INFLUENZA VIRUS INFECTION IN THE HAMSTER*

A Study of Inapparent Virus Infection and Virus Adaptation

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Viruses may mulitply in a host without producing manifest illness or gross evidence of a pathological process. It is well known, for example, that inapparent or subclinical infections commonly occur in such virus diseases of man as mumps, influenza, and poliomyelitis. Infections of this sort often assume great importance in the epidemiology of a virus disease and in calling forth immunity in the individual. One of the most interesting examples of inapparent virus infection is seen in the process of adapting a virus to a new host by serial blind passage. Infection may not be apparent during repeated passages until the virus, presumably as a result of variation, acquires the capacity to produce lesions in the host. Although the method of serial passage has been used extensively in adapting viruses to experimental animals, or in altering the properties of a virus, little is known regarding the fundamental mechanism of this process.

The hamster has been generally regarded as having only limited susceptibility to influenza virus infection. Although intranasal inoculation of influenza virus calls forth specific serum antibodies, lung lesions usually have not been observed even after repeated passage (1). The immune response of hamsters has been used as an indirect method for detecting influenza virus in throat washings (2) and it has been reported that the hamster is as effective as the ferret for this purpose (3). The present paper describes further studies on the behavior of influenza virus in the hamster, undertaken in an attempt to learn more about the factors involved in an inapparent virus infection and in the adaptation of a virus to a new host.

Methods

Virus Strains.—Two strains of influenza A virus (PR8 and Ga. 47) and one strain of influenza B virus (Lee) were used in the present study. The Ga. 47 strain was isolated from a patient in Atlanta during the influenza A epidemic of 1947 by intraamniotic inoculation of a throat washing into chick embryos. It was subsequently maintained by allantoic passage in 11-day-old embryos.

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Hamster Passages.—Syrian golden hamsters (*Cricetus auratus*) were used in all the experiments. The virus suspensions were inoculated intranasally in 0.3 cc. amounts into four hamsters under light ether anesthesia. Two of the hamsters were sacrificed after 2 to 4 days and the lungs removed with aseptic precautions. The lungs were weighed, ground in a mortar with alundum, and suspended in 0.9 per cent saline to a concentration of 20 per cent. The extract was finally cleared by centrifugation at about 2500 R.P.M. for 15 minutes. For serial passage an equal volume of 20 per cent horse serum broth was added to the extract and 0.3 cc. of the mixture inoculated intranasally into four normal hamsters. Two of the inoculated hamsters in each passage were observed for a period of 10 days. Rectal temperatures were recorded daily and the lungs of all hamsters dying during this period were examined for the presence of lesions; the survivors were sacrificed at the end of 10 days and lung lesions recorded.

Tests for Virus in Hamster Lung Extracts

(*a*) *Hemagglutination.*—The saline extracts prepared as described above were tested for their capacity to agglutinate chicken, human, and guinea pig red blood cells. The chicken cell titrations were read after 30 minutes, and the human and guinea pig cell titrations after 45 minutes at room temperature. The end-point was taken as the highest dilution causing complete agglutination (4).

(*b*) *Complement Fixation.*—Serial twofold dilutions of the saline extracts were tested in mixture with 1½ units of guinea pig complement and a 1:30 dilution of human serum containing a high titer of either influenza A or B antibodies. The complement was titrated in the presence of the serum as previously described (5) except that the end-point was taken as the highest dilution of complement producing complete hemolysis. A PR8 mouse lung extract containing a high titer of complement-fixing antigen was included in each test. This extract was preserved at −76°C. between tests and its titer remained remarkably constant throughout the experiments. Thus, the differences in titer of the hamster lung extracts obtained during passage cannot be attributed to variation in sensitivity of the test. In addition to the usual anticomplementary controls, a normal hamster lung extract was included in each test. The titer of complement-fixing antigen was recorded as the highest dilution of extract causing a ++ or better fixation of complement.

(*c*) *Titration in Mice.*—The extracts were diluted in tenfold steps with 10 per cent normal horse serum broth and each dilution was inoculated intranasally in 0.05 cc. amounts into four to six mice. All mice dying within 10 days were examined for specific lung lesions and the 50 per cent mortality end-points were calculated (6).

(*d*) *Titration in Embryonated Eggs.*—Tenfold dilutions of the lung extracts in 10 per cent horse serum broth were inoculated in 0.1 cc. amounts into the allantoic sac of 11-day-old chick embryos. Six eggs were used for each dilution. Two hundred units of streptomycin and of penicillin were inoculated into each egg to prevent bacterial contamination. The eggs were incubated at 37°C. for 48 hours and then placed at 4°C. overnight. The allantoic fluid from each egg was tested for agglutination of chicken red cells (7) and the 50 per cent infectivity titer calculated according to the method of Reed and Muench (6).

EXPERIMENTAL

Multiplication of Influenza Virus in the Hamster Lung

1. Ga. 47 Strain.—The recently isolated influenza A strain, Ga. 47, was selected for study in the initial experiment. A freshly prepared allantoic fluid suspension of this virus was inoculated intranasally into hamsters and

carried through eleven serial passages as described under Methods. The original virus suspension agglutinated red cells in high titer, fixed complement in dilution of 1:160, and had mouse mortality and egg infectivity titers of $10^{-4.5}$ and $10^{-8.6}$ respectively.

The results obtained with this virus strain are summarized in Table I. During the first six passages, no lung lesions or fatalities were observed in the

TABLE I

Serial Passage of Ga. 47 Influenza Virus in Hamsters

Passage No.	Tests for virus in hamster lung extracts*						Pathogenicity of virus in hamsters‡	
	Titration in		Complement fixation titer	Hemagglutination titer Red cells				
	Eggs	Mice		Chicken	Guinea pig	Human	Lung lesions	Mortality
0§	8.6	4.5	160	512	1024	1024		
1	6.2	2.5	80	—	—	—	0, 0	0/2
2	4.8	1.5	80	—	—	—	0, 0	0/2
3	6.1	1.3	80	—	—	—		
4	5.4	1.3	80	—	—	—	0, 0	0/2
5	6.6	3.2	80	—	—	—	0, 0	0/2
6	6.0	4.5	640	—	—	—	±, ±	0/2
7	7.5	4.6	320	—	20	160	++++, ++++	2/2
8	7.2	5.3	640	—	640	320	++++, ++++	2/2
9	7.0	4.6	640	—	160	320	++++, ++++	2/2
10	8.5	5.6	640	—	320	1280	++++, ++++	2/2
11	8.5	5.6	640	80	1280	1280	++++, ++++	2/2

* Titers expressed as the reciprocal of the dilution end-points (see Methods). — = less then 1:20.

‡ Two hamsters in each passage observed for 10 days following inoculation.

Lung lesions recorded as relative degrees of consolidation of total lung. ++++ = complete consolidation.

Under mortality, numerators indicate the number of hamsters dying within 10 days after inoculation, and the denominators the number of hamsters inoculated.

§ Allantoic fluid suspension of the Ga. 47 strain.

hamsters followed for a period of 10 days. Nevertheless, virus was present in high titer as shown by egg infectivity tests with extracts of the lungs obtained 3 to 4 days after inoculation. Virus was also demonstrable in the extracts by mouse mortality tests, but in lower titer. It will be seen that the total amount of virus present in the lungs as determined by titration in eggs and mice decreased during the first two passages. By the sixth passage, however, the mouse mortality titer was comparable to that of the original virus suspension, while the egg infectivity titer was $10^{-6.0}$ as compared with the original titer of $10^{-8.6}$. Complement-fixing antigen was present in extracts

of the lungs of all the passages, but showed a sharp increase on the sixth passage. The extracts during these early passages failed to cause agglutination of chicken, guinea pig, or human red blood cells. Occasionally a weak agglutination of human or guinea pig red blood cells was observed with a 1:20 dilution of the lung extracts, but the reaction was too indefinite to record.

On the seventh hamster passage a sudden change in the course of events was observed. Extensive pulmonary consolidation occurred and the hamsters died on the 6th day after inoculation. The most striking finding in the tests for virus in the lungs was the appearance of agglutinins for human and guinea pig red cells for the first time. The egg infectivity titer increased from $10^{-6.0}$ to $10^{-7.5}$, but no change in mouse mortality titer was observed. The latter, together with the titer of complement-fixing antigen, had increased during the preceding passage.

The lethal character of the virus persisted on subsequent passages. Agglutination of chicken red blood cells, however, was not observed until the eleventh passage. A further increase in the total amount of virus in the lungs, as determined by titration in mice and eggs, occurred on the tenth passage. The content of complement-fixing antigen remained constant at a titer of 1:640. The lung extract of the thirteenth passage (not shown in the table) produced complete pulmonary consolidation and death in hamsters in dilutions through $10^{-6.0}$.

2. PR8 Strain.—In the next experiment the PR8 strain of influenza A virus was inoculated intranasally into hamsters and carried through twelve serial passages. The virus suspension used for inoculation consisted of a freshly prepared PR8 mouse lung extract at a concentration of 10 per cent.

Table II shows the results of this experiment. Infectivity tests in eggs were not carried out in this experiment. In general, the results are similar to those obtained with the Ga. 47 strain (Table I). Lung lesions were not observed during the first six passages and extracts of the lungs failed to agglutinate red blood cells. Nevertheless, virus was demonstrable in all of the passages by mouse mortality tests and also by means of the complement fixation test. On the seventh hamster passage a fatal disease with pulmonary consolidation was produced, together with the appearance of hemagglutinins in extracts of the lungs for guinea pig and human red cells, but not for chicken red cells. At this time the mouse mortality and complement fixation titers of the lung extracts increased. On the eighth and ninth passages, however, the hamsters did not die and lung lesions were not observed. Extracts of the lungs then failed to agglutinate guinea pig red cells and showed a decreased capacity to agglutinate human red blood cells. During these passages the mouse mortality titer decreased, but no change in the titer of complement-fixing antigen occurred. By the tenth passage a fatal disease was again produced and on the eleventh passage, hemagglutinins for chicken, guinea pig, and human red blood

cells were present in high titer. A sharp increase in the mouse mortality titer occurred on the eleventh passage, coinciding with the first appearance of agglutinins for chicken red cells.

As in the preceding experiment, a close correlation between the capacity of the extracts to produce lung lesions in the hamster and hemagglutination of human and guinea pig cells was evident. In both experiments lung lesions and a fatal disease appeared suddenly on the seventh hamster passage.[1] With the PR8 strain, for some unknown reason, the lethal character of the virus

TABLE II

Serial Passage of PR8 Influenza Virus in Hamsters

Passage No.	Tests for virus in hamster lung extracts					Pathogenicity of virus in hamsters	
	Mouse mortality titer	Complement fixation titer	Hemagglutination titer Red cells				
			Chicken	Guinea pig	Human	Lung lesions	Mortality
0* ML	6.5	320	1280	640	640		
1	4.0	20	—	—	—	0, ±	0/2
2	3.5	80	—	—	—		
3	3.3	80	—	—	—	0, 0	0/2
4	3.6	40	—	—	—	0, 0	0/2
5	3.6	80	—	—	—	0, 0	0/2
6	4.0	80	—	—	—	0, ±	0/2
7	4.5	320	—	160	640	++++, ++++	2/2
8	4.3	320	—	—	160	0, 0	0/2
9	2.3	320	—	—	40	0, +	0/2
10	3.0	320	—	—	—	++++, +	1/2
11	6.3	640	320	640	640	++++, ++++	2/2
12	6.5	640	1280	1280	1280	++++, ++++	2/2

* 10 per cent PR8 mouse lung extract.

See footnotes under Table I.

was lost on the eighth and ninth passages but reappeared on the tenth passage. An unusual opportunity was thus afforded for correlating the pathogenicity of the virus with its other properties.

3. Lee Virus.—For comparison with the results obtained in the preceding experiments with influenza A viruses, the Lee strain of influenza B virus was selected for a passage series in the hamster. A freshly prepared allantoic fluid suspension of Lee virus was inoculated intranasally into hamsters and extracts of the lungs were passed serially at 2 day intervals.

[1] In order to rule out a bacterial component in the production of the lung lesions, cultures of the lung extracts were taken throughout these experiments. Except for occasional contaminants, the cultures were negative.

The results are summarized in Table III. The course of events differed markedly from that observed with the influenza A virus strains (Tables I and II). Lung lesions and fatalities occurred with the first passage and persisted through the series of eleven passages. There was no apparent increase, however, in the capacity of the virus to produce pulmonary lesions on repeated passage in the hamster. Extracts of the lungs from all passages caused agglutination of chicken, human, and guinea pig red cells. The mouse infectivity titer and complement fixation titer of the lung extracts remained re-

TABLE III

Serial Passage of Lee Influenza Virus in Hamsters

Passage No.	Tests for virus in hamster lung extracts						Pathogenicity of virus in hamsters	
	Titration in		Complement fixation titer	Hemagglutination titer Red cells				
	Eggs	Mice		Chicken	Guinea pig	Human	Lung lesions	Mortality
0*	6.5	3.6	160	512	128	512		
1	5.6	3.3	320	640	640	1280	++++, ±	1/2
2	4.3	3.0	160	40	320	160	+++, ++	1/2
3	6.3	3.6	160	160	160	320	+, 0	0/2
4	5.3	4.5	640	160	640	160	++++, ++	1/2
5	4.6	3.5	320	160	320	320	++++, 0	1/2
6	6.3	2.6	320	40	160	160	++, 0	0/2
7	3.5	3.3	640	80	160	80	++++, ++	1/2
8	4.5	3.5	320	80	160	320	++++, +++	1/2
9	5.0	3.6	640	160	320	320	++++, +	1/2
10	4.0	3.3	320	640	640	640	+, ++	0/2
11	5.4	3.5	320	160	320	320	++++, ++++	2/2

* Allantoic fluid suspension of Lee influenza virus.

See footnotes under Table I.

markably constant throughout, differing little from the titers of the original virus suspension used for inoculation.

Effect of Incubation at 37°C. on Chicken Red Cell Agglutination with Hamster Lung Extracts.—The preceding experiments demonstrated that influenza A virus in hamster lung extracts from the early passages failed to agglutinate red blood cells, even though virus was readily demonstrable by infectivity or complement fixation tests. After six serial passages, however, agglutinins for human and guinea pig red cells appeared in high titer, but agglutinins for chicken red cells either did not occur or produced only an indefinite reaction until the eleventh passage. This finding is of interest in view of the observation of Burnet and Bull (8) that freshly isolated strains of influenza virus in the chick embryo agglutinate mammalian red cells in higher titer than fowl

red cells. A further study of this phenomenon in extracts of hamster lungs was therefore undertaken.

The hamster lung extracts of the sixth, seventh, tenth, and eleventh passages of the Ga. 47 strain of influenza virus (see Table I) were selected for study. Portions of the 10 per cent saline extracts, which had been previously cleared by low speed centrifugation, were spun at about 12,000 R.P.M. for 30 minutes to sediment the virus. The supernatant fluids were discarded and the sediments were resuspended in the original volume of 0.9 per cent saline. The suspensions were then placed in a 37°C. water bath for 6 hours. Following incubation, the suspensions thus treated and portions of the original extracts kept at 4°C. were tested for capacity to agglutinate chicken red cells. The end-point was recorded as the highest dilution of the extract that produced complete agglutination of the red cells.

Table IV shows the results of these tests. It will be seen that the procedure of high speed centrifugation and incubation at 37°C.[2] was effective in bringing

TABLE IV

Effect of Incubation at 37°C. on the Hemagglutination Titer of Hamster Lung Extracts

Hamster passage No.	Agglutination titer of lung extracts with chicken red cells	
	Before incubation	After incubation at 37°C. for 6 hrs.*
6	<20	<20
7	<20	40
10	<20	640
11	80	640

* Extracts spun at 12,000 R.P.M. for 30 minutes, sediment resuspended in original volume of 0.9 per cent saline, then incubated at 37°C. for 6 hours (see text).

out agglutinins for chicken red cells in those extracts which originally caused agglutination of only human and guinea pig red cells (compare Table I). Furthermore, the titers of chicken red cell agglutinins in the extracts thus treated were approximately the same as the titers of human and guinea pig red cell agglutinins obtained with the untreated extracts. In additional tests (not shown in the table) it was found that the titers of agglutinins for the mammalian types of red cells were not increased by the method of centrifugation and incubation at 37°C. The implications of these findings will be considered in the discussion.

Effect of Influenza Virus Infection on the Temperature of the Hamster.— During the serial passages of the influenza A virus strains already described, rectal temperatures were taken daily on the hamsters during the 10 day observation period. It was found that a precipitous drop in temperature oc-

[2] Incubation of the extracts at 37°C. for 6 hours without preliminary centrifugation was also effective in calling forth chicken cell agglutinins. The titers obtained, however, were not as high nor was the reaction as definitive following centrifugation as outlined.

curred on the 2nd to 4th day after inoculation of either the Ga. 47 or PR8 strains of virus. During the early passages of these viruses, before lung lesions were produced, this temperature response was observed in only about half of the inoculated hamsters and the temperatures usually returned to normal

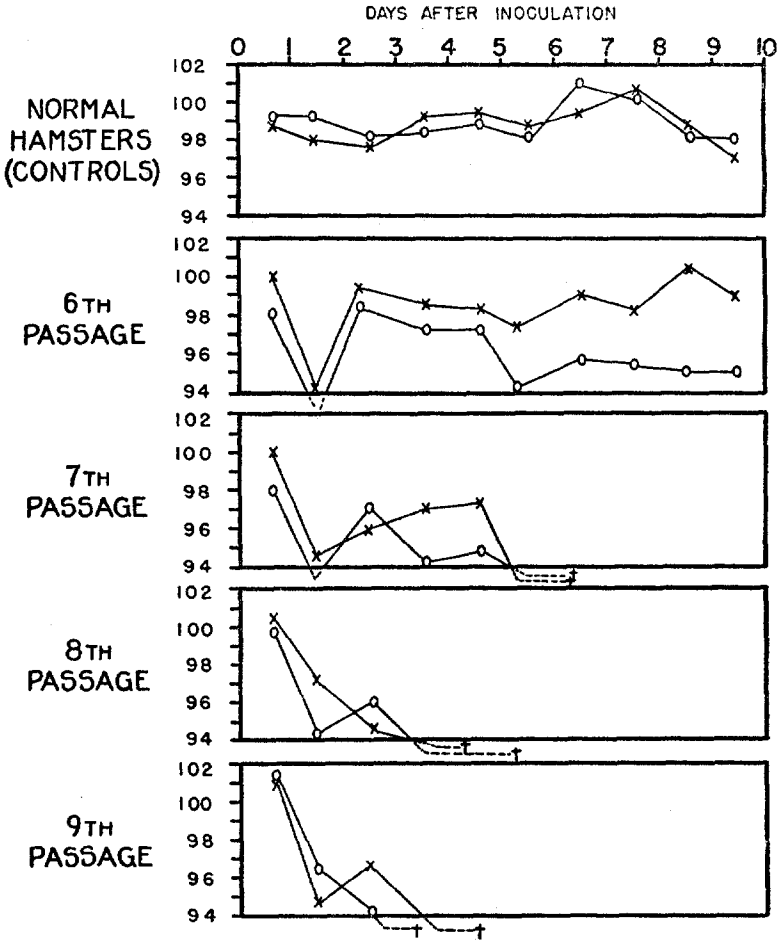

CHART 1. Temperature response in hamsters to Ga. 47 influenza virus.

levels within 24 to 48 hours. After the sixth passage, however, the drop in temperature occurred consistently and subnormal levels persisted until death.

The temperatures of the hamsters inoculated with the Ga. 47 strain from the sixth to the ninth passage are shown in Chart 1. The temperatures of two normal hamsters are included as controls. Temperatures less than 94°F. are indicated by dotted lines, since the thermometers used did not record below

this level. On the sixth passage the temperatures of both hamsters dropped sharply to 94°F. or less 24 hours after inoculation, but returned to normal limits within 48 hours. In one hamster the temperature then remained normal, while in the other it gradually fell to subnormal levels during the 10 day period. The hamsters in this passage did not die and only minimal lung lesions were observed (see Table I). In the subsequent passages the temperature either remained low or showed a rise followed by a second drop to less than 94°F. It will be noted also that the interval between inoculation and death decreased with passage.

DISCUSSION

The course of events following the inoculation of influenza virus into hamsters herein reported may represent an unusual instance of virus activity in a new host. Regardless of whether this is the case or not, the findings shed some light on the behavior of a virus during inapparent infection and on the process whereby a virus assumes new characteristics as a result of serial passage. First of all, it is clear that the influenza A strains used in these experiments multiplied readily in the hamster lung. Although illness and pulmonary lesions were not observed in the early passages, virus was demonstrable from the start in extracts of the lungs by infectivity tests in mice and in embryonated eggs, or by means of the complement fixation test. The findings are similar to those of Hirst (9) who recently reported that maximum multiplication of virus occurs in mouse lungs following the inoculation of egg-adapted influenza A virus strains, before lung lesions are produced as a result of serial passage. These results emphasize the fact that no correlation may exist between the pathogenic potentialities of a virus and its capacity to multiply in a host. If the conditions are such that the virus, though actively multiplying in the host, fails to acquire pathogenic qualities, even after repeated passage, the infection might be overlooked. Obviously, such findings have practical implications regarding the isolation of viruses in experimental animals.

In the present experiments the inapparent virus infection in the hamster, which persisted for six serial passages, changed dramatically to a severe disease characterized by extensive pulmonary consolidation and death. The various tests utilized for studying the content of virus in the lungs during passage revealed certain findings of interest, which accompanied the abrupt change in pathogenicity of the virus. Agglutinins for human and guinea pig red blood cells appeared and a marked increase occurred in the amount of complement-fixing antigen. Although the tests for infectious virus are less accurate and more difficult to interpret, there was a definite tendency toward an increase in the total amount of virus in the lungs. In both experiments with influenza A strains the titer of virus in the lungs after eleven serial passages was as high or higher than the titer of the original inoculum. The findings as a whole

indicate that the virus population in the lungs was undergoing both quantitative and qualitative changes during serial passage. If the new character of the virus was due to a variant which replaced the original type of virus, it either appeared suddenly in the virus population or was being fostered during the complex process of adaptation to the hamster lung, but was unable to manifest itself during the early passages.

The simultaneous appearance of lethal effects of the virus and agglutinins for mammalian red blood cells, but not fowl cells, was observed with both strains of influenza A virus tested. · The difference in capacity of the hamster-adapted virus to agglutinate mammalian and fowl types of red cells is a characteristic of recently isolated human influenza strains in the chick embryo (8) and egg-adapted influenza virus strains during serial passage in the mouse (9). A study of this phenomenon in the hamster lung has revealed that agglutinins for chicken red cells are actually present in titers comparable to those obtained with the mammalian types of red cells, but are not manifest until the extracts are heated at 37°C. for about 6 hours (Table IV). It will be recalled that influenza virus can be eluted from red cells by incubation at 37°C. (10) and that this procedure is also effective in dissociating virus from the inhibitory substance found in extracts of red cells (11, 12). Evidence has been previously presented (11) indicating that the substance in extracts of red cells and normal tissues responsible for inhibition of virus hemagglutination is identical with the virus receptor substance. It appears, therefore, that the hamster lung extracts contain an inhibitor, similar to the virus receptor substance, which masks the chicken cell hemagglutinins. The inhibitory substance, however, was not effective in masking the hemagglutinating activity of the virus for mammalian types of red cells. Furthermore, elution at 37°C. failed to increase the titer of the mammalian cell hemagglutinins. Finally, it should be noted that with further passage in the hamster, the difference in capacity of the virus to agglutinate chicken and mammalian cells became less obvious and in one experiment (Table II) the titers were identical.

It is well known that influenza A and influenza B viruses are distinct antigenically and differ in certain other properties. A comparison of the results obtained with these viruses in hamsters provides additional evidence of their individuality. Pulmonary lesions were produced with the influenza B strain on the first passage and no apparent increase in titer or pathogenicity of the virus occurred during eleven serial passages. Furthermore, hemagglutination was produced by the virus in all of the passages, without significant difference in titer for the fowl and mammalian types of red cells.

SUMMARY

A study of influenza virus infection in the hamster has yielded the following results:

1. Two influenza A strains (Ga. 47 and PR8) multiplied readily in the hamster lung, although no lung lesions were produced during six serial passages. On further passage both viruses abruptly acquired the capacity to produce pulmonary consolidation and death of the animals.

2. Extracts of the lungs during the early passages contained complement-fixing antigen and infectious virus, as revealed by titration in mice and embryonated eggs. Agglutinins for chicken, human, and guinea pig red cells, however, were not demonstrable at this time. With further passage a close correlation was observed between the capacity of the virus to produce lung lesions in the hamster and to agglutinate mammalian types of red cells. In addition, quantitative changes in the virus population were demonstrated in the lung extracts by complement fixation tests and titrations in mice and eggs.

3. Incubation at 37°C. was effective in bringing out agglutinins in high titer for chicken red cells in lung extracts, which originally failed to agglutinate chicken cells but agglutinated mammalian red cells. This method did not increase the titers of mammalian cell agglutinins.

4. The body temperature of the hamster was found to decrease within 1 to 4 days after inoculation of influenza virus. In the early passages the temperature returned to normal within 24 hours, but with the development of the pathogenic strain of virus the temperature remained at subnormal levels until death.

5. The Lee strain of influenza B virus produced pulmonary lesions in the hamster on the first passage and no increase in pathogenicity of the virus occurred during eleven serial passages. Virus was demonstrable in extracts of the lungs by all the methods used and no difference was observed in its capacity to agglutinate fowl and mammalian types of red cells.

The implications of these findings are considered briefly in the discussion.

BIBLIOGRAPHY

1. Taylor, R. M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 541. Taylor, R. M., and Dreguss, M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 100.
2. Taylor, R. M., and Parodi, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 105.
3. Eaton, M. D., Corey, M., van Herick, W., and Meiklejohn, G., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 6.
4. Salk, J. E., *J. Immunol.*, 1944, **49**, 87.
5. Friedewald, W. F., *J. Exp. Med.*, 1943, **78**, 347.
6. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.
7. Hirst, G. K., *J. Immunol.*, 1942, **45**, 285.
8. Burnet, F. M., and Bull, D. R., *Australian J. Exp. Biol. and Med. Sc.*, 1943, **21**, 55.
9. Hirst, G. K., *J. Exp. Med.*, 1947, **86**, 357.
10. Hirst, G. K., *J. Exp. Med.*, 1942, **76**, 195.
11. Friedewald, W. F., Miller, E. S., and Whatley, L. R., *J. Exp. Med.*, 1947, **86**, 65.
12. Bovarnick, M., and de Burgh, P. M., *Science*, 1947, **105**, 550.