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INFLUENZA A VIRUSES
Laboratory Studies, with Special Reference to European Outbreak of 1950-1

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Influenza A was widespread in Europe between October 1950 and February 1951. A general account of the epidemic has been published by Freyche & Klimt,[11] and two of the present authors (Isaacs & Andrewes [15]) have briefly described the epidemiological and laboratory findings from this epidemic. The tentative conclusion was drawn that the epidemic had a twofold origin : (*a*) from the activation of latent influenza in Scandinavia ; and (*b*) from virus spreading to Europe from the southern hemisphere. In this paper fuller details will be given of the laboratory findings, and they will be further discussed in relation to the epidemiology of this visitation. The viruses characteristic of the Scandinavian focus are referred to as Scandinavian (S) subtype; those believed to have spread from the south, and to have notably attacked Liverpool, as Liverpool (L) subtype.

Materials and Methods

Viruses. Virus strains were received by the World Influenza Centre (WIC) from many WHO influenza laboratories and from elsewhere ; some arrived in the dried state, others packed in thermos containers in dry ice.

On receipt in the laboratory, a virus strain was passaged allantoically and the first passage material stored at −70°C and used as seed virus. Where strains were investigated in greater detail, the virus was stored at −70°C in capillary-tubes which could be used singly and then discarded. Under these conditions the infectivity titre of a virus remained unaltered over long periods. Passage of virus strains was avoided where possible ; when it was carried out, high dilutions of seed were used and the harvested

fluids checked serologically. All egg-inoculations were carried out in a room where no other work was in progress and every attempt was made to minimize the risk of laboratory contamination of strains. Strains were designated as previously described (Chu, Andrewes & Gledhill [7]), e.g., A/London/1/51, except where a strain was sent to us already named, when the laboratory designation was kept in order to avoid confusion.

Sera. Sera were prepared from ferrets bled 12 days after intranasal infection with 1 ml of a 10^{-2} dilution of freshly harvested allantoic fluid. The animals were kept under strict isolation. To each serum 1/10,000 merthiolate was added, and the sera were then stored at 2°C.

Red cells. Fowls were bled from the wing vein and the blood collected in citrate. The cells were washed thrice in saline, stored as packed cells at 4°C, and used for two to three days. A 0.5% suspension was prepared each day from freshly washed cells and standardized in a photo-electric absorptiometer.

Receptor-destroying enzyme. The receptor-destroying enzyme (RDE) was prepared from the 4Z strain of *Vibrio cholerae* by the method of Burnet & Stone.[5] Crude agar filtrates were partially purified by adsorption of the enzyme on to 5% fowl cells for 1 minute at 2°C and elution into saline for 30 minutes at 37°C. This RDE preparation was stored at 2°C, and maintained its titre over a prolonged period.

Inoculation of chick embryos. Primary isolation of virus was carried out by inoculation of garglings into the amniotic cavity of 13-day-old chick embryos (Beveridge & Burnet [1]). Penicillin (200 units per egg) and streptomycin (20 μg per egg) were added to prevent bacterial growth. The amniotic fluids were harvested after five days' incubation at 35°C. Further passages were carried out by allantoic inoculation of 10-day-old embryos which were then incubated for two to three days at 35°C.

Mouse inoculation and passages. Mice were inoculated intranasally with 0.05-ml volumes of fluid under light ether anaesthesia. For passage, the lungs of each mouse were harvested separately, ground individually in Tenbroeck tissue-grinders with a small amount of powdered glass, and suspended in 2 ml of broth-saline. These suspensions were either used for further mouse passage or were mixed with penicillin and streptomycin before allantoic inoculation of chick embryos.

Haemagglutination titrations. Titrations were carried out in plastic plates (Salk [20]). Serial twofold dilutions (0.25 ml) of virus were prepared in normal saline and an equal volume of 0.5% fowl cells added. Readings were made by the pattern method and the end-point taken as partial (50%) agglutination. The 50% end-point is determined by observing the pattern

made by a 1 in 2 dilution of virus showing just complete agglutination. Where partial (50%) agglutination did not show in any tube, the end-point was determined by interpolation. Titres are expressed as the initial dilution of virus present at the end-point, and the amount of virus present at the end-point is defined as one agglutinating dose.

Antihaemagglutinin test. Ferret antisera were treated with red-cell eluates of *V. cholerae* filtrates containing RDE, as described by Isaacs & Bozzo,[16] in order to remove non-specific inhibitors of haemagglutination. RDE treatment was unnecessary for fowl or mouse antisera, which were heated at 56°C for 30 and 60 minutes respectively.

In routine antihaemagglutinin tests, serial twofold dilutions of serum were made in saline. Since several strains and sera were usually tested at one time, the serum dilutions were made up in bulk and distributed in 0.25-ml volumes in plastic plates. 0.25-ml volumes of 0.5% fowl cells were then added, followed immediately by an equal volume of virus containing eight agglutinating doses. The virus suspension was usually made up before the actual tests, titrated to see if the dilution was accurate, and adjusted in strength if necessary. A second control of the number of agglutinating doses in each virus suspension was carried out during the test proper. Where errors in the number of agglutinating doses were found, the final antihaemagglutinin titres were adjusted accordingly. Readings were made by the pattern method, partial agglutination being taken as the end-point and interpolation being made where necessary. The titres of the sera as shown in the tables refer to the reciprocals of the initial dilution of serum present at the end-point.

When carried out in this way the test showed a high degree of reproducibility, and the effects of small variations in experimental conditions, e.g., susceptibility of red cells, room temperature, were eliminated. Replicate titrations of a single serum against 48 separate antigen dilutions of homologous virus showed that a twofold difference in antihaemagglutinin titre between two titrations was unlikely to occur as a result of technical error more than once in a hundred titrations. (A normal distribution of the logarithms of antihaemagglutinin titres occurring as a result of random technical errors was assumed.)

Complement-fixation tests. Complement-fixation tests of the virus particle antigen were carried out on perspex sheets by the method of Fulton & Dumbell.[12] For tests of soluble antigen, a modification of Hoyle's [14] method was carried out with an extract of infected chorio-allantoic membrane as the source of antigen.

Egg-neutralization tests. Sera were inactivated by heating at 56°C for 30 minutes and then diluted serially in 50% broth-saline. To each tube an equal volume of virus, diluted in broth-saline and containing

100 50%-infective doses (ID_{50}) per 0.05 ml, was added. The mixtures were kept at 4°C for 15 minutes, and then 0.05-ml volumes were inoculated allantoically into groups of from four to six 10-day-old chick embryos. After incubation for three days at 35°C the allantoic fluids were harvested and tested for agglutinins. 50% end-points were calculated by the method of Reed & Muench [19] and the titres in the tables are expressed as the reciprocals of the initial dilution of serum present at the end-point.

Limiting infective dilution. In studies on the variation in behaviour of strains grown in the allantoic cavity in the presence of antiserum or on passage in mice, it was necessary to use " pure clones " of virus. These were obtained by passage of virus allantoically in eggs at a dilution where the vast majority of eggs (usually 80%-90%) showed no growth of virus. The purity of the resulting clones was tested (" plating out ") by a further passage at a dilution where 50%-75% of eggs would show no growth of virus, and the positive fluids were then harvested and tested individually.

Passage of virus in eggs with serum. Virus was diluted 10^{-2} and a preliminary neutralization test with homologous ferret antiserum was carried out in eggs. The amount of serum present at the 50% end-point is referred to as one neutralizing dose. In the test proper, virus was diluted 10^{-2}, mixed with 0.5-0.75 neutralizing doses of serum, and then inoculated into the allantoic cavity of groups of one to two dozen eggs. The eggs were incubated at 35°C and re-inoculated at 18, 24, and 42 hours with 4, 16, and 32 times the amount of antiserum used initially. After a total of three days' incubation at 35°C, the fluids were harvested and tested individually for their serological behaviour.

General Characters of 1950-1 A-prime Viruses

The general characters of the 1950-1 viruses were similar to those of the A-prime viruses isolated between 1946 and 1949. The three viruses isolated in the National Institute for Medical Research were in the O-phase (Burnet & Bull [3]) on first passage in the amniotic cavity of 13-day-old chick embryos, and were readily adapted to the allantoic cavity by passage at low dilution. Similar results were found by Dr. B. P. Marmion, Colindale, London, and Svedmyr, von Magnus & Freundt [21] with the strains which they isolated.

Mouse pathogenicity. Twenty-six strains selected at random were tested for their mouse pathogenicity by inoculation of freshly harvested allantoic fluid intranasally into lightly anaesthetized mice. Some strains produced slight lesions when inoculated undiluted into mice, presumably the result of a toxic action of virus in high concentration. No strains produced lesions when inoculated at 10^{-2} dilution.

Ferret pathogenicity. Antiserum was prepared against 17 strains by intranasal instillation of 1 ml of 10^{-2} dilution of freshly harvested allantoic fluid into lightly anaesthetized ferrets. Strict isolation of the ferrets was maintained throughout. In most cases a moderate temperature response —104°F (40°C)—occurred on the second day, together with the usual symptoms of respiratory catarrh. In some a diphasic temperature-curve was found.

Electron microscopy. Our colleagues, the late Dr. W. J. Elford and Dr. C. E. Challice, kindly carried out an electron microscopic examination of 17 strains selected at random. Virus from freshly harvested allantoic fluid was adsorbed on to red-cell " ghosts " by the method described by Dawson & Elford.[9] All strains showed numerous filamentous forms along with elementary bodies, a picture similar to that found with 1949 A-prime viruses.

Preliminary serological tests. A small number of strains was examined by complement-fixation tests of the soluble antigen. " A " antigen was found present to the expected titre.

Haemagglutination-inhibition tests with ferret antisera to strains of the four main serological types of influenza A virus were carried out against the first 20 strains received at the World Influenza Centre from the outbreak in Europe. Representative results are shown in table I.

TABLE I. HAEMAGGLUTINATION-INHIBITION TESTS WITH STOCK A ANTISERA AND REPRESENTATIVE 1950-1 A-PRIME VIRUSES

Virus	Serum			
	Swine 15	WS	PR8	FM1
Swine 15	1280	< 10	< 10	< 10
WS	< 10	960	60	20
PR8	< 10	70	5120	35
FM1	< 10	< 10	< 10	1280
A/Swe/3/50	< 10	< 10	< 10	60
A/Ned/3/51	< 10	15	< 10	240
A/Mad/2/51	< 10	< 10	< 10	160
A/Belfast/2/51	< 10	< 10	< 10	160

All the freshly isolated strains were most closely related to FM1 virus and are therefore A-prime viruses. In only a few cases were weak cross-reactions with WS and PR8 antisera found.

Cross-haemagglutination-inhibition tests with antisera to 1950-1 viruses. Table II shows the results of an experiment in which 9 viruses were tested in haemagglutination-inhibition tests with ferret antisera to 5 of the 1950-1 strains.

TABLE II. HAEMAGGLUTINATION-INHIBITION TESTS WITH REPRESENTATIVE 1950-1 A-PRIME VIRUSES AND SERA

Virus	Subtype *	Serum				
		A/Eng/1/51	A/Mad/3/51	A/Swe/3/50	A/Dan/2/50	A/Dan/3/50
A/Eng/1/51	L	640	1280	25	60	120
A/Mad/3/51	L	320	640	12	30	60
A/Swe/3/50	S	10	10	30	20	35
A/Dan/2/50	S	40	20	100	160	200
A/Dan/3/50	S	80	200	80	160	200
A/Swe/1/51	S	20	20	10	50	40
A/Ned/3/51	S	60	100	20	60	60
A/Naples/1/51	S	15	10	30	70	90
A/Lon/3/51	L	640	1280	60	240	320

* L = Liverpool subtype ; S = Scandinavian subtype

As already mentioned, two main serological subtypes of virus can be recognized. The first, the " Scandinavian ", or S, subtype is represented by A/Sweden/3/50 virus. The second, the " Liverpool ", or L, subtype is represented by A/England/1/51 (Liverpool) virus. The L strains were indistinguishable from one another serologically whereas the S strains were much less homogeneous.

All the strains received from WHO influenza laboratories were tested in haemagglutination-inhibition tests with typical S and L antisera. The subtype of each strain, as indicated by this test, is shown in Annex 1 (see page 309). Ninety-six strains were A-primes and 15 were influenza B viruses. Of the A-primes, 47 were of the Liverpool (L) subtype, 46 were classified as of the Scandinavian (S) subtype, and 3 showed intermediate properties.[a]

Strains A/Sweden/3/50 (S) and A/England/1/51 (L) differ in at least two respects. First, the strains differ antigenically, and, secondly, they differ in the amount of antibody response which they produce following intranasal inoculation of ferrets. In order to be sure that the poor antibody response to A/Sweden/3/50 virus was not due to the unusual behaviour of an individual animal, four ferret antisera to this virus were prepared on

[a] Since this paper was submitted for publication, seven strains of virus isolated in Tokyo between December 1950 and February 1951 were received from Dr. Kono. All these strains were A-primes ; six were of the Liverpool subtype and one has not yet been fully identified.

TABLE III. HAEMAGGLUTINATION-INHIBITION TESTS WITH 4 DIFFERENT FERRET ANTISERA TO A/SWE/3/50 VIRUS

Virus	Sub-type *	A/Swe/3/50 serum			
		No. 1	No. 2	No. 3	No. 4
A/Swe/1/50	S	80	40	80	70
A/Swe/2/50	S	15	< 10	20	20
A/Swe/3/50	S	40	40	70	70
A/Swe/4/50	S	40	50	70	70
A/Eng/1/51	L	30	30	30	20
A/Mad/3/51	L	30	30	35	20
A/Dan/2/50	S	100	140	140	100
A/Naples/1/51	S	120	120	160	60

* L = Liverpool subtype ; S = Scandinavian subtype

different occasions. The results of haemagglutination-inhibition tests with these sera are shown in table III.

The uniformity in the behaviour of the four antisera makes it clear that the poor antibody response is due to a peculiarity of the virus.

Relationship of 1950-1 to 1947-9 A-prime viruses

Table IV shows the results of cross-haemagglutination-inhibition tests with current strains and previously isolated (1947 and 1949) A-prime viruses. Both S and L viruses were distinguishable from, although closely related to, the 1947 and 1949 A-primes.

TABLE IV. HAEMAGGLUTINATION-INHIBITION TESTS WITH 1947-51 A-PRIME VIRUSES AND ANTISERA

Virus	Year	Serum						
		FM1	Barratt	A/Paris/ 1/49	A/Ned/ 1/49	A/Swe/ 3/50	A/Eire/ 1/51	A/Eng/ 1/51
FM1	1947	1280	640	640	640	40	160	560
Barratt	1947	960	480	480	480	30	120	480
A/Paris/1/49	1949	70	70	80	100	10	15	80
A/Ned/1/49	1949	80	120	80	200	10	15	80
A/Swe/3/50	1950(S)*	30	20	< 10	30	20	15	20
A/Eire/1/51	1951(S)	100	40	15	60	20	80	40
A/Eng/1/51	1951(L)*	160	200	70	140	20	20	800

* L = Liverpool subtype ; S = Scandinavian subtype

However, nine South African strains and one Australian (1950) strain isolated from influenza outbreaks which preceded the European outbreak by six months were of the L subtype. Three South African strains and one from Australia isolated at the same time were S. Three Indian strains isolated in August 1950 were of the L subtype. One virus isolated from Ocean Island, in the South Pacific, in October 1950 was L. Table V shows the relationship between A/Johannesburg/7/50 (L), A/Cape Town/1/50 (S), and representative 1947-51 A-prime viruses and antisera. A/Cape Town/1/50 (S) closely resembles A/London/1/49, isolated 18 months earlier, whereas A/Johannesburg/7/50 is of the Liverpool subtype.

TABLE V. HAEMAGGLUTINATION-INHIBITION TESTS WITH SOUTH AFRICAN 1950 AND 1947-51 A-PRIME VIRUSES AND SERA

Virus *	Serum					
	Mercer	A/Lon/ 1/49	A/Swe/ 3/50	A/Cape Town/ 1/50	A/Jo'burg/ 7/50	A/Lon/ 2/51
Mercer (1947)	160	320	30	280	40	80
A/Lon/1/49	10	70	< 10	40	10	10
A/Swe/3/50 (S)	< 10	< 10	40	15	< 10	10
A/Cape Town/1/50 (S)	15	60	< 10	30	< 10	15
A/Jo'burg/7/50 (L)	80	200	40	240	2560	1280
A/Lon/2/51 (L)	30	30	15	40	640	640

* L = Liverpool subtype ; S = Scandinavian subtype

Serum neutralization tests in ovo

Typical S and L subtype strains were compared by egg neutralization tests with ferret antisera. Table VI shows the results of one such experiment; for comparison, the haemagglutination-inhibition titres with the same sera and viruses are shown alongside. The greater reactivity of the L subtype virus with homologous antiserum is evident in both tests.

TABLE VI. EGG-NEUTRALIZATION AND HAEMAGGLUTINATION-INHIBITION TITRES OF A/SWE/3/50 AND A/MAD/3/51 SERA AND VIRUSES

Virus	Subtype *	Egg-neutralization test †		Haemagglutination-inhibition test	
		A/Swe/3/50 serum	A/Mad/3/51 serum	A/Swe/3/50 serum	A/Mad/3/51 serum
A/Swe/3/50	S	18	13	30	10
A/Mad/3/51	L	60	200	12	640

* L = Liverpool subtype ; S = Scandinavian subtype

† The titres under the egg-neutralization test show the dilution of serum at the 50% neutralization end-point.

Strain-specific complement-fixation tests

Strain-specific complement-fixation tests were carried out according to the method of Fulton & Dumbell [12] with four viruses—FM1 (1947), A/Paris/1/49, A/Sweden/3/50 (S), and A/Belfast/1/51 (L)—and the corresponding mouse antisera. The results, shown in table VII, suggest less antigenic difference between the four strains than was found in haemagglutination-inhibition tests (see Chu et al.[7]).

TABLE VII. RELATIONSHIP BETWEEN FOUR A-PRIME STRAINS (1947-51) AS SHOWN BY STRAIN-SPECIFIC COMPLEMENT-FIXATION TESTS

	FM1	A/Paris/1/49	A/Swe/3/50	A/Belfast/1/51
FM1	1.0	0.83	0.56	0.45
A/Paris/1/49		1.0	0.88	0.62
A/Swe/3/50 (S) *			1.0	0.62
A/Belfast/1/51 (L) *				1.0

* L = Liverpool subtype ; S = Scandinavian subtype

Complement-fixation tests were carried out by the method of Fulton & Dumbell.[12] The figures express the extent of relationship between two strains. 1.0 indicates complete serological identity between two strains ; the smaller the fraction, the more distant is the serological relationship.

Such a difference might be due to the use of mouse antisera ; as is shown later, different animal sera give a different picture from ferret antisera of the serological relationships between the A-prime strains. Alternatively, this result might suggest that the two tests do not measure the same virus antigens. In this connexion it is interesting to note that an eluate of A/Paris/1/49 virus, which had been stored at 2°C for nearly two years, showed a pronounced drop in complement-fixing titre without any corresponding drop in haemagglutinin titre. Thus the ratio between the complement-fixing and haemagglutinin titres of this preparation and that of a freshly prepared eluate of the same strain differed more than eightfold (see table VIII).

TABLE VIII. RATIO BETWEEN VIRUS COMPLEMENT-FIXING AND HAEMAGGLUTININ TITRES OF AN OLD AND A FRESH RED-CELL ELUATE OF A/PARIS/1/49

Preparation of A/Paris/1/49	Complement-fixing titre *	Haemagglutinin titre	$\frac{HA}{CF}$ ratio
Stored at 2°C for 2 years	< 1:1	10,240	> 10,240
Freshly prepared	1:2	2,560	1,280

* The complement-fixing titre is expressed as the dilution of the preparation which gave specific fixation of 1 mm^3 of complement when titrated with excess homologous antiserum.

Antihaemagglutinin tests with A/Paris/1/49 ferret antiserum gave the same titre with both preparations of virus. This result emphasizes the necessity (Fulton & Dumbell [12]) of storing antigens required for strain-specific complement-fixation tests at −70°C rather than at 2°C.

Other tests

In an attempt to find methods, other than serological, which would differentiate the S and L viruses, tests were carried out of the fowl red-cell receptor gradient (Burnet, McCrea & Stone [4]) and of the heat stability of the virus haemagglutinin. No differences were found between viruses of the two subtypes. Similarly, no differences were noted between S and L viruses in tests of mouse pathogenicity or on electron microscopic examination.

Antigenicity of S and L subtype strains

The differences found between strains which were highly reactive and those which were poorly reactive in haemagglutination-inhibition tests appeared to be due partly to differences in combining power of virus with antibody and partly to differences in antigenicity in ferrets. Tests of the antigenicity of the two subtypes following parenteral injection in mice were therefore carried out. Mice were bled 14 days after a single intraperitoneal inoculation of 1,280 agglutinating doses of virus, and their sera were titrated individually for their antihaemagglutinin content. The results of an experiment are shown in table IX.

TABLE IX. ANTIHAEMAGGLUTININ TESTS WITH SERA FROM MICE IMMUNIZED WITH A/SWE/3/50 AND A/MAD/3/51

Virus *	Number of mice	Sera from mice immunized with	
		A/Swe/3/50	A/Mad/3/51
A/Swe/3/50 (S)	16	12	12
A/Mad/3/51 (L)	18	14	32

* L = Liverpool subtype ; S = Scandinavian subtype
The table shows the geometric-mean agglutinin titres of individual mouse sera.

The results show that A/Madrid/3/51 (L) is a significantly better antigen ($p < 0.001$) in mice than A/Sweden/3/50 (S). However, there was very little antigenic difference between the two viruses in tests with these antisera. A similar experiment in which mice were immunized with 3,840 agglutinating doses of A/Cape Town/1/50 (S) and A/Johannesburg/7/50 (L) viruses showed that the latter was a better antigen.

Serology of L and S viruses with immune fowl and ferret sera

The difference between the serological results obtained with convalescent ferret sera and immune mouse sera in tests with L and S subtype strains suggested an investigation of sera from other species.

Adult fowls were inoculated intravenously with 7,680 agglutinating doses of A/Sweden/3/50 (S) and A/Madrid/3/51 (L) viruses ; two birds were inoculated with each virus. The resulting antisera did not differentiate the two viruses in cross-serological tests. Furthermore, fowl antiserum to A/Madrid/3/51 (L) did not differentiate A/Johannesburg/7/50 (L) and A/Johannesburg/7/50 variant viruses (see table XVII, page 305) ; this suggests that the A/Madrid/3/51 fowl serum does not contain antibody to the L antigen. Hilleman [13] found that he could not detect serological differences between L and S subtype viruses by rooster antisera.

In order to see whether the differences between ferret and fowl antisera were due to the different species or to the route of inoculation, two ferrets were immunized by intraperitoneal inoculation of 3,840 agglutinating doses of A/Sweden/3/50 (S) and A/Madrid/3/51 (L) viruses. Table X shows cross-serological tests with these viruses using ferret " immune " and ferret " convalescent " sera.

TABLE X. HAEMAGGLUTINATION-INHIBITION TESTS WITH " IMMUNE " AND " CONVALESCENT " FERRET ANTISERA TO A/SWE/3/50 AND A/MAD/3/51 VIRUSES

Virus *	Serum			
	immunized ferret		convalescent ferret	
	A/Swe/3/50	A/Mad/3/51	A/Swe/3/50	A/Mad/3/51
A/Swe/3/50 (S)	< 10	10	80	40
A/Mad/3/51 (L)	< 10	40	20	960

* L = Liverpool subtype ; S = Scandinavian subtype

The result of tests with " immune " ferret sera is similar to that obtained with " immune " mouse sera (table IX). It is clear that the use of animal sera from different species may give quite different results and it is hoped that this matter may be elucidated as a result of a detailed investigation now in progress at the World Influenza Centre. Meanwhile, it is interesting to note that sera from ferrets convalescent after intranasal infection with virus are the most specific in these tests ; this suggests the possibility that the production of high-titred antihaemagglutinin to the L subtype viruses is a response to a multiplying virus. This might also indicate that the use of convalescent ferret antisera was preferable in studies of the antigenic characters of A-prime viruses.

P-Q Variation

Van der Veen & Mulder [23] have reported that influenza strains may show three types of behaviour in reacting with antisera, as shown in table XI;

TABLE XI. DIAGRAMMATIC REPRESENTATION OF RESULTS OF HAEMAGGLUTINATION-INHIBITION TESTS BETWEEN P, Q, AND R VIRUSES AND ANTISERA

Virus	Serum		
	P	Q	R
P	+++	+	+
Q	+	+	+
R	+++	+++	+++

From van der Veen & Mulder [23]

they have described strains on this basis as P, Q, or R. P strains have their haemagglutinating activity inhibited to high titre by homologous sera only ; Q strains are inhibited to low titre by all sera including homologous ones; R strains are inhibited to high titre by homologous and also by other related sera. As will appear later, it may be possible to convert a Q strain into a P, and conversely, and it seems that a reversible phase-variation may be involved, this being a phenomenon distinct from the antigenic differences under discussion. Nevertheless, this P-Q variation enters into the story. The facts suggest that nearly all the Scandinavian-type viruses are in the Q phase, only a few being " P " and two " R ". On the other hand, the Liverpool viruses are all P (highly reactive specific strains).

It should be emphasized that antigenic differences between P strains are more easily recognized than between Q strains, which give low titres with all antisera. Thus, Scandinavian P strains were readily distinguished from Liverpool P strains. On the other hand, Q strains isolated from different parts of the world were hard to distinguish from one another. Since we have not found any naturally occurring L strains which were clearly in the Q phase, the provisional labelling of the S strains served to indicate the serological behaviour rather than the epidemiological source of a virus.

Behaviour of Q viruses in antihaemagglutination tests

Examples will be found in tables II, III, IV, and V of antisera to Q viruses which inhibit heterologous viruses to higher titres than they do the parent strain : attempts were made to find an explanation for this.

Tests of homogeneity. One possible explanation which was considered was that Q strains were inhomogeneous. A mixture of two or more viruses might not be easily neutralized in haemagglutination-inhibition tests but yet give rise to antihaemagglutinin active against heterologous viruses. Tests of this hypothesis were made by " plating out " virus cultures by passage at limiting infective dilution. Under these conditions there is a high probability that many of the cultures will have originated from single virus particles. Strain A/Sweden/3/50, a Scandinavian Q virus, was passed at limiting dilution; at $10^{-7.5}$ dilution, 4 out of 12 inoculated eggs were positive, and at 10^{-8} dilution, 2 out of 12 eggs were positive. All six positive fluids behaved in the same way as the original virus in antihaemagglutinin tests. This is evidence against any gross inhomogeneity in the culture. Inhomogeneity has been found in three of the 1950-1 virus strains (see below), but does not apparently explain the low antihaemagglutinin titres with Q viruses. Further evidence against the hypothesis of inhomogeneity comes from the fact that haemagglutination-inhibition tests with A/Sweden/3/50 (S) virus and mixed antisera against a number of A-prime strains did not show a high inhibition titre. Controls showed that a mixture of equal parts of FM1 (1947) and A/Madrid/3/51 (L) viruses was inhibited to higher titre by a mixture of the two corresponding antisera than by either serum alone.

Further tests have shown that when A/Sweden/3/50 virus was deposited by high-speed centrifugation, the virus in the deposit and in the supernatant (10% of the original) reacted alike in the antihaemagglutinin test. The same applied to virus which was adsorbed on to red cells and eluted into saline and to the unabsorbed virus in the supernatant. When A/Sweden/3/50 (previously passaged once amniotically and three times allantoically) was passaged twice in the amniotic cavity, six times in the allantoic cavity at high dilution (10^{-7}), or twelve times in the allantoic cavity at low dilution (10^{0}), no alterations in antigenic behaviour were found.

Use of different red cells. The use of guinea-pig, human, or pigeon cells in place of fowl cells led to higher haemagglutination-inhibition titres in antibody tests with Q strains but not with P strains. The results of a test of this type are shown in table XII.

Table XII shows that A/Sweden/3/50 virus gives higher haemagglutinin-inhibition titres with homologous and heterologous antiserum in tests with guinea-pig cells ; A/England/1/51 behaves in the same way in both tests. Thus the antigenic ratio between the two viruses is not altered by the use of guinea-pig cells. This result could not be explained by any difference in the haemagglutinin titre of Q viruses with the two types of cell, nor to demonstrable agglutinins for only one type of cell. Absorption of A/Sweden/3/50 virus by either fowl or guinea-pig cells removed agglutinin for both types of cell.

TABLE XII. HAEMAGGLUTINATION-INHIBITION TESTS WITH FOWL AND GUINEA-PIG CELLS

Virus *	Cells	Serum	
		A/Swe/3/50	A/Eng/1/51
A/Swe/3/50 (S)	fowl	30	10
A/Eng/1/51 (L)	fowl	25	640
A/Swe/3/50	guinea-pig	120	60
A/Eng/1/51	guinea-pig	20	960

* L = Liverpool subtype ; S = Scandinavian subtype

No explanation has been found for the higher titres obtained with human, guinea-pig, and pigeon cells. These are the cells which are agglutinated by influenza virus in the O-phase (Burnet & Bull [3]), but no connexion between the two phenomena is apparent. There is no evidence that Q viruses have a higher affinity than P viruses for fowl cells. The fowl red-cell receptor gradient, which is taken as a measure of the affinity of different virus strains for red cells, showed no significant differences between P and Q viruses. An experiment in which equal amounts of A/Sweden/3/50 and A/Madrid/3/51 viruses were absorbed with varying amounts of fowl red cells showed no preferential absorption of A/Sweden/3/50 virus.

Inhibitors in normal sera. Table XIII shows some results of haemagglutination-inhibition tests with A/Sweden/3/50 and A/Madrid/3/51 viruses and normal animal sera.

TABLE XIII. HAEMAGGLUTINATION-INHIBITION TESTS WITH A/SWE/3/50 AND A/MAD/3/51 AND NORMAL SERA

Virus *	Serum †		
	normal ferret	normal rabbit	normal fowl
A/Swe/3/50 (S)	30	240	< 10
A/Mad/3/51 (L)	30	240	< 10

* L = Liverpool subtype ; S = Scandinavian subtype
† The sera were heated at 56°C for 30 minutes before use.

In spite of the considerable differences in combining power of the viruses with specific antibody, both viruses were inhibited by normal sera to the same titre. In other experiments the same inhibitory titre with normal sera was found when both viruses were tested with fowl, guinea-pig, or pigeon cells.

Possible inhibitors to combination of specific antibody with Q virus. No evidence of the existence of an inhibitor to the combination of Q virus with specific antibody has been found. A/Sweden/3/50 virus heated at 56°C for 30 minutes gave the same inhibition titres with specific antibody as did unheated virus. A/Sweden/3/50 was treated with the receptor-destroying enzyme of *V. cholerae*, on the assumption that chorio-allantoic membrane inhibitor might be attached to the virus particles : no effect was noted on the agglutination-inhibition titre with specific antibody.

Elford & Chu [10] have found that, following treatment with ether, influenza virus loses its infectivity and that a " soluble " haemagglutinin, much smaller than the virus haemagglutinin, is released. The " soluble " haemagglutinin can be readily separated from the virus by high-speed centrifugation. It was interesting to see whether the " soluble " haemagglutinin of Q viruses might be readily inhibited by antiserum to the intact virus. A/Sweden/3/50 and A/Madrid/3/51 viruses were treated with ether and the " soluble " and virus haemagglutinins were used as antigens in antihaemagglutinin tests with ferret antisera to the viruses. The results are shown in table XIV.

TABLE XIV. HAEMAGGLUTINATION-INHIBITION TESTS WITH VIRUS AND " SOLUBLE " (ETHER TREATMENT) HAEMAGGLUTININS

Strain *	Antigen	Serum	
		A/Swe/3/50	A/Mad/3/51
A/Swe/3/50 (S)	virus	50	10
	" soluble " haemagglutinin	1280	320
A/Mad/3/51 (L)	virus	30	960
	" soluble " haemagglutinin	320	5120

* L = Liverpool subtype ; S = Scandinavian subtype

A rise in antihaemagglutinin titres occurred when virus haemagglutinins were replaced by " soluble " haemagglutinin. However, this happened with both P and Q viruses so that no evidence of inhibition for Q viruses has been detected in this way.

Stability of P and Q viruses

In a recent publication (Isaacs & Andrewes [15]), a description was given of the changes produced in P viruses by passage in the presence of homologous immune serum and in Q viruses by one or two mouse passages. In these experiments, and in those which are described below, all viruses were passaged in eggs at limiting dilution to obtain " pure " cultures before

any attempts to produce variants were carried out. In some experiments, tests of the purity of these cultures were made by passaging a second time at limiting dilution and testing the characters of the individual virus fluids obtained, i.e., " plating out ". These measures are important to exclude the possibility that any apparent changes produced in a virus culture are not the result of selection of one component of a mixed-virus population.

Change of P to Q virus. In preliminary experiments, a P virus (L subtype) was passaged four or five times in eggs in the presence of gradually increasing concentrations of homologous antiserum. By this means variants are produced which are less well neutralized by antiserum than the original culture (Isaacs & Edney [17]) both in egg-neutralization and haemagglutination-inhibition tests. In later experiments, variants were readily produced in a single passage by increasing the concentration of serum during growth of the virus (see page 290). These variants maintained their properties on further passage in eggs in the absence of serum.

The results of cross-haemagglutination-inhibition tests with ferret antisera to two parent and variant strains are shown in table XV.

TABLE XV. CROSS-HAEMAGGLUTINATION TESTS WITH SERUM PASSAGE VARIANTS AND PARENT VIRUSES

Strain *	Virus	Serum	
		parent	variant
A/Mad/3/51 (L)	parent	2560	640
	variant	640	160
A/Jo'burg/7/50 (L)	parent	1920	1920
	variant	60	960

* L = Liverpool subtype

The results in table XV show that, on passage with antiserum, A/Madrid/3/51 (L) has become more like a Q virus, giving lower titres with sera from parent or variant virus. The A/Johannesburg/7/50 variant, on the other hand, shows low reactivity with parent antiserum (and other L antisera) but not with homologous antiserum, while the variant antiserum contains high titre antibody to parent viruses. The possibility that this was due to the use of a mixed culture of virus in preparing the ferret antiserum was excluded by passing A/Johannesburg/7/50 variant at limiting dilution and " plating out ", by a second limiting dilution pass, as a test of homogeneity. The A/Johannesburg/7/50 variant might be described as Q in regard to the parent (L) antigen. Such a result might occur if passage in antiserum had led to the multiplication of a variant virus which contained L antigen in a site where it did not affect the results of haemagglu-

tination-inhibition tests, but was nevertheless able to induce antibody formation. In conformity with the language of bacteriology, one might suggest that A/Johannesburg/7/50 virus had L antigen on the virus surface whereas the variant contained L antigen " within " the virus particle. A large number of variants have been obtained from these two strains by passage with antiserum. In the case of A/Johannesburg/7/50 (L) virus, which has been extensively investigated, five different limit-dilution fluids have been passaged in eggs with homologous antisera, and four of these fluids have produced variants. One variant obtained from A/Johannesburg/7/50 was more like a Q strain than that shown in table XV and gave low inhibition titres with S and L antisera and also with the A/Johannesburg/7/50 variant antiserum shown in table XV. Variants obtained from A/Madrid/3/51 and A/Johannesburg/7/50 were compared with one another in haemagglutination-inhibition tests and were found to be quite distinct serologically.

Other strains were tested by passage in homologous immune serum. The Lee (influenza B) strain gave rise to a Q variant which showed low inhibition titres with parent and with Crawley (England, 1946, B) antisera. PR8 and A/Johannesburg/7/50 variant strains showed no alteration in behaviour on growth in immune serum (three experiments with each strain).

Change of Q to P virus. A considerable increase of reactivity of strains with antiserum was obtained by making one or two passages by intranasal inoculation of mice followed by re-inoculation of mouse-lung suspensions into eggs. In preliminary experiments in which the age of mice, amount of virus inoculated, and day of harvest were varied, it was found that the change could be most readily produced by passages of undiluted virus in six-week-old mice at 48-hour intervals. In this way significant changes have been produced in A/Sweden/3/50 (S), A/Cape Town/1/50 (S), A/Eire/1/51 (S), A/Melbourne/2/50 (S), and A/England/1/51 (L) ; all viruses had been passaged at limiting dilution in eggs before inoculation into mice. The effect of seven mouse passages of A/Sweden/3/50 (S) virus on its behaviour in antihaemagglutinin tests is shown in table XVII ; a pronounced increase in antihaemagglutinin titre occurred with all the sera tested. The results with other strains are described in the next subsection.

A/England/1/51 was the only L strain tested in this way. Table XVI shows the results of haemagglutination-inhibition tests with unadapted virus and with virus after 2, 8, and 15 mouse passages ; the virus was fully adapted to kill mice after 15 passages.[b] The titre with homologous serum was unaltered but the titre with Scandinavian (A/Eire/1/51) antiserum was greatly increased, even after two passages. Tests of fifteenth-passage

[b] The adaptation of A/Sweden/3/50 and A/England/1/51 to mice was carried out by our colleague, Dr. Janet S. F. Niven, in the course of separate studies.

TABLE XVI. HAEMAGGLUTINATION-INHIBITION TESTS WITH A/ENG/1/51 VIRUS AFTER VARIOUS MOUSE PASSAGES

Virus *	Number of mouse passages	Serum *		
		A/Eng/1/51 (L)	A/Eire/1/51 (S)	unheated normal mouse
A/Eng/1/51 (L)	0	1280	20	800
A/Eng/1/51	2	960	120	320
A/Eng/1/51	8	1280	320	160
A/Eng/1/51	15	1920	480	< 10

* L = Liverpool subtype ; S = Scandinavian subtype

A/England/1/51 virus with other A-prime antisera showed greatly increased inhibition titres to 1947, 1949, and 1951 (S) antisera. According to the terminology of van der Veen & Mulder,[23] this would be described as change of P to R virus. Table XVI also shows, in agreement with Chu,[6] that unheated normal mouse serum contains a heat labile inhibitor, which can be conveniently called " Chu inhibitor ", and which is much more active against unadapted than against mouse-adapted influenza A viruses. The table shows that this and the P-R changes occur independently of one another.

The A/Johannesburg/7/50 variant described above was passaged four times in mice, but no serological changes occurred.

" *Plating out* " *tests.* During these studies ten strains have been passaged at limiting dilution and the resulting fluids tested serologically in order to find evidence of any gross serological inhomogeneity in the virus cultures. Inhomogeneity has been found in three strains :

(*a*) A/Eire/1/51 (S) virus : Seven of 8 limiting-dilution fluids gave a haemagglutination-inhibition titre of 120 with homologous serum ; the eighth gave a titre of 30. This virus, which was called A/Eire/1/51 (Q), was passaged twice in mice and the resulting virus was then found to resemble closely the original A/Eire/1/51 in serological tests with four A-prime antisera. " Plating out " tests of A/Eire/1/51 (Q) showed no evidence of inhomogeneity.

(*b*) A/Melbourne/2/50 (S) : Twelve virus fluids (from three limiting-dilution tests) were poorly reactive with A-prime antisera (Q), while one fluid gave haemagglutination-inhibition titres significantly (four times) higher.

(*c*) A/Cape Town/1/50 (S) : Two components were found in this strain by passage at limiting dilution, one Q and one P (see table XVII). After four mouse passages, the Q strain closely resembled the P strain in its serological behaviour. The Q strain has shown no evidence of inhomogeneity on " plating out ". A ferret antiserum prepared against the purified Q

strain again reacted to a much higher titre with P virus than with the homologous Q virus.

The possibility of laboratory contamination of strains was kept constantly in mind during these studies. However, the evidence of inhomogeneity in these strains rather favours the hypothesis that the change of Q to P virus has been occurring in nature during passage from one human being to another, just as the change can be readily demonstrated in the laboratory by passage in mice.

Relationship between S and L viruses

The above experiments on P and Q viruses were carried out to test the possibility that L and S viruses isolated from the same country might be alternate phases of a single virus rather than two serological subtypes. However, no evidence of interconvertibility of the L and S subtypes has been found. The relationship between the two subtypes can be appreciated from the results of cross-haemagglutination-inhibition tests with A/Johannesburg/7/50 (L), A/Cape Town/1/50 (S), and their derivatives, along with some related A-prime viruses and sera.

TABLE XVII. HAEMAGGLUTINATION-INHIBITION TESTS WITH A/CAPE TOWN/1/50 AND A/JO'BURG/7/50 VIRUSES AND SOME VARIANTS DERIVED FROM THEM

Virus *	Serum						
	FM1	A/Paris/ 1/49	A/Swe/ 3/50	A/Cape Town /1/50	A/Jo'burg/ 7/50	A/Jo'burg/ 7/50 variant	A/Lon/ 1/51
FM1 (1947)	1920	320	80	640	70	1280	240
A/Paris/1/49	240	160	20	480	60	560	60
A/Swe/3/50 (S)	50	20	70	15	< 10	30	50
A/Swe/3/50 M7 †	480	160	280	200	200	480	160
A/Cape Town/1/50-Q	10	40	< 10	< 10	< 10	20	10
A/Cape Town/1/50-P	280	120	10	480	40	560	40
A/Jo'burg/7/50 (L)	240	120	70	140	1920	960	120
A/Jo'burg/7/50 variant	320	120	40	200	80	800	50
A/Lon/1/51 (S)	160	80	80	70	30	320	320

* L = Liverpool subtype ; S = Scandinavian subtype

† After 7 mouse-passages when the virus was adapted to kill mice.

Table XVII shows that A/Cape Town/1/50 (S) and A/Johannesburg/7/50 (L), at first sight quite dissimilar serologically, have some interesting points of resemblance. The P derivative of A/Cape Town/1/50 closely

resembles A/Paris/1/49 virus in its reactions with the seven sera shown. Similarly, the A/Johannesburg/7/50 variant virus closely resembles both these viruses. A/Johannesburg/7/50 (L) virus differs from the other three viruses mainly in its reactions with A/Johannesburg/7/50 (L) antiserum, to which it alone of the four viruses reacts to high titre.

There is a general serological similarity, allowing for differences in avidity for antibody, between the 1947, 1949, and 1950-1 S A-prime viruses. Superimposed on this antigenic structure is the L antigen, which so far as present results show is a new antigen, just as the A-prime viruses were quite different antigenically from previously isolated influenza A viruses. Since its first recognized appearance, the Liverpool subtype virus has been recovered from a large number of places (see Annex 1, page 309) and it has shown very little antigenic alteration during its travels.

Influenza B Viruses

Strains of influenza B virus were received from a number of laboratories during 1950-1. These were examined by Dr. A. Bozzo, working at the World Influenza Centre, and the results of his investigations have been published separately (Bozzo [2]). The strains received are listed in Annex 1 (page 309).

Discussion

The work just described indicates that viruses concerned in the 1950-1 outbreak were of the A-prime type, which has been prevalent all over the world since 1946-7. The viruses have shown, further, variation of two different kinds. First, there are the relatively stable, perhaps progressive, antigenic changes which seem to go on among influenza viruses all the time. These may result from adaptation of viruses to growth in a population immune to unmodified virus. There are suggestions that influenza keeps going by virtue of " continually emerging antigenic novelty ". Thus Anderson (1950, personal communication) has found that human sera taken at any time during recent years will tend to neutralize viruses which had appeared in years previous to the date of bleeding, but will fail to neutralize " future viruses ", i.e., those appearing after that date. On this basis, small but definite differences between the 1947, 1949, and 1951 viruses, all of which have become prevalent over whole continents, are found among A-prime viruses. The antigenic character of the L subtype is that particularly characteristic of 1951.

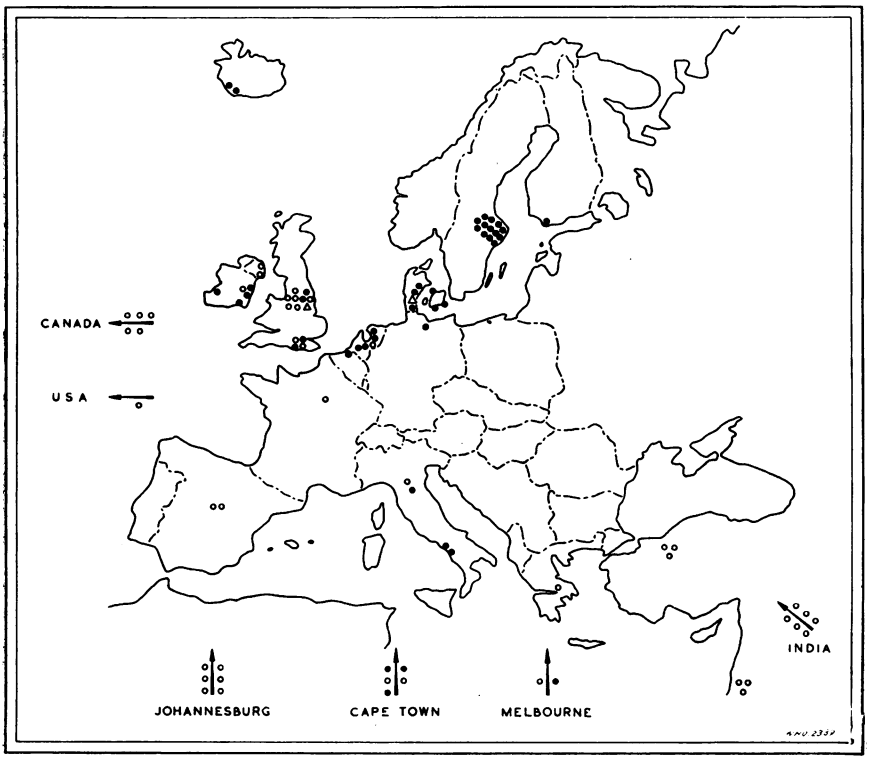
The constancy of antigenic type of L viruses isolated from different parts of the world could be taken as evidence for the evolution of this virus on a single occasion, followed by subsequent spread of a new serological subtype. This would support the suggestion put forward previously (Chu et al.[7]) that epidemic influenza may be the result of the true spread

of a single strain of virus. On the other hand, influenza outbreaks may also arise by activation of latent virus ; some work, e.g., the United States Commission on Influenza,[22] Chu et al.,[7] and the present results with the Swedish viruses of 1950-1, strongly supports this. It is most interesting, therefore, that the serological evidence suggests that S viruses may have evolved from 1949 A-prime viruses. This process might have occurred independently in different parts of the world, thus accounting for the greater inhomogeneity among S viruses isolated early in 1950 than among L viruses.

Apart from this fairly well-known tendency to antigenic modification is the P-Q phase variation which has been discussed. This seems to have the character of a fairly easily reversible phase-change within an antigenic type. Thus, the 1950-1 S viruses were nearly all Q-phase, yet, when modified to a P-phase by mouse passage, they did not become identical with L subtype viruses, but had antigenic characters of their own, much less uniform than those of L subtype viruses. On the other hand, when P-phase Liverpool viruses were made to acquire Q properties in the laboratory, they were hard to distinguish from Scandinavian Q viruses. One may recall how rough pneumococci derived from several serological types are very similar. It seems likely that most of the S viruses remained in the Q-phase, while L ones, while actively spreading, were all P. At some times and places apparently unconnected with the European focus, Q viruses have appeared along with Liverpool strains. It is not yet possible to decide definitely whether they were derived from L strains, or were their precursors, or were epidemiologically unrelated. The laboratory studies reported here do not support either of the first two hypotheses. One is left with the possibility that the same factors favoured the simultaneous appearance of S and L viruses in Australia and South Africa. This is not very satisfying intellectually, but comfort may be taken from the many reported simultaneous occurrences of influenza A and B infections in a community.

Such a picture makes intelligible the map given in fig. 1 (page 308). The Scandinavian focus, including Iceland, yielded us 23 strains which were mainly Q. As discussed earlier (Isaacs & Andrewes [15]), this outbreak is likely to have originated from activation in November 1950 of virus which had been lost to sight after local activity in June 1950. Such an occurrence has been noted on several previous occasions. A parallel and presumably independent origin of a spread of Q viruses was in southern Ireland (Meenan & Clarke [18]). From these two centres S subtype viruses spread over the North Sea to Great Britain and the Low Countries, and northward in Ireland, respectively. The L virus which caused high mortality in and near Liverpool in January 1951 appeared almost simultaneously in Belfast, Northern Ireland, and thence spread to other parts of the United Kingdom. At about the same time, or soon after, it was recognized in France, Greece, Israel, Italy, Spain, and Turkey. Discovery of antigenic

FIG. 1. MAP OF EUROPE SHOWING DISTRIBUTION OF 1950-1 A-PRIME VIRUSES



● = S (Scandinavian subtype) ; O = L (Liverpool subtype); △ = SL (intermediate subtype)

identity between these viruses and some received from the southern hemisphere (Australia and South Africa) and from India from outbreaks in June and August 1950 made it seem possible that their origin was from south of the equator or from the east. Where in the southern hemisphere the L subtype originated we cannot guess ; its first known appearance in time was in Australia (June 1950) and it was found in India, South Africa, and Ocean Island (South Pacific) soon after. A few strains received from Canada and one from the USA were all of L subtype ; they were obtained at a time (February 1951) when westward spread of virus across the Atlantic seemed not improbable. It is of interest that the larger " peaks " of mortality in the USA were in the States of the north-eastern seaboard (Collins & Lehmann [8]) in February 1951.

The findings suggest that it will be worth noting in the future whether Q viruses tend to prevail at the first emergence of an outbreak and P viruses when the epidemic has gained momentum. One wonders what would have happened to the Scandinavian virus if it had not so soon encountered another strain across its epidemic path.

The laboratory methods we have used make possible an interpretation —we hope, a true one—of epidemic findings. It must be emphasized, however, that workers in other laboratories use, in many instances, different techniques—for example, fowls, rabbits, or hamsters may be used for preparing immune sera. We have already referred to the fact that immune fowl sera fail to distinguish between L and S viruses. It is essential in the future to use various techniques in studies such as those we have reported, to discover whether they all lead to similar conclusions. It is quite possible that one technique for comparing viruses may prove to be best for epidemiological studies and another one for selection of strains to make vaccines.

ACKNOWLEDGEMENTS

We should like to thank the workers in many countries who have helped by sending us strains which they have isolated; their names are recorded in Annex 1. We should also like to thank Mr. E. Owen for his most valuable technical help.

ANNEX 1. INFLUENZA STRAINS ISOLATED IN 1950-1

Country of origin	Designation of strain	Place and approximate date of isolation	Worker	Serological type *	
Australia	A/Melbourne/1/50 (Chom)	Melbourne, June 1950	Burnet	A-prime	L
	A/Melbourne/2/50 (Phil)	Melbourne, June 1950	"	"	S
Belgium	A/Belgium/1/51	Brussels, February 1951	Nihoul	"	S
Canada	A/Halifax/1/51	Halifax, Nova Scotia, February, 1951	Nagler	"	L
	A/Montreal/1/51	Montreal, February 1951	"	"	L
	A/Newfoundland/1/51	St.John's, Newfoundland, February 1951	"	"	L
	A/Newfoundland/2/51	St.John's, Newfoundland, February 1951	"	"	L
	A/Regina/1/51	Regina, Saskatchewan, February 1951	"	"	L
Denmark	A/Danmark/1/50	Copenhagen, November 1950	von Magnus	"	S
	A/Danmark/2/50	Copenhagen, November 1950	"	"	S
	A/Danmark/3/50	Copenhagen, November 1950	"	"	S
	A/Danmark/4/50	Copenhagen, November 1950	"	"	S

ANNEX 1. INFLUENZA STRAINS ISOLATED IN 1950-1 (continued)

Country of origin	Designation of strain	Place and approximate date of isolation	Worker	Serological type *	
Denmark (*continued*)	A/Danmark/5/50	Copenhagen, November 1950	von Magnus	A-prime	SL
	A/Danmark/6/50	Copenhagen, November 1950	"	"	S
	A/Danmark/7/50	Copenhagen, November 1950	"	"	S
Finland	A/Finland/1/51	Helsinki, January 1951	Penttinen	"	S
France	A/Paris/1/51	Paris, January 1951	Cateigne	"	L
	B/France/2/51	Paris, March 1951	"	B	
Germany	A/Germany/4/51	Hamburg, March 1951	Lippelt	A-prime	S
Greece	A/Athens/1/51	Athens, March 1951	Pavlidis	"	L
Iceland	A/Reykjavik/1/51	Reykjavik, February 1951	Sigurdsson	"	S
	A/Reykjavik/2/51	Reykjavik, February 1951	"	"	S
India	A/India/1/50 (Rao)	Coonoor, August 1950	Menon	"	L
	A/India/2/50 (Walsh)	Coonoor, August 1950	"	"	L
	A/India/3/50 (Bheeman)	Coonoor, August 1950	"	"	L
	A/India/2/51 (Pearl)	Coonoor, December 1951	"	"	L
	A/India/3/51 (Jesudas)	Coonoor, December 1951	"	"	L
	A/India/4/51 (Chinna Nanjan)	Coonoor, December 1951	"	"	L
Ireland	A/Eire/1/51	Dublin, January 1951	Meenan	"	S
	A/Eire/2/51	Dublin, January 1951	"	"	S
	A/Eire/3/51	Limerick, January 1951	"	"	S
	A/Eire/4/51	Dublin, January 1951	"	"	L
	A/Eire/5/51	Ennisculty, south-east Ireland, January 1951	"	"	S
Israel	A/Jerusalem/1/51	Jerusalem, January-February 1951	Bernkopf	"	L
	A/Jerusalem/3/51	Jerusalem, January-February 1951	"	"	L
	A/Jerusalem/4/51	Jerusalem, January-February 1951	"	"	L
Italy	A/Florence/1/51	Florence, February 1951	Davoli	"	L
	A/Florence/2/51	Florence, February 1951	"	"	S
	A/Florence/4/51	Florence, February 1951	"	"	S
	A/Florence/5/51	Florence, February 1951	"	"	S

ANNEX 1. INFLUENZA STRAINS ISOLATED IN 1950-1 (continued)

Country of origin	Designation of strain	Place and approximate date of isolation	Worker	Serological type *	
Italy (*continued*)	B/Florence/1/51	Florence, March 1951	Davoli	B	
	B/Florence/2/51	Florence, April 1951	"	B	
	B/Milan/3/51	Milan, April 1951	Monaci	B	
	A/Naples/1/51	Naples, February 1951	Benzoni	A-prime	S
	A/Naples/2/51	Naples, February 1951	"	"	S
Japan	B/Japan/1/50 (T7)	Tokyo, early 1950	Kono	B	
	A/Japan/2/50 (T9)	Tokyo, early 1950	"	A-prime	SL
	B/Japan/3/50 (T14)	Tokyo, early 1950	"	B	
Netherlands	A/Nederland/1/51	Leyden, January 1951	Mulder	A-prime	S
	A/Nederland/3/51	Flushing, January 1951	Verlinde	"	S
	A/Nederland/4/51	The Hague, February 1951	"	"	L
	A/Nederland/5/51	Deventer, February 1951	"	"	S
	A/Nederland/6/51	Rotterdam, January 1951	"	"	S
	B/Nederland/11/51	Holland, April 1951	"	B	
	B/Nederland/12/51	Holland, April 1951	"	B	
Northern Ireland	A/Belfast/1/51	WIC, London, January 1951	Isaacs	A-prime	L
	A/Belfast/2/51	WIC, London, January 1951	"	"	L
Ocean Island (South Pacific)	A/Ocean Island/1/50	Melbourne, October 1950	French	"	L
South Africa	A/Cape Town/1/50 (Valkenberg)	Cape Town, August 1950	van den Ende	"	S
	A/Cape Town/2/50 (Groote Schuur)	Cape Town, August 1950	"	"	L
	A/Cape Town/3/50 (Hill)	Cape Town, August 1950	"	"	S
	A/Cape Town/4/50 (DC3)	Cape Town, August 1950	"	"	S
	A/Cape Town/5/50 (RBHS3)	Cape Town, August 1950	"	"	L
	B/Cape Town/1/51	Cape Town, November 1951	"	B	
	A/Nyasaland/1/50 (Gresham)	Johannesburg, August 1950	Gear	A-prime	L
	B/Johannesburg/1/50 (Davis)	Johannesburg, August 1950	"	B	

ANNEX 1. INFLUENZA STRAINS ISOLATED IN 1950-1 (continued)

Country of origin	Designation of strain	Place and approximate date of isolation	Worker	Serological type *	
South Africa (*continued*)	A/Johannesburg/2/50 (George)	Johannesburg, August 1950	Gear	A-prime	L
	A/Johannesburg/3/50 (James)	Johannesburg, August 1950	"	"	L
	A/Johannesburg/4/50 (Oliff)	Johannesburg, August 1950	"	"	L
	B/Johannesburg/5/50 (Sedgley)	Johannesburg, August 1950	"	B	
	A/Johannesburg/6/50 (Tom)	Johannesburg, August 1950	"	A-prime	L
	A/Johannesburg/7/50	Johannesburg, August 1950	"	"	L
	A/Johannesburg/8/50	Johannesburg, August 1950	"	"	L
Spain	A/Madrid/2/51	Madrid, January 1951	Gallardo	"	L
	A/Madrid/3/51	Madrid, January 1951	"	"	L
Sweden	A/Sweden/1/50	Stockholm, June 1950	Svedmyr	"	S
	A/Sweden/2/50	Stockholm, June 1950	"	"	S
	A/Sweden/3/50	Stockholm, June 1950	"	"	S
	A/Sweden/4/50	Stockholm, June 1950	"	"	S
	A/Sweden/5/50	Stockholm, December 1950	"	"	S
	A/Sweden/6/50	Stockholm, December 1950	"	"	S
	A/Sweden/7/50	Stockholm, December 1950	"	"	S
	A/Sweden/8/50	Stockholm, December 1950	"	"	S
	A/Sweden/9/50	Stockholm, December 1950	"	"	S
	A/Sweden/10/50	Stockholm, December 1950	"	"	S
	A/Sweden/11/50	Stockholm, December 1950	"	"	S
	A/Sweden/12/50	Stockholm, December 1950	"	"	S
	A/Sweden/1/51	Stockholm, February 1951	"	"	S
Turkey	A/Ankara/1/51	Ankara, January 1951	Payzin	"	L
	A/Ankara/3/51	Ankara, February 1951	Erzin	"	L
	B/Ankara/4/51	Ankara, March 1951	"	B	

ANNEX 1. INFLUENZA STRAINS ISOLATED IN 1950-1 (concluded)

Country of origin	Designation of strain	Place and approximate date of isolation	Worker	Serological type *
Turkey (*continued*)	B/Ankara/5/51	Ankara, March 1951	Erzin	B
	A/Ankara/6/51	Ankara, March 1951	"	A-prime L
	A/Ankara/7/51	Ankara, November 1951	"	" L
United Kingdom	A/England/1/51	Liverpool, January 1951	Marmion	" L
	A/England/2/51 (Greer)	Liverpool, February 1951	Stuart-Harris	" L
	A/England/3/51 (Johnson)	Sheffield, February 1951	"	" S
	A/England/4/51	Manchester, February 1951	Tobin	" L
	A/England/5/51 (Heeley)	Sheffield, February 1951	Stuart-Harris	" SL
	A/England/6/51 (Heron)	Sheffield, February 1951	"	" L
	A/England/7/51 (Perkins)	Sheffield, February 1951	"	" L
	A/England/8/51 (Potts)	Sheffield, February 1951	"	" L
	A/England/10/51	Bradford, February 1951	Marmion	" S
	A/London/1/51	London, January 1951	"	" S
	A/London/2/51	WIC, London, January 1951	Bozzo	" L
	A/London/3/51	London, January 1951	Marmion	" L
	A/London/4/51	London, January 1951	"	" S
United States of America	Gilmore	New York, January 1951	Rose	" L
Yugoslavia	A/Yugoslavia/1/51	Zagreb, early 1951	Vesenjak	" S
	B/Yugoslavia/1/51	Belgrade, March 1951	Terzin	B
	B/Yugoslavia/2/51	Belgrade, March 1951	"	B

* L refers to Liverpool and S to Scandinavian subtype; SL describes strains with characters intermediate between L and S.

SUMMARY OF SEROLOGICAL SUBTYPES

A-prime			B	Total
S	SL	L		
46	3	47	15	111

SUMMARY

During the 1950-1 influenza epidemic, 111 strains of virus were received at the World Influenza Centre (WIC). Of these, 96 were influenza A-prime and 15 were influenza B viruses. The significance of the few A viruses received has been discussed in an earlier paper by Isaacs & Andrewes.[15] The epidemic is believed to have been mainly due to A-prime viruses, and the present paper is concerned with antigenic studies of the A-primes.

Two main serological subtypes of virus were recognized by means of haemagglutination-inhibition tests. Forty-seven strains were closely related to A/England/1/51 virus, isolated from Liverpool, and they have been called Liverpool (L) subtype strains. Forty-six strains were less homogeneous serologically and were more closely related to A/Sweden/3/50 virus than to the Liverpool subtype viruses; they have been called Scandinavian (S) subtype viruses. Three strains showed intermediate properties. The S strains were in general distinguishable from, but closely related to, 1947 and 1949 A-prime viruses from which they may have evolved. The L subtype apparently shows the evolution of a new antigen, which had not been recognized previous to June 1950. The L viruses were all in the P phase. The S strains, particularly those isolated early in the epidemic, were mainly in the Q phase. These differences may be related to whether an epidemic occurs by spread from a neighbouring focus or by activation of latent virus; this point will be watched carefully in future outbreaks. Laboratory experiments suggest that P and Q phases of a virus may be reversible, and some evidence is presented that similar changes are occurring in nature. No evidence of a change from S to L subtype, or vice versa, was found in the laboratory.

The epidemiological implications of these findings are briefly discussed.

RÉSUMÉ

Durant l'épidémie de grippe 1950-51, le Centre Mondial de la Grippe (CMG) a reçu 111 souches de virus; 96 d'entre elles appartenaient au type A-prime, 15 au type B. La présence de quelques souches A parmi les envois a été discutée dans un travail antérieur de Isaacs et Andrewes. [15] On estime que l'épidémie a été provoquée essentiellement par des virus A-prime. Ce travail est consacré à l'étude de leurs propriétés antigéniques.

Les épreuves d'inhibition de l'hémagglutination ont permis de distinguer deux sous-types sérologiques. Quarante-sept souches étaient étroitement apparentées au virus A/England/1/51 provenant de Liverpool; elles ont été désignées comme appartenant au sous-type Liverpool (L). Quarante-six étaient moins homogènes sérologiquement; elles étaient plus proches du virus A/Sweden/3/50 que du sous-type Liverpool. Elles ont été considérées comme appartenant à un sous-type dit scandinave (S). Trois souches présentaient des caractères intermédiaires. Les souches S étaient en général distinctes des virus A-prime de 1947 et 1949, mais leur étaient cependant étroitement apparentées; elles en sont peut-être issues. Avec le sous-type L est apparu un nouvel antigène qui n'avait pas été observé avant juin 1950. Les virus L étaient tous en phase P. Les souches S, en particulier celles qui avaient été isolées au début de l'épidémie, étaient en phase Q. Il est possible que ces différences soient en relation avec la diversité d'origine de l'épidémie : propagation à partir d'un foyer voisin ou activation d'un virus latent. Il y aura lieu de suivre attentivement cette question au cours de futures épidémies. Des expériences de laboratoire permettent de penser que les phases P et Q d'un virus peuvent être réversibles et quelques faits semblent indiquer qu'il en est ainsi dans la nature. Un changement des sous-types S en L et vice-versa n'a pas été constaté en laboratoire.

Les conséquences que ces observations peuvent avoir en épidémiologie sont brièvement examinées.

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