Comparison of Quantity of Egg and Mouse-Adapted Influenza Viruses Required to Infect Each Host. (20606)

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Certain characteristics of strains of influenza viruses, adapted to the chick embryo and to the mouse lung, have been considered to provide an inherent basis for differences in their biological behavior. Properties have been ascribed to mouse-adapted agents, that distinguish them from their relatives which, although "unadapted," can nevertheless multiply extensively in the mouse lung. Mouse-adapted strains propagating in the mouse lung have been reported to possess: 1) a more rapid multiplication rate(1); 2) a shorter "lag" phase of the growth cycle(2); 3) the capacity to synthesize more virus(2); and 4) the ability to destroy the inhibitor of hemagglutination in mouse lung(3). In addition, differences have been demonstrated in strains of viruses in their capacity to produce cell damage(4) and the effect of this injury on subsequent viral multiplication has been studied(4). In every instance the comparable quantity of each agent employed was determined solely by infectivity titrations in chick embryos whereas the properties of the influenza viruses were investigated in the mouse. In order to interpret these studies adequately and to carry out further experiments comparing influenza viruses it was important to test the validity of the assumption that comparable quantities of 2 strains measured in the chick embryo would maintain the same quantitative relationship in a second host, *i.e.* the mouse. Studies by Knight indicated that with the mouse-adapted PR8 strain of influenza A virus, mouse-passage preparations were about 100 times as infectious for mice as egg-passage preparations of this agent(5). It seemed possible, therefore, that the relative quantity of adapted virus necessary to infect the mouse would be considerably less than the concentration of unadapted agent essential to initiate viral multiplication. If so, this would provide another differentiating property of mouse-adapted influenza viruses. Moreover, it appeared that the quantitative capacity to infect a given host might vary from strain to strain, mouse or egg adapted. The results of a study of the relative quantity of mouse adapted and unadapted influenza viruses essential to infect the chick embryo allantoic membrane and the mouse lung are here described.

Materials and methods. Viruses. A number of different chick embryo- and mouse-adapted strains of influenza A virus and the mouse-adapted Lee strain of influenza B virus were employed. In Table I are indicated the source, animal passage history and whether or not they are adapted to the mouse lung. The strain names of these agents will be used for reference in this paper. Viruses were cultivated by inoculation of 0.2 ml of a 10^{-4} dilution of each into the allantoic sac of 10-11 day old chick embryos(6). Infected allantoic fluids were stored in sealed glass ampules at —70°C in a cabinet which contained solid CO_2. *Animals.* Three to 4 week old albino Swiss mice, CFW strain, were obtained from Carworth Farms for mouse infectivity titrations. Fertile white eggs received from a local dealer were incubated at 39°C for 10 to 11 days and then employed for *in ovo* infectivity titrations. *Viral infectivity titrations.* Dilutions of virus were prepared in 0.5 $\log_{10}$ increments in tryptose phosphate broth containing 500 units of penicillin and 0.5 mg of streptomycin per ml. Dilutions were kept in an ice-water bath, and each inoculated as quickly as possible into mice and embryonated eggs. *In ovo titrations.* Each dilution was inoculated intra-allantoically in 0.1 ml volumes into groups of 4 chick embryos. Inocu-

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TABLE I. Descriptive Data Concerning the Strains of Influenza A and B Viruses Employed.

Virus	Isolated by	Passage history‡	Mouse adapted
Influenza A—PR8	Francis	Unknown§ E1	Yes
—CAM*	Anderson	E210	No
—WRU 51-51	Jordan	E8	"
— " " "	"	E5; M13; E1	Yes
—WRU 52-51	"	E4	No
— " 53-51	"	E3	"
—Rhodes†	Francis	F3; E9	"
— " †	"	F4; M127; E1	Yes
Influenza B—Lee	"	Unknown§ E1	"

* Obtained from Dr. John Y. Sugg, Cornell University Medical College.
† Obtained from Dr. Fred M. Davenport, School of Public Health, University of Michigan.
‡ E = No. of chick embryo passages; M = No. of mouse passages; F = No. of ferret passages.
§ Isolated in ferrets; followed by numerous mouse and chick embryo passages.

lated eggs were incubated for 44-48 hours at 35°C, followed by 16-18 hours at 4°C. The presence of virus in the allantoic fluid of each egg was determined by the hemagglutination reaction(7): to undiluted and 1:10 dilution of each fluid was added an equal volume of 1% chicken erythrocytes and the pattern of RBC was observed after 60 minutes at room temperature. *Mouse infectivity titrations.* A combination mouse-egg infectivity technic was employed in that the presence of unadapted influenza viruses in mouse lung cannot be demonstrated directly. Mice were inoculated intranasally under light ether anesthesia with 0.05 ml of the diluted material to be tested; groups of 4 mice per dilution were employed. Mice were killed 3 days after inoculation, and the lungs removed asceptically from each group starting with mice which had received the highest dilution. A 10% suspension of lung from each mouse was prepared individually by grinding in a mortar and pestle with 1.5 ml of tryptose phosphate broth which contained penicillin and streptomycin. Suspensions were centrifuged at 2000 rpm for 2 minutes, and each supernate was inoculated into the allantoic sac of each of 3 embryonated eggs. The inoculated eggs were incubated for 44-48 hours at 35°C and the presence of virus determined exactly as described under *in ovo* infectivity titrations. A mouse was considered to be infected if 1 or more of the 3 allantoic fluids had virus present. The results of titrations were expressed as 50% egg ($E.I._{50}$) or mouse ($M.I._{50}$) infectivity titers (8).

Experimental. The infectivity titer of an organism may be defined as the smallest number of particles of the agent which can infect a given host. This quantitative expression then indicates the relative amount of the organism in one infectious unit. By means of titrations in 2 hosts with the same dilutions of a single virus, the comparative quantity of the agent required to initiate infection in each host may be determined. Or expressed somewhat differently, one may estimate the relative susceptibility of each host to the agent. Experiments were designed to determine with chick embryo and mouse-adapted influenza viruses the relative quantity of virus in a single infectious unit for each of the 2 hosts.

The results of an experiment carried out with mouse-adapted and chick embryo-adapted lines of the WRU 51-51 strain of influenza A virus are presented in detail in Table II. The egg infectivity titer was 2.5 log units higher than the mouse infectivity titer when the strain which had been passed only in embryonated eggs was employed. In sharp contrast to this finding were the data obtained with the WRU 51-51 virus which had been adapted to the mouse lung by serial passages. With this strain the dilution of virus which infected the mouse was almost identical with that essential to initiate viral propagation in the allantoic sac of the chick embryo. These findings suggest that to infect a mouse approximately 100 times as much egg-adapted as mouse-adapted influenza virus is required.

To determine if the quantity of virus in an

TABLE II. Results of Simultaneous Egg and Mouse-Egg Infectivity Titrations Carried Out with Mouse-Adapted and Egg-Adapted Strains of WRU 51-51 Influenza A Viruses.

Virus titered	Dilution inoc.	Egg infectivity titration		Mouse-egg infectivity titration						
				Egg infectivity score†				Mouse lung score	Titer, $M.I._{50}$§	Ratio $E.I._{50}$/ $M.I._{50}$, log
		Score†	Titer, $E.I._{50}$‡	1*	2	3	4			
WRU 51-51, egg-adapted	$10^{-5.0}$			3/3	3/3	3/3	3/3	4/4		
	$10^{-5.5}$			0/3	"	2/3	0/3	2/4	$10^{-5.6}$	
	$10^{-6.0}$			3/3	0/3	0/3	"	1/4		2.5
	$10^{-6.5}$	4/4		0/3	"	"	"	0/4		
	$10^{-7.0}$	"		"	"	"	"	"		
	$10^{-7.5}$	"		"	"	"	"	"		
	$10^{-8.0}$	2/3	$10^{-8.1}$							
	$10^{-8.5}$	0/4								
WRU 51-51, mouse-adapted	$10^{-5.0}$			3/3	3/3	3/3	3/3	4/4		
	$10^{-5.5}$	4/4		"	"	"	"	"		
	$10^{-6.0}$	"		"	"	"	2/3	"		
	$10^{-6.5}$	"	$10^{-6.6}$	"	"	0/3	0/3	2/4	$10^{-6.5}$	.3
	$10^{-7.0}$	0/4		0/3	0/3	"	"	0/4		
	$10^{-7.5}$	1/4		"	"	"	"	"		
	$10^{-8.0}$	0/4		"	"	"	"	"		
	$10^{-8.5}$	"								

* Indicates the 10% mouse lung suspension inoc. intra-allantoically into 3 chick embryos.
† Numerator denotes No. infected; denominator denotes total No. per group.
‡ $E.I._{50}$ = 50% egg infectivity titer.
§ $M.I._{50}$ = " mouse " " .

infectious unit as measured in mice and chick embryos was relatively constant for mouse-adapted and chick embryo-adapted influenza viruses several strains of each were employed. The results are summarized in Table III. It is strikingly clear that a marked distinction exists in every instance between unadapted and adapted viruses in relation to the comparative quantities of each essential to induce infection in a mouse. Whereas, with unadapted viruses approximately 80 to 270 times more virus was present in a mouse infectious unit than in an egg infectious unit, with 3 of the mouse-adapted strains approximately the same quantity of virus composed the infectious unit for either host. Even greater contrast was noted with mouse-adapted Rhodes virus which manifested the capacity to infect the mouse with one-tenth the quantity of this agent required to produce infection in the embryonated egg. The marked discrepancy between infectivity titers of chick embryo-adapted viruses and the similarity of titers of mouse-adapted viruses as determined in both hosts cannot be attributed to the quantity of virus in the infected allantoic fluid tested. It will be noted in Table III that the chick embryo infectivity titer of viruses, unadapted or adapted to the mouse, varied markedly but the quantitative relationship described was maintained.

Discussion. The experimental evidence presented indicates that strains of influenza A virus which have been cultivated only in chick embryos required approximately 100 times more virus to infect mice than chick embryos. The ratio of egg infectivity titer to mouse infectivity titer (E:M) was of the same order of magnitude, *i.e.,* 2.0 log units for each of the strains tested. In marked contrast, influenza A and B viruses adapted to the mouse lung infected each host with approximately the same quantity of virus; the E:M infectivity ratio approached unity in 3 out of 4 examples. With the Rhodes strain of influenza virus the chick embryo was a less sensitive host than the mouse.

The relatively constant E:M infectivity ratio of egg-adapted viruses and probably a relatively constant but different ratio for mouse-adapted agents allow members of each group to be compared with others of similar host-adaptation characteristics by the use of infectivity dosages determined in embryonated eggs. In contrast, comparison in mice of unadapted with adapted strains in experiments in which the infectious dose of each agent is determined solely by titrations in the chick

TABLE III. Comparative Chick Embryo and Mouse Infectivity Titers Obtained by Parallel Titrations with Several Mouse and Chick Embryo Adapted Strains of Influenza A and B Viruses.

Virus titered	Mouse adapted	Infectivity titer— Chick embryo, $E.I._{50}$	Mouse, $M.I._{50}$	Ratio $E.I._{50}/M.I._{50}$, log
IAV—WRU 51-51	No*	$10^{-8.1}$	$10^{-5.6}$	2.5
— " " "-M	Yes	$10^{-6.8}$	$10^{-6.5}$	.3
—Rhodes	No	$10^{-8.1}$†	$10^{-6.5}$†	1.6
— " -M	Yes	$10^{-7.1}$†	$10^{-8.1}$†	−1.0
—CAM	No	$10^{-5.3}$	$10^{-6.0}$	2.3
—WRU 52-51	"	$10^{-6.7}$	$10^{-4.7}$	2.0
— " 53-51	"	$10^{-6.4}$	$10^{-4.6}$	1.8
—PR8	Yes	$10^{-8.2}$	$10^{-8.7}$	− .5
IBV—Lee	"	$10^{-6.0}$	$10^{-5.8}$	.2

* Chick embryo-adapted. † Geometric mean of results of 2 experiments.

embryo is probably a hazardous technic. Rather the more accurate but cumbersome procedure of combination mouse-egg titration should probably be employed.

Data have been presented by Friedewald and Pickels to indicate the extreme sensitivity of chick embryos to infection with exceedingly small quantities of egg-adapted influenza virus(9). The results of the experiments reported suggest that with properly adapted influenza virus the mouse is an equally sensitive host to infection with this agent. Infection of mouse lungs with relatively small amounts of adapted strains is even more surprising when one considers the relative inefficiency of intranasal inoculation of mice. For example, with PR8 virus it was estimated that of the order of only 18% of the inoculated virus actually initiated progressive infection in the mouse lung(10).

The factors which permit an agent to adopt the cellular environment of a new host for its biological activities are not clear. Since unadapted viruses multiply extensively in the mouse lung(11), adaptation of an influenza virus to this organ implies the acquisition by that agent of the property to inflict cell damage which results in pulmonary consolidation. Certain characteristics have been ascribed to these mouse-adapted viruses which distinguish them from unadapted strains(1-3). That mice can be infected with considerably less adapted than unadapted virus suggests at least a partial explanation for some of the previously described multiplication characteristics(1,2) of mouse-adapted influenza virus. In addition, this finding may be considered a further biological property of the mouse-adapted agent.

Summary. Parallel titrations to determine the dilution of virus essential to initiate infection in the allantoic membrane of the embryonated egg and the mouse lung were carried out. In every instance with mouse-adapted viruses, the relative quantity essential to infect the mouse and the chick embryo was approximately the same; with egg-adapted viruses approximately 2 logs more virus was required to initiate multiplication in the mouse lung than in the allantoic sac These data describe another property of mouse-adapted influenza viruses which distinguish them from unadapted strains.

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