. In the center there was infiltration of numerous neutrophiles with a marked tendency towards degeneration and destruction. The surrounding connective tissue was edematous and there were vascular stagnation and perivascular infiltration especially with neutrophiles.

b. One week after the insertion.

A conglomeration of neutrophiles and mononuclear cells was seen around the masses of dead bacilli.

In contrast to the experiments with living bacilli, reported in the previous study¹⁾, there was no tendency for the lesion to spread even after administration of cortisone.

In sections treated with Van Gieson's stain fibroblasts and the formation of new collagenous fibers were less than in lesions of the control group.

c. Three weeks after the insertion.

The infiltrating cells around the masses had already been absorbed at this stage and the encapsulating connective tissues looked thinner and coarser than those of the control group and included a few capillaries.

Epithelioid cells and giant cells were very rare around the focus and Van Gieson's stain showed that collagenous fibers were fewer than in the control group.

3) The MAD administered group.

a. Three days after the insertion.

There was no specific change when compared with the control group. There were infiltration of numerous neutrophiles into the central area, marked degeneration and destruction of these cells, and intense perivascular cellular infiltration. There was no formation of new connective tissue at this stage.

b. One week after the insertion.

There was a characteristic formation of the surrounding connective tissue in the MAD administered group. The production of fibroblasts and collagenous

fibers was marked with formation of capillaries at this stage. Although many neutrophiles and their destroyed granules were observed in the central area, there was already a tendency towards encapsulation.

In the productive area, mononuclear cells and many cells with the typical shape of epithelioid cells and also giant cells of the Langhans' type were observed.

c. Three weeks after the insertion.

Masses of dead bacilli and destroyed cellular fragments had already been absorbed from the central area leaving round or oval spaces surrounded by some neutrophiles. The connective tissue showed characteristic findings, namely the empty spaces were surrounded by large and also fine collagenous fibers and a circle of neutrophiles as above mentioned. Some circular fibers were also present in the outer zones of the foci. Some fibroblasts were hypertrophic. In this productive granuloma, there were many mononuclear cells some of which were identical with epithelioid cells. There were also some lymphocytes, plasma cells, Langhans's type of giant cells and neutrophiles.

The histopathological findings in the tissues which occurred following the insertion of dead tubercle bacilli and the administration of cortisone or MAD are summarized as follows. In the early stage, the nonspecific phenomena of acute inflammation such as separation of subcutaneous tissue, bleeding, vascular stagnation and migration of leucocytes, especially of neutrophiles, could be seen. Most of these migrated leucocytes rapidly degenerate and are destroyed probably because of the toxicity of dead bacilli.

The central area of the lesion was occupied by masses of inserted dead bacilli, neutrophiles and their fragments. One week after the insertion, mononuclear cells such as monocytes increased gradually in proportion to the diminution of the acute inflammatory process. Some cells changed their shape to become epithelioid cells. Production of fibroblasts and collagenous fibers, and also formation of blood capillaries in the outer zones of foci appeared gradually. Giant cells of the Langhans' type could be seen at the same time. This granulation tissue spread gradually.

Three weeks after the insertion, neutrophiles were diminished and could be observed only in the central area, and most of the cells in the outer zone of the lesion were epithelioid cells, fibroblasts and some mononuclear cells. Circular collagenous fibers appeared at the outermost area and there was also irregular granulation tissue within the foci. Following cortisone administration there was a characteristic inhibition of the fibroblastic response but the invasive tendency of lesions caused by living bacilli was not observed.

The collagenous fibers in both the inner and outer zones of the foci remained

thinner than in the controls. On the other hand, in the MAD administered group, there were a characteristic fibroblastic response and encapsulation ; production of fibroblasts and collagenous fibers and formation of blood capillaries were marked. The proliferation of epithelioid cells was not so conspicuous. No caseation occurred in any case.

Comment

The first investigator about the tissue reactions induced by dead tubercle bacilli was Maffuci (1890)¹²⁾. He reported the abscess formation in the subcutis of guinea pigs by dead bacilli (heated at 70°C). Then after many investigators have studied about this problem, and it was generally recognized that the formation of a tubercle like in that of tuberculous lesions and also noticed the appearance of epithelioid cells and formation of giant cell of Langhans type (Vissman,¹⁴⁾ Sternberg,¹⁵⁾ Lewis & Sanderson,¹³⁾ Itogawa,¹⁶⁾ Nakamura & Arai,¹⁸⁾ ect.)

With regard to the mechanism of the tubercle formation, Maffuci thought that it would result by an action of toxin. The endotoxin might remain in the bacilli by heat and caused the reaction after contact with the living tissues. Against this, Vissman¹⁴⁾ Baumgarten¹⁷⁾ has assumed it as only a foreign body reaction and maintained dead bacilli as only a foreign body. Then, many investigations about the chemical fractions of bacilli, have emphasized that the characteristic of tubercle is responsible to some phosphatid fractions.

In general, the tissue reactions around dead bacilli differ from those around living bacilli ; the latter causes the migration of leucocytes continuously while dead bacilli cause a more rapid leucocyte migration with degeneration and destruction in early stages after which epithelioid cells and other mononuclear cells predominate in the foci. Ordinarily living bacilli do not stimulate formation of blood capillaries. Dead bacilli cause marked proliferation of fibroblasts and collagenous fibers tend to form capsules.

According to Nakamura and Arai,¹⁸⁾ the principal histological differences between the reactions to dead bacilli and those caused by living bacilli are, (1) the former may form many blood capillaries, (2) there is marked conglomeration of leucocytes, (3) fibroblasts are numerous, (4) although some lesions look caseous macroscopically, they are really abscesses and there is no genuine caseation, (5) the granulation tissue contains many more round cells than epithelioid cells and does not form any tubercle like nodules.

These findings coincide with the author's at many points except in regard to the epithelioid cells. The author found that many epithelioid cells appeared in the lesions, but their shape was more round and hypertrophic and differed from those in human tuberculosis. However, these cells are common in experimental animal tuberculosis.

The tissue reactions caused by dead bacilli are characterized by extensive leucocyte migration, their rapid degeneration and destruction, formation of tubercle-like nodules which contain epithelioid cells and Langhans type giant cells and the tendency of connective tissue to form capsules without genuine caseation.

Following cortisone administration the production of surrounding connective tissue was inhibited markedly and the encapsulating process was delayed. The spread of the lesion, which could be seen in the experiment with living bacilli, was not observed.

In other words cortisone, one of the glucocorticoids, showed a characteristic inhibition of fibroblastic response as well as the formation of blood capillaries following insertion of dead bacilli. (Selye¹⁹⁾ 1953)

The first report showed that cortisone indirectly caused the tuberculous lesions to spread by inhibiting fibroblastic response not by any direct action on the bacilli. There was also a marked inhibition of fibroblastic response in lesions caused by dead bacilli.

In contrast, MAD, one of the synthetic steroid hormones, promotes fibroblastic response and healing by granulation. This experiment confirmed the characteristic actions of MAD, namely, the increased production of fibroblasts, collagenous fibers, and formation of blood capillaries.

Thus, it was proved that the tissue reactions of an inflammatory lesion, even one caused by a specific agent could be characteristically modified by changes in the hormonal environments.

Summary

Tubercle bacilli of the human type (H37Rv) killed by heating and drying were inserted into the subcutis of albino rats and the tissue reactions were examined. These were compared with tissue reactions of rats receiving cortisone or MAD (Methylandrosterone). The lesions which developed around the bacilli were examined histologically 3 days, 1 week, and 3 weeks after the insertion. The findings can be summarized as follows.

1. An enormous migration of polymorphonuclear leucocytes and their rapid degeneration and destruction were observed in the early stage, and then this migration decreased.

2. In the next stage, mononuclear cells such as monocytes appeared as leucocytes disappeared, and the masses of dead bacilli were phagocytized and destroyed cellular fragments appeared. Some of these cells changed their shape to look like epithelioid cells. Langhans type of giant cells also appeared. Thus, tuberculosis like lesions developed but no caseation was found.

3. The production of fibroblasts and collagenous fibers and the formation of blood capillaris could be seen in the outer zones of lesions. A tendency towards encapsulation followed.

4. Cortisone caused a characteristic inhibition of fibroblastic response and the general cellular reaction was decreased. In spite of these alterations, the lesion does not spread in contrast to that caused by living bacilli.

5. In the case of MAD administration, there were a characteristic fibroblastic response and tendency towards capsule-formation.

References

- 1) Amatsu, M. : *Acta Tub. Jap.* **6** : 49, 1956.
- 2) Sabin, F. R. and C. A. Doan : *J. Exp. Med.* **46** : 645, 1927.
- 3) Sabin, F. R., et al. : *Ibid.* **62** : 751, 1935.
- 4) Sabin, F. R., et al. : *Ibid.* **68** : 563, 1938.
- 5) Smithburn, K. C. and F. R. Sabin : *Ibid.* **68** : 641, 1938.
- 6) Sabin, F. R. and A. L. Joyner : *Ibid.* **68** : 659, 1938.
- 7) Sabin, F. R. : *Ibid.* **68** : 837, 1938.
- 8) Sabin, F. R. and A. L. Joyner : *Ibid.* **68** : 853, 1938.
- 9) Sabin, F. R. : *Am. Rev. Tub.* **94** : 415, 1945.
- 10) Roulet, F. : *Virchows Arch. Path. Anat.* **294** : 262, 1935.
- 11) Roulet, F. and K. Bloch : *Virchows Arch. Path. Anat.* **298** : 311, 1937.
- 12) Maffucci, A. : *Cbl. f. All. Path. u Path. Anat.* **1** : 825, 1890.
- 13) Lewis, P. A. and E. S. Sanderson : *J. Exp. Med.* **45** : 291, 1927.
- 14) Vissman, W. : *Virch. Arch. f Path. Ana.* **129** : 136, 1892.
- 15) Sternberg, C. : *Cbl. f. All. Path. u Path. Anat.* **13** : 753, 1902.
- 16) Itogawa, S. : *Keioigaku in Jap.* **7** : 99, 1927.
- 17) Baumgarten, : *Verh. d Deut. Path. Ges.* **4** : 99, 1927.
- 18) Nakamura, U. and T. Arai : *Hokkaidoigaku in Jap.* **8** : 1664, 1930.
- 19) Selye, H. : *J.A.M.A.* **152** : 1207, 1953.

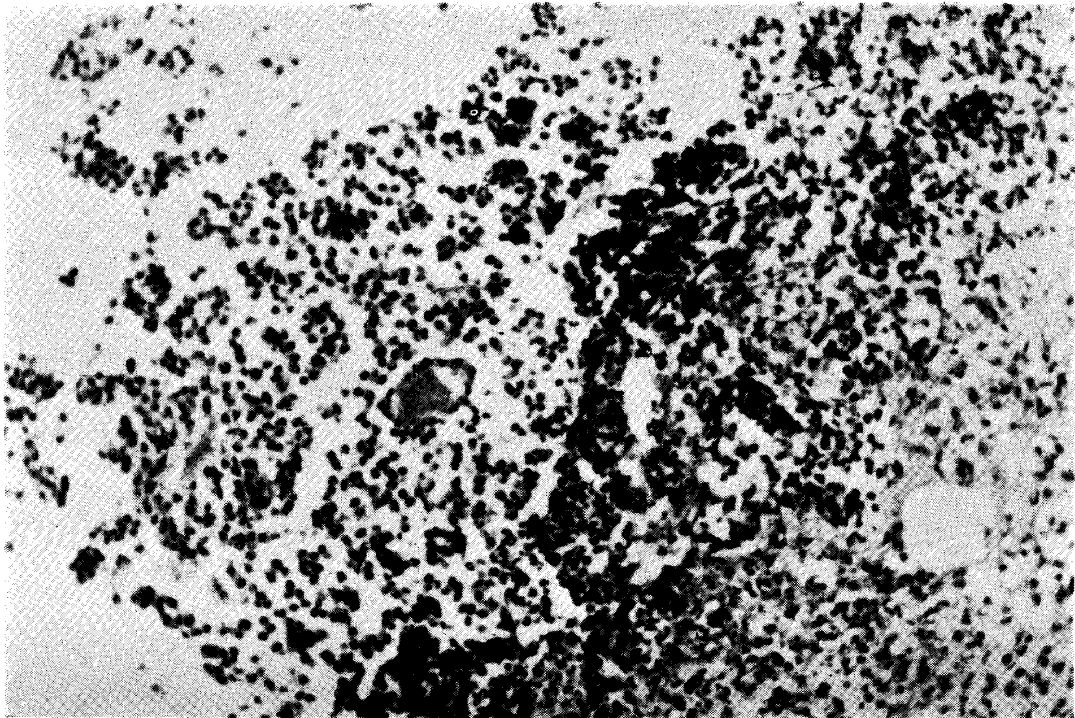


Fig. 1. The control group. Three days after insertion of dead tubercle bacilli. The bacillary mass is observed at the center, with many migrating polymorphonuclear leucocytes. H. E. stain. ($\times 200$)

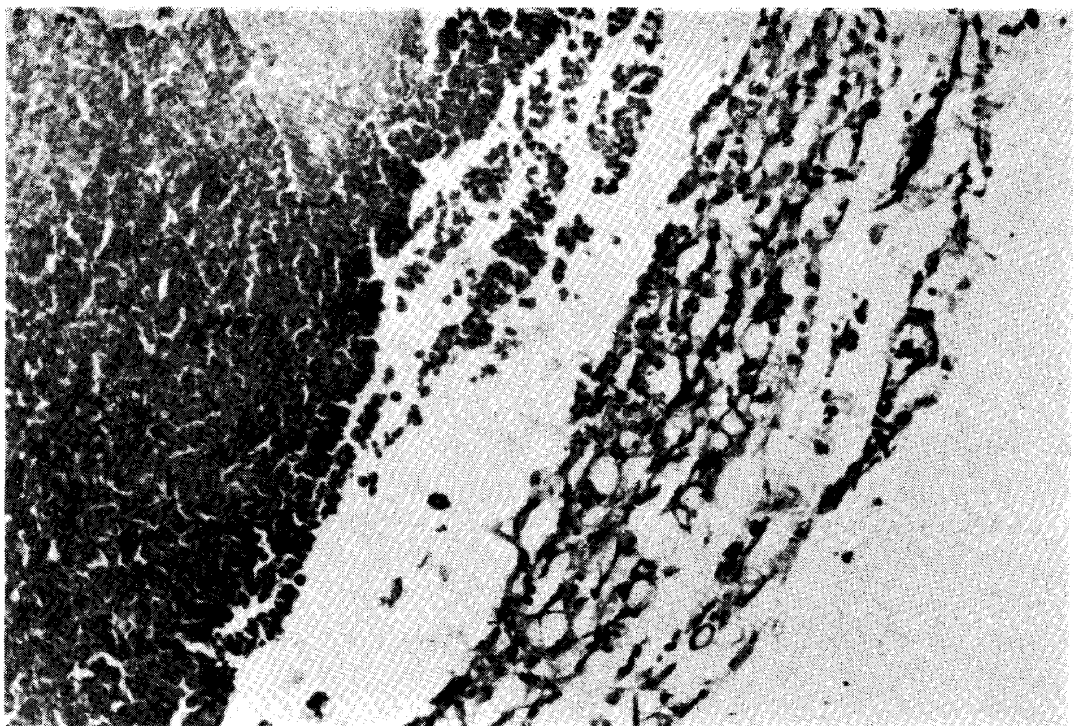


Fig. 2. The cortisone administered group. One week. The migration of leucocytes around the bacillar mass is marked. The production of connective tissue is almost negligible. Van Gieson stain. ($\times 200$)

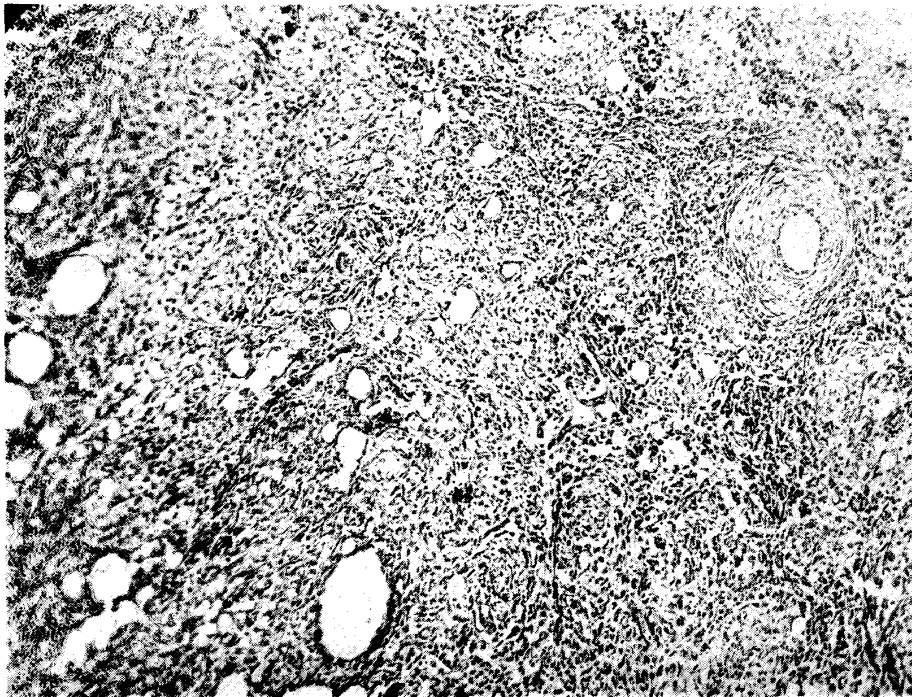


Fig. 3. The control group. Three weeks. The empty spaces show the sites in which bacillar mass existed. Some leucocytes are seen around the space. Granulation tissue, containing giant cells is proliferating. H. E. Stain. ($\times 100$)

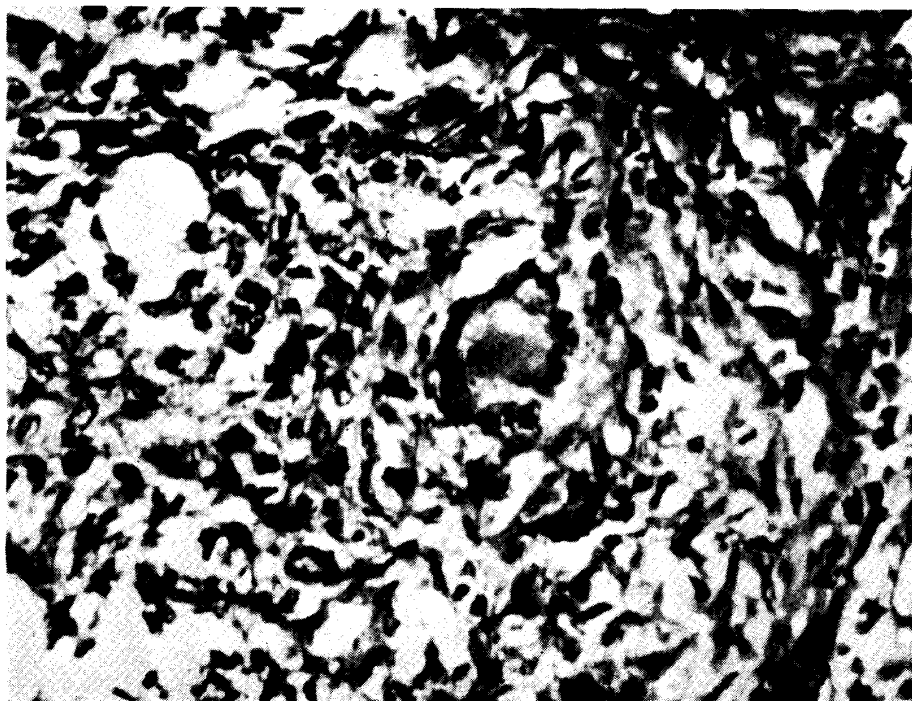


Fig. 4. The control group. Three weeks. A giant cell, many fibroblasts and macrophages are seen. (high magnification.) ($\times 400$)

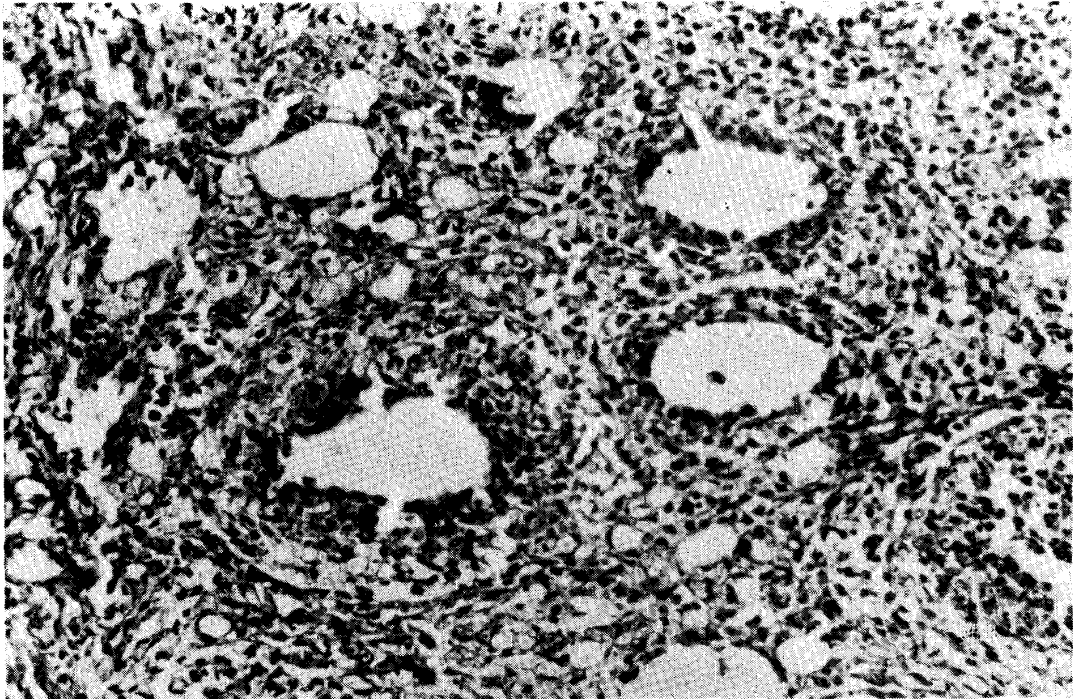


Fig. 5. The control group. Three weeks. Gros sand fine collagenous fibers surround the spaces. Some leucocytes are also seen around the spaces. Van Gieson stain. ($\times 200$)

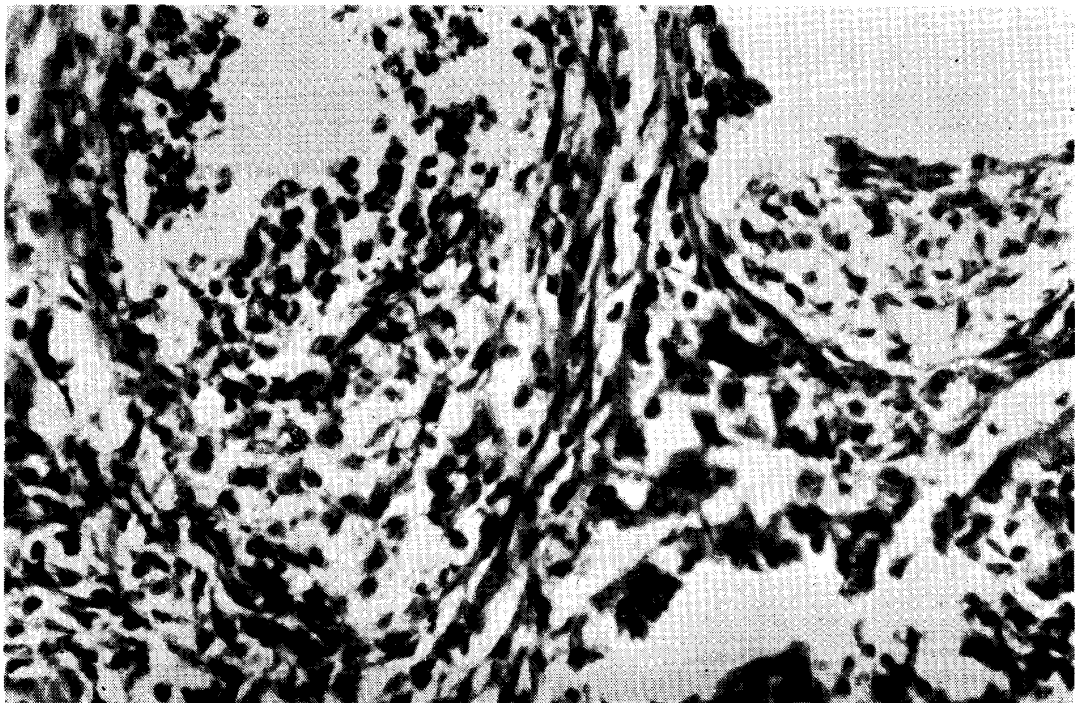


Fig. 6. The control group. Three weeks. collagenous fibers and leucocytes are indicated by Van Gieson stain. ($\times 400$)

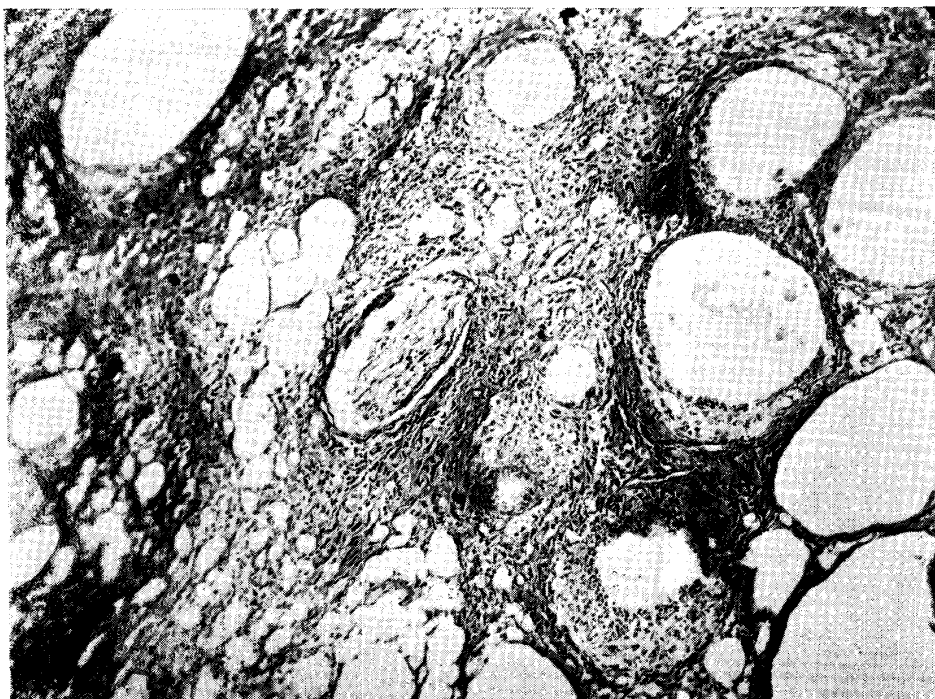


Fig. 7. The MAD administered group. Three weeks. The production of collagenous fibers is shown by Van Gieson stain. Some residues of the bacillar masses are seen in the space. ($\times 100$)



Fig. 8. The MAD administered group. Three weeks. There is marked production of connective tissue. Compare fig 5, 6, 9, and 10. Van Gieson stain. ($\times 400$)

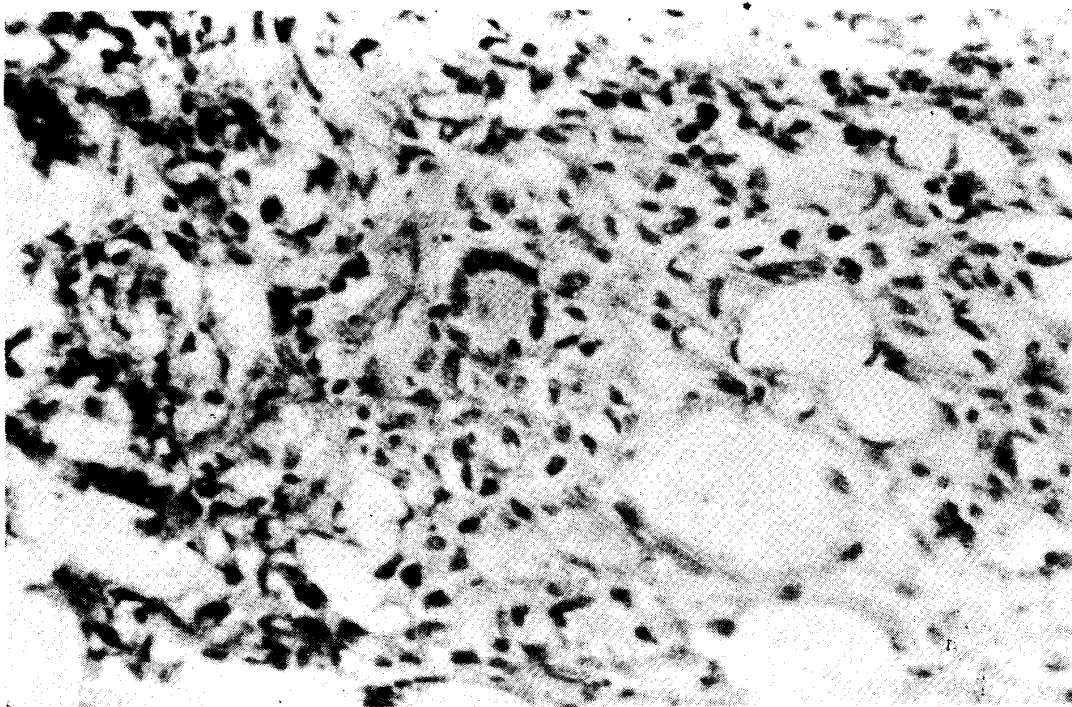


Fig. 9. The cortisone administered group. Three weeks. There are few collagenous fibers and fibroblasts. A giant cell is seen in the center. Van Gieson stain. ($\times 400$)

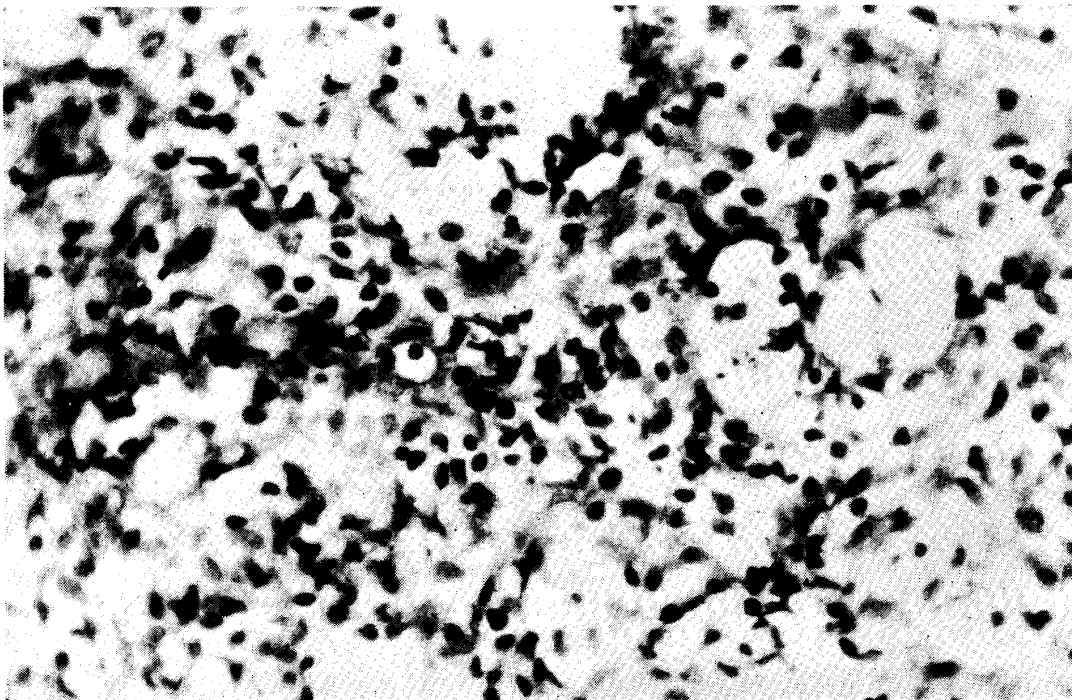


Fig. 10. The cortisone administered group. Three weeks. The production of collagenous fibers is almost negligible. Compare Fig. 5, 6, and 8. Van Gieson stain. ($\times 400$)