A Sensitive Method for the Detection and Isolation of Recombinants of Foot-and-Mouth Disease Virus

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SUMMARY

Recombination between temperature-sensitive (ts) mutants of foot-and-mouth disease (FMD) virus was examined, using an infectious centre technique that was more sensitive (approx. 30-fold) than the conventional virus yield test. The test involved a brief incubation of the mixedly infected cells at the permissive temperature to allow recombination to occur followed by assay at the restrictive temperature to select for those cells in which recombination had occurred. With crosses involving widely separated mutations, as many as 28% of the infected cells produced presumptive recombinant plaques. Since each plaque was the result of an independent event, large numbers of different presumptive recombinants could be isolated for further study. Analysis of presumptive recombinant plaques from a variety of crosses showed that, in general, the virus produced had the properties expected of recombinants. An approximate correlation was found between genetic distance, as determined in the yield recombination test, and the percentage of recombinant infectious centres observed. The phenomenon was very sensitive to the balance between the input multiplicities of the two parent viruses and occurred very early in virus replication. The test has considerable potential for the study of genetic interactions in FMD virus, but it would be surprising if this potential was limited to picornaviruses.

INTRODUCTION

Evidence of recombination in RNA viruses with non-segmented genomes is limited to picornaviruses and it is notorious for its variability and rarity (Cooper, 1968; Pringle, 1968; Mackenzie et al., 1975; Lake et al., 1975; McCahon et al., 1977). Most of the work on recombination has used the virus yield recombination test, i.e. cells mixedly infected with the two parent viruses are incubated at the permissive temperature for a sufficient time to permit the production of good yields of virus which are then assayed at the restrictive temperature to detect recombinants. Since recombination events are rare, the recombinants are only a tiny fraction of the total virus yield (generally 0.5% or less for picornaviruses). This has given rise to some of the variability and to difficulties in isolating even a few recombinants for biological and biochemical examination. An alternative test for recombination, using an infectious centre technique, has been used in a few experiments on foot-and-mouth disease (FMD) virus (Pringle, 1965) and influenza virus (Hirst, 1973). In these experiments the mixedly infected cells were not incubated at the permissive temperature after infection but rather were assayed immediately in an infectious centre assay incubated at the restrictive temperature. In both cases evidence was found for genetic recombination (or, more precisely, reassortment of segments, in the case of influenza) under these conditions.

In this paper we describe a method based on infectious centres which consists of recombination at the permissive temperature followed by a selection procedure for the
detection of recombination events within the infectious centres. This method was more sensitive than the conventional method and we have used it to study some of the factors affecting recombination.

**METHODS**

*Cell cultures.* The description and culture of the BHK cells used in this work were as described previously (Lake et al., 1975).

*Mutants.* The 13 mutants used in these studies were selected because the location of their *ts* mutation on the genetic (McCahon et al., 1977) and biochemical (Sangar et al., 1977; Doel et al., 1978) maps of the genome had been fairly well established. The location of the *ts* mutation in eight of the mutants (22, 33, 40, 58, 103, 107, 109 and 115) had been identified on specific gene products by electrofocusing studies and this had led to an alignment of the genetic and biochemical maps of the genome (King et al., 1980; A. M. Q. King, unpublished observations). Four mutants (O3, 16, 19 and 49) had been used in previous genetic studies as standards (Lake et al., 1975; McCahon et al., 1977) and therefore their position in relation to each other and to the eight electrophoretic mutants was well established. The remaining mutant (201) was a spontaneous cold-sensitive (*cs*) mutant isolated by the temperature shift technique (Lake & Mackenzie, 1973) from the standard mutant, O3. The isolation and preparation of seed and working stocks of the mutants have been described previously (McCahon et al., 1977).

*Efficiency of plating (e.o.p.) or 'leak'.* This was the ratio of the number of plaques produced at the restrictive temperature compared to the number of plaques produced at the permissive temperature. This measurement reflected both the defective growth of the mutant under restrictive conditions, i.e. 'leak', and the occurrence of spontaneous revertants to the non-defective state. However, since it was generally not possible to identify these two components separately, it was regarded as being a measure of 'leak' since that was the major component.

*Virus assays.* Infectivity was assayed on confluent BHK monolayers in 90 mm Petri dishes or by the modified version (Lake & Mackenzie, 1973) of the agar cell suspension technique of Cooper (1961). For both types of assay a modified form of Eagle's medium was used, principally to obtain a good discrimination between guanidine-sensitive (gs) and guanidine-resistant (gs+) viruses. The medium used was that normally used to culture BHK cells (Macpherson & Stoker, 1962) except that (i) choline was omitted, (ii) the normal buffer, bicarbonate, was reduced in concentration to 10 mM and (iii) the more efficient HEPES buffer substituted at a final concentration of 40 mM. The pH of the medium was adjusted at room temperature to 7.4 (for assays containing no guanidine) or 7.8 to 7.9 (for assays containing guanidine). This medium kept cells in good condition, yet maintained its pH well under the conditions of incubation used. High pH in our experience was the single most important factor in obtaining good inhibition of the virus by guanidine.

Assay plates were incubated in plastic boxes immersed in water baths with efficient water circulation and sensitive temperature control (±0.1 °C). A temperature of 36.5 to 36.7 °C was used as the permissive temperature for all mutants, 30.5 to 31.0 °C as the restrictive temperature for the *cs* mutation and 40.8 to 41.3 °C as the restrictive temperature for the *ts* mutations. The temperature ranges reflect deliberate variation between experiments involving different mutants and not the error due to temperature control of the water baths. A level of 700 to 800 µg guanidine hydrochloride/ml in pH 7.8 medium at 36.6 °C was used as the restrictive condition for the gs marker.

*Isolation and examination of recombinants.* All recombinants were initially identified as clear plaques in an agar–cell suspension assay incubated at the restrictive temperature. Well-isolated plaques were picked and, where cloning was performed (see Results on analysis
Infectious centre recombination test

Infectious centre recombination test

of presumptive recombinant infectious centres), this was done in an agar cell suspension assay at the restrictive temperature. Plaque material (either original isolate or first or second cloning) was generally examined directly for biological markers, but for biochemical examination the virus was grown in BHK cells. Virus purification and electrofocusing analysis of the virus structural polypeptides were as described by King & Newman (1980).

RESULTS

General description of the infectious centre test and controls

In this test the cells were infected, usually at 36.6 °C, either as a suspension or as a monolayer with a mixture of the two parent viruses (multiplicity of infection 10 to 30 p.f.u./cell). After approx. 1 h at 36.6 °C the cells were washed with acid buffer (pH 6.2) to remove inoculum virus and, if necessary, a suspension prepared by using trypsin and versene. The cells were then assayed as infectious centres on monolayers or in the agar cell suspension assay at the permissive and restrictive temperatures. The titre at the restrictive temperature was expressed as a percentage of the titre of the cell suspension at the permissive temperature (% recombinant infectious centres, r). Plaques produced at the restrictive temperature were regarded as presumptive recombinants that had arisen from a mixedly infected cell. Routine microscopic examination of the cell suspensions before assay showed that approx. 80 to 90% were single cells. In addition, in several experiments (see Fig. 2) the percentage of presumptive recombinants (r) exceeded the percentage of clumped cells observed in these experiments (approx. 10%). That the presumptive recombinant plaques did not arise from clumping of two cell populations each infected with a parent virus was checked by mixing the singly infected controls used in the chequerboard experiment shown in Table 1. The singly infected controls produced only small numbers of plaques at the restrictive temperature (<0.2% of the titre at the permissive temperature) and there was no enhancement of the figures when the cell populations were mixed before assay. Cell suspensions were also checked for free virus that might have survived the acid treatment and might in some way have given rise to the recombinant plaques observed. No free virus was ever detected by assaying the supernatant fluids of cell suspensions at 41 °C unless the incubation was prolonged beyond 70 min.

The relative sensitivity of the two methods of assaying infectious centres was compared in several experiments. In every case the monolayer technique was two to four times more sensitive than the agar cell suspension technique. However, as observed previously (Slade & Pringle, 1971), only about 30% of the cells could be infected productively, even at the high multiplicities used in Table 1. This low frequency probably reflects the success rate of infected cells in producing a plaque rather than differences in the cell population, since many other observations such as cytopathic effect and fluorescent antibody studies suggest that 100% of the cells are producing virus. The apparent low infection rate did not affect the calculation of results since the recombinant infectious centres were expressed as a percentage of the total number of successful infectious centres (permissive temperature assay).

Effect of multiplicity of infection

Two crosses were studied extensively to determine the effect of using varying amounts of each parent virus on the efficiency of recombination. The results were the same for both crosses and the results from a typical experiment are shown in Table 1. The major conclusion was that the optimum balance between the multiplicities of the two parent viruses was more important than the actual multiplicities. In the experiment shown in Table 1 the input multiplicity of each parent virus was varied over a 1000-fold range yet the percentage of presumptive recombinant infectious centres only varied between 0.76 and 9.21% (see centre diagonal of Table 1). By contrast, when the parent viruses were in a ratio of 7.2:1 (lower
Table 1. Effect of varying multiplicity on the production of recombinant infectious centres from the cross 19 x 49*

<table>
<thead>
<tr>
<th>Added multiplicity of ts 49 (p.f.u./cell)</th>
<th>72</th>
<th>18</th>
<th>4.5</th>
<th>1.12</th>
<th>0.28</th>
<th>0.07</th>
<th>0.018</th>
</tr>
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<tbody>
<tr>
<td>40</td>
<td>8.00</td>
<td>10.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>3.46</td>
<td>5.71</td>
<td>5.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>2.49</td>
<td>9.21</td>
<td>5.86</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.63</td>
<td>-</td>
<td>-</td>
<td>2.45</td>
<td>5.08</td>
<td>4.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.49</td>
<td>6.72</td>
<td>4.42</td>
<td>-</td>
</tr>
<tr>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.75</td>
<td>4.14</td>
<td>0.95</td>
</tr>
<tr>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.89</td>
<td>0.76</td>
</tr>
</tbody>
</table>

* Aliquots of a cell suspension were infected with either or both viruses at the multiplicities indicated, incubated for 1 h at 36.5 °C, acid washed and assayed at 36.5 and 41 °C.
† Calculated from titration on same day of actual inocula.
‡ Number of infectious centres which produced plaques at the restrictive temperature expressed as a percentage of the number of infectious centres which produced plaques at the permissive temperature.
§ -, Not done.

diagonal of Table 1), the percentages observed were consistently lower than those obtained when the viruses were more nearly balanced, as in the centre diagonal (1.8 : 1) and in the upper diagonal (1:1.8). Similar results were obtained with several other crosses as a simple form of this experiment was often used to check for optimum recombinations in each cross, e.g. in the construction of Fig. 2.

It is also clear from an inspection of Table 1 that efficient recombination has occurred at very low multiplicities, suggesting that apparently non-infectious particles may be giving rise to recombinants.

Effect of time of incubation at the permissive temperature

In these experiments the time of incubation of the mixedly infected cells at the permissive temperature, before assay for infectious centres at the restrictive temperature, was varied. Two crosses were examined and the results were very similar for both crosses (Fig. 1). In these experiments the viruses and cells were prewarmed and mixed at 42 °C, i.e. slightly above the restrictive temperature, to ensure that the conditions were restrictive. In this way it was hoped to synchronize the cell population at the same stage of virus infection before incubating at the permissive temperature for precise times. Some recombination was seen at time zero but after an initial lag of about 30 min there was a steady increase up to approx. 75 min. Beyond this time it was difficult to measure the frequency accurately because of the infectious virus that was released from about 90 min onwards and which was not completely removed by the acid-washing treatment (see general description of the test). Therefore, incubation for 60 to 70 min was used in all subsequent experiments.

Sensitivity of the infectious centre test

In these experiments the sensitivity of the infectious centre test was compared with that of the virus yield recombination test. Table 2 shows a selection of crosses using mutants whose ts mutations have been defined by electrofocusing studies (King et al., 1980; A. M. Q. King, unpublished results). In each case the ts mutation has been shown to co-vary with an electrophoretic mutation in one of the virus-induced polypeptides (22 and 115 in p56, 33 and 58 in VP2, 107 in VP3 and 40 in VP1). The maximum and minimum possible molecular
Infectious centre recombination test

Fig. 1. Effect of incubation time at the permissive temperature on the appearance of recombinant infectious centres for the cross 19 × 49. Prewarmed (42 °C for 10 min) aliquots of cell suspension and virus stocks were mixed and incubated at 42 °C for 10 min (multiplicities: cross, 6 p.f.u./cell of each virus; self cross, 12 p.f.u./cell of either virus). Cells were then washed twice with ice-cold acid buffer, resuspended in medium and incubated at 36.5 °C. At each time point, three aliquots were removed, washed twice with ice-cold acid buffer and assayed at 36.5 and 41 °C.

Table 2. Comparison of the sensitivities of the infectious centre and conventional yield tests for detecting recombination between ts mutants

<table>
<thead>
<tr>
<th>Cross description</th>
<th>Infectious centre test†</th>
<th>Conventional yield test‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plaques observed at R.T. (%)§</td>
<td>Plaques observed at R.T. (%)§</td>
</tr>
<tr>
<td></td>
<td>Parent A</td>
<td>Parent B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>33</td>
<td>4·1-6·7</td>
</tr>
<tr>
<td>22</td>
<td>40</td>
<td>3·1-5·2</td>
</tr>
<tr>
<td>40</td>
<td>33</td>
<td>0·6-1·8</td>
</tr>
<tr>
<td>40</td>
<td>107</td>
<td>0·1-2</td>
</tr>
<tr>
<td>58</td>
<td>107</td>
<td>0·1-2</td>
</tr>
<tr>
<td>115</td>
<td>22</td>
<td>&lt;1·3</td>
</tr>
</tbody>
</table>

* This is the maximum and minimum possible molecular distance between loci based on the known location of the mutations used as determined by electrofocusing (see text).
† As described in the text, using multiplicities of 10 to 30 p.f.u./cell and monolayer assay at 36·7 and 41 to 41·3 °C to detect recombinant infectious centres.
‡ Aliquots of the same infected cell suspension or replicate monolayer cultures were incubated for a total of 6 h at 36·7 °C. Frozen and thawed and then assayed for released virus by monolayer assay using the same conditions as those used for the infectious centre assay.
§ Plaque titre at 41 to 41·3 °C expressed as a percentage of the titre at 36·7 °C; R.T., restrictive temperature.
‖ These conclusions were based on seeing a significant increase (×3) in the percentage of plaques at the restrictive temperature in the cross compared to the controls (+, recombination; −, no detectable recombination). The figures in parentheses in the last column are the recombination frequencies calculated by the usual formula (McCahon et al., 1977).

distance between loci was based on the coding sequence for the genome as determined by biochemical means (Sangar et al., 1977; Doel et al., 1978). The crosses were performed as described for the infectious centre test but, in addition, an aliquot of the mixedly infected cell suspension or replicate mixedly infected monolayers were incubated at the permissive temperature for a further 5 h rather than being assayed as infectious centres. The virus yields from such cultures were then assayed at the permissive and restrictive temperature and the results represent the yield recombination test.
The incidence of recombinant infectious centres was much higher (approx. 30-fold) than the incidence of recombinants in the yield test. In addition, the infectious centre test was able to detect recombination in crosses where no recombination could be detected in the yield test. The limit of sensitivity of the infectious centre method needs to be explored further but on the basis of these results it was able to detect recombination between loci that were separated by no more than 1.3 kilobases (kb).

**Correlation between genetic map distance (d) and percentage of recombinant infectious centres (r)**

Fig. 2 shows the results obtained with 23 different crosses in infectious centre experiments (r) compared with the genetic map distance between loci (d) as determined previously in yield recombination tests (McCahon et al., 1977). There is a correlation between d and r (correlation coefficient 0.76, significant at the 0.001% level) but the exact nature of the relationship is difficult to define, principally because of the great variability in r as d increases. The same approximate correlation can also be seen in Table 2.

**Analysis of presumptive recombinant infectious centres**

**Temperature sensitivity of presumptive recombinants**

125 plaques were picked from seven crosses and assayed without cloning at the permissive and restrictive temperatures. A total of 121 isolates were indistinguishable from ts+ and four were slightly temperature-sensitive (e.o.P.41 10^{-1} to 10^{-2}). In addition, 23 cloned ts+ isolates from the cross 22 x 58 were examined by electrofocusing of the virus proteins (J. Newman & A. King, unpublished results). Both parents possessed an electrophoretic mutation (e) that had been shown to co-vary with the ts mutation in reversion studies (King et al., 1980; A. King & J. Newman, unpublished results) and were therefore considered to be different phenotypes of the same mutation (referred to as a ts/e mutation). None of the 23 clones...
Infectious centre recombination test

![Diagram of infectious centre recombination test](image)

Fig. 3. A five-factor cross (201 x 109) performed in the infectious centre test. Aliquots of a cell suspension were infected with either or both viruses at 25 p.f.u./cell. The infectious centres were assayed at 36.5 and 41 °C (r = 17.2%) and presumptive recombinant plaques were picked from the 41 °C plates. The figures in parentheses, e.g. P34, refer to the physical sites of the mutations on the genome as determined by biochemical methods (see text).

Table 3. Analysis of presumptive recombinants isolated at 41 °C from a five-factor cross*

<table>
<thead>
<tr>
<th>Presumptive recombinant plaques</th>
<th>Number</th>
<th>Phenotype</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 uncloned isolates†</td>
<td>8</td>
<td>ts⁺ gs⁺ cs</td>
<td>Recombinant or ts⁺ Revertant of 201</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>ts⁺ gs cs⁺</td>
<td>Recombinant or ts⁺ Revertant of 109</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>ts⁺ gs⁺ cs⁺</td>
<td>Recombinant for at least one unselected marker</td>
</tr>
<tr>
<td>7 cloned isolates‡</td>
<td>1</td>
<td>ts⁺ gs⁺ cs VP1⁺</td>
<td>Recombinant or ts⁺ Revertant of 201</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ts⁺ gs cs VP1 109</td>
<td>Recombinant or ts⁺ Revertant of 109</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ts⁺ gs⁺ cs⁺ VP1⁺</td>
<td>Recombinant for at least one unselected marker</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ts⁺ gs⁺ cs⁺ VP1⁺</td>
<td></td>
</tr>
</tbody>
</table>

* Illustrated in Fig. 3.
† These were plaques produced at 41 °C in the infectious centre assay which were then tested directly for possession of ts, gs and cs phenotypes. Only those plaques that appeared to be homogeneous (see text) are shown.
‡ These clones were derived at the permissive temperature from four of the apparent mixtures identified in the initial screening tests (see uncloned isolates above). The VP1 marker was identified by electrophoresis of the purified structural proteins. All the clones had ts⁺-like VP2 and VP3, i.e. like 201.

possessed either of the electrophoretic mutations that were believed to be responsible for the temperature sensitivity of the parents. This indicated that they were recombinants or true revertants rather than suppression revertants, which were seen occasionally in studies on ts⁺ revertants of these mutants (King et al., 1980).

Analysis of a five-factor cross

This cross is illustrated in Fig. 3. The physical site on the genome for four of the mutations (indicated in parentheses, e.g. P34) has been established in relation to the biochemical map of the genome derived from the use of protein synthesis inhibitors (Sangar et al., 1977; Doel et al., 1978). The locations of the gs⁺/e mutation of 201 and the e and ts/e mutations of 109 are based on electrophoresis evidence (King et al., 1980; K. Saunders & A. King, unpublished results). The location of the ts mutation of 201 is based on its juxtaposition on the genetic map to two mutations which have ts/e mutations in P56a and on the temperature sensitivity of the isolated P56a from this mutant (i.e. 201) in an RNA polymerase assay (Lowe & Brown, 1981; Lowe et al., 1981). The location of the cs mutation of 201 is only approximate and was deduced from the results of this experiment.
After the initial 1 h incubation under permissive conditions (36.5 °C), the infected cells were assayed at 41 °C to look for ts+ recombinants. 17.2% of the infected cells (based on the infectious centre titre at 36.5 °C) produced plaques at 41 °C. Thirty of these presumptive recombinant plaques were picked and the plaque material was examined directly for possession of the ts, cs and gs+ markers. All the isolates were ts+ as expected, since that was the selected phenotype. Fifteen recombinants could be clearly categorized as regards the cs and gs+ markers (see uncloned isolates in Table 3) but 15 recombinants were apparently mixtures, as judged by a mixture of plaque sizes in the cs assay and/or intermediate sensitivity in the gs assay. Four of these apparent mixtures were cloned at the permissive temperature and three clones from each were examined for biological and biochemical (electrofocusing of structural polypeptides as in King et al., 1980) markers. Four different types of ts+ virus were found in this analysis (see cloned isolates in Table 3) and in three cases two different types were isolated from the same infectious centre. Ten of the presumptive recombinants isolated from this cross (five uncloned and five cloned isolates) cannot be explained on the basis of simple reversion of the ts phenotype of one of the parents and, therefore, they support the idea that recombination was responsible for the production of some of the presumptive recombinant infectious centres.

DISCUSSION

Several lines of evidence have been presented which are consistent with the hypothesis that recombination is an integral part of this test, in particular the analysis of recombinant plaques, the kinetics of the appearance of recombinant genomes and the correlation between genetic map distance (d) and percentage of recombinant infectious centres (r).

The analysis of the presumptive recombinant plaques demonstrated that the plaques were due to the presence of ts+ virus and not to complementing mixtures of the two ts parent viruses. The analyses of the electrophoretic mutant crosses and the five-factor cross suggested that the ts+ viruses isolated were recombinants and not revertants. Recent work involving double mutants possessing both a ts and a non-ts electrophoretic mutation has demonstrated that recombinant viruses having electrophoretic mutations derived from both parents are produced, at least on some occasions (A. M. Q. King, D. McCahon & W. R. Slade, unpublished observations).

In earlier work on the kinetics of recombination (Pringle, 1968; Mackenzie et al., 1975; Lake et al., 1975), recombinant virions were first detected at 90 min and the amount increased up to 150 min post-infection. However, since the infectious centre method measures the appearance of recombinant genomes rather than mature virions, we were able to detect an increase in recombinant genomes as early as 30 min and the amount increased up to 90 min post-infection. This would suggest that the mechanism involves a very early event in virus replication, such as replication of the RNA. A small amount of recombination was observed in both crosses at zero time (see Fig. 1) but this was probably due to the choice of parent viruses. In each cross one parent had a mutation in a structural protein and was able to make some viral RNA at the restrictive temperature (R. Priston, unpublished results). The other parent virus had a mutation in the P56a protein, which is the major component of the purified virus polymerase (Newman et al., 1979; Lowe & Brown, 1981; Lowe et al., 1981), and was unable to make significant amounts of viral RNA at the restrictive temperature (R. Priston, unpublished results). It seems likely that some complementation could have occurred in the assay plates and this gave rise to the low level of recombination observed at zero time.

The relationship between d and r is difficult to define precisely, partly because of the errors in the estimation of both parameters and partly owing to the nature of the test. The test is basically qualitative in that it detects cells in which a recombination event or events has occurred rather than the total number of recombinant viruses produced. Therefore, one would
expect to see only an approximate correlation between \( d \) and \( r \). Since there is a reasonably good correlation between the genetic and biochemical maps (King et al., 1980; A. M. Q. King, unpublished observations), it follows that there ought to be a relationship between the physical distance separating loci and \( r \). Some indication that such a relationship does exist was seen in crosses using mutants whose mutations had been defined fairly precisely by electrofocusing studies (Table 2).

While the yield recombination test suggests that recombination is a rare event (generally 0.5% or less of the virus yield from infected cells), the infectious centre test demonstrates that it can occur in a large proportion of cells, e.g. 28% of the infected cells in the 22 × 33 cross shown in Table 2. Therefore, it should be possible to study the phenomenon more precisely using the infectious centre method and to use it to produce recombinants, each resulting from an independent recombination event. In addition, because of the sensitivity of the method, it should be possible to separate closely linked mutations and possibly even determine their order on the genome.

Clearly, the method has considerable potential for the study of genetic interactions in picornaviruses but it would be surprising if it could not be applied to other RNA viruses. The usefulness of the method in re-examining the possibility of recombination in other RNA viruses could well be limited by the efficiency of complementation in those viruses. If that is the case, then the togaviruses would seem to be the best candidates for re-examination, since they show relatively inefficient complementation (Pfefferkorn, 1977). In viruses where efficient complementation (e.g. rhabdoviruses and paramyxoviruses) or efficient reassortment of gene segments (e.g. reoviruses, bunyaviruses and myxoviruses) occurs, then the method might still be useful in looking for intragenic recombination.

We would like to thank, in particular, Dr A. King for access to his unpublished results on electrofocusing, which have enabled us to estimate the distance between genetic loci (Table 2), and Mr J. Newman for running the electrofocusing gels referred to in the analysis of recombinant plaques. We would also like to thank Miss Frances Mortimer and Mrs Helen Cooper for excellent technical assistance.

REFERENCES


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