THE RELATION OF INFECTIOUS AND HEMAGGLUTINATION TITERS TO THE ADAPTATION OF INFLUENZA VIRUS TO MICE*

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A number of serial passages is necessary before recently isolated strains of influenza virus can be adapted to mice. Little is known of the fundamental mechanism involved in the process of adaptation of the virus to mice. Hirst (1) reported that egg-adapted influenza A virus (Ala. 41, Kil. 41, N. Y. 43) while inducing no pulmonary lesions in mice, multiplied to the same maximum egg-infectious titer as the mouse-adapted passage of the same virus. The materials used by Hirst consisted of mouse lung preparations harvested 3 or 4 days after the virus was introduced intranasally. Thus the results, if correct, represent only the conditions occurring at the peak of virus growth. Since the adaptability of a virus may be more closely related to events taking place in the early stage rather than in the later stage of its growth, it was of interest to determine the earlier life history of the virus in mice. The present study is a report of observations on the rate of growth and the infectious titer of unadapted and adapted lines of a strain of influenza type A prime virus in the lungs of mice at various time intervals after intranasal introduction.

Methods

The influenza viruses used in the present experiments were two lines of the Rhodes strain (2), one adapted and the other unadapted to mice although both are propagated readily to approximately the same titers in the chorio-allantois of embryonated eggs. Antigenically they are the same, but their adaptability to mice is entirely different, one being pathogenic (adapted), and the other non-pathogenic (unadapted). At the time this study was instituted, the adapted line had been subjected to four ferret and fourteen mouse passages, and the unadapted line had been subjected to three ferret, two amniotic, and four allantoic passages.

Fresh preparations of equivalent concentrations of unadapted Rhodes (in the form of allantoic fluid) and adapted Rhodes (in the form of mouse lung suspension) virus were each inoculated intranasally (0.05 ml.) into similar groups of 20 to 24 young Swiss mice. The mice used in each experiment were of the same breed and of about the same size. Four mice from each similar group were sacrificed at 0, 4, 12, 24, 48, and 72 hour intervals after inoculation, and the lungs thus obtained at each interval from each group were ground and sus-

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pended in 10 per cent normal horse serum-saline. These mouse lung suspensions were centrifuged at 2000 R.P.M. for 5 minutes to remove the gross sediments. Tenfold dilutions of the fresh mouse lung suspensions were made in broth and 0.1 ml. amounts were inoculated into the allantoic sacs of three 10-day embryonated eggs. The eggs were incubated (35°C.) for 48 hours and then chilled at 4°C. overnight. The allantoic fluids were then tested individually

TABLE I

Rate of Multiplication of Unadapted and Adapted Lines of Rhodes Virus in Mouse Lung

Experiment No.	Rhodes viruses	Mice used	Dilution	Volume inoculated	Route of inoculation	Method of titrating virus	Titer at intervals in hours after I.N. inoculations*					
							0	4	12	24	48	72
				ml.								
I	Unadapted	20	10^{-2}	0.05	I.N.	E. $I._{50}$	0	$10^{-0.5}$	$10^{-3.4}$	$10^{-3.5}$	$10^{-6.5}$	N.D.
						Hemagglutinin	0	0	0	<20‡	<20‡	"
	Adapted	20				E. $I._{50}$	$10^{-2.0}$	$10^{-1.8}$	$10^{-7.0}$	$10^{-7.5}$	$10^{-7.4}$	"
						Hemagglutinin	0	0	0	1,280	1,280	"
II	Unadapted	24	10^{-2}	0.05	I.N.	E. $I._{50}$	$10^{-2.0}$	$10^{-1.5}$	$10^{-3.8}$	$10^{-5.3}$	$10^{-6.5}$	$10^{-6.7}$
						Hemagglutinin	0	0	0	0	<20‡	0
	Adapted	20				E. $I._{50}$	$10^{-3.5}$	$10^{-4.0}$	$10^{-6.7}$	$10^{-7.8}$	$10^{-7.6}$	N.D.
						Hemagglutinin	0	0	<20‡	1,280	640	"

N.D., not done.
I.N., intranasal.
E.$I._{50}$, 50 per cent egg-infectious titer.
* Lungs of four mice were pooled at each interval.
‡ Hemagglutination ± at 1/20.

for hemagglutinating titer by a pattern method (3), and the 50 per cent egg infectious titer calculated (4). The mouse lung suspensions at each time interval were also tested for hemagglutinating titers.

EXPERIMENTAL

Two separate experiments were conducted by similar procedures as described. The data are recorded in Table I and Figs. 1 and 2.

It will be observed that the growth of the line of unadapted Rhodes in mice is much slower than that of the line of adapted Rhodes. At 4 and 12 hours

after inoculation, the egg-infectious titer ($E.I._{50}$) of mouse lung suspensions of the unadapted line is low ($10^{-3.8}$ or less), and the peak of growth is not reached until 48 hours. On the other hand, the adapted line multiplied rapidly after its introduction into mice, and nearly attained its peak of growth within 12 hours ($E.I._{50}$: $10^{-6.7}$ to $10^{-7.0}$). The latter finding is in agreement with Taylor (5) who found that the PR8 strain of influenza virus reached a maximum M.L.D. titer in mouse lungs within 24 hours after intranasal introduction. In

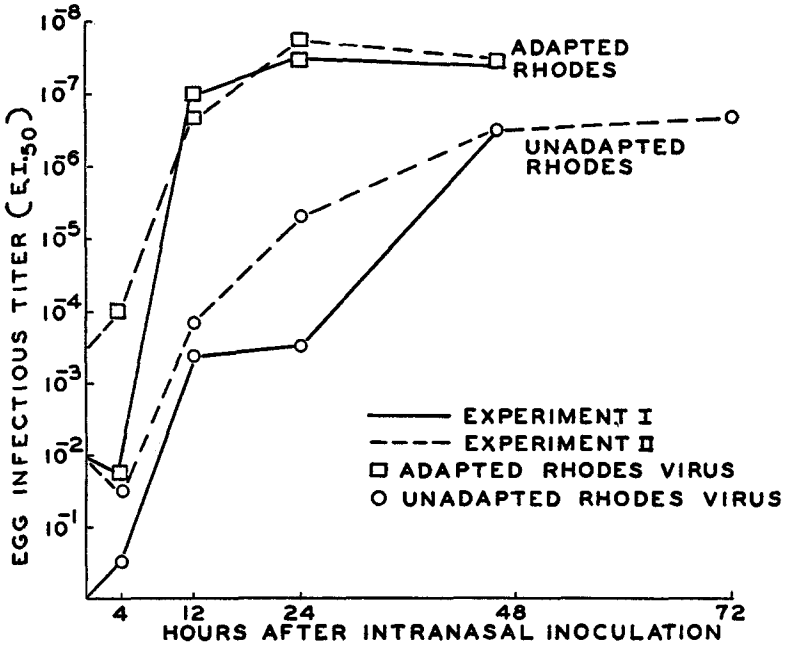

FIG. 1. Rate of multiplication of mouse-adapted and unadapted lines of Rhodes virus in mouse lung expressed as 50 per cent end-point infectious titer in eggs.

addition, the hemagglutination titers obtained from the multiplication of these two lines of virus in mouse lung are also different. This is clearly shown in Fig. 2. The hemagglutination produced by the unadapted line is exceedingly low (less than 20) even when the virus has reached its peak of growth at the end of 48 or 72 hours, while the adapted line gives a titer of 1,280 as early as 24 hours after inoculation. Since hemagglutinin is not readily separable from the influenza virus particles (6, 7), it would tend to indicate that there have been more virus particles produced by the adapted line than by the unadapted line at the peak of growth in the pulmonary tissue of mice.

DISCUSSION

It is of considerable interest to note that the line of mouse-unadapted Rhodes strain (ferret-egg passage), though completely non-pathogenic to mice, can

multiply readily in the pulmonary tissue of these animals. This finding is in agreement with that of Hirst (1). In contrast to Hirst's results, however, was the fact that the titer of virus concentration of the unadapted line was much lower than that of the adapted line (ferret-mouse passage). Hirst reported that the mouse-unadapted and adapted passages of influenza virus multiplied to the same maximum titer in the pulmonary tissue of mice. The discrepancy may be accounted for by the facts that (*a*) Hirst did not sacrifice his animals until 3 or 4 days after virus inoculation while mice were sacrificed at repeated

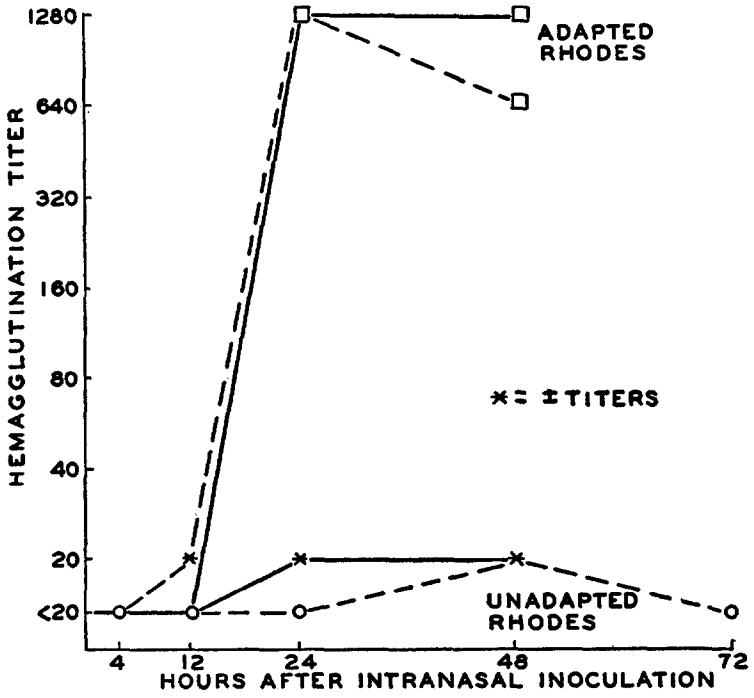

FIG. 2. Hemagglutination titers of the same mouse lung virus preparations as in Fig. 1

intervals in the present study; (*b*) a comparison of different passages of the same strain was made by Hirst while two different lines (unadapted and adapted) of the Rhodes strain in simultaneous passage were used in the present experiments. Whatever the explanation, the fact remains that the mouse-adapted line reaches a much higher hemagglutination titer (1,280) at the peak of its growth than does the unadapted line (less than 20). Since hemagglutination is intimately associated with the particles of influenza virus (6, 7), a higher hemagglutinating titer would logically imply a higher virus titer. It is to be pointed out, however, that the egg-infectious titers attained by these two lines of virus at the peak of their growth are not markedly different, $10^{-7.5}$ to

$10^{-7.8}$ for the adapted, *versus* $10^{-6.5}$ for the unadapted line. The discrepancy between egg-infectious and hemagglutination titers was also observed with the adapted strain in eggs after 12 and 48 hours of incubation (35°C.). The egg infectivity closely approached the peak at 12 hours ($10^{-6.7}$ to $10^{-7.0}$) when no hemagglutination was detectable, while another 12 hours of incubation raised the hemagglutination titer from less than 20 to 1,280 without appreciably increasing the egg-infectious titer.

It seems that the virus particles in mouse lungs infected with the adapted line present two characteristics: one being infective for eggs, and the other being capable of agglutinating chicken red cells in high titer. The line unadapted for mice on the other hand, produced a pattern of high egg infectivity and low hemagglutinating titer. This tends to indicate that the capacity of the virus to agglutinate chicken red cells to high dilution is a better measure of its pathogenicity for mice than the egg-infectious capacity.

It is also significant to note that the mouse-pathogenic virus (adapted Rhodes) had a much more rapid growth rate in mice than that of the non-pathogenic line. This difference in growth rates might well explain why some variants of one virus may easily be adapted to mice while with others greater difficulty is encountered. The adaptation of a virus to mice or to other animals through passages might be the result of an actual natural selection of the more rapidly growing variants of the same virus.

SUMMARY

The non-pathogenic (unadapted) line of the Rhodes strain of influenza virus multiplied readily in the mouse lung, but its rate of multiplication was slower and its hemagglutination titer attained at the peak of growth was much lower than that of the adapted line of the same virus. The implication of these findings to mouse adaptability of the virus is discussed.

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