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Journal of the Royal Army Medical Corps.

Original Communications.

TYPHUS FEVER IN IRAN AND IRAQ, 1942-43
A REPORT ON 2,859 CASES.

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(D) PATHOLOGICAL ASPECTS.

(1) Morbid Anatomy.

Post-mortem examinations were carried out on nearly all fatal military cases, but were not permissible on Iranian civilians.

The post-mortem changes are indeterminate and it is not possible to make a diagnosis on the macroscopic appearances alone. Findings vary with the duration of disease prior to
death, i.e. whether the patient died during the pyrexial period or convalescence. In the former the changes are those associated with acute toxemia. There are, however, certain findings which may suggest a diagnosis of typhus fever in the presence of an epidemic.

The lungs are haemorrhagic, oedematous and exude a blood-stained frothy fluid. Varying degrees of collapse or consolidation may be found. The air passages are congested and pink in colour. Haemorrhagic patches are sometimes present in the walls of the trachea and bronchi.

It would appear that there is a particular liability to haemorrhages. Petechial and larger haemorrhages occur, not only in the skin but in different organs, and are also found below the serous membranes. Subserous haemorrhages into the wall of the cæcum and subepicardial haemorrhages are fairly constant findings.

Acute abdominal symptoms were present in three out of a series of 60 cases. 2 of these were operated on—one for acute appendicitis two weeks prior to his death; the other survived the exploratory operation. In the fatal case no evidence of healing of the wound was found either macroscopically or microscopically. The findings in two fatal cases with symptoms of "acute abdomen" were not unlike those associated with Henoch's purpura. Both had massive subepicardial haemorrhages and haemorrhages into the wall of the cæcum.

In some cases, particularly dark-skinned individuals, the rash appeared more obvious after death.

When death has occurred during convalescence, the findings are those usually associated with cardiac failure—the heart muscle being thin and flabby.

(2) Morbid Histology.

The histological changes found are described in some detail, as only scanty information is given in the standard textbooks of pathology consulted, and some new material is included.

The microscopic changes are more definite than the macroscopic, but even these are liable to variation in different cases. It would appear that this apparent variation does in reality represent progressive stages in the disease. The description which is given is based on the study of sections stained with hæmatoxylin and eosin and Giemsa's stain after either formalin or Zenker's fixation.

As emphasized by different workers, and by Wolbach et al. (1922) in particular, the essential and characteristic change found microscopically in typhus fever is a proliferative lesion of the endothelium of the blood-vessels. This observation has been confirmed in Iran and Iraq by histological examinations made from cases dying on various days of the disease.

(a) Vascular Changes (see Plate I, figs. 1, 2 and 4).—The characteristic vascular changes are best seen in the small blood-vessels of the mid-brain, particularly in the region of the upper pons, but they may equally readily be studied in the skin, heart, kidney or other organs.

The primary change is a proliferation of the endothelial cells lining the vessels. This proliferation may affect the whole circumference of the vessel or be a small papilliform outgrowth of endothelial cells into the lumen. Associated with the proliferation is a swelling of the endothelial cells around the lumen, each cell becoming rounded and hyperplastic instead of having the normal flattened shape. In some vessels the lumen is almost completely obliterated by a uniform proliferation of the endothelial cells and in the finest capillaries occlusion is complete. This partial blocking combined with the disturbance of the endothelial cells results in thrombosis which leads to complete occlusion of the vessel. If the lumen is incompletely thrombosed, the hyperplastic endothelial cells sometimes appear to be covered with a fibrinous exudate which prevents contact with the circulating blood. These findings are characteristic of the vascular changes found in cases dying early in the disease.

Should the patient survive into the second and third weeks before death, characteristic nodules described by Wolbach et al. (1922) are found in the brain, heart and kidneys. The nodules when examined under the high power are found to consist of perivascular accumulations of mononuclear and occasionally polymorphonuclear cells. It is believed that these mononuclear cells are chiefly derived from the adventitia and periadventitial elements. As far as can be ascertained these nodules do not appear until after the vascular changes described
above have occurred. The typical Wolbach nodules are tubercle-like collections of cells surrounding an apparently necrotic centre, but which is in reality the lumen of an occluded vessel. They stain rather more intensely than the surrounding tissue.

(b) The Central Nervous System (see Plates I and II, figs. 3, 4, 6 and 8).—The cerebral cortex, hypothalamus, thalamus, pons, medulla and the cerebellum have been examined and show the typical vascular changes. Thrombosis occurs very frequently in the smaller arteries and capillaries.

In addition to the typical Wolbach nodules found scattered throughout the brain and associated with the vascular lesion, other nodules have been seen which, by means of serial sections, have been shown to have no apparent connexion with the vascular system. One possible explanation is that some may represent minute areas of ischaemic necrosis and are probably collections of neuroglial or microglial cells.

It is well known that collections of mononuclear cells in the brain substance are not peculiar to typhus fever, but may be found in any type of encephalitis due to virus and some spirochaetal infections. In these diseases the usual vascular change is perivascular cuffing with lymphocytes and plasma cells but without occlusion of the vessels by endothelial hyperplasia.

Another change noted is degeneration of the neuron cells. This is shown by chromatolysis, loss of nuclear structure and rounding off of the cell contour.

It is interesting to note that in some cases dying late in the disease the only change found is the presence of the nodules. The vascular endothelium appears to have recovered from the hyperplasia and the cells have resumed their normal flattened shape.

(c) Heart (see Plate I, figs. 1 and 2).—Generally there is a diffuse well-marked infiltration by mononuclear cells between the muscle fibres of the myocardium. Small areas of necrosis have been observed, and varying degrees of toxic changes are also present in the muscle fibres.

But the most characteristic finding is the appearance of nodules between the muscle bundles. These at first sight under the low power appear to resemble Aschoff nodes. On examination with the high power, these are seen to be typical perivascular collections of cells and form part of the general infiltration of the whole musculature.

(d) Lungs (see Plate II, fig. 9).—The earliest change found is œdema and congestion of the alveolar walls. Varying degrees of pneumonitis and bronchopneumonia are always present. An infiltration of the alveolar walls with the same type of mononuclear cells found in other organs is present, but there does not appear to be the same degree of endothelial proliferation or obstruction to the blood flow which is found elsewhere. Thrombosis is sometimes present in many of the smaller arterioles and in the perialveolar capillaries.

(e) Liver (see Plate II, fig. 10).—The findings have by no means been constant. The portal canals have a marked cellular infiltration and their arterioles show signs of endothelial hyperplasia, but typical nodules have not been observed. Generally the liver cells show some degeneration, but surprisingly little in view of the vascular changes. Focal necrosis mainly in relation to the central vessels has been observed.

Hyaline material has been seen in the sinusoids. But, when present, the most striking finding is the reaction of the Kupffer cells. These are swollen and lie free in the sinusoids. Occasionally they are in mitosis and sometimes contain débris or cells.

(f) Kidneys (see Plate I, figs. 5 and 7).—Glomeruli: The earliest change observed is proliferation of the endothelial cells of the afferent vessels to the glomeruli. This proliferation then appears to extend into the tufts which show a marked cellular hyperplasia. In the later stages the glomerular capillaries contain hyaline material which is suggestive of intraglomerular thrombosis.

Perivascular accumulations of mononuclear cells and some polymorphonuclear leucocytes are generally related to the glomerular vessels.

Tubules: There is much destruction of the cells of the convoluted tubules; in many of which the lumen contains an acidophilic-staining fibrinous exudate and desquamated tubule cells. Perivascular collections of cells are related to the intertubular capillaries and are most
numerous in the medulla. The intertubular blood-vessels are obliterated by endothelial proliferation. Mononuclear cells then appear to migrate and, in addition to the perivascular accumulations, invade one or several tubules surrounding the vessels, giving rise to fairly large collections of cells which stain more intensely than the surrounding tissues.

(g) The Skin.—Lesions in the skin are present early in the disease. The capillaries show marked endothelial proliferation and perivascular collections of mononuclear cells. Thromboses are present.

In the other organs and the endocrine system, some degree of vascular proliferation similar to that already described and the changes associated with toxæmia are present. Some cases show the maximum changes in the internal organs like the spleen and heart, with the presence of only slight changes in the brain-stem. But in the majority of these cases changes in the pontine region predominate. Another feature well shown in the cases that have been collected is that the histological changes precede the existence of a positive Weil-Felix reaction.

(3) Demonstration of Rickettsiae in Human Tissues

(see Plates II and III, figs. 11, 12, 13 and 14).

Gram's, Macchiavello's, Castanedo's, Giemsa's and Leishman's stain were tried in Paiforce. The most successful results were obtained with Giemsa. Since then I have had the opportunity of examining the same material and have obtained satisfactory results by using a slight modification of Wolbach's (1922) technique for Giemsa stain.

Details of the technique used are as follows:

1. It is essential for tissues to be fixed in Zenker's fluid for twenty-four hours. Should tissues be embedded without prior fixation in Zenker's fluid, cut sections should be post-fixed for an hour in this fluid. It is also important to wash the section in ether prior to staining.
2. Sections 4 µ in thickness are cut from tissues embedded in paraffin.
3. Xylol ➔ Ether ➔ Alcohol (various dilutions).
4. The corrosive sublimate is removed by washing with Gram's iodine for five minutes, after which the section is washed with 0·5% sodium thiosulphate for ten to fifteen minutes to remove the iodine.
5. Sections are washed well in running water for ten minutes, followed by further washing in distilled water.
6. Stain for twelve to eighteen hours in alkaline Giemsa solution prepared as detailed below:

<table>
<thead>
<tr>
<th>Distilled water</th>
<th>100 c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0·8% soda bicarbonate</td>
<td>2 to 4 drops</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>3 c.c.</td>
</tr>
<tr>
<td>Giemsa stain</td>
<td>2·5 c.c.</td>
</tr>
</tbody>
</table>

7. Differentiate in acetone, controlling under the microscope.
8. Dehydrate, clear, and mount in cedarwood oil.

Using this technique the globular masses of rickettsiae stain a purplish colour, and can be differentiated from the acidophilic and basophilic-staining granules present in leucocytes of the granulocyte series.

Rickettsial smears made from the vaccine and infected yolk sac of developing chick embryos obtained from Van Rooyen have been used as controls. Globular masses of coccoid bodies morphologically resembling rickettsiae have been demonstrated in the swollen endothelial cells of the smaller blood-vessels of the kidney, liver, brain, heart, alveolar walls of the lungs, and in skin clips.

Pairs or short chains and occasionally bipolar-staining organisms were also present.

In addition to rickettsiae, blue-staining inclusion bodies in cells have sometimes been seen in the endothelial cells of blood-vessels in human cases. Their significance is not known.

The importance of control fixation and staining of normal tissues has been very evident as organisms found in the brain and other organs examined from patients dying of quite different diseases may be mistaken for rickettsiae.
Typhus Fever in Iran and Iraq, 1942–43

PLATE II.

Rickettsiae in Human Tissues and Culture
NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS

PLATE I.

Fig. 1.—Myocardium. Death tenth day. Shows a diffuse infiltration by mononuclear cells between muscle fibres. There is a small necrotic area above a typhus nodule which resembles an Aschoff nodule under the low power. × 100 diameters.

Fig. 2.—Myocardium. High-power photomicrograph of the lesions in fig. 1. × 275 diameters.

Fig. 3.—Pons. Shows the presence of two collections of mononuclear cells which form the characteristic nodules described by Wolbach. × 60 diameters.

Fig. 4.—Pons. Two blood-vessels are present which show marked endothelial proliferation with papilliform ingrowths of cells into the lumen of the vessels. × 60 diameters.

Fig. 5.—Kidney. Shows vascular proliferation, which is related to the intertubular blood-vessels. Mononuclear cells appear to have migrated and invaded several tubules forming a large cellular nodule. The cells lining the tubules show degenerative changes. × 60 diameters.

Fig. 6.—Pons. Death tenth day. Shows an early nodule of the type described by Wolbach. × 400 diameters.

Fig. 7.—Kidney. Death tenth day. Some hyalinization is present, suggesting intraglomerular thrombosis. Lining cells of Bowman’s capsule appear to be desquamating. Approximately × 250 diameters.

PLATE II.

Fig. 8.—Pons. High-power photomicrograph of fig. 3. Approximately × 220 diameters.

Fig. 9.—Lung. Death tenth day. Shows the presence of oedema and cellular infiltration of the alveolar walls. Signs of early bronchopneumonia are seen on the left. × 110 diameters.

Fig. 10.—Liver. Death tenth day. Kupffer cells are swollen and lie free in sinusoids. One near the top of the field is in mitosis. Another in the centre contains cells. × 400 diameters.

Fig. 11.—Photomicrograph of a Giemsa-stained smear made from a developing chick embryo yolksac inoculated with the Addis Ababa strain of Rickettsia prowazekii. Specimen supplied by Major C. R. Van Rooyen. × 1,050 diameters.

Fig. 12.—Photomicrograph of the alveolar wall which shows the presence of two globular masses of rickettsiae below the centre of the field. Death tenth day. × 1,200 diameters (stained by Giemsa).

PLATE III.

Fig. 13.—Photomicrograph of another area in the same lung section as fig. 14, showing rickettsiae in pairs. × 1,200 diameters (stained by Giemsa).

Fig. 14.—Photomicrograph of heart muscle fibre in the region of a blood-vessel shows a fusiform collection of globular masses of rickettsiae. × 1,050 diameters (stained by Giemsa).
(E) Laboratory Investigations.

(1) Serological Investigations.

(a) Weil-Felix Reaction.—Up to the present time the Weil-Felix reaction, still an empirical test, has been the standard laboratory method for diagnosing typhus fever and was carried out by all military laboratories. In interpreting the Weil-Felix reaction it was accepted that the serum of a case which agglutinated a suspension of Proteus 0 X19 in a dilution of 1 : 100 or more could be regarded as suggestive of typhus fever and a diagnosis could be made with more confidence if the agglutinin titre rose rapidly as the disease developed.

Blood was taken on various days of the disease from all patients suspected of suffering from typhus fever—the first specimen being taken about the fifth day, the second between the eleventh and fourteenth, the third between the eighteenth and twenty-first, and the fourth about the twenty-eighth.

All sera were tested with 0 suspensions of Proteus X19 and X2 sent from Kasauli, India. Suspensions of Proteus 0 XK were frequently found to be auto-agglutinable. As no case with a diagnostic agglutinin titre for Proteus 0 XK had been found previously, and as the suspensions available were frequently unsatisfactory, it was decided to discontinue testing for the presence of these agglutinins.

In the majority of laboratories Dreyer’s technique and tubes were used. The tubes were incubated in a water bath at 50° to 52° C. for two hours and after standing at room temperature overnight the results were read.

At Teheran Felix’s technique was used. Tubes were incubated at 37° C. for two hours and after standing at room temperature overnight the results were read.

Over a thousand sera were sent to Van Rooyen for confirmatory tests, and also for the rickettsial agglutination reaction.

(i) Laboratory Findings: The serological findings from cases diagnosed as typhus fever are analysed and tabulated below (only denominators of dilutions are given). As no significant differences were found in the percentage mortality rates or the agglutinin response in sera, British, and Indian troops are considered as one group. A total of 2,424 sera were examined.

<table>
<thead>
<tr>
<th>Table I.—Details of Titres Obtained During Different Weeks of the Illness. Figures Represent Numbers of Sera Examined Weekly.</th>
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</thead>
<tbody>
<tr>
<td>A. British and Indian troops.</td>
</tr>
<tr>
<td>Below</td>
</tr>
<tr>
<td>1st Week</td>
</tr>
<tr>
<td>2nd Week</td>
</tr>
<tr>
<td>3rd Week</td>
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<tr>
<td>4th Week</td>
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<tr>
<td>Less than Total</td>
</tr>
<tr>
<td>125</td>
</tr>
<tr>
<td>1st Week</td>
</tr>
<tr>
<td>2nd Week</td>
</tr>
<tr>
<td>3rd Week</td>
</tr>
<tr>
<td>B. Coolies.</td>
</tr>
<tr>
<td>Not present Below present</td>
</tr>
<tr>
<td>1st Week</td>
</tr>
<tr>
<td>2nd Week</td>
</tr>
<tr>
<td>3rd Week</td>
</tr>
<tr>
<td>4th Week</td>
</tr>
<tr>
<td>C. Iranian Civilians.</td>
</tr>
<tr>
<td>Not present Below present</td>
</tr>
<tr>
<td>1st Week</td>
</tr>
<tr>
<td>2nd Week</td>
</tr>
<tr>
<td>3rd Week</td>
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<tr>
<td>4th Week</td>
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</table>

Table II.—Mean Average Weekly Titres of Sera.

<table>
<thead>
<tr>
<th>Table II.—Mean Average Weekly Titres of Sera.</th>
<th>British and Indian Troops</th>
<th>Coolies</th>
<th>Iranians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nos. Mean</td>
<td>Nos. Mean</td>
<td>Nos. Mean</td>
<td></td>
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<tr>
<td>1st Week</td>
<td>66</td>
<td>114</td>
<td>43</td>
</tr>
<tr>
<td>2nd Week</td>
<td>98</td>
<td>843</td>
<td>79</td>
</tr>
<tr>
<td>3rd Week</td>
<td>28</td>
<td>960</td>
<td>9</td>
</tr>
<tr>
<td>4th Week</td>
<td>13</td>
<td>1031</td>
<td>—</td>
</tr>
</tbody>
</table>
(ii) Analysis of Laboratory Findings: Other diseases were prevalent at the same time as typhus fever. Cases of typhoid, relapsing fever, measles and hemorrhagic smallpox also had an exanthematous eruption. The symptoms in others like sandfly fever and various respiratory infections resembled those of the early stages of mild cases of typhus fever. As far as possible no case was diagnosed typhus fever unless the cases were clinically typhus fever irrespective of the serological findings or mild and atypical cases showing a diagnostic rising titre. It is therefore probable that some mild or abortive cases producing Felix's (1944) low titre curve were not diagnosed typhus fever. This controlled diagnosis was necessary to prevent numerous non-typhus fever cases being diagnosed as such.

The agglutinin titres obtained are illustrated in the two graphs which follow:

Graph illustrating the rise in Proteus OX 19 agglutinins during the course of the disease in different groups of patients.
and between 80 and 90 per cent of the cases produced a diagnostic high titre curve. The significant and high titres were obtained between the eleventh and seventeenth days. The high titres usually persisted for at least fourteen days after the defervescence of the temperature. The highest percentage of all sera showing a titre above $1 : 1,000$ was found in this group: titres of $1 : 5,000$ and above were only present in 3.9 per cent of cases.

**Iranian civilians.**—The mortality rate was 12.0 per cent. These individuals were undernourished and came from an area where typhus fever is always endemic and likely to become epidemic.

In this group the highest percentage, viz. 50 per cent of all sera taken in the first week, showed a titre above $1:100$. The titre rose as the disease progressed, but not to the same height as in the military cases. High agglutinins did not persist to the same degree after the defervescence of the temperature. The high titre diagnostic curve was present in 72 per cent of cases. This is slightly lower than the preceding group. Less than 10 per cent of the sera had a titre above $1:640$ and under 2 per cent went beyond $1:2,560$.

**Coolie labourers.**—The mortality rate was 37.9 per cent. The patients were either old men or young boys who were undernourished and in poor physical condition. They came from areas where typhus fever is always endemic.

Only 27 per cent of the sera of this group showed a diagnostic titre by the end of the second week. The highest titre obtained was $1:1,000$, but less than 4 per cent of sera gave this titre.
The majority of the cases did not produce titres of diagnostic significance until late in the disease, usually about the twelfth day.

The agglutinin titre produced was not necessarily indicative of the course of the disease. Of 28 sera from fatal military cases, 14 showed titres ranging from 1:125 to 1:5,000. The proportion of low titre curves obtained from moderately severe Iranian cases is higher than expected.

The age of the patient was not found to have any bearing on the agglutinin titre obtained nor was there a significant difference between the different age-groups.

The percentage of all sera examined from Polish and Iranian patients showing a titre above 1:100 is similar. The percentage mortality in both groups is almost the same, and both came from countries where typhus fever is endemic.

(iii) Proteus OX2 Agglutinins.—The presence of agglutinins to Proteus OX2 suspensions is interesting and cannot be explained unless it is to be regarded as characteristic of the type of disease prevalent. One apparently genuine case belonging to the Proteus X2 group did occur. The patient was an I.A.M.C. officer, who was Officer Commanding an Indian Field Hygiene Section. He was not protected by vaccine and had a very severe attack which was complicated by bronchopneumonia and femoral thrombosis. The Weil-Felix test findings are given below:

<table>
<thead>
<tr>
<th></th>
<th>10th day</th>
<th>13th day</th>
<th>16th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>O X2</td>
<td>1:25</td>
<td>1:250</td>
<td>1:250</td>
</tr>
<tr>
<td>O X19</td>
<td>nil</td>
<td>1:125</td>
<td>1:300</td>
</tr>
</tbody>
</table>

The analysis of Proteus O X2 agglutinins obtained from 333 tests carried out on military and coolie cases is given below:

<table>
<thead>
<tr>
<th></th>
<th>Below 1:125</th>
<th>1:250</th>
<th>1:500</th>
<th>Above 1:500</th>
</tr>
</thead>
<tbody>
<tr>
<td>10th day</td>
<td>265</td>
<td>38</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>13th day</td>
<td>1:25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16th day</td>
<td>1:250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O X19</td>
<td>1:500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It was frequently noted that a transient rise in Proteus O X2 agglutinins preceded Proteus O X19 but, as the titre of the latter increased, the Proteus O X2 agglutinins assumed a corresponding lower level.

In the Iranian cases it was found that in 10 per cent of the sera examined the Proteus O X2 agglutinins showed significant titres equal to and in a number even higher than Proteus O X19.

(b) Rickettsial Agglutination Reaction.—In addition to the Weil-Felix reaction, agglutination tests were carried out with rickettsial suspensions on sera from Paiforce by Major C. R. Van Rooyen, R.A.M.C., in his laboratory at Cairo.

This rickettsial agglutination reaction has been fully described by Van Rooyen and Bearcroft (1943).

Over 1,000 sera were sent to Van Rooyen from Iranian civilians treated in the military hospital at Teheran, from Professor Beattie at Baghdad, and from military and civilian cases in other areas of Paiforce.

Van Rooyen (in a personal communication) made the following comments:

(1) During January to April, 1943, there was a widespread epidemic of human typhus fever which affected the whole of Iraq, Iran and Transjordan.

(2) The Weil-Felix tests performed in military laboratories were invariably identical with his own Weil-Felix results.

(3) The rickettsial agglutination reaction showed complete correlation with the Weil-Felix tests.

(4) In a series of 81 sera tested, 75 were epidemic and only 6 murine.

(5) Independent confirmation of the above cases from two sources, i.e. the high death-rate and the results of guinea-pig inoculations.

(6) When considering the rickettsial agglutination reaction, it is important to note that the epidemic component of Craigie’s vaccine was made up of one or other of two antigens, either the classic Brieni strain isolated in Prague many years ago or the Madrid 4 strain...
isolated by Major Snyder from a political prisoner at the Commandores Prison in Madrid during the Spanish Civil War. Both these antigens were tested and the following observations were made. While the Egyptian sera agglutinated both to some extent, the Paiforce sera agglutinated the Brienl strain rather better but the Madrid 4 strain very strongly indeed. The reason one may never know—but it brings out the great importance of verifying the authenticity of strains of antigens to be employed for proposed field immunization work in any particular area.

The right type of antigen must be used for the right place at the right time. Having surveyed the terrain of Paiforce Van Rooyen had no doubt that the Craigie vaccine containing Madrid 4 and murine antigens is the correct immunizing agent to use.

(c) Wassermann and Kahn Tests.—(i) Wassermann Reaction.—The Wassermann reaction was carried out on too few typhus fever sera to allow any conclusions to be made. A number of Wassermann positive results has been recorded.

(ii) Kahn Test.—646 Weil-Felix positive sera from Iranian patients were examined: only 134 (or 20·7 per cent) gave a positive Kahn reading. Positive findings did not depend on the titre of the O X19 agglutinins present in the serum. Of 614 control sera from patients other than clinical typhus fever examined during the same period for syphilis infection, an almost identical percentage (21·4 per cent) was Kahn test positive. As there is a relatively high incidence of syphilis among the civilian population it is not considered that 20 per cent positives of all typhus fever bloods prove the Kahn test to be of any real diagnostic value in typhus fever.

An interesting observation recorded in this connexion was that in nearly one-quarter of the Kahn positive typhus fever sera the O X2 agglutinins were either higher in titre or at least equal to the O X19.

(d) Gel Tests.—As the characteristic attack of typhus fever affects the endothelial cells, if not the entire reticulo-endothelial system, various gel tests were carried out.

The “Formogel” test with formaldehyde in serum, associated with the antimony reaction of Chopra in kala-azar, failed to give any interesting results. Of 92 Weil-Felix positive sera treated with formalin, 22, or nearly one-quarter, showed definite gel, but clear without opacity, and this did not increase appreciably on standing.

(e) The P.C. or Precipitin Colloid Test (Platinum Chloride).—Major L. E. Elkerton, I.M.S., in charge of a military laboratory in Teheran, evolved a test which gives an immediate serum reaction. He has called this “the Precipitin Colloid Test (Platinum Chloride)” or as a short title “the P.C. Test.”

(i) Requirements for the Test:

1. 0·664 per cent solution of platinum chloride.—This is eight times the strength of the solution supplied in the poison-testing case.

2. Distilled water pH 6·6.—The diluent must be distilled water. The precipitate will not form in saline, in any buffered solution, or in the presence of any other electrolyte, and thus citrate or similar anticoagulants cannot be used to facilitate a bedside reading.

3. Rickettsial vaccine.—The clear supernatant fluid obtained either when the vaccine has been standing for some time or after centrifuging is used. The vaccines employed were Canadian, South African and American supplied for protective inoculation of troops.

4. Control sera or suitable comparator, e.g. Brown’s opacity tubes.—The sera were known positives from typhus fever cases and negative from other cases.

5. Patient’s serum.—The serum must not be heated in a water bath prior to use as it was found that at temperatures from 56° to 60°C, although strong P.C. positive sera were relatively unaltered, weak positives became negative.

Apparatus:

1. Small test-tubes, 3 in. by ⅜ in., thin glass, rimmed.
(2) Dropping pipette.—This pipette is used throughout the test, and is marked for a volume of 18 drops, which is the standard one volume for determining the relative proportions of all agents. The exact volume is not important; that generally used was—1 volume = 0·4 to 0·5 c.c. (approximately).

(ii) Preparation of Antigen.—Four volumes of rickettsial vaccine are added to three volumes of platinum-chloride solution. The antigen is ready for immediate use and is sufficient for 100 tests.

(iii) Technique.—This is a one tube test only, and all unknowns and controls are set up in the same way and at the same time without any delay.

One volume (18 drops) distilled water is placed in each test tube, and one drop of serum is then added giving approximately a 1 : 20 dilution. The tubes are well shaken and one drop of antigen is pipetted into each. They are shaken again, and a further one volume of distilled water is added giving approximately a final dilution of 1 : 40. Readings are then taken.

(iv) Readings.—The grade of initial turbidity is estimated, in comparison with controls, i.e. 0 ; < + ; + ; ++ ; +++ ; +++. Slight opacity on first addition of the serum to the distilled water and early appearance of a precipitate may be associated with stronger sera.

(v) Findings.—Sera from typhus fever cases and control sera from non-typhus fever cases have been examined by both the P.C. and Weil-Felix tests and the results compared.

**Analysis of Findings from 1287 Sera from Clinical Typhus Fever Cases**

<table>
<thead>
<tr>
<th></th>
<th>Complete agreement with Weil-Felix positives</th>
<th>Complete agreement with Weil-Felix negatives</th>
<th>P.C. test stronger than Weil-Felix</th>
<th>P.C. test weaker than Weil-Felix</th>
<th>P.C. test negative, when Weil-Felix positive</th>
<th>P.C. test positive, when Weil-Felix negative</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) N.A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42·0</td>
</tr>
<tr>
<td>(2) S.A.K.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9·5</td>
</tr>
<tr>
<td>(3) A.Y.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25·3</td>
</tr>
<tr>
<td>(4) Y.A.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14·3</td>
</tr>
<tr>
<td>(5) T.I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2·7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6·2</td>
</tr>
</tbody>
</table>

A table is given below of 5 cases in which the precipitin colloid test was positive earlier than the Weil-Felix.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age</th>
<th>Day Fever</th>
<th>O X2</th>
<th>O X19</th>
<th>P.C. Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) N.A.</td>
<td>19</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>320</td>
<td>640</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>320</td>
<td>640</td>
<td>+++</td>
</tr>
<tr>
<td>(2) S.A.K.</td>
<td>45</td>
<td>2</td>
<td>80</td>
<td>80</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>80</td>
<td>640</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>320</td>
<td>2,560</td>
<td>+++</td>
</tr>
<tr>
<td>(3) A.Y.</td>
<td>22</td>
<td>2</td>
<td>80</td>
<td>80</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>80</td>
<td>80</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>320</td>
<td>640</td>
<td>++</td>
</tr>
<tr>
<td>(4) Y.A.</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>40</td>
<td>80</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>160</td>
<td>160</td>
<td>+++</td>
</tr>
<tr>
<td>(5) T.I.</td>
<td>24</td>
<td>3</td>
<td>40</td>
<td>80</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>160</td>
<td>80</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>40</td>
<td>80</td>
<td>++</td>
</tr>
</tbody>
</table>

It was noted that as a rule a typhus fever serum will give a positive P.C. test by the third day, while agglutinins do not appear on an average before the fifth day, and then not in diagnostic titre until later.

**Analysis of findings from 670 control sera from Cases Other than Typhus Fever.**

It has been found that some supposedly normal sera give a measurable reading with the P.C. test. During the investigation from military patients sent for routine Kahn tests and others from a variety of diseases were examined as controls. Of 614 bloods assumed to be normal 56 (9 per cent) gave a doubtful or weak positive P.C. reaction. Blood from typhoid, malaria, sandfly fever, tuberculosis and short fever cases appeared to give consistently negative reactions. One leprosy and a query kala-azar case gave weak positives.
It seems probable that group precipitins, which are similarly sensitive to the colloids, account for these unrelated reactions.

(vi) Commentary.—The ease, simplicity and immediate reading of the test commends it to the clinician. Findings appear to show that the P.C. test can be a useful adjunct to the Weil-Felix, particularly as differences are in favour of the former.

The precipitate formed during the P.C. test appears to be independent of the proteus agglutinins. The Weil-Felix test can be carried out after the precipitate has been removed without change in titre. Conversely the P.C. test can be performed on a serum diluted 1:160 after the removal of the proteus agglutinins. It would appear that the precipitin may be more closely related to the antibodies of the specific infective agent than the proteus agglutinins.

(f) Non-specific Reactions.—1,562 sera from Iranian typhus fever cases were examined by Major L. E. Elkerton, I.M.S., for the presence of B. typhosus agglutinins. The findings are summarized below:

<table>
<thead>
<tr>
<th>Titres of T O agglutinins</th>
<th>Nil</th>
<th>1:40</th>
<th>1:80</th>
<th>1:160</th>
<th>1:320</th>
<th>1:640</th>
<th>1:1280</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sera with agglutinins</td>
<td>1,176</td>
<td>269</td>
<td>71</td>
<td>31</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T O agglutinins were present in 386 sera (24.7%).

<table>
<thead>
<tr>
<th>Titre of O X19 agglutinins</th>
<th>Nil</th>
<th>1:160</th>
<th>1:320</th>
<th>1:640</th>
<th>1:1280</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera with T O agglutinins</td>
<td>41</td>
<td>87</td>
<td>49</td>
<td>73</td>
<td>88</td>
<td>48</td>
</tr>
<tr>
<td>186</td>
<td>262</td>
<td>223</td>
<td>230</td>
<td>196</td>
<td>79</td>
<td>1,176</td>
</tr>
<tr>
<td>Sera without T O agglutinins</td>
<td>186</td>
<td>262</td>
<td>223</td>
<td>230</td>
<td>196</td>
<td>79</td>
</tr>
</tbody>
</table>

In assessing the significance of these findings it is necessary to remember that although the civilian population in Iran is not protected with T.A.B. inoculation, typhoid fever is prevalent. The figures do show that a greater proportion of the non-specific T O agglutination was found in sera with a high proteus O X19 agglutinin titre.

(2) Leucocyte Counts.

The results obtained from total and differential leucocyte counts in both fatal and non-fatal cases were so variable as to be of neither diagnostic nor prognostic significance, irrespective as to what day of the disease they were carried out. The majority of the counts performed on fatal cases were within normal limits.

(3) Animal Transmission Experiments.

The animal transmission experiments were carried out by a typhus fever research team which was placed under the command of Major J. Bowie, I.M.S. This team carried out investigations during the spring in the Mosul area in Northern Iraq and at Teheran in Iran. Major C. R. Van Rooyen co-operated and carried out confirmatory transmission experiments in Egypt with material flown over from Bowie. Details of the experimental work were correlated by him.

The investigation had to be carried out in tents under active service conditions. As travelling was done mainly by air, equipment was limited. Four weeks were spent by the team in Northern Iraq and five in Teheran. Material for animal inoculation was obtained from 54 typhus fever patients, 49 of whom were Iraqi or Iranian civilians. The patients selected varied widely, but 52 out of the 54 cases were either febrile or convalescent from typhus fever. About 240 guinea-pigs were used in the experiment—a series of at least two to six animals being employed on each case.

(a) Technique.—(i) Patient to guinea-pig.—Venous blood was withdrawn from 52 typhus fever patients and injected into 52 series of guinea pigs, which are divided into four groups according to the type of inoculum used. The disease was successfully transmitted from 32 patients.
Group I. In 31 cases 10 ml. of blood was withdrawn and allowed to clot in the ice-box. Within six hours the clot was macerated in 3 ml. of sterile normal saline and guinea-pigs injected intraperitoneally with 2 ml. of the suspension. Eight series of animals were successfully infected. As this technique proved disappointing other methods were tried.

Group II. In seven cases guinea-pigs were injected at the bedside with 2 ml. of whole blood immediately it had been withdrawn from the vein. Three series of animals were successfully infected.

Group III. In ten cases a suspension of leucocytes was used as the inoculum, 20 ml. of blood were withdrawn into 4 ml. sterile sodium citrate, centrifuged at 2,000 r.p.m. for twenty minutes and the supernatant plasma was pipetted off the leucocyte cream. Within four hours of taking the blood, guinea-pigs were injected intraperitoneally with 2 ml. of leucocyte and upper red blood cell layers. Five series of animals were successfully infected.

Group IV. In four cases a 2 ml. dose of leucocyte was followed next day with the same volume of suspended macerated clot in saline. Three series of animals were successfully infected.

From the results obtained it would appear that 2 ml. of patient’s macerated blood clot suspended in saline is not a satisfactory inoculum in attempting to produce experimental typhus fever in guinea-pigs and that an inoculum consisting of leucocytes is more likely to be effective. It was also found that the chances of success in transmitting the infection are greatest in the first week of the disease, slightly less in the second and very slight in the third.

(ii) From lice to guinea-pig.—It was assumed that about eight days must elapse before lice became infective after feeding on febrile patients. Fifteen to twenty days after onset of fever lice were gathered from clothes of 5 typhus fever patients who had evaded admission to hospital. Within three hours of collection the lice were rapidly washed in ether to sterilize the surface as far as possible, ground up with sterile saline in a mortar and guinea-pigs were inoculated intraperitoneally with 2 ml. of the resulting suspension.

Only one strain was isolated from lice. It was established in guinea-pigs by repeating passage and despatched to Middle East. The histological lesions produced by this strain were very definite from the first animal onwards.

(iii) From guinea-pig to guinea-pig.—The animal was killed on the second or third day of continuous fever, usually between the tenth and fourteenth day after injection. Using strict aseptic precautions, one half of the cerebrum was removed from the cranial cavity, ground up in a sterile mortar, suspended in 5 ml. sterile normal saline and 2 ml. injected intraperitoneally into the next passage animal.

(b) The Febrile Reaction in Guinea-pigs.—Experimental exanthematic typhus fever in guinea-pigs is not a severe disease and the animals do not appear to be ill. Great difficulty was first experienced in determining the presence or absence of a specific febrile reaction in guinea-pigs. The essential feature is the registration of intra-abdominal as opposed to rectal temperatures. It is the only accurate method by which the correct internal temperature in guinea-pigs may be ascertained. The use of this method is also referred to in the Official History of the War, Medical Services, Pathology (1923). Only by this can the characteristic curve be observed in typhus fever-infected guinea-pigs. This difference between the abdominal and rectal temperatures is illustrated in temperature Charts 2 and 3.

Among healthy guinea-pigs the abdominal temperature was found to vary from 101 to 102° F. between 0600 and 0800 hours and before the morning feed. Prior to their use as passage animals daily temperatures were taken for three days. To exclude subclinical natural disease, guinea-pigs showing a greater variation in daily temperatures of more than 2° F. or with a maximum morning temperature of more than 102° F. were not used in the investigation.

The characteristics observed in the temperature charts of typhus fever-infected guinea-pigs were as follows: (1) An incubation period of six to eight days (extremes, five to nine days), (2) a sharp rise and fall in one day, followed by (3) an afebrile interval of two to four days,
then (4) a second rise between the ninth and twelfth day which is sustained as a continuous temperature for four to six days at least 2°F. above the animal's average normal temperature, and, finally (5) the temperature falls by crisis.

TEMP. CHART NO. 1

TEMP. CHART NO. 2

TEMP. CHART No. 3
The common form of the characteristic temperature curve is shown in Curve 1. Charts 1 and 2 are the actual records of animals which had typical febrile reactions.

Particularly among passage animals, what may be termed a "shift to the left," was observed in some temperature charts. Here the onset and course of the febrile reaction is rapid and the temperature during the period of continuous fever may reach 107°F. The first sharp
A rise and fall in one day occurs early and may be of such short duration that either it is missed altogether where temperatures are registered only once daily (Curve 3 and Chart 4) or it is only observed during the rise or fall, so that the rise appears small (Curve 2).

A "shift to the right," usually occurs in the first animal of a passage series. The primary rise and fall may appear as late as the ninth day and the following afebrile period may be as long as four days, so that the period of continuous temperature only begins on the thirteenth day. But when the inoculum consisted of a suspension of leucocytes, the curve of the febrile reaction even in the first guinea-pig is almost without exception as shown in Curve 1.

It would appear that a "shift to the left" may occur where the strain is more usually virulent for susceptible animals or where the infecting dose is large, but a "shift to the right" may indicate low virulence or small infecting dose.

(c) Morbid Anatomy.—With the exception of the tunica reaction, the macroscopic changes are not remarkable and are confined to the serous cavities. The peritoneum loses its smooth glistening appearance and becomes flushed and thickened. There is free fluid in the peritoneal cavity and often there is a delicate, thin, milky exudate over the spleen which is not appreciably enlarged. The mesentery and bowel are hyperoemic. Small petechial hemorrhages may be present below visceral and parietal peritoneum and in the pericardium. The membranes over the surface of the brain are hyperoemic, dull and thickened.

One of the main objects of the team was to ascertain whether scrotal reactions occurred. This phenomenon may be observed in guinea-pigs infected with the murine strain. It then consists of an inflammation in the scrotal wall which may become gangrenous. In animals infected with exanthematic typhus fever, the genital lesion is milder and limited to the immediate coverings of the testicle, i.e. the tunica reaction.

No scrotal reactions but six tunica reactions were observed at post-mortem examination of experimental animals infected in Iran and Iraq.

In the presence of the tunica reaction, due to typhus fever infection, the testicle becomes fixed within its muscular diverticulum, the wall of which becomes swollen and more firmly bound down in its scrotal bed. The sac is therefore no longer able to extrude the testicle into the abdomen. Except in patches which are occupied by a cellular exudate, the two layers of the tunica vaginalis become adherent and anchor the testicle even more firmly. Externally the scrotum appears slightly swollen and flushed.

When the testicle is forcibly withdrawn from its coverings, the surface is raw; this is due to irregular laceration of the tunica albuginea and internal spermatic fascia which have become continuous through the adhesions between the visceral and parietal tunica vaginalis.

During the greater part of the investigation, it was not realized that the tunica reaction may be of a transient nature. It may pass off between the tenth and thirteenth day after
injection and should therefore be examined for daily by digital pressure over the scrotum at the time temperatures are registered. This had not been done; consequently the fact that only six tunica reactions were observed at post-mortem examination of 48 febrile male guineapigs is probably of little significance as an index of the actual frequency of this reaction.

(d) Morbid Histology.—It would appear that as in human cases the essential change in the organs is an inflammation of small blood-vessels and their supporting areolar tissue. The findings described below are not those usually associated with epidemic typhus fever infection in guinea-pigs.

The Brain.—The hyperplastic activity begins with the rise in temperature, about the seventh or eighth day after injection. This is at first generalized but most pronounced in the grey matter of the cortex and basal ganglia at the junction with the white, being particularly obvious in the comparatively acellular molecular lamina immediately below the pia-mater. Over the whole surface of the brain, the areolar tissue of the membranes is hyperplastic and the pia-arachnoid spaces are crowded with young endothelial cells, mononuclear cells, plasma cells and leucocytes; the cells may extend for some distance into the brain along the perivascular spaces.

No histological change was found in the choroid plexus or ependyma lining the ventricles. With the onset of the continuous temperature period, young endothelial cells are aggregated in the form of Wolbach nodules with prominent dilated capillaries as their focal points. At first, the aggregations are large and loose at their periphery, as though endothelial cells were infiltrating areas of particularly active hyperplasia. Later, the characteristic nodule is formed in which the cells are more densely packed. With the exception of a few eosinophilic leucocytes, the new cells appear to originate from vascular endothelium or associated areolar tissue cells. Pre-existing nerve and neuroglia cells involved in the nodule show no obvious histological change. Gold and silver stains were not available in this investigation.

About the second day of continuous temperature, the generalized hyperplasia passes off and a few cells, near the centre of the nodule, show karyorrhexis of their nuclei.

It is probable that blood-flow through capillaries in the nodule ceases at this time. Owing to the dense cellularity, the capillary outline may be indistinguishable. Thrombosis is not a feature of exanthematic typhus fever in guinea-pigs. Occasional dilated capillaries in the brain appear to be blocked by masses of endothelial cells but no organizing thrombi were found in any of the brains examined.

After the third day of continuous fever, cells in the nodules disperse and when the temperature has reached normal no nodules remain and there is no gliosis.

Lungs.—In the walls of the alveoli throughout the lung, hyperplasia of capillary lining can be distinguished. In the larger vessels, the supporting areolar tissue of the adventitia is hyperplastic and the ground substance and lymph channels of this tissue are crowded with young endothelial cells, large mononuclear cells, plasma cells, a few lymphocytes and occasional eosinophilic leucocytes. Among these cells, as in the nodules of the brain, there are pyknotic globules, varying in size from 1μ to 3μ, the nature of which is not clear.

Heart.—In the heart, a cellularity of the areolar tissue in the periphery of vessels separates the bundles of muscle fibres, but some increase in size and number of muscle cell nuclei occurs. The cells present are endothelial, large mononuclear cells, plasma cells with occasional lymphocytes and eosinophilic leucocytes.

Lymph channels accompanying vessels in the cellular areas are distended with cells and phagocytosed pyknotic cell debris.

Kidney.—In the kidney, the lesions are scanty and consist of occasional localized areas of hyperplasia in connective tissue.

The most common site is just external to the malpighian body. The nodule begins in connexion with the areolar tissue coat of vessels to and from the glomerulus, just external to Bowman’s capsule.

The condition may be termed an interstitial proliferative glomerulitis.
The Male Generative Organs.—In the testicles there is an inflammatory hyperemia, cellularity and thickening of the tunica vasculosa under the tunica alba. The cremaster and external spermatic muscle laminae share in this change and the thickening is marked. In the interstitial tissue of these structures, associated with engorged vessels, there is an increase of cells of the same type as those found in the hyperplastic interstitial tissue of the heart and lungs. Related lymph channels, numerous in the genital organs, are dilated; among the cells within the lymph channels, there are pyknotic globules, often without macrophages.

The remaining laminae of the testicular coverings and subscrotal fascia show a marked increase in connective tissue cells.

In spaces between the adhesions of visceral and parietal tunica vaginalis, there is a cellular exudate identical in character with that in the peritonea.

In the epididymis, there is a generalized increase of areolar tissue cells and a nodular periarteritis in the fine terminal branches of the internal spermatic artery which supplies the epididymis.

It would appear, from the macroscopic and microscopic appearances found in guinea-pigs infected with typhus fever in Iraq and Iran, that the genital lesion is a modified scrotal reaction and that the so-called "tunica reaction" is in reality a misnomer.

(e) Demonstration of Rickettsiae in Animal Tissues.—Smear preparations from the peritoneum and tunica vaginalis were examined in more than 120 guinea-pig post-mortem examinations. For routine purposes, Giemsa’s stain and, in selected cases, Macchiavello’s method was used.

In many febrile guinea-pigs numerous acidophilic extracellular bodies, variable in size and pleomorphic, were seen throughout the exudate. These bodies were coccical, diplococci, bacillary, with and without granules, and lanceolate with bipolar staining. These structures were not only seen in peritoneal smears from animals later proved histologically typhus fever, but in smears from histologically negative cases. In three infected animals, the staining of the coagulated protein of the exudate resembled smear preparations of Craigie’s concentrated vaccine.

On four occasions, fine filamentous structures about 0.5 μ in diameter were seen within macrophages: They stained red by Macchiavello’s method.

Sections of normal human and guinea-pig tissues and many sections of granulomatous inflammations were examined. It was found that cells of connective tissues, definitely not eosinophil leucocytes, may show granules similar to those described as rickettsiae. Such granules exist in normal and pathological tissues more than was realized.

(f) Commentary.—Thirty one animals infected with strains of epidemic typhus fever isolated in Mosul, Suleimanyia and Teheran were sent to Major C. R. Van Rooyen in Egypt. He has made the following observations:

(i) There were certain pathological differences between the Egyptian and the Iraq-Iran types. The latter are more virulent and cause more intense lesions in the guinea-pigs with marked scrotal congestion (never true orchitis, adhesions, or matting) and petechial hemorrhages which were just like a typhus fever rash in a guinea-pig’s peritoneum. No Egyptian strain encountered by him or Colonel Plotz (U.S. Army Medical Corps), who isolated some 70 or more, has behaved in this way.

(ii) He believed that the epidemic typhus fever of Iraq-Iran is of a type peculiar to those countries, characterized by minute peritoneal hemorrhages in guinea-pigs; also by frequent nodules in these animals’ brains which he has never found in over 200 guinea-pigs studied in the Egyptian disease.

(iii) That this disease is true epidemic typhus fever is based on the following facts: That not only the human patient but also the injected guinea-pig from the same case gave high titrte epidemic rickettsial agglutination.
General Commentary and Summary

This is a record of an investigation carried out by personnel of the R.A.M.C. and the I.A.M.C. under the direction of Major-General J. G. Gill, C.B.E., D.S.O., M.C., the Director of Medical Services, Paiforce. Full details are given of a comprehensive survey of the outbreak of typhus fever which occurred during the first seven months of 1942 in Iran and Iraq. The disease has been investigated in cases which occurred among British and Indian troops, Polish soldiers, Iranian civilians and coolie labourers. It was mainly due to the fact that the disease was spread over a very extensive area and covered a period of seven months that it was possible to carry out such an extensive investigation. Cases of typhus fever continued into the really hot weather of June and July, a time when heatstroke was occurring in Southern Iran and Southern Iraq. In these areas, pathologists were working in shade temperatures of over 110°F. Morbid histology could only be done with difficulty as no microtome for cutting frozen sections was available. In reviewing the results obtained under trying active service conditions, and without any special equipment, pathologists and their laboratory assistants must be given full credit for their accomplishments.

A summary of the findings follows:

(1) Epidemiology.—Conditions in Iran were especially favourable to an epidemic of typhus fever. The economic state of the poorer classes was deplorable. The wheat crop was inadequate for the needs of the country and the price of bread, the staple article of food, soared to unprecedented heights. The starving population, ill-clad and verminous, wandered from town to town and across the frontier into Iraq in search of food and work. The winter of 1942–43 in Northern Iran was unusually severe. The spread of the disease followed the trade routes. The maximum incidence occurred during April, May and June. Conditions were very similar to those prevailing when typhus fever occurred during the Mesopotamia Campaign of 1914–18. The mortality rates varied in the military forces and the different groups of the civilian population.

(2) Preventative measures have been discussed and were chiefly aimed at the prevention of louse infestation and checking the spread of the disease. The low incidence of typhus fever among military personnel, living under unfavourable conditions, is proof of the efficacy of the measures adopted.

(3) Prophylactic inoculation was carried out but, as this was given during the course of an epidemic, it is difficult to assess its true value. The incidence of the disease and the mortality rates were, however, less among the inoculated than the non-inoculated.

(4) Clinically the disease did not differ in any marked degree from the textbook description of typhus fever. The prognostic value of certain signs and symptoms has been discussed.

(5) Pathology.—Tissues from typical cases of typhus fever have been examined both abroad and at Edinburgh University. Full details of the observations made are given and illustrated by microphotographs. These in the main conform to those described by Wolbach et al. (1922). Some additional findings are also recorded. The presence of rickettsiae in human tissues has also been discussed.

(6) Treatment.—No new advances have been made. Convalescent serum was not available. Sulphanilamide proved to be of no value in influencing the course of the disease.

(7) Laboratory diagnosis.—(a) The Weil-Felix test was used extensively as the standard method of laboratory diagnosis but, owing to the relatively late appearance of significant high titre agglutinins, was considered to have only a limited value except in doubtful or atypical cases, since the clinical diagnosis was generally obvious before a positive serological result could be obtained. It was found that the agglutinin response varied in different groups of individuals, and appeared to depend on whether they came from countries where typhus fever was endemic or not. Findings have been fully analysed, and the presence of Felix's high and low titre curves discussed. (b) Wassermann, Kahn and Gel tests were carried out, but no information of any diagnostic significance was obtained. (c) Rickettsial agglutination reactions were carried out by Van Rooyen in Egypt. He showed that the Iran-Iraq typhus.
fever was the true epidemic type and related serologically to the Madrid 4 strain. (d) The precipitin-colloid test is a new precipitin test evolved by Major L. E. Elkerton, I.M.S. He used as his antigen a mixture of rickettsial vaccine and platinum chloride solution. Full details of the test are given in the text, but its value must remain sub judice until further work has been carried out. (e) Leucocyte counts, both differential and total, were carried out. Findings were variable but, in the majority of cases, were within normal limits and of no diagnostic or prognostic value.

(8) Animal transmission experiments were investigated by Major J. Bowie, I.M.S., with the assistance of Major C. R. Van Rooyen, R.A.M.C. These showed that the Iran-Iraq strain differed from and was more virulent than the Egyptian, producing a tunica reaction in guinea-pigs but not true orchitis. Frequent nodules were present in the brains of these animals. Van Rooyen had not seen similar nodules in the brains of guinea-pigs infected with the Egyptian strain.

(G) Acknowledgments.

I must acknowledge the help and advice given by Major-General J. G. Gill, C.B.E., D.S.O., M.C., D.M.S., Persia-Iraq Force, without which this investigation would not have been possible, and I must emphasize that in addition to my own observations, which were largely concerned with pathology, diagnosis, epidemiology, immunization and general conclusions, the information contained in this paper is based on the investigations carried out by the aforementioned medical officers, without whose assistance and co-operation the official report would not have been possible. Brigadier F. M. Lipscomb, Consultant Physician; Lieut-Colonel J. D'Arcy Champney, R.A.M.C., Assistant Director of Hygiene; Majors J. C. Bell, F. Livesey, J. M. Macfie, H. Spencer, A. L. Latner and C. R. Van Rooyen, R.A.M.C.; Majors J. Bowie, L. Elkerton and M.S. Rao, I.M.S.; and Professor C. P. Beattie, Professor of Bacteriology, Royal Faculty of Medicine, Baghdad, and all pathologists, medical officers, and laboratory personnel who co-operated under trying conditions to make this investigation a success.

Particular thanks are due to the clerks both at Home and Abroad for their willing help.

I must acknowledge my deep gratitude to Mr. T. C. Dodds, of the Department of Pathology, Edinburgh University, for preparing photomicrographs of specimens brought by medical personnel and the reproduction of the majority of the plates and photographs of the graphs and temperature charts, and to Messrs. Kodak, Ltd., London, for the high-power photomicrographs which appear in Plate II.

Finally, I must record that this report is submitted with the kind permission of Lieut-General Sir Alexander Hood, K.C.B., C.B.E., Director-General of the Army Medical Services, and Major-General L. T. Poole, C.B., D.S.O., M.C., Director of Pathology, the War Office, London.

REFERENCES.


Official History of the War, Medical Services, Pathology (1933). H.M.S.O., p. 347.


Official History of the War, Medical Services, Casualties and Medical Statistics (1931). H.M.S.O., p. 238.

Shawbacker, et al. (1943). Lancet, 1, 739.


