COMPARISONS OF INFLUENZA VIRUS STRAINS FROM THREE EPIDEMICS

By GEORGE K. HIRST, M.D.

(From the Laboratories of the International Health Division of The Rockefeller Foundation, New York)

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In the course of numerous field studies of influenza made in the past few years, a considerable amount of information has been accumulated on the behavior of virus strains isolated at different times. This information is of some immediate practical value, since much of it concerns difficulties encountered in virus isolation, which are important in making a laboratory diagnosis. The observations cover the characteristics of influenza A strains isolated during the outbreaks of 1940–41 and 1943–44 and of influenza B virus isolated in 1945–46. A number of the strains were tested for antigenic differences, the results of which may have some bearing on the selection of viruses for human vaccination.

Materials and Methods

Virus Strains.—Four strains of influenza A virus were used. Strain 1741 was obtained from Dr. E. R. Rickard, then at Minneapolis, Minnesota, and is herein referred to as the Minn. 43 strain. Strain ICE49 was obtained from Dr. W. H. Hale and is referred to as the Iowa 43 strain. The Martin strain of influenza A virus was obtained from Dr. J. E. Salk and is called the Mich. 43 strain. The Olson strain, received from Dr. M. D. Eaton, will be referred to as the Calif. 43 strain. All of these strains were isolated during the outbreak of influenza A occurring between mid-November, 1943, and February, 1944. They are named for the states in which they were isolated. All were initially isolated in chick embryos, and none had any passages in other animals. All other strains examined were isolated in this laboratory except strain Mid-Pac. which will be referred to later.

Throat Washings.—Specimens for virus isolation were obtained by having influenza patients gargle with 15 cc. of a 5 per cent normal horse serum in phosphate buffer. The washings were all taken during the acute stage of clinically typical epidemic influenza, usually while the patient was still febrile and within 4 days or less of the onset. Unless the washings were tested immediately they were stored on dry ice.

Detection of Virus in Throat Washings.—For attempts at virus isolation in chick embryos, throat washings were mixed with penicillin (165 units per cc.). For the amniotic method 0.3 cc. of washing was inoculated into the sacs of 13-day-old chick embryos by a modification of the method described by Burnet (1). The eggs were incubated for 4 days at 35°C., and the amniotic fluid was tested for chicken and guinea pig red cell agglutinins by the pattern method. Where repeated passages were performed by the amniotic route the lungs and tracheas of embryos were removed and ground in amniotic fluid for passage.

Virus isolations by the allantoic route were performed by the inoculation of 0.2 cc. of throat washing–penicillin mixtures into the sacs of 11-day-old chick embryos, followed by 2 or 3 days' incubation at 35°C. Tests for hemagglutinins were carried out by the pattern method.

Tests for virus were carried out in ferrets by inoculating these animals intranasally under light ether anesthesia with 1 cc. of untreated throat washing. Daily temperatures were taken, and sera obtained 2 weeks after inoculation were tested for antibody by the agglutination inhibition method (3).

Strain Difference Tests.—Cross inhibition tests for strain differences were carried out by the methods previously used (4). Antisera were obtained by infecting ferrets with a 10^{-3} dilution of allantoic fluid, and sera were taken 2 weeks later. Simultaneous titrations of all immune sera against 4 agglutinating units of all the viruses were carried out by the agglutination inhibition tests using a photoelectric densitometer (3).

EXPERIMENTAL

Titration of Influenza Virus in Throat Washings by Several Methods

Although delicate methods of detecting influenza virus have been available for years, very little has been done to determine what concentrations of virus may be expected in the secretions of influenza patients. The frequent absence of detectable amounts of virus in throat washings of serologically proven cases of influenza has led to the assumption that the concentration is probably low. The development of new methods of virus detection in chick embryos provided a good means of investigating this problem.

The discovery by Rickard *et al.* (5) of the feasibility of inoculating the embryo by the allantoic route for primary virus isolation has led to the wide use of this method. The amniotic method of inoculation, which was developed earlier, has been used very little in the United States. While work at this laboratory has shown that with a group of throat washings the amniotic method gives a much greater number of positive isolations than the allantoic method (2), nothing so far has shown what the actual difference in sensitivity of virus detection might be.

Of forty-five throat washings from persons ill of influenza A in the 1943–44 epidemic, eight specimens yielded virus on inoculation into chick embryos by the allantoic route. These washings presumably had the highest virus titers and were investigated further. Two washings were found by amniotic titration to contain very high concentrations of virus, and these were tested by two other methods.

From one of these two washings (973), virus was obtained by amniotic inoculation even in a dilution of 10^{-6} of the garglings (Table I). This result was confirmed by repetition and by subinoculation of the first-passage positive embryos. With the same washing, positive serological results were obtained with ferrets that had been inoculated with a throat-washing dilution of 10^{-5} But titration of washing 973 in chick embryos by the allantoic route gave positive results only through a 10^{-2} dilution. A second throat washing (1004) was lower in titer but showed the same differences by the three methods of titration. Two other washings were titrated in the chick embryo amniotic sac with positive results in dilutions of 10^{-3} and 10^{-4}. It should be emphasized

that these high levels are exceptional and as a group made up less than 10 per cent of the total. The results confirm what was inferred from previous experience, that the amniotic method is definitely the most sensitive for the detection of virus. Ferret titration is a little less sensitive while, with the 1943 material, isolation by allantoic inoculation required of the order of 10,000 times more virus than isolation by the amniotic technique. It ahould be mentioned in connection with later comparisons with influenza B that these washings had been stored for 12 months on dry ice before being finally tested.

TABLE I

Comparative Titers of Virus in Throat Washings by Three Different Methods

Dilution of throat washing	Throat washing 973 (1943-A)			Throat washing 1004 (1943-A)		
	Allantoic sac	Amniotic sac	Ferret-immune response	Allantoic sac	Amniotic sac	Ferret-immune response
10^{0}	6/6	4/5	1:256	4/4	—	
10^{-1}	2/4	3/3	—	1/6	—	1:700
10^{-2}	2/4	3/3	—	0/6	—	1:1024
10^{-3}	0/6	5/5	1:475	—	4/4	1:350
10^{-4}		—	1:525		4/6	1:300
10^{-5}		—	1:300		1/5	1:32
10^{-6}		3/6	1:32		0/5	—
10^{-7}		0/4	—		—	

In the chick embryo tests the numerator indicates the number of fluids showing positive chick cell agglutinins and the denominator the number of inoculated embryos that survived the incubation period. The titers given under the ferret test indicate the agglutination inhibition titer of serum taken 2 weeks after inoculation for 4 units of the PR8 strain. Inhibition titers of 1:32 are within *normal* inhibitory levels.

Isolation of Virus by the Amniotic Route

Of forty-five throat washings obtained in the influenza A epidemic in 1943–44, 74 per cent were found to contain virus when tested by the amniotic technique. However, in the influenza B epidemic of 1945–46, eighty-two throat washings were examined, of which only twenty-five (30 per cent) yielded virus. While it is conceivable that some of this difference may have been the result of variation in case sampling, this is unlikely since almost all of the later washings were from serologically positive cases. Other evidence makes it seem likely that the difference was due to the nature of the strains concerned.

In the influenza A epidemics it was unusual to obtain positive results from serial amniotic passage when the initial passage was negative, while in the influenza B epidemic 44 per cent of the strains isolated were first detected only on second passage, indicating that B virus took longer for detectable growth in the amniotic sac. A similar result was obtained by Burnet, Stone, and

Anderson (6), who state that with influenza B virus a 5-day incubation period gave better results than a 4-day one.

Attempts to isolate influenza B virus in dilutions of throat washings beyond 10^0 and all attempts to culture the virus initially in the allantoic sac completely failed.

Detection of Virus in Ferrets

In influenza A epidemics the ferret has been successfully used for the detection of virus in numerous instances, but in our experience influenza B throat washings (1945–46) consistently failed to elicit any serological response in this animal. Eleven throat washings, proved by amniotic isolation to contain influenza B virus, gave completely negative results in the ferret. Only a suspension of lung tissue from a patient dying of hemorrhagic tracheobronchitis (strain Sinai 45) consistently infected these animals. However influenza B virus isolated in the egg and then administered to ferrets produced a good serological response and sometimes a febrile reaction; and the infection could be readily passed by the use of turbinate, but not lung, suspension.

Stability of Strains on Freezing and Storage

In general, strains of influenza A virus are quite stable on prolonged storage at low temperature. In this laboratory influenza virus has been isolated from throat washings and stored for 5 years in cellulose nitrate tubes at −72°C. The influenza B strains, on the other hand, are apparently very sensitive to freezing and storing at low temperatures. It was observed that if throat washings containing B virus were inoculated into eggs before freezing better results were obtained than if they were first frozen. Six washings were tested for virus before and after freezing, and the results recorded in Table II show that all washings became negative for virus after 4 months or less of storage at −72°C. Tests were undertaken with egg-passage B virus stored under similar conditions and tested after several intervals. Table III shows the marked loss of titer with storage under these conditions. The same strain stored in sealed glass ampules did not lose titer over an even longer period, so that it seemed possible that the inactivation was due to diffusion of CO_2 through the cellulose nitrate storage tubes. A comparison of the pH stability of this strain of B virus and the Lee strain showed no difference however. It seems reasonably clear that the B strains of 1945–46 were especially susceptible to deterioration with freezing and storage at low temperature.

From what has been presented so far in relation to the isolation of influenza B virus from throat washings, the conclusion would be justified that the difficulties encountered were due essentially to the low titer of virus in the garglings. However, in other respects these strains were so peculiar that it is

felt that the difficulty was more probably one of adaptation of the agent to various media.

TABLE II

Isolation of Influenza B Virus before Freezing and after Freezing and Storage at Low Temperature

Throat washing	Before freezing		Frozen and stored 1 wk.		Frozen and stored 4 mos.	
	1st passage	2nd passage	1st passage	2nd passage	1st passage	2nd passage
1	*3/3	—	‡0/5	*1/5	‡0/5	‡0/4
2	*3/4	—	‡0/4	*4/4	‡0/3	‡0/4
3	*3/4	—	‡0/5	‡0/5	—	—
4	*2/6	—	‡0/5	‡0/5	—	—
5	‡0/6	*	—	—	‡0/4	‡0/3
6	‡0/5	*5/5	—	—	‡0/5	‡0/3

* Successful isolation.
‡ Negative isolation attempt.
—, no test.
Numerator = number of positive (hemagglutinin) embryos.
Denominator = total number of surviving embryos tested.

TABLE III

Allantoic Titrations on a Strain of Egg Passage Influenza B Virus (Strain Sinai 45) before and after Storage at Low Temperature

Dilution of allantoic fluid	Before freezing	Stored in lusteroid tubes at −72°C. for			Stored in sealed glass for 60 days
		3 days	7 days	14 days	
10^{0}				6/6	
10^{-1}				2/6	
10^{-2}		5/6	5/6	1/6	
10^{-3}		5/5	3/6	0/6	
10^{-4}	6/6	4/4	1/6	0/6	6/6
10^{-5}	4/5	2/4	0/6		4/4
10^{-6}	3/6	2/4			4/4
10^{-7}	2/6	2/4			1/4
10^{-8}	0/5				0/4

Numerators indicate the number of embryos with allantoic fluid yielding positive tests for hemagglutinin, and denominators the number of embryos inoculated that survived the incubation period.

Adaptation of Viruses to the Allantoic Sac

When influenza A strains, isolated in the amniotic sac, are subsequently titrated in both the allantoic and amniotic sac it is common for the two titrations to have similar end points, as shown in Table IV. In this laboratory

all A strains tested have adapted rapidly to the allantoic sac in the first passage, even the O form described by Burnet and Bull (7). The B strains of 1945, when detected in amniotic fluids were almost always present in high titer (1:500 or more), as tested by the pattern method. In spite of this, subculture of such fluids in the allantoic sac was difficult and adaptation was very slow. Titrations of successive allantoic passages of strain Sinai 45 gave ir-

TABLE IV

Comparative Amniotic and Allantoic Titrations of an Influenza A and an Influenza B Virus Strain on Primary Isolation and after Passage in Chick Embryos by the Allantoic Route

Dilution of inoculum	Strain 1004 (1943-A)				Strain Sinai (1945-B)							
	Titration of throat washing		Passage 1		Titration of lung suspension		Passage No. 1		6		10	
	Amniotic	Allantoic	Amniotic	Allantoic	Amniotic	Allantoic	Amniotic	Allantoic	Amniotic	Allantoic	Amniotic	Allantoic
10^{0}		4/4										
10^{-1}		1/6			2/3*	0/5				4/4		
10^{-2}		0/6			0/5			5/6		4/4		
10^{-3}	4/4							4/6		3/4		
10^{-4}	4/6							0/6		2/4		3/5
10^{-5}	1/6*		4/4	6/6				1/6		0/4	4/5	3/5
10^{-6}	0/5		4/5	3/6			2/5	0/6	3/4		5/5	0/6
10^{-7}			4/5	1/6			0/6		5/5		5/6	
10^{-8}			0/4	0/6					0/5		2/4	
Approximate amniotic allantoic titer ratio...	10,000		10		—		1000		1000		1000	

Strain 1004 was obtained from throat washings and strain Sinai from a human lung.

* Indicates the amniotic fluids used for second passage, and titration; thereafter allantoic fluid was used for passage.

regular and spotty results, with unpredictable ups and downs, and a large number of passages was required to bring about full allantoic adaptation.

This was further shown by the simultaneous titration of different allantoic passages of strain Sinai 45 in both the amniotic and allantoic sacs (Table IV). With A strains, as previously noted, the amniotic/allantoic titer ratio of 10,000/1 on initial isolation rapidly changed to 10/1 on titration of first passage material, while with B strains the ratio persisted in the neighborhood of 1,000/1 for ten passages. One influenza B strain (Mid-Pac.) was not fully adapted to the allantoic sac after forty passages. Allantoic fluids yielded hemagglutinin titers (pattern method) of 1:8, while amniotic subinoculation at the same passage level yielded amniotic fluids with titers of 1:500 and over.

Attempts to adapt egg-passage strains of influenza A and B virus to mice are described in another paper (8). It is sufficient to point out here that A strains in general adapt readily, while the B strains of 1945–46 could not be maintained in mice without prior ferret passage. And even after such treatment the B strains did not increase much in virulence after many mouse passages.

Isolation of O Forms of Virus from Throat Washings

Burnet and Bull have described what they refer to as the O (original) form of influenza virus, encountered on initial isolation in the chick embryo by the amniotic route (7). The O form is notable primarily for its high capacity for agglutinating guinea pig cells, with a much lower agglutinin titer for chicken cells. This type of virus disappears promptly on further passage in chick embryos unless special precautions are taken to preserve it. It is also characterized by good adaptation to growth in the amniotic sac and poor growth in the allantoic sac. Mice were found to be relatively insusceptible to infection with the O form of virus. Most of the work reported has been in connection with influenza A strains, but in a special study by Burnet *et al.* (9) the form was described in a strain of influenza B virus.

Many of the A strains isolated from the 1940–41 and 1943–44 epidemics showed O characteristics on initial isolation. Numerous attempts were made to maintain some of these strains in the O form by passage *via* the amniotic route in limiting dilutions. All of the techniques described by Burnet *et al.* (9) for maintenance of O strains were carefully followed. Each passage was carried out in a series of dilutions, lung and trachea were dissected out and tested for chick cell and guinea pig cell agglutinins, and only material showing a high guinea pig/chick cell titer ratio was passed. Before passage the chicken cell agglutinins were adsorbed out with chicken cells, and incubation was restricted to 2 or 3 days. In spite of all these precautions it was not found possible to maintain the O form through more than one or two passages without complete reversion to the D (derivative) form. Even in those instances in which the O form was briefly maintained it occurred in only a few embryos out of many and then not always in the dilutions near the end point. The results were further obscured by the tendency of O forms to agglutinate chicken cells in such a manner that the end points were long drawn out, with many consecutive dilutions showing a low grade of pattern formation. This made it difficult to assign reproducible chicken cell titers to a suspension.

Of the twenty-five influenza B strains isolated from the 1945–46 epidemic not one showed the slightest tendency toward the high guinea pig/chicken cell titer ratio on initial passage which is characteristic of the O form. In spite of the absence of this evidence of the O form, these strains had a uniformly high degree of adaptation to growth in the amniotic sac, poor adaptation to the allantoic cavity, and low infectivity for mice. These characteristics have all been described by Burnet and Bull (7) as corollaries of the O phenomenon,

and yet they exist in recently isolated B strains without the primary requisite of the O form, namely a high guinea pig/chicken cell titer ratio.[1]

It is frequently difficult to type freshly isolated strains of influenza virus because of the high degree to which their agglutination is inhibited by normal

TABLE V

Summary of the Differences in Behavior of Influenza Virus Strains from Two Epidemics of Influenza A and One of Influenza B

	Influenza A 1940–41 1943–44	Influenza B 1945–46
Per cent of throat washings yielding virus after amniotic inoculation	74	31
Per cent of washings yielding virus after allantoic inoculation	11	0
Ferret-immune response as indication of presence of virus in throat washing	Not quite as satisfactory as amniotic method	Gave no positive results, except with human lung material
Adaptation of virus to the amniotic sac	Very rapid	Very rapid
Value of repeated amniotic passage for initial isolation of virus	Little value, most strains take on first passage or not at all	44 per cent of strains isolated were first detectable only after two passages
Adaptation of virus to growth in allantoic sac	Very rapid, maximal adaptation on initial passage	Slow, poor, and difficult. Titrations irregular and titer often poor even after repeated allantoic passages
In ovo amniotic/allantoic titer ratios of passage strains	Usually 1/1 or 10/1	Often 1000/1 even after repeated allantoic passage
Mouse adaptation of egg passage virus	Easy, rapid. Maximum titer of virus occurs on initial passage	Difficult, and detectable virus disappears after one or two passages
O forms, guinea pig/chicken cell agglutinin titer ratio	O form fairly common on initial passage	No O forms seen
Stability at −72°C.	Very stable	Rapid loss of titer
Strain differences after embryo isolation	None between strains of same year and very slight between the two outbreaks	No strain differences found

serum. This finding was equally true of both influenza A and B virus. After repeated passage in the allantoic sac this high non-specific inhibition tends to disappear.

[1] Burnet *et al.* (6), investigating an epidemic of influenza B (1945) in Australia, also failed to find O forms.

Some of the differences in behavior of influenza A and B strains are summarized in Table V.

Study of Strain Differences by Agglutination Inhibition

Ever since the original descriptions of differences among strains of influenza A virus (10–12) these antigenic variations have loomed as a potentially important factor in the epidemiology of influenza, in the natural resistance of populations to infections, and in resistance induced by vaccination.

Differences have been described as occurring among strains isolated in the same epidemic. In some instances the differences were great though they defied any very detailed classification into antigenic types. This early work was done with cross-protection tests in mice. More recently it has been demonstrated (4) that the antigenic differences among many of these early strains could be confirmed by cross-tests using the *in vitro* agglutination inhibition technique. After showing that the *in vitro* test was capable of demonstrating antigenic differences, it was found that strains isolated by the amniotic method from the same epidemic (1940–41) showed no detectable antigenic differences even when taken from widely separated points in the epidemic. On the other hand two strains isolated in ferrets and passed in mice did show definite antigenic differences from each other and from the embryo-isolated strains. This suggested that the strain differences first described in 1935 might be the result of ferret and mouse adaptation, a possibility which has actually been shown to occur (8). It therefore seemed profitable to study further the occurrence of strain differences by the *in vitro* technique.

The performance of cross-tests by the *in vitro* method is simple, and the results for each serum are expressed as a ratio of the homologous over the heterologous titer. An empiric correction is applied to the results. This has been fully discussed previously (4) and it is sufficient to emphasize that it serves mainly to render differences between strains reciprocal and to eliminate the paradox of sera which give higher than homologous titers with heterologous strains. The limit of error of the test is about one-half of a twofold difference on a logarithmic scale.

In the first cross-test performed six strains of influenza B were compared.

The strains include the Lee strain (13) isolated in 1940 in ferrets and mice and five strains isolated during 1945 from widely separated geographic areas. An influenza B epidemic occurred in the Territory of Hawaii during the months of June and July, 1945, during which time roughly 10 per cent of the population was affected. A strain (TW39) was isolated from a soldier on Oahu during June, 1945, by members of the virus section of the 18th General Medical Laboratory (14). It was isolated in chick embryos by the amniotic route and was carried through thirty-five passages in the allantoic sac before it was received at this laboratory. Even after this treatment the strain did not show consistently good growth on further allantoic passage. It is referred to here as strain Mid-Pac. Strains NH 1 and NH 2 were isolated from student military personnel stationed at Yale during December, 1945, also by the amniotic

route. Strain Cox was obtained from a patient at the New York Boys Vocational School, in November, 1945, and strain Sinai 45 was obtained from the lung of a patient who died of hemorrhagic tracheobronchitis at Mt. Sinai Hospital, New York City, also during November, 1945. All of the 1945 strains were passed from eight to forty times in the allantoic sac before use. The hemagglutinin titers of two strains (NH 2 and Mid-Pac.) were so low in allantoic fluid that they could not be used for the test, and it was necessary to grow the strains in the amniotic sac to obtain higher titer material.

The serum titers and the results of the cross-tests are shown in Fig. 1. These are very clear cut and show that none of the 1945 B strains studied differed

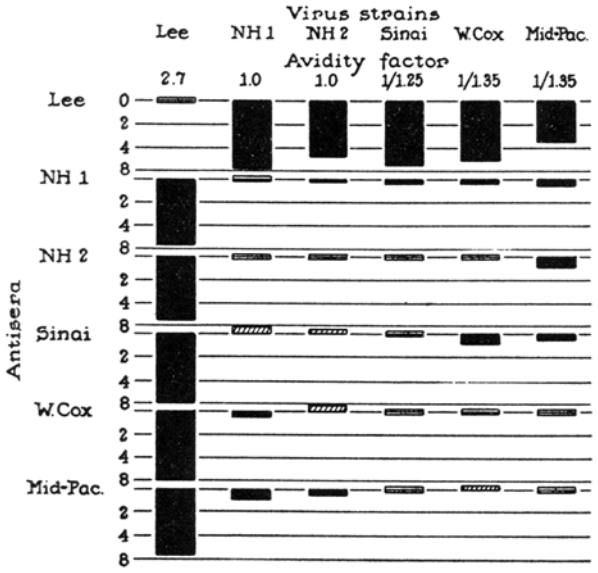

FIG. 1. Cross-tests between strains of influenza B virus. The results of agglutination inhibition tests between various strains of influenza B virus and their respective ferret antisera are expressed for a given serum in terms of the ratio of homologous over heterologous titers. The height of the black bars indicates the degree of difference between strains.

Corrections for avidity factor are included at the top of the figure. This renders the strain differences more nearly reciprocal and may be made necessary by varying degrees of aggregation in virus suspensions. All of the strains were isolated in 1945 except Lee, which was obtained in 1940.

significantly from any of the others, not even the Mid-Pac. strain, which came from a case occurring 6 months earlier than the others. All the 1945 strains show distinct, and within the error of the method, equal differences from the mouse-adapted Lee strain. The corrections applied were small and affected mainly the distribution of differences with the Lee strain. This test with influenza B virus confirms the result previously obtained with influenza A strains of 1940–41 in that there appears to be very little strain variation evident in virus from widely separated sources.

In Fig. 2 are shown the results of a similar test with strains from four different states, obtained during the influenza A epidemic of 1943–44. While

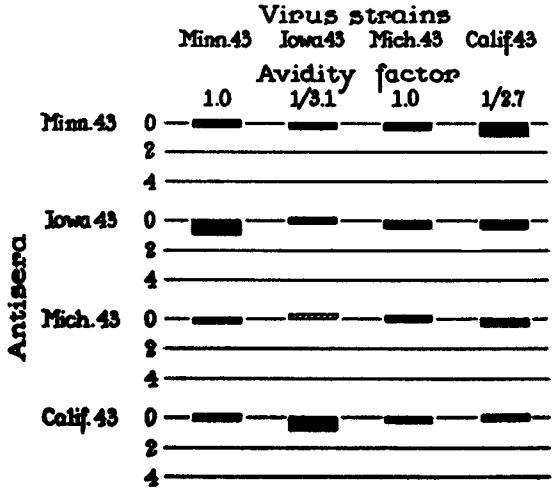

FIG. 2. Cross-tests between influenza A strains of 1943–44. The strains tested were all obtained from the influenza epidemic of 1943–44 and were isolated initially in chick embryos.

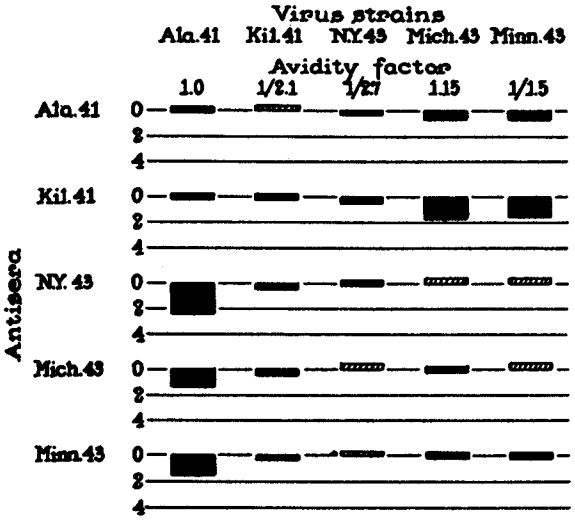

FIG. 3. Cross-tests of influenza virus strains from two epidemics. A comparison of strains of influenza A virus isolated in the epidemics of 1940–41 and 1943–44.

the corrections are large, no essential or significant differences were found between strains obtained from these four areas. All were egg-isolated strains, primarily obtained in the allantoic sac.

A cross-test was also performed between A strains of 1940–41 and of 1943–44, the latter including two strains shown in Fig. 2 and an additional one from New York (Fig. 3). The 1940–41 strains were both obtained from different places in Alabama. The similarity of strains isolated in the same year is striking, while there seems to be some slight change in antigenic pattern between the 2 years. This latter antigenic difference is small and in many crosses it is not significant in degree, but it is so consistently present in all the 1940–41 *vs.* 1943–44 cross-tests that it probably represents a small but real change.

DISCUSSION

The work here described further underlines the advantages of chick embryos over ferrets for isolation of influenza virus even from the standpoint of sensitivity in virus detection. More marked, however, is the demonstration of the advantage of inoculation by the amniotic route over allantoic inoculation, especially in the case of influenza B virus, which in this laboratory was successfully isolated only by the amniotic route. Success of virus isolation is heightened when washings are directly inoculated into eggs shortly after they are obtained. If freezing is necessary, it seems better that washings be stored in glass ampules rather than in lusteroid tubes. The difficulty of maintaining some B strains in the allantoic sac is evident, and material for serological work can sometimes be more advantageously obtained by amniotic passage.

From the epidemiological standpoint the occasional occurrence of influenza patients with exceptionally high virus titers in their throat secretions is of interest. While the exact dilution of the throat secretions in the gargling mixture is unknown, a tenfold dilution would be a rough estimate. This would place the minimum number of infectious particles in throat secretions in one instance in the neighborhood of $10^{7.5}$ per cc. It may well be that individuals with such concentrations of virus may be the ones mainly responsible for disseminating the disease. It is also possible that the occurrence of such high levels is more common than is indicated and that the peak level is maintained for only a short time.

The O phenomenon described by Burnet and Bull (7) has been confirmed in regard to its occurrence on initial isolation, but it has not been found possible to maintain this form on passage. Strains may differ in their tendency to shift to the D form, and it may be that those with a strong tendency to do so cannot be maintained in the O state. At present the importance of the O phenomenon seems somewhat obscure. The O-D shift apparently does not represent a loss of any potentialities but mainly a gain in chicken cell hemaglutinins. The difficulty of maintaining the virus in the O form hampers detailed study very greatly.

At the risk of some repetition it might be well here to restate the present position in regard to strain differences within the influenza A and B groups:

(1) Antigenic differences have been found between strains of the A, B, and swine types of influenza virus; in some instances the differences have been trivial but in others large, even between strains occurring in the same epidemic. (2) In every instance in which differences have been described they have occurred in strains which have been adapted to mice. (The cross-tests have been carried out by cross-neutralization in mice.) (3) These differences have been confirmed by cross-tests by the agglutination inhibition method, showing this test to be capable of detecting small changes in antigenic pattern. (4) With the use of this *in vitro* technique of comparison it has been shown that during the process of mouse adaptation the antigenic pattern shifts; and the shift in structure is not always in the same direction, so that two similar strains may become quite different after passage in mice. This observation alone would make the significance of the initial observations on differences questionable as regards their meaning for human strains. (5) Adaptation of human virus to the egg is apparently a less drastic procedure than mouse adaptation, and when egg-adapted viruses have been used for comparison of strains from epidemics, no significant antigenic differences have been revealed. This was the case in the influenza A epidemics of 1940–41 and 1943–44 and in the influenza B epidemic of 1945–46. Even a comparison between the 1940–41 and 1943–44 strains revealed only slight differences.

These observations uniformly point to the fact that strain differences in a given epidemic are negligible. At first glance this would seem to be unlikely when one realizes the enormous number of human passages some of these similar strains must have been subjected to, and especially in view of their antigenic lability with relatively few mouse passages (9). If this view of antigenic similarity is accepted it must mean that both the A and B influenza viruses have achieved considerable antigenic stability. An analogous finding was described by Magill and Francis, who observed little difference between the thirtieth and the two hundred eighty-fifth passage of the PR8 strain of influenza A virus in mice, indicating that antigenic stability was achieved before the thirtieth passage.

These findings also have a practical bearing on influenza prophylaxis by subcutaneous vaccination. The current preparation of vaccine authorized for distribution, and widely tested in the field, contains only mouse-adapted strains of virus. Among these strains is PR8, which has been most widely studied and differs from the egg-adapted A strains by a two- to fourfold margin. The second A strain in the vaccine is Weiss, on which no antigenic studies have been published; and finally there is the Lee strain of influenza B virus, which differs considerably from the 1945–46 type. It would seem logical, in preparing vaccines, to substitute for these strains others which are as similar as possible to strains ordinarily current during epidemics in man. Experience in this laboratory suggests that egg-adapted strains would be a more logical

choice, providing they proved to be satisfactory in other ways, such as yielding high titer in eggs and good antigenic response in man. From the wide selection of such strains now available it should not be difficult to obtain a number which have the desired characteristics.

SUMMARY

Some of the peculiarities of strains of influenza A and B virus from two epidemics have been described. The influenza B virus of 1945–46, when compared with influenza A virus, proved to be much more difficult to isolate from human sources by any known means. Its adaptation to the chick embryo (by any route) or to mice was much slower than that of A virus. It did not keep nearly as well on storage at −72°C. either in throat garglings or as passage material. Its adaptation to amniotic growth was usually much better than to allantoic growth even after repeated allantoic passages. It failed to show primary evidence of occurring in the O form, although many of the secondary O characteristics were present and persisted. Its titer in throat washings was not demonstrably high as compared with certain strains of A virus, which were demonstrated in garglings at dilutions of 10^{-5} and 10^{-6}

The antigenic patterns of influenza A strains from two epidemics were compared. No antigenic differences of significant degree were found among the strains of either epidemic and the difference between the strains of the two epidemics was very slight. A similar study was made of the influenza B strains of the epidemic of 1945–46. This also showed complete lack of significant strain differences. The implications of these findings for influenza prophylaxis are discussed.

Addendum.—More recent experience with the influenza strains isolated from the A epidemic of early 1947 in the United States indicates that these strains show considerable antigenic differences from examples of the earlier outbreaks. The 1947 strains also differ from the earlier ones in that they are much more difficult to adapt to the allantoic sac and are readily neutralized in *in ovo* tests by normal ferret and normal rabbit sera. The occurrence of strain differences within the epidemic is being investigated.

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