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AN INVESTIGATION INTO THE BASIC VIRUS-ANTIBODY NEUTRALIZATION REACTION, WITH SPECIAL REGARD TO THE REACTION IN THE CONSTANT-VIRUS/VARYING-SERUM NEUTRALIZATION TEST*

By

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BITSCH, V.: *An investigation into the basic virus-antibody neutralization reaction, with special regard to the reaction in the constant-virus/varying-serum neutralization test.* Acta vet. scand. 1978, *19*, 110—128. — A study of the basic reaction in neutralization of virus (V) by virus-neutralizing antibody (VNA) was performed with infectious bovine rhinotracheitis virus and serum collected from naturally and experimentally infected cattle after the primary immunization phase. In constant-virus/varying-serum neutralization tests a direct proportionality between VNA titer and length of preincubation was observed and found to be in accordance with basic laws of neutralization. A deviation from this direct proportionality, which was partly attributed to the presence of a dissociable V-VNA complex, was seen with relatively short preincubation. Expressing a relationship between VNA titer, length of preincubation, and virus dose under conditions where a dissociable V-VNA complex can be ignored, a log. VNA/log. V equivalence factor of neutralization was introduced. A linear relationship was found between VNA titer, taken logarithmically, and preincubation temperature. A rise in temperature by 10°C gave an increase in VNA titer of approx. 1.2 in $\log_2$. Formulae are presented for the neutralization rate factor corrected for a demonstrated invalidity of the percentage law, and for the relation between the neutralization rate factor and VNA titer. It is concluded that the results presented have elucidated the possibilities of improving the sensitivity of neutralization tests.

virus-antibody reaction; neutralization test.

Burnet et al. (1937) found that neutralization of virus in a virus-serum mixture was proportional to the antibody concentration, and that the proportion of unneutralized virus was in-

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dependent of the initial virus concentration, the percentage law (*Andrewes & Elford* 1933). *Dulbecco et al.* (1956) confirmed these conclusions. The initial virus neutralization was linearly related to time, and a neutralization rate factor, defined by the formula $K = \frac{D}{T} \ln \frac{V_O}{V_T}$*, was introduced for expression of neutralizing activity of a serum. *Burnet et al.* had developed an identical formula for the phage-antibody reaction.

Dissociability of the virus-antibody complex under physiological conditions has been a subject of discussion since 1928, when *Andrewes* reported that the combination product of vaccinia virus and antibody was dissociable on dilution. *Andrewes* (1930) later found that after prolonged incubation demonstration of dissociation was more difficult. *Burnet et al.* maintained that the virus-antibody complex was almost completely dissociable on dilution and that the neutralization would lead to an "equilibrium state". *Dulbecco et al.* found little or no dissociation at 37°C under physiological conditions and attributed the "equilibrium state" to a fraction of virus being resistent to neutralization. *Gard* (1955) concluded that the virus-antibody complex was initially dissociable but stable after a prolonged reaction period, the length of which was dependent on the temperature. A similar point of view was presented by *Lafferty* (1963). *Brunner & Ward* (1959) found different dissociation rates in acute and convalescent phase serum, and later studies have shown that the neutralization reaction is of a complex nature, as different immunoglobulin classes may be involved. Late in the course of immunization, however, the reacting antibody would predominantly be of one class, i. e. IgG (cf. reviews by *Svehag* 1968 and *Cowan* 1973).

The neutralization rate factor has proved to be of limited importance as expression of the neutralizing activity of sera, and the constant-virus/varying-serum method has become the test usually employed. The test conditions, especially the temperature and length of incubation, have been chosen empirically rather than according to basic laws of neutralization.

In the present work a study was made of the basic and uncomplicated virus-serum neutralization reaction with a special

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* D is the serum dilution factor, T the reaction period, and V_O and V_T titers of unneutralized virus after O and T min. of reaction.

view to disclosing determinatives of the constant-virus/varying-serum neutralization test. The model chosen was the relatively heat-resistent infectious bovine rhinotracheitis virus and antiserum collected from cattle relatively long time after natural or simulated natural infection.

Abbreviations and definitions

IBR: Infectious bovine rhinotracheitis.

D: Dilution factor of serum. When applied to virus-serum mixtures, D is the dilution factor of serum before the mixing, in equal amounts, with the virus suspension.

Preincubation: Incubation of virus-serum mixtures before inoculation of tissue cultures.

S: Serum titer.

T: (Pre)incubation period for virus-serum mixtures (hours).

TCID50: Fifty % tissue culture infective dose(s).

V: Virus, virus titer (TCID50 per 0.1 ml of the original virus suspension (virus dose) or per 0.2 ml of the virus-serum mixture); V_O and V_T: virus titer after O (also virus dose) and T hours of incubation, respectively.

K: Neutralization rate factor, K_{10}: the factor related to a virus dose of 10 TCID50; K_{st}: standard neutralization rate factor. (When D is recorded by half its actual value in a virus-serum mixture, the value of K will be reduced likewise).

VNA: Virus-neutralizing antibody.

VNA test, or cv/vs test: Constant-virus/varying-serum neutralization test.

vv/cs test: Varying-virus/constant-serum neutralization test.

cv/vs neutralization curve: The relationship shown graphically on a logarithmic base between VNA titer and preincubation period.

P_6^{37} test: VNA test, with indication of preincubation temperature (37°C) and period (6 hrs.).

Overneutralization: Neutralization more advanced than expected merely from first order neutralization kinetics.

MATERIAL AND METHODS

Virus. The Danish IBR virus strain DaB69 was employed.

Sera. Late antisera from two bulls infected experimentally with the above virus strain (*Bitsch* 1973) and serum of a naturally infected bull were used. The sera from the experimental bulls were collected 116 (Sera A_1 and B_1), 121 (Serum B_2) and 122 weeks (Serum B_3) after infection. Serum of the naturally infected bull was taken seven years after infection (Serum D). All sera were stored at —20°C after inactivation at 56°C for 30 min.

Tissue cultures. Primary or secondary calf kidney cell cultures in roller tubes were used throughout the study. Maintenance medium was Earle balanced salt solution with 0.05 % lactalbumin hydrolysate, 0.01 % yeast extract, 2 % SPF calf serum, 100 i.u. of penicillin and 0.1 mg of streptomycin per ml. Each tube contained 1.8 ml medium.

Test procedures. Hanks balanced salt solution with 2 % SPF calf serum and 50 µg neomycin per ml was used for virus suspensions and serum dilutions, which in all experiments were preheated to the actual temperature of reaction. Unless otherwise mentioned, tissue cultures were inoculated shortly after change to maintenance medium. Final readings were taken after seven days at 37°C. Titers of virus suspensions were recorded as TCID50 per 0.1 ml, but of unneutralized virus in virus-serum mixtures as TCID50 per 0.2 ml. VNA titers were recorded as the serum dilution factors corresponding to the 50 % endpoints of neutralization. All titers were calculated after the Spearman-Kärber method. The following two tests were employed:

T e s t I. To examine the kinetics of neutralization in a virus-serum mixture serial twofold or tenfold dilutions with diluent cooled to 4°C were made after appropriate periods of reaction. Infectivity was tested by inoculation of four tissue culture tubes from each dilution. The dose inoculated was 0.2 ml.

T e s t I I. To examine the relation between the VNA titer and the preincubation period of the cv/vs test, i. e. the progression of the neutralization, twofold dilutions of the test serum were mixed with equal amounts of a virus suspension of known titer. After appropriate periods of reaction, infectivity was assayed by inoculation of four tissue culture tubes from each virus-serum mixture. The dose inoculated was 0.2 ml. — Unless otherwise stated, the titer of the virus suspension used was checked by inoculating six tissue culture tubes from each of a series of tenfold dilutions of the virus suspension. The dose inoculated was 0.1 ml.

RESULTS

Experiment I. The relation between VNA titer and duration of preincubation in tests with extended preincubation

Test II was used on Serum B_1. The preincubation temperatures were 4, 22 and 37°C, and the periods ranged from 0.75 to

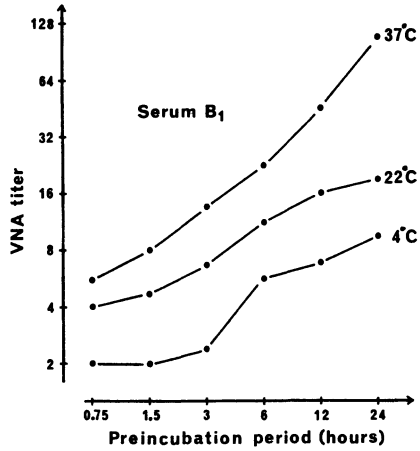


Figure 1. The relation between virus-neutralizing antibody (VNA) titer and duration of preincubation at varying temperatures (Expt. I).

24 hrs. The titer of the virus suspension was approx. 100 TCID50 per 0.1 ml.

VNA titers and preincubation periods showed an almost linear relationship when plotted on logarithmic scales (Fig. 1), but the curves obtained at 22 and 4°C tended to be less steep than that obtained at 37°C.

Experiment II. The relation between VNA titer and duration of preincubation in tests with short preincubation

Test II was used on Sera B_1, B_2 and B_3. The virus suspension contained approx. 100 TCID50 per 0.1 ml. In tests with Serum B_3, besides four tubes with maintenance medium, four tubes without medium were inoculated from each virus-serum mixture. Maintenance medium was then added after 3 hrs. at 37°C.

Again an almost linear relationship could be demonstrated between logarithmic values of the VNA titers and the preincubation periods (Fig. 2). Neutralization progressed similarly with all three sera, but the slope of the curves was less than after preincubation for longer periods. A higher degree of neutralization was observed after inoculation of tubes without medium. The difference was most pronounced with short preincubation and tended to disappear when preincubation was extended to abt. 1 hr.

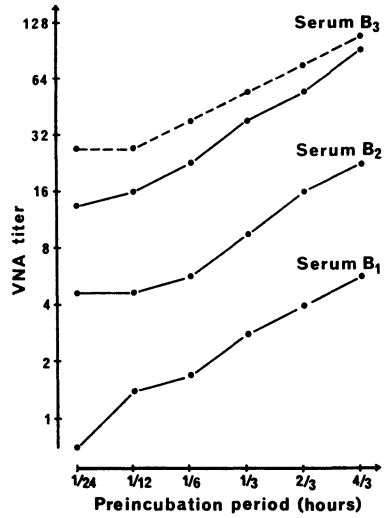


Figure 2. The relation between virus-neutralizing antibody (VNA) titer and duration of preincubation at 37°C. The dotted line shows the results obtained when cultures were inoculated before medium was added (Expt. II).

Experiment III. The kinetics of neutralization in a virus-serum mixture with low antibody concentration

Test I was used, with a virus suspension with approx. 100 TCID50 per 0.1 ml. The incubation temperature was 37°C. Serum B_1 was used in dilutions 1/16 and 1/64. Twofold dilutions of the mixtures were made. A VNA-negative control serum was included.

Only negligible quantities of the virus was inactivated by heat during the 24 hrs. of incubation at 37°C (Fig. 3) and the two serum dilutions showed a linear relationship between incubation time and titer of unneutralized virus, when plotted semilogarithmically. The stronger serum dilution gave a neutralization rate of practically four times that of the weaker.

Experiment IV. The kinetics of neutralization in a virus-serum mixture with relatively high antibody concentration

Test I was used with approx. 100 TCID50 per 0.1 ml of the virus suspension and an incubation temperature of 37°C. Tissue cultures without medium were inoculated, maintenance medium

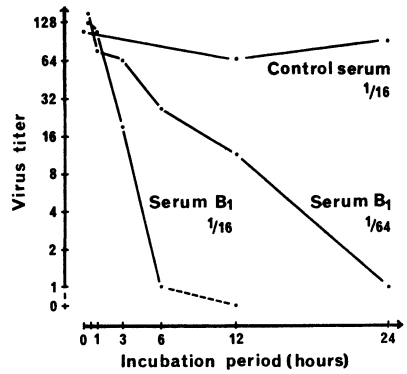


Figure 3. The kinetics of neutralization in virus-serum mixtures with low antibody concentration (Expt. III).

being added after 3 hrs. at 37°C. Serum A_1 was used in dilution 1/8.

The results (Table 1) demonstrate an overneutralization phenomenon in the undiluted virus-serum mixture and in the lower dilutions thereof.

Table 1. Kinetics of neutralization in a virus-serum mixture with relatively high antibody concentration (Expt. IV). The table gives the number of tubes, out of four, exhibiting growth of virus after inoculation from dilutions of the virus-serum mixture.

Period of incubation (min.)	Dilutions of the virus-serum mixture									
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
0	4	4	4	4	4	4	4	4	2	0
5	4	4	4	4	4	4	3	2	1	0
10	4	4	4	4	4	4	2	1	1	0
20	3	4	4	4	4	3	2	0	1	0
30	1	2	3	4	3	3	1	0	0	1
50	1	2	3	2	3	0	3	1	1	0

Experiment V. Overneutralization in a cv/vs test with short preincubation

Tests II and I were used with preincubation at 37°C. Serum D was tested against a virus dose of approx. 1000 TCID50. In Test II dilutions of serum were made at intervals of 0.5 in $\log_2$. After 10 min. twofold dilutions were made of each virus-serum

mixture, and cultures without medium were inoculated immediately from these dilutions (Test I). Maintenance medium was added after 3 hrs. at 37°C.

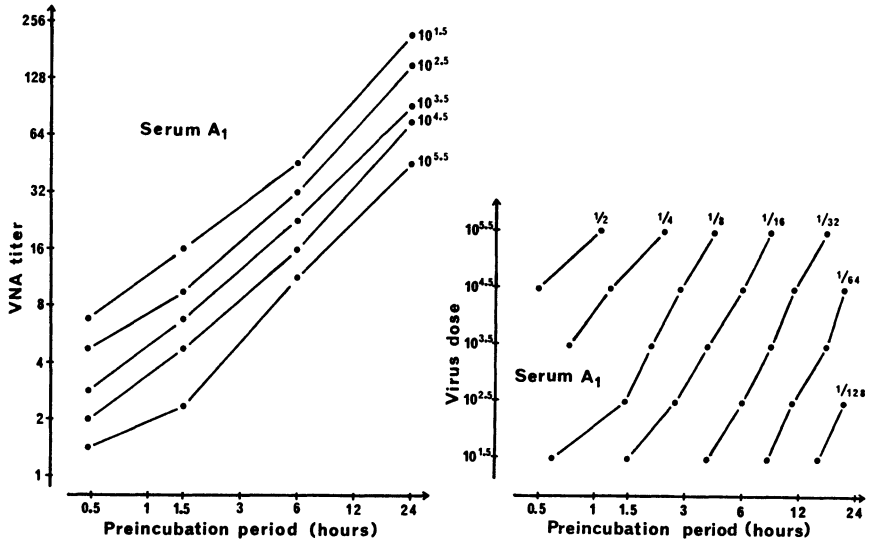
Table 2. Overneutralization in a constant-virus/varying-serum neutralization test with a short period of reaction (Expt. V). The table gives the number of tubes, out of four, exhibiting growth of virus after inoculation from the dilutions of the virus-serum mixtures.

Dilutions of the virus-serum mixtures incubated for 10 min.	Virus-serum mixtures defined by the serum dilutions tested						
	1/1	1/1.4	1/2	1/2.8	1/4	1/5.6	1/8
1/1 (undiluted)	0	0	0	0	0	3	4
1/2	0	0	0	0	1	3	4
1/4	0	0	0	1	3	3	4
1/8	0	0	1	2	2	3	4
1/16	0	0	0	3	4	4	4
1/32	0	1	0	2	4	4	4
1/64	0	1	2	2	1	1	3
1/128	0	0	0	3	0	4	4
1/256	0	0	0	0	1	1	2
1/512	0	0	0	0	1	1	1
1/1024	0	0	0	0	0	0	.
Sum of tubes with growth of virus	0	2	3	13	17	27	34

The results (Table 2) clearly demonstrate overneutralization in lower dilutions of the virus-serum mixtures, the neutralization endpoints being higher than should be expected from the results of inoculation from higher dilutions of the virus-serum mixtures.

Experiment VI. The relation between VNA titer, length of preincubation and virus dose.

Test II was employed with virus suspensions containing from about $10^{1.0}$ to $10^{5.5}$ TCID50 per 0.1 ml. In one part of the experiment Sera A_1 and D were tested with preincubation at 37°C for up to 24 hrs. On testing Serum D a study was also made of the kinetics of neutralization in mixtures of the virus suspension of highest titer and serum dilutions 1/4 and 1/16 (Test I). In the second part Serum D was tested with preincubation at 4°C for periods ranging from 12 to 192 hrs. The VNA titers after preincubation at 37°C were recorded simultaneously.

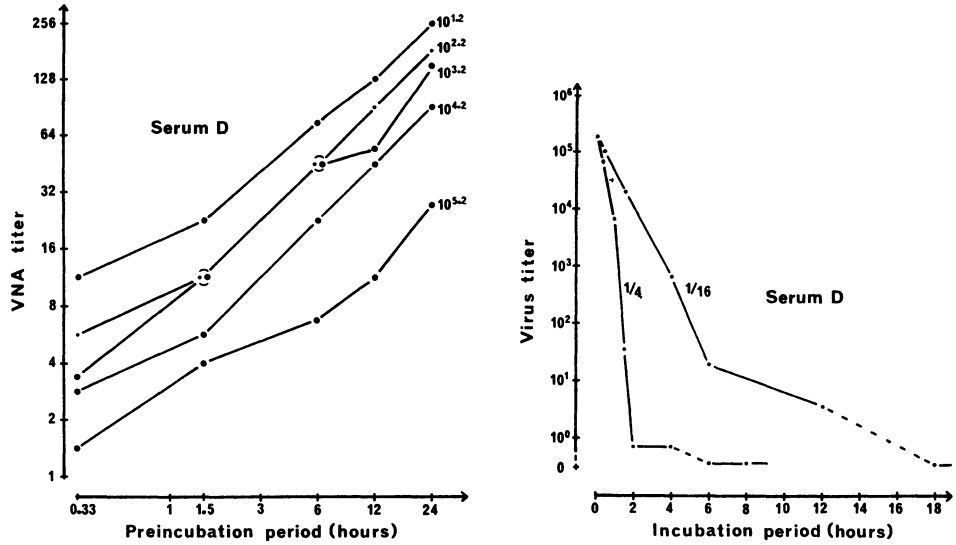


Figures 4a and 4b. The relation between virus-neutralizing antibody (VNA) titer, length of preincubation at 37°C, and virus dose in the constant-virus/varying-serum neutralization test. An example with a regular progression of neutralization. The abscissa and ordinate in Fig. 4b are for practical reasons shown with scales based on different logarithmic units. Antibody concentrations are shown by the serum dilutions employed (Expt. VI).

The slopes of the cv/vs neutralization curves with Serum A_1 (Fig. 4a) all appear to be identical and to be approaching 1 with increasing length of preincubation.

The preincubation periods required for the various serum dilutions to reduce the virus titers of the mixtures to 1 TCID50 per 0.2 ml can be read from the curves in Fig. 4a. When, again logarithmically, these incubation periods are plotted against virus doses (Fig. 4b), a linear relationship is found with incubation periods exceeding 2—3 hrs. This relationship illustrates the progression of neutralization in the vv/cs neutralization test.

The neutralization curves obtained with Serum D (Fig. 5a) are identical with those obtained with Serum A_1 (Fig. 4a) apart from the fact that VNA titers recorded with the highest virus dose were relatively low, especially after long preincubation (low antibody concentration). In accordance with this latter observation the curves in Fig. 5b show that a residual part of the virus was neutralized at a lower rate, especially in the mixture with highest serum dilution (1/16).



Figures 5a and 5b. The relation between virus-neutralizing antibody (VNA) titer, length of preincubation at 37°C, and virus dose in the constant-virus/varying-serum neutralization test (Fig. 5a), and the kinetics of neutralization of the highest virus dose with two serum dilutions (Fig. 5b). An example with an irregular progression of neutralization (Expt. VI).

The neutralization curves obtained in the second part of the experiment (Fig. 6) are practically all linear with slopes of 1, though with a tendency to lower values with low virus doses after preincubation for less than 24 hrs. and with the highest virus dose after preincubation for more than 96 hrs. The $P_{24}^{3\,7}$ titers obtained with the various virus doses are compatible with the results in Fig. 5a.

Experiment VII. The relation between VNA titer and preincubation temperature in tests with extended preincubation

Test II was used on Serum D with approx. 1000 TCID50 per 0.1 ml of the virus suspension. Preincubation took place at 4, 15, 26 and 37°C and lasted 12 to 192 hrs.

With so long preincubation all neutralization curves (Fig. 7) showed a slope of 1, irrespective of the temperature. The results furthermore suggest a linear relationship between preincubation temperatures within 4 to 37°C and logarithmic values of VNA titer, a temperature increase of 10°C corresponding to an increase in VNA titer of 1.2 in $\log_2$.

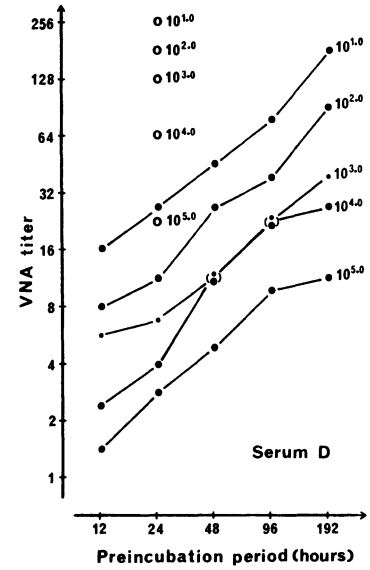


Figure 6. The relation between virus-neutralizing antibody (VNA) titer, length of preincubation at 4°C, and virus dose in the constant-virus/varying-serum neutralization test with extended preincubation. The symbol o shows VNA titers obtained simultaneously with preincubation at 37°C (Expt. VI).

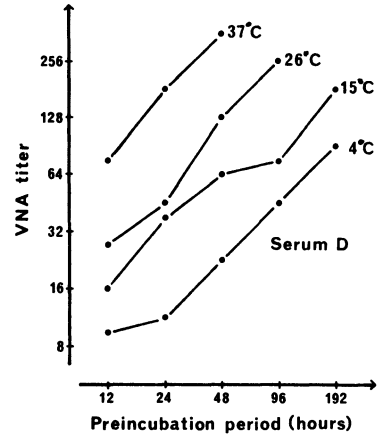


Figure 7. The relation between virus-neutralizing antibody (VNA) titer and preincubation temperature in constant-virus/varying-serum neutralization tests with extended preincubation (Expt. VII).

DISCUSSION

Relations between variables of the cv/vs test

The relation between VNA titer and duration of preincubation

The basic neutralization of virus in a virus-serum mixture occurs at a rate $k = \frac{1}{T} \log. \frac{V_O}{V_T}$, which is proportional to the antibody concentration. In a series of virus-serum mixtures of a cv/vs test the time required for neutralization of $\frac{V_O}{1}$ TCID50 should therefore expectedly be proportional to the serum dilution factor, and the relation between VNA titer (S) and preincubation period (T) should be of first order:

$$(I) \quad S = S_1 T,$$

where S_1 is the VNA titer after preincubation for 1 hr. If S and T were plotted on logarithmic scales, the resultant curve should show a slope of 1:

$$(II) \quad \log_2 S = \log_2 T + \log_2 S_1.$$

This simple relationship does not seem to have been pointed out in literature.

The relations found with extended preincubation, and shown in Figs. 1, 4a, 5a and 7 (37°C) and in Fig. 6 (4°C), are essentially in accordance with Equation II, and the results shown in Fig. 3 seem to confirm that such progression is associated with first order neutralization kinetics. Deviations from the relationship given in Equation II were observed in some experiments, as either advanced (overneutralization) or retarded neutralization.

Tables 1 and 2 demonstrate that the deviation related to short preincubation (Figs. 1, 2, 4a and 5a) can be characterized as an overneutralization effect. With short preincubation neutralization will most probably continue to some degree after inoculation of tissue cultures, but neutralization resulting from V-VNA combinations that are dissociable on dilution is an additional explanation. Tables 1 and 2 give examples of no growth of virus in cultures inoculated from undiluted virus-serum mixtures or lower dilutions thereof, in spite of the fact that growth was obtained from higher dilutions (Table 1: dilutions from 1/8 onwards after 30 min. of reaction; Table 2: dilutions from 1/16

onwards of virus-serum mixtures 1/2.8 and 1/4). This was observed whether cultures were inoculated with or without medium, and results shown in Fig. 2 even indicate enhanced overneutralization after inoculation of cultures without medium.

Overneutralization thus appears to be a combined effect of neutralization going on after inoculation, and formation of V-VNA complexes which are dissociable on dilution. At 37°C overneutralization will be negligible after preincubation exceeding 3 hrs., and an experiment not described here has indicated that at 26, 15 and 4°C the same will be the case after preincubation for more than 6, 12 and 24 hrs., respectively. This is consistent with the early observation by *Gard* (1955) that dissociability of V-VNA complexes is dependent not only on time but also on temperature.

Retarded neutralization was noted with Serum D in Experiment VI, most pronounced at 37°C (Figs. 5a and 6), and obviously related to high virus doses. The investigation of the kinetics of neutralization of the highest virus dose at 37°C (Fig. 5b) confirmed that the phenomenon was connected with a residual fraction of virus being neutralized at a lower rate: a persistent (*Dulbecco et al.* 1956) or protected fraction (*Lafferty* 1963). Retarded neutralization is therefore suggestive of a complicated process, while overneutralization must be considered a regular feature of neutralization.

The general validity of the demonstrated relationship between VNA titer and length of preincubation is substantiated by previous studies (*Bitsch* 1973, *Bitsch & Eskildsen* 1976) on the rise in VNA titer related to a prolongation of preincubation from 1 to 24 hrs. at 37°C. Forty bovine sera with antibody to IBR virus gave titer rises that were judged to be identical (*Bitsch* 1973) and in an examination of 126 porcine sera with antibody to Aujeszky virus (*Bitsch & Eskildsen*) seemingly identical improvements were observed for all the sera, namely approx. 4.2 in $\log_2$, which is approx. 0.5 less than the value of 4.59 expected from Equation II. Also sera from early stages of experimental immunization with these viruses have followed this pattern (unpubl. data).

The relation between VNA titer and virus dose. The log. VNA/log. V equivalence factor of neutralization (the q factor)

The same linear relationship between VNA titer (S) and virus dose (V_0) as illustrated in Fig 4a was demonstrated by *Burnet et al.* (1937) and has been found valid by many later authors (*Tyrrell & Horsfall* 1953). With preincubation long enough to eliminate overneutralization this relation can be expressed by the formula:

$$\text{(III)} \quad \log_2 S = -q \log_2 V_0 + \lim_{V_0 \to 1} \log_2 S.$$

The factor q could be designated the log. VNA/log. V equivalence factor of neutralization.

With the same preincubation period an increase of 1 in $\log_{10}$ of virus dose will give a decrease of q in $\log_{10}$* of VNA titer. Since the VNA titers shown along the ordinate in Fig. 4a represent antibody concentrations inversely proportional to these titers, this involves that with an increase of q in $\log_{10}$ of antibody concentration a dose of virus 10 times higher can be neutralized within the same period of reaction: $\frac{d(\log. VNA)}{d(\log. V)} = q$, where VNA is used to express antibody concentration.

The factor q was found to be 0.15 after preincubation at 37°C, but 0.24 at 4°C. This indicates that q is dependent on temperature.

The relation between VNA titer and preincubation temperature

In Fig. 6, with virus dose 10^2 TCID50 the VNA titer was 4 in $\log_2$ higher in a $P\frac{37}{24}$ test than in $P\frac{4}{24}$ test. Fig. 7 shows a linear relationship between preincubation temperature and logarithmic VNA values, and in consistency with the above observation a change in VNA titer of approx. 1.2 in $\log_2$ is recorded in response to a change in temperature of 10°C, implying a change in neutralization rates by a factor of $2^{1.2} = 2.3$. The relationship thus appears to be consistent with the general finding of direct proportionality between reaction temperature and logarithmic va-

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* Multiplying Equation III on both sides by $\log_{10}2$ yields

$$\log_{10} S = -q \log_{10} V_0 + \lim_{V_0 \to 1} \log_{10} S.$$

lues of neutralization rates (*Dulbecco et al.* 1956, see also review by *Svehag* 1968).

If, as assumed, the neutralization curves in Fig. 6, with preincubation from 24 to 96 hrs., reflect regular kinetics of neutralization*, the response in VNA titer to a variation in preincubation temperature will be virus dose dependent. Clarification of this question will require further studies.

Further implications of the results obtained with respect to regular neutralization

The vv/cs neutralization rate

Fig. 4b shows the regular progression of neutralization in a vv/cs neutralization test. Negligible overneutralization assumed, the relation between virus dose (V_0) and length of preincubation is given by the equation:

$$\text{(IV)} \quad \log_2 V_0 = \frac{1}{q} \log_2 T + \log_2 V_1,$$

where V_1 is the virus dose irreversibly neutralized to 1 TCID50 after 1 hr.

That the slope illustrating the progression of neutralization is 1/q, is obvious from Fig. 4 a. The cv/vs neutralization curves for irreversible neutralization will have slopes of 1, and an increase of 1 in $\log_{10}$ of virus dose will correspond to a decrease of q in $\log_{10}$ of VNA titer, when T is constant. For a given serum concentration an increase of 1 in $\log_{10}$ of virus dose will then correspond to an increase of q in $\log_{10}$ of T: $\frac{d(\log. V)}{d(\log. T)} = \frac{1}{q}$.

The percentage law

According to the percentage law the rate of irreversible neutralization in a V-VNA mixture, $k = \frac{1}{T} \log. \frac{V_0}{V_T}$, is independent of the virus titer V_0. If, however, the virus titer is increased by a factor of 10^x, while the serum concentration is unchanged, the incubation period required to reduce the virus titer to 1 will, on the assumption of negligible overneutralization, be increased by a factor of 10^{xq} (Fig. 4a and Equation IV), and the neutra-

* In an examination not described here, a virus dose of $10^{2.5}$ TCID50 was neutralized linearly and completely at 4°C within 28 hrs. by Serum D in dilution 1/10.

lization rate $\frac{\log. V_0}{T}$ will be changed to $\frac{\log. V_0 + x}{T\ 10^{xq}}$ by the *variable* factor $\frac{\log. V_0 + x}{\log. V_0\ 10^{xq}}$. This shows that the validity of the percentage law is limited.

The variation factor for the neutralization rate related to an increase in virus titer by a factor of 10 is $\frac{\log. V_0 + 1}{\log. V_0\ 10^q}$. With a q value of 0.15 this factor will be 1.4, 1.1, 0.88 and 0.83 for $V_0 = 10$, 10^2, 10^4 and 10^6, respectively, and be approaching 0.71 ($\frac{1}{10^q}$) with further increasing values of V_0. But according to the percentage law it should have a constant value of 1.

The neutralization rate factor and its relation to the VNA titer

The limited validity of the percentage law implies that the accepted formula for the neutralization rate factor will be inaccurate for expression of neutralization activity, as it does not include the initial virus titer. From the results obtained, however, it is possible to give a corrected formula and even to express a relationship between corrected neutralization rate factors and antibody titers obtained in a cv/vs test.

Using virus titer V_0 and expressing neutralizing activity by the neutralization rate factor related to a virus dose of 10 TCID50 (K_{10}) — and adhering to the above elucidation of the relation between neutralization rate and virus dose ($x = 1 - \log. V_0$) — we can form the following equation:

$$(V) \quad K_{10} = \frac{D}{T} \log. \frac{V_0}{V_{T_1}} \frac{10^{(\log. V_0 - 1)q}}{\log. V_0}.$$

(Note that in this and the following formulae for neutralization rate factors D is recorded by half its actual value in the virus-serum mixtures. See also the definition of virus titer).

With a VNA titer of S obtained against virus dose V_0 in a cv/vs test with regular neutralization and negligible overneutralization, and Equation V applied to the mixture of virus and serum dilution 1/S, we have:

$$K_{10} = \frac{S}{T} 10^{(\log. V_0 - 1)q} \text{ or}$$

$$(VI) \quad K_{10} = \frac{S\ V_0{}^q}{T}.$$

An identical formula can be derived simply from Equations I, III and IV:

$$\text{(VII)} \quad K_{st} = \frac{S\,V_0^{q}}{T},$$

where K_{st} is a constant characteristic of the serum tested and equal to $K_{10}10^{q}$. From the relation between K and V_0 given above is seen that $K_{st} = K_{10}10^{q}$ is the limit of K as V_0 approaches 1.

When in Equation V $K_{st}\,10^{-q}$ is substituted for K_{10}, a slightly simpler formula for the neutralization rate factor appears:

$$\text{(VIII)} \quad K_{st} = \frac{D}{T} \log.\frac{V_0}{V_T} \frac{V_0^{q}}{\log. V_0}.$$

Equation VII will now be seen to express the relationship between this standard neutralization rate factor and VNA titers obtained in tests with extended preincubation and regular progression of neutralization.

K_{st} will be dependent on the temperature of reaction, as is probably also the factor q, but more knowledge of this dependence is needed before a complete formula including all independent variables can be given.

The results recorded in this paper do not seem to be conflicting with results reported by other investigators of the virus-antibody neutralization reaction. As furthermore the present findings are in accordance with fundamental laws of neutralization, it is implied that the conclusions concerning the basic neutralization reaction with IBR virus may be of general validity. From the results obtained it should be feasible to elaborate neutralization tests with high sensitivity.

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SAMMENDRAG

Undersøgelser over den basale antigen-antistof-reaktion med særligt henblik på reaktionen i konstant-virus/varierende-serum-neutralisationstesten.

Den basale reaktion mellem virus (V) og virusneutraliserende antistoffer (VNA) er undersøgt ved anvendelse af infektiøst bovint rhinotracheitisvirus og serum af naturligt og eksperimentelt inficeret kvæg opsamlet lang tid efter smitte. I konstant-virus/varierende-serum-neutralisationstests fandtes ligefrem proportionalitet mellem VNA-titer og præinkubationstiden, hvilket kunne findes at være i overensstemmelse med basale love for virusneutralisation. Ved relativt korte præinkubationstider sås en afvigelse fra den fundne simple relation som delvis kunne tilskrives dissocierbare V-VNA-forbindelser. Relationen gældende under forhold, hvor sådanne forbindelser kan negligeres, mellem VNA-titer, præinkubationstiden og virusdosis gives. Herved introduceres en log.VNA/log.V-ækvivalensfaktor for neutra-

lisationsforløbet. Der fandtes en lineær relation mellem den logaritmiske værdi for VNA-titer og præinkubationstemperaturen, idet en forhøjelse af temperaturen med 10°C modsvarede en forøgelse af VNA-titeren med en faktor på 2,3. Der gives formler for neutralisationshastighedsfaktoren korrigeret for en fundet begrænset gyldighed af „the percentage law" og for relationen mellem denne størrelse og VNA-titeren. Det konkluderes, at undersøgelsesresultaterne belyser mulighederne for forbedring af følsomheden af neutralisationstests.

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