Clinical and Experimental Aspects of Exanthematous Typhus in the Tropical Regions of America[1]

Aids to Diagnosis

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AN INCREASING interest in the study of the Rickettsioses, especially of exanthematous typhus, has been evident, not only in countries where the latter disease constitutes an important sanitary problem, such as in the United States, Mexico, Bolivia, and Chile, but also in those parts of the world where it has passed unrecognized because of the scant number of clear-cut cases. Typhus, which had previously been limited to the cold and temperate zones, has extended into frankly tropical regions.

In Mexico, typhus occurs endemically in the two prevailing climatic regions of that country. In the highlands, where the climate is temperate or cold, three varieties are found: one caused by the pure murine virus, another identical to the classical European typhus, and a third of intermediate type, characterized by presenting the properties of classical typhus in the first passage in guinea pigs and by reverting later to the murine type.[2] It is quite possible that this diversity is nothing else than the result of a constant transformation of murine into classical typhus through adaptation to a different host. The lowlands of Mexico belong climatically to the tropical and subtropical zones. In this connection, we wish to refer to certain investigations[3] carried out in Zacoalco, in the province of Jalisco, where typhus occurs endemically in a mild, atypical form, so that it is usually mistaken for malaria and even for typhoid-paratyphoid infections. In that region murine typhus was found both in man and in the rats captured. The subtropical climate of this province is not very favorable to the development of *Pediculus vestimenti*, for which reason transmission from man to man seems to be rather

1. Received for publication July 2, 1942.

2. M. Ruiz Castañeda, *J.Exper.Med.*, LII (1930), 195.
H. Mooser, G. Varela, and H. Pils, *J.Exper.Med.*, LIX (1934), 137.
M. Ruiz Castañeda and Roberto Silva G., *Pub.Health Rep.*, LIV, No. 29 (1939), 1337.

3. M. Ruiz Castañeda, *Rev.Med.Hospital General*, I (1939), 513. Roberto Silva G. and R. Reyes Ochoa, *Medicina*, XXI (1941), 148.

infrequent. In the tropical provinces of Nayarit and Sinaloa on the Pacific coast, the number of febrile cases with a serology suggestive of typhus is steadily increasing but, so far, the type has not been determined, although this is probably murine. In the port of Acapulco, also on the Pacific coast, we saw a case that had undoubtedly contracted the infection there and from which we isolated typical murine virus.

The epidemiological aspects of typhus in Mexico are much more complex than we had thought up to the year 1930. In addition to the classical typhus described by Veintemillas[4] in Bolivia and by Groot[5] in Colombia, there is evidence for the belief that murine typhus also exists in Chile,[6] Argentina,[7] and Brazil.[8] Future studies will undoubtedly disclose a wider spread of this last type, perhaps the only one that can prevail in tropical zones where the lack of man-to-man vector restricts the source of infection to rats, as in the greater part of the United States. Nonetheless, it is highly significant that, in Cuba, Curbelo *et al*[9] should have discovered recent cases of atypical typhus with classical serology giving rise to inapparent infections in the guinea pig, even though bodies identical to those of *Rickettsia prowazeki* were found in that animal. Through the courtesy of Dr. Curbelo we were able to study convalescent sera, finding that they agglutinated a Rickettsial antigen and had the power of immunizing against Mexican murine typhus.

Because of the above observations we have thought it indicated to review all the data which, in our experience, may guide the student of typhus foci in tropical countries where, because of climatic conditions, the disease is acquired directly from the rat and where the human infection is generally mild and atypical, as compared with classical European typhus.

II

Since the typical clinical case of the stuporous and exanthematous variety seems not to occur in the tropical regions of America, it would be rather difficult to describe its clinical aspects in a manner that would facilitate recognition of the average case. We must

4. F. Veintemillas, Suplemento Inst. Nacional Bacteriología, 1935, La Paz, Bolivia.
5. H. Groot, *Pub.Lab.Hig.de Nariño* (1942), pp. 37, Pasto, Colombia.
6. M. G. Contreras Macaya and A. Macchiavello Varas, Antofagasta, Chile, Tip. Droguett y Cía., 1932.
7. E. A. Molinelli and M. Rissel, *Bol.San.Depart.Nac.Hig.*, IV (1940), 209.
8. J. Lemos Monteiro and F. da Fonseca, *Compt.rend.Soc.de biol.*, CXII (1933), 402.
9. A. Curbelo and E. Marrero Vela, *Vida Nueva*, XLVII (1941), 53.

mention, however, the three fundamental characteristics which rarely fail to appear in cases of typhus, no matter how mild they be: fever, headache, and an exanthem.

The fever is usually continuous and lasts from ten to twenty days, generally two weeks. Ordinarily, it has a gradual beginning, rising to a maximum toward the end of the first week and decreasing slowly to normal. Very seldom will there be an abrupt onset or end. The headache is in most cases rather troublesome but may be temporary and, sometimes, does not even appear. The exanthem is usually mild, yet it can be as intense as in the typhus of the highlands, whether of the murine or epidemic type. On the other hand, this last aspect may pass unnoticed, either because of its mildness or because of the dark complexion of the inhabitants of these tropical regions. We have seen an ambulatory case in a white-skinned individual which presented no eruption. This patient had a moderately high temperature but a very intense headache.

As seen in the United States and in tropical Mexico, typhus of murine origin begins its course with symptoms that are diagnosed as influenza, malaria, or a gastrointestinal disturbance, during the first week. As I have already said, it was only recently that attention was called to the cases in our midst because of a positive Weil-Felix reaction. It is therefore recommended that all cases of undetermined fever be systematically investigated by means of agglutination tests with Proteus X-19 and, if possible, with specific antigen. This mode of attack has been particularly useful to us in dealing with patients, not only from tropical regions, but also from Mexico City, where the infection may be so atypical that the diagnosis can only be established through serological studies.

III

The serological diagnosis of typhus has become so simple and dependable that it allows us to reach a definite diagnostic conclusion in a few minutes. The classical Weil-Felix reaction continues to be the principal basis of diagnosis, but this has been so modified and simplified that it eliminates possible causes of error due to modification of the antigen. The method which we have followed for some years consists in preparing fixed antigens that maintain their sensitivity for long periods and allow a careful standardization both in an ordinary laboratory and in those with more ample facilities. Furthermore, our antigen has the advantage of facilitating a rapid diagnosis at the bedside with only one drop of whole blood.

This test is performed on a slide by mixing one drop of the antigen with a loopful of blood and then moving the slide back and forth to help the process of agglutination. Since the antigen is stained blue, the clumps stand out prominently against the red background, when the reaction is positive. When the test is negative, on the other hand, the mixture maintains a uniform coffee color. Readings are made within one minute. It is interesting to note that the sensitivity of the test has been so adjusted that reactions are positive only when the titer of agglutination is above 1:50. Lower titers do not yield a positive test, thus saving the investigator from considering such reactions that are of no significance, as far as the diagnosis is concerned.

The preparation of the antigen is simple enough.[10] One must select a strain of Proteus X-19 free from motile organisms and of sufficient sensitivity when mixed with the serum of typhus patients. Twenty-four- to forty-eight-hour cultures are suspended in saline solution and filtered through cotton; formalin is then added to make a final concentration of 10 percent. The volume of emulsion may be 50 to 100 cc. for each Roux bottle of media. The formalin is then allowed to act for seventy-two hours, after which the organisms are washed, at least twice, by centrifuging and resuspending in distilled water. To the washed suspension there is now added sufficient methylene blue to give the antigen an intense blue color, which is of great aid in the agglutination test. After removing the excess dye by another centrifugation, the organisms are suspended in a small volume of isotonic sodium citrate solution and so diluted that 0.1 cc., when added to 25 cc. of water, will yield a turbidity of 3 cm. with Gates' loop.

The antigen should be standardized by means of serum of known titer or, lacking this, by means of dilutions of serum corresponding to titers between 1:50 and 1:100. The antigen is mixed with saline in different proportions, such as, for example, 0.9 to 0.5 and 0.1 to 0.5. With these dilutions, tests are carried out on a slide, drop by drop, utilizing a loop with a diameter of 5 mm. One selects the mixture yielding the best reactions with the dilutions of serum that correspond to titers between 1:50 and 1:100. In accordance with the results obtained, the volume of antigen can then be corrected by adding the necessary amount of citrate solution. If the antigen is

10. M. Ruiz Castañeda, Roberto Silva G. and A. Monnier *Rev.Med.Hospital General*, VIII (1940), 382.

prepared carefully, it is not necessary to add a preservative. It may happen that the mixture loses its color from contamination, in which case, we not only keep the antigen in the icebox but also add formalin in a proportion of 0.2 percent, or merthiolate (1:10,000). Our experience with the method is not yet sufficient to tell us what the end effect of such a preservative on the sensitivity of the antigen may be, but this antigen has been very useful in the diagnosis of many cases of typhus, confirmed in the classical way.

A frankly positive test is of as much value as a Weil-Felix of above 1:100. Negative reactions have the same significance as a Weil-Felix test that is negative, or of a titer lower than 1:50. The latter, however, does not eliminate the possibility of typhus being present, for which reason it is convenient to obtain serum from the patient and carry out a second test by mixing equal parts of serum and antigen in the manner recommended for the process of standardization. A positive reaction with these mixtures may be of value, but one should await confirmation by the specific test, or by obtaining a new blood sample. It should be remembered that, in many cases of typhus, the titer is not above 1:100 or even 1:50. Agglutinins against Proteus X-19 may appear early with appreciable titers even on the fourth day, but, as a rule, these should not be expected until the fifth day.

Rapid specific diagnosis. The use of Rickettsial suspensions for agglutination tests with typhus serums was first carried out in 1931 with Weigl antigen (emulsion of infected lice), and with the murine antigen prepared by Zinsser and Castañeda. These authors[11] were able to carry out agglutination tests in tubes with practically pure suspensions of formalinized Rickettsiae. However, it was not possible to obtain antigen on a practical scale until the Rickettsiae were grown in the lungs of rats.[12] With phenolized Rickettsiae prepared in our laboratory, Hudson[13] was able to obtain numerous agglutination reactions in test tubes in a manner similar to that used for Widal reaction. His results revealed the great specificity of the test, as shown by the fact that typhus serums could agglutinate in dilutions, at times, of 1:1,000, while the serums of persons from regions like Ohio, where typhus fever is not encountered, were negative even in a dilution of 1:2. An interesting observation revealed from the above work is that the serum of vaccinated persons, or of those who

11. H. Zinsser and M. Ruiz Castañeda, *J.Exper.Med.*, LVI (1932), 455.
12. M. Ruiz Castañeda, *Am.J.Path.*, XV (1939), 467.
13. N. P. Hudson, *J.Infect.Dis.*, LXVII (1940), 227.

have had typhus, will not usually agglutinate to titers higher than 1:10, a fact that should be of practical value, since the agglutination titer in cases of typhus is always above that dilution.

We have tried to simplify the specific agglutination reaction by preparing sufficiently sensitive antigens that will enable us to carry out the test on a slide by only mixing equal drops of serum and antigen. This antigen consists of a phenolized suspension of approximately twenty billion Rickettsiae per ccm. The sensitivity of such a suspension is really perfect and can yield a frank agglutination test to a titer of 1:640 with typhus serums.

Agglutinations with serum diluted at 1:10 are of diagnostic significance. The test is performed in the following manner: the serum to be studied is submitted to a preliminary test by mixing, on a glass slide and with a loop 3 mm. in diameter, one drop of antigen and one of serum. The slide is moved back and forth and examined over a light or in front of a window. If the mixture remains uniform, the results are negative. If clumps form, one must obtain the titer by making up serum dilutions from 1:5 to 1:160 in a series of test tubes. One then places two or more drops of antigen on a glass slide, conveniently apart. With a loop, drops of the first two or three dilutions are taken up, mixed with the antigen, and the loop flamed after each mixing. The results are read and, if necessary, the process is repeated each time with a larger dilution, until the highest titer is reached. Ordinarily, a glance at the slide is all that is needed, but a magnifying glass may be useful at times. However, we have noted that the agglutinating titer, obtained in this manner, does not usually correspond to the results obtained in test tubes, though a slide agglutination may be considered to correspond to dilutions four times as high as those carried out in test tubes. We have therefore adopted it in our routine because of its specificity and the rapidity with which it can be carried out.

In summing up the steps necessary to carry out a rapid serological diagnosis for typhus, we have:

1. A rapid bedside test with Proteus X-19, utilizing whole blood. If positive, it is equivalent to a Weil-Felix reaction of over 1:100; if negative, it has the value of a Weil-Felix of 1:50, or less.

2. Blood serum is obtained for confirmation of the results of agglutination with Proteus X-19.

3. The preliminary test with Rickettsial antigen is practiced. If positive, the titer is then determined.

Since the Proteus X-19 is standardized so as to yield minimal

agglutination reactions at 1:50, one may obtain the approximate Weil-Felix titer by mixing this antigen drop by drop with the same dilution utilized in obtaining the agglutination titer for Rickettsiae. The titer will correspond, approximately, to the highest dilution multiplied by fifty. Of course, the great difference in sensitivity of both antigens, when mixed with the same serum, is worthy of attention.

Interpretation of results with the agglutination tests. A positive test, be it with whole blood or serum and X-19 antigen and confirmed by a positive agglutination test with Rickettsiae, even at a low titer, may be considered indicative of typhus fever, its diagnostic value increasing in proportion to the intensity of the reactions. It is unusual for reactions to be positive to the X-19 and negative to the Rickettsiae, yet we have encountered one such case. Nonetheless, both reactions were positive in a subsequent test.

It is a frequent finding that, during the first days of the infection, the reaction to X-19 remains negative while that to the Rickettsiae is positive, at times, even to a high titer. In these cases, agglutinins for Proteus X-19 usually appear later. Lastly, when both reactions remain negative with different samples, obtained at intervals of two or more days, the possibility of typhus fever can be totally discarded.

There is always the risk of having low agglutination reactions with Proteus X-19 and Rickettsiae, without being able to confirm the diagnosis of typhus. In these cases, the disease may prove to be something else, or the previous history of the patient may clear up the diagnosis, but there have been times when we have not been able to reach a definite conclusion. However, there are no important variations in the agglutination titer, as have occurred in typhus fever when repeated tests are performed throughout the course of the illness.

The advantages afforded by these tests are great, particularly since one is dealing with procedures that are easy and quick to perform, but their reliability depends on the care with which the antigens are prepared.

The phenomena of opsonization and complement fixation, reported previously,[14] have not yet been applied to the practical diagnosis of typhus. The technique of these tests is complicated, and their great sensitivity may give rise to erroneous interpretations.

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14. M. Ruiz Castañeda, *J.Immunol.*, XXXI (1936), 227, 285.

However, an interesting possibility exists as regards the opsonic phenomenon; considerable difference has been noted in the opsonic activity of the serum of animals having recuperated from typhus and from Rocky Mountain spotted fever. While this activity is very intense in typhus, it is only mild in the serum of animals convalescing from spotted fever. It is well known that the serum will agglutinate the Proteus X-19 and the *Rickettsia prowazeki*[15] in both infections.

Another immunological reaction, applicable to the diagnosis of typhus, is the test for allergy. This is performed with a suspension of formalinized Rickettsiae,[16] injected into the dermis and producing toxic and allergic reactions in the patient. Toxic reactions are observable in individuals who have not had typhus fever; these are negative in the immune. Allergic reactions are apparent in persons immune to typhus and are negative in normal people, and at the beginning of the disease. These phenomena have not been sufficiently studied but, in special cases, have been of aid in determining a previous typhus infection.[17]

IV

Determination of the type of virus. Up to now, the type of virus could only be determined by inoculating susceptible animals. However, the more intensely one studies the nature of the virus, the greater is the conviction that it was originally murine and that, on passage to a new host and through transmission under conditions different from the natural ones, it has been undergoing modification until it has acquired properties entirely at variance with those of the original virus. In general terms, its pathogenicity for the rat has been diminishing while increasing, at the same time, for the guinea pig. It is therefore well to keep these points in mind when trying to isolate the virus from an infected patient.

Following the findings of Mooser and, upon trying to isolate murine, intermediate, and even European virus, we have obtained good results by inoculating rats intraperitoneally with citrated blood taken from the sixth to the twelfth day of the illness. These rats are sacrificed on the fifteenth day and male guinea pigs are inoculated, also intraperitoneally, with a brain emulsion made from

15. M. Ruiz Castañeda and Roberto Silva G., *J.Immunol.*, XLII (1941), 1.
16. M. Ruiz Castañeda, *J.Exper.Med.*, LXIV (1936), 701.
J. Vargas Curiel, Tesis, México, 1939.
17. F. Veintemillas, *J.Immunol.*, XXXVI (1939) 339.

the rats. The murine virus will give rise to fever and scrotal reactions and Mooser cells and Rickettsiae will be found in smears of the tunica vaginalis; the latter will occur both extracellularly and phagocytized by polymorphonuclear leukocytes. Typhus of the intermediate type gives rise to fever of eight to ten days' duration, but scrotal reaction is established only through successive passage in the guinea pig. European typhus maintains indefinitely a purely febrile reaction.

When the infection is expected to be of the European type, it is well to immediately inoculate guinea pigs with blood from the patient, as rats are less sensitive to this virus than to murine. It must be remembered that the female guinea pig contracts an inapparent infection when inoculated with murine typhus, while it reacts with fever when inoculated with the European type.

Once the virus is implanted in the guinea pig, it may be kept by serial passages, either by inoculating washings of tunica vaginalis or by brain emulsions, according to the type of infection. In both instances, the diagnosis has to be corroborated by histological examination and by protection tests with known strains. The different serological tests should also be tried.

The histological examination reveals numerous brain lesions of the type described by Wolbach,[18] when the virus is of the European type, but these same lesions are very infrequent in murine infections. Rickettsiae may be observed in the tunica vaginalis of guinea pigs infected with the European type, but this occurs only during the first days of the infection. However, by x-raying the guinea pigs, one can find Rickettsiae quite easily, especially in fatal infections; hemorrhages in the testicles are also frequently observed.

Since no agglutinins are formed against Proteus X-19 in the guinea pig infected with typhus, the agglutination reaction must be carried out with Rickettsial antigen. If one desires to observe the formation of agglutinins for Proteus X-19, rabbits may be inoculated. In the case of murine typhus, we have obtained fairly high agglutination titers, appearing early, when the virus was inoculated into lambs.

Cultures in ordinary media, from the brain and the tunica vaginalis, must always remain sterile. When the virus is being isolated directly from wild rats, one must bear in mind the frequency with which a scrotal reaction may be provoked by Sodoku, for which

18 B. S. Wolbach, J. L. Todd, and F. W. Palfrey (Cambridge, Mass.: Harvard University Press, 1922).

reason we recommend that dark-field examinations be carried out constantly in such work.

The protection tests are of great diagnostic value since both murine and European typhus give rise to crossed immunization to one another but not to the virus of Rocky Mountain spotted fever. When known strains of typhus or Rocky Mountain spotted fever are not available, we recommend that the serum from convalescent animals be sent to other laboratories for serological and passive protection tests.

SUMMARY

The author describes practical ways of diagnosing typhus, especially in tropical regions of America where the infection, apparently of murine type, is clinically atypical.

The author gives simplified tests for facilitating a specific diagnosis in man, and indicates the minimal data necessary for identification of the type of virus, when this is inoculated into laboratory animals.