Quantal and graded dose-responses of bluetongue virus: a comparison of their sensitivity as assay methods for neutralizing antibody*

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INTRODUCTION

For the quantification of virus-neutralizing antibodies, various procedures for obtaining quantal, graded or enumerative responses have been employed (Bryan, 1957). When an enumerative response, based on quantitative pock or plaque-counting methods, is not available, a 50 % quantal response, recording an all-or-none reaction, is commonly used. In such a response, based on extinction dilution titrations, each animal or test tube provides only a positive or negative reading. In contrast, in a graded response, which uses a continuous variate such as survival times, each host unit allows a numerical estimation of the virus, or indirectly antibody quantity (Smith & Westgarth, 1957). Therefore, almost twice as many host units are required for bioassays of a certain accuracy in the quantal as in the graded response from which the quantal response is derived.

The purpose of the present study was first to compare the sensitivity of quantal and graded responses to bluetongue virus for the detection of homologous antibody. Secondly, we wanted to analyse these responses to three bluetongue virus variants and in particular to determine the adherence of the quantal responses to the one-or-more particle curve of the Poisson distribution. When comparing the sensitivity of the two responses for antibody detection, the data were analysed by the probit (Bliss, 1952; Finney, 1952) and rankit (Ipsen & Jerne, 1944) methods.

MATERIALS AND METHODS

Viruses

The test virus, California isolate BT_8, was obtained from Dr C. J. York, Indianapolis, Indiana, at the 80th passage level as a suspension of infected chicken embryos. White Leghorn eggs were supplied from a single hatchery.

Embryonated eggs, incubated at 37° C. for 7 days, were inoculated by the yolk-sac or stab method (Gorham, 1957) with a virus suspension containing $10^{2\cdot7}$ egg lethal doses for 50 % (ELD 50). After incubation at 34° C. for 72 hr., whole embryos

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were harvested, and a 1/5 tissue suspension (v/v) was prepared in Difco nutrient broth pH 6·8 containing 250 i.u. penicillin and 100 μg. streptomycin per ml. The suspension was clarified by low-speed centrifugation and the supernatant fluid stored at $-60°$ C. in sealed glass ampoules. This stock virus preparation represented the 180th embryonated egg passage.

The egg-propagated bluetongue virus (EBTV) was adapted to the intracerebral route in unweaned mice (Svehag, 1962). For preparation of a mouse-adapted bluetongue virus (MBTV) stock, Webster Swiss albino mice of 3–4 days of age were inoculated intracerebrally with an estimated $10^{2·7}$ mouse lethal doses for 50 % (MLD 50) of virus and killed when moribund. A 1/5 dilution of brain material was prepared in nutrient broth, the suspension was clarified and the supernatant stored at $-60°$ C. The stock virus used in this study represented the 53rd serial mouse passage.

Infectivity assays

Two inoculation techniques were used for the chicken embryos: the stab method (Gorham, 1957), in which several embryonic structures received virus, or the yolk-sac route. The eggs were inoculated with 0·2 ml. virus and incubated at 37° C. for 7 days, at which time all eggs were opened and inspected so that non-specific deaths could be differentiated from deaths caused by the virus. Embryos succumbing to EBTV infection usually have a cherry-red colour. For plotting the graded response infected eggs were candled at 6 hr. intervals from 30 to 180 hr. post-inoculation (p.i.). Infectivity titres were calculated by the method of Reed & Muench (1938) or by probit analysis (Bliss, 1952; Finney, 1952) and are expressed in $\log_{10}$ ELD 50 per ml. undiluted chicken embryo homogenate.

Mouse-adapted virus was propagated by inoculation (0·02 ml.) into the left cerebral hemisphere of 3- to 4-day-old mice. In determining the graded response to MBTV, the mortalities were recorded every 4th hour from 36 to 124 hr. p.i. The quantal response as recorded by death or survival of inoculated mice was obtained 9 days p.i. The methods of Reed and Muench or probit analysis were used for calculating 50 % end-points and titres are expressed in $\log_{10}$ MLD 50 per ml. undiluted mouse brain homogenate. The virus stock had a geometric mean titre in $\log_{10}$ MLD 50 per ml. of 8·5 and the standard error of the mean was 0·056.

Sera

Antiserum was prepared in a sheep by three subcutaneous inoculations of about 50,000 MLD 50/dose of MBTV. The animal was bled before immunization and 2 weeks after the final virus injection. The sera were stored at $-25°$ C. without preservatives.

EXPERIMENTS AND RESULTS

I. *Analysis of quantal and graded dose-responses to bluetongue virus*

(1) *Quantal dose-responses for bluetongue virus variants.*

Quantal dose-responses obtained with BTV in three different *in vivo* systems were compared with theoretical dose-response curves, constructed according to the Poisson distribution for the random variation of particles in virus inocula

(Fig. 1). The latter curves represent the frequencies of samples containing at least 1, 2, 3, 4 and 5 virus particles for various average numbers of particles per inoculum. The responses in the three *in vivo* systems are plotted so that the fitted curves coincide at 50 %.

The response data fit best to the 'one-or-more particle' Poissonian curve. The results obtained with mouse-adapted virus show a high degree of goodness of fit to this curve when the differences between observed and expected frequencies are
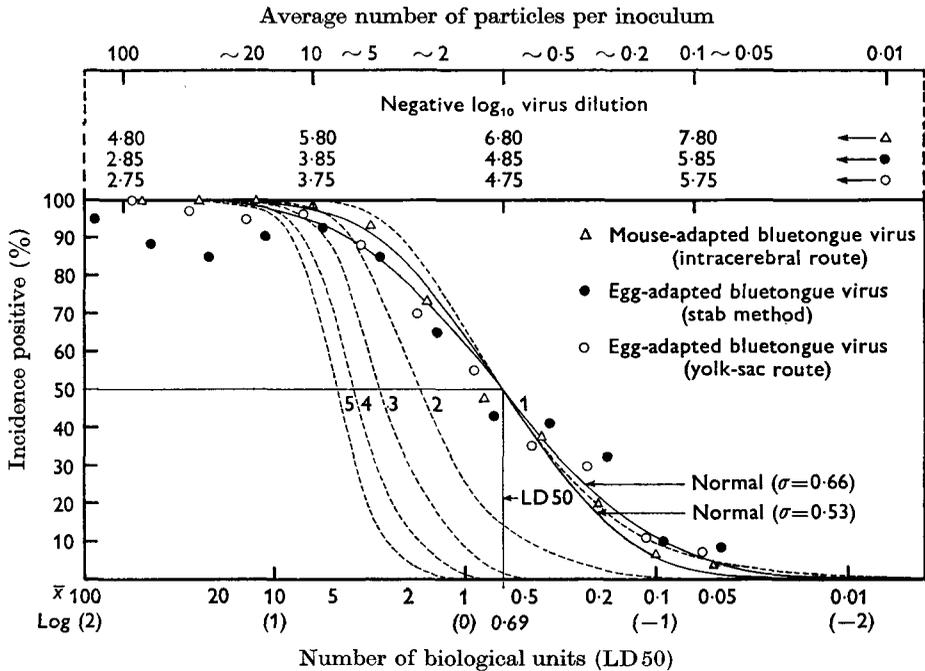

Fig. 1. Quantal dose-response curves of three bluetongue virus variants compared with the theoretical Poisson curves for samples containing at least 1, at least 2, etc., virus particles and with integrated normal distributions with standard deviations (σ) of 0·53 and 0·66. Number of animals: ($\triangle$) 165, ($\bullet$) 180, ($\bigcirc$) 176.

tested with the χ^2-test. However, a satisfactory approximation to the same data is also given by a cumulative normal distribution curve with a standard deviation of about 0·5 in $\log_{10}$ dose units. The curves fitted to the responses of the other two virus variants are flatter and the responses are more irregular. Moran (1954 *a*, *b*) has described a test specifically designed for the detection of heterogeneity. This test is particularly useful when twofold dilution series, which cover a response range from zero to nearly 100 %, are employed. When this test is applied to the data for the yolk-sac route, the fit to the theoretical 'one particle' curve is not acceptable ($T = 334$, $E(T) = 240$, S.E. $(T) = 35\cdot14$ and $\{T-E(T)\}/$S.E. $(T) = 2\cdot7$; $P < 0\cdot01$). However, the results obtained with the two egg-adapted virus variants show a good fit to normal distribution curves with slightly greater standard deviations (0·66 for the yolk-sac route and 0·76 for the stab method).

The response to virus propagated by the stab method fits a normal curve only within the response range 0–80 %.

A good fit to the first term of the Poisson distribution, as illustrated by data for MBTV, has usually been interpreted to indicate that a single virus particle is capable of initiating infection (Bryan, 1959). The assumption is made that the response curve only reflects the distribution of virus particles in the inoculum and is unaffected by variations in virus strains or host factors. An experiment was designed to test the hypothesis that the chance variation of one-or-more virus particles in inocula is the only random variable that can account for a curve of this type.
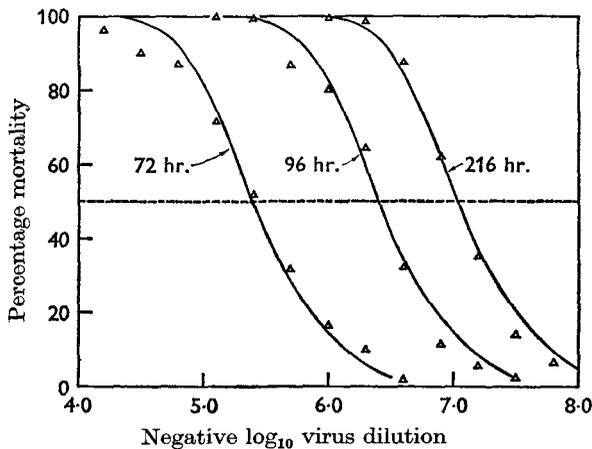

Fig. 2. Quantal responses ($\triangle$) to mouse-adapted bluetongue virus recorded at 72, 96 and 216 hr. post-inoculation. Solid lines represent Poisson 'one-or-more' particle curves superposed at the observed 50 % mortality levels. Number of mice: 143.

The quantal response (percentage mortality) to serial twofold dilutions of MBTV, inoculated intracerebrally in 143 mice, was recorded at 72, 96 and 216 hr. after inoculation (Fig. 2). The character of the response curve was essentially the same at these successive readings although the 50 % infectivity end-point increased with time. The concept that the lower tail of the response is due to the rare distribution of single virus particles in the inoculum does not seem to be compatible with this gradual change in the position of the response curve. For instance, the 72 hr. reading shows that the probability of having at least 1 virus particle in an inoculum from the 10^{-6} dilution is quite low, while according to the 216 hr. reading this occurs with a probability of 1 (causing a 100 % mortality). Therefore, the curves cannot be explained by the random variation of one-or-more virus particles in virus inocula.

The main data obtained with all three virus variants are fitted by integrated normal distribution curves. Such curves can be converted to linear form by the use of probits as response metameter (Fig. 3). The curves for the egg-adapted virus variants are more shallow and the responses more irregular than for the mouse-adapted variant. Rather than fit a curvilinear regression to the data of the egg-adapted variant (yolk-sac), the curve was divided into two linear segments to

obtain a so-called truncated normal distribution (Bliss, 1937; Ipsen, 1949; Bryan, 1956; Svehag, 1962). Possible explanations for the truncation are the presence of a few highly refractory animals in an otherwise uniformly susceptible group of
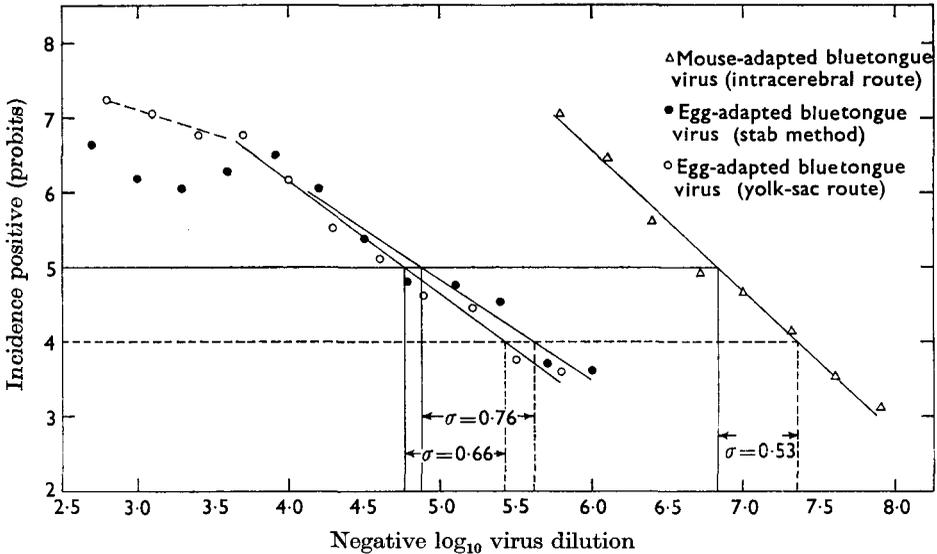

Fig. 3. Quantal dose-response curves of three bluetongue virus variants converted to linear form by the use of probit transformation. σ = standard deviation. Number of animals: ($\triangle$) 120, ($\bullet$) 180, ($\bigcirc$) 176.
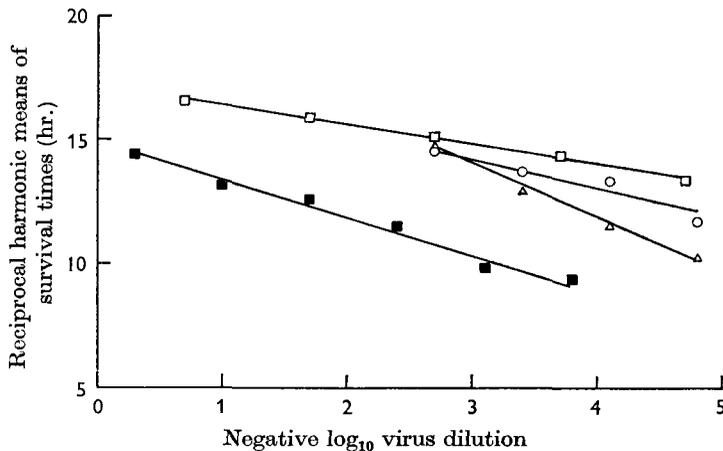

Fig. 4. Graded dose responses for two bluetongue virus variants plotting reciprocal harmonic means of survival times against virus dilutions. ■, Egg-adapted virus (yolk-sac route); □, mouse-adapted virus; ○, mouse-adapted virus and normal serum ($10^{-5.5}$); $\triangle$, mouse-adapted virus and antiserum ($10^{-5.5}$). Number of animals: (■) 75, (□) 95, (○) 60, ($\triangle$) 60.

animals or of reversible aggregates of virus in the lower virus dilutions. The second possibility is less likely since the mouse-adapted virus variant, when assayed in eggs (yolk sac), also gave a truncated distribution as did a partly purified EBTV

preparation. Thus, the truncation appeared to be associated with the assay system and was best explained by the existence of a few highly resistant chicken embryos.

(2) *Graded dose-responses for bluetongue virus variants.*

Before we could proceed with the experiments described in section II, it was necessary to analyse the graded responses with respect to survival times to the virus variants. Fig. 4 illustrates such graded responses to varying amounts of virus. In an attempt to detect a function of the response which would yield a straight line, the plotting of reciprocal harmonic mean times against log virus dilution (Gard, 1940; Mandel & Racker, 1953) was found to give the best fit to such a line. A rectilinear relationship simplifies statistical analysis, and the slope of the response lines was a necessary parameter for the subsequent studies.

Table 1. *Summary of results graphically illustrated in Figs. 5 and 6*

Test	Serum dilution	No. animals	No. positives*
Virus† + IOS	$10^{-4.3}$	48	9
	$10^{-4.6}$	47	18
	$10^{-5.5}\ 10^{-5.8}\ 10^{-6.1}$	48	30
	$10^{-6.4}$	48	31
Virus + NOS	$10^{-4.3}$	48	29
Virus‡ + IOS	$10^{-5.5}$	95	25
Virus + NOS	$10^{-5.5}$	60	16

* At five different dilutions of the virus-serum mixtures.
† Test dose: 100 MLD 50.
‡ Test dose: 2 MLD 50.
IOS, immune ovine serum; NOS, normal ovine serum.

II. *A comparison of the sensitivity of quantal and graded responses for the detection of antibody*

(1) *The quantal response.*

The approach used was simply to find the lowest dilution of a reference antiserum that showed no antibody activity in an assay based on a quantal response and then retest this particular dilution by the graded response method. It was therefore important that the quantal response data be obtained under conditions which were optimal for the detection of antibody.

An ovine antiserum was used as reference serum. Eight serial twofold dilutions of this serum ($10^{-4.3}$ to $10^{-6.4}$) were each incubated with 100 MLD 50 of virus at 37° C. for 24 hr. The virus control consisted of a pre-immunization serum sample from the sheep (diluted $10^{-4.3}$) and 100 MLD 50 of virus (Table 1). After incubation all virus-serum mixtures were placed in an ice bath, diluted in six twofold increments and immediately titrated in unweaned mice using eight animals per dilution.

The resultant quantal responses to the different virus-serum mixtures were compared when the percentage death was transformed into probits and plotted against the logarithm of the dilution of the virus-serum mixtures (Fig. 5). The reasonably good fit to straight lines in the probit analysis indicated that the

response to each virus-serum mixture reflected a host susceptibility that followed a normal distribution. This was in agreement with the results for the MBTV preparation in Fig. 3. The regression coefficients (b) of the lines in Fig. 5, and the corresponding standard deviations of the tolerance distributions (σ) were determined graphically and found not to be significantly different. Therefore, the response lines could be considered parallel and differences in virus titres directly given by
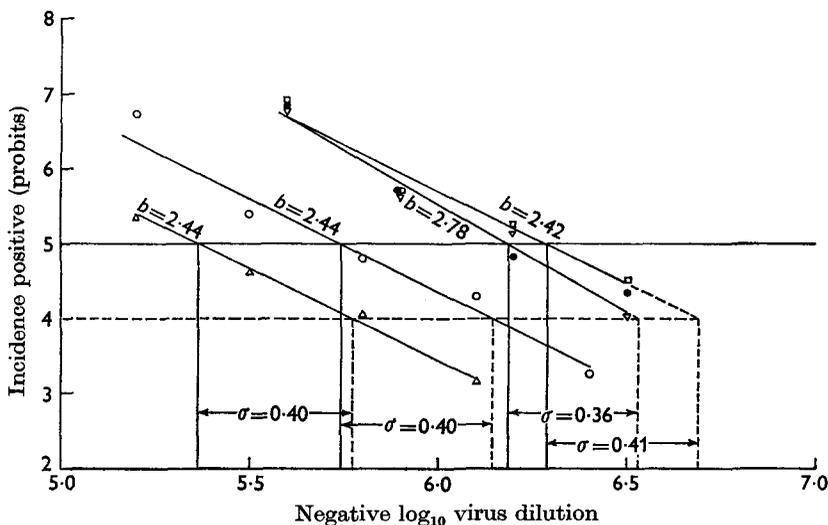

Fig. 5. Probit analysis of quantal response data to seven mouse-adapted bluetongue virus-serum mixtures. $\triangle$, Virus and antiserum ($10^{-4.3}$) $\bigcirc$, virus and anti serum ($10^{-4.6}$), $\triangledown$ virus and antiserum ($10^{-5.5}$, $10^{-5.8}$, $10^{-6.1}$); $\square$, virus and antiserum ($10^{-6.4}$); $\bullet$, virus and normal serum ($10^{-4.3}$); σ = standard deviation. The virus test dose was 100 MLD 50. Number of mice: ($\triangle$) 48, ($\bigcirc$) 47, ($\triangledown$) 48, ($\square$) 48, ($\bullet$) 48.

the displacements of the lines at the probit value 5. The probit slope values of all four responses were higher than those expected according to the independent active theory (Meynell, 1957) as under this theory the slopes should be equal to or less than 2.

In the presence of the lowest dilution of antiserum ($10^{-4.3}$) virus did not produce a 100 % positive response and the 50 % infectivity endpoint was as low as $10^{-5.3}$. With higher dilutions of antiserum the response lines moved closer to the virus control (virus plus normal serum). For the sake of clarity, the lines representing virus plus the serum dilutions $10^{-4.9}$ and $10^{-5.1}$ were not included in the figure but fell between the $10^{-4.6}$ and $10^{-5.5}$ lines. The straight line representing the best fit to the responses of virus plus antiserum of the final dilutions $10^{-5.5}$, $10^{-5.8}$ and $10^{-6.1}$ was almost identical with the line for the virus control. Thus, these virus-serum mixtures evoked the same percentage death and the graphically estimated 50 % end-points were the same, indicating that in the quantal response antibody was not detectable when the antiserum was diluted equal to or more than $10^{-5.5}$.

The test dose of virus used in quantal responses influences the sensitivity of the test for detection of antibody; a smaller virus dose increasing the sensitivity. The $10^{-5.5}$ dilution of antiserum was therefore retested by the quantal response method

against an estimated 2 MLD 50 of virus (Table 1). This experiment was run as a concurrent control with the graded response test described in the next section of this paper. A probit analysis of the results (Fig. 6) revealed no differences between the responses to this virus dose and normal serum and antiserum of the $10^{-5.5}$ dilution respectively. Thus, with this small test dose of virus no neutralizing antibody was demonstrable in this serum sample by the quantal response method.
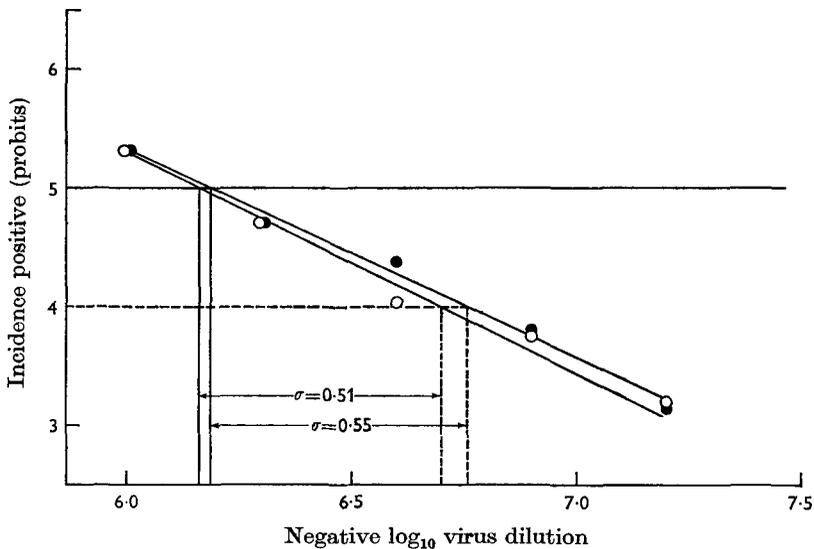

Fig. 6. Probit analysis of quantal response data to two mouse-adapted bluetongue virus-serum mixtures. ○, Virus and antiserum ($10^{-5.5}$); ● virus and normal serum ($10^{-5.5}$). The virus test dose was 2 MLD 50. σ = standard deviation. Number of mice: (○) 95, (●) 60.

(2) The graded response.

The $10^{-5.5}$ dilution of the antiserum, saved in a frozen state from the previous experiments, was also tested for the presence of viral neutralizing antibody by the graded response method. Four fivefold virus dilutions were each divided into two portions and incubated for 24 hr. at 37° C. with antiserum and normal serum (both diluted $10^{-5.5}$) respectively. After incubation all serum-virus mixtures were placed in an ice-bath and immediately assayed in unweaned mice using sixteen animals per reaction mixture. The time of mouse death was recorded at 4 hr. intervals until all mice had succumbed. To allow a graphical and statistical evaluation, the data had to be tested to ascertain whether all the graded responses to time after inoculation with serum-virus mixture showed normal and similar distributions. Therefore, the arithmetic means and standard deviations of the survival times for the responses to virus plus normal serum or antiserum were calculated and multiples of the standard deviations were used as abscissae for two histograms (Fig. 7). Two other histograms representing normal distributions with the same standard deviations and number of observations as in the two test groups were superimposed upon the experimental data. When the observed frequencies were compared in the
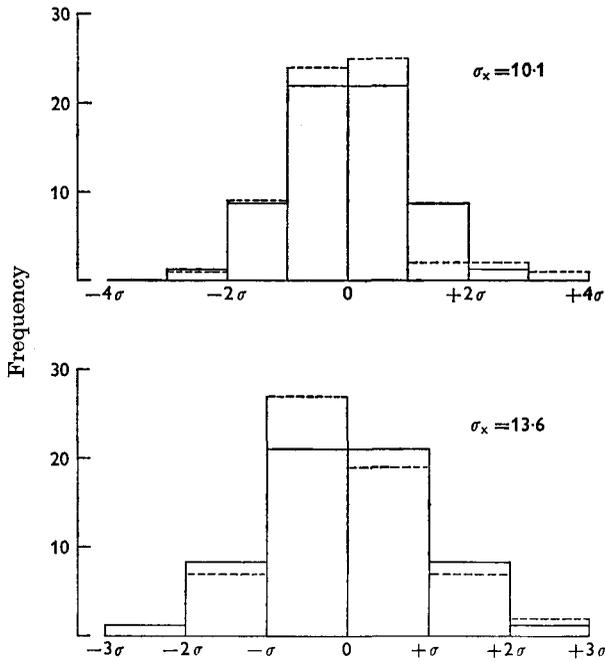
Fig. 7. Variation of susceptibility between mice. The histograms show the distributions of survival times to mouse-adapted virus and normal serum (top part) and virus and antiserum (bottom part) respectively. The solid lines represent normal distributions with the same standard deviations (σ) and number of observations as in the two test groups. Number of mice: top histogram 64, bottom histogram 62.

Fig. 8. Rankit analysis of survival times to different dilutions of mouse-adapted virus and normal serum ($10^{-5\cdot5}$). $\bigcirc$, Virus dilution $10^{-2\cdot7}$; $\triangle$, dilution $10^{-3\cdot4}$; $\square$, dilution $10^{-4\cdot1}$; $\triangledown$, dilution $10^{-4\cdot8}$. Total number of mice 63.

χ^2-test with those expected according to the corresponding normal distributions, there was no significant difference ($\chi^2_{(3)} = 4.3$; $0.3 > P > 0.2$ and $\chi^2_{(3)} = 2.7$; $0.5 > P > 0.4$) indicating that the survival times followed distributions indistinguishable from normal distributions. The mean survival time for responses to virus plus antiserum was 5 hr. longer than for virus plus normal serum, a difference which was significant at the 5 % level ($t = 2.34$; $0.05 > P > 0.01$). The response to virus and antiserum showed the highest standard deviation.
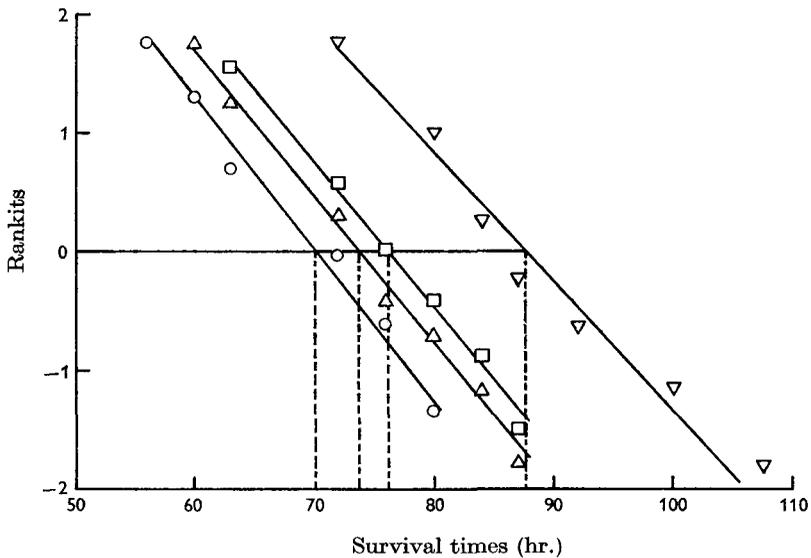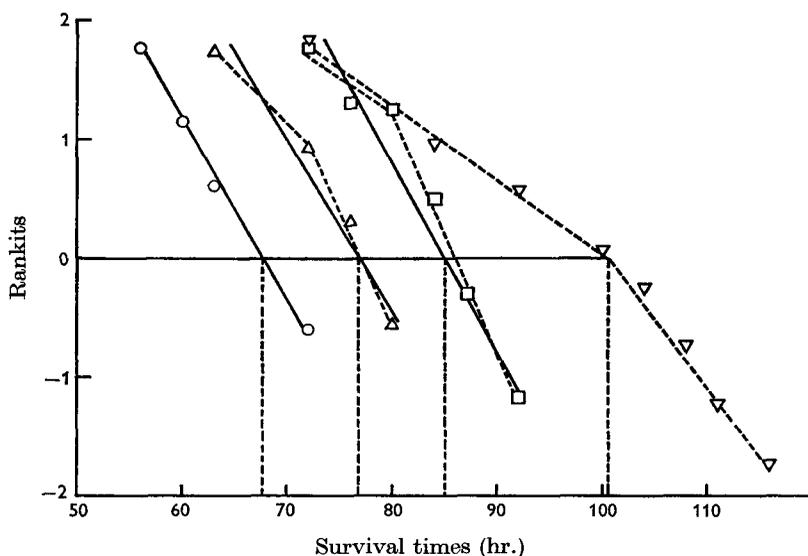

Fig. 9. Rankit analysis of survival times to different dilutions of mouse-adapted virus and antiserum ($10^{-5.5}$). $\bigcirc$, Virus dilution $10^{-2.7}$; $\triangle$, dilution $10^{-3.4}$; $\square$, dilution $10^{-4.1}$; $\bigtriangledown$. dilution $10^{-4.8}$. Total number of mice 63.

It was then tested whether the graded response data within varying dilutions of virus also reflected normal distributions of susceptibility and if a certain virus antiserum mixture was mainly responsible for the change in the overall mean survival time. Therefore, a rankit-analysis (Ipsen & Jerne, 1944) of the time-frequency responses to virus plus normal serum or antiserum was performed (Figs. 8, 9). For virus plus normal serum (Fig. 8), the relationships between time and rankit appeared to be linear, showing that the times were approximately normally distributed. Further, since the standard deviations of the different responses were similar (parallel lines), the means of the survival times could be directly compared. In the analysis of the responses to virus and antiserum (Fig. 9), dilute virus inocula appeared to give slightly truncated response curves. This suggested change from a linear to a truncated response indicated a change in the action of the inoculum. At the $10^{-4.8}$ dilution, the range and the variance of the response was much greater than at the lower virus dilutions. With this virus dose, the first death occurred as early as in the corresponding group in the control with normal serum (Fig. 8), but most animals exhibited distinctly prolonged survival times. Table 2 gives the number of observations, the mean survival times, their

standard errors and the standard deviations of the survival times. When the mean survival times of the responses to virus plus normal and immune serum respectively were compared by the *t*-test, significantly prolonged survival times were observed in response to the two highest virus dilutions plus antiserum.

In the graded response to time with mouse-adapted bluetongue virus, a linear relationship exists, over an intermediate dose range, between the reciprocal harmonic means of survival times and virus dilutions (Svehag, 1962). Also, in this kind of plot (Fig. 4), the effect of the antiserum was observed only in the higher virus dilutions.

Table 2. *Analysis of the survival times to various test doses of bluetongue virus and dilute normal and immune sheep serum respectively*

($\bar{Y}$, Mean survival time; σ, standard deviation; $\sigma_{\bar{Y}}$, standard error of the mean; N.S., not significant; S., significant.)

Log$_{10}$ virus dilution	Virus plus normal sheep serum				Virus plus immune sheep serum				*t*-test for $\bar{Y}_1 = \bar{Y}_2$
	No. observa-tions	$\bar{Y}_1$	σ	$\sigma_{\bar{Y}_1}$	No. observa-tions	$\bar{Y}_2$	σ	$\sigma_{\bar{Y}_2}$	
−2·7	16	70	8	2	16	68	7	1·8	N.S.*
−3·4	16	73·5	7·5	1·9	16	77·5	7	1·8	N.S.**
−4·1	16	76	8	2	16	85	6·5	1·6	S.**
−4·8	16	87·5	9	2·3	15	101	8·5	0·94	S.***

DISCUSSION

When samples are taken at random from a homogeneous virus suspension and every sample containing at least one infectious virus particle produces a visible sign of infection, the quantal dose response curve is expected to be Poissonian. The fact that only a fraction of the virus particles may be infectious does not affect the Poissonian character of the curve. The assumption is made that the host system is uniformly susceptible.

Deviation of observed quantal dose-response curves from the theoretical one-or-more particle Poisson curve have, however, been observed in some virus-host systems (Bryan & Beard, 1940; Meynell, 1957; Fazekas de St Groth & Cairns, 1952; Armitage & Spicer, 1956). In particular, the slopes have been significantly less than that of the Poisson curve. Parker's response curves with vaccinia virus (1938) also showed shallower slopes than those expected from the Poisson distribution. In contrast, his data gave a very good approximation to normal distribution curves. Similar results were obtained with the two egg-adapted variants of blue-tongue virus in the present study.

Factors considered responsible for the deviations from the theoretical exponential curve were reversible aggregation of virus particles and variations in susceptibility among host units. It has been suggested that a shallow slope could be due to the presence of reversible aggregates of virus particles that dissociate into smaller aggregates on stepwise dilution (Price, 1946). This explanation was unlikely in the

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present study since one and the same MBTV preparation yielded data which in one system (intracerebral route) agreed well with the theoretical 'one particle curve', while in another (yolk-sac route) the response curve was significantly shallower. Further, both ultracentrifugally purified and unpurified EBTV gave curves with slopes less than the Poisson curve when administered by the yolk-sac route.

The type of truncation illustrated in Fig. 3 is probably due to the existence of a few more resistant chicken embryos. The presence of individuals with increased resistance will show up particularly in the upper tail of the curve as 100 % mortality is expected here. This heterogeneity in the test population will mask the effect of the statistical distribution of infectious virus particles. Had an interferon effect been involved, this would have been expected to affect the response curve in the high dilution range.

There are only a few reports in the literature of dose-response curves which are steeper than the 'one particle curve'. They have been associated with complicating factors in the assay system, e.g. toxic effects of influenza (Henle & Henle, 1946) and adenovirus preparations (Pereira & Kelly, 1957) in mice and HeLa cell cultures respectively. Thus, the theoretical one-or-more particle curve came to be generally recognized as the limiting curve of error for quantal responses to viruses. Yet, too much stress should not be laid on the fact that good agreement exists between an experimental dose-response curve and a theoretical curve based on the assumption that at least one infectious virus particle is necessary to produce visible signs of infection. Factors other than the statistical distribution of virus particles may be responsible for or affect the observed distribution. The finding in the present study that the character of the quantal responses to MBTV was the same at three successive readings even though the infectivity end-point gradually increased did not support the assumption that the response curves only reflected the distribution of virus particles in inocula. The data are, however, consistent with the more general version of the independent active theory (Meynell, 1957) which assumes that each particle has a probability P of initiating an infection. The different curves in Fig. 2 would correspond to different values of P. Pereira & Kelly (1957) similarly reported that the character of quantal dose-response curves to adenovirus in HeLa cell cultures remained the same at three successive readings but varied in position with time.

The quantal dose-response curves to the EBTV variants had the appearance of cumulative normal distribution curves and could consequently be converted to linear forms by the use of probits (probability units) as the response metameter. Probit transformation (Bliss, 1952) is merely a statistical device for transforming the bell-shaped or sigmoidal normal distribution curve into a straight line by the proper choice of coordinates. The probit method permits good utilization of the data, including the 5–95 % response range and easy graphic estimation of the 50 % dose effect and the standard deviation of the distribution. Further, the slope of the response line represents a valuable new parameter.

Normality must be assumed when probits are used. Although the MBTV data showed no exact fit to a normal distribution, the approximation was sufficiently

close to justify the application of probit transformation also to results obtained with this virus variant. The relatively steep probit line obtained with MBTV was advantageous as the accuracy of a dose-response assay largely depends on the steepness and reproducibility of the probit-regression line—a steep slope permitting more precise quantification. Therefore, in the comparison of the sensitivity of quantal and graded responses to BTV for the detection of specific antibody, the response to the MBTV variant was employed.

It is advantageous for statistical analysis to work with response data which are linearly related to dosage or dosage transformed to other units. When the percentage response to MBTV was expressed as probits and virus doses as their logarithms, the relationship was linear. In the graded response a linear relationship was obtained when the rank order of observed data was transformed into rank units (Ipsen & Jerne, 1944). The plotting of reciprocal harmonic mean times against the logarithms of the virus doses was also found to result in a linear relationship. For comparing the sensitivity of quantal and graded responses, the rankit transformation was preferred.

The distributions of individual graded responses about their mean value are frequently unsymmetrical and the frequency distribution may tail out toward the higher measurement values as in Fig. 7. The survival times to MBTV, however, did not deviate significantly from a normal distribution. Further, as the standard deviations of the responses to different doses of virus were similar, the amount of antibody present in a certain virus-serum mixture could be estimated by reference to a control preparation of virus and normal serum. The average test dose of virus used in the quantal response (100 MLD 50) was less than that in the graded response (equal to or more than 1000 MLD 50) which might be expected to render the quantal response more sensitive for the detection of antibody. In spite of this, the graded response to time was found to be superior for the detection of minor amounts of antibody to BTV.

SUMMARY

The sensitivity of quantal and graded responses to mouse-adapted bluetongue virus for the detection of neutralizing antibody was compared using probit and rankit analysis. The graded response, based on survival times, allowed the demonstration of antibody in highly dilute serum, in which antibody was not detected by the quantal response recording percentage death.

Quantal responses to bluetongue virus variants were compared with theoretical dose-response curves constructed according to the Poisson distribution for the random variation of virus particles in inocula. Of these theoretical curves the first term in the Poisson distribution gave the best approximation to the experimental data but the fit to normal distribution curves was better. The quantal responses to bluetongue virus did not appear to reflect the random variation of one-or-more infectious virus particles in inocula.

In graded responses to bluetongue virus, a rectilinear relationship was observed between reciprocal harmonic means of survival times and log virus dilutions.

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REFERENCES

ARMITAGE, P. & SPICER, C. C. (1956). The detection of variation in host susceptibility in dilution counting experiments. *J. Hyg., Camb.*, **54**, 401.

BLISS, C. I. (1952). *The Statistics of Bioassay*. New York: Academic Press Inc.

BLISS, C. I. (1937). The calculation of the time mortality curve. *Ann. appl. Biol.* **24**, 815.

BRYAN, W. R. & BEARD, J. W. (1940). Host influence in the characterization of response to the papilloma protein and to vaccinia virus. *J. infect. Dis.* **67**, 5.

BRYAN, W. R. (1956). Biological studies on the Rous sarcoma virus. IV. Interpretation of tumor-response data involving one inoculation site per chicken. *J. natn. Cancer Inst.* **16**, 843.

BRYAN, W. R. (1957). Interpretation of host response in quantitative studies on animal viruses. *Ann. N.Y. Acad. Sci.* **69**, 698.

BRYAN, W. R. (1959). Quantitative biological experimentation in the virus and cancer fields. *J. natn. Cancer Inst.* **22**, 129.

FAZEKAS DE ST GROTH, S. & CAIRNS, H. J. F. (1952). Quantitative aspects of influenza virus multiplication. IV. Definition of constants and general discussion. *J. Immun.* **69**, 173.

FINNEY, D. J. (1952). *Probit Analysis,* 2nd ed. Cambridge University Press.

GARD, S. (1940). Encephalomyelitis of mice. II. A method for the measurement of virus activity. *J. exp. Med.* **72**, 69.

GORHAM, J. R. (1957). A simple technique for the inoculation of the chorioallantoic membrane of chicken embryos. *Am. J. vet. Res.* **18**, 691.

HENLE, G. & HENLE, W. (1946). Studies on the toxicity of influenza viruses. I. The effect of intracerebral injection of influenza viruses. *J. exp. Med.* **84**, 623.

IPSEN, J. Jr. & JERNE, N. K. (1944). Graphical evaluation of the distribution of small experimental series. *Acta path. microbiol. scand.* **21**, 342.

IPSEN, J. Jr. (1949). A practical method of estimating the mean and standard deviation of truncated normal distributions. *Hum. Biol.* **21**, 1.

MANDEL, B. & RACKER, E. (1953). Inhibition of Theiler's encephalomyelitis virus (GD VII strain) of mice by an intestinal mucopolysaccharide. *J. exp. Med.* **98**, 399.

MEYNELL, G. G. (1957). The applicability of the hypothesis of independent action to fatal infections in mice given *Salmonella typhimurium* by mouth. *J. gen. Microbiol.* **16**, 396.

MORAN, P. A. P. (1954a). The dilution assay of viruses. *J. Hyg., Camb.*, **52**, 189.

MORAN, P. A. P. (1954b). The dilution assay of viruses. II. *J. Hyg., Camb.*, **52**, 444.

PARKER, R. F. (1938). Statistical studies of the nature of the infectious unit of vaccine virus. *J. exp. Med.* **67**, 725.

PEREIRA, H. G. & KELLY, B. (1957). Dose-response curves of toxic and infective actions of adenovirus in HeLa cell cultures. *J. gen. Microbiol.* **17**, 517.

PRICE, W. C. (1946). Measurement of virus activity in plants. *Biometr. Bull.* **2**, 81.

REED, L. J. & MUENCH, H. (1938). A simple method of estimating 50 percent endpoints. *Am. J. Hyg.* **27**, 493.

SMITH, G. C. E. & WESTGARTH, D. R. (1957). The use of survival time in the analysis of neutralization tests for serum antibody surveys. *J. Hyg., Camb.*, **55**, 224.

SVEHAG, S-E. (1962). Quantitative studies of blue tongue virus in mice. *Arch. ges. Virusforsch.* **12**, 363.