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# VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS: DETERMINATION OF INHALATION $LD_{50}$ FOR GUINEA PIGS AND MICE

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*Summary.* — Mouse brain-passed Venezuelan equine encephalomyelitis (VEE) virus was used in estimating inhalation $LD_{50}$ for guinea pigs and mice. One inhalation $LD_{50}$ for guinea pigs corresponded to 3.5 guinea pig intracerebral (ic) $LD_{50}$. One inhalation $LD_{50}$ for mice corresponded to 100 guinea pig ic $LD_{50}$ or 135 mouse ic $LD_{50}$. The size of the inhalation median lethal dose probably was equivalent to the inhalation infective dose, since no antibody was detected in any of the guinea pigs and mice that survived exposure to infectious aerosol.

## *Introduction*

The determination of the size of the inhalation dose of VEE virus was the subject of several investigations. Victor *et al.* ((1956) expressed the susceptibility to VEE virus aerosol of guinea pigs, mice, rabbits and monkeys in mouse ic $LD_{50}$. Miller (1966) gave an estimate of the minimal inhalation infective dose for pigeons. Kuehne *et al.* (1962) studied the inhalation infective dose for guinea pigs, mice and monkeys in an attenuated strain of VEE virus.

In the present work we attempted a more exact determination of the inhalation $LD_{50}$ of VEE virus for guinea pigs and mice. The results obtained were used in further studies on the experimental infection with VEE virus, which will be reported later.

## *Materials and Methods*

*Virus.* The VEE virus strain "Venezuela" was obtained from the Institute of Virology, U.S.S.R. Academy of Medical Sciences, Moscow, where it was kept by ic mouse passages since 1944, when supplied by the Rockefeller Institute, New York. The passage history is unknown; in our laboratory the virus had undergone 2 mouse brain passages. It was kept in the form of a 10% brain suspension on dry ice.

*Animals.* Guinea pigs weighing 300—380 g and H strain mice weighing 15 g were employed.

*Virus titration* was carried out by ic inoculation of guinea pigs and mice with 0.1 and 0.03 ml volumes, respectively.

*Inhalation infection.* The animals were exposed to infectious aerosol in a steel chamber of 600 litre capacity. The polydisperse aerosol contained a majority of particles 1 μ in diameter. Air was passed through the chamber at a flow rate of 100 litres/min. The relative humidity was kept at 65—75% and the temperature at 20—21° C. The infectious aerosol was caught up in the chamber into glass impingers; the flow rate through the impingers was manometrically controlled. The virus caught up into medium 199 (Institute of Sera and Vaccines, Prague)

containing 3% calf serum was titrated in guinea pigs. The time of action of the impingers was calculated so that the amount of virus determined in the liquid (0.1 ml) corresponded to the amount of virus inhaled by one animal. The following formulae were employed:

$$Q_1 \times \tau = \frac{V_1 \times v \times t \times 3.5^*}{1000^*} \text{ for guinea pigs, and}$$

$$Q_1 \times \tau = \frac{V_1 \times v \times t \times 1.3^{**}}{100^{**}} \text{ for mice, where}$$

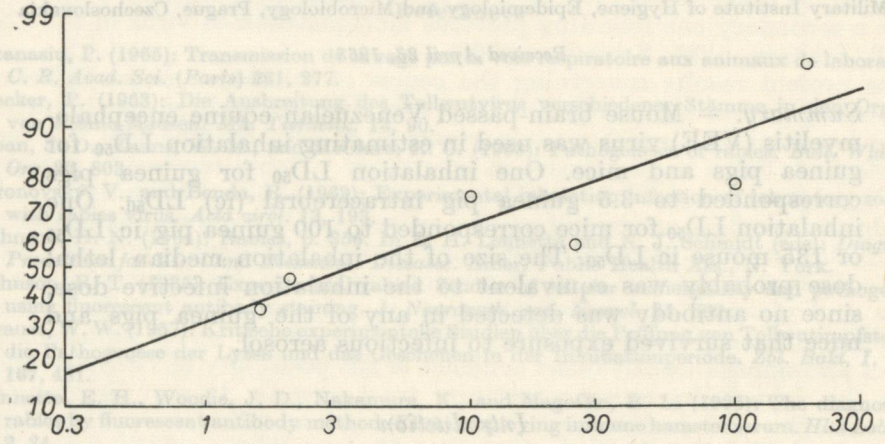


**Fig. 1.**

Calculation of inhalation $LD_{50}$ for guinea pigs expressed by guinea pig ic $LD_{50}$
Abscissa: amount of guinea pig ic $LD_{50}$ inhaled; ordinate: probit (%)
Note. The goodness of fit of the straight lines in Figs 1—3 was tested by the chi square test with a risk of 0.05. The upper and lower limits of $LD_{50}$ were calculated for a 95% confidence limit.

**Table 1. Determination of the inhalation $LD_{50}$ for guinea pigs**

| Dose inhaled in guinea pig ic $LD_{50}$ | Mortality | | p − P (%) | $\chi^2$ test |
|---|---|---|---|---|
| | expected* P (%) | established p (%) | | |
| 1.6 | 37.5 | 35 | 2.5 | 0.003 |
| 2.1 | 42 | 45 | 3 | 0.004 |
| 10 | 66 | 75 | 9 | 0.038 |
| 25 | 78 | 60 | 18 | 0.192 |
| 100 | 91 | 80 | 11 | 0.145 |
| 180 | 94 | 100 | 6 | 0.062 |

* Read from Fig. 1.
Calculation of the median lethal dose (r = number of classes; n = number of animals in the class; f = degrees of freedom (= r − 2); N′ = number animals used with doses from $LD_{16}$ to $LD_{84}$; $\chi^2$ calculated = $\Sigma\chi^2$ from nomograms × n):

$LD_{50}$ = 3.5 r = 6 $\chi^2$ calculated = 6.880 f $LD_{50}$ = 2.25
$LD_{16}$ = 0.29 n = 20 $\chi^2$ tabulated = 9.488 $LD_{50}$ lower limit = 1.6
$LD_{84}$ = 44 N′ = 80 $LD_{50}$ upper limit = 7.9

$Q_i$ = actual flow of air through the impinger nozzle, in litres/min,
$\tau$ = time of action of the impinger in minutes,
$V_i$ = volume of the liquid in the impinger,
v = weight of the animal in grams,
t = time of exposure of the animals to the aerosol,
* and ** — respiratory volume in litres per gram body-weight of a guinea pig (*) or mouse (**) per minute (Guyton, 1947).

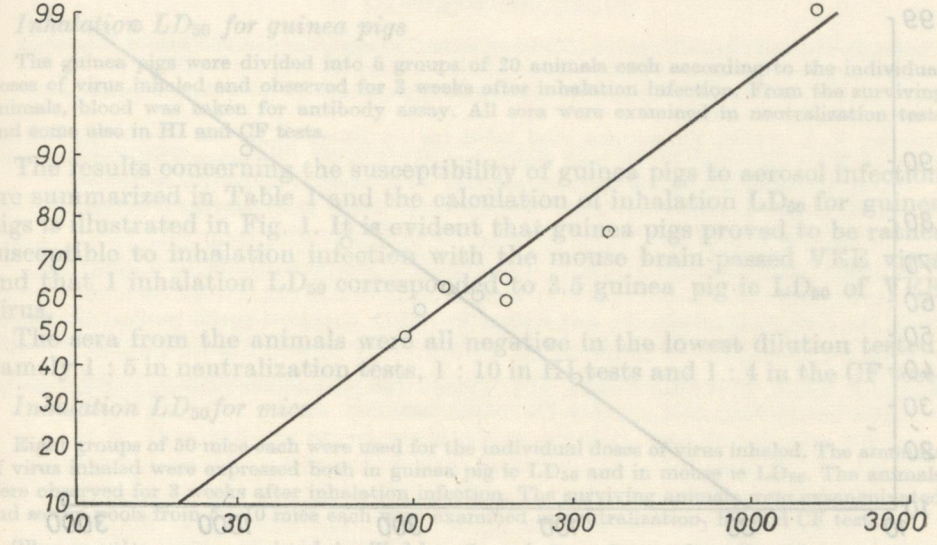


**Fig. 2.**
Calculation of inhalation $LD_{50}$ for mice expressed by guinea pig ic $LD_{50}$
Abscissa and ordinate as in Fig. 1.

**Table 2. Determination of the inhalation $LD_{50}$ for mice (expressed in guinea pig ic $LD_{50}$)**

| Dose inhaled in guinea pig ic $LD_{50}$ | Mortality | | p − P (%) | $\chi^2$ test |
|---|---|---|---|---|
| | expected* P (%) | established p (%) | | |
| 62 | 35.5 | 38 | 2.5 | 0.003 |
| 95 | 48 | 48 | 0 | 0.000 |
| 125 | 56 | 62 | 6 | 0.015 |
| 190 | 67 | 58 | 9 | 0.033 |
| 190 | 67 | 64 | 3 | 0.004 |
| 385 | 82.5 | 76 | 6.5 | 0.030 |
| 770 | 92.5 | 92 | 0.5 | 0.000 |
| 1600 | 97.4 | 100 | 2.6 | 0.026 |

* Read from Fig. 2.
Calculation of the median lethal dose (for explanation see Table 1):

| | | | |
|---|---|---|---|
| $LD_{50}$ = 100 | r = 8 | $\chi^2$ calculated = 5.550 | f $LD_{50}$ = 1.25 |
| $LD_{16}$ = 24 | n = 50 | $\chi^2$ tabulated = 14.950 | $LD_{50}$ lower limit = 80 |
| $LD_{84}$ = 415 | N′ = 250 | | $LD_{50}$ upper limit = 125 |

The $LD_{50}$ values were determined by the graphical probit method (Roth *et al.*, 1962). The calculations were carried out by the graphical modification of the probit method for estimation of $LD_{50}$ values and their confidence limits; the linearity test using nomograms was included.

*Serological reactions.* The haemagglutination inhibition (HI) and complement-fixation (CF) tests were carried out according to Clarke and Casals (1958) and Ilyenko (1953), respectively. The neutralization test was done in chick embryo cells as follows. Equal volumes of serum

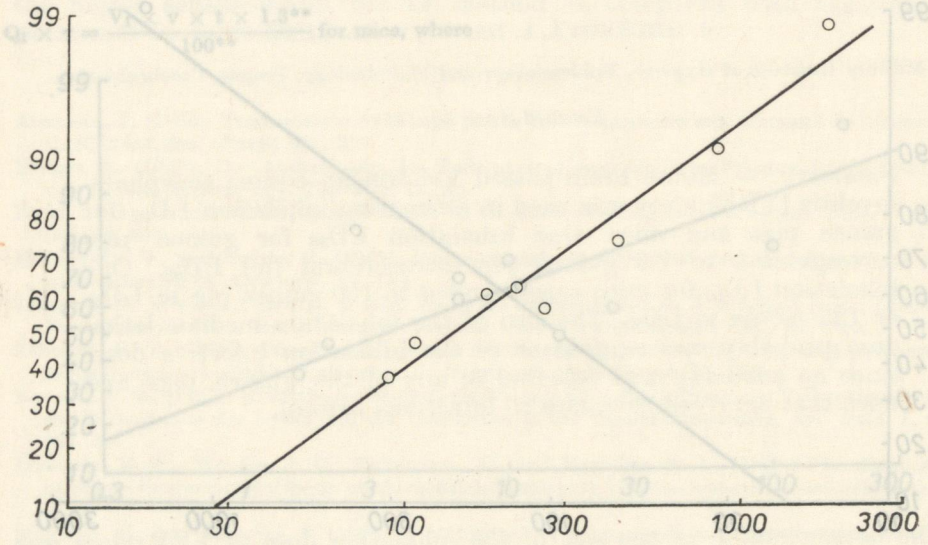


**Fig. 3.**

Calculation of inhalation $LD_{50}$ for mice expressed by mouse ic $LD_{50}$
Abscissa: amount of mouse ic $LD_{50}$ inhaled; ordinate: probit (%)

**Table 3. Determination of the inhalation $LD_{50}$ for mice (expressed in mouse ic $LD_{50}$)**

| Dose inhaled in mouse ic $LD_{50}$ | Mortality | | p − P (%) | $\chi^2$ test |
|---|---|---|---|---|
| | expected* P (%) | established p (%) | | |
| 90 | 37 | 38 | 1 | 0.000 |
| 108 | 43 | 48 | 5 | 0.011 |
| 175 | 58 | 62 | 4 | 0.009 |
| 215 | 64 | 64 | 0 | 0.000 |
| 260 | 70 | 58 | 12 | 0.070 |
| 430 | 82 | 76 | 6 | 0.024 |
| 860 | 93 | 92 | 1 | 0.001 |
| 1800 | 98 | 100 | 2 | 0.021 |

* Read from Fig. 3.

Calculation of the median lethal dose (for explanation see Table 1):

$LD_{50} = 135$ r = 8 $\chi^2$ calculated = 6.800 f $LD_{50} = 1.53$
$LD_{16} = 39$ n = 50 $\chi^2$ tabulated = 12.592 $LD_{50}$ lower limit = 88
$LD_{84} = 420$ N′ = 250 $LD_{50}$ upper limit = 207

dilutions and virus (100—500 $TCD_{50}$/0.1 ml) were mixed and the mixtures incubated for 1 hour at 37° C. Then 0.2 ml of the mixtures were inoculated into 0.8 ml of chick embryo cell suspension. The results were read after 3 days of incubation at 37° C. The highest serum dilution that prevented the cytopathic effect of virus was taken for antibody titre.

## *Results*

### *Inhalation $LD_{50}$ for guinea pigs*

The guinea pigs were divided into 6 groups of 20 animals each according to the individual doses of virus inhaled and observed for 3 weeks after inhalation infection. From the surviving animals, blood was taken for antibody assay. All sera were examined in neutralization tests and some also in HI and CF tests.

The results concerning the susceptibility of guinea pigs to aerosol infection are summarized in Table 1 and the calculation of inhalation $LD_{50}$ for guinea pigs is illustrated in Fig. 1. It is evident that guinea pigs proved to be rather susceptible to inhalation infection with the mouse brain-passed VEE virus and that 1 inhalation $LD_{50}$ corresponded to 3.5 guinea pig ic $LD_{50}$ of VEE virus.

The sera from the animals were all negative in the lowest dilution tested, namely 1 : 5 in neutralization tests, 1 : 10 in HI tests and 1 : 4 in the CF test.

### *Inhalation $LD_{50}$ for mice*

Eight groups of 50 mice each were used for the individual doses of virus inhaled. The amounts of virus inhaled were expressed both in guinea pig ic $LD_{50}$ and in mouse ic $LD_{50}$. The animals were observed for 3 weeks after inhalation infection. The surviving animals were exsanguinated and serum pools from 5—10 mice each were examined in neutralization, HI and CF tests.

The results, summarized in Tables 2 and 3 and graphically illustrated in Figs 2 and 3, showed that 1 inhalation $LD_{50}$ for mice corresponded to 100 guinea pig ic $LD_{50}$ or 135 mouse ic $LD_{50}$.

None of the sera contained antibody when tested in the same dilutions as given above for guinea pig sera.

## *Discussion*

The available reports suggest that VEE virus may easily cause air-borne infection in laboratory personnel. An experimental study on VEE virus aerosol showed that guinea pigs are highly and mice somewhat less susceptible to inhalation infection (Victor *et al.*, 1956). The reports, however, contained no exact data on the properties of aerosol, the experimental conditions and documentation of the obtained values of inhalation $LD_{50}$. In principle, our present results are in accordance with those reported in the literature. In our experiments, however, the susceptibility of mice to aerosol infection with VEE virus proved to be about tenfold higher.

As far as the reported data make possible a comparison of the susceptibility and sensitivity of animals to experimental inhalation infection with VEE virus and other arboviruses, the effect of VEE virus in the form of aerosol cannot be considered as an exceptionally high one. Miller *et al.* (1963) obtained infection of monkeys with yellow fever virus and of hamsters with Rift Valley fever virus following much lower doses of aerosol.

The results of our experiments on the estimation of inhalation $LD_{50}$ for mice and guinea pigs themselves cannot offer an explanation, why the risk of laboratory work with VEE virus, according to general experience, is much higher than that with other arboviruses.

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