WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

EXPERT COMMITTEE ON YELLOW FEVER

Entebbe, 9-15 March 1971

INDEXED

VIR/YF/71.10

ENGLISH ONLY

RECENT ADVANCES IN DIAGNOSTIC METHODS FOR ARBOVIRUSES AND THEIR POSSIBLE APPLICATION TO YELLOW FEVER

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One of the major handicaps to effective yellow fever surveillance in South America and Africa has been the difficulty in obtaining and utilizing rapid, reliable and inexpensive diagnostic laboratory procedures. The isolation of yellow fever strains by present methodology requires the availability of a suitable mouse colony and serological diagnosis necessitates the use of multiple reagents and serological test procedures. None of the above procedures have been effectively adapted to widespread field use thus requiring the preservation and shipment of fresh material to central laboratories often located hundreds of miles away from field areas.

Viscerotomy was successfully utilized in Brazil as a practical method of alleviating some of the difficulties outlined above (Soper, Rickard & Crawford, 1934). Unfortunately, up to the present time, this potent epidemiological tool has not found wide acceptance in Africa.

The search for new diagnostic procedures which might be more adaptable has continued. Some of these procedures will be outlined below along with their potential application. It should be kept in mind that as of the present time none of them have been developed to the point where they can replace existing procedures.

The isolation of yellow fever virus by mouse inoculation and subsequent identification utilizing mouse neutralization tests is a lengthy procedure requiring at least 2-4 weeks resulting in considerable delay when a quick answer is most often necessary. Recently, agar gel diffusion and immunofluorescence (IF) have been applied to the diagnosis of arbovirus isolates obtained by inoculation of suckling mice. Crude mouse brain harvests can be used in the agar gel diffusion test for positive and relatively specific identification of isolates within 24 hours of harvest (Calisher & Maness, 1970). This system has been successfully applied to several arbovirus groups but needs further study within arbovirus group B to increase specificity. The direct IF test has been used in a limited way utilizing tagged immune mouse fluids and appears to be rapid and specific (Emmons, pers. comm., 1970). This test requires further refinement and has the disadvantage of requiring expensive equipment and well trained staff.

The emergence of cell culture systems for the isolation and characterization of arbovirus isolates does hold promise of a less expensive and more rapid isolation system. It should be possible to develop vertebrate or invertebrate cell cultures which will show cytopathic effect (CPE) upon inoculation with yellow fever in a manner analogous to the CPE seen in Aedes albopictus with dengue and West Nile viruses. The IF test could be developed to specifically identify yellow fever isolates in the early stages of CPE. The application of this methodology might shorten the time required for isolation and identification to less than a week.

Of perhaps greater potential significance has been the demonstration by Emmons & Lenette (1968) that direct IF could be used on blood smears from patients with Colorado tick fever (CTF). CTF is known to be largely cell associated. Previous work has demonstrated that some yellow fever is also cell associated and certainly this technique should be attempted in yellow fever diagnosis. The obvious advantages to such methodology are that very rapid diagnosis is possible and field collection and handling of specimens would be considerably easier than with present techniques.

One of the major drawbacks to current serological procedures is the lack of specificity of the presently available reagents in such rapid and relatively inexpensive test as the haemagglutination-inhibition test. Currently, several groups are working on the purification and fractionation of arbovirus antigens in the hopes of producing specific reagents. Such efforts require sophisticated biochemical techniques which have been successfully applied in other areas of virology.

There continues to be a need for quicker and more reliable serological techniques for immunity surveys. It is evident that plaque reduction neutralization tests in cell cultures have major advantages in time and cost saving over the existing mouse protection test. Plaque assay systems have been developed for many of the group B viruses in various hampster, monkey and pig kidney cell cultures. The experience of several different investigations indicates that the cell culture plaque reduction system is as sensitive as the mouse protection test and that by using micromethods, hundreds of sera can be quickly screened against several viruses. Further adaptation of this neutralization test should make it possible to test finger-stick capillary blood collected on filter paper. Such specimens are much easier to collect than the venous blood needed for the mouse protection test.

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