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Effect of crystalline penicillin and bicillin-1 on experimental syphilis in the rabbit

Electron microscope study

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In continuing the study of the mode of action of penicillin, it was decided to compare the therapeutic effect of single massive doses of crystalline penicillin and of bicillin-1 (benzathine penicillin, which produces therapeutic serum levels for 2 to 4 weeks).

Evaluation of the effectiveness of these two preparations was based on clinical observation of the resorption of the chancres, the disappearance of the treponemes, the transfer of tissue from the lesions in the treated rabbits to healthy rabbits, and serial serological tests.

At the same time, the authors carried out electron microscope examinations of fragments of chancre tissue with a view to studying the fine processes of interaction between the chancre cells and the treponemes. The study showed, that, despite the high concentrations of penicillin in the serum after one injection of crystalline penicillin, the rabbits were not cured, whereas after administration of bicillin cure was achieved in the majority of the animals. This experiment confirmed that a prolonged concentration of penicillin is essential for therapeutic effectiveness. At the same time it showed that treponemes become more subject to phagocytosis by the cells and to subsequent digestion, mainly by macrophages. In lymphocytes, endothelial cells, and plasmocytes, endocytobiosis apparently takes place. Consequently, in penicillin therapy of syphilis, a therapeutic effect is obtained by the combined action on the treponemes of the antibiotic and the macro-organism.

Despite the great successes achieved in the treatment of syphilis with penicillin, a number of aspects of its mode of action are still obscure.

In selecting a preparation for treating syphilis, doctors are guided by various considerations. Some believe that the higher the concentration of penicillin in the blood stream the better, and, on that basis, they administer penicillin every 2 or 3 hours in quite high doses. Others, in view of the inconvenience of 3-hourly injections, treat their patients with long-acting preparations of the bicillin type. A therapeutic concentration of penicillin persists for 2 to 4 weeks after administration of bicillin-1, depending on the dose of the preparation administered, but the concentration is always low. In view of this, some propose the administration of bicillin-3 (or similar preparations, such as panbiotic) which achieves a short-lived, high concentration of penicillin and then maintains a low but therapeutic concentration of the preparation over a long period. As can be seen, in selecting a preparation, every doctor follows his own ideas in his efforts to achieve the maximum effect.

To elucidate the significance of high concentrations of penicillin in treatment, Ovčinnikov and Korbut (1961) administered to rabbits with syphilomas or with latent syphilis a single intramuscular injection of crystalline penicillin in doses of 42,000, 84,000, and 168,000 units per kg. body weight, *i.e.* the whole dosage for a course of treatment was given at once. After this, the treponemes disappeared on the average after 168, 216, and 268 hours respectively and the chancres were resorbed after 32, 28, and 37 days. The serological reactions did not become negative. Transfer of lymph nodes to healthy rabbits after 4½ to 6 months gave positive results, but the number of such results was inversely proportional to the dose of the preparation.

It became clear from the experiments that a single administration of crystalline penicillin, even in very high doses, did not cure the rabbits, but that it did cure them if given in equal doses every 2 hours for 7 days, the same total dosage being spread over the whole course of treatment (84,000 and 168,000 units per kg.). After bicillin-1 was administered in a single dose at these levels, despite the low concentration of penicillin in the serum, the therapeutic results were

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considerably better than with crystalline penicillin, the success being proportional to the size of the dose. A dose of 42,000 units per kg. was not large enough to cure syphilis, and clinical and serological relapses occurred.

The results also depended on the stage of syphilis at which treatment was begun. In the case of a hard chancre, one injection of bicillin-1 was enough to cure the rabbit concerned, whereas in the case of latent syphilis one injection was inadequate. After treatment with bicillin-1, lymph nodes from the treated rabbits were transferred to 22 fresh rabbits, and in the first passage a hard chancre developed in one of them. After treatment with bicillin-3, a hard chancre developed in two out of 41 so treated. After treatment with 168,000 units bicillin per kg., no clinical or serological relapses occurred.

In comparing the short-term and long-term results of treatment with bicillin and crystalline penicillin administered every 2 hours for a week, the authors obtained the best results with the latter.

In view of the need to carry out electron microscope investigations, we decided to repeat, with certain modifications, the experiment of administering crystalline penicillin and bicillin-1 in single doses.

Experimental method

Crystalline penicillin

Three rabbits with negative serological reactions were infected intradermally on both sides of the scrotum with a suspension of treponemes of Strain 1; 49 days after infection syphilomas containing a large number of *Treponema pallidum* had formed in the rabbits and the Wassermann reaction had become positive.

Crystalline penicillin in a dose of 84,000 units per kg. body weight was administered intramuscularly to the three rabbits. After 3, 6, 24, 30, and 48 hours, chancres were removed for electron microscope examination and dark-field examination was carried out at the same time. Suspensions prepared from chancre fragments were transferred to healthy rabbits, in all of which chancres developed after the normal period, and the standard serological reactions became positive.

Consequently, a single administration of crystalline penicillin failed to cure the rabbits.

Bicillin-1

Experiments were carried out on thirteen rabbits, six of whom were infected with Strain 1 while the other seven were infected with Strain 8 of the Central Institute for Research on Skin and Venereal Diseases. The bicillin was administered 40 to 80 days after infection.

Eleven rabbits were given a single dose of 84,000 units per kg. and two were given two doses each of 84,000 units, the second 2 weeks after the first. Material was taken for electron microscope examination from rabbits given a single dose of bicillin at 3, 24, 48, and 72 hours, and 7 and 14 days after administration of the preparation, and from rabbits given two doses of bicillin at 48 hours and 14 days after the second administration.

At the same time, we transferred to the scrotum in twelve healthy rabbits a suspension prepared from fragments of chancre or, in cases where there was no chancre, from scrotal skin and inguinal lymph node tissue. In rabbits to which tissue fragments from bicillin-treated animals had been transferred, no clinical manifestations developed. In one rabbit, positive results were obtained on one occasion in the standard serological tests but not in the immobilization test, and later all serological reactions became negative. In one rabbit, weakly positive results in the standard serological tests but a negative *Treponema pallidum* immobilization test occurred on three occasions. Subsequently, all the serological reactions became negative. In all the remaining rabbits, the standard serological tests and the TPI test were negative on all the many occasions on which they were made.

After 5 to 6 months, the original rabbits (those given treatment) and the rabbits to which the first transfer had been made were re-inoculated with the same strain or with the Nichols strain.

Chancres did not develop in any of the treated rabbits after re-inoculation. In three animals, the standard serological tests became positive and in three others the TPI also became positive (100 per cent. immobilization) with a gradual increase in titre. In another, the TPI alone became positive. In one rabbit, the TPI from being slightly positive became negative at a second test. Of the twelve rabbits which had had a first transfer of tissue and were re-inoculated, nine developed chancres and three died before chancres could develop. Popliteal lymph nodes had been taken from these nine rabbits before re-inoculation and transferred to healthy rabbits. Syphilomas did not develop in the healthy rabbits, but serological reactions in two rabbits were found to have become positive after repeated examinations.

This experiment shows that, despite the very high concentration of penicillin found in the serum of rabbits after a single administration of massive doses of penicillin in an aqueous solution, the rabbits were not cured.

Meanwhile bicillin-1, administered in the same doses, which produces a low but persistently therapeutic concentration, had a good therapeutic effect.

According to investigations by Ovčinnikov and Korbut (1965), the concentration of penicillin in the blood 30 minutes after administration of 84,000 units penicillin per kg. body weight in rabbits averaged $120{\cdot}8 \pm 35{\cdot}3$ units per ml. The maximum concentration was 256 and the minimum 64 units. After administration of bicillin-1 in the same dose, the mean concentration was $1{\cdot}04 \pm 0{\cdot}34$, with a maximum of 8 and a minimum of 0·25 unit.

After an aqueous solution of penicillin has been administered, it persists in the blood for not more than 6 hours, whereas after administration of bicillin-1

that substance is still found in some rabbits 216 hours later in a concentration of 0·12 ± 0·01 per ml.

Consequently, our experiments confirmed the long-established fact, that, for a therapeutic effect to be obtained, penicillin must remain in the blood for a long period in therapeutic concentrations. The presence of high concentrations for short periods does not lead to cure.

Electron microscope studies

It was of interest also to determine what changes occurred in the morphology of *Treponema pallidum* in the syphiloma itself and the interrelationships which arise with cellular elements in the chancre.

In earlier electron microscope studies by Ovčinnikov and Zelikova (1951) on treponemes exposed to penicillin *in vitro* in concentrations of 1,000, 5,000, and 25,000 units for 2½ or 5 hours, no essential morphological changes could be found except for the appearance of granular areas in the body of the treponeme.

Vjaseleva (1958) found single motile treponemes even after 24 hours' exposure to a solution containing 50,000 units penicillin, but these treponemes failed to infect rabbits.

Following 5 hours' exposure to penicillin (5,000 units), certain changes were observed in the body of the treponemes. Dark-field examination showed that such treponemes had remained motile, but the electron microscope examinations made at that time must now be considered inadequate, primarily because ultrathin sections were not examined. In addition, changes in treponemes in rabbit tissue are of greater interest than changes *in vitro*. For that reason, we decided to carry out comparative electron microscope examinations of ultrathin sections of tissue fragments from syphilomas from rabbits given crystalline penicillin and bicillin in the above-mentioned doses.

The material for the electron microscope examinations was prepared in the following way:

At appropriate intervals after administration of the penicillin and bicillin, the hard chancre was cut away and the material used for examination under the ordinary light microscope and the electron microscope. Immediately after removal part of the hard chancre was placed in a buffered glutaraldehyde solution, cooled to + 4°C., and stored in the refrigerator at this temperature for 1 hour. The material was then washed in chilled phosphate buffer of pH 7·2 for 15 minutes, cut into pieces measuring 0·5 by 1 mm., and transferred into a buffered solution of osmium tetroxide cooled to + 4°C. After 2 hours' fixation, the fragments were rinsed in a 5 per cent. saccharose solution and dehydrated by the standard Luft method. Dehydration was carried out in ethyl alcohol and the material was transferred from the absolute alcohol into absolute acetone, then into a mixture of acetone and a working mixture of Epon 812, the working mixture being changed twice. Polymerization was carried out at 45°C. for 24 hours, with a subsequent increase in temperature to 60°C. before the final polymerization (48 to 72 hours in all). Since it is difficult to find *Treponema pallidum* under the electron microscope after long-standing infection, we shall describe only the data obtained when treponemes were examined earlier, although material was also taken after longer periods had elapsed.

In comparing the electron microscopic changes in rabbits given crystalline penicillin and bicillin, the following points should be noted:

(1) Most but not all of the treponemes outside the cells are broken down 3 hours after administration of the penicillin. In Fig. 1, of material taken 3 hours after administration of penicillin, the treponemes are situated among the collagen fibres. In the cross-cut treponemes in the electron micrograph (Figs 1 and 1a), part of the outer bunch of fibrils (F) has been preserved, and the outer wall (me) covering the bundle, the cytoplasmic membrane (Cm), and the deep layer of fibrils (F′) have been partly preserved, while the cytoplasm contains a small number of ribosomes (r). Around the treponemes is a clearer area containing no collagen. Compared with the treponemes not exposed to penicillin, the most marked feature in the early stages after administration of penicillin is the partial or complete absence of the outer treponemal wall. No appreciable changes can be seen in the protoplasmic cylinder. A longitudinally-cut treponeme (T′) (Fig. 1) is contained, as it were, in a two-layered casing standing out some distance from the body of the treponeme itself. The collagen fibres are pressed close against this casing. The treponeme has preserved its cytoplasmic membrane and fibrils, and occasional ribosomes are visible.

(2) 3 hours after a single administration of penicillin in a dose of 84,000 units per kg. body weight, substantial morphological and functional changes can be seen in the cellular elements of the hard chancre, primarily in the macrophages. The number of pseudopodia rose sharply, thus considerably increasing the surface area of the cells (Fig. 2). The number of lysosomes, food vacuoles (Fv), and mitochondria (M) also increased. The phagocytic activity of the cells rises sharply.

(3) The treponemes in the phagosomes of the macrophages (Figs 3 and 3a) undergo greater changes during this time than those situated outside the cells. At first, they became more electron dense,

the outlines of the membrane become blurred, and the ribosomal apparatus loses its granular appearance and becomes less clear-cut until it can hardly be distinguished. The phagosome of the macrophage (PH) contains a treponeme (T) which has been partly lysed. The structure of the fibrils persists for quite a long time since, in general, they are more resistant to penicillin. Near the phagosomes containing treponemes, the number of food vaucoles (Fv) increases; these fuse and the treponemes are lysed further. Fig. 4 shows a macrophage with a large number of pseudopodia and 4a shows a treponeme inside a phagosome which has kept its superficial and deep-lying fibrillar bundles and cytoplasmic membrane (Cm). Also inside the phagosome, there is a layered structure with amorphous contents. The outline of the treponeme is blurred. In some macrophages (Figs 5, 5a, and 6), the treponemes are almost completely lysed and it is possible to see clearly the increase in the number of food vacuoles (Fv) with residual bodies in them. The membrane (m) of the food vacuoles (Fig. 6) has two clear-cut electron

FIG. 1 *Ultrathin section of chancre tissue from rabbit infected with* Treponema pallidum *Strain* 1. *Material taken* 3 *hours after administration of* 84,000 *units crystalline penicillin. There are no collagen fibres round the cross-cut treponemes (T). The superficial bundles of fibrils (F) and outer wall (me) are partly preserved; the deep-lying (F') bundles of fibrils, the cytoplasmic membrane (Cm), and the cytoplasm (C) are preserved. The ribosomes (r) are well-defined. Round a longitudinally-cut treponeme (T'), there is a two-layered casing and collagen fibres (KL) are pressed close against the treponeme casing.* ×18,000

FIG. 1*a* *Detail* × 58,000

FIG. 2 *Ultrathin section of chancre tissue from a rabbit infected with* Treponema pallidum *Strain* 1. *Material taken* 3 *hours after administration of* 84,000 *units crystalline penicillin. Intensification of macrophage activity. Large numbers of pseudopodia (Ps), food vacuoles (Fv), lysosomes (L), and mitochondria (M). The extra-cellular treponemes (T) among the collagen fibres have undergone no appreciable change. N = nucleus.* × 5,000

FIG. 3 *Ultrathin section of rabbit chancre tissue* 45 *days after infection with* Treponema pallidum *Strain* 1. *Material taken* 3 *hours after administration of* 84,000 *units crystalline penicillin. Macrophage with a large number of pseudopodia (Ps). M = mitochondria. Treponemes (T) inside phagosome (PH) partly lysed. Fibrils preserved. Round the phagosome there are food vacuoles (Fv) with welldefined membranes.* × 5,000

FIG. 3*a* *Detail* × 50,000

FIG. 4 *Ultrathin section of hard-chancre tissue taken* 3 *hours after administration of crystalline penicillin. Macrophage with large number of pseudopodia (Ps). Inside the phagosome is a cross-cut treponeme with the superficial (F) and deep-lying (F') bundles of fibrils still preserved; Cm = cytoplasmic membrane. Inside the phagosome (PH) is a layered structure with amorphous contents.* × 10,800

FIG. 4*a* *Detail* × 45,800

FIG. 5 *Ultrathin section of chancre tissue from a rabbit infected with* Treponema pallidum *Strain* 1. *Material taken* 3 *hours after administration of crystalline penicillin. Macrophage with a large number of pseudopodia (Ps).* Treponema pallidum *(T) inside a phagosome (PH) is in a state of lysis. There are many food vacuoles (Fv).* × 5,000

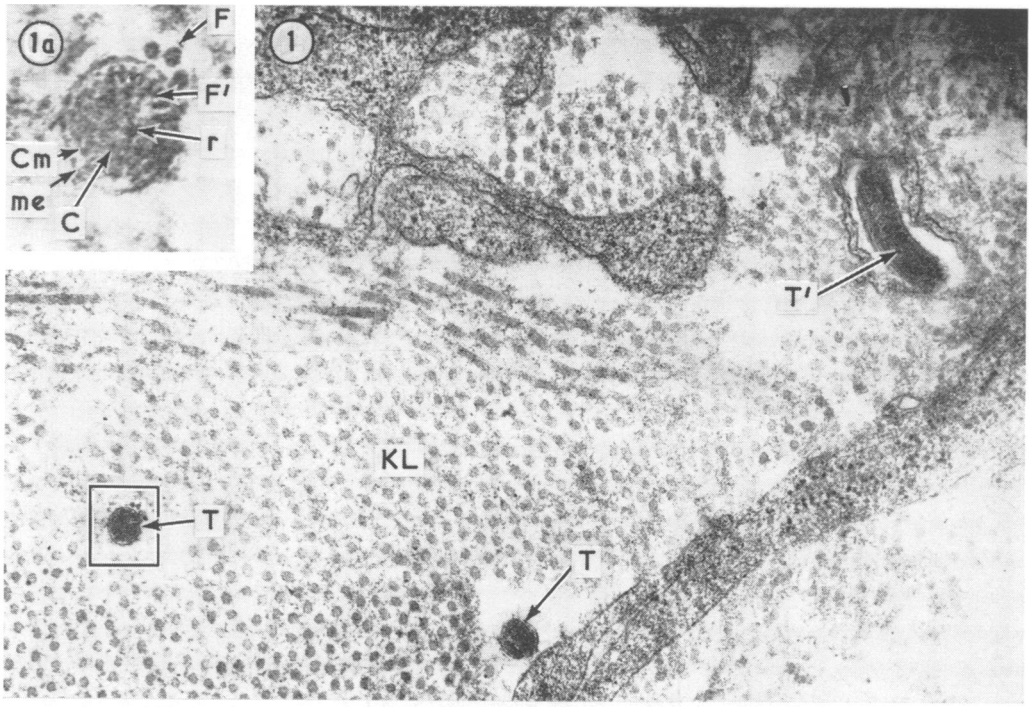
FIG. 5*a* *Detail* × 30,000

FIG. 6 *Ultrathin section of chancre tissue taken from a rabbit* 3 *hours after administration of crystalline penicillin.* Treponema pallidum *(T) inside a macrophage phagosome. Two electron dense layers in the membrane of the phagosome (mPH) and food vacuoles (Fv) are clearly visible; r = ribosome. The treponeme is at the lysis stage.* × 51,000

FIG. 7 *Phagocytosed treponeme (T) in a state of lysis.* × 30,000

FIG. 8 *Ultrathin section of hard-chancre tissue* 45 *days after infection. Material taken for examination* 6 *hours after administration of crystalline penicillin. Outer wall (me) of extracellular treponeme (T) is homogeneous in structure and highly osmiophilic. Phagocytosed treponeme less altered. Superficial (F) and deep-lying (F') bundles of fibrils and cytoplasmic membrane (Cm) are still preserved; PH = phagosome; mPH = phagosome membrane.* × 150,000

FIG. 9 *Extracellular treponeme (T) from a chancre* 18 *hours after administration of* 84,000 *units crystalline penicillin. Ribosomes not distinguishable. Fibrils (F') preserved.* × 16,500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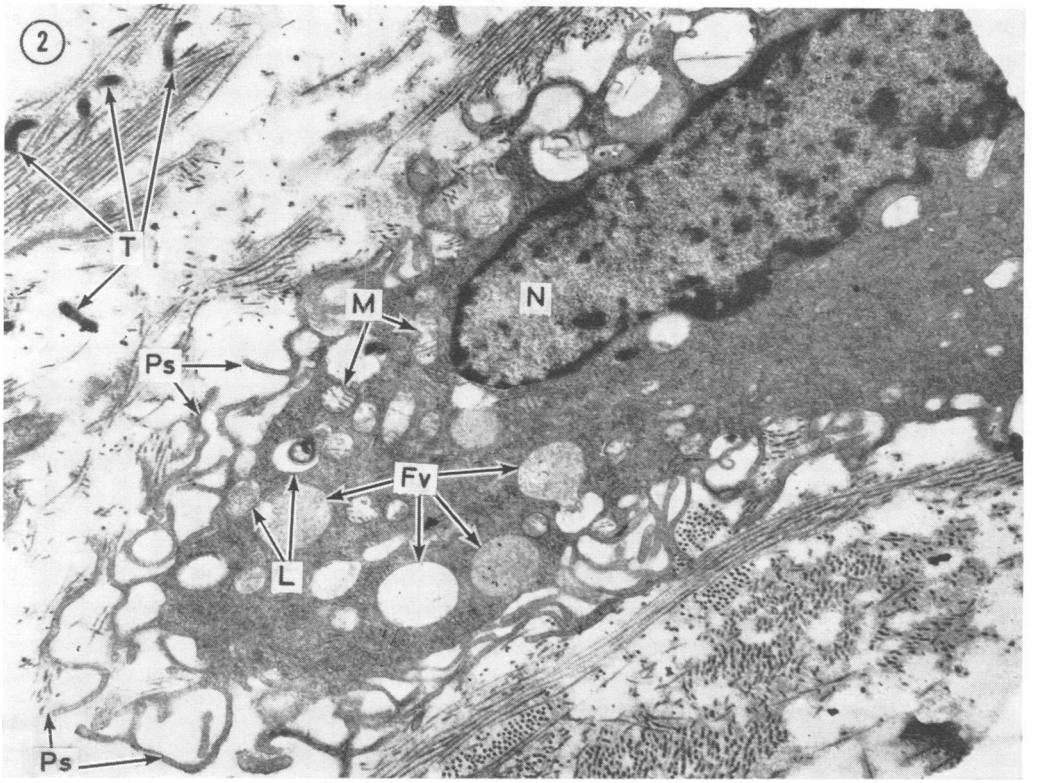
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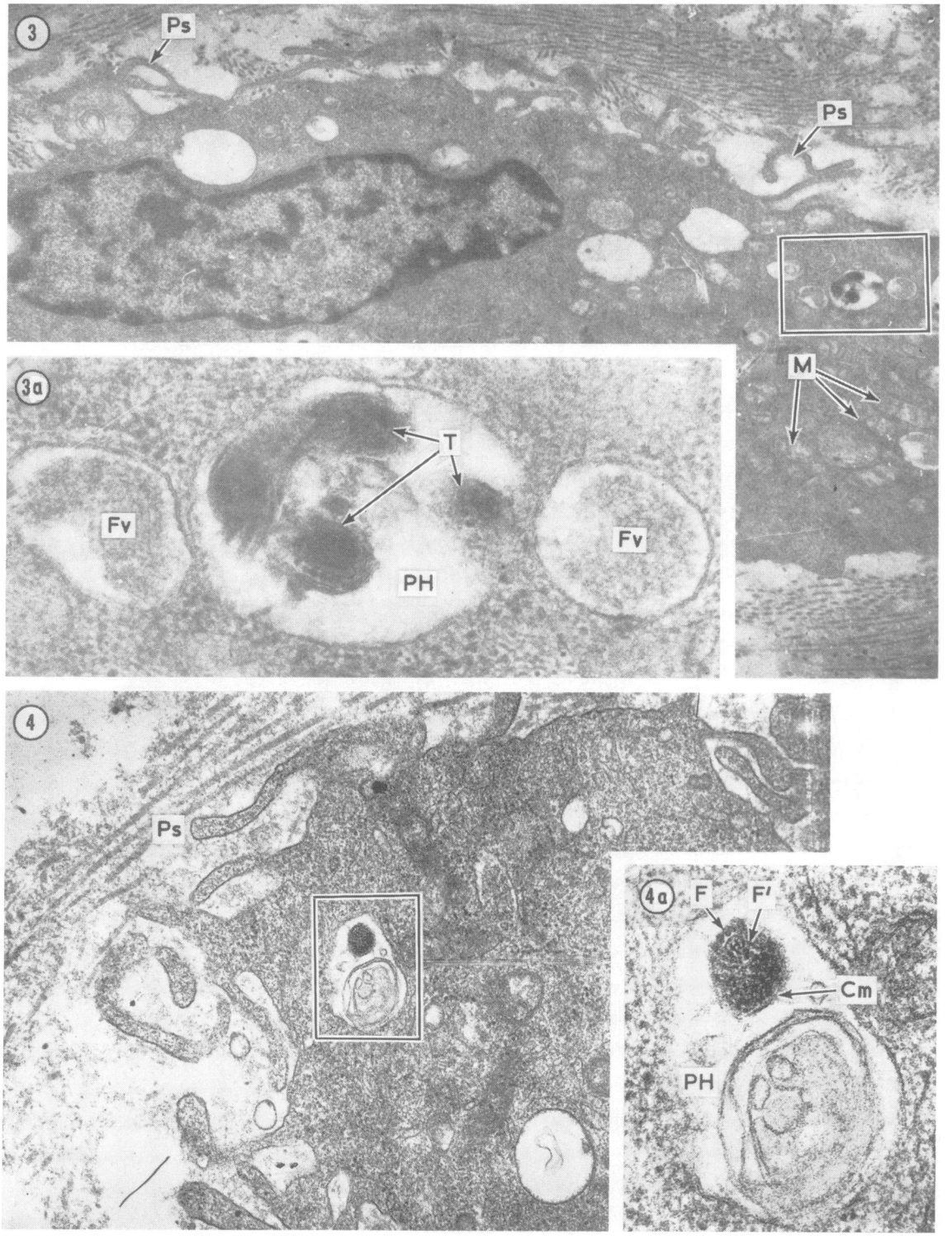
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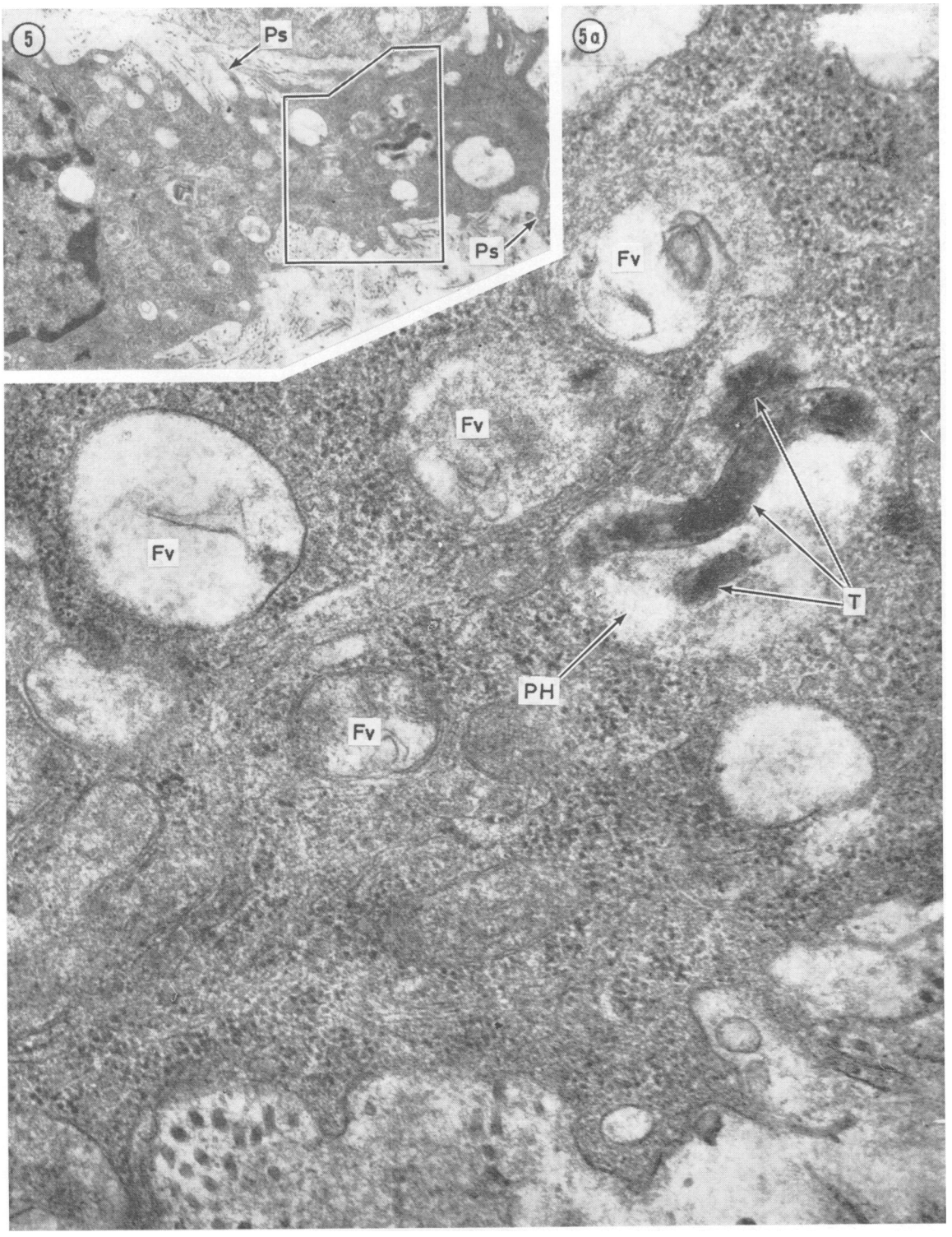
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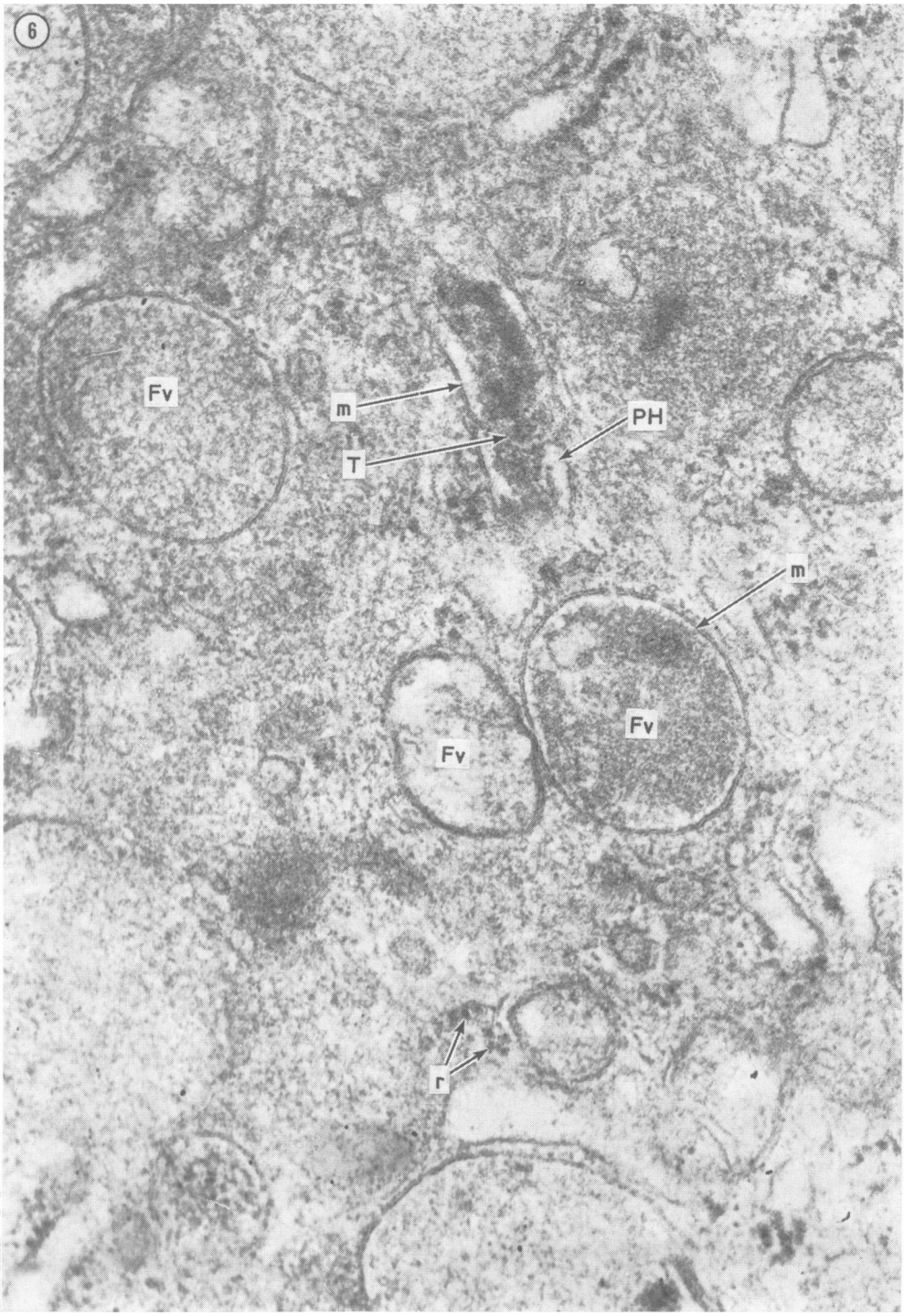
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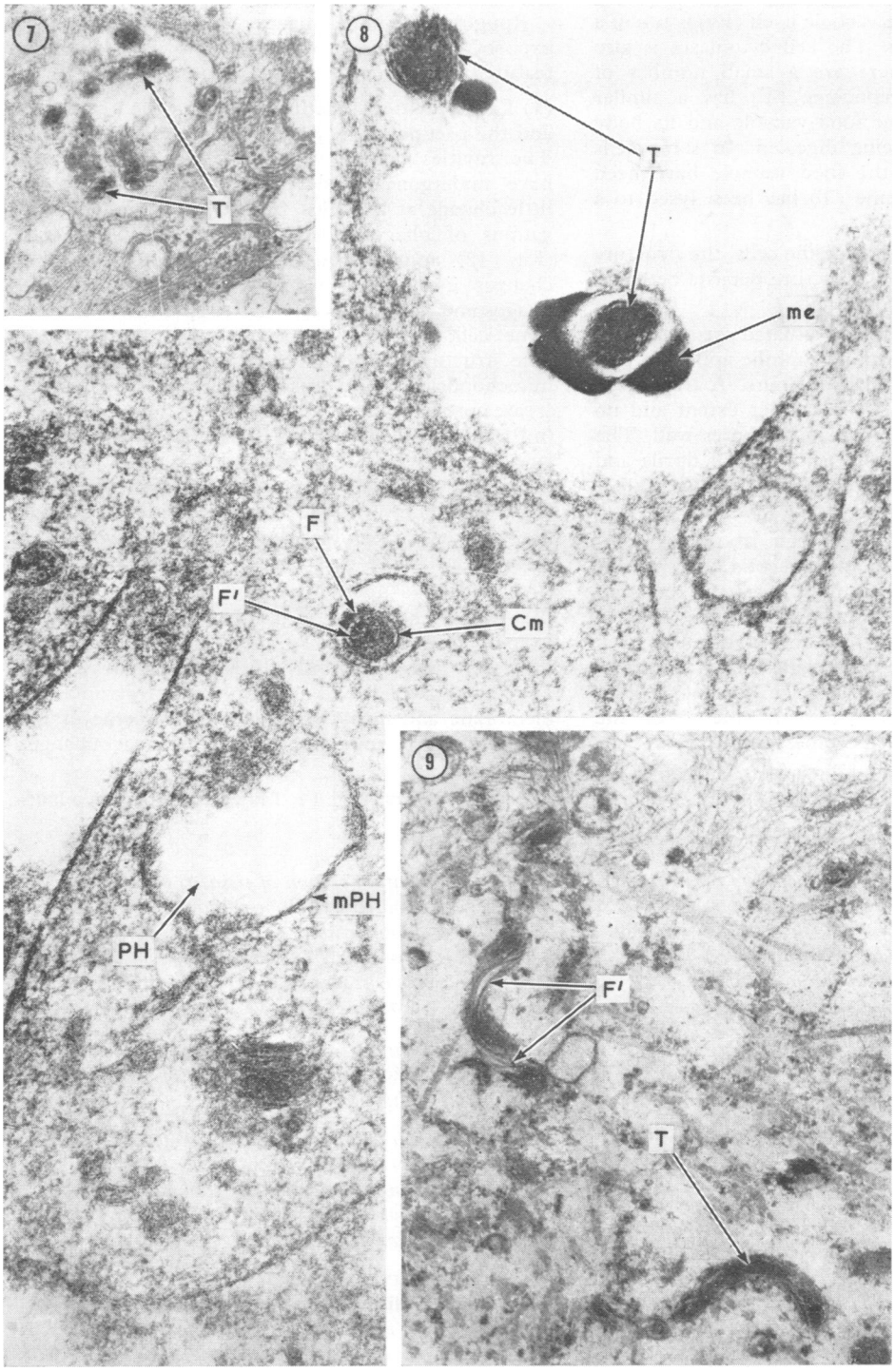
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dense layers with one electron transparent layer between them, while the vacuole itself (Fv) is full of a fine granular substance. The cell cytoplasm is also finely granular and there are a small number of ribosomes (r). The treponeme (T) has a similar membrane to that of the food vacuole and its body is in the process of being digested. In some cells (Fig. 7), the walls of the food vacuole have been destroyed. The treponeme (T) has been lysed to a considerable extent.

(4) In some treponemes inside the cells, the structure is preserved. Treponemal structure persists better in plasmocytes and endothelial cells.

(5) In some of the treponemes situated extracellularly there is a marked uniform osmiophilic appearance of the outer wall (Fig. 8) after 6 hours. A treponeme inside a cell is changed to a smaller extent and no osmiophilic area can be seen in the outer wall. The superficial (F) and deep (F') bundles of fibrils and the cytoplasmic membrane (Cm) are clearly visible.

(6) After longer intervals (18 hours—Fig. 9), the extracellular treponemes lose their ribosomes completely and the cytoplasm appears solid and smooth; part of the cytoplasmic membrane can be seen and the fibrils show up better than anything else (Fig. 9). It must be assumed that these treponemes had been in a cell which has been destroyed. This is shown by the considerable number of elements of cellular origin around the treponeme: fragments of the endoplasmic reticulum, ribosomes, etc.

It was not possible to follow all the changes in treponemes caused by the penicillin, because of their small number and the consequent difficulty in finding them under the electron microscope.

Roughly the same changes occur in treponemes exposed to bicillin-1, with the following additional features:

(1) Large cavities sometimes occur in the histiocytes and the pseudopodia (villi) are directed inside them. The cavities contain treponemes, some of which have undergone considerable changes and others little change at all (Figs 10, 10a, 11). Some aggregations of phagocytosed treponemes can be seen (Fig. 12) and they have undergone considerable changes even after 3 hours, particularly in the phagosome (PH). Occasional treponemes in the same cell, however, are considerably less altered. The structure of the cell itself is also changed. The mitochondria (M) are broken down and the ribosomes (r) are not clearly outlined. The phagosome membrane (mPH) is two-layered and well-defined. In places, large aggregations of treponemes can be seen in the chancre 24 hours after administration of bicillin (Fig. 13). They may be situated inside or outside the cell and some of them have preserved their morphology quite well, whereas others are in a stage of advanced lysis.

(2) After 48 hours, the cells themselves took on the form of an extremely fine granular mass (G) in which there were treponemes in a half-destroyed state (*cf.* Fig. 14), although a few had a well-preserved cytoplasmic membrane and fibrils. In this process, some of the chancre cells also apparently undergo extensive changes and die.

(3) After 3 hours (Figs 14, 14a), treponemes in a half-destroyed

FIGS 10, 10a, *and* 11 *Macrophage taken from rabbit chancre 3 hours after administration of* 84,000 *units bicillin-1. Fig.* 10 × 4,000, *Fig.* 10a × 33,000, *Fig.* 11 × 16,500. *Abundance of pseudopodia (Ps), food vacuoles (Fv), and lysosomes (L). N = nucleus. There is a large clear cavity (phagosome?), towards the interior of which numerous pseudopodia are directed (Ps), and treponemes are also to be found there in different stages of preservation (T). The fibrils are clearly visible (F). Mitochondria (M) food vacuoles (Fv), and endoplasmic reticulum (Er) are also present; r = ribosomes*

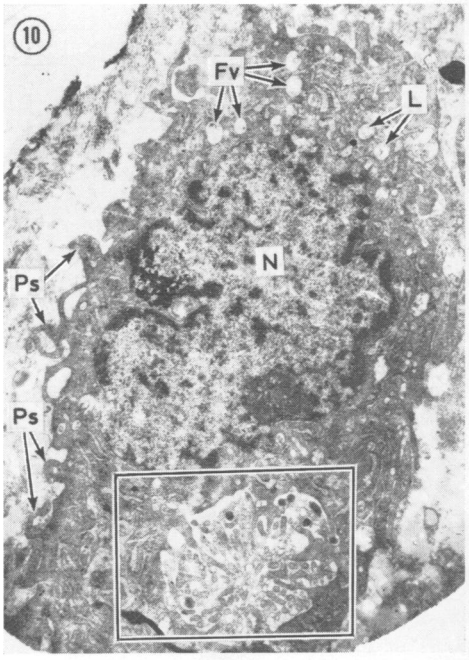
FIG. 12 *Ultrathin section of chancre tissue taken from a rabbit 3 hours after administration of* 84,000 *units bicillin-1. Treponeme (T) inside phagosome (PH) almost lysed, but some treponemes still preserved; mPH = phagosome membrane. The mitochondria (M) have been broken down; r = ribosomes.* × 48,000

FIG. 13 *Ultrathin section of tissue from a rabbit chancre 60 days after infection. Material taken 24 hours after administration of* 84,000 *units bicillin-1. Numerous treponemes (T) situated inside and outside the cells in various stages of preservation. Fv = food vacuoles.* × 14,000

FIG. 14 *Ultrathin section of material taken from a chancre 3 hours after administration of bicillin-1. Ps = pseudopodia.* × 6,000

Fig. 14a *shows a polynuclear with a treponeme inside the phagosome in a state of lysis, nuclei (N) of the polynuclear, granular area on the polynuclear (G), a treponeme (T), and a phagosome (PH); mPH = phagosome membrane.* × 18,000

FIG. 15 *Ultrathin section of chancre material.* Treponema pallidum *round blood vessels. Treponeme (T), erythrocyte (E), polynuclear (PL).* × 4,500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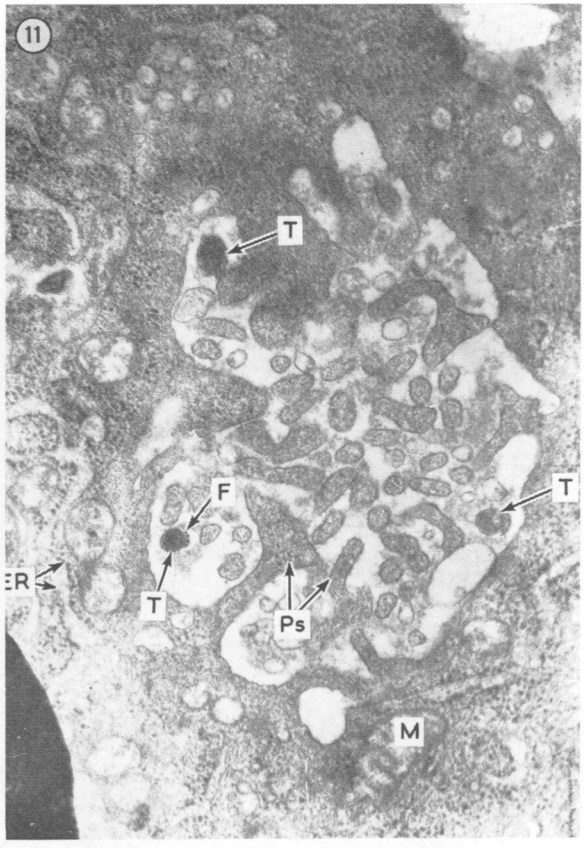
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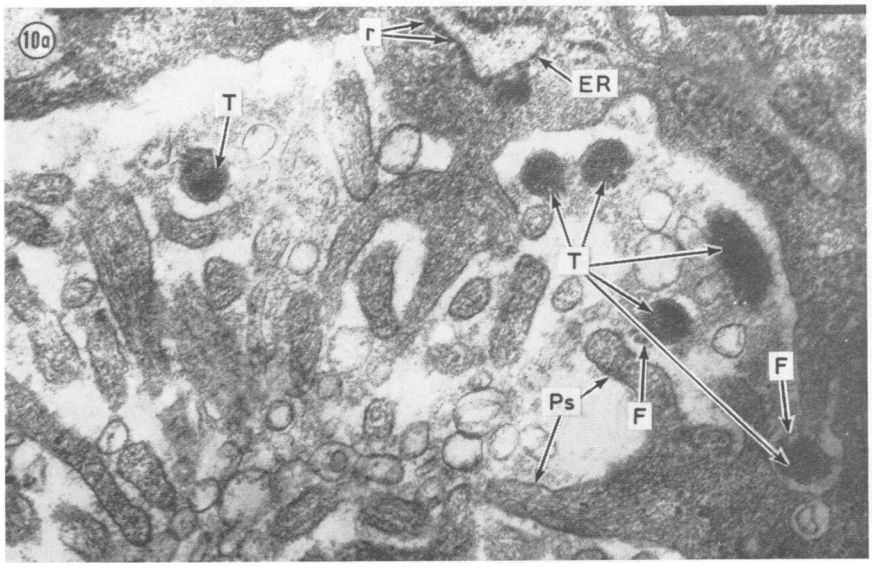
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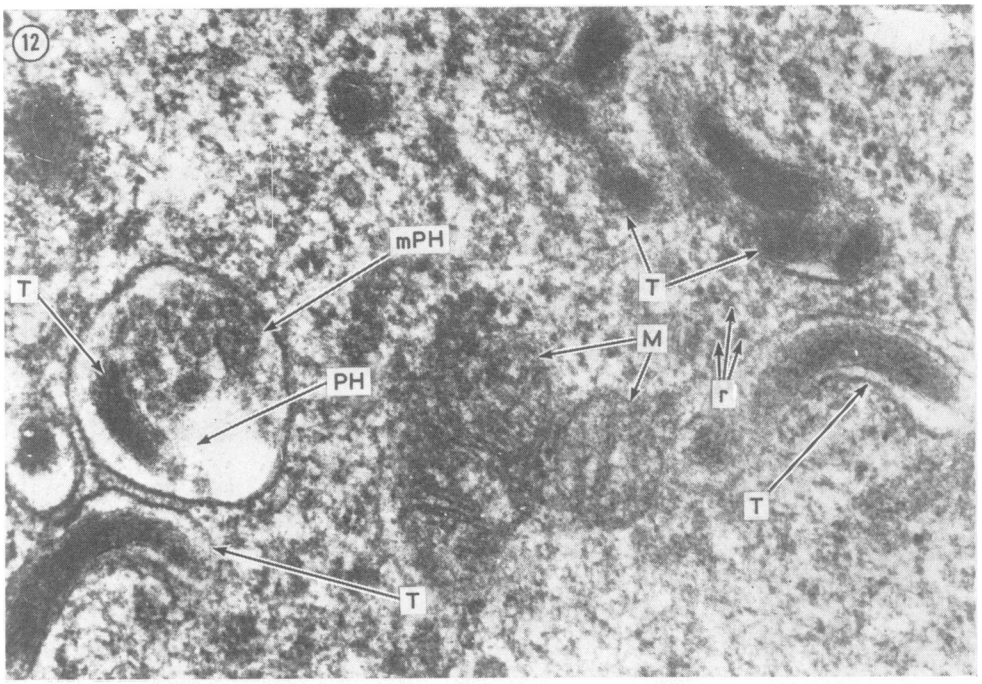
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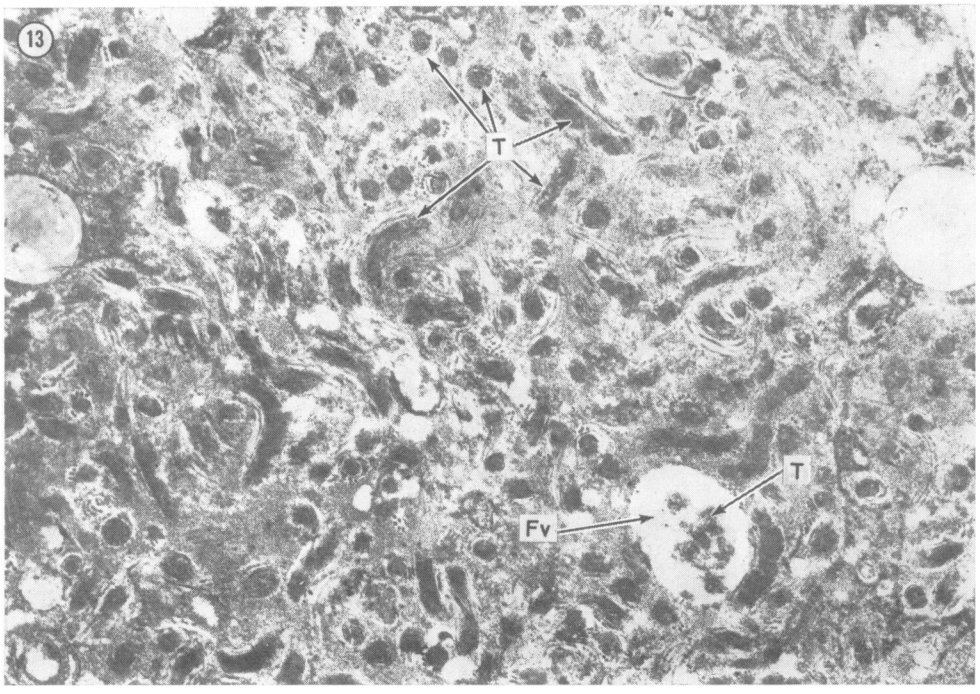
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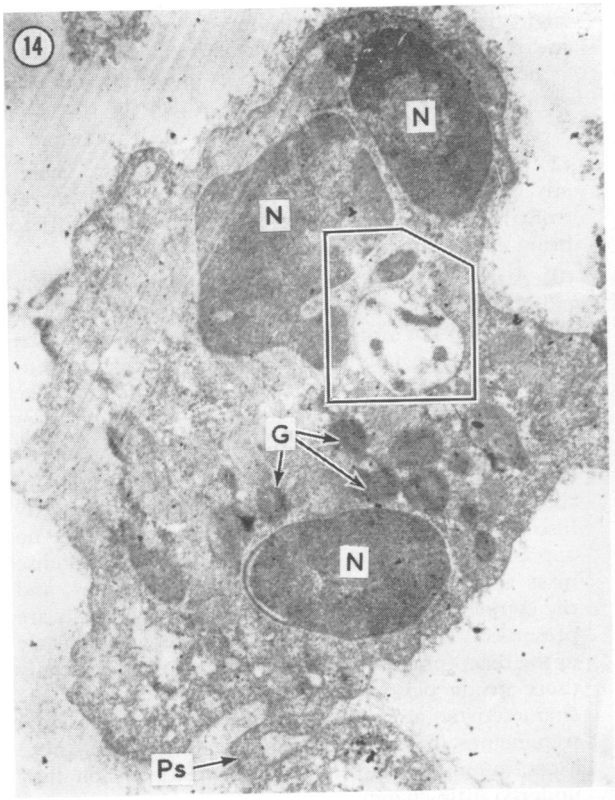
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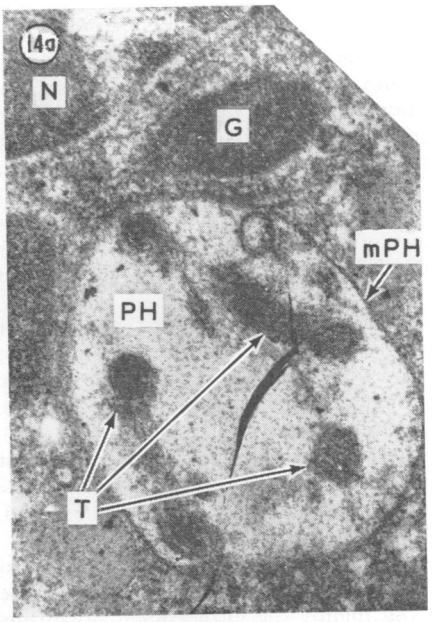
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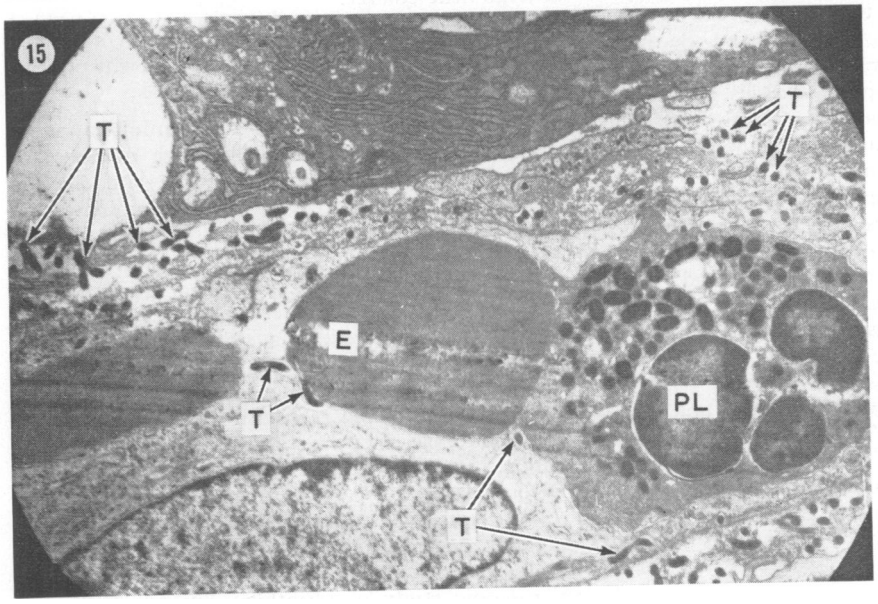
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state can be seen inside the phagosomes in polynuclears, but there is no certainty that these treponemes were phagocytosed by the polynuclear when they were alive and not when they were already altered or killed by the penicillin.

In the first few hours after the administration of penicillin and bicillin, the morphological changes in the treponemes did not differ appreciably. The outcome of the struggle depends not on high concentrations of penicillin killing the treponemes direct but on a combination of a weakening of the vital capacities of the treponemes following exposure to penicillin and an enhancement of the phagocytic capacity of the body cells. Penicillin makes the treponeme susceptible to the effect of specific and non-specific immune factors and, at the same time, in small doses, activates the phagocytic properties of the cellular elements.

It should be noted that in the first 3 hours the treponemes were found in considerable numbers round the blood vessels, in the vascular walls or in the lumen itself, in cells surrounding the blood vessels, and in the expanded intercellular spaces (Fig. 15). Later it is chiefly the extracellular treponemes that disappear, whilst the autophagic processes of the cellular elements in the hard chancre are intensified. The amount of collagen also decreases.

It must be assumed that after exposure to penicillin the bulk of the treponemes die, but that some of the extracellular, but mainly the intracellular, organisms remain alive, protected by their casing, the body's reaction to such treponemes being reduced. Because the treponemes are in the walls of the blood vessels or in the lumen of the lymphatic vessels, and have in some cases penetrated into the lumina of the capillaries, it may be assumed that they can move along lymph and blood pathways to distant organs and can there exist for a very long time inside the plasmocytes and lymphocytes, which are particularly long-lived. We are not yet fully convinced that these treponemes are alive, as even when dead they may persist in the cells for a long period. Since the plasmocytes are the main cells in which antibodies are formed, they may produce varying quantities of antibodies, particularly those determined by the *Treponema pallidum* immobilization test and the fluorescent antibody test. This may explain the persistently positive results obtained in serological reactions, despite the administration of penicillin and the absence of clinical manifestations.

Conclusions

(1) After a single administration of crystalline penicillin in a dosage of 84,000 units per kg., very high blood levels are produced but the rabbits are not cured. Transfer of chancres from 3 to 48 hours after the administration of penicillin induces syphilis in the healthy rabbits to which the transfers are made.

(2) Bicillin-1, administered in the same dose, generally cures the rabbits, and the transfer of chancres from bicillin-treated rabbits to healthy ones fails to bring about infections.

(3) After the administration of both types of penicillin, electron microscope examination of ultrathin sections of fragments of chancres from treated rabbits reveals heightened cellular activity, particularly in the macrophages. The number of pseudopodia, lysosomes, food vacuoles, and mitochondria increases and phagocytic activity is enhanced.

(4) In the first 3 hours after the administration of the preparations, the extracellular treponemes partly lose their outer wall, but the cytoplasm shows no appreciable change. After 6 hours, an electron dense mass appears around some of the treponemes, and the cytoplasm shows signs of lysis, but the fibrils are preserved. The intracellular treponemes, particularly in the macrophages, are lysed, but in some, especially those inside plasma cells and endothelial cells, the characteristic morphology is preserved. Occasional treponemes, both inside and outside the cells, have a continuous two-layered casing, inside which they undergo little change.

(5) It may be assumed that the preservation of treponemes in the plasma cells may be the reason why positive serological reactions persist for so long.

(6) In the first 6 hours after the administration of penicillin, there are particularly large numbers of treponemes in the pericapillary zone and the organisms are seen to penetrate into the lumina of the capillaries.

Summary

The therapeutic effect of crystalline penicillin and bicillin-1 administered in a single dose of 84,000 units/kg. was tested experimentally in rabbits, with subsequent transfer of chancre fragments and lymph nodes from the treated rabbits to healthy rabbits. At various intervals after the administration of penicillin, chancre fragments were examined under the electron microscope.

It was established that a single dose of crystalline penicillin does not cure rabbits of syphilis, but that bicillin-1 in the same dose does do so. Electron microscopic study of ultrathin sections of chancre fragments showed enhanced cellular activity, particularly in the macrophages. In the first 3 hours after

the administration of the preparations, extracellular treponemes partly lose their outer wall, and 6 hours later, the cytoplasm shows signs of lysis. The intracellular treponemes, particularly in the macrophages, are at different stages of lysis. In some of the treponemes, particularly those in plasma cells and endothelial cells, the structure is well preserved.

In the first 6 hours after administration of penicillin, particularly large numbers of treponemes are found in the pericapillary zone.

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Effet de la pénicilline crystalline et de la bicilline-1 sur la syphilis expérimentale du lapin
Etude expérimentale au microscope électronique

SOMMAIRE

L'effet thérapeutique de la pénicilline crystalline et de la bicilline-1 donnée à la dose unique de 84.000 U/kg. a été étudiée expérimentalement chez le lapin par la méthode des transferts des fragments de chancre et des ganglions lymphatiques des lapins traités à des lapins sains. A intervalles variés après administration de la pénicilline, des fragments de chancres furent examinés au microscope électronique.

On put établir qu'une dose unique de pénicilline ne guérit pas la syphilis chez le lapin alors que la même dose de bicilline-1 y parvient. L'étude en microscopie électronique de sections ultrafines de fragments de chancres montre une augmentation de l'activité cellulaire, en particulier pour les macrophages. Dans les 3 premières heures après l'administration des médicaments, les tréponèmes extra-cellulaires perdent partiellement leur membrane extérieure et, 6 heures après, le cytoplasme présente des signes de lyse. Les tréponèmes intra-cellulaires, spécialement dans les macrophages, se montrent à des stades différents de lyse. Pour quelques uns des tréponèmes, en particulier pour ceux qui sont dans les plasmocytes ou dans les cellules endothéliales, la structure est bien conservée.

Dans les 6 premières heures après l'administration de pénicilline, un nombre très élevé de tréponèmes se trouve rassemblé autour des capillaires.

KEY TO THE FIGURES

C	cytoplasm
Cm	cytoplasmic membrane
E	erythrocyte
ER	endoplasmic reticulum
F	fibril
F′	deep-lying fibril
Fv	food vacuole
G	granular area
KL	collagen fibres
L	lysosome
M	mitochondria
m	cell membrane
me	outer wall of treponeme
mPH	membrane of phagosome
N	nucleus
PH	phagosome
PL	polynuclear
Ps	pseudopodia
r	ribosome
T	Tp
T′	longitudinal section Tp