STUDIES OF THE INFECTIOUS UNIT OF MYXOMA

BY ROBERT F. PARKER, M.D.

(From the Department of Medicine of Western Reserve University School of Medicine, and the Lakeside Hospital, Cleveland)

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Studies of the immunological and chemical characteristics of the larger viruses have been greatly facilitated by the development of techniques which make it possible to work with suspensions of virus largely free from extraneous material, both soluble and particulate. These methods have facilitated the study of the relationship which exists between the virus and the susceptible animal, by allowing quantitative concepts to be introduced in describing phenomena which have seemed at some times in the past to be wholly disorderly. But quantitative description of the mechanisms of infection and resistance presuppose that an accurate assay of the concentration of the pathogenic agent is possible, and it is with this problem that the present series of investigations has been concerned. The unit of viral activity is commonly taken as the "infectious unit," defined as the least amount of virus capable of giving rise to infection under suitable conditions. From our knowledge of the physical characteristics of certain of the viruses it is evident that a given volume of viral suspension contains a finite number of particles, which in turn must make up the infectious unit. In exact studies of infection and resistance it is important to know whether many or few particles are required to initiate infection, and in order to obtain this information for certain viruses, we have applied inferential methods in an effort to define the infectious unit of virus in terms of viral particles, and have in a previous publication given an account of the technique (1).

Since the theoretical bases of the method have been fully described, detailed consideration will be omitted here. The method depends in essence upon determining the relation which exists between the dilution of a viral suspension and the probability that the inoculation of an aliquot sample of it will give rise to a lesion. The percentage of inoculations giving positive results is determined experimentally for a number of appropriate dilutions of virus, and a curve constructed. This is compared with curves derived mathematically, on the assumption that one or more, that two or more, etc., particles are required to initiate recognizable infection. The theoretical curve is then selected which most closely resembles the experimental curve, and its goodness of fit is evaluated.
by appropriate statistical methods. From these results the number of viral particles composing the infectious unit is deduced. Haldane (2) has pointed out that this procedure is not as accurate as an algebraic method which he describes, which allows of a much more refined estimate of the degree of agreement between expectation and result. In the present series of investigations, however, inherent inaccuracies in the original data are such that the graphic method of analysis seems to be adequate, and hardly justify the use of the more elaborate, although more exact algebraic study.

**EXPERIMENTAL**

For the present experiments, myxomatous virus was used in the form of a suspension of elementary bodies, and the activity was measured by titration in the skin of rabbits.

**Methods and Materials**

The strain of myxomatous virus used was obtained from Dr. T. M. Rivers, and is one originally isolated by Sanarelli (3). It has been maintained until recently by successive passages in the skin of rabbits. Since the appearance of the description by Rivers and Ward (4) of the technique of elementary body preparation, the virus has been used only in the form of washed elementary bodies, and has been passed serially in rabbits, using the elementary body suspension as seed. No change in the pathogenicity of the virus has been apparent, although it is our impression that the yield of elementary bodies has risen with continued passage. At the present time ten serial passages have been accomplished. The virus has been stored at 4-6°C. suspended in a mixture of equal parts of glycerine and 0.004 M citric acid phosphate buffer pH 7.0-7.2, in glass stoppered bottles. Under these conditions the activity of the virus does not diminish appreciably over a period of 1 to 2 months. The virus was titrated by intradermal inoculations of rabbits, inoculating a standard volume of 0.25 cc. The sites of inoculation were observed daily, and readings were terminated on the day before secondary lesions appeared. Under the conditions of our experiment, evidence of generalization of infection was present usually by the 6th day, but when a number of sites had been inoculated with concentrated suspensions it might be as early as the 5th. The prompt appearance of secondary lesions increased to some extent the difficulty of performance of titrations, but did not seriously impair their accuracy. In order to avoid cross infection with vaccinia, only rabbits immune to this disease were employed.

For the initial experiment, a suspension of elementary bodies was used which was prepared from rabbits inoculated with virus in the form of an emulsion of infected tissue.

**Experiment 1.**—The elementary body suspension was prepared according to the technique of Rivers and Ward and its purity was confirmed by microscopic examination of a stained smear. This revealed that only a moderate amount of amorphous debris was present, and that there was no appreciable number of clumps of elementary bodies. Preliminary titrations of the suspension, using four inoculations of serial tenfold dilu-
tions, indicated that a dilution of suspension of approximately $10^{-6.2}$ should give an equal number of positive and negative results if a single particle suffices to induce infection. A suspension was accordingly prepared in a dilution of $10^{-5.9}$. From this serial tenfold dilutions were made, and multiple (38 to 40) inoculations of each were made in rabbits. Each inoculation was classified on the 5th day as positive or negative.

The results are given in Table I, and the same data are presented graphically in Fig. 1. Inspection of Fig. 1 indicates that the experimental bears a close resemblance to the theoretical curve, which is derived on the assumption that a single particle is adequate to initiate infection. A second theoretical curve, derived on the assumption that two particles are required for initiation of infection has a greater slope, and therefore deviates more widely from the experimental one.

In a second experiment virus which had undergone nine successive passages in the form of elementary bodies was used.

Table I

<table>
<thead>
<tr>
<th>Logarithm of virus dilution</th>
<th>Number of inoculations</th>
<th>Number positive (x)</th>
<th>If 1 particle initiates infection</th>
<th>If 2 particles initiate infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number expected positive (x)</td>
<td>$\frac{(x - \bar{x})^2}{\bar{x}}$</td>
</tr>
<tr>
<td>5.0</td>
<td>38</td>
<td>38</td>
<td>38.0</td>
<td>0.00</td>
</tr>
<tr>
<td>5.3</td>
<td>39</td>
<td>34</td>
<td>39.0</td>
<td>0.64</td>
</tr>
<tr>
<td>5.6</td>
<td>40</td>
<td>32</td>
<td>37.5</td>
<td>0.81</td>
</tr>
<tr>
<td>5.9</td>
<td>40</td>
<td>25</td>
<td>29.7</td>
<td>0.74</td>
</tr>
<tr>
<td>6.2</td>
<td>40</td>
<td>19</td>
<td>20.0</td>
<td>0.05</td>
</tr>
<tr>
<td>6.5</td>
<td>40</td>
<td>10</td>
<td>11.7</td>
<td>0.25</td>
</tr>
<tr>
<td>6.8</td>
<td>40</td>
<td>8</td>
<td>6.4</td>
<td>0.40</td>
</tr>
<tr>
<td>7.1</td>
<td>40</td>
<td>5</td>
<td>3.3</td>
<td>0.88</td>
</tr>
<tr>
<td>7.4</td>
<td>40</td>
<td>4</td>
<td>1.7</td>
<td>3.11</td>
</tr>
</tbody>
</table>

$\chi^2 = 6.88$, $P = 0.55$, $<0.01$
FIG. 1. Experiment 1. Relation between percentage of inoculations positive and dilution of virus. The solid line is derived theoretically on the assumption that one particle initiates infection. The dots are experimentally determined points.

FIG. 2. Experiment 2. Relation between percentage of inoculations positive and dilution of virus. The solid line is derived theoretically on the assumption that one particle initiates infection. The dots are experimentally determined points.

TABLE II

<table>
<thead>
<tr>
<th>Logarithm of virus dilution</th>
<th>Results of experiment</th>
<th>Number of inoculations</th>
<th>Number positive (x)</th>
<th>Number expected positive (( \hat{\beta} ))</th>
<th>((x - \hat{\beta})^2)</th>
<th>Number expected positive (( \hat{\beta} ))</th>
<th>((x - \hat{\beta})^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>40</td>
<td>39</td>
<td>40.0</td>
<td>0.03</td>
<td>40.0</td>
<td>0.23</td>
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<tr>
<td>5.5</td>
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<td>37</td>
<td>39.8</td>
<td>0.20</td>
<td>39.0</td>
<td>0.41</td>
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<tr>
<td>5.8</td>
<td>40</td>
<td>35</td>
<td>36.8</td>
<td>0.09</td>
<td>36.8</td>
<td>0.06</td>
<td></td>
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<tr>
<td>6.1</td>
<td>40</td>
<td>29</td>
<td>29.2</td>
<td>0.01</td>
<td>30.0</td>
<td>0.49</td>
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<td>6.4</td>
<td>40</td>
<td>17</td>
<td>18.7</td>
<td>0.15</td>
<td>18.0</td>
<td>0.06</td>
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<tr>
<td>6.7</td>
<td>40</td>
<td>9</td>
<td>10.9</td>
<td>0.33</td>
<td>10.0</td>
<td>0.26</td>
<td></td>
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<tr>
<td>7.0</td>
<td>40</td>
<td>8</td>
<td>5.8</td>
<td>0.83</td>
<td>5.0</td>
<td>15.25</td>
<td></td>
</tr>
<tr>
<td>7.3</td>
<td>40</td>
<td>4</td>
<td>3.0</td>
<td>0.33</td>
<td>2.0</td>
<td>21.60</td>
<td></td>
</tr>
<tr>
<td>7.6</td>
<td>40</td>
<td>0</td>
<td>1.3</td>
<td>1.30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2 \] = 3.27, \( P = 0.91 \) for 2 degrees of freedom; \( \chi^2 \) = 38.30, \( P = 0.01 \) for 3 degrees of freedom.

In this experiment, as in the first, the resemblance of the experimental to the theoretical curve is evident, a conclusion which is supported by the
statistical treatment of each set of data. There is in this experiment, as in
the first one, a systematic tendency toward departure of the experimental
from the theoretical curve, in that at low dilutions there is a consistent
excess of negative results, while at high dilutions the reverse tends to be the
case. An explanation of this is not immediately apparent. It is possible
that it is the expression of some fundamental relation between host and
virus, but a more probable explanation is that some technical factor is
responsible.

DISCUSSION

In the present study an effort has been made to define the infectious
unit of myxomatous virus in terms of viral particles, presumably elementary
bodies. This has been done inferentially by comparing the results of
multiple inoculations of viral suspensions with results to be expected from
theoretical considerations. The method depends upon the primary as-
sumption that if the virus is particulate its distribution in a dilute suspen-
sion should be governed by Poisson's law of small numbers, and therefore
if the concentration of particles is known, the probability of obtaining a
particle in a single sample may be derived mathematically. The results
obtained in the foregoing experiments appear to be explained best by the
assumption that a single particle of virus is capable of causing infection
of the rabbit skin. While the data do not necessarily indicate that
each infective particle reaches a susceptible cell and infects it, they do
indicate that the chance of a single particle entering a susceptible cell is
a regular one, and the simplest explanation would appear to be that if only
one or two particles are inserted into the skin, they remain at the site of
inoculation, and are engulfed by cells capable of supporting the multiplica-
tion of virus.

The results presented indicate, moreover, that since chance enters so
largely into the result when dilute viral suspensions are injected, accurate
titration of the virus requires that multiple inoculations of proper dilu-
tions be made, and that the data obtained be treated statistically. It
would appear to be most advisable to use as an end point that dilution of
virus which gives rise on inoculation to 50 per cent of positive results. A
simple method for doing this has been described by Muench and Reed (5, 1).

CONCLUSIONS

The virus of infectious myxoma of rabbits behaves on inoculation into
the skin as if infection were initiated by a single viral particle.
INFECTION UNIT OF MYXOMA

BIBLIOGRAPHY