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That certain viruses and rickettsiae can infect animals and man by the respiratory route has been known for many years. Aerobiological research, however, has been concerned primarily with only a few agents, such as influenza virus, which are obviously implicated in transmission cycles involving the respiratory tract. Evidence for infectivity of other viruses by inhalation derives from studies of laboratory infections, summarized recently by Sulkin (1).

It is of particular interest that arthropod-borne viruses have been implicated in respiratory infections in man. For example, Slepushkin (2) reported that a large group of laboratory personnel was infected by aerosols from an accidentally dropped vial of dry Venezuelan equine encephalitis virus.

Reliable experimental data are required on the infectivity of respiratory virus and of agents normally thought to be transmitted by insect vectors. Further, information concerning the resistance of viral aerosols to various atmospheric environments is also needed. The present study was conducted with the viruses of yellow fever and Rift Valley fever to obtain estimates of stability in the airborne state and of infectivity by inhalation for susceptible animal hosts.

MATERIALS AND METHODS

Agents. Three harvests of the pantropic strain of Rift Valley fever virus were subjected to aerosol tests to study the influence of the virus source and suspending fluid on aerosol characteristics. A plasma harvest, obtained from suckling lambs, contained $10^{10.5}$ mouse intraperitoneal LD_{50} ($MIPLD_{50}$) units per ml. Two tissue-culture harvests (hamster kidney cells and Chang's human liver cells) contained $10^{7.8}$ and $10^{6.3}$ $MIPLD_{50}$ units per ml, respectively.

The Asibi strain of yellow fever virus was grown in HeLa cell tissue cultures employing mouse-brain inoculum. To the harvest was added 20 per cent egg yolk from 6-day-old embryonated eggs. The final titer of this suspension was $10^{7.4}$ mouse intracerebral LD_{50} ($MICLD_{50}$) units per ml.

Aerosol methods. The cloud chambers were similar to those described by Wolfe (3). Aerosol samplers were liquid impingers of the type described by Miller et al. (4). Aerosol ages which samples were obtained varied with the agent under study and with the circumstances of animal exposure. The infectivity of the aerosol was estimated at fixed ages to determine the rate of decay. When the desired inhalation doses were below

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[2] In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

[3] The assistance of Dr. A. N. Gorelick and numerous personnel of the Viral and Rickettsial Division, Fort Detrick, is gratefully acknowledged for advice on virus techniques and for production of agent suspensions.

assessable limits, doses were estimated by extrapolation from earlier cloud age estimates.

Aerosols were produced by a spray system capable of emitting a particle mass with a median diameter of about 4 microns.

Animals were exposed to aerosols by inserting their heads through rubber diaphragms into a cloud chamber. Subsequently they were held for the required incubation periods in ventilated cabinets (5). A similar arrangement was employed for holding animals inoculated for assay purposes. The number of organisms which exposed animals could have inhaled was estimated by Guyton's equations for respiratory volume (6).

Assay procedures. Rift Valley fever virus aerosol samples were collected and diluted in beef heart infusion broth (Difco). Appropriate dilutions were prepared in duplicate and 0.1-ml aliquots inoculated intraperitoneally into 18- to 22-gram Swiss-Webster strain mice. Ten mice were employed per dilution. Total deaths were recorded over a 7-day period.

Aerosol samples of yellow fever virus were collected in beef heart infusion containing egg yolk as stabilizer (20 per cent). Beef heart infusion broth alone was employed for diluting samples. The assay animals were 8- to 10-gram Swiss-Webster strain mice, 10 per dilution, inoculated with 0.03 ml by the intracerebral route. Deaths occurring during the period 72 hours through 20 days after inoculation were regarded as specific responses to yellow fever virus.

Parameters. Aerosol data are expressed herein as per cent recovery and decay rate. The former is the proportion × 100 of the number of LD_{50} units recovered as aerosol to the number of LD_{50} units disseminated. Decay rate is approximately the linear decrease of the natural logarithm of concentration with time, expressed as per cent per minute. Such decay rates, estimated only by the changing viral concentrations at different sampling periods, are termed "total" decay. When changes in concentration of a physical tracer are observed, the resulting estimate is a "physical" decay. The difference between these two parameters is "biological" decay or death rate. With Rift Valley fever virus, uranine (Fisher Scientific Company) was employed as the physical tracer (1.0 milligram per ml of virus suspension). The general concept was described by Miller et al. (7).

Replication and analysis. Treatments were tested in 6 to 8 replicate aerosol trials. A probability of less than 5 per cent that an observed difference between treatments was due to chance alone was defined as statistically significant.

Results

Table 1 presents the initial aerosol concentrations (4 minutes aerosol age) and the biological decay rates with each of the 3 harvests of Rift Valley fever virus. Means did not differ significantly in either comparison, thus suggesting that the source of virus is relatively unimportant to the characteristics of the aerosol. The trials were conducted at a relative humidity of 50 per cent. However, previous work involving trials at 80 per cent relative humidity yielded the same responses, indicating that the virus is not affected by humidities over the range of 50 to 80 per cent.

The decay rates for Rift Valley fever virus indicate a very stable virus. After 1 hour in the aerosol form about 25 per cent of the original airborne virus is still effective.

The corresponding results with yellow fever virus are presented in table 2. This virus was more resistant to the

TABLE 1

*Aerosol per cent recovery and biological decay rate of pantropic Rift Valley fever virus**

Harvest	Initial aerosol recovery (per cent)†	95 per cent conf. limits	Biological decay rate (per cent/min)†	95 per cent conf. limits
Lamb plasma	9.25	2.37–36.1	1.14	0–2.3
Hamster kidney tissue culture	9.42	2.41–36.8	2.95	0.5–5.3
Chang liver tissue culture	19.4	4.95–75.7	3.62	0.9–6.2

* Trial conditions: 50 per cent relative humidity at 75 F.
† Means of 8 replicate trials.

dissemination process than was Rift Valley fever virus, since almost 70 per cent of the original material was viable 4 minutes after dissemination. Thereafter, however, the loss in virus activity was at least as high as shown previously for Rift Valley fever virus. The decay rates in this instance are total, including components of both biological decay and physical decay. Although a tracer was not employed to detect the latter component, the Rift Valley fever studies with tissue-culture harvests of similar physical properties (viscosity, surface tension) indicated a physical decay rate of 1.5 per cent per minute. Subtraction of this value from the 5.9 per cent per minute mean resulted in a biological decay rate of about 4.5 per cent per minute, a value probably not different from that found for the Rift Valley fever virus.

TABLE 2

*Aerosol per cent recovery and total decay rate of pantropic yellow fever virus**

	Mean†	95 Per cent confidence limits	
		Lower	Upper
Initial aerosol recovery (%)	67.2	35.1	128
Total decay rate (%/min)	5.9	3.58	8.20

* Trial conditions: 50 per cent and 80 per cent relative humidities at 75 F.
† Over-all mean from 6 trials at each humidity.

The results shown for yellow fever virus were averages over the two relative humidities tested (50 and 85 per cent). There was no significant effect of humidity on the basis of either level of initial concentration or decay rates, indicating again lack of sensitivity to humidity changes at the levels tested.

Susceptible animal species were exposed to graded respiratory doses of each virus. Tests with lamb plasma harvest of Rift Valley fever virus were conducted with hamsters and with rhesus monkeys (*Macaca mulatta*). The criterion of infection in the hamsters was death from days 1 through 7 after exposure. The results are presented in table 3. The low lethal dose for hamsters (LD_{50} of 0.525 $MIPLD_{50}$ units) is remarkable but not necessarily invalid. The mouse is probably not as susceptible following intraperitoneal inoculation as is the hamster by the respiratory route.

It was not expected that rhesus monkeys would succumb to Rift Valley fever infection and therefore other criteria were established for evidence of infection in this species. These criteria were (*a*) viremia, (*b*) fever and (*c*) height of serum neutralization titers. Tests for viremia were made on blood samples taken at 4, 6, 8 and 10 days after exposure. These samples were diluted 1 in 10 and 1 in 100 in heart infusion

TABLE 3

Response of hamsters to graded doses of pantropic Rift Valley fever virus

Inhaled dose ($MIPLD_{50}$)	Response dead/total	LD_{50}	Probit slope
14	23/24		
2	20/24		
0.7	14/24	0.525 $MIPLD_{50}$	1.400 probits/log dose
0.2	6/24	(0.334–0.824)	(0.827–1.97)

broth and 0.1-ml aliquots of each were inoculated intraperitoneally into groups of 10 mice each. All deaths were recorded over a 7-day observation period. Fevers were estimated by rectal temperature measurements taken daily for 10 days after exposure. Samples for serum neutralization titers were taken prior to exposure and again 21 days after exposure.

Sixteen monkeys were divided into 4 groups of 4 each. Two additional monkeys were held as controls. Each group received different inhaled doses achieved by aging the virus aerosol. Results are presented in table 4.

The mean doses achieved were 2,820, 275, 145 and 76 $MIPLD_{50}$ units. Although there were differences among individual animals in the time required to develop viremia and also differences in duration of viremia, it is obvious that these were random with respect to dose. Almost all animals were viremic at some point and all developed high levels of neutralizing antibodies. While some animals had obvious temperature elevations, others were at borderline levels difficult to distinguish from the normal fluctuations seen in monkeys. The ED_{50} dose cannot be reliably estimated, but seemed to be very low.

Only rhesus monkeys were exposed to aerosols of yellow fever virus. As before, attempts were made to obtain graded doses by exposing groups of 6 animals at various cloud ages. Since these doses were at levels below assessment capabilities, they were estimated by extrapolation from the early cloud age decay curve.

Monkeys were held for 30 days after exposure and then survivors, as well as controls, were sacrificed for gross pathologic examination.

Criteria of infection included (*a*) fever, with rectal temperature measurements taken daily, beginning the third day after exposure; (*b*) viremia as indicated by death of mice following intracerebral inoculation with blood (1-in-10 and 1-in-100 dilutions) taken on the fifth and on the seventh day of exposure; and (*c*) death, if yellow fever infection was confirmed by gross pathologic examination. Pathologic signs included degenerative lesions in the liver, kidney, spleen and lymph nodes.

The results are summarized in table 5. The mean doses achieved were 38.0, 16.0 and 6.0 $MICLD_{50}$ units inhaled. Monkey response fractions in terms of death were 5/5, 4/6, and 4/6. It can be stated only that the LD_{50} was something less than 6 $MICLD_{50}$ units. Of the 13 monkeys that died, all but one (no. 4633) showed viremia on day 5 or 7 after exposure. The exception was an animal in the low dose group which survived longer than the other test animals. Considering these factors, one is led to the conclusion that the dates chosen for tests of viremia were inappropriate in this case.

Monkey no. 4636 had a viremia on day 7, but did not succumb. Further,

TABLE 4

Responses of rhesus monkeys exposed to various inhaled doses of Rift Valley fever virus

Monkey no.	Est. dose MIPLD$_{50}$	Viremia*					SN tests†		Fever (degrees F)	
		Dilution	Day 4	Day 6	Day 8	Day 10	Pre	Post	High	Low
4502	2820	10^1	5/10	9/10	0/10	8/10	<1.0	>9.0	104.2	97.6
		10^2	0/10	9/10	0/10	3/10				
4767		10^1	8/10	9/10	7/10	7/10	<1.0	>9.0	103.8	101.4
		10^2	1/10	8/10	5/10	3/10				
4604		10^1	10/10	10/10	4/10	4/10	<1.0	>9.0	104.8	101.0
		10^2	10/10	10/10	2/10	1/10				
4616		10^1	10/10	9/10	0/10	1/10	<1.0	>9.0	104.0	100.6
		10^2	10/10	10/10	0/10	0/10				
4503	275	10^1	10/10	10/10	2/10	0/10	<1.0	>9.0	103.6	98.0
		10^2	0/10	10/10	0/10	1/10				
4768		10^1	6/10	4/10	0/10	0/10	<1.0	>9.0	103.8	99.2
		10^2	0/10	3/10	0/10	0/10				
4610		10^1	10/10	9/10	7/10	0/10	<1.0	>9.0	104.0	101.4
		10^2	9/10	10/10	0/10	1/10				
4501		10^1	10/10	10/10	9/10	0/10	<1.0	‡	104.0	98.4
		10^2	10/10	7/10	8/10	0/10				
4619	145	10^1	10/10	10/10	9/10	2/10	<1.0	>9.0	104.4	100.4
		10^2	0/10	10/10	6/10	0/10				
4617		10^1	10/10	10/10	8/10	0/10	<1.0	>9.0	105.2	101.6
		10^2	0/10	10/10	0/10	0/10				
4603	145	10^1	2/10	10/10	9/10	3/10	<1.0	‡	105.0	101.0
		10^2	5/10	10/10	10/10	1/10				
4620		10^1	10/10	10/10	6/10	0/10	<1.0	>7.2	103.2	101.2
		10^2	10/10	8/10	0/10	0/10				
4766	76	10^1	10/10	10/10	3/10	0/10	<1.0	>9.0	105.2	101.2
		10^2	0/10	7/10	0/10	0/10				
4602		10^1	7/10	9/10	0/10	0/10	<1.0	>9.0	104.0	101.2
		10^2	0/10	9/10	0/10	0/10				
4618		10^1	10/10	10/10	9/10	2/10	<1.0	>9.0	104.0	101.0
		10^2	10/10	9/10	1/10	1/10				
4600		10^1	8/10	10/10	5/10	2/10	<1.0	>6.7	104.0	101.4
		10^2	6/10	8/10	2/10	1/10				

* Mouse response (dead/total) to inoculation with monkey blood on indicated days after inoculation exposure.

† Log neutralization units. Determined on sera collected 21 days after exposure.

‡ Animals died of other causes.

TABLE 5

Responses of rhesus monkeys exposed to various inhaled doses of yellow fever virus

Monkey no.	Inhaled dose ($MICLD_{50}$)	Day of death after exposure	Viremia*			Fever (degrees F)	
			Dilution	Day 5	Day 7	High	Low
4997	38.0	10	10^1	3/10	10/10	104.2	102.2
			10^2	0/10	10/10		
4995		8	10^1	10/10	10/10	103.8	101.4
			10^2	3/10	10/10		
4624		10	10^1	1/9	10/10	103.2	102.4
			10^2	1/10	10/10		
4636		survived	10^1	3/10	9/9	102.8	101.2
			10^2	1/10	9/9		
4631		9	10^1	10/10	10/10	104.6	97.0
			10^2	10/10	10/10		
4632		7	10^1	10/10	10/10	103.6	101.2
			10^2	10/10	10/10		
4996	16.0	10	10^1	3/10	10/10	104.2	101.2
			10^2	2/10	10/10		
4994		8	10^1	10/10	10/10	104.4	101.8
			10^2	10/10	9/9		
4634		8	10^1	4/10	4/10	103.4	86.0
			10^2	1/10	4/10		
4622		survived	10^1	2/10	2/9	102.6	101.2
			10^2	1/9	2/10		
4612	16.0	survived	10^1	1/10	1/10	102.6	100.6
			10^2	1/10	1/10		
4628		8	10^1	3/10	10/10	104.0	101.4
			10^2	1/10	10/10		
5000	6.0	10	10^1	7/10	9/9	104.4	102.2
			10^2	3/10	10/10		
4637		8	10^1	10/10	9/9	104.6	101.0
			10^2	2/10	10/10		
4640		survived	10^1	1/10	1/9	102.8	100.0
			10^2	3/10	2/9		
4633		13	10^1	1/10	2/8	103.0	102.0
			10^2	0/10	1/9		
4629		survived	10^1	0/10	3/10	102.8	101.2
			10^2	2/10	3/10		
4630		12	10^1	1/10	5/10	102.6	97.0
			10^2	0/10	0/10		

* Mouse response (dead/total) to inoculation (IC) with monkey blood on fifth and seventh days post exposure

there was no evidence of yellow fever infection upon gross pathologic examination. Accordingly, this animal was not included among those that responded.

Higher temperature peaks were generally associated with dying animals but were not infallible indicators of infection. Similar conclusions were reported by Stokes et al. (8) in his early studies with over 90 rhesus monkeys.

All monkeys that died, and only these, showed hepatic lesions on gross pathologic examination.

Early work by Bauer and Hudson (9) indicated that rhesus monkeys may be infected by applying virus to the intact skin of the abdomen. This poses a question as to the actual portal of entry of the virus in these studies. Certainly, head exposure of our animals leads to the possibility of viral particles on the skin or in the eyes. However, the aerosol was extremely dilute at the time of our exposures because of the low dose levels desired. Considering that Bauer was unable to show transmission except in instances where he believed the dose to be quite high, we are of the opinion that infections in our studies did not result from external absorption of virus.

Discussion

These studies were conducted to provide quantitative data on the infectivity of two Arbor viruses, one Casal's group B, one ungrouped, for susceptible animal species with administration of the virus by the respiratory route. Further, it was intended to determine the stability of these viruses in aerosol form.

The results indicate that both viruses are quite capable of surviving aerosolization and once airborne exhibit a marked durability. It should be noted again that the conditions of the test included only higher relative humidities (50 and 80 per cent at 75 F). The viruses were exposed only to incandescent lighting and the effects of ultraviolet light or sunlight were not estimated.

The results indicate extreme infectivity by inhalation and have several important implications for any laboratory engaged in studies with these two viruses. One, the possibility of infecting laboratory workers with chance aerosols becomes seriously high if man exhibits a degree of susceptibility comparable to that of the animals tested. The possibility of infections with small respiratory doses has been suspected by some workers reporting laboratory-acquired illnesses with, in fact, both yellow fever and Rift Valley fever implicated in this category. The findings reported here are, however, the first known to support these suspicions.

With these indications of high invasiveness by the respiratory route and stability in the aerosol form, one is led to speculate on the importance of aerosols in natural infections. A basic requirement for this nonentomologic role is the release of virus aerosol by the host, and in the case of yellow fever this is not likely to occur. Taylor (10), in his review of the epidemiology of yellow fever, indicated that concentrated virus probably occurs only in the blood of the host and infers that the disease is noncontagious because there is no natural means of virus release except through the insect vector. This theory is, no doubt, valid for natural infections in man; but laboratory infections occur without insect vectors and it is believed that these, in many instances, result from inhaled virus. It has been observed that infections are frequently restricted to only those workers handling infectious blood or tissues (11), operations which are known in many instances to result in aerosols (12).

With Rift Valley fever there is a much greater chance that virus will be released naturally without vectors during the course of viremia because of the hosts. This disease affects sheep and cattle herds and commonly results in death to lambs, cows and ewes and in frequent abortions. The morbidity rate among herdsmen and others handling infected tissues is extremely high. A high morbidity rate is also the rule among nonimmunized laboratory workers. In many of the observed cases, the possibility of mosquito transmission, normal for animal infections, can be eliminated, leaving skin contact and inhalation as routes of infection (13). In view of the results of this study, the latter is worthy of further investigation as an important natural route in human beings.

Summary

Yellow fever virus and Rift Valley fever virus were found to be highly stable in aerosol form at temperatures of 75 F and at relative humidities of either 50 or 85 per cent. Loss of infectivity ranged between 1 and 5 per cent per minute. Yellow fever virus appeared to be more resistant to the aerosol production process than did Rift Valley fever virus although in both instances extremely potent and durable aerosols were produced. Yellow fever virus was found to be highly infective for rhesus monkeys by the respiratory route, with data indicating that less than 6 mouse $ICLD_{50}$ units constituted an LD_{50}.

Both hamsters and rhesus monkeys were exposed to Rift Valley fever virus aerosols. An LD_{50} for hamsters of 0.5 mouse $IPLD_{50}$ units was established. Monkeys were exposed to doses as low as 76 $MIPLD_{50}$ units inhaled and all developed viremias with subsequent positive serology.

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