STUDIES ON THE MECHANISM OF IMMUNITY IN TYPHUS FEVER*

I. Rickettsia prowazeki in the Different Stages of the Typhus Lesion

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Plates 38 to 40

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The demonstration of histopathological changes in typhus fever was first presented by Fraenkel who, in 1914, described microscopic lesions in the skin of typhus patients. Prowazek, Fraenkel, Otto and Dietrich, Spilmeyer, and others found lesions in the central nervous system and in other organs. Such lesions were extensively studied by Wolbach, Todd, and Palfrey, whose work is a classic on the subject (1).

The fundamental picture of a typhus lesion, regardless of its location, consists in a cellular infiltration around blood vessels whose endothelium has been damaged. Such damage depends on the actual multiplication of the typhus virus in the cells of the endothelium of the affected vessel.

The finding of Rickettsia prowazeki in the typhus lesion has been a matter of considerable difficulty. After the work of Prowazek and da Rocha-Lima (1916) it was considered necessary to find intracellular organisms in mammals, similar in appearance to those found in the cells of the infected louse.

The first direct demonstration of Rickettsiae in the cells of typhus infected men and guinea pigs was presented by Wolbach and co-workers (1920–22). They showed that the swollen cells of the endothelium of small vessels contained numerous organisms morphologically resembling the intracellular Rickettsiae of von Prowazek and

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da Rocha-Lima. A second important demonstration was presented by Mooser (2), who found, in the tunica vaginalis of guinea pigs infected with Mexican typhus, cells of mesothelial origin parasitized by a large number of organisms identical with the Rickettsia prowazeki. Pinkerton (3) duplicated this finding in guinea pigs infected with European typhus. The last mentioned author (4) also found, after considerable search, Rickettsia bodies in the cells of the small vessels of the scrotal sac. From the cytological changes observed by Mooser in the tunica vaginalis of typhus infected guinea pigs (5), Mooser and Dummer (6) inferred that Rickettsiae were digested by polymorphonuclear leukocytes, which in turn were then taken up by the mononuclears. The nodules formed by the accumulation of the latter cells represent according to these authors a healing stage of the typhus lesion.

As a direct application of these findings Zinsser and Castaneda (7) developed a method by which Rickettsiae have been cultivated within mesothelial cells in the peritoneal cavity of rats, from which large quantities of organisms were obtained. The purified suspensions of Rickettsiae have been successfully used in active immunization against typhus fever. With rich vaccines a serum has been prepared in horses which has prophylactic and therapeutic value (8).

The purpose of this paper is to present a systematic study of the lesions produced in the skin of guinea pigs by intradermal and intracardial inoculation of the typhus virus. The position of the Rickettsia bodies in the lesions is followed at the different stages of the tissue reactions.

**Material and Technique**

1. The virus used for inoculum was obtained from the tunica vaginalis of guinea pigs and from the peritoneum of rats infected with Mexican typhus.

The tunica vaginalis of guinea pigs killed on the 7th day of the disease was washed in a 1/10 dilution of guinea pig serum in saline. The washings were centrifuged at low speed for a few minutes and the cell-free supernatant inoculated as soon as possible.

Rats were submitted to short wave X-ray radiations, then inoculated with typhus virus, and were killed on the 5th day after this injection (7). The peritoneal cavity was washed with saline or diluted guinea pig serum. The washings were centrifuged at low speed and the supernatant fluid, usually very rich in Rickettsiae, was diluted before inoculation. A rough titration of the rat inoculum
was made by smearing a standard loop on a measured surface. The fixed smears were stained and the number of organisms counted per oil immersion field.

The inoculum for the intracardial inoculations was prepared in the same manner except for using an isotonic solution of sodium citrate to prevent clotting of the peritoneal exudate.

2. White adult guinea pigs were shaved on the sides and inoculated intradermally with 0.2 cc. of the inoculum. The intracardial injections were made under ether anesthesia.

3. The *Rickettsia* bodies were demonstrated by staining the preparations according to the following methods.

(a) Smears from scrapings of the tissues were treated by the methylene blue-safranin method described elsewhere (9).

(b) The extracellular *Rickettsiae* were readily stained with the following mixture.

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\begin{align*}
\text{Phosphate buffer solution pH 7.6 (same as that used in (a))} & \quad 50 \\
\text{Formalin (40 per cent)} & \quad 2.5 \\
\text{1 per cent alcoholic solution of methyl violet} & \quad 1
\end{align*}
\]

The smears were fixed by heat and stained for 1 to 5 minutes and washed.

(c) Staining sections. The tissues are fixed in Regaud's fluid (one part of formalin to five parts of a solution containing 2.5 per cent potassium dichromate and 1 per cent sodium sulfate) for 24 hours, washed, and imbedded in paraffin in the usual way. Sections cut as thin as possible are stained by a Giemsa solution prepared as follows: To a 2.5 to 5 per cent solution of formalin in distilled water sufficient acetic acid is added to bring the pH to about 5.5. Usually, for neutral formalin, 0.1 cc. of a 1/10 solution of glacial acetic acid is enough for 100 cc. of the solution. The acidity of commercial formalin is sometimes such that no addition of acetic acid is required. Add to 100 cc. of the acidified formalin solution 2 cc. of Giemsa solution and stain the sections for periods of time lasting from 6 to 18 hours. After staining, the sections are rinsed with water and the excess moisture wiped off. They are then rapidly dehydrated with absolute alcohol followed by xylol and mounted in cedar oil.

In our experience, many trials are often necessary in order to obtain good staining solutions.

The acid Giemsa solutions are stable and for this reason one bath is enough for the entire period of staining. This formalinized Giemsa acts, by its acidity, as a restraint upon the tendency of ordinary Giemsa to stain the tissues blue. The formalin also serves as a mordant for the *Rickettsia* bodies which are easily detected in the faintly stained cytoplasm. An excess of acidity increases the red staining with poor results.
The sections stained for 6 hours show *Rickettsia* bodies stained blue. The nuclei of the cells stain blue. Connective and muscle tissues are red and the blood elements retain their ordinary reactions. The granulations of mast cells take a brilliant purple. The longest periods of staining reveal a similar picture but the blue effect becomes deeper and purplish.

If necessary, the sections may be restained, after washing with xylol and alcohol, by a 2 hours immersion in the formalinized Giemsa solution.

*Microscopic Lesions Produced by Intradermal Inoculation of Mexican Typhus Virus*

With the exception of a few guinea pigs which did not react after the intradermal inoculation of tunica washings from typhus guinea pigs, the rest of the animals, treated with guinea pig or rat material, showed reactions at the site of inoculation, which varied in intensity according to the numbers of *Rickettsiae* inoculated. A moderate inoculum, as in the case of guinea pig tunica scrapings, produces an immediate congestive reaction which invariably fades in the next few hours. However, from 24 to 48 hours later the local inflammatory reaction becomes definitely conspicuous doubtless due to its progress, which continues to develop in intensity for the subsequent 2 to 4 days. This is followed by an induration of the skin, which may last for about 2 weeks after the inoculation. In mild cases the lesions may disappear within a week, but in strong reactions the inflamed skin may become ulcerated in the center, which retards the healing of the lesions. The size of the swollen and congested wheals varies from 1 to 2.5 cm. in diameter.

When the inoculum is prepared from typhus infected rats, the reactions are usually of the more intense type and are apparent 24 hours after the inoculation.

The lesions described above have been found in a large number of guinea pigs. Such lesions have not been observed after the inoculation of brain or serum from typhus infected animals.

*General Symptoms.*—In addition to the local reaction at the site of the injection of typhus virus, the guinea pigs show an elevation of temperature which may appear as early as 48 hours after the inocula-
tion. The temperature is usually moderately elevated and maintained for short duration. A few animals had fever which lasted over a week, while others did not show fever at all, particularly those presenting mild skin reactions.

**Microscopic Findings**

The peritoneal washings of typhus infected rats were the inoculum used for the production of the skin lesions described in this study.

White guinea pigs were shaved on the sides and inoculated intradermally in several different places, usually four to six, and the site of cutaneous inoculation was removed at intervals of 24 hours. Two guinea pigs were usually prepared for each series of skin lesions. Part of the removed skin was scraped, smeared, and stained by the methylene blue-safranin method for direct examination. The rest of the skin was fixed in Regaud’s fluid and prepared by the usual methods for subsequent sectioning and staining with the formalinized Giemsa solutions according to the technique already described.

**Results of the Direct Examination.**—The examination of the smears made from scrapings of the removed skin showed few or no *Rickettsiae* 24 to 48 hours after inoculation. After 72 to 96 hours, intracellular and extracellular *Rickettsia* bodies were found in relatively large numbers. Polymorphonuclear leucocytes, some of which contained phagocyted *Rickettsiae*, were also found, an observation frequently seen in smears from the tunica of guinea pigs and the peritoneum of rats infected with typhus. From the 5th day on, the finding of *Rickettsiae* in the scrapings of the skin lesions was more and more difficult until their complete disappearance on about the 8th day.

**Examination of the Sections.**—At the site of the inoculation, the epidermis is interrupted and its place is infiltrated by a considerable number of polymorphonuclear leucocytes. This focal infiltration may be seen as early as 24 hours after the inoculation and it gradually increases in intensity to be subsequently displaced by the usual reparative processes. In other skin lesions studied, no local infiltration appeared in the epidermal region. There is no evidence of *Rickettsia* bodies in the zone of infiltrating polymorphonuclears at the epidermis. These cells appear mostly degenerated.

The corium, subdermis, and usually the muscularis, show various

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1 Under ether anesthesia.
degrees of inflammatory response. The type of reaction in the first 48 hours manifests considerable cytological variation. This may be in the form of a moderately diffuse infiltration by mononuclear phagocytes interspaced by few polymorphonuclear leucocytes or the diffuse infiltration may be characterized by preponderance of polymorphonuclear cells. There is some extravasation of red cells particularly in the subcutis. As early as 24 to 48 hours after the inoculation, there is evidence of swelling of the endothelial lining of the capillaries and small blood vessels, in which not infrequently one may find cells packed with *Rickettsiae*. Around larger vessels, there is a perivascular infiltration of mononuclear phagocytes.

As the lesions progress, the perivascular infiltrations become more intense, with prevalence of mononuclear type of cells. 48 to 72 hours after the inoculation of the virus, the lymphatics may be seen occluded by a delicate reticulum of fibrin containing polymorphonuclear leucocytes. Some lymphatics are simply dilated and show fibrin in the lumina. The blood vessels present changes due to marked swelling of the endothelium and early thrombus formation. The small arteries may be occluded by the swollen and vacuolated cells of the intima.

On the 3rd to 4th day, the infiltration by mononuclear phagocytes is conspicuous in all layers of the skin, particularly in the lower portion of the corium. When the vessels are cut transversely the perivascular infiltration displays nodular formations of various sizes, depending on the caliber of the vessels involved. The walls of the vessels within the nodules may or may not be damaged. When one searches for *Rickettsiae* in such nodules it may be possible to find the organisms within the endothelial cells of a small vein, but it is more frequent to observe the typical Mooser's cells outside the larger vessels. When serial sections are followed, such cells can be traced to the capillaries within the nodular formation.

The best places to look for *Rickettsia* cells is the capillary wall, which is easily followed by a swollen endothelial lining. The cells are usually found packed with *Rickettsia* bodies in the same way as those seen in smears from the tunica vaginalis of typhus infected guinea pigs as described by Mooser. There is no cellular reaction around such cells at this stage. Amidst the dense mononuclear phagocytic infiltration of the subdermis, cells containing *Rickettsiae*
are frequently found, also small vessels with their lumen occluded by a swollen cell filled with *Rickettsiae*.

The criteria for the diagnosis of *Rickettsiae* in our sections have consisted either in the finding of typical Mooser's cells or the cells of blood vessels showing the organisms sufficiently clearly to exclude the possibility of confusion with granular material of cellular origin.

We have been unable to obtain any evidence of *Rickettsia* bodies in arteries and lymphatic vessels.

It is interesting to note that one frequently finds veins occluded to variable degrees by mural thrombi. Such thrombi are formed by fibrin lined by a layer of endothelium. The search for *Rickettsiae* in such veins has not been successful.

On the 4th and particularly on and after the 5th day subsequent to inoculation there are recurrences of polymorphonuclear infiltration. Here and there one may see small foci of these cells on the 4th day. When observed under oil immersion, one frequently finds such foci adjacent to swollen cells of the capillaries.

Some of the infiltrations around small vessels may show a prevalence of polymorphonuclear leucocytes or may be entirely formed by these cells. In such cases the vessels are found greatly degenerated.

Interspaced areas of polymorphonuclear leucocytes of considerable size may be found within the mononuclear phagocytic infiltration of the subdermis. (Sometimes one may find veins surrounded by mononuclear phagocytes showing within their lumina a number of polymorphonuclears adhering to the walls of the vessels.)

In searching for *Rickettsiae*, the organisms are more frequently found in places with little or no polymorphonuclear infiltration. With the increase in the polymorphonuclears there is diminution in the numbers of *Rickettsia*-containing cells. When a nodular formation shows an abscess-like appearance *Rickettsiae* are seldom found.

After the 5th day, the mononuclear phagocytic infiltration again predominates and remains as such thereafter. It is difficult to follow the evolution of the nodular formations. However, in view of the considerable phagocytosis of polymorphonuclear leucocytes by macrophages, one may infer that the polymorphonuclears are partly disposed of by phagocytosis and some are returned to the circulation by way of the lymphatics which, as was pointed out, are found to be filled with cells as early as the 4th day after the inoculation.
Intracardial Inoculation of Mexican Typhus Virus into Guinea Pigs

The inoculation of large doses of extracellular *Rickettsiae* by intracardial route has produced the following results. The removal of fragments of skin at daily intervals, as well as the study of the organs at various intervals after inoculation has given relatively few positive findings. On the 3rd day after inoculation it was found, in one animal, that a fragment of the skin contained capillaries with swollen cells filled with *Rickettsia* bodies. The involved vessels were found immediately under the epidermis. In the same animal a small vein was also found in the subdermis that contained *Rickettsiae* within its endothelial cells. No cellular infiltration was noticed around or near such vessels. In another animal, which was killed on the 3rd day, a number of infected cells was found in the veins of the polar fat of the testis. The organisms were rather large and there was some infiltration by mononuclear phagocytes and polymorphonuclears in the intervascular spaces.

Up to the present time we have not made a more intensive search for *Rickettsiae* in the organs of typhus infected guinea pigs, but according to the results obtained in the few animals thus far studied, the detection of *Rickettsiae* outside of the tunica, peritoneum, and local skin lesions, is a difficult task.

**SUMMARY**

This study of the lesions produced in the skin of guinea pigs inoculated intracutaneously with Mexican typhus virus, shows that there is an early polymorphonuclear response at the point of inoculation. As early as 24 hours after the virus is given, a mononuclear phagocytic infiltration, which is more pronounced around the larger vessels of the corium, vascularis, and muscularis, replaces the polymorphonuclear infiltration. The endothelial cells of capillaries and small vessels swell up, thus partially occluding the lumina. *Rickettsia* bodies are found in the swollen cells, in numbers which remind one of the intracellular *Rickettsiae* of the tunica in typhus infected guinea pigs. The mature Mooser's cells are found in abundance on the 3rd to the 4th day after inoculation. They are found in various positions as follows: (a) in the endothelial cells of capillaries, particularly in places of little or no infiltration; (b) within the mononuclear nodules.
formed around the larger vessels and within the dense infiltration of the vascularis (the parasitized cells are usually traced to a capillary wall); and (c) less frequently the organisms are found within the swollen cells of arterioles and small veins. The organisms disappear gradually from the zones of increasing polymorphonuclear infiltrations, suggesting that the presence of such polymorphonuclears is due to the bursting of the infected cells.

In the artificial lesions produced in the skin by the inoculation of considerable numbers of *Rickettsiae*, the tissue reactions are abnormally enhanced. One can see in the same slide different stages of the development of the lesions and their relationship to the infecting agent. The early perivascular infiltration by mononuclear phagocytes does not seem to be related to an actual infection of the endothelial lining by the inoculated virus, but seems rather, when properly controlled, to be primarily due to a nonspecific type of response. The capillaries or small vessels within these infiltrated zones may become parasitized and call forth a polymorphonuclear reaction which may thus transform the cytological picture of the nodule. The subsequent migration of macrophages terminates the histological sequence.

In capillaries apart from areas of cellular infiltrations, the polymorphonuclear reaction is first to appear, when the *Rickettsiae* are liberated from the cells.

One cannot safely generalize from the results observed in an artificial typhus lesion, but in the light of these observations, it is probable that the *Rickettsia* bodies are difficult to find in typhus patients or infected animals because they disappear rapidly from the nodules, or perhaps because some nodules are not necessarily related to an infected endothelium. At any rate, a late typhus lesion is not likely to reveal *Rickettsiae* which most probably have been removed by the early polymorphonuclear invasion.

CONCLUSIONS

The Mexican typhus virus is capable of producing a local inflammatory reaction when injected intradermally into guinea pigs.

*Rickettsia* bodies are easily found in the skin lesion, particularly in the walls of capillaries, in places of little or no cellular reaction, in the early stages of the disease.
The Rickettsia bodies are less frequently seen in places of increasing polymorphonuclear infiltrations.
In the mononuclear phagocytic nodules, characteristic of the typhus lesion, Rickettsiae are rarely found. This may perhaps be due to an early destruction by polymorphonuclear phagocytes.

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Note.—After this paper had been prepared for publication, there appeared an article by Baltazard, M., Bull. Soc. path. exot., 1936, 29, 403. The author observed skin lesions following intradermic injections with murine typhus virus.

BIBLIOGRAPHY

EXPLANATION OF PLATES
Formalinized Giemsa was used to stain all sections.

PLATE 38

FIG. 1. Rickettsiae in a capillary from the dermis of a guinea pig 72 hours after the intracardial injection of Mexican typhus virus. Notice the absence of cellular infiltration. ×2000.

FIG. 2. Bursting Mooser cell in a capillary. Local skin lesion 4 days after intradermal inoculation with Mexican typhus. ×2000.


PLATE 39

The photomicrographs of Figs. 4 to 6 and those from Figs. 7 and 8 were made from sections of the skin lesions produced by intradermal inoculation of Mexican virus.
Fig. 4. Perivascular infiltration by mononuclear phagocytes on the 4th day. Capillaries containing Rickettsia bodies are marked with arrows at the periphery of the infiltration. ×600.

Fig. 5. Nodule on the 5th day. Notice increase in polymorphonuclear leukocytes. The small vessel (arrow) is swollen and occluded. No Rickettsiae were found. ×400.

Fig. 6. Nodule on the 6th day. Notice the damage of the blood vessels and cellular elements. There is phagocytosis of polymorphonuclear leukocytes by macrophages. ×400.

Plate 40

Fig. 7. Mononuclear phagocytic nodules in the subdermis. 10th day lesion. No Rickettsiae were found. Low power magnification.

Fig. 8. 4th day lesion. Dense infiltration by mononuclear phagocytes and polymorphonuclears. Notice large vein with mural thrombus of fibrin covered by proliferating endothelium. No Rickettsiae were found in the cells of this vein.
(Castaneda: Mechanism of immunity in typhus fever. 1)
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