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THE SPREAD OF INFLUENZA

EVIDENCE FROM 1950-1951

BY

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Despite rapid advances in the knowledge of the properties and behaviour of influenza viruses in the laboratory, most of the major epidemiological problems of influenza remain unsolved. There are three problems which constantly trouble the minds of all epidemiologists concerned with influenza. First, is there a true spread of virus from one country to another, or is there merely an apparent spread due to successive activation of endemic virus in different countries? Secondly, how does the influenza virus survive in nature in the long interepidemic periods? Thirdly, what factors are responsible for the sudden storm of influenza which arises from an apparently calm sea, and which may do as little damage as it did in 1950-1 or as much as it did in 1918-19?

During the 1948-9 and 1950-1 epidemics, workers in W.H.O. influenza laboratories have attempted to isolate influenza viruses which they have sent to the World Influenza Centre in London for typing. It was hoped that following an investigation of this kind answers would be forthcoming to at least some of the epidemiological problems of influenza, and that at the same time information required in the preparation of influenza vaccines would become available. In the present paper influenza epidemiology is discussed in the light of experiences of the 1950-1 influenza epidemic. No attempt has been made to describe the investigations in detail in this account; a report which is being prepared for the *Bulletin of the World Health Organization* will include these details. We hope to acknowledge in that report the kind help of individual workers in many W.H.O. influenza centres. Without their co-operation this work would not have been possible.

The 1950-1 Influenza Epidemic

In May and June, 1950, small outbreaks of influenza occurred in a number of places in Sweden, particularly in the southern and central parts. Outbreaks were also reported from Norway. Small summer outbreaks of influenza have been previously observed to foreshadow epidemics starting in the same area in the following winter (Andrewes, 1950). In this case Dr. A. Svedmyr, in Stockholm, was able to isolate four strains of virus, which he kindly sent to the World Influenza Centre. The strains proved to be A-prime strains which were slightly but recognizably different serologically from previously isolated strains of influenza virus. We com-

municated with Dr. Svedmyr, suggested that there might be a further outbreak of influenza in the following winter, and asked him to keep a close look-out for any cases. However, in spite of a systematic search for evidence of virus infections no influenza was detected until some months later when influenza occurred, first in Denmark and then in Norway and Sweden. From Denmark and Sweden, strains of virus were recovered which we have found to be closely related serologically to the summer Swedish strains. (Unfortunately no strains of virus were isolated from Norway.) Here seems to be another and very striking example of the latent period preceding an epidemic, during which the virus is presumably present in the community but is not readily detectable.

The important epidemiological characters of influenza in 1950-1 are best appreciated by a consideration of the course of infection in Scandinavia, Holland, and the United Kingdom and Ireland.

Scandinavia

Towards the end of October an increase in influenza notifications was observed in the islands of Lolland and Falster, in Southern Denmark, and on November 4 an attack rate of about 75% was reported in various schools and factories. Only two days later an equally high incidence was reported in two other districts—northern Jutland and the island of Fyen—both far away from Lolland and Falster. Apparently, then, the epidemic in Denmark started simultaneously at a number of parts of the country. The epidemic reached its peak in Denmark towards the end of November and had declined greatly by the end of the year.

In Sweden influenza was first observed towards the end of November as a local outbreak in a school in the extreme north of the country. Between December 7 and 10 a number of medical officers reported epidemics arising in the east, south-west, and south of Sweden. The epidemic reached its peak in late December and early January.

In Norway the peak incidence of influenza was in November, and the epidemic had died down by January. Throughout Scandinavia the incidence of influenza was high, but the disease was mild and the mortality very low. Further details will be found in a paper by Svedmyr, von Magnus, and Freundt (1951), to whom we are greatly indebted for much valuable information.

In Iceland, Dr. Sigurdsson informed us, the infection was introduced on the eastern seaboard in January, possibly by fishermen coming from England. Travel was much reduced at this time by climatic conditions, and the peak incidence in Reykjavik occurred in February. The spread of infection apparently followed normal lines of communication. Thus in one remote district in the north-east with poor communications with the rest of the country the first cases were observed in July. As in Scandinavia, the outbreak was mild and no deaths from influenza were reported.

Holland

The epidemic in Holland started in Flushing, on the isle of Walcheren, about Christmas, 1950. About the New Year, influenza appeared in Rotterdam, The Hague, and IJmuiden. In the middle of January evidence of spread eastwards and northwards was noted. The outbreak reached its peak at the end of January; the influenza was mild, and the overall incidence in Holland was approximately 9%. The evidence strongly suggests that infection was introduced into Holland from the west and spread eastwards.

United Kingdom and Ireland

In view of the widespread influenza in Scandinavia, it was not surprising that the first reports of influenza in this country came about Christmas-time from Newcastle. Unfortunately, no strains of virus were isolated from Newcastle, so that the probability of spread from Scandinavia must rest on epidemiological grounds only.

In the Merseyside area deaths from influenza increased sharply at the beginning of January, and in Liverpool alone there were 216 deaths from this infection in the second week of January. The corresponding figure for the maximum number of deaths per week in 1918 was 230; this may give some idea of the severity of the outbreak in this area. Most of the deaths occurred in the older age groups, however, in contrast with the high incidence in young adults during the 1918-19 influenza epidemic. The majority of the influenza deaths in England occurred in the north, and, although the influenza virus was present in force in the south of England and in the London area, the outbreaks were much less

severe than in the north. Bradley (1951) has shown that coincidentally with the peak of influenza deaths in England there was an equally sharp but much larger peak of deaths not actually certified as due to influenza. Over a period of five weeks the weekly deaths from all causes in the 126 great towns were roughly double (10,000 against 5,000) those of a normal year such as 1948. This finding, which is not unprecedented, emphasizes the relatively serious character of the influenza in this country as compared with elsewhere in Europe.

In Scotland cases were reported towards the end of December from Aberdeen and Edinburgh. Local outbreaks occurred in a number of parts of Scotland during January, but in general the disease was much less severe than in Northern England.

In Northern Ireland influenza broke out quite sharply

in Belfast towards the end of December and gradually spread southwards. In a most careful report, Meenan and Clarke (1951) concluded on epidemiological grounds that the outbreak in Ireland had two distinct foci of origin—one in Northern Ireland and the second in the Ballybunion area, in the south-west. Our serological studies of the viruses isolated strongly support their views, virus strains isolated from the two foci being recognizably distinct serologically (see accompanying Map).

In the northern half of the country Meenan noted definite evidence of local spread advancing south, with few areas becoming involved unless those to the north

Map showing distribution of influenza A viruses isolated in 1950-1. The circles indicate Scandinavian subtype strains; the crosses indicate Liverpool subtype strains.

had first been affected. Curiously enough, Dublin, the centre of communications in Ireland, was only secondarily and mildly involved, and did not apparently play an important part in spreading the infection. The analogy with London is an interesting one. The general pattern in Ireland was one of spread by continuity of district.

Further details of the epidemiology of influenza in 1950-1 will be found in a report prepared for the World Health Organization by Freyche and Klimt (1951).

These accounts show two patterns of spread in influenza epidemics. First, there is the type found in Sweden and Denmark, in which influenza first appeared in the summer and then, following a latent period of four to five months, a number of small outbreaks occurred more

or less simultaneously in widely scattered areas. The suspicion of pre-seeding of virus in these areas is extremely high. Secondly, there are the accounts of influenza in Iceland, Holland, England, and Northern Ireland, where there is presumptive epidemiological evidence that virus was introduced from elsewhere and that spread by continuity of district followed. There is the further contrast between the low mortality in Scandinavia and the large number of deaths found in Northern England and particularly in Merseyside. An attempt to correlate these features with antigenic studies of the strains isolated proved particularly interesting.

Methods

We have used the haemagglutination-inhibition test with ferret antisera in most of our antigenic studies. No attempt will be made in this paper to describe details of the techniques used. However, it should be mentioned that greater clarity was introduced into the results of these tests by the use of a modification (Isaacs and Bozzo, 1951) of the method recommended by van der Veen and Mulder (1950) for freeing sera from non-specific inhibitor of haemagglutination by means of the receptor-destroying enzyme from *Vibrio cholerae*. Full details of the methods used will be found in a forthcoming publication (being submitted to the W.H.O. Bulletin).

Antigenic Studies of the 1950-1 Strains

Preliminary Typing.—The first task with the present strains was to determine their antigenic relationship with previously isolated strains. It was soon obvious that the 1950-1 viruses were more closely related to the FM1 (1947) strain than to any other standard influenza A virus. An example of the relationship found in haemagglutination-inhibition tests between strains A/England/1/51, isolated from Liverpool, and FM1 is shown in Table I.

TABLE I.—Haemagglutination-inhibition Tests with FM1 and A/England/1/51 Viruses and Antisera

Virus	Serum	
	FM1	A/England/1/51
FM1	1,280	560
A/England/1/51	160	800

Similar comparisons between the 1950-1 strains and the standard WS (1933) and PR8 (1934) strains showed only the weakest cross-reactions. It is concluded, therefore, that the 1950-1 epidemic was caused by viruses of the A-prime type. However, the 1950-1 viruses were recognizably different from previously isolated (1947 and 1949) A-prime strains.

Cross-comparisons Among the 1950-1 Viruses.—It was of great interest to see whether the 1950-1 strains were antigenically uniform. Chu, Andrewes, and Gledhill (1950) found that the strains responsible for the 1948-9 epidemic in Europe were serologically identical with one another although slightly different from previously isolated strains. It seems a reasonable inference, therefore, that the 1948-9 epidemic had a single source, probably Sardinia. A similar comparison of the 1950-1 strains showed that they could be divided into two serological subtypes.

We have called the first the "Scandinavian" subtype; it is represented by strains from Sweden, Denmark,

Belgium, Germany, Finland, and Iceland. The second has been called the "Liverpool" subtype, and it is represented by strains from Northern Ireland, France, Spain, Greece, Turkey, and Israel. Both subtypes of strain were found in Eire, England, Holland, and Italy. The distribution of the strains is shown in the Map.

The Liverpool strains were all very close to one another serologically; all were neutralized to high titre by homologous serum or homotypic sera. Even strains isolated thousands of miles apart were almost indistinguishable, as the example in Table II illustrates.

TABLE II.—Haemagglutination-inhibition Tests with A/Ankara/1/51 and A/England/1/51 Viruses and Antisera

Virus	Serum	
	A/Ankara/1/51	A/England/1/51
A/Ankara/1/51	560	640
A/England/1/51	960	960

On the other hand, the Scandinavian strains were more heterogeneous; many of them were very poorly neutralized, even by homologous antiserum. In this they resemble the Q strains described by van der Veen and Mulder (1950). The relationship between a Liverpool and a Scandinavian subtype strain is shown in Table III.

TABLE III.—Haemagglutination-inhibition Tests with A/Sweden/3/50 and A/England/1/51 Viruses and Antisera

Virus	Serum	
	A/Sweden/3/50	A/England/1/51
A/Sweden/3/50 (Scandinavian)	30	10
A/England/1/51 (Liverpool)	25	640

Relationship with South African and Australian 1950 Strains.—A few months before the outbreak in Europe, influenza was prevalent in South Africa (July-August, 1950) and in Australia (June, 1950). A number of the infecting strains were available, and were compared with the 1950-1 European viruses. Six strains from Johannesburg were identical with the Liverpool strains. Two strains from Capetown and one from Melbourne were also of the Liverpool subtype, and three strains from Capetown and one from Melbourne were poorly neutralized by antiserum and resembled Scandinavian Q strains.

Complement-fixation Tests.—In addition to haemagglutination-inhibition tests, experiments were carried out with strain-specific complement-fixation tests, according to the method of Fulton and Dumbell (1949). The results suggested less antigenic difference between strains FM1 (1947), A/Paris/1/49, A/Swe/3/50 (Scandinavian), and A/Belfast/1/51 (Liverpool) strains than was revealed by haemagglutination-inhibition tests. A similar observation was made by Chu, Andrewes, and Gledhill (1950) in their comparisons of 1947 and 1949 A-prime viruses.

The P-Q Variation

Van der Veen and Mulder (1950) described influenza viruses which behaved in three different ways in haemagglutination-inhibition tests. Viruses of what they called P type reacted to high titre with homologous serum only; the Q type gave low titres with all sera, including homologous serum; and the R type reacted to high titre with all sera of the same subtype. These

authors represented diagrammatically the relationship between P, Q, and R strains by a table which is reproduced in Table IV.

TABLE IV.*—Diagrammatic Representation of Results of Haemagglutination-inhibition Tests Between P, Q, and R Viruses and Antisera

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

* Reproduced from van der Veen and Mulder (1950).

All three types of virus have been encountered among the 1950-1 strains. An example of the relationship between P and Q strains from South Africa (1950) is shown in Table V.

TABLE V.—Haemagglutination-inhibition Tests with A/Capetown/1/50 and A/Johannesburg/7/50 Viruses and Antisera

Virus	Serum	
	A/Capetown/1/50	A/Johannesburg/7/50
A/Capetown/1/50 (Q) ..	30	<10
A/Johannesburg/7/50 (P) ..	240	2,560

In this example the Capetown virus is poorly neutralized by its own antiserum, even although that serum contains antibody, as shown by its reaction with the Johannesburg virus.

The occurrence of P and Q viruses side by side in a number of countries in different parts of the world raised the possibility that they might be alternative phases of a single virus. So far as is known, the main defence of human beings against influenza is the production of specific antibody. Q viruses, being poorly inhibited by antiserum in agglutination-inhibition tests and poorly neutralized by antiserum in neutralization tests in eggs, might be better adapted than P and R viruses to survive in an immune population.

Such a hypothesis implies that when P-phase virus encounters increasing amounts of specific antibody as it passes through partially immune human beings it is transformed into the Q phase, which is better adapted to survive under these conditions. An attempt was made to imitate this sequence of events in the laboratory.

Transformation of P into Q Phase.—P-phase virus was passaged in eggs along with a low concentration of homologous antiserum. In each successive passage the concentration of antiserum was increased. After three or four passages it was found that virus would grow in the presence of a much greater concentration of serum than controls similarly passaged in the absence of serum. An example of the results of haemagglutination-inhibition tests between the P strain, A/Madrid/3/51, and A/Madrid/3/51 variant, obtained after six passages in homologous immune serum, is shown in Table VI.

TABLE VI.—Haemagglutination-inhibition Tests with A/Madrid/3/51 Variant and A/Madrid/3/51 Viruses and Antisera

Virus	Serum	
	A/Madrid/3/51 Variant	A/Madrid/3/51
A/Madrid/3/51 variant ..	160	640
A/Madrid/3/51 ..	640	2,560

The A/Madrid/3/51 variant strain has become less well neutralized by specific antiserum without apparently having undergone any fundamental antigenic change—that is, the P phase has changed to the Q phase.

These experiments are laborious because of the number of egg passages involved. However, it has recently been possible to produce this change consistently in a single passage by adding increasing concentrations of immune serum to the eggs at intervals during the post-inoculation period. Following an initial injection of virus with a sub-inhibitory dose of serum, the concentration of serum in the eggs was increased fiftyfold by giving further injections at 18, 24, and 42 hours. In this way, strain A/Johannesburg/7/50, which was originally inhibited by homologous serum to a titre of 1,920, gave a variant which was inhibited to a titre of 120.

Slight evidence in support of the idea that change of P to Q phase can occur in nature comes from the fact that some 1949 A-prime strains show evidence of Q type of behaviour compared with 1947 A-prime strains, although the change is not so far advanced as with the 1950-1 Q strains. An example of this behaviour is shown in Table VII with strain A/Paris/1/49 and the FM1, 1947, strain.

TABLE VII.—Haemagglutination-inhibition Tests with A/Paris/1/49 and FM1 Viruses and Antisera

Virus	Serum	
	A/Paris/1/49	FM1
A/Paris/1/49	80	70
FM1	640	1,280

Transformation of Q into P Phase.—In support of the idea that Q-phase viruses might be better adapted than P viruses to survive in an immune human population is the fact that all four strains received from the Swedish summer (June, 1950) outbreak, which preceded the winter epidemic, were in the Q phase. However, many strains isolated six months later in Sweden and in other parts of Northern Europe showed an apparent change to the P phase. If a change of Q to P phase was occurring during the passage of virus from case to case it was of interest to see whether a similar change could be induced in the laboratory on passage in mice.

Experiments soon showed that a change of Q to P phase could be readily demonstrated after one or two intranasal passages of virus in mice, followed by reinoculation into eggs. Examples of the results of haemagglutination-inhibition tests with some variants obtained after passage of A/Sweden/3/50 virus (Q) in mice are shown in Table VIII. For comparison, strain A/London/1/51, a Scandinavian, and A/Madrid/3/51, a Liverpool, strain in the P phase, were tested at the same time.

The second variant shown in Table VIII is similar, serologically, to the naturally occurring A/London/1/51 (Scandinavian) strain, and is distinctly different from the Liverpool subtype strains. Van der Veen and Mulder (1950) have shown that, following adaptation to mice (12 to 20 mouse passages), four influenza A strains were much more highly inhibited by antiserum than were the original egg strains. The changes described here after one or two mouse passages appear essentially similar to those of van der Veen and Mulder.

It is worth emphasizing that, although the present evidence does not conflict with the idea that the change

TABLE VIII.—*Haemagglutination-inhibition Tests with A/Sweden/3/50 Variants and Scandinavian and Liverpool Subtype Viruses and Antisera*

Virus	Treatment	Serum			
		A/Sweden/3/50	A/Eire/1/51	A/London/1/51	A/Madrid/3/51
A/Sweden/3/50 (Scandinavian Q)	Nil—control	40	10	50	10
A/Sweden/3/50 variant 1	2 mouse passages	60	35	240	15
A/Sweden/3/50 variant 2	1 mouse passage	120	60	360	25
A/Sweden/3/50 variant 3	1	100	160	640	60
A/London/1/51 (Scandinavian P)	Nil—control	60	60	320	60
A/Madrid/3/51 (Liverpool P)	" "	30	20	240	1,280

of P to Q phase is a reversible one, it may prove that naturally occurring P and Q viruses differ in some other property from the laboratory variants. In the meantime, however, a reversible P-Q change seems an attractive hypothesis. In its reversibility the P-Q change differs from the O-D mutation in influenza A viruses described by Burnet and Bull (1943). Presumably both Scandinavian and Liverpool subtypes of A-prime can exist in either the P or the Q phase, but the naturally occurring Scandinavian viruses were mainly Q. The naturally occurring Liverpool subtypes were apparently all P, but this is less certain as Q phases of different subtypes would be hard to distinguish.

Every precaution has been taken in these experiments to eliminate the possibility of laboratory contamination of strains. Although this is a possibility which cannot be easily excluded, to account for the appearance of a single variant, it may be pointed out that some of the variants obtained are serologically quite distinct from other strains in our laboratory. In general, the reproducibility of the results excludes laboratory contamination as an explanation of the phenomena.

Dangers of Laboratory "Pick-ups"

Besides the 80 to 90 A-prime strains isolated in 1950-1, there have been a few, coming from laboratories in two countries, indistinguishable from the classical PR8 strain. The question arises of whether these are genuine isolations or laboratory contaminants ("pick-ups").

We need to be quite sure concerning a most interesting and important point in the epidemiology of influenza A: Have the A-prime viruses completely supplanted the classical A strains (those related to WS or PR8), or are the latter still sporadically causing influenza in man? The matter is not merely of great theoretical interest to the epidemiologist, but has a bearing on policy, for it may decide what strains should be incorporated in a vaccine. Complete disappearance of classical A strains would be perhaps the last thing one would expect: the evidence suggests, however, that this has in fact happened.

Since 1946 A-prime strains have been isolated in hundreds, first in Australia (1946), and then in North America and Europe, and since then repeatedly in Europe, Asia, America, Africa, and Oceania. Several claims to have isolated classical A strains have been published since 1947 (Nagler *et al.*, 1949; van Rooyen *et al.*, 1949; Ward and Eddy, 1950; Hilleman *et al.*, 1950), but certain evidence suggests that these should be received with caution. In 1944 it was pointed out (Andrewes *et al.*) that influenza virus could easily turn up as a laboratory contaminant or "pick-up." This danger still does not seem to be sufficiently appreciated, and is ignored, or at any rate not mentioned, by the workers just quoted. In earlier years of work on influenza the virus strain most widely current in European

laboratories was WS, and the strains "isolated" under suspicious circumstances were mostly indistinguishable from WS. Recently PR8 has been the laboratory strain almost universally studied, and the A strains isolated since 1947 have mainly been identical, or almost so, with PR8.

There is no *a priori* reason why strains antigenically very close to WS or PR8 should not keep turning up anew, nor is it a light matter to cast doubt on the findings of serious workers. Reasons for suspecting "pick-ups" must therefore be extremely cogent to be publicly discussed. They are as follows. During the decade (1937-46), when PR8-like strains were dominant all over the world so far as we know, they were never pathogenic for mice on first isolation but had to be adapted by several serial passages to infect that animal visibly. This was so whether primary isolation was in ferrets or eggs. Further, they were all serologically related to PR8 but hardly ever identical with it. Now that such strains have been generally supplanted as causes of human influenza by A-primes, the exceptional A strains described as appearing have all shown two remarkable characteristics. We have had an opportunity of examining most of the strains described, and have found that they are pathogenic for mice without need for any adaptation, and that they are antigenically and in other ways indistinguishable from PR8 or other strains currently under study in the same laboratory. In one instance such a PR8-like strain was found to differ to a minor degree from our own PR8 but to be indistinguishable from the slightly different substrain of PR8 carried in the laboratory concerned.

In this connexion the term "pathogenic for mice" needs to be defined. Some strains, especially of B, will kill mice when high-titre allantoic fluids are inoculated intranasally, but serial passage in mice is hard to achieve. Also, some A-prime strains will produce minor lung lesions in mice without prior mouse passage, but will not kill. Primarily mouse-pathogenic viruses, as we use the term now, are those which kill mice after intranasal inoculation both at first inoculation and regularly on further mouse passage.

A third possibly useful criterion is now available to distinguish between a "laboratory strain" and a "wild strain." All recently isolated A-prime strains examined show, on dark-ground microscopy or electron micrography of chick embryonic fluids, numerous filamentous forms. Laboratory-adapted strains such as PR8 show under comparable conditions almost wholly spherical forms; it is, of course, true that under other conditions filaments can be found in preparations of PR8. Further, classical A strains isolated as long ago as 1937 (Talmey) and 1943 (Weiss), if they have been dried and stored without many mouse or egg passages, also show, when revived and passed in eggs, largely filamentous forms. (This information was kindly supplied by our colleague

W. J. Elford.) On the other hand, all the classical A strains "isolated" since 1947 have shown predominantly round forms, as do laboratory strains.

Finally, wherever a careful examination has been made it has been possible to show that recently isolated influenza A strains are in the O phase (Burnet and Bull, 1943). The laboratory strains PR8 and WS are in the D phase. We have not seen any example of a recently isolated PR8- or WS-like strain in the O phase.

Laboratory "pick-ups" of influenza viruses have been proved to occur. They are a menace also to workers with neurotropic viruses. Workers with foot-and-mouth disease prefer to work with one strain at a time to avoid risk of inadvertently supplanting one type by another. There seems no justification, therefore, for ignoring the danger in the influenza field. It seems to us far easier to believe that "pick-ups" explain the few recent isolations of classical A strains than to imagine that these are genuine. For, if they are so, there is the treble coincidence that all are antigenically like currently used laboratory strains, all are primarily pathogenic for mice, and all exist predominantly as spherical forms.

The danger needs to be appreciated *now*, for nowadays many influenza viruses are studied after as few passages as possible following isolation and without mouse-adaptation; they may fail, therefore, to acquire the stigmata of a laboratory strain. If they turn up as contaminations their illegitimate origins will be harder to trace than in similar occurrences in the past.

Discussion

With every epidemic of influenza we seem to learn a little more of its ways. The epidemic of 1950-1 followed some earlier precedents in one respect: minor outbreaks in Scandinavia in the early summer of 1950 preceded a larger epidemic in the same area in the following autumn. On this occasion it was possible to obtain virus from the precursor as well as the bigger epidemic and to show that they were serologically identical. It is hard to escape the inference that virus had been latent in the area in the months between June and October, when its presence could not be detected.

Once activated, in October, influenza apparently spread to neighbouring countries—the Low Countries, Germany, Finland, Iceland, and probably Britain. Its almost simultaneous apparently multi-focal origin in Scandinavia recalls Magrassi's description of the beginnings of the 1949 epidemic in Sardinia (Magrassi, 1949). The Scandinavian virus was an A-prime, but had special serological characters permitting its recognition. A separate focus of infection with a similar virus may have appeared in Southern Ireland. These two areas of apparent activation of dormant viruses occurred on the fringes of the 1949 epidemic, for both Sweden and Ireland were just touched in that year but not seriously affected. We have elsewhere discussed possible reasons why outbreaks might logically be expected to start on the fringes of previous epidemic areas (Andrewes, 1951).

While the Scandinavian virus, especially in its countries of origin, caused negligible mortality, the behaviour of influenza elsewhere was more disturbing. The outbreak in Liverpool in December, 1950, caused a rather high mortality amongst old people; it appeared almost simultaneously in Belfast. Strains from Belfast and Liverpool, though A-primes, were readily distinguished serologically from the Scandinavian viruses. Viruses identical with them were obtained from France, and all

along the Mediterranean from Spain to Israel. Viruses of both subtypes occurred in Ireland, England, Holland, and Italy: attack from two directions seems to have occurred in those countries.

An unexpected finding was antigenic identity between the Liverpool-Mediterranean subtype of virus and several strains from an outbreak in South Africa occurring in July-August, 1950. One strain from Australia obtained in the same period was also identical. The possibility therefore arises that spread had occurred from the epidemic in the 1950 southern hemisphere winter across the Equator to the Mediterranean and to Britain. This is made somewhat more probable since immediately both in time and in space there was influenza in Spanish and French Guiana, Nigeria, and the Cameroons in September and October, and in French equatorial Africa in November (Freyche and Klimt, 1951). However, before this question can be settled we require to know more about the Q-phase viruses from Capetown and Australia; these are now under study.

A surprising feature is that spread has seemed to be directly geographical, rather than along main lines of communication. One would hardly have expected virus from South Africa to come north via West Africa and along the length of the Mediterranean when sea communications direct to northern Europe are so considerable. Again, spread from Europe seems to have occurred across the Atlantic, first involving the nearest area, Newfoundland, and then Canada. Similar "geographical spreads" were noted also within Ireland, England, and Holland, in circumstances where movement of traffic was not very great.

The finding of P and Q strains and their apparent interconvertibility in the laboratory has raised a number of interesting possibilities. The lability of influenza viruses is well known, and it is tempting to suppose that the change of P to Q phase represents an adaptive response of the virus to an immune population. However, much more study of the phenomenon is required before its significance outside the laboratory can be assessed. In particular, attention will be directed to observing whether, as in the present epidemic, Q strains are particularly common in mild summer outbreaks, and whether P strains are generally associated with more severe outbreaks than Q strains. From the practical point of view it appears that P strains function as better antigens than Q strains in animal tests; the possibility of their greater suitability in influenza vaccines is now under study.

Summary

An account is given of some important aspects of the epidemiology of influenza in 1950-1. Two apparent types of origin of the 1950-1 influenza epidemic were distinguished. (a) Activation of latent influenza in Scandinavia following an earlier summer outbreak. (b) Introduction of infection from without, possibly from the southern hemisphere, followed by rapid spread.

The causal strains of the 1950-1 outbreaks were serologically A-primes which were related to but distinguishable from previously isolated (1947-9) strains. Two serological subtypes were recognized—the "Scandinavian" and "Liverpool."

Both P and Q phase viruses (van der Veen and Mulder) were found among the strains isolated. Laboratory experiments suggest that the two may be interconvertible forms of one virus. The association in 1950-1 of Q strains with the first type and P strains with the second type of origin of influenza epidemics (described above) is noted.

The reported claims of recent isolations of classical influenza virus A strains are considered. The evidence suggests that these claims should be viewed with great caution.

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REFERENCES

- Andrewes, C. H. (1950). *New Engl. J. Med.*, **242**, 161.
 — (1951). *Proc. roy. Soc. Med.*, **44**, 803.
 — Glover, R. E., Himmelweit, F., and Smith, W. (1944). *Brit. J. exp. Path.*, **25**, 130.
 Bradley, W. H. (1951). *Proc. roy. Soc. Med.*, **44**, 789.
 Burnet, F. M., and Bull, D. R. (1943). *Aust. J. exp. Biol. med. Sci.*, **21**, 55.
 Chu, C. M., Andrewes, C. H., and Gledhill, A. W. (1950). *Bull. World Hlth Org.*, **3**, 187.
 Freyche, M. J., and Klimt, C. (1951). *World Hlth Org., Epidem. and Vital Statist. Rep.*, **4**, 141.
 Fulton, F., and Dumbell, K. R. (1949). *J. gen. Microbiol.*, **3**, 97.
 Hilleman, M. R., Mason, R. P., and Buescher, E. L. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 829.
 Isaacs, A., and Bozzo, A. (1951). *Brit. J. exp. Path.* In press.
 Magrassi, F. (1949). *Minerva med., Torino*, **40**, 565.
 Meenan, P. N., and Clarke, M. (1951). *J. Irish med. Ass.*, **29**, 3.
 Nagler, F. P., van Rooyen, C. E., and Sturdy, J. H. (1949). *Canad. J. publ. Hlth*, **40**, 457.
 Svedmyr, A., von Magnus, P., and Freundt, E. A. (1951). *Acta path. microbiol. scand.*, **29**, 96.
 van Rooyen, C. E., McClelland, L., and Campbell, E. K. (1949). *Canad. J. publ. Hlth*, **40**, 447.
 Veen, J. van der, and Mulder, J. (1950). "Studies on the Antigenic Composition of Human Influenza Virus A Strains." Stenfort Kroese, Leyden.
 Ward, T. G., and Eddy, B. E. (1950). *Science*, **112**, 501.

RELATION OF ADRENAL CORTICAL FUNCTION TO SCURVY IN GUINEA-PIGS

PRELIMINARY EXPERIMENTS

BY

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It has been known for many years that the adrenal gland contains a very high concentration of ascorbic acid (Szent-Györgyi, 1928), and more recently the increasing amount of information on adrenal steroids has centred interest on the relationship between ascorbic acid and adrenal steroid metabolism.

Following the work of Sayers *et al.* (1944, 1946) it has become well known that pituitary adrenocorticotrophic hormone, which stimulates the release of adrenal steroids, also causes a fall in adrenal ascorbic acid. This is true for the rat, which can synthesize ascorbic acid, and for the guinea-pig, which is unable to do so (Sayers *et al.*, 1946; Long, 1947a, 1947b) and therefore is dependent upon an adequate dietary intake. In the absence of ascorbic acid the guinea-pig develops scurvy with all its well-known manifestations (Bessey *et al.*, 1934).

The present investigation was undertaken to determine the effect of scurvy on urinary 17-ketosteroid excretion.

These excretory products are generally thought to arise from the adrenal cortex and, to a less extent, from the testes in the male (Fraser *et al.*, 1941). Observations have also been made on the effect of A.C.T.H. and cortisone in scorbutic guinea-pigs, with particular reference to the effect on development of symptoms and the excretion of 17-ketosteroids.

17-Ketosteroid Excretion During Untreated Scurvy

Methods

Male and female guinea-pigs of varying weights (300 to 590 g.) obtained from two registered breeders were kept in groups of two or three in metabolism cages. They were maintained on the scorbutic diet described by Harris and Ray (1932), which was supplemented by cabbage *ad lib.* where healthy pigs were required. Tap-water and the scorbutic diet were supplied *ad lib.* but the amount eaten each day was usually measured. Body weights were determined daily.

Daily, 24-hour specimens of urine were collected over concentrated A.R. hydrochloric acid, except on Sundays. Urinary 17-ketosteroids were determined at frequent intervals. After acid hydrolysis and carbon tetrachloride extraction, the Zimmerman reaction as described by Callow *et al.* (1938) was carried out, and a colour correction was finally applied according to Talbot *et al.* (1942). Of each group of animals one was killed when moribund and the organs were sectioned. All animals were examined at death, and the diagnosis of scurvy was confirmed. In some healthy and scorbutic animals the ascorbic acid content of the adrenal glands was determined by the method of Roe and Kuether (1943).

Results

In normal male and female guinea-pigs the daily 17-ketosteroid excretion varied between 0.09 and 0.30 mg./24 hours.

In Table I a series of results is given for four separate groups of animals. This shows the daily average body weight in grammes, and the average 17-ketosteroid excretion in milligrams per 24 hours. In addition, for group I, details of daily food and daily urine volume are given. In Table II the day on which a fall in body weight began and the day of death are given.

1. All animals showed a fall in body weight beginning on the 9th to the 15th day after starting the scorbutic diet.

2. The daily food intake began to fall rapidly about the 12th to the 16th day after the scorbutic diet began.

3. Death from scurvy occurred in all animals on the 21st to the 29th day of the experiment.

4. In all groups of animals the urinary 17-ketosteroid excretion showed a gradual but definite increase of varying degree as the animals became scorbutic, and excretion reached a peak in the terminal phases.

5. When not required for histological examination the right adrenal gland was weighed to the nearest 0.1 mg. Scorbutic glands showed an increase in weight over glands from healthy pigs, and the mean ratio of right adrenal gland (mg.) to body weight (g.) was 0.40 ± 0.03 . Corresponding figures for healthy pigs are 0.30 ± 0.03 .

6. The ascorbic acid content of the adrenal glands of healthy pigs varied between 106 and 164 mg./100 g. adrenal tissue. Ascorbic acid was not detected chemically in the glands of grossly scorbutic animals, neither could it be demonstrated histologically.

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