associated with deposits of hard calculus, particularly in those regions of the denture inaccessible to cane friction, the development of other procedures is required.

My thanks are due to Sir Edward Mellanby, Secretary, Medical Research Council, for advice and criticism; to Dr. J. L. Simonsen, Director, Colonial Products Research, for the supplies of sugar-cane; to Dr. E. B. Hughes and Dr. J. H. Bushill, J. Lyons and Co., for canning of the cane; to Dr. H. E. Magee, Medical Officer, Ministry of Health, and Dr. H. C. Gilmore, Chief Medical Officer, Dr. Barnardo's Homes, for arrangements and permission to carry out the investigations; to Dr. W. J. Martin, Medical Research Council Statistics Committee, for his analysis and evaluation of some of the results; to Mr. R. F. Wheatley, head master, William Baker Technical School, Hertford, and his assistant masters for supervision of the sugar-cane distribution and consumption; and to the Medical Research Council for the expenses of the investigation.

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SEROLOGICAL DIAGNOSIS OF EPIDEMIC INFLUENZA BY THE COMPLEMENT-FIXATION REACTION

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Laboratory tests play an important part in the investigation of an outbreak of epidemic influenza, not only in confirming the clinical diagnosis but also in determining the type of the infecting virus. There are two methods: (1) the isolation of the virus by culture or animal inoculation, and (2) serology. The direct isolation of influenza virus from cases of human influenza by inoculation of the ferret or the fertile hen's egg is by no means always successful. Serological methods of diagnosis are therefore of great importance and have been much used. The virus neutralization test is too laborious and technically difficult for extensive use, but two other methods are available: (1) the complement-fixation test of Fairbrother and Hoyle (1937), and (2) the red-cell agglutination inhibition test of Hirst (1941, 1942). These two tests differ immunologically and do not always give comparable results. In recent years it has become evident that the complement-fixation test is more satisfactory than the Hirst test for the diagnosis of epidemic influenza, and the present paper summarizes the results of over ten years' experience of the former.

The agglutination of fowl red blood cells by the influenza virus is due to the virus particle itself and is inhibited by sera containing antibody against the particle. Individual strains of influenza virus A show differences in antigenic structure, and convalescent serum may inhibit red-cell agglutination by the infecting strain but have much less or no inhibiting effect on agglutination by other strains of virus A. The Hirst test thus exhibits considerable strain specificity. By contrast the complement-fixation test gives consistent results with all strains of virus A. Hoyle and Fairbrother (1937b) showed that the effective antigen in the mouse-lung extracts used in the fixation test was a soluble substance which could be easily separated from the infective virus particle. This observation has been repeatedly confirmed (Lennette and Horsfall, 1940; Friedewald, 1943; Henle, Henle, Groupe, and Chambers, 1944). Suspensions of tissues infected with influenza virus usually contain antigenic particles of two different sizes, which sediment at different rates in the high-speed centrifuge. The larger particle, of diameter 80–100 mμ, having a sedimentation constant of 600S, is the infective virus particle, while the smaller particle, approximately 10 mμ in diameter, with a sedimentation constant of 30S, appears to be identical with the soluble antigen (Henle and Wiener, 1944; Wiener, Henle, and Henle, 1946). Both particles will give complement fixation with convalescent sera, but the fixation differs in character (Hoyle, 1945; Wiener, Henle, and Henle, 1946). The fixation given by the virus particle shows strain-specific phenomena comparable with those encountered in neutralization or red-cell agglutination inhibition tests, while that due to the soluble antigen does not. The soluble antigens of all strains of influenza virus A appear to be identical. For practical purposes it is desirable to use in complement-fixation tests an antigen consisting predominantly of soluble substance, since this avoids the strain-specific phenomena shown by the virus particle. Antigens made from infected mouse lungs or infected egg chorio-allantoic membrane fulfil this requirement, while antigens derived from the allantoic fluid of infected hen's eggs contain large amounts of infective virus and only small amounts of soluble antigen (Friedewald, 1943) and are therefore less suitable.

The work described here has been done with the mouse-lung antigen first described by Fairbrother and Hoyle (1937). The original technique has been used with minor modifications (Hoyle, 1945). The complement-fixation reaction was applied to influenza virus B by Francis (1940), and the results are similar to those given by virus A. Both the red-cell agglutination inhibition test and the complement-fixation test differentiate sharply between virus A and virus B, there being no antigenic relationship between either the virus particles or the soluble antigens of the two viruses.

Complement-fixation Titres of Norman Human Sera

The results of complement-fixation tests against the soluble antigen of influenza virus A given by random samples of normal human sera collected during inter-epidemic periods over ten years are shown in Table I,

TABLE I.—*Complement-fixation Titres of Normal Human Sera against Influenza Virus A*

Titre	Series 1: Leningrad, 1936 (Fairbrother and Hoyle, 1937)	2: Manchester, 1936 (Hoyle and Fairbrother, 1937a)	3: Manchester, 1938 (Martin, 1940)	4: Manchester, 1939 (Martin, 1940)	5: Northampton, 1942 (Hoyle, 1942)	6: Northampton, 1943	7: Northampton, Jan., 1946 (Hoyle, 1946)	8: Northampton, Autumn, 1946	9: Manchester, Autumn, 1946	Total	
1:32 ..	0	0	1	1	0	0	0	1	0	3	
1:16 ..	0	2	4	2	1	3	1	0	1	14	
1:8 ..	2	15	26	20	17	3	1	3	5	92	Total positives 718 = 46%
1:4 ..	2	60	77	75	29	10	6	24	14	297	
1:2 ..	1	63	56	53	49	9	8	58	15	312	
Doubtful	0	64	55	63	44	6	5	17	10	264	
(1:2) Negative	0	96	81	86	69	15	24	147	62	580	Doubtful + negative 844 = 54%
Total..	5	300	300	300	209	46	45	250	107	1,562	

which includes two series of results obtained by Martin (1940) using the same technique. It will be seen that the various series of results show a close correspondence. Of 1,562 sera examined, 46% gave positive readings and 54%

TABLE II.—*Complement-fixation Titres of Normal Human Sera against Influenza Virus B*

Titre	Series 1 Northampton, 1943 (Hoyle, 1944)	2 Northampton, Jan., 1946 (Hoyle, 1946)	3 Northampton, Autumn, 1946	4 Manchester, Autumn, 1946	Total	
1:16	0	0	1	2	3	
1:8	4	2	3	5	14	Total positives
1:4	8	7	33	9	57	139 = 31%
1:2	6	1	40	18	65	
Doubtful (1:2) ..	9	3	16	18	46	Doubtful + negative
Negative	19	32	157	55	263	309 = 69%
Total	46	45	250	107	448	

doubtful or negative readings. Only about 1% of the sera gave titres of 1 : 16 or higher. Table II shows the results of similar tests with virus B. Of 448 sera examined, 31% gave positive and 69% doubtful or negative readings. Less than 1% of sera gave titres of 1 : 16 or higher.

The age distribution of antibody against virus A was studied by Fairbrother and Hoyle (1937) and by Martin (1940). It was shown that children under 6 months old showed a similar antibody distribution to adults (presumably maternal antibody is transmitted to the infant), but that after 6 months it was unusual to find antibody in the sera of young children. The incidence of positive sera increased with advancing age.

Complement-fixation Titres of Convalescent Sera

The complement-fixation titres of convalescent sera in five epidemics of influenza due to virus A and one epidemic due to virus B are shown in Table III. Similar results were

TABLE III.—*Complement-fixation Titres of Influenza Convalescent Sera*

Titre	Virus A Epidemics 1 Leningrad, 1936 (Fairbrother and Hoyle, 1937)	2 Manchester, 1937 (Hoyle and Fairbrother, 1937)	3 Northampton, 1943 (Hoyle, 1944)	4 Hardingstone, 1946 (Hoyle and Lyell, 1946)	5 Northampton and Manchester, 1947	Virus B Epidemic 6 Northampton and Manchester, 1946	Total		
1:256 ..	0	0	1	0	1	0	2		
1:128 ..	0	9	1	0	2	3	15	Positive	
1:64 ..	0	13	1	3	5	4	26	over 1:16	Total
1:32 ..	1	14	2	2	3	2	24	88 = 64%	positives
1:16 ..	2	10	2	3	4	0	21		132 = 96%
1:8 ..	2	11	4	2	6	1	26		
1:4 ..	0	8	2	0	4	0	14		
1:2 ..	0	0	1	0	3	0	4		
Doubtful (1:2)	0	0	0	2	0	1	3	Doubtful + negative	
Negative ..	0	0	0	1	2	0	3	6 = 4%	
Total ..	5	65	14	13	30	11	138		

obtained in all six outbreaks. Over 95% of convalescent sera gave positive complement fixation with the infecting virus, and 64% gave titres above the normal limits—that is, 1 : 16 or higher. The test differentiated sharply between virus A and virus B; thus in the A epidemics the convalescent sera showed an increased titre to virus A antigen but titres against virus B were within the normal range, and the converse was true in the B epidemic. Fairbrother and Martin (1938) studied the rate at which the high titres in convalescent sera returned to normal. A rapid fall in titre took place in the six months following infection, and by one year all the titres were within the normal range.

The above results show that the complement-fixation reaction can be a valuable diagnostic test in influenza. The test has been positive in over 95% of 138 convalescent sera, and it is interesting to note that the few negative or doubtful results almost all occurred in the very mild outbreaks of 1946 and 1947. The severe epidemics of 1937 and 1943 gave 100% of positive results. In fact a negative complement-fixation reaction in a convalescent serum tends to exclude the diagnosis of epidemic influenza. Over 60% of convalescent sera give titres of 1 : 16 or higher, while these titres are found in less than 1% of normal sera, so that a titre of 1 : 16 or higher is practically diagnostic of recent infection.

It is to be noted that figures comparable with the above cannot be obtained by the red-cell agglutination inhibition test. Both normal and convalescent sera show enormous variations in titre by the Hirst test according to the strain of virus used, so that no normal base-line can be drawn and it is impossible to decide whether a given titre in a convalescent serum is diagnostic of infection. To make a diagnosis of influenza by the Hirst test it is essential to examine two samples of serum—one collected at the onset of disease and the other during recovery—in order to demonstrate a rise in antibody titre. The test is therefore much less convenient and reliable than the complement-fixation test.

Comparison of the Complement-fixation Test and the Red-cell Agglutination Inhibition Test

The Hirst test has been much used in the diagnosis of epidemic influenza, but it became evident in recent years that the results were inferior to those of the complement-fixation test. It was at first thought that this inferiority was due to antigenic differences between the strain of virus used in the Hirst tests and that responsible for the infection, and that better results would be given if the homologous virus could be used. Experience in 1946–7, however, has shown not only that this is not so but also that the homologous virus may in fact be less suitable for use in the Hirst tests than other strains.

During the last year paired samples of blood serum from acute and convalescent cases of supposed influenza have been examined at the Northampton Public Health Laboratory. Some of the samples were received before the mild outbreak of influenza which occurred in the winter and were almost certainly not from cases of influenza. The sera were tested by complement fixation and by red-cell agglutination inhibition, using six different strains of virus A. The Salk (1944) modification of the Hirst test was used A fourfold increase in antibody titre was taken as diagnostic of infection. The results are given in Table IV.

TABLE IV.—*Comparison of Complement-fixation and Red-cell Agglutination Inhibition Tests in the Diagnosis of Epidemic Influenza (Virus A)*

Total pairs of sera examined	32
Total positives (i.e., positive by any one or more tests)	18
Positive by complement-fixation test	16
Positive by red-cell agglutination inhibition tests with:	
P.R.8 virus	13
Swine virus	12
Barratt virus	11
Melbourne virus	11
D.S.P. virus	10
W.S. virus	8

The complement-fixation test shows an obvious superiority over the red-cell agglutination inhibition test. In the Salk tests the best results were given by the P.R.8

and swine viruses. The Barratt strain, received from Dr. C. H. Andrewes, was actually isolated from the mild epidemic of 1947, but gave results inferior to P.R.8 and swine viruses. It is of interest to note that the worst results in the Salk tests were given by the W.S. strain of virus; it was from this strain that the preparation of complement-fixing antigen used in the fixation tests was made. Table V

TABLE V.—*Quantitative Increase in Antibody Titre in* 18 *Cases of Influenza Virus A Infection as Measured by Complement-fixation and by Red-cell Agglutination Inhibition Tests*

Increase in Titre	Complement-fixation Test	Red-cell Agglutination Inhibition Tests					
		P.R.8	Swine	Barratt	Melbourne	D.S.P.	W.S.
×128 ..	4	0	1	0	0	0	0
× 64 ..	2	0	1	0	0	0	0
× 32 ..	2	1	3	1	1	0	1
× 16 ..	4	2	0	3	2	0	2
× 8 ..	2	5	4	0	3	3	2
× 4 ..	2	5	3	7	5	7	3
× 2 ..	1	1	4	2	2	0	4
No increase	1	4	2	5	5	8	6

shows the quantitative increase in titre of the positive sera measured by the complement-fixation test and by the six different Salk tests. In general the increase in titre as a result of infection was greater in the complement-fixation test than in the red-cell agglutination inhibition tests.

Increase in Antibody Titre as a Result of Vaccination

The rise of antibody titre as a result of the prophylactic inoculation of a vaccine containing a concentrated suspension of virus A of Australian origin was tested in 16 individuals, using the complement-fixation test and Salk tests with P.R.8 and Melbourne viruses. The results are shown in Table VI. Considerable individual differences

TABLE VI.—*Quantitative Increase in Antibody Titre in* 16 *Individuals Vaccinated against Influenza A Virus*

Increase in Titre	Complement-fixation Test	Red-cell Agglutination Inhibition Tests	
		P.R.8 Virus	Melbourne Virus
×128	0	0	1
× 64	0	1	1
× 32	0	1	0
× 16	0	2	0
× 8	1	3	3
× 4	4	4	6
× 2	5	2	1
No increase ..	6	3	4

occurred in the response to the vaccine. The rise of antibody titre was greater when measured by the Salk test than by the complement-fixation test. The antibody response to vaccination therefore differs from that due to infection. In infection the body is exposed to large amounts of both virus and soluble antigen and reacts to both stimuli. The vaccine, however, contains only small amounts of soluble antigen, and the antibody response is therefore small when measured by the fixation test.

Discussion

The most striking feature of the red-cell agglutination inhibition test is its strain variability. Results obtained with one strain of virus A may differ completely from those obtained with a different strain. This phenomenon is even more marked with strains of virus B. This strain variation renders the test unsuitable for the serological diagnosis of epidemic influenza, since the results are dependent on a chance antigenic relationship between the infecting virus and that used in the tests. By contrast the complement-fixation test is species-specific, distinguishing sharply between virus A and virus B but giving identical results with individual strains of virus A or virus B. On theoretical grounds, therefore, the complement-fixation test should be more suitable for the serological diagnosis of epidemic influenza, and the work described in this paper shows that this theoretical superiority is in fact observed in practice.

In view of the considerable differences in antigenic structure between different strains of both virus A and virus B, the question arises what degree of difference from the classical strains would lead to a virus being regarded as a new species. We would suggest that the essential species characteristic is the soluble antigen. Any virus having a soluble antigen identical with that of the classical W.S. strain should be regarded as a strain of virus A, while any virus having soluble antigen identical with that of the Lee strain should be regarded as a strain of virus B.

The position of the swine virus is of interest in this connexion. On serological grounds there is no reason to regard the swine virus as anything other than a strain of virus A. Its soluble antigen is identical with that of the W.S. virus, and even in red-cell agglutination inhibition tests it is at least as satisfactory an antigen for the diagnosis of human infections as any human strain available. It might be maintained that the swine virus differs from other strains of virus A in being avirulent for man, but in this respect it does not differ from the majority of laboratory strains, which as a result of adaptation to mice or fertile eggs have lost human virulence. If the soluble antigen is regarded as the essential species character, then the complement-fixation test becomes the essential criterion for the diagnosis of influenza outbreaks. It has obvious advantages for this purpose. The range of complement-fixation titres of normal human sera is remarkably constant at a low level, while the majority of convalescent sera show titres above the normal range. It is advisable to examine two sera (acute and convalescent) from each patient, but a diagnosis of epidemic influenza can often be made by the examination of convalescent serum only.

The increase in antibody titre as a result of infection is generally greater when measured by the complement-fixation test than by the red-cell agglutination inhibition test, while the complement-fixation titres of normal sera are not greatly influenced by prophylactic vaccination. Moreover, the complement-fixation test presents no special technical difficulties, and we have obtained strictly comparable results even when working in different laboratories. Results obtained over a period of ten years also seem to be exactly comparable.

Summary

The results of complement-fixation tests on normal sera and the sera of influenza convalescents over a period of ten years are described.

The complement-fixation titres of normal human sera are very constant at a low level, while the majority of convalescent sera show titres above the normal range.

The complement-fixation test is species-specific, distinguishing between virus A and virus B, but shows no strain specificity such as is seen in the red-cell agglutination inhibition test. The complement-fixation test is therefore superior to the red-cell agglutination inhibition test for the serological diagnosis of epidemic influenza.

The complement-fixation test is not suitable to assess the antibody response to vaccination with influenza virus.

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THE GENERAL PRACTITIONER AND THE INFLUENZA PROBLEM

BY

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The general medical practitioner undoubtedly is closely concerned with the problem of human sickness attributable directly or indirectly to the operation of the influenza viruses. Long before the statistician has decided that an influenza epidemic has begun in the population, the practitioners in at least some parts of the country have begun to experience the well-recognized phenomena of crowded surgeries and long lists of sick people who require to be visited. There is no doubt that, if a solution of the problem of influenza could have been reached by mere clinical observation alone, someone concerned in actual general practice would have been the most likely person to suggest the remedy. As it is, however, mere clinical observation failed to distinguish any clear light in the darkness which enveloped the whole subject of influenza prior to the nineteen-thirties, when the first clues of the influenza viruses of animals and of man were picked up in the laboratory.

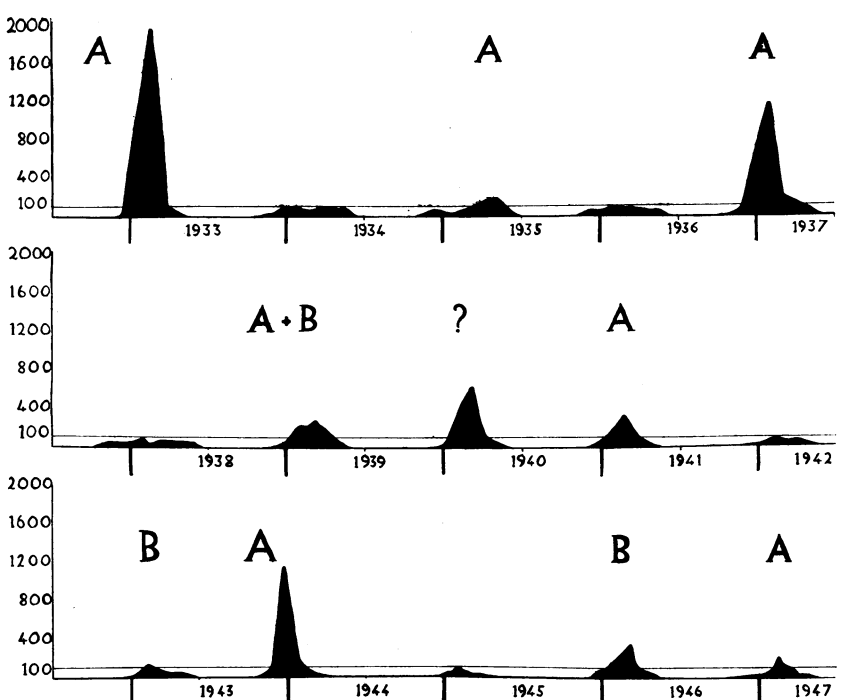

Chart showing number of influenza deaths in the great towns from 1933 to 1947, together with the causative virus.

Now, laboratory workers concerned with the influenza viruses have always recognized the fact that influenza is a disease which lies peculiarly within the province of the general practitioner. Whenever opportunity has presented, the laboratory findings and problems, both solved and unsolved, have been laid before practitioners in the traditional manner. If it had not been for the great practical difficulties connected with the laboratory technique needed to prove the existence of human influenza virus infection, patients under the care of general practitioners would have been chosen for investigation instead of individuals in the Services, school-children, and others who were in fact investigated. Nevertheless, though few direct tests have ever been made on subjects from a general practice, as knowledge has grown it has become clear that information furnished by the practitioners of this country has an important part to play in the general study of the epidemiology of influenza. The information referred to is that supplied in the form of notifications of deaths from influenza in the great towns of England and Wales. The manner in which this information has been correlated with the isolation of influenza virus from actual outbreaks of influenza or with serological tests has been referred to in detail on several occasions (Stuart-Harris, 1945, 1947). Briefly, the facts are that in the last ten years, whenever practitioners have notified 100 or more deaths from influenza in one week, laboratory workers have been able to obtain evidence that one or other of the influenza viruses is concerned in the causation of outbreaks of the disease in what are usually termed semi-isolated communities—i.e., schools, hospitals, or Service establishments. The years when the deaths never rose above 100 a week have also failed to supply the laboratory with evidence of virus infection. Furthermore, the laboratory worker has been able to correlate the type of curve obtained from plotting the figures of deaths from influenza with the type of virus concerned in the outbreak. Thus the sharp peaks reaching 1,000 or more in a week have been associated with influenza virus A. The lesser peaks between 100 and 500 have been associated with either or both of the two viruses A and B. These facts are shown in graphic manner in the accompanying chart.

Such facts would at once suggest that influenza virus infection in man is clinically recognizable. At the same time it has become clear to some of us who have studied patients from actual outbreaks that influenza virus infection in the individual is not recognizably different clinically from other acute infections of the respiratory tract. Furthermore, the cases which are notified as deaths from influenza are fatalities, whereas influenza is in the vast majority of instances a benign disease. Thus it has come about that those of us who have been concerned with research on the problem of influenza in this country are continually puzzled, first of all, why fatal cases of the human disease should be correlated with influenza virus infection, and, secondly, to know what sort of disease is constituted by this notification of a "death from influenza." The puzzle has not been merely that of the armchair speculative type but has concerned us because of the imperfection of our knowledge of the extent of the damage caused by the influenza viruses. We know that the virus is causative of that type of clinical picture commonly