

## **Studies of the Rickettsias of the Typhus- Rocky - Mountain - Spotted - Fever Group in South Africa.**

---

### **IV.—Discussion and Classification.**

---

By J. H. MASON and R. A. ALEXANDER, Section of  
Protozoology and Virus Diseases, Onderstepoort.

---

WE are in full agreement with Pijper and Crocker (1938) when they say that "Rickettsiosis is now an important and recognized chapter of pathology". However, a perusal of the literature indicates that a state of very considerable confusion exists and that this confusion is probably more marked when considering the South African rickettsioses; these have not received the same careful attention of the numbers of independent workers engaged on the subject in other countries.

In the preceding articles (this journal) details have been given of the experimental work carried out with a number of rickettsias isolated from several different sources. In this portion of the study, it is our intention to summarize the findings with a view to classifying the diseases and to use as a basis the criteria outlined by Pinkerton (1936).

At the outset it must be emphasised that we appreciate fully that our data are by no means complete. The work was incidental to a study of *Rickettsia ruminantium*, the cause of heartwater of cattle, sheep, and goats, and was initiated primarily to serve as a positive control for certain technique and experimental procedures which had produced negative results; but the findings are recorded as it is believed that some, at least, are of considerable importance and may be of value to other workers in the field.

#### *1. Cross-immunity Tests.*

It is agreed that for practical purposes the result of a reciprocal cross-immunity test is the most important single criterion for the differentiation of rickettsias. Care, however, must be exercised in the interpretation of results and it is again emphasized that an

adequate number of animals must be included in a series of tests before definite conclusions are drawn. When dealing with strains of high virulence and approximately equal infectivity for guinea-pigs, clear-cut results are usually obtained, but with strains of low infectivity considerable caution must be exercised in the final interpretation. In these cases, it is believed that the use of high-titre chorio-allantoic-membrane-cultures as the test inoculum is a decided advantage.

In table 7, part III, the results of the cross-immunity tests with the five strains investigated have been summarized. With the single exception of one guinea-pig out of 23 which reacted to *fièvre boutonnaise* and subsequently proved susceptible to the homologous virus, each strain produced a solid immunity against itself. Further, strains "Hare", "Robertson", *fièvre boutonnaise*, and "Appleton" (the latter not tested against "Robertson" and *fièvre boutonnaise*) showed practically complete reciprocal cross-immunity. These results are in striking contrast with those recorded by Pijper (1936). On the other hand, the results of the tests with the murine typhus strain were not so clear cut. Rat typhus broke down the immunity of 55 (52 completely and 3 partially) out of 59 guinea-pigs recovered from infection with any one of the other strains, i.e. only 4 out of 59 did not react at the heterologous immunity test. On the other hand, these strains produced a definite reaction in only approximately 20 per cent. of the rat-typhus-recovered guinea-pigs. These results agree partially with those recorded by Pijper and Dau (1932) and are in almost complete agreement with those recorded by Gear (1938). The significance of these results obtained with cross-immunity experiments with rat typhus is not entirely clear. One is inclined to interpret them as being due to the existence of an antigen common to the two groups. Pinkerton makes a point of issuing a warning against the too rapid acceptance of this interpretation; he obtained similar results when investigating a strain of mild spotted fever from Minnesota and he quotes Nicolle who reported that a considerable number of guinea-pigs are refractory to typhus following vaccination with *B. paratyphosus* B or the bacillus of pseudo-tuberculosis. When viewed in the light of our final conclusions that the rat typhus strain belongs to one group (the typhus group) and all the other strains belong to another group (Rocky Mountain spotted fever group), this discrepancy in the cross-immunity tests indicates the danger of using a single criterion when comparing the different rickettsias.

## 2. Study of Smear Preparations of the Scrotal-sac Exudate.

The general nature of the cellular reaction produced by each strain was practically identical with all the strains studied, but the morphology, location, and especially the distribution of the organisms showed marked differences.

In the case of murine typhus the rickettsias were longer, thinner, and more delicate with a definite tendency towards the formation of threads; they were found in large numbers only in serosa cells though single organisms were seen in neutrophils and monocytes,

probably as a result of phagocytosis. On many occasions, large numbers of infected cells were found in preparations and there was always a tendency for the rickettsias to be aggregated in clumps; heavily infected cells contained uncountable numbers of parasites which distended the cell and displaced the nucleus. Intranuclear forms were never seen.

With all the other strains the rickettsias were shorter and plumper, more commonly diplo-bacillary or diplo-coccal and never showed any tendency to form threads. Infected cells were always rare and these included, in addition to serosa cells, monocytes, the degree of infection excluding the probability of simple phagocytosis. There was never any tendency for the organisms to be aggregated in clumps; on the contrary they were always scattered throughout the cytoplasm and the degree of infection of individual cells was never so great that the number of parasites could not be counted with comparative ease. Again, intranuclear forms were not encountered.

These observations agree with those of Gear except that he records the presence of intranuclear forms in the case of his tick-bite fever strains.

### 3 and 4. *Histopathology, and Location and Morphology of Organisms in Sections.*

No data are available on these two points.

### 5. *Study of the Organism in Tissue Culture.*

Pinkerton deals exclusively with the *in vitro* cultivation of rickettsias in serum and Tyrode in the presence of surviving cells from the tunica vaginalis of guinea-pigs. In our studies, the use of the chorio-allantoic membrane of the developing chick embryo has been explored and there appears to be no valid reason why the results obtained by the two methods are not strictly comparable.

Again, our strains divided themselves into two groups. On morphological grounds, it was exceedingly difficult to differentiate the rickettsias of these two groups; the impression was gained that large numbers of minute cocco-bacillary forms were more common in the rat-typhus cultures and that, in cultures of the other strains, the individual organisms were distinctly larger. With rat-typhus cultures individual cells were frequently distended with a dense mass of rickettsias but intranuclear forms were never found in spite of the most diligent search. The picture seen when the other strains were examined was quite different. The cytoplasm of individual cells was never distended with parasites; these were scattered in a disorderly manner, though they were present in far larger numbers than in any preparation of the scrotal-sac exudate of guinea-pigs. In addition, intranuclear forms were common. The intranuclear parasites did not differ morphologically from the intracytoplasmic, and varied in number from single individuals to dense masses which gave the distended nucleus an almost homogeneous appearance. This intranuclear habitat was a constant differentiating feature.

The rat-typhus strain, although its multiplication resulted in the death of the embryo, appeared to multiply more slowly and showed a decided tendency to die out on serial passage. This tendency was not apparent with the other strains, although a sufficiently detailed investigation was not carried out to allow of a definite opinion.

#### 6. *Studies of Organisms in the Arthropod Vector.*

No information whatever is available on the morphology, distribution or location of any of the South African strains of rickettsia of the typhus-Rocky-Mountain-spotted-fever group in arthropod vectors.

In the case of rat typhus, apart from the negative feeding experiments of Gray (1931), no record appears in the literature of any attempt to determine experimentally whether fleas are the vectors of the typhus-like-condition harboured by rats. Apparently it has been the practice to label as murine or endemic typhus a strain of rickettsia isolated from that source. This was actually done in the case of the strain with which we worked, though fortunately we believe that our data, together with those of Gear (1938) and Gear and Becker (1938), certainly place this strain in the typhus group (Gear's and our strain originated from the same source). The fact that we have shown experimentally that the rat is susceptible to both groups of rickettsia illustrates the danger of this practice since the larva of *Amblyomma hebraeum* is well known for its extremely ubiquitous feeding habits. This tick is a known transmitter of the tick-bite fever.

In the case of tick-bite fever very little has been done experimentally even to identify the arthropod vector. Brumpt (1927) states that *Amblyomma hebraeum*, *Rhipicephalus simus*, and *Boophilus decoloratus* are transmitters of tick-bite fever but does not quote any work in support of this statement. Pijper and Dau (1934-1935) isolated a strain by the injection of an emulsion of *Rhipicephalus appendiculatus* larvae collected from an immune man. They failed, however, to transmit the disease with larvae, nymphae or adults of *Amblyomma hebraeum*. Gear and Douthwaite (1938) reported the isolation of tick-bite fever from an engorged adult dog tick, *Haemaphysalis leachi*. We isolated a rickettsia, identical with Gear's tick-bite fever strains, by feeding on a guinea-pig *Amblyomma hebraeum* nymphae collected from a hare. As far as we are aware this represents the whole of the published experimental work on the transmission of tick-bite fever by ticks.

*The Weil-Felix Reaction.*—According to Pijper and Crocker (1938), the titres of the sera of patients convalescent from South African epidemic typhus, South African sporadic typhus, and tick-bite fever are nearly always lower than those obtained with the sera of people who have suffered from European typhus. Pijper and Dau (1934, 1935), and Pijper and Crocker (1938) report that proteus OX2 is agglutinated almost as well as OX19, and OXK agglutination is not uncommon. In an outbreak of tick-bite fever (12 patients) recorded by Gear and Bevan (1936) low titres were obtained; the

titre for OX19 was, except in one instance, higher than that for OX2 and one serum only agglutinated OXK (1:50). Gear (1938), in a larger investigation, again shows that low titres were obtained in tick-bite fever, that OX2 and OX19 agglutinins were of equal significance and that OXK was not constantly agglutinated and never to a high titre. In an extensive series of tests of the sera of patients infected with South African epidemic (louse-borne) typhus Gear (1938) shows that OX19 is generally agglutinated to a higher titre than OX2, that high OX19 titres are not uncommon (1:3,200–1:25,600), and that OXK agglutination is irregular and occurs no more frequently than in febrile conditions other than typhus.

In our hands, 10 rat-typhus-infected rabbits produced agglutinins for OX19 and, although low, the titres were definite. Two rabbits produced OXK, in addition to OX19, agglutinins but in no instance did OX2 agglutinins appear.

The strain "Hare", which had much in common with tick-bite fever, stimulated the formation of both OX2 and OX19 agglutinins in one rabbit, of OX19 agglutinins only in another, and of none at all in 7 others. The human tick-bite fever strain (Robertson) itself caused the formation of just detectable amounts of OX19 agglutinins in one of seven rabbits inoculated; the remaining six were negative.

In button fever (Durand, 1932) and in Rocky Mountain spotted fever (Maxcy, 1936) high titres are not common and OX2 and OX19 are of equal significance; however, OXK is not agglutinated. This absence of OXK agglutination in these diseases would, on Pijper's positive findings in tick-bite fever, differentiate these diseases from tick-bite fever. But there is little doubt that the Weil-Felix reaction in button fever, spotted fever and tick-bite fever is a group, as opposed to a specific, agglutination; thus one is not fully justified in stressing the OXK agglutination in tick-bite fever. If the OXK agglutination is to be used as one means of separating tick-bite fever from button fever and spotted fever, then it must also be used to group South African epidemic and endemic typhus and tick-bite fever together. Whilst, on the one hand, we have results which show that a murine strain of South African typhus probably shares an antigen with tick-bite-fever (see "cross-immunity tests in guinea-pigs"), on the other hand, we have other results which clearly separate these two diseases (see "the morphology and location of the rickettsias" and "cross-immunity tests in guinea-pigs"). Thus, until a *proteus* X strain specific for tick-bite fever is isolated, we consider that the Weil-Felix reaction should not be used as an important test in differentiating tick-bite fever from button fever and spotted fever.

### 8. *The Clinical Picture in the Guinea-pig.*

*Rat Typhus.*—In common with Dr. J. H. S. Gear, of the South African Institute for Medical Research, who gave us the strain, we have had no difficulty whatsoever in maintaining rat typhus in the guinea-pig for two-and-a-half years by brain to peritoneum

passage at 8 to 10 day intervals. In addition, we have been maintaining another murine strain, placed at our disposal by Dr. G. Blanc, of the Pasteur Institute, Casablanca, for the last six months without any difficulty. Pijper and Crocker (1938) noted that endemic typhus, in their hands, always tended to die out in the guinea-pig; however, in an earlier publication [Pijper and Dau (1935)] they make no mention of this point.

We made no observations that have not already been recorded. The incubation period, the course of the fever, the scrotal reaction and the post-mortem picture agree closely with the findings of other workers. Whilst appreciating fully that a scrotal swelling can be caused by agencies other than a typhus infection, we would mention that a temperature reaction *plus* a swelling constitutes, in our experience, a surer sign of a typhus infection than a temperature reaction alone.

*Tick-bite Fever and Allied Diseases* ("Robertson", "Hare", "Appleton" and *fièvre boutonneuse*).—The maintenance of these infections (with the possible exception of "Hare") in the guinea-pig caused the greatest difficulty. By carrying "Robertson" (human tick-bite fever) in duplicate, we were able to hold it for about fifty generations, but at no time were we happy about it. Not infrequently, one line would die out and a fresh start would have to be made with the other line. However, there was seldom any fear of losing the strain; in addition to passaging it in guinea-pigs, we cultured it on the chorio-allantoic membrane of the chick embryo. The Appleton strain behaved in almost the same way, except that we never tried to adapt it to the egg-membrane.

With infected *fièvre boutonneuse* brain as inoculum, we never really succeeded in producing satisfactory reactions in guinea-pigs and very early in the work had to have recourse to the egg-membrane. With this, good results were nearly always obtained. "Hare" gave satisfactory results for about three years (90 passages) by the brain to peritoneum method in guinea-pigs and then died out.

Thus, a difference of importance but perhaps not of first importance, is shown in the behaviour of the viruses of rat typhus and of the *fièvre boutonneuse*—tick-bite fever group in the guinea-pig.

#### 10. *The Clinical Picture in Other Animals.*

It is questionable whether the rat, mouse, dog or sheep are of value in differentiating murine typhus from the other four typhus-like diseases with which we worked. The rat was a suitable animal in which to maintain the murine strain but, on the other hand, both "Appleton" and "Robertson" could be passaged in this animal. On the contrary, we did not succeed in adapting "Hare" to the rat. Sufficient work was not carried out in the other animals to permit a definite statement, but no indication was got that they were of value in differentiating one disease from another.

The association of the dog with tick-bite fever is an aspect of this problem which merits discussion. Pijper insists that tick-bite fever has no association with dogs except in so far as they may act as mechanical carriers of infected ticks to the habitation of man, the ticks having picked up their infection by feeding on some unidentified reservoir in a previous developmental stage. Gear, on the other hand, emphasizes the association of his cases with the deticking of dogs, but this observation throws no light upon the susceptibility of the dogs. The fact that a strain of rickettsia was isolated from the dog tick, *H. leachi*, also is of little value since the larvae of this tick are known to feed on an exceedingly wide variety of hosts. In these studies we have pointed out our failure to prove experimentally the susceptibility of the dog, but the value of this negative finding in a small number of experiments is largely discounted by our inability to be certain of the initial susceptibility of our animals. The final elucidation of this point therefore must await the completion of work on dogs, born and bred, preferably for two generations, under tick-free conditions. On the other hand, it must be borne in mind that our strain "Appleton" was isolated from the blood of a dog showing clinical symptoms of a febrile disease.

#### 11. *Clinical Picture in Man.*

This criterion is included in this paper merely for the sake of completeness. From a perusal of the literature it is apparent that, apart from the primary sore which may be regarded as an almost pathognomonic symptom of tick-bite fever, it would be exceedingly difficult to differentiate this disease from typhus except in classical cases.

#### DISCUSSION.

Our final conclusions may be open to the criticism, possibly justifiable, that we have incorrectly labelled the various strains of rickettsia as the causal organisms of murine typhus, tick-bite fever and *fièvre boutonneuse* respectively. In the absence of "type cultures" isolated from classical cases of the diseases this is a possibility, but not, in our opinion, a probability. Our strain of rat typhus was that used by Gear in his studies, the infectivity for man and the course of the disease resulting from accidental laboratory infection being described in detail by Gear and Becker (1938). Our "Hare" and "Appleton" strains proved identical with Gear's "Robertson" strain isolated from a human being suffering from what he considered to be tick-bite fever. The strain of *fièvre boutonneuse* came from the same source as that used by Gear and was isolated from ticks in a manner similar to that described by Pijper (1936). Consequently we do not feel that any unjustifiable claims for the correct identification of the respective rickettsias have been made.

The five strains of rickettsia appear to fall into two distinct groups when due consideration is given to the sum-total of those criteria which have been the subject of detailed study. In the one group falls the murine, endemic or rat-typhus strain which has

been compared with epidemic louse-borne typhus by Gear. In the other group fall strains "Robertson" (tick-bite fever of man), "Appleton" (from a dog), "Hare" (from ticks collected from a hare) and *fièvre boutonneuse* (from ticks collected from dogs at the Pasteur Institute, Tunis). It is not suggested that the name tick-bite fever should be replaced by either the French or the American term, since the name tick-bite fever has rightly received international recognition as that applied to a specific rickettsial disease in South Africa and has come into popular usage in the country. However, it is suggested that, from a biological and etiological point of view, the relationship of the various diseases should be clearly recognized or understood. Bearing in mind Pinkerton's classification of the etiological agents of the rickettsial diseases, it is proposed that the name "*Rickettsia prowazeki* var. *mooseri*" be retained for the causative agent of South African endemic typhus and that the name "*Dermacentor. renus rickettsi* var. *pijperi*" be given to the causative agent of South African tick-bite fever. The variety name "*pijperi*" is suggested in honour of Dr. A. Pijper of Pretoria who is unquestionably the pioneer in the investigation of rickettsial diseases of man in South Africa.

If this nomenclature is adopted it is apparent that certain additional modifications will become necessary; for instance, the name "*D. rickettsi* var. *conori*" should be the name given to the rickettsia causing *fièvre boutonneuse*. Further, the generic name "*Rickettsia*" cannot be allowed for *Rickettsia ruminantium*, *R. boris*, *R. ovina* or *R. canis*, although the general names "rickettsia" and "rickettsiosis" may be retained since all are specific genera of the family *Rickettsiaceae*. These latter rickettsias differ so markedly morphologically and biologically from those of the typhus-spotted-fever group that the separation is merited, but no generic name is suggested pending the completion of adequate comparative studies.

#### SUMMARY.

1. The five strains of rickettsia are discussed and compared according to the criteria suggested by Pinkerton (1936).
2. It is concluded that they fall into two groups:
  - (a) Typhus group—endemic, murine or rat typhus.
  - (b) Rocky Mountain spotted fever group—*fièvre boutonneuse*, strains "Robertson", "Appleton" and "Hare", which are similar and show only minor strain differences.
3. A proposed nomenclature is discussed.

#### ACKNOWLEDGEMENTS.

We have much pleasure in acknowledging our indebtedness to Dr. J. H. S. Gear, of the South African Institute for Medical Research, for the rat-typhus and tick-bite-fever strains and for much helpful criticism and advice, and to Dr. L. Balozet, of the Pasteur Institute, Tunis, for a supply of *fièvre-boutonneuse*-infected ticks.



## REFERENCES.

- ALEXANDER, R. A. (1938). Studies on the neurotropic virus of horse-sickness VI. Propagation in the developing chick embryo. *Onderstepoort Jnl. of Vet. Sc. and An. Ind.*, Vol. 11, pp. 9-19.
- BARYKINE, W., KOMPANESY, A., BOTCHAROWA, A. and BAVER, H. (1938). Nouvelle méthode de culture du virus du typhus exanthématique. *Off. Internat. Hyg. publ.*, Vol. 30, pp. 326-331. (Abstract *Zentralb. f. Bakt.* 1. Ref., Vol. 130, p. 212.)
- BENGTSON, I. A. and DYER, R. E. (1935). Cultivation of the virus of Rocky Mountain spotted fever in the developing chick embryo. *U.S. Pub. Health. Rep.*, Vol. 50, pp. 1489-1498.
- BRUMPT, E. (1927). Précis de parasitologie. Masson and Cie, 4e édition, Paris p. 872.
- COMBIESCO, D. and ANGELESCO, J. (1933). Recherches expérimentales sur le typhus exanthématique chez le chien. *C.R. Soc. Biol.*, Vol. 113, pp. 497-498.
- COWDRY, E. V. (1926). Cytological studies on heartwater. I. The observation of *Rickettsia ruminantium* in the tissues of infected animals. *11th and 12th Reports, Director of Vet. Ed. and Res. Union of S.A.* pp: 161-177.
- DA CUNHA, A. M. (1934). Sur la culture des *Rickettsia* du typhus exanthématique de Sao Paulo dans la membrane chorio-allantoïde de l'embryon de poulet. *C.R. Soc. Biol.* Vol. 117, pp. 392-394.
- DURAND, P. (1932). La réaction de Weil-Felix dans la fièvre boutonneuse. *Arch. Instit. Pasteur, Tunis.*, Vol. 20, pp. 395-421.
- DURAND, P. (1933). Etude expérimentale de quelques virus exanthématiques chez le chien. *Arch. Instit. Pasteur, Tunis.*, Vol. 21, pp. 484-503.
- GEAR, J. H. S. and BEVAN, C. (1936). An outbreak of tick-bite fever. *S.A. Med. J.*, Vol. 10, pp. 485-488.
- GEAR, J. H. S. (1938). South African typhus. *S.A.J. Med. Sci.*, Vol. 3, pp. 134-160.
- GEAR, J. H. S. and BECKER, L. H. (1938). A case of typhus fever contracted in the laboratory from a virus isolated from rats. *S.A. Med. J.*, Vol. 12, pp. 57-60.
- GEAR, J. H. S. and DOUTHWAITE, M. (1938). The dog tick *Haemaphysalis leachi* as a vector of tick typhus. *S.A. Med. J.*, Vol 12, pp. 53-55.
- GIRAUD, P. and TANNENBAUM, S. (1937<sup>1</sup>). Faits concernant la réaction de Weil et Felix. *C. R. Soc. Biol.*, Vol. 125, pp. 668-670.
- GIRAUD, P. and TANNENBAUM, S. (1937<sup>2</sup>). Le comportement du cobaye vis-à-vis de la réaction de Weil et Felix peut-il éclairer la pathogénie de cette réaction. *Arch. Instit. Pasteur, Tunis*, Vol. 26, pp. 671-675.
- GRAY, F. C. (1931). A note on preliminary investigations of the Port Elizabeth type of fever. *J. Med. Ass. of S.A.*, Vol. 5, pp. 814-816.
- HASS, G. M. and PINKERTON, H. (1936). Spotted fever II. An experimental study of fièvre boutonneuse. *J. Exp. Med.*, Vol. 64, pp. 601-623.
- MAXCY, K. F. (1936). The Weil-Felix reaction of the rabbit in the diagnosis of Rocky Mountain spotted fever. *J. Infect. Dis.*, Vol. 58, pp. 288-292.
- MONTEIRO, J. L. (1934<sup>1</sup>). Etude comparative du " typhus exanthématique de Sao Paulo " (*Rickettsiose neutrophique*) et du " typhus exanthématique " d'autres lieux basée sur l'épreuve de protection par le sérum de convalescents. *C. R. Soc. Biol.*, Vol. 115, pp. 1360-1362.

STUDIES OF THE RICKETTSIAS IV.

- MONTEIRO, J. L. (1934<sup>2</sup>). Etude comparative entre le "typhus exanthématique de Sao Paulo" (Rickettsiose néotropical) et le "typhus exanthématique de Chili" (Rickettsiose épidémique) par l'épreuve de protection avec des serums de convalescents. *C.R. Soc. Biol.*, Vol. 116, pp. 1131-1132.
- NICOLLE, CH. AND LAIGRET, J. (1933). Conservation du virus typhique dans le cerveau des rats et des cobayes inoculés avec ces virus. *Arch. Institut. Pasteur, Tunis.*, Vol. 21, pp. 357-380.
- NICOLLE, CH. (1934). Sur les conditions de la recherche des infections inapparentes chez les animaux qui ne réagissent pas visiblement à l'inoculation du virus d'une fièvre exanthématique ou d'un produit dans lequel on suppose sa présence. *Arch. Institut. Pasteur, Tunis.*, Vol. 23, pp. 19-31.
- PARKER, R. R. AND DAVIS, G. E. (1933). Protective value of convalescent sera of Sao Paulo exanthematic typhus against virus of Rocky Mountain spotted fever. *U.S. Pub. Health Rep.*, Vol. 48, pp. 501-507.
- PHILIP, C. B. AND PARKER, R. R. (1938). The persistence of the viruses of endemic (murine) typhus, Rocky Mountain spotted fever and bouton-neuse fever in tissues of experimental animals. *U.S. Pub. Health Rept.*, Vol. 53, pp. 1246-1251.
- PIJPER, A. AND DAU, H. (1932). Cross immunity between South African typhus and tick-bite fever. *Brit. J. Exp. Path.*, Vol. 13, pp. 33-35.
- PIJPER, A. AND DAU, H. (1934). Die fleckfieberartigen Krankheiten des südlichen Afrika. *Zentralb. f. Bakt. 1. Orig.*, Vol. 133, pp. 7-22.
- PIJPER, A. AND DAU, H. (1935). South African typhus. *J. Hygiene.* Vol. 35, pp. 116-124.
- PIJPER, A. AND DAU, H. (1936). Étude expérimentale comparée de la fièvre bouton-neuse et de la tick-bite fever. *Arch. Institut. Pasteur, Tunis.*, Vol. 25, pp. 388-401.
- PIJPER, A. AND CROCKER, C. G. (1938). Rickettsiosis of South Africa. *S.A. Med. J.*, Vol. 12, pp. 613-630.
- PINKERTON, H. (1936). Criteria for the accurate classification of the rickettsial diseases (Rickettsioses) and of their aetiological agents. *Parasitology.* Vol. 28, pp. 172-189.
- ZIA, S. H. (1934). The cultivation of Mexican and European typhus Rickettsiae in the chorio-allantoic membrane of the chick embryo. *Amer. J. Path.*, Vol. 10, pp. 211-218.
- ZIA, S. H. AND WU, C. J. (1936). Serum treatment of experimental typhus. *Chinese Med. J. Suppl.* 1, February, pp. 270-274.
- ZINSSER, H. AND CASTANEDA, M. R. (1930). Studies on typhus fever, II. Studies on the etiology of Mexican typhus fever. *J. Exper. Med.*, Vol. 52, pp. 649-659.
- ZINSSER, H. AND CASTANEDA, M. R. (1933). Studies on typhus fever, XI. A report on the properties of the serum of a horse immunized with killed formalinized rickettsia. *J. Exp. Med.*, Vol. 57, pp. 391-398.