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EXPERIMENTAL INHALATION INFECTION OF LABORATORY RODENTS WITH RABIES VIRUS

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Summary. — The R-205 strain of street rabies virus adapted to primary dog kidney cell cultures caused a reproducible inhalation infection in laboratory animals. On calculating the doses of inoculum per unit of body-weight, guinea pigs (especially suckling animals), rabbits and mice proved to be the most, less and least susceptible, respectively. The mean incubation periods after inhalation of lethal doses of virus were on the average twice those after intracerebral infection. Pilot experiments demonstrated the possibility of an air-borne infection of mice and guinea pigs with the CVS fixed rabies virus in the form of brain suspension aerosol. The median inhalation lethal (or infective) doses of either virus strain were very high for all the animal species tested.

Introduction

The possibility of an air-borne rabies infection of susceptible animals was experimentally proved by Constantine (1962) in the course of investigations on rabies in bats in Texas caves. Studies on bat rabies have been continued by a number of investigators whose most interesting findings were the variability of bat strains with frequent changes in virulence, differences in species pathogenicity and the existence of latent infections and long-term persistence of virus in hibernating bats (Sulkin *et al.*, 1966; Constantine, 1966; Baer and Bales, 1967). Experimental respiratory infection of laboratory animals with wild and fixed strains which originated from usual reservoir animals was studied by Atanasiu (1965) and recently by Selimov *et al.* (1969). These works suggested a connection between in vitro propagation of rabies virus and its increased pathogenicity after respiratory infection.

To obtain further information about this problem, we studied the relationship between pathogenicity after inhalation infection and the kind and character of rabies virus and attempted to determine differences in susceptibility of, and median inhalation lethal doses for, certain species of laboratory rodents.

Materials and Methods

Virus strains. The original street rabies virus strain R-205, isolated from a badger, was used in the form of supernatant fluids from mouse brain suspensions (9th—14th ic mouse passage) with titres from 10^4—10^5 ic mouse LD_{50}/0.03 ml. Most inhalation experiments were done with the R-205 strain adapted by consecutive passaging to growth in primary dog kidney cell cultures (Hronovský *et al.*, 1968). This adapted virus was used in the form of culture medium from the

Table 1. Approximate estimates of inhalation LD_{50} for laboratory rodents of cell culture-adapted rabies virus

Dose of virus inhaled (ic mouse LD_{50})	Mice			Suckling guinea pigs			Adult guinea pigs			Rabbits		
	Mortality		Mean incubation (days)	Mortality		Mean incubation (days)	Mortality		Mean incubation (days)	Mortality		Mean incubation (days)
	Abs.*	%		Abs.*	%		Abs.	%		Abs.	%	
$\leqq 10^2$	2/36	5.5	24.0	—	—	—	—	—	—	—	—	—
$>10^2-5\times10^2$	5/36	13.9	14.6	5/12	41.6	22.5	—	—	—	—	—	—
$>5\times10^2-10^3$	12/30	40.0	14.4	11/17	**64.7**	16.5	2/23	8.7	18.0	—	—	—
$>10^3-5\times10^3$	18/35	**51.4**	13.2	19/25	76.0	13.3	9/23	39.1	18.2	—	—	—
$>5\times10^3-10^4$	21/24	87.5	13.7	14/14	100.0	12.5	12/19	**63.2**	17.8	0/8	0	—
$>10^4-5\times10^4$	—	—	—	24/24	100.0	12.5	21/23	91.3	15.1	3/15	20.0	40.3
$>5\times10^4-10^5$	—	—	—	—	—	—	20/20	100.0	13.4	4/12	33.3	23.0
$>10^5-10^6$	—	—	—	—	—	—	—	—	—	7/12	**58.3**	18.5

* Number of dead over number of exposed animals.
— = Not done.

22nd—40th passage with titres from 10^4—$10^{7.5}$ ic mouse LD_{50}/0.03 ml. A few pilot experiments were carried out with mouse brain suspensions infected with the CVS strain of fixed virus with titres from $10^{7.5}$—$10^{8.5}$ ic mouse LD_{50}/0.03 ml. The CVS (Challenge Virus Standard) strain, fixed and maintained by ic passaging in mice, was obtained from the Institute of Sera and Vaccines, Prague. In our laboratory it underwent 7 ic mouse passages with an average incubation of 4—5 days; its previous passage history is unknown. It is highly pathogenic for all laboratory rodents after ic inoculation.

Inhalation infection. White mice weighing 10—14 g, guinea pigs weighing 250—300 g, 2—4-day suckling guinea pigs weighing 60—80 g and rabbits weighing 2—2.5 kg were employed. The animals were exposed to infective aerosol produced by a direct metal nebulizer in a hermetic chamber (volume approx. 600 litres) at an air flow of 50 litres/min, and under standard physical conditions. The virus doses were varied by changing either the time of exposure from 20 to 80 minutes or the virus concentration in the infectious materials. For details of the technique and calculation of the virus doses inhaled see Daneš *et al.* (1962) and Benda *et al.* (1964).

Specificity of the infection was checked by examining brains from dead or sick animals by the direct fluorescent antibody (FA) technique or, in equivocal cases, by parallel isolation experiments in mice. Antirabic antibody in infected guinea pig sera was assayed by the indirect FA technique with smear preparations from infected mouse brains and commercial anti-guinea pig conjugate. Virus neutralization tests were carried out in mice by testing serial serum dilutions with 100 LD_{50} of CVS virus (Johnson, 1964).

Results

Ten negative experiments on the R-205 strain passed in mice revealed that the relatively low titres reached in the brain street virus were not sufficient to induce a detectable inhalation infection in any of the animal species tested. The calculated doses of virus in these experiments, expressed in ic mouse LD_{50}, were 500 in mice, 1000 in suckling guinea pigs, 3000 in adult guinea pigs and up to 20000 in rabbits.

By contrast, the same R-205 strain adapted to cell cultures due to its high infective titres made it possible to prepare substantially more concentrated aerosols and, on inhalation infection, proved to be pathogenic in a different degree for all the animal species examined. Table 1 summarizes the results of 20 experiments carried out in different periods of time with various doses of adapted virus. These results made it possible to estimate the approximative median inhalation lethal dose and to reach certain further conclusions.

The direct relationship between the specific mortality rate and the dose of virus inhaled was obvious. In Table 1, the inhaled doses were schematically distributed within given dose limits. The inhalation LD_{50} for mice, suckling guinea pigs, adult guinea pigs and rabbits were in the range of $1-5\times10^3$, $5\times10^2-10^3$, $5-10\times10^3$ and 10^5-10^6 ic mouse LD_{50}, respectively. When calculating the doses per gram of body-weight, guinea pigs, especially suckling ones, proved to be the most susceptible; rabbits were less and mice the least susceptible. The absolute inhalation lethal doses which were reached in newborn and adult guinea pigs were about 10 times greater than the respective median lethal doses. There was also a clear relationship between the dose of virus inhaled and the length of the incubation period: the greater the dose, the shorter the mean incubation period. The mean death time on intracerebral inoculation of the cell culture-adapted R-205 virus was 7 days in mice and guinea pigs and 12 days in rabbits. On inhalation infection with lethal doses of virus this period was about twice longer.

For comparison, we carried out 5 pilot experiments on air-borne infection of mice and guinea pigs with various doses of the CVS strain. This virus, fixed by adaptation to mouse brains, showed a comparatively high pathogenicity after inhalation infection. Its inhalation LD_{50} for mice was in the range from 500—1000 ic mouse LD_{50}, thus lower that of the cell culture-adapted R-205 virus. Less susceptible to inhalation infection with the CVS strain were guinea pigs, in which the inhalation LD_{50} corresponded to $5—10 \times 10^4$ ic mouse LD_{50}, i.e. 10 times more than with the cell culture adapted R-205 virus. The mean incubation periods with the CVS strain were on inhalation twice longer than on intracerebral inoculation.

The clinical picture of inhalation rabies caused by street virus, apart from a delayed onset, did not differ from the classical modes of infection. The course was comparatively rapid with the typical sequence of symptoms: restlessness, irritability, tremor and seizures. Death (in mice often with seizures, otherwise in the paralytic stage) occurred in mice and guinea pigs 24—48 hours and in rabbits within 3 days after the onset of symptoms.

The infected animals were observed for 10—16 weeks. Transmission of the infection from the young to the uninfected mothers, as reported by Atanasiu (1965) in hamsters infected by inhalation, was not seen in our experiments on suckling guinea pigs. Significant levels of specific antibody were found in about 50% of suckling guinea pigs that survived doses in the proximity of the median lethal dose without showing disease symptoms.

Discussion

Adaptation of the R-205 strain of street rabies virus to cells of non-neural origin made it possible to obtain the virus in the form of relatively pure culture fluids, its infectivity for mice being high after cerebral and somewhat lowered after extraneural infection (Hronovský *et al.*, 1968). The present results showed that this material was able to cause a reproducible aerogenous infection in mice, guinea pigs and rabbits. The positive results of experiments on inhalation infection with brain suspension of the fixed CVS strain which, like the cell culture-adapted R-205 strain, is highly infectious for laboratory animals by the cerebral route and shows a lowered extraneural virulence, suggest that pathogenicity on inhalation infection cannot be regarded as a manifestation of changed virus properties resulting from its adaptation to cell cultures. By contrast, it seems that inhalation infection may be experimentally produced in susceptible animals with rabies virus strains of different origin, in so far as the strains are sufficiently virulent with respect to the central nervous system and in so far as their infectious titres make it possible to reach a sufficiently great concentration of the aerosol.

The discrepancy between the pathogenicity of the cell culture-adapted R-205 virus after inhalation infection and its lowered extraneural (intramuscular and intraperitoneal) infectivity is only seeming. The results of our further investigations (Hronovský and Benda, 1969) namely showed that inhalation infection in principle represents only a peculiar form of the common experimental neural rabies. From this point of view it is also possible to

understand the higher ability of the mouse brain-adapted CVS virus to produce inhalation infection just in mice.

The finding of antibody in sera from guinea pigs that survived sublethal inhalation doses of virus is of interest. It offers an indirect evidence of the existence of inapparent rabies infection.

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