Studies on the Development of Inflammatory Lesions

III. Influence of Cortisone and Methylandrostendiol on the Tissue Reactions Caused dy Fatty Acids and Turpentine.

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Introduction

In the previous reports of these serial studies[1)2)], the auther described specific influence of the steroid hormones cortisone and methylandrostendiol (MAD) experimental tuberculosis and on the tissue reactions induced by dead tubercle bacilli inserted into the subcutis of rates, especially with regard to the fibroblastic response.

It was shown that cortisone and MAD characteristically modified the histopathological patterns of the lesions through the homonal environment ; cortisone has a specific inhibiting action on the production of connective tissue and granulation tissue, and causes the extention of tuberculous lesions, while MAD promotes the production of connective and granulation tissue with the inducement of tuberculous lesions to the productive form.

The auther has also studied the influence of the hormones on nontuberculous or non-specific inflmmation induced by higher saturated fatty acids and turpentine. It is understandable that the histopathological pattern in lesions caused by dead tubercle bacilli is simpler than in tuberculous infecttion. As is well known, dead bacilli, however, are not simple compounds but are composed of proteins and various ohter substances, some of the chemical structures of which are still unknown.

In regard to the tissue reactions caused by each of these complex chemical bacillary components, Sabin and many other investigators[3)-13)14)] have reported their studies in dettail and, especially, described the important role of lipid compounds in the formation of tubercle-like lesions.

This author has studies the influence of the steroid hormones cortisone and MAD on the tissue reactions to stearinic acid* and superinic acid,** inserted

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***Superinic acid.

into the subcutis of albino rats.

Turpentine was also used as a stimulus. It is desirable to use liquid substance for the study of foreign body reactions, since solid materials may cause some nonspecific influences such as stretching of tissues by their insertion itself. Many liquid materials, however, are not suitable for the study of topical reactions, because of their easy absorption, excessive stimulation or negligible reaction.

Turpentine may causes restricted granulomas in the subcutis without influencing the general condition (in the amount used), although some of it may be absorbed.

Thus, an understanding of the influence of cortsone and MAD the tissue reactions due to simpler materials such as fatty acids and turpentine may serve to further elucidate complicated inflammatory lesions and hormonal actions.

Materials and Methods

Male albino rats (Wister strain), weighing 110~130gm were used. Stearinic acid and superinic acid,* of high purity, and turpentine were used as sources of inflammation. The solid fatty acid (about 10 mg) were inserted into the subcutis of the rats with a small trocar and turpentine (0.5cc) was injected as aseptically as possible.

The animals were divided into 3 groups.

1) The first group received daily subcutaneous injections of cortisone (4.0 mg /kg).
2) The second group received daily subcutaneous injections of MAD (2.0 mg/kg).
3) The third group received no treatment and served as controls.

They were sacrificed 3 days, 2 week and 3 weeks after the beginnig of the experiment. (Table I).

The further steps were the same as in the previous study.[2)]

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

* Solid fatty scids of high purity were generously furnished by Prof. Dr. Y. Inouye, Faculty of Agriculture, Kyoto University.

Results

1. Tissue reactions to solid fatty acids.

1) The control group.

a. Three days after the insertion.

Acute inflammatory processes has occured after the insertion of strearinic acid and superinc acid. Transparent particles of the fatty acids were scattered in the central area without being staind by hematoxylin or eosin. Numerous neutrophilic leucocytes had migrated around the material, and the surrounding subcutaneous connective tissue was edematous. The blood vessels were dilated and stagnated, and perivascular infiltration was marked. Some leucocytes were degenerating and destoryed nuclear fragments were observed as gross fibers stained with hematoxylin. New formation of collagenous fibers had not yet occurred.

b. One week after the insertion.

The materials were gathered in a mass and encapsulated with thin connective tissue. Neutrophiles had decreased markedly and, in their place many mononuclear cells such as monocytes had migrated around the material and penetrated into the mass along the crevices.

Some of the cells had degenerated and had piknotic nuclei. However, direct phagocytosis by these cells was not noted. It is interesting that many giant cells were formed in various shapes; both the foreign body type and the Langhans type were observed. Some large cells with two or more oval nuclei were also seen. They were belived to be in a transitional stage of giant cell formation but there was no sign of plasmodium or syncitium. Superinic acid seemed to be more effectived than stearinic acid in causing the formation of giant cells.

c. Three weeks after the insertion.

Some fat still remained in the central area although it had been moderately absorbed.

In general, a granuloma had formed containing the residue of the material. The remaining fat was solitarily surrounded by collagenous fibers. In some cases, the material was still gatheced in a mass the central parts of which showed almost no alteration.

The surrounding connective tissues had increased still further and many capillaries had been formed. Most of the cells were macrophages but there also a few leucocytes. They were generally pale-staining and large. Giant cells had increased and most of them were of the Langhans type.

Thus, acute inflammatory processes had been caused by the insertion of two

kinds of solid fatty acids, which were then absorbed by migration macrophages such as monocytes and by other factors, and then enacapsulated by productive connective tissues.

Thse were accompanied by the characteristic phenomena of giant cell formation, especially of the Langhans type. This tendency was more marked in the case of superinic acid.

2) The cortisone administered group.

The cortisone administered group showed no specific difference 3 days after the insertion as compared with the control group. Merely acute inflammation with migration of leukocytes and their degeneration was observed. After one week the migration and proliferation of macrophages and the production of collagenous fibers, which could also be observed following the diminution of the acute inflammatory process in the control group, were markedly weak.

These phenomena were more marked at 3 weeks. The surrounding collagenous fibers were shown by Van Gieson's stain to be slight thin and coarse and almost no fibers penetrated into the crevices of the masses. Migration and proliferation of macrophages were also slight.

The materials remained as a somewhat large mass. Some giant cells were observed but of an atypical smaller type.

Thus, the abministration of cortisone apparently inhibited the formation of connective tissue and cellular reactions, and delayed the absorption of the inserted materials.

3) The MAD administered group.

No characteristic effect of the hormone was observed 3 days after the insertion, at which tissue acute inflammation was present.

After one week, however, the development of surrounding connective tissues was marked, and they were thicker and denser than in the control group. The Van Gieson's stain showed fine collagenous fibers even in the deep parts of the masses along the crevices, which could not be observed in the control group.

Three weeks after insertion, most of the materials had been absorbed though small particles remained scattered and surrounded by connective tissue. The production of collagenous fibers was marked and they formed a thick capsule. Many fibroblasts were quite large.

The formation of giant cells was also marked, especially by the insertion of superinic acid.

This study shows that the production of connective tissue and the encapsulation process in parallel with the absorption of the materials are specifically promoted by the administration of MAD.

II. Tissue reactions to turpentine.

1) The control group.

a. Three days after the injection.

The area injected with turpentine showed extensive necrosis, with marked edema and numerous leucocytes in the surrounding area. Many leucocytes had also migrated into the muscle layers and subcutaneous tissues near the lesion.

Van Gieson's stain, showed the central necrotic area as homogeneously yellow. Large red collagenous fibers were observed irregularly encirculing the necrotic area, and most of them were assumed to be pre-existing fibers.

b. One week after the injection.

The lesion was divided into three layers as follows; 1) the central injected area composed of homogenous eosin-stainable necrosis, 2) a thick surrounding leucocyte layer, 3) a thick layer in the outermost zone which contained connective tissue and capillaries.

Most of cells in this outermost layer were generally bipolar shape. Connective tissue stain showed that some pre-existing collagenous fibers remained in the necrotic area and new fine fibers centripetally penetrated into the leucocyte layer from the outer-most layer.

c. Three weeks after the injection,

The central necrosis was almost absorbed and displaced by the granulation tissue composed of connective tissue, capillaries and some cells, but in some cases a little necrotic material remained.

With regard to the cells, fibroblasts and macrophages increaed as well as leucocytes, and some macrophages were swollen, large, and pale. Lymphocytes were also present. Fibroblasts generally had small long or round nuclei. Van Gieson's stain, showed many gross and fine collagenous fibers forming a surrounding annular capsule, but they were very scarce in the central residual necrotic lesion.

2) The cortisone administered group.

a. Three days after the injection.

The area injected with turpentine showed a wide homogenous necrosis with many pre-existing fibers irregularly arranged as in the control group.

The surrounding area was infiltrated with many leucocytes and was very edematous.

b. One week after tne injection.

The fundamental pattern at this stage resembled that of the control group with three layers, although the leucocyte and connective tissue layers were much thinner. The connective tissue was generally edematous. The formation of new fine collagenous fibers was scarcely apparent even by Van Gieson stain.

c. Three weeks after the injection.

The homogeneous necrosis remained in the central area in most of the cases. The production of connective tissue was observed in the surrounding area but there was less than in the control group. The reaction of connective tissue was not uniform in the lesion ; much less in the dermal area and somewhat more in the viscerl area.

In some cases, some eosinophiles infiltrated the connective tissue. Connective tissue cells were generally long and bipolar.

3) The MAD administered group.

a. Three days after the injection.

There was no specific difference in the histological pattern between this group and the control group. In some cases, the muscle layer was also involed in the necrosis and, in other cases, many cells in the intermuscular area were markedly degenerated and destroyed. Almost no formation of connective tissue was observed.

b. One week after the injection.

At this stage, the formation of surrounding granulation tissues was marked. Homogeneous necrosis with degenerating and destroyed leucocytes about it existed in the central area, and many macrophages with foamy vacuoles surrounded it.

Fibroblasts and blood capillaries were markedly increased in the outer zone. The fibroblasts were generally large with somewhat rounded nuclei and abundant cytoplasm.

Van Gieson's stain showed that large collagenous fibers had penetrated into the necrotic area and also were increased in the outer zone, where they formed a surrounding ring.

c. Three weeks after the injection.

The three layered structure still remained ; central necrosis, destroyed leucocytes and large foamy macrophages, and connective tissues. Fibroblasts were large.

Thus, the subcutaneous injection of turpentine caused a topical acute inflammation with necrosis. The injected material, necrotic substance and des-

troyed cellular substance were gradually absorbed, although some residue was observed three weeks after the injection.

The formation of granulation tissue followed the absorption, and encapsulation progressed.

In the cortisone administered group, the fibroblastic response was inhibited and there was a tendency towards edema formation.

In the MAD administered group, on the contrary, the fibroblastic response was promoted. The production of collagenous fibers increased and most fibroblasts were large and foamy.

Comments

In the first and second reports of these serial studies, the author described tissue reactions due to living and dead tubercle bacilli, and discussed the resemblances and difference of the histological patterns of the reactions due to these somewhat different agents. The modification of the reactions through changes in hormonal environment by the administration of the steroid hormones cortisone and MAD was especially studied.

Langhans giant cells and epithelioid cells observed in genuine tuberculous lesions are not specific cells for tubercle bacilli but may be reactions to some chemical substances.

Sabin[3)-11)] reported that high molecular fatty acids derived from tubercle bacilli might form a nodule resembling that of genuine tuberculosis, and this has been confirmed by many investigators. Tsutsui and Takeuchi[15)] upheld the possibility that Langhans giant cells could be formed by parmitinic acid, stearinic acid and tristearin emulsified by saline solution, which they injectied into the subcntis of ginuea pigs.

Koita[16)] and his coworkers reported that sodium salts of parmitinic acid, arachinic acid and lignoserinic acid might also form tubercles and that their ability paralleled their molecular weights. The present author used free saturated higher fatty acids such as stearinic acid and superinic acid, and confirmed the fact that these materials could from nodules with many giant cells of both the Langhans and the foreign body type at the inserted area. This tendency was more marked in the case of superinic acid. These studies may be significant in the use of highly purified free fatty acids. With regard to epithelioid cells, a definite conclusion could not be obtained from the present studies, although some cells resembled epithelioid cells.

Moreover, the author studied the usual type of foreign body inflammation. Hitherto, various materisls have been used for these studies : Glass plastes, glass sticks, glass balls, India ink, neutral red (Sato, Mitsunaga et al),[17)-19)] polyvinyl

chloride-powder (Matsuura et al[20]) and polyvinyl-sponge (Okuda[21]). Taubenhaus [22][23] had an experiment in which he produced abscess by injecting turpentine into the subcutis of albino rats which were castrated, adrenalectomized or hypophysectomized. The author used turpentine because it could cause sufficient topical inflammation without influencing the general condition when injected in moderate quantityies, although it was gradually absorbed.

The present study indicates that the tissue reactions caused by solid higher fatty acids and turpentine are divided into two processes ; the first is absorption and exclusion of the materials by various cells and other factors, and the second is an encapsulation mechanism by productive connective tissue. These reactions can be modified characteristically by the administration of cortisone or MAD. Following the administration of cortisone, cellular reeactions, especially fibroblastic response, were decreased and the formation of collagenous fibers was inhibited. This tendency was most marked at one week after the beginning of the experment, and it seemed to be weakened at three weeks. On the other hand, the administration of MAD promoted fibroblastic response and formation of collagenous fibers. Each fibroblast also was hypertrophied. These phenomena may be comprehended as due to the specific actions of cortisone or MAD. Thus, the serial studies have investigated the influences of steroid hormones on various inflammatory lesions, and shown that inflammation, whatever the cause of it may be, is characteristically affected and modified by these hormones.

Previous studies on the influence of steroid hormones upon connective tissue have been restricted to the relationships of sex hormones and the genital glands. However, since it has recently claimed that cortisone inhibits the formation of mesenchymal tissues and has a significant affect on various lesion, and has investigated in detail the relationships between inflammation and hormones.

Selye (1936)[24] studies the actions of hormones on the fibroblastic respons by direct injection into a "granuloma pouch" and remarked that close relationships existed between the systemic stress response and the topical phenomena of inflammation. He also[25][26] emphasized that both the general adaptation syndrome and inflammation represent nonspecific responses to a variety of apparently quite unrelated agents, and, in both phenomena, certain pituitary and adrenal hormones such as ACTH, STH and corticoids exerted an important regulatory role.

However, he reported that corticoids such as cortisone did not directly effect pathogens as "antiphlogistic corticoids", but acted mainly by interfering with the development of the granulomatous barriers. More recently, the reports that Selye (1946)[27] experimentally created periarteritis, nephrosclerosis and arthritis by DOCA, and Hench et al (1949)[28] remedied clinical rheumatoid arthritis and allied disorder by compound E have attracted intense interest to the actions of adrenococtical hormones upon the mesenchymal tissue. It is generally recognized

that adrenocorticotrophic hormones delay wound healing (Ragan et al[19]) and hypophysectomy causes insufficient growth of granulation tissue in the turpentine necrosis.

In the other hand, anterior growth hormone is regarded as a significant promoting factor for the formation of granulation tissue (Taubenhaus et al[23] 1950).

With regard to adrenocortical hormones, it is well known that cortisone inhibits all reactions of connective tissue, including the formation of blood vessels. Blunt et al[30] (1950) reported that healing of bone fractures and absorption of hematoma were markedly delayed by the administration of cortisone. It is obvious that the delay in wound healing coused by the administration of ACTH results from the production of adrenocortical glucocorticoids, while DOCA, one of mineralcorticoids, stimulates granulation tissue (Taubenhaus 1949)[22].

Thus, it may be recognized that both the anterior pituitary and the adrenocortical gland regulate fibroblastic response by two reverse factors, and the hormonal actions in inflammation are very complex.

Alrich et al[31] (1951) suggested that wound healing was not affected by adrenalectomy or delayed by the administration of cortisone and excessive ACTH.

There are various opinions about the cause of the characteristic inhibition of fibroblblastic response by cortisone. According to Blunt[30] (1950), it may be due to poor nutrition through an inadequate blood supply or may be due to the action of a circulating material possibly cortisone itself, brought to the area through new vessels. Lattes. (1955) remarked that cortisone in adequate dose problably blocked the release from injured tissues of chemical substances which were necessary for the series of events known as inflammation and repair. The substances which fail to be released in the injured tissue under the effect of cortisone are probably mucopolysaccharide in nature (Lattes et al 1953, 1954[31)-34)]).

In the other hand, the stimulation action of DOCA on the fibroblastic response was attributed to changes in water and mineral metabolism (Taubenhaus 1949[22]).

Both testosterone-propionate and estradiol dipropionate cause poor granulation response to turpentine. These hormonal actions may be divided into two mechanisms: a systemic action through the inhibition of hypophyseal function, and a topical action on granulation tissue itself (Taubenhaus 1949[22]).

Thus, connective tissue reactions to various inflammatory lesions are surely regulated by the hormonal environment. The author observed that cortisone (one of the glucocorticoids) and MAD, which has male hormonal action, had evident different effects on defensive mechanisms, especially on fibroblastic response.

The charatceristic inhibition of fibroblastic responses by the administration of

cortisone was most marked about one week after the beginning of the experiments. However, it was not conspicious at later periods, because the effects of cortisone probably do not continue for so long a time. Moreover, MAD markedly promoted the fibroblastic response in these serial studies, although Taubenhaus reported opposite results with sex hormones. The swelling or hypertrophy of each fibroblast observed in the MAD administered group is probably due to the direct action of MAD as well as growth hormones.

Conclusion

In the 3rd report of the serial studies, purified solid stearinic acid and superinic acid as free high fatty acids and turpentine as a usual stimulant were used. These materials were inserted or injected into the subcutis of albino rats, and the resulting tissue reactions were histologically investigated up to 3 weeks after the beginning of the experiments. The modification of the histological pattern of the lesions by the administration of cortisone and MAD was also studied.

It was proved that fatty acids cause formation of granulation tissue with many giant cells of the Langhaus type, and turpentine also causes it with necrosis. These materials were gradually absorbed. Cortisone characteristically inhibited the fibroblastic response in these tissue reactions, while, MAD markedly promoted it.

Finally the influences of various hormones on inflammation were discussed in connection with these serial studies.

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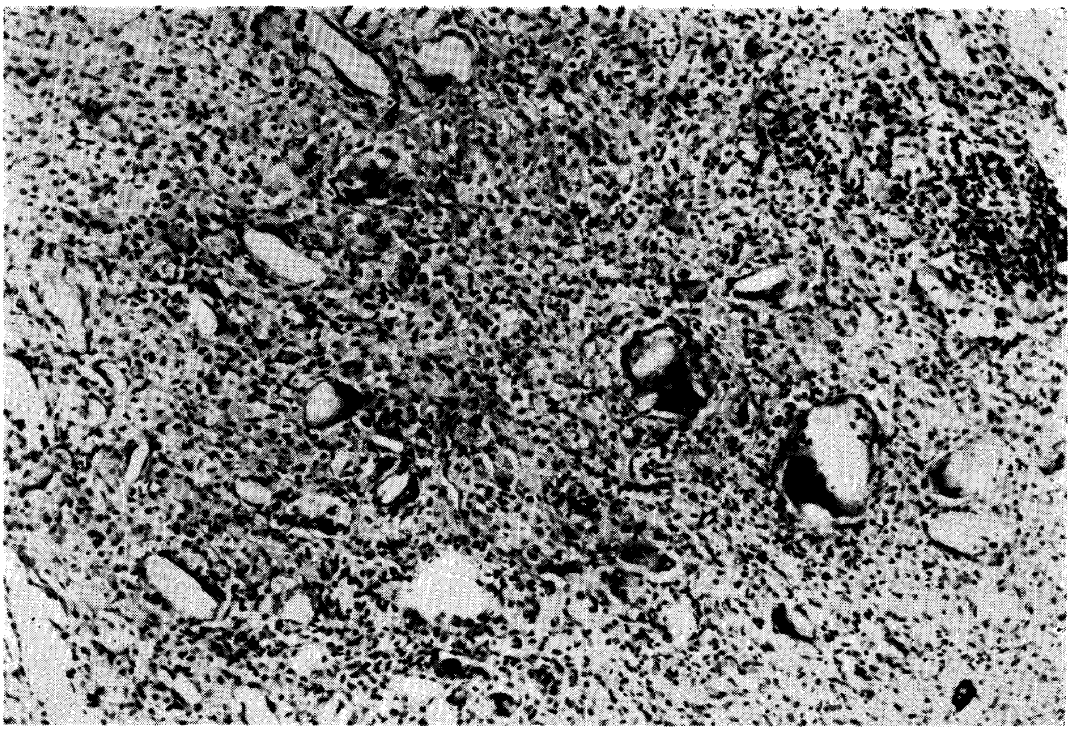

Fig. 1 Sperinic acid. Three weeks. Control group. The scattered lucid spaces show the presence of the material. Many giant cells of various shapes are seen. H. E. stain. (× 100)

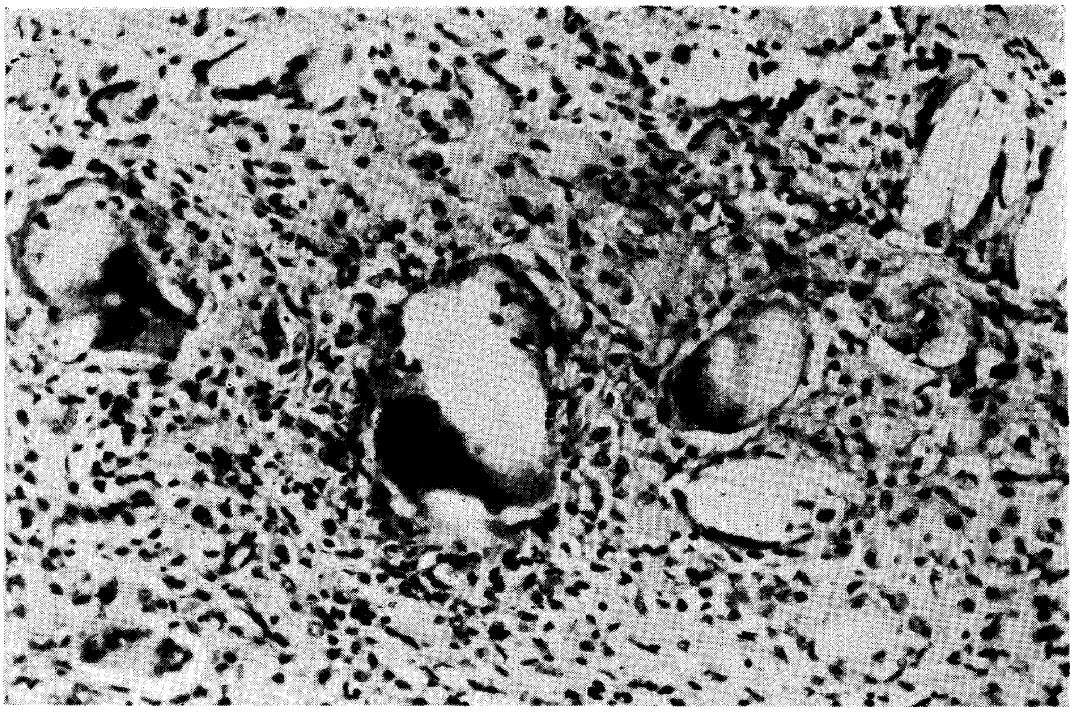

Fig. 2 Higher magnification of Fig. 1. This shows the lucid spaces and giant cells. (× 200)

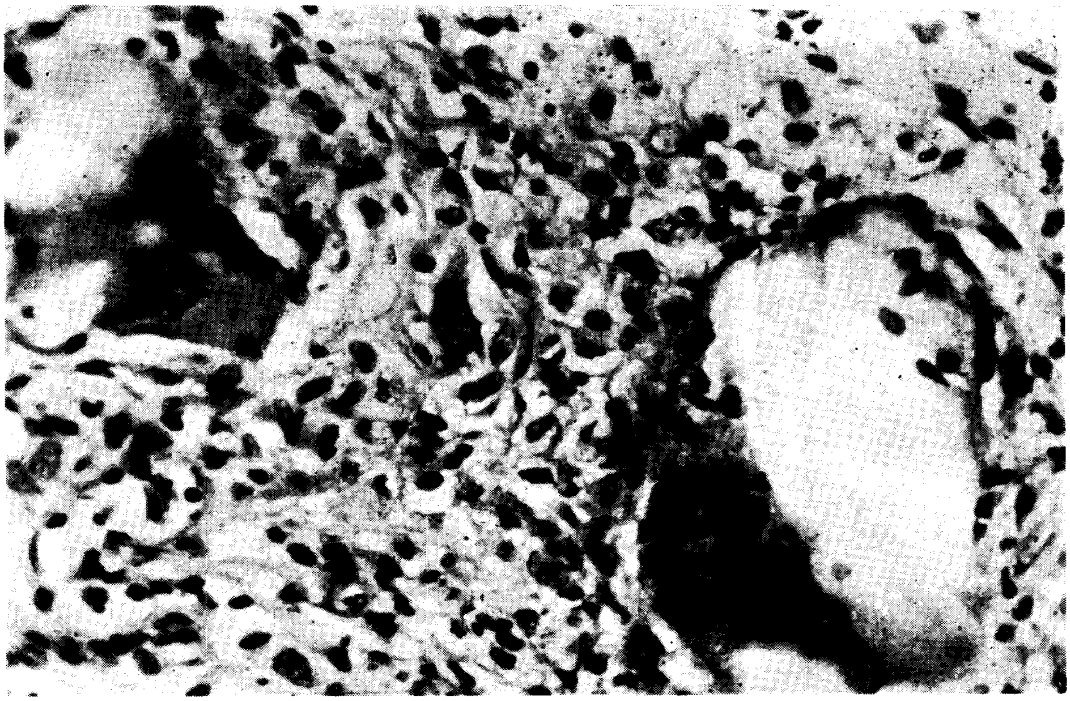

Fig. 3 Higher magnification of Fig. 2. Two giant cells are in contact with lucid spaces of which the border is not clear. (× 400)

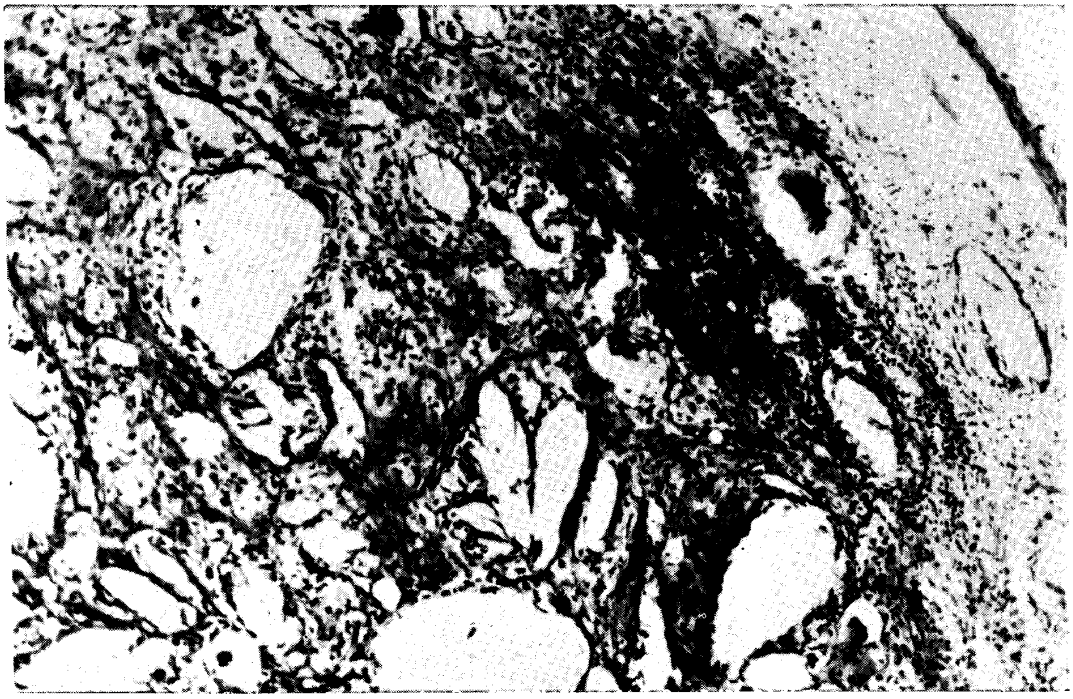

Fig. 4 Stearinic acid. One week. The MAD administered group. The production of collagenous fibers is shown. Van Gieson stain. (× 100)

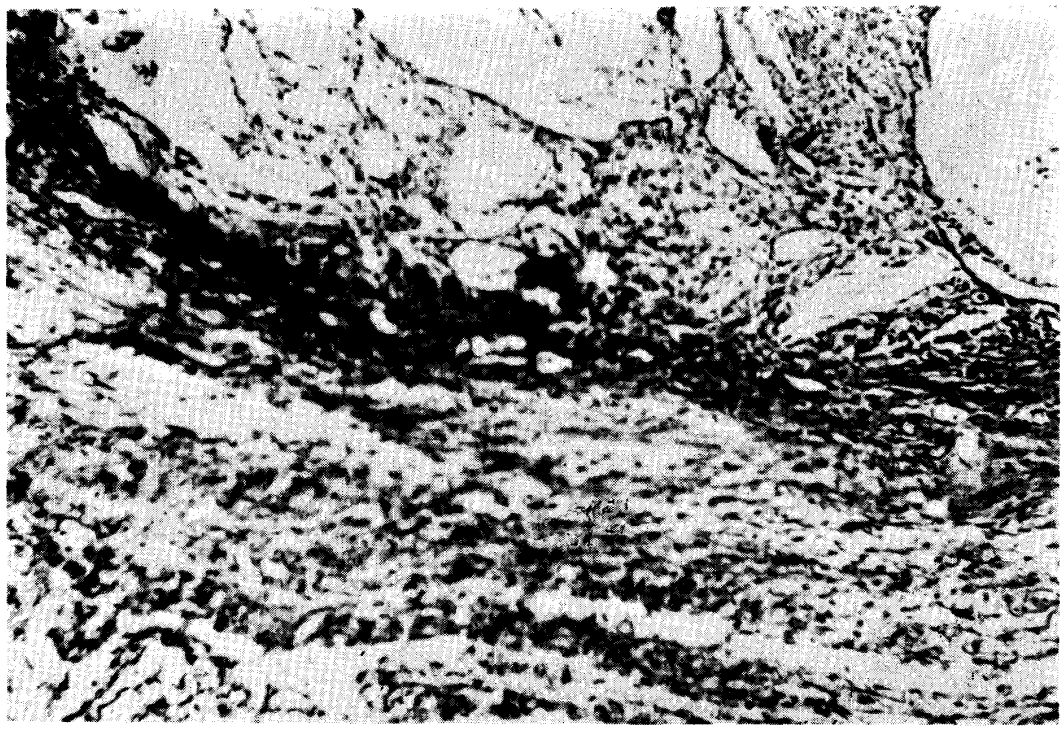

Fig. 5 Sperinic acid. One week. The MAD administered group. Many collagenous fibers are formed and encapsulate the material shown at the upper site. Van Gieson stain. (× 200)

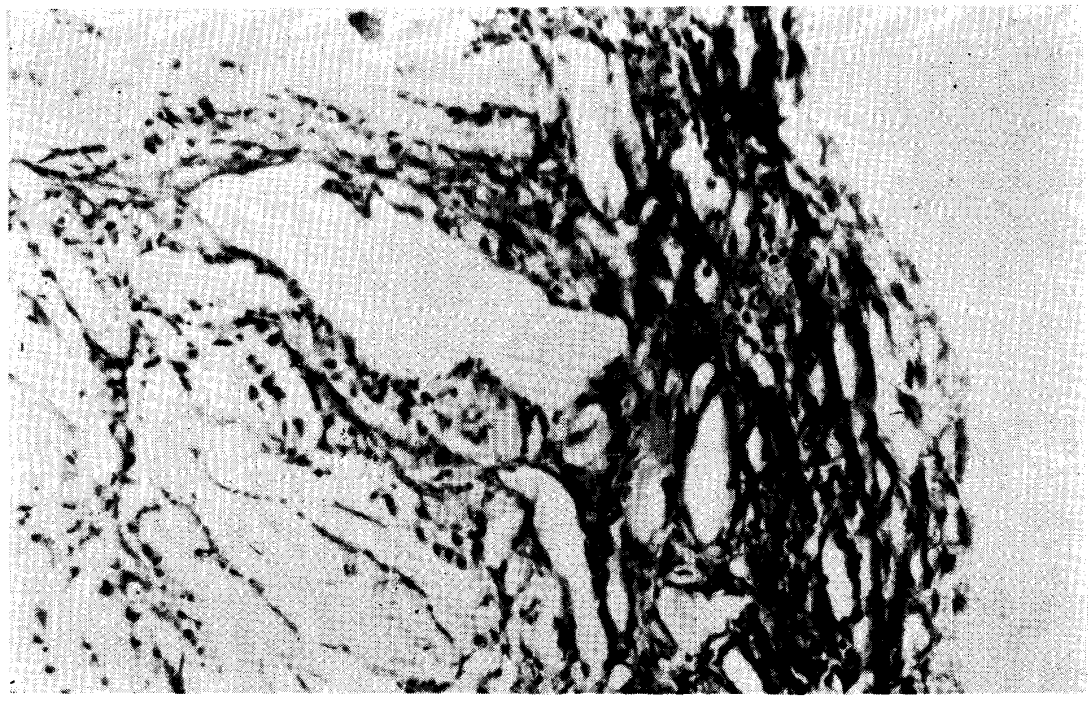

Fig. 6 Sperinic acid. Three weeks. The cortisone administered group. The poor formation of collagenous fibers may be compared with Fig. 5. Van Gieson stain. (× 200)

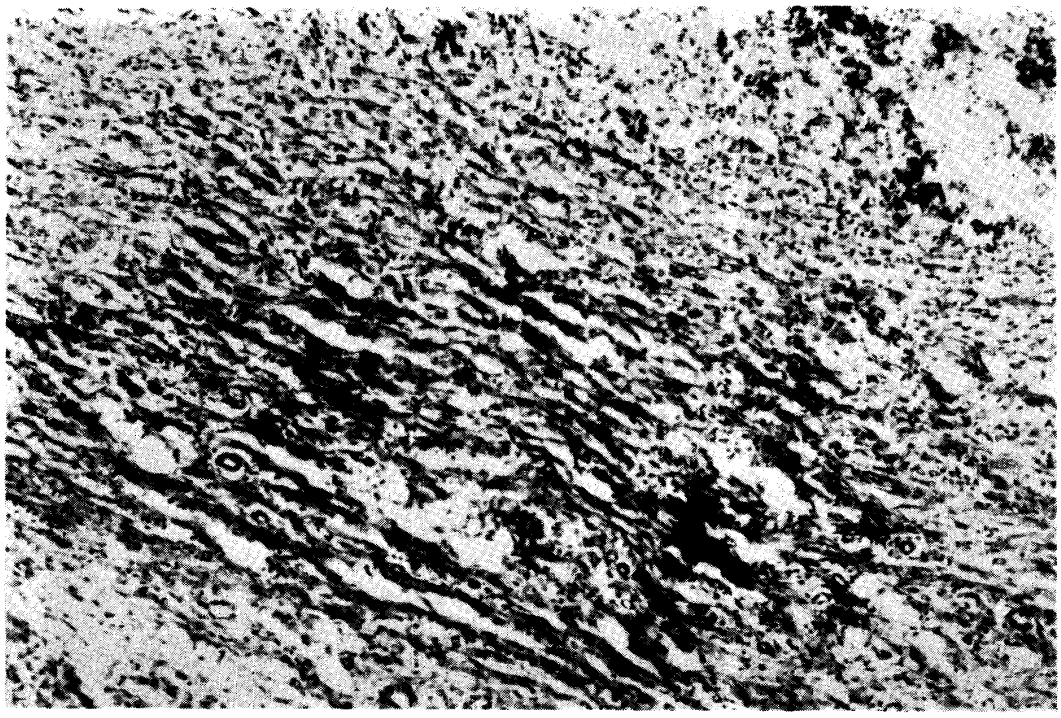

Fig. 7 Turpentine. Three weeks. The control group. The formation of granulation tissue is shown. Van Gieson stain. (× 200)

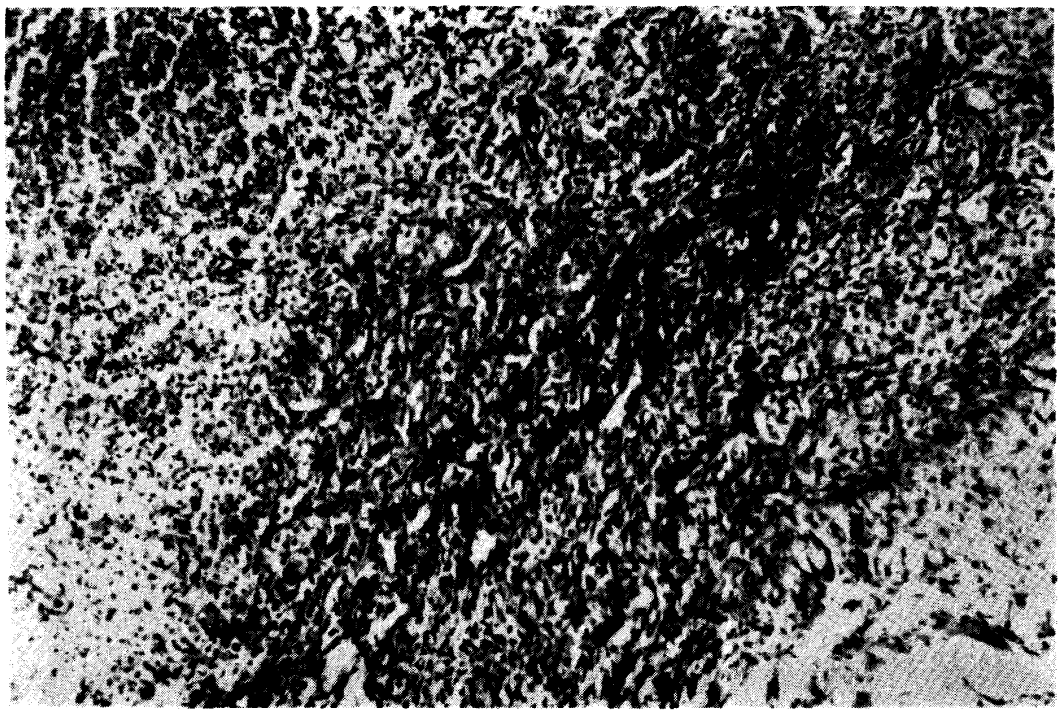

Fig. 8 Turpentine. One week. The MAD administered group. The marked formation of granulation tissue may be compared with Fig. 7. Van Gieson stain. (× 200)

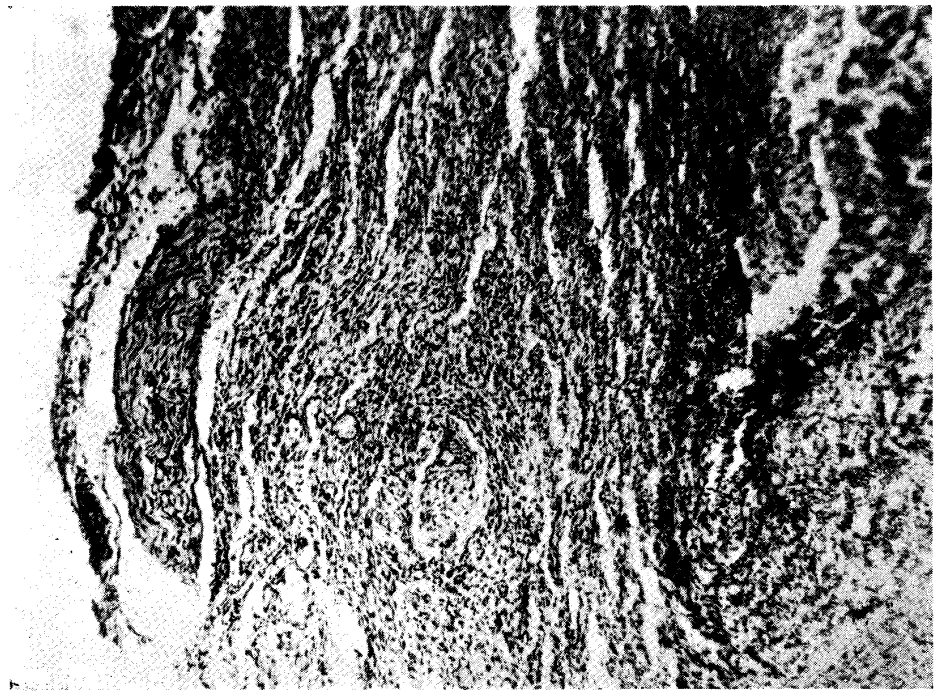

Fig. 9 Turpentine. Three weeks. The MAD administered grouup. The encapsulating collagenous fibers are shown. Van Gieson stain. (× 100)

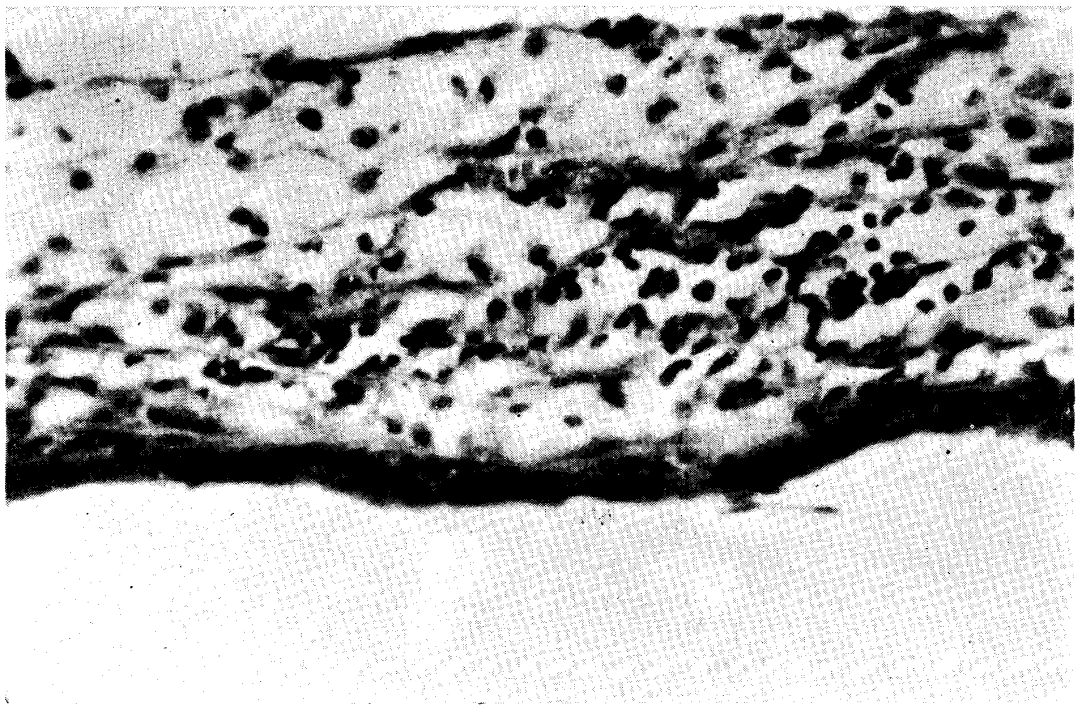

Fig. 10 Turpentine. Three weeks. The cortisone administered group. The poor formaion of collagenous fibers is compared with Fig. 9 Van Gieson stain. (× 400)