

The proper technique seems to be as follows: (1) injection of a total of 175 000-200 000 sporozoites in 5-6 inoculations and (2) bleeding within a month of the last immunizing inoculation. In further research we shall try to standardize a method which will give optimum results.

Staining sporozoites of plasmodia with fluorescent antibodies opens the way to research which may have practical importance. The fluorescent antibody staining appears to be specific and it may therefore prove possible to identify sporozoites found in mosquitos captured in nature.

Serological Tests for the Confirmation of Plague Infections: A Preliminary Communication

by K. F. MEYER, M.D., *George Williams Hooper Foundation, University of California, San Francisco, Calif., USA*

A rural plague outbreak in Ovamboland, South-West Africa, in 1962 and 1963 offered the opportunity of examining the sera of persons who had recovered from the disease after treatment with streptomycin. For the performance of these tests the technical procedures originally developed by Chen and co-workers^{a, b} and recommended by an international group of experts (see Baltazard et al.,^c) have been refined by the use of a highly purified *P. pestis* Fraction 1 (F₁) antigen and have been adapted to the microtitre equipment described by Sever.^{d, e} Initial tests were made with serum specimens collected at intervals ranging from 6 to 55 days after onset of the disease.

Out of 25 clinically suspected Ovambos (11 of whom had been bacteriologically proved to suffer from plague) 20 reacted positively in haemagglutination (HA) tests and 13 in complement-fixation (CF) tests with the purified F₁ antigen. The HA macroscopic serum titres ranged from 1:512 to 1:32 768, those in the complement-fixation tests from 1:4 to 1:256.

The five 7-18-year-old persons who reacted negatively in the HA tests were stated to have suffered respectively from "typical" femoral or inguinal buboes, atypical or mild plague, non-specific lymphadenitis, and a "furuncle" near the umbilicus.

A re-examination of the sera of the positively reacting individuals 7-9 months after onset of the disease revealed CF titres varying from zero to 1:8, while the HA titres dropped, as a rule, to one-half to one-sixteenth of their original level. In three persons, two of whom were under 10 years of age, the originally significant HA titres (1:32, 1:2048 and 1:8192) had even declined to zero within eight months. However, in marked contrast to these findings, a woman who had suffered from a culturally confirmed plague attack and had shown an extraordinarily high initial HA titre (1:32 768) still had a CF titre of 1:16 and an HA titre of 1:8192. Since X-ray examination showed a chronic pathological process in her left hip joint suspected to be due to an unresolved plague infection, she was once more intensively treated with streptomycin. Marked clinical improvement ensued but it has not yet been possible to retest her serum.

During a personal inspection tour made by the author eight months after the outbreak, 16 additional serum specimens were collected from Ovambos, in 14 of whom a diagnosis of plague had been made on clinical grounds, while in two the presence of the disease had been bacteriologically confirmed. In seven members of this group HA tests gave a positive

^a Chen, T. H., Quan, S. F. & Meyer, K. F. (1952) *J. Immunol.*, **68**, 147.

^b Chen, T. H. & Meyer, K. F. (1954) *J. Immunol.*, **72**, 282.

^c Baltazard, M., Davis, D. H. S., Devignat, R., Girard, G., Gohar, M. A., Kartman, L., Meyer, K. F., Parker, M. T., Pollitzer, R., Prince, F. M., Quan, S. F. & Wagle, P. (1956) *Bull. Wild Hlth Org.*, **14**, 457-509.

^d Sever, J. L. (1962) *J. Immunol.*, **88**, 320.

^e A full final report giving details of the test procedures will appear in due course.

result with titres ranging from 1:4 to 1:4096. Out of two individuals in whom insignificantly low HA titres were found, one had suffered from a culturally confirmed plague attack while in the other the presence of the disease had been merely suspected.

When an attempt is made to evaluate these findings, it seems at first glance disconcerting that a part of the sera of persons supposed to have suffered from plague showed no HA antibodies eight months after onset of their illness. It deserves attention, however, that eight of the tested persons were 3-11 years old, thus possibly having suffered in part from non-specific affections of their lymph-nodes that are often met in childhood. It must also be seriously doubted whether all the adults recorded as plague patients actually suffered from this disease. Indeed, four of them had been listed with such vague entries as "particulars incomplete" (2); "questionable" and "lues or lymphogranuloma".

Nevertheless, though instances are on record in which HA tests gave positive results even three years after plague attacks (see, for instance, Payne and associates^f), it has to be admitted that it is not always possible to arrive at a retrospective diagnosis of the disease through serological examinations made eight months after recovery. It has to be emphasized, however, that even under such unfavourable circumstances the serological tests gave significant results. Out of the 83 patients, with 7 deaths, officially recorded as the toll of the 1962-63 outbreak, 41 patients were available for careful study. Only 15, or 36%, of the 41 could be confirmed through bacteriological examinations, whereas a retrospective serological diagnosis was obtained in 27 instances, or 78%. HA and CF tests made two to three months after onset of the disease confirmed the cultural findings.

The difficulties that often stand in the way of an adequate investigation of rural plague manifestations are well known. Attempts to collect and transport specimens of bubo fluids or blood cultures are apt to fail, particularly during the hot season, and as a consequence the clinical diagnoses are frequently not confirmed through bacteriological tests. It is therefore often the practice to report in the course

of an outbreak every person with lymphadenitis or other suggestive signs as a plague patient and afterwards to sort out the real cases through a study of the records. However, it is difficult to obtain reliable results when analysing the often sketchy histories of the patients in a purely inductive manner. The now proposed method of serological tests might go a long way to overcome this difficulty. Therefore, no effort ought to be spared to procure serum specimens for examination, preferably on the day the patients are discharged from treatment in the hospitals or isolation centres. The specimens of non-haemolysed serum will have to be sent to a central laboratory capable of performing HA and CF tests with standardized F₁ antigens. That such a transport of serum specimens is within the realm of practical possibilities has been shown in the course of the above-recorded investigation.

Studies in progress indicate that the new serological tests may be also of outstanding value in the conduct of surveys to detect the presence of rodent plague, supplementing, or even in time replacing, the laborious method of autopsies followed by the inoculation of organ pools or the likewise tedious method of animal experiments with flea pools. Thus far tests with over 1000 East African rodents belonging to different species have yielded between 5% and 32% of serum specimens which proved positive in HA tests. *Arvicanthis* sp. and *Rattus natalensis* showed titres as high as 1:512 and 1:2048, while *R. rattus* and *R. kaiseiri* in towns frequently reacted in dilutions of 1:32 and 1:512. As shown by comparative observations on artificially infected laboratory animals, it would appear that the free-living rodents showing HA antibodies in their sera had recovered from sublethal plague infections.

No doubt large-scale investigations will be necessary to confirm this assumption and to ascertain when the height of the HA reactions is reached and how long the positive findings persist. It may be claimed, however, that the results obtained thus far give reason to hope that this expedient method of serological sampling of the rodent populations will prove of practical value. Such serological testing would be particularly useful in the absence of manifest epizootics when it has to be decided whether plague has altogether disappeared or continues to exist in an inapparent form, difficult to detect with the aid of the methods available hitherto.

^f Payne, F. E., Smadel, J. E. & Courdier, J. (1956) *J. Immunol.*, 77, 24.