A Simple Plaque-Inhibition Test for the Study of Arthropod-borne Viruses

J. S. PORTERFIELD

The plaque-neutralization method developed by Dulbecco and co-workers for the study of the virus of western equine encephalomyelitis in cultures of chick fibroblasts, though probably unequalled for accuracy, has not been adopted for general use in laboratories engaged in research on the arthropod-borne viruses, for two reasons: (a) the belief that the technique would be suitable only for viruses with a powerful cytopathic effect on chick-embryo cells, and (b) the laborious procedures involved.

The simple plaque-inhibition test described in this paper was devised with the requirements of the field or diagnostic laboratory in mind; while less precise than Dulbecco’s method, this test is sufficiently accurate for many purposes and is far less laborious. The range of applicability of the technique is discussed, the author claiming that it can be used both as a qualitative test for the presence of neutralizing antibodies to a particular plaque-producing virus and as a test for the identification of unknown plaque-producing viruses.

The plaque technique of Dulbecco (1952) was first applied to the titration of an arthropod-borne virus, the western equine encephalomyelitis (WEE) virus, in cultures of chick fibroblasts, and a refined technique for the study of the kinetics of virus neutralization was later described for the same virus (Dulbecco, Vogt & Strickland, 1956). Whilst these methods are probably unequalled for accuracy, they have not come into general use in laboratories where the study of arthropod-borne viruses is a primary interest. This has been due in part to the belief that the plaque technique was applicable only to those viruses, such as WEE, which produce a powerful cytopathic effect on chick-embryo cells, and in part to the fact that the labour involved in plaque-neutralization tests is considerable.

It has been shown elsewhere that yellow fever and a number of other group-B arthropod-borne viruses could be titrated in chick-embryo cells by a modified Dulbecco technique (Porterfield, 1959a, 1959b). A technique in current use at the National Institute for Medical Research, London, for the assay of antibiotics by their diffusion through agar (Humphrey & Lightbown, 1952) has been adapted for use with the plaque technique to demonstrate the presence of yellow fever neutralizing antibodies (Porterfield, 1959c) and has also been used to measure the antiviral effects of interferon (Porterfield, 1959d). The technique has been devised with the requirements of the field or diagnostic laboratory in mind. Whilst the results are less accurate than those obtained by the Dulbecco, Vogt & Strickland procedure for plaque neutralization, the accuracy obtained is sufficient for many purposes and the labour involved is very much less. This paper describes the technique in detail and indicates some of the ways in which it may be applied. A more detailed analysis of the quantitative aspects of the method will be presented elsewhere.

MATERIALS AND METHODS

Media

Gey’s balanced salt solution. This was made according to the formula of Gey & Gey (1936), modified by Dr H. G. Pereira (personal communication, 1959).

Stock solution A:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>70.0 g</td>
</tr>
<tr>
<td>KCl</td>
<td>3.7 g</td>
</tr>
<tr>
<td>Na₂HPO₄, 12H₂O</td>
<td>3.01 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.237 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Phenol red (0.1%)</td>
<td>100 ml</td>
</tr>
<tr>
<td>Ion-exchange (deemineralized) water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

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1 Member of the scientific staff, National Institute for Medical Research, London, England.
This 10-times-concentrated solution is diluted 1 : 9 and sterilized by autoclaving at a pressure of 10 pounds per square inch (p.s.i.) (0.7 atm.) for 10 minutes.

Stock solution B:
- MgCl₂, 6H₂O .......... 0.42 g
- MgSO₄, 7H₂O .......... 0.14 g
- CaCl₂ .......... 0.34 g
- Phenol red (0.1%) .......... 1.00 ml
- Ion-exchange water to 100 ml

Stock solution C:
- NaHCO₃ .......... 2.25 g
- Phenol red (0.1%) .......... 1.00 ml
- Ion-exchange water to 100 ml

Adjust to pH 7.9 by bubbling CO₂ through the solution. Autoclave at 10 p.s.i. (0.7 atm.) for 10 minutes.

Standard Gey’s solution was made by adding 5% of solution B and 5% of solution C to 90% of solution A.

Tris (tris-(hydroxymethyl)-aminomethane). This was obtained from C. F. Boehringer & Soehne, Mannheim, Germany. A stock 0.05 M solution of pH 7.6 was made by dissolving 2.42 g in approximately 100 ml of Gey’s solution (A+B, no C). To this was added 76.8 ml of 0.2 M HCl and the volume was made up to 400 ml with further Gey’s solution (A+B, no C).

Tris Gey’s solution. This contained 90% of solution A, 5% of solution B, and 5% of stock 0.05 M tris, pH 7.6.

Chick-embryo extract. After removal of the limbs, viscera, beak and eyes, 10-day-old chick embryos were homogenized with 1 ml of standard Gey’s solution per embryo, using an M.S.E. Micromixer run at full speed for two minutes. The extract was centrifuged at 2000 r.p.m. for 30 minutes at 4°C. The supernatant fluid was stored at -10°C and was centrifuged again at 2000 r.p.m. for 10 minutes immediately before use.

Antibiotics. Penicillin (100 units per ml), streptomycin (100 μg per ml) and mycostatin (20 units per ml) were incorporated in the growth medium and in the overlay medium.

Tris growth medium:
- Gey’s solution A, 90%
- " B, 5%
- " C, 1%
- 0.05 M tris pH 7.6, 4%
- Calf serum .......... 5%
- 0.5% lactalbumin hydrolysate .......... 5%
- Chick-embryo extract .......... 1%

Tris overlay medium:
- Gey’s solution A (10-times concentrated but without phenol red) 20%
- Gey’s solution B .......... 10%
- 0.05 M tris pH 7.6 .......... 10%
- 0.5% lactalbumin hydrolysate .......... 10%
- Calf serum .......... 10%
- Neutral red (1 in 1000) .......... 10%
- Distilled water .......... 30%

To the above mixture is added chick embryo extract 5%.

Preparation of chick fibroblast cultures

This follows the basic Dulbecco (1952) technique, with a number of modifications. The process is represented schematically in Fig. 1. Ten-day-old chick embryos are removed aseptically. The limbs, viscera, eyes and beaks are removed and the remainder is washed in two changes of tris Gey’s solution. The tissue is then placed in a stainless-steel cylinder closed at one end by a perforated disc and at the other end by a plunger which may be screwed down forcing the tissue through the holes. The minced tissue is transferred to a trypsinizing flask containing 4 ml of 0.5% Light’s trypsin per embryo and the whole is agitated by means of a magnetic stirrer for 30 minutes in a warm room at 35°C. The suspension is next filtered through stainless-steel gauze to remove undigested fragments and the cells are washed twice in tris Gey’s solution. The final cell deposit is resuspended in tris growth medium, using 2 ml per embryo, and the suspension is again filtered through metal gauze. The cell concentration is determined by counting in a haemocytometer and the cells are diluted in growth medium to 8-10 million cells per ml. The cell suspension is filtered through a grade I sintered-glass pad. This stage improves the quality of the cell sheets by removing aggregates without materially reducing the cell count. The final suspension is distributed into borosilicate glass Petri dishes or large trays, using 8 ml per 60-mm dish, 20 ml per 90 mm dish or 150 ml per large tray approximately 250 mm square. The preparations are incubated at 37°C overnight. Accurate levelling of large trays is achieved by standing them on a levelling platform during incubation.

Virus strains

Mayaro, Chikungunya, yellow fever (French neurotropic strain), West Nile (Egypt 101 strain), Wesselsbron, and dengue (Trinidad 1751 strain) viruses were received from Dr M. Theiler (New
York, USA). Eastern and western equine encephalomyelitis (EEE and WEE), Semliki Forest (SF), Uganda S, Zika and Japanese B viruses were received from Professor G. W. A. Dick (Belfast, Northern Ireland). Louping-ill virus was received from Dr D. G. ff. Edward (Beckenham, England). Ilesha virus was received from Dr F. N. Macnamara (Lagos, Nigeria). Tahyna virus was received from Dr V. Bardos (Bratislava, Czechoslovakia). All viruses were inoculated into 4-day-old mice by the intracerebral route. When the animals were sick, brains were removed and homogenized in 10% calf serum in saline to give a 10% suspension. This was centrifuged at 10 000 r.p.m. for 1 hour at 4°C and the supernatant fluid preserved in 0.1-ml volumes in capillary tubes or in 0.5-ml volumes in ampoules stored at -70°C.

Immune sera

Rabbits were given 3 intravenous inoculations of 1 ml of 10% mouse-brain suspension prepared as described above at intervals of 7 days. The animals were bled out 7-10 days after the third injection. Sera were inactivated at 56°C for 30 minutes and were stored at −10°C or were lyophilized and stored at 4°C.

Infection of cell sheets

After the growth medium had been removed, virus dilutions in 5% calf serum in tris Gey's solution were pipetted on to cell sheets, using 0.5 ml per 60-mm dish, 1 ml per 90-mm dish and 15 ml per large tray. The preparation were next incubated at 37°C for 2 hours, with occasional rocking to ensure an even distribution of virus.

Agar overlay

At the end of the adsorption period the fluid was removed and the cells were covered with an agar overlay prepared by mixing equal quantities of 1.8% washed agar in distilled water at 42°C and overlay medium at 37°C. The volumes used have been 6 ml per 60-mm dish, 10 ml per 90-mm dish and 100 ml per large tray. Some care was necessary to achieve a satisfactory result with the large trays. The molten agar and overlay medium were mixed in a 250-ml conical flask fitted with a side-arm near the base to which is attached a length of silicone rubber tubing of 5-mm internal diameter. The tray
was removed from the incubator, the residual virus was pipetted off, and without delay, whilst the tray was still at approximately 37°C, the end of the rubber tubing was placed in one corner of the tray and the overlay medium was allowed to flow over the cell sheet by gradually raising the reservoir of liquid in the flask. During the pouring and until the agar had hardened, the tray was placed on a levelling platform, which was accurately adjusted so that the agar hardened to form a sheet of equal thickness over the entire plate.

**Application of beads**

"Fish-spine" electrical insulating beads, size no. 2, were used. The methods of washing, sterilizing and filling the beads were taken from those described by Humphrey & Lightbown (1952) and Lightbown & Sulitzeanu (1957). The volume of serum taken up by a bead was approximately 0.025 ml with less than 2% variation between beads. With Petri dish preparations one to four beads dipped in antiserum were applied to each dish. To minimize the risk of contamination, beads were applied to large trays under the cover of a sterile hood. The tray was then covered with a glass sheet which prevented evaporation but did not produce an air-tight seal. The preparations were then incubated at 37°C.

**Reading the results**

The optimal time for reading the results varies with different viruses, from 48 hours with WEE to the fifth or sixth day with yellow fever virus. Zone diameters were measured with a pair of callipers. With viruses which produce complete lysis of cells beyond the zone of inhibition more accurate readings may be made by placing the plate in an enlarging viewer which magnifies the image ten times.

**RESULTS**

Petri dish preparations were seeded with tenfold dilutions of yellow fever virus which was allowed to adsorb for two hours at 37°C. A bead dipped in a yellow fever immune rabbit serum was placed on one plate for each dilution of virus tested and a parallel series of plates were treated with a normal rabbit serum. Control plates gave uncountable plaques in the 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions and 30 plaques in plates seeded with 0.5 ml of the 10⁻⁶ dilution of virus, when read on the fifth day. There was virtually no effect with either serum in the plates seeded with 10⁻⁸ dilutions, but zones of approximately 15-mm and 20-mm diameter were produced by the immune serum in plates infected with the 10⁻³ and 10⁻⁴ dilutions of virus, respectively. Photographs of plates seeded with 10⁻⁴ dilution of virus are shown in Plate 1. Minute plaques can be seen within the area of protected cells, but the edge of the zone of plaque inhibition is fairly sharp and the zone diameter can be measured without difficulty. At the next dilution, 10⁻⁵, the zone of inhibition was larger but less accurately measurable since the transition from complete inhibition in the centre, through a zone of minute plaques, to discrete plaques approximately 2 mm in diameter at the periphery was more gradual.

Fig. 2 shows the relationship between the diameter of the zone of inhibition and the dose of virus in five experiments with four different viruses. With rapidly growing agents such as WEE and SF virus the dose-response curve is relatively flat, a hundredfold difference in virus titre altering the diameter of the zone of inhibition by only 3 or 4 mm. The slope is considerably steeper with yellow fever virus, two separate experiments giving almost parallel slopes. With this virus most satisfactory results were obtained with between 1000 and 10 000 plaque-forming units (PFU) of virus per dish. With less than 100 PFU of any virus the effect produced by an immune serum could be seen without difficulty, but accurate measurement of inhibition zones was impossible.

**Effect of serum dilution on size of zone of inhibition**

This may be demonstrated by setting up a number of replicate plates, each of which is treated with a different dilution of serum. Alternatively, a large tray may be used. In an experiment with yellow fever virus illustrated in Plate 2, the first, third and fifth rows represent serial fourfold dilutions of a rabbit immune serum, and the second, fourth and sixth rows represent similar dilutions of a human serum taken four years after yellow fever vaccination. The zone diameters were measured and the results were plotted in the graph shown in Fig. 3 in which the squares of the radii are set out against the serum dilution on a logarithmic scale. Measurable inhibition zones developed with the rabbit immune serum as far as a 1:256 dilution; with the human serum no inhibition was demonstrable beyond a 1:4 dilution. In this experiment the arrangement of the beads on the plate followed the order of the serum dilutions. A better course is to randomize the order in which the beads are applied.
PLATE 1
PETRI DISH PREPARATIONS INFECTED WITH 3900 PFU OF YELLOW FEVER VIRUS TO WHICH BEADS DIPPED IN NORMAL SERUM (A) AND YELLOW FEVER IMMUNE SERUM (B) HAVE BEEN APPLIED
PLATE 2
LARGE TRAY TECHNIQUE APPLIED TO YELLOW FEVER VIRUS
PLATE 3
LARGE TRAY TECHNIQUE APPLIED TO BUNYAMWERA VIRUS
PLATE 4

PLATE INFECTED WITH 1000 PFU OF MAYARO VIRUS TO WHICH EEE, SF, MAYARO, AND YELLOW FEVER ANTISERA HAVE BEEN APPLIED

A = EEE; B = SF; C = Mayaro; D = Yellow fever
This was done in an experiment with Bunyamwera virus illustrated in Plate 3. The sharper demarcation of the zones produced by a virus which has a more definite cytopathic effect is clearly seen.

The effect of a period of preliminary incubation upon the size of the zones of inhibition could be detected when serum beads were applied 24 hours after the cells had been covered with agar and incubated at 37°C. When the time interval was increased to 48 hours an effect was still demonstrable, but when the beads were applied after 72 hours' incubation no inhibition developed.

If, after the beads had been applied to the agar, plates were first incubated at 30°C for 24 hours before being incubated at 37°C, larger zones were produced than when the preparations were incubated at 37°C from the beginning. Thus in one experiment with Bunyamwera virus, preparations incubated at 37°C gave inhibition zone diameters of 20.5-22 mm, whilst replicate plates which were first held at 30°C for 24 hours gave zones of 25-26 mm after an equivalent time at 37°C.

Application of technique to study of cross relationships between viruses

The following experiment was designed to test the specificity of the technique and to see if it could be applied to demonstrate serological cross relationships. Plates were seeded with dilutions of four...
different group-A arthropod-borne viruses, overlaid with agar and subsequently spotted with beads, using four beads per 60-mm Petri dish. Six different group-A antisera were tested, together with a group-B immune serum and a control. The diameters of the zones of inhibition produced with different viruses are shown in Table 1.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Virus</th>
<th>SF</th>
<th>WEE</th>
<th>Mayaro</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>18</td>
<td>0</td>
<td>19</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mayaro</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>West Nile</td>
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<td>0</td>
<td>15</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Sindbis</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chikungunya</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Diameter (mm) of Zone of Inhibition Produced with Different Viruses and Antisera

WEE virus was inhibited by WEE antiserum and to a lesser extent by Sindbis antiserum. SF virus was inhibited by homologous antiserum only. Mayaro virus was inhibited by its homologous antiserum and also by SF antiserum (see Plate 4). Chikungunya virus was inhibited by Chikungunya, Mayaro and SF antisera. These results are consistent with the already observed subdivision of group-A viruses into two sub-groups—one containing Chikungunya, Mayaro and SF viruses and the other WEE and Sindbis viruses (Olitsky & Casals, 1959).

Less extensive tests with group-B viruses have shown even greater specificity. Plaque production with yellow fever virus has been inhibited by yellow fever immune rabbit sera and by human sera following 17D vaccination, but not by rabbit sera prepared against West Nile, Uganda S, Japanese B, Zika, Trinidad 1751 dengue or loping-ill viruses. Tests with West Nile virus have shown similar specificity.

Effect of varying thickness of agar

In experiments designed to determine the optimal thickness of the agar overlay, plates were seeded with WEE virus and were overlaid with different volumes of agar. Beads dipped in WEE antiserum were applied and inhibition zones were measured 48 hours later. Decreasing from 6 ml to 3 ml the volume of the overlay applied to a 60-mm diameter Petri dish increased the zone diameter from 11 mm to 13 mm. Similar results were obtained with Bunyamwera virus. However, the survival of the cells was less satisfactory under the smaller volume of agar, and although 3 ml was sufficient for use with rapidly growing viruses such as WEE, more satisfactory results were obtained with 5 or 6 ml when more slowly growing viruses were used.

Range of applicability of technique

Table 2 lists those arthropod-borne viruses which have been shown to produce plaques by the present technique and those which have also been examined by the plaque-inhibition test.

It is of interest that the size and time of appearance of plaques appears to be independent of the serological sub-group within the group-A viruses to which the virus belongs. WEE and EEE viruses both produce plaques which appear in 24-36 hours, reach 4-6 mm in diameter at 48 hours and continue to enlarge thereafter. SF virus plaques appear slightly later and are somewhat smaller. Mayaro virus plaques do not appear until the third day and remain in the range of 2-3 mm in diameter. Chikungunya virus plaques appear at the same time as Mayaro plaques, but are slightly smaller and less well defined.

Eight group-B viruses have been studied. The time of appearance, size and clarity of the plaques produced by these viruses depend to a considerable extent upon the media employed. Different batches of calf serum have given somewhat variable results. The addition of 5% chick-embryo extract to the basic overlay medium has removed much of this variability and has greatly improved the results, particularly with loping-ill virus. It was reported earlier that this virus produced rather indistinct plaques on the seventh day of incubation (Porterfield, 1959a). When 2% chick-embryo extract was incorporated in the overlay medium (making a final concentration of 1% when mixed with agar) plaques could be detected on the fifth day and with 5% embryo extract (2.5% final concentration) these were considerably more distinct and measured 3-4 mm in diameter.

With the exception of dengue (Tr. 1751 strain) the other group-B viruses all gave clear distinct
TABLE 2
ARTHROPOD-BORNE VIRUSES WHICH HAVE BEEN EXAMINED FOR PLAQUE PRODUCTION AND PLAQUE INHIBITION

<table>
<thead>
<tr>
<th>Virus</th>
<th>Plaque production</th>
<th>Plaque inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EEE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SF</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mayaro</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Group B:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uganda S</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>West Nile</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Louping-ill</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zika</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>Japanese B</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>Wesselsbron</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>Dengue (Tr. 1751)</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td><strong>Group C:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None examined</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td><strong>Others:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bunyamwera</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ilesha</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tahyna</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive result.
0 = negative result.
NT = not tested.

plaque, particularly with 5% embryo extract in the overlay medium.

The dengue strain gave indefinite plaques in the first experiment with this virus, but two subsequent experiments failed to reproduce this result. In view of the improvement experienced with louping-ill virus it seems quite possible that a satisfactory technique could be devised by paying attention to the composition of the media. This point is under investigation.

Bunyamwera virus and a serologically related virus, Ilesha, isolated by Dr F. N. Macnamara in Nigeria, have been examined. Both produce well-defined plaques on the third day, increasing in size to 4-5 mm in diameter. Tahyna virus (Bardos & Danielova, 1959) gave rise to plaques which appeared on the third day and remained between 2 mm and 3 mm in diameter on the fourth and fifth days.

Further studies with other arthropod-borne viruses are planned, but the results so far obtained indicate that the technique has a wide application.

DISCUSSION

In its simplest form, the technique described in this paper may be used as a qualitative test for the presence of neutralizing antibodies against a particular plaque-producing virus. Tests with yellow fever virus and a number of different antisera have shown that the inhibition is highly specific, and the cross reactions demonstrated within the sub-group of the group-A viruses are no more than have been demonstrated by conventional neutralization tests carried out in mice. The sensitivity of the test depends upon the particular virus concerned and upon the conditions of incubation. A yellow fever immune serum with a neutralization index of 1500, as determined by the intracerebral inoculation of mice with mixtures of undiluted serum and dilutions of virus, may be diluted 1 : 256 and still produce a measurable zone of inhibition. The sensitivity of the test may be increased by allowing a preliminary period of incubation of 30°C before placing the cultures at 37°C.

It has been shown that the area of the zone of inhibition is directly proportional to the logarithm of the concentration of antiserum applied to the agar, thus agreeing with the theoretical considerations of Humphrey & Lightbown (1952). This result is at variance with the findings of de Somer & Prinzie (1957) and Farrell & Reid (1959), who used an analogous system (filter-paper discs dipped in antiserum) for the titration of poliomyelitis antibodies and found that relationship was to the radius of the zone of inhibition, not to the area or the radius squared. Humphrey & Lightbown (1952) pointed out that the expression which they derived was apparently applicable to their system but was not necessarily applicable to the diffusion of substances applied on filter-paper discs.

The technique may be used in the identification of unknown viruses, if they are capable of producing plaques. Standard antisera, or pools of sera, could be used in screening tests to determine whether or not the strain could be shown to be similar to an already known virus.
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Since this paper was submitted for publication two more group-A viruses, Middleburg (Kokernot et al., 1957) and Uruma (Schmidt et al., 1959), and one further group-B virus, Ilheus, have been examined by both plaque and plaque-inhibition techniques with satisfactory results.

ACKNOWLEDGMENTS

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RÉSUMÉ

L'étude des virus par la méthode très précise de neutralisation des plages sur cultures de fibroblastes de poulet par l'antisérum correspondant, élaborée par Dulbecco et ses collaborateurs pour les recherches sur le virus de l'encéphalite équine, n'a pas été appliquée de façon générale à l'étude des autres virus, car elle est d'une exécution ardue, et l'on pensait qu'elle ne convenait qu'aux virus ayant un fort pouvoir cytopathogène.

L'auteur de cet article a mis au point une méthode simple d'inhibition de formation des plages destinée à l'établissement du diagnostic, sur le terrain et en laboratoire, de viroses transmises par les arthropodes. Elle n'a pas la précision de la méthode type précitée, mais est, en revanche, plus facile à exécuter. La technique est fondée sur l'inhibition de formation des plages — en comparaison d'un témoin non additionné de sérum — sur cultures de fibroblastes de poulet en plaques de Petri, par des perles de porcelaine trempées dans l'antisérum correspondant, préparé sur le lapin.

Sous sa forme la plus simple, la méthode peut être employée comme test qualitatif, dans la recherche d'un certain virus. L'étude de l'action de divers antisérums sur le virus de la fièvre jaune a montré que la méthode était hautement spécifique. Les réactions croisées au sein du groupe A des virus ne sont pas plus nombreuses qu'avec la méthode conventionnelle de neutralisation.

La superficie de la zone de cellules protégée par l'antisérum est proportionnelle, dans les conditions types, au logarithme de la concentration du sérum, de sorte que cette technique peut être utilisée pour déterminer la concentration d'un antisérum. Elle peut être exécutée avec un seul échantillon de sérum par boîte de Petri. Une modification de la méthode, comportant l'emploi de cuves de plus grandes dimensions permet d'examiner parallèlement jusqu'à 36 échantillons de sérum.

L'auteur l'a appliquée à l'étude de 15 virus des groupes A et B. Elle permet également de déceler l'existence de virus jusqu'alors inconnus, pour autant qu'ils ont le pouvoir de former des plages.