A SEROLOGICAL INVESTIGATION INTO THE EPIDEMIOLOGY OF INFLUENZA WITH PARTICULAR REFERENCE TO SPORADIC CASES

By A. E. MARTIN
Sir H. Royce Research Fellow

From the Department of Bacteriology and Preventive Medicine, University of Manchester

It has been repeatedly noted that the virus of human influenza may be isolated only during widespread and typical outbreaks of the disease. All attempts to isolate the virus during minor or localized epidemics have failed (Fairbrother & Hoyle, 1937; Stuart-Harris and others, 1938). During non-epidemic periods it is not known whether a small proportion of apparent clinical influenza is actually caused by the virus or whether carriers, atypical and subclinical cases of the virus disease exist. No reservoir of the virus during non-epidemic times has as yet been demonstrated, nor has an explanation of the explosive nature of influenza outbreaks and their occurrence at periodic intervals been forthcoming.

The duration of immunity in man following an attack of influenza is unknown, but second attacks appear to be frequent. In this connexion two distinct hypotheses have been formulated; one holding that a decline in immunity takes place following the first attack so that eventually the individual again becomes susceptible to infection with the virus; the other maintaining that the attacks are caused by different strains of virus, the strain causing the first attack not giving much protection against infection by the strain causing the second.

With these problems in view an epidemiological investigation has been conducted in the Manchester district during the past two years. Isolation of the influenza virus is a somewhat uncertain laboratory procedure, particularly during inter-epidemic periods, and is not therefore likely to be a profitable method of investigation. It has, however, been shown that specific neutralizing and complement fixing antibodies to the influenza virus are produced in human sera as a result of attacks of the disease, and, further, that persons with a low influenzal antibody titre are more liable to experience attacks of the disease than persons with a high (Smith, Andrewes & Laidlaw, 1933; Hoyle & Fairbrother, 1937*a*).

Fluctuations in antibody titre give an indication of recent infection by the virus and consequently have been used as a basis for this investigation which has been conducted along the following lines:

(1) Repeated serological examinations of (*a*) samples of serum selected from the general community of the district, and of (*b*) a selected closed community as represented by certain of the residential hostels of the University of Manchester.

(2) Close clinical observation of all cases of upper respiratory tract infections occurring in the latter closed community during a period of 18 months.

(3) Serological examinations of the serum from cases of apparent clinical influenza collected from as many sources as possible in the district during this period.

TECHNIQUE

Neutralization test. This was carried out in the usual manner. Serum suitably diluted with saline was mixed with an equal volume of virus suspension containing approximately 10,000 minimal infecting doses, and after standing at room temperature for 15–30 min. the mixture was given intranasally to mice under ether anaesthesia. The animals were killed at the end of 4 or 5 days and the lungs closely examined for lesions. Recent work by Smith & Andrewes (1938) and Magill & Francis (1938) has demonstrated a number of antigenic factors by the neutralization test, and since the relative proportions of these may vary considerably in different strains, the test was largely abandoned during this investigation in favour of the complement fixation test.

The *Complement Fixation Test* was performed in the manner described by Fairbrother & Hoyle (1937). The antigen was a saline suspension of mouse lung lesions and $2\frac{1}{2}$ M.H.D. of complement were used. The titre was taken as the dilution of serum in the tube with the highest dilution showing complete fixation of complement. As opposed to the neutralization test, only one complement fixing antigen has been demonstrated in all strains of influenza virus so far investigated (Hoyle & Fairbrother, 1937*b*).

Isolation of virus was attempted from saline broth naso-pharyngeal washings of several typical cases of apparent influenza. Isolation was attempted both in ferrets by the method described by Hoyle & Fairbrother (1937*a*) and direct from man to mice by the method of Francis & Magill (1937).

RESULTS

The normal distribution of influenza complement fixing antibodies in the community

Previous workers have investigated the distribution of influenzal antibodies in the community, chiefly by means of the neutralization test. The most important work of this nature was by Andrewes, Laidlaw & Smith (1935) and by Francis & Magill (1936) who demonstrated the incidence of influenzal neutralizing antibody titres according to age, and found that infants had negative or low titres, that antibodies were usually acquired during adolescence or early adult life, and that high titres such as were found in convalescent cases of epidemic influenza were rare. Fairbrother & Hoyle (1937) showed that a similar distribution occurs with influenzal complement fixing antibodies.

At the commencement of the present investigation it was decided that some knowledge of the distribution of influenzal complement fixing antibodies in the general population of the district was necessary. At intervals, therefore, during

the past two years, a period in which epidemic influenza was not prevalent, samples of Wassermann sera were selected at random from a routine laboratory and tested. Although not a true sample in a statistical sense, the results demonstrated satisfactorily the general distribution of influenzal antibodies in the population.

Table 1. *Distribution of influenzal complement fixing titres according to age and sex*

Age group	No. of sera in each group	Titres: Negative and doubtful		Positive (titres 1 in 2 and 1 in 4)		Strongly positive (titres 1 in 8 and over)	
		No. of sera	%	No. of sera	%	No. of sera	%
All ages	900	398	44·3	373	41·4	129	14·3
0–1	6	6	100	0	0	0	0
1–9	32	24	75·0	8	25·0	0	0
10–19	58	33	56·4	20	34·1	5	9·4
20–29	290	110	37·9	120	41·4	60	20·7
30–39	183	70	37·2	87	46·5	26	16·3
40–49	118	49	41·5	50	42·4	19	16·1
50–59	134	59	43·9	62	46·2	13	9·9
Over 60	79	47	59·4	26	32·9	6	7·7
Sex:							
Males	453	196	43·3	194	42·8	63	13·9
Females	447	202	45·1	180	40·3	65	14·6

The results are summarized in Table 1, and it is verified that children have low complement fixing titres, that the highest proportion of strongly positive titres occurs in the age group 20–29, and that the lowest proportion of negatives is in the age group 30–39. It is also noticeable that there is a steady fall in titre in old age, and that there is no significant difference in distribution of titres in the two sexes. The figures agree very closely with similar ones published by Francis & Magill (1936) showing the normal distribution of neutralizing antibody titres to the influenza virus in the population of several large American cities.

In another group of individuals whose serum was investigated, histories were obtained of the date of the last attack of clinical influenza—the diagnosis resting on the individual's own description. The results have been classified in three groups in Table 2: (*a*) those who asserted that they had never had influenza,

Table 2. *Influenzal complement fixing titres of* 245 *adults correlated with clinical histories of "influenza"*

	Total no. of cases	Titres: Negative or doubtful		Positive (1 in 2 or 1 in 4)		Strongly positive (1 in 8 or over)	
		No. of cases	%	No. of cases	%	No. of cases	%
History of never having had "influenza"	71	46	62	22	31	3	5
History of "influenza" prior to Nov. 1936 or after March 1937	140	42	30	78	55	20	15
History of "influenza" between Nov. 1936 and March 1937	34	12	35	12	35	10	30

https://doi.org/10.1017/S0022172400027674 Published online by Cambridge University Press

(*b*) those who had had "influenza" at dates before November 1936 or after March 1937, and (*c*) those who had had it when true epidemic influenza was known to be prevalent (November 1936 to March 1937). It is noteworthy that a significantly high percentage in the third group had strongly positive titres as compared with the other two groups.

Variations in titre of samples of the general population during the period 1936 *to* 1939

Hoyle & Fairbrother (1937*a*) published a table comparing the titres of 300 sera selected at random from a routine laboratory with 63 sera from cases convalescent from the 1937 influenza epidemic. Similar random selections have been made at periodic intervals since this date and the results compared with those of Hoyle & Fairbrother (Table 3). It is seen that in a sample of 300 sera

Table 3. *Groups of sera, selected at periodic intervals and tested by complement fixation arranged to demonstrate their relationship to the* 1937 *influenza epidemic*

Titres	1 Nov. to Dec. 1936	2 Convalescent cases from the 1937 epidemic	3 Oct. to Nov. 1937	4 Oct. to Nov. 1938	5 March 1939
1 in 128	0	9	0	0	0
1 in 64	0	13	0	0	0
1 in 32	0	14	6	1	1
1 in 16	2	10	10	4	2
1 in 8	15	11	59	26	20
1 in 4	60	8	62	77	75
1 in 2	63	0	50	56	53
±1 in 2	64	0	42	55	63
Negative	96	0	71	81	86
Total	300	65	300	300	300

Columns 1 and 2 reproduced from Hoyle & Fairbrother (1937*a*).

selected in the autumn of 1936, immediately before the influenza epidemic of 1936–7, the proportion of high titres was low. A year later, in the autumn of 1937, six months after the widespread epidemic from which the influenza virus had been frequently isolated, a similar sample of sera selected from the same source demonstrated a very definite upward shift in the influenzal antibody titres of the population. In the autumn of 1938 another sample demonstrated a fall in titre of the population; a further sample in the spring of 1939 showed that this gradual fall in titre had continued and that the distribution of titres was approaching that of the pre-epidemic sample in 1936.

Variations in individual antibody titre during the period November 1937 *to March* 1939

Whilst periodic examinations of groups of sera from a routine laboratory were sufficient to show the general trend of the antibody level of the population in the district during the period under investigation, more precise knowledge was needed concerning the variations in individuals. Students from the resi-

dential hostels of the University of Manchester were therefore asked to volunteer, and an examination of the blood of each student was made during each of the university terms from November 1937 to March 1939. The variations in titre of each student from term to term were noted and the results summarized. Table 4 shows the results of 648 such observations carried out on a total of 267 students during the period. The titres of the majority of students remained

Table 4. *Analysis of changes in complement fixing antibody titres of* 267 *students each tested and retested at intervals of* 3–6 *months, giving a total of* 684 *serological observations*

		Variations from the original titre after a period of 3–6 months						
			Increase			Decrease		
Original titres at the commencement of each observation period of 3–6 months	*Total no. of observations*	No change	One dilution	Two dilutions	Three dilutions	One dilution	Two dilutions	Three dilutions
Negative	222	207	11	3	1	—	—	—
1 in 2	160	131	18	4	0	7	—	—
1 in 4	126	95	9	0	0	22	0	—
1 in 8	117	88	1	0	0	21	7	0
1 in 16	19	14	0	0	0	5	0	0
1 in 32	4	1	0	0	0	2	1	0
Totals	648	536	39	7	1	57	8	0

Examples

A student having a titre of 1 in 8 in Nov. 1937 and 1 in 2 in Jan. 1938 is entered as showing a decrease of 2 dilutions from the original titre of 1 in 8. Similarly a rise from negative to 1 in 8 as an increase of 3 dilutions from an original titre of negative.

An increase or decrease of one dilution is regarded as being within the margin of experimental error. An increase or decrease of more than one dilution is definite evidence of an actual change in antibody concentration in the individual concerned.

constant, but there were a few decreases in the higher dilutions and a few increases among the lower titres. An increase or decrease of one dilution may be regarded as being within the experimental error of the test, so that the results indicate that there was very little variation in the titres of individuals when considered over unit periods of from three to six months. It was noteworthy that the majority of decreases occurred shortly after the 1937 epidemic at a time when it has already been shown that there were more of the higher titres in the population than at periods subsequent to this.

As far as possible students were retained under observation for a consecutive period of at least eighteen months, and in Table 5 a comparison is shown between the titres of 116 students at the beginning of this period and of the same students at the end of the period. In this table it is more obvious that the titres of most of the students remained constant. It was, however, noted that when a fall in titre occurred, it was gradual and continued over many months, whereas when a rise in titre occurred it was generally abrupt, and after the rise the titre usually remained constant at the higher level. Such rises in titre were, however, invariably small when compared with similar abrupt rises which occurred in patients convalescent from true epidemic influenza. Thus the

largest was from negative to a titre of 1 in 8, whereas Hoyle & Fairbrother (1937*a*) frequently found rises such as 1 in 2 to 1 in 64 during the epidemic of February 1937.

Table 5. *Analysis of the changes in complement fixing antibody titres of* 116 *students each tested in November* 1937 *and retested after a period of* 18 *months*

Original titre in Nov. 1937	Total no. of observations	Variations from the original titre after a period of 18 months: No change	Increase: One dilution	Increase: Two dilutions	Increase: Three dilutions	Decrease: One dilution	Decrease: Two dilutions	Decrease: Three dilutions
Negative	44	37	2	4	1	—	—	—
1 in 2	25	18	6	1	0	0	—	—
1 in 4	21	13	4	0	0	3	1	—
1 in 8	20	10	1	0	0	5	2	2
1 in 16	5	2	0	0	0	2	1	0
1 in 32	1	0	0	0	0	1	0	0
Totals	116	80	13	5	1	11	4	2

Explanation: similar to Table IV.

Tables 4 and 5 have demonstrated variations in titre of the students without consideration of any illnesses from which they might have suffered during the period they were under observation. The next step in the investigation was to attempt to correlate changes in titre with any respiratory ailments which the students experienced. For the purpose of comparison Table 6 was drawn up showing the variations in titre of 182 students who did not suffer from any illnesses whatsoever during unit periods of from 3 to 6 months. A total of 261 such observations were made and it is again to be noted that while most of the titres remained constant a few fluctuations occurred, thus demonstrating that increases in titre may occur in apparently healthy individuals.

Table 6. *Analysis of changes in complement fixing antibody titres during* 261 *observations on* 182 *students each tested and retested at intervals of* 3–6 *months. Only students who remained healthy and free from all respiratory ailments included*

Original titres at the commencement of each observation period of 3–6 months	Total no. of observations	Variations from the original titre after a period of 3–6 months: No change	Increase: One dilution	Increase: Two dilutions	Increase: Three dilutions	Decrease: One dilution	Decrease: Two dilutions	Decrease: Three dilutions
Negative	83	79	2	2	0	—	—	—
1 in 2	73	57	8	1	0	7	—	—
1 in 4	46	34	7	1	0	4	0	—
1 in 8	50	34	0	0	0	12	4	0
1 in 16	8	4	0	0	0	4	0	0
1 in 32	1	0	0	0	0	1	0	0
Total	261	208	17	4	0	28	4	0

Explanation: similar to Table IV.

https://doi.org/10.1017/S0022172400027674 Published online by Cambridge University Press

The relationship between changes in antibody titre and the various common respiratory illnesses

Stuart-Harris (1937, 1938), basing his conclusions on the failure to isolate the influenza virus from all except typical cases of epidemic influenza, attempted to demonstrate a difference in clinical symptomatology between cases caused by the influenza virus and cases caused by other agents.

Actual isolation of the virus is, however, a difficult and uncertain procedure and is not a suitable method for the investigation of mild or atypical cases. Serological methods and, in particular, investigation by means of the complement fixation test offer alternative methods which should indicate any activity of the influenza virus in many of these milder conditions. Detailed records were therefore kept of all respiratory ailments suffered by the 267 students under observation during the present investigation. The various conditions were classified into groups according to symptomatology, and each group correlated with the changes in antibody titre of the individuals in that group. By this means it was hoped to demonstrate if any case or group of cases was etiologically associated with infection by the influenzal virus, and to show whether the virus exists in non-epidemic times as a cause of vague endemic respiratory disease or whether the influenza virus produces disease only in typical influenza epidemics.

Each group of respiratory ailments suffered by the students is considered in turn and the relationship of each to the influenza virus discussed.

(*a*) *The common cold.* A total of 303 attacks of common cold were studied. Of these 253 showed exactly the same complement fixing titres both before and after the attack, forty-eight showed insignificant variations, and only two showed an increase of titre which was outside the experimental error of the test. This was actually less than the number of significant increases which by reference to Table 6 would be expected in a group of healthy students of similar number, who experienced no respiratory illnesses during corresponding periods of time. It may, therefore, be concluded that the experiencing of a common cold is not in general associated with a rise in influenzal complement fixing titre, and as a further indication that there was usually no serological relationship it was observed that there was no greater tendency for people with low antibody titres to suffer from common colds than people with high titres.

(*b*) *Acute afebrile catarrhs.* This group included afebrile catarrhal inflammations of the pharyngeal, tonsillar, peritonsillar and laryngeal regions but excluded uncomplicated cases of "common cold". One hundred and twenty-seven attacks were investigated and of these only one showed a significant rise in titre following the attack. In this case the complement fixing antibodies which were negative before the attack rose to positive in a dilution of 1 in 4 after the attack. Since it has already been shown that similar spontaneous rises occurred in four out of a group of 261 observations on healthy students experiencing no

illnesses, it is doubtful if the respiratory catarrh in this one case was actually responsible for the rise in titre.

The diagnosis of acute afebrile catarrh of the upper respiratory tract is a vague one and doubtless includes conditions due to a number of etiological agents. The various common catarrhal bacteria are almost certainly responsible for many of the cases, but it would be expected that if the virus of epidemic influenza is also a cause of a small proportion of endemic disease and if carriers and atypical and subclinical cases exist, then evidence might reasonably be expected to be found in this group. The results appear to indicate that if such cases do occur, then they are either so mild that they do not cause a rise in antibody titre, or that such cases are comparatively rare.

(*c*) *Cases of febrile catarrh and apparent influenza.* One hundred and twenty-one attacks of febrile illnesses associated with varying degrees of inflammation of the upper respiratory tract were studied. These were all of the type which would commonly be diagnosed as influenza upon clinical grounds. The cases were considered as a group in the first place, and when classified according to the complement fixing titre of the convalescent sera it is seen that the general distribution of titres is not raised above that of a similar group of normal healthy individuals (Table 7). The titres are in marked contrast to a group of typical convalescent sera studied by Hoyle & Fairbrother after the 1937 influenza epidemic. It is evident that most of the cases, although similar in symptomatology, were not cases of influenza produced by the virus.

Table 7. *Influenzal complement fixing titres of convalescent sera of* 121 *cases of febrile catarrh and apparent influenza, compared with the distribution in a group of healthy adults and with a small group of convalescent cases from the* 1937 *epidemic*

Titres	1 Convalescent sera of cases of febrile catarrh and apparent influenza	2 Normal distribution of titres in a group of healthy adults	3 Convalescent sera from the 1937 influenza epidemic
1 in 128	0	0	9
1 in 64	0	0	13
1 in 32	0	1	14
1 in 16	4	8	10
1 in 8	14	50	11
1 in 4	33	46	8
1 in 2	37	73	0
±1 in 2	10	21	0
Negative	23	62	0
Totals	121	261	65

Column 3 reproduced for comparison from Hoyle & Fairbrother (1937*a*).

For more precise etiological information individual cases were studied and the complement fixing titres both before or during the early stages of the attack and later during convalescence were compared. Fifty-three cases were studied in this manner; in fifty-two of these cases there was no rise in antibody titre as a result of the attack and in only one case was a rise in titre noted, and this, a rise n titre from 1 in 4 to 1 in 8, was within the experimental error of the test. Thus

again there was a marked contrast to the findings in cases of true influenza, such as were experienced during the 1937 epidemic, when a sudden rise in titre was noted in almost all cases following infection by the influenza virus (Hoyle & Fairbrother, 1937*a*).

The results were verified by means of the neutralization test in twenty-four cases, and with this test also no sudden rises in titre were detected, neither were the convalescent titres raised above normal.

In fourteen cases efforts were made to isolate the virus by inoculating ferrets and mice with the nasopharyngeal washings from typical cases. In no case was the influenza virus isolated. Bacteriological cultures were made as a routine upon all nasopharyngeal washings, with the object of excluding, as far as possible, cases primarily due to the common catarrhal bacteria.

Discussion

This investigation has demonstrated that influenzal complement fixing antibodies exist to a low titre in the sera of 50–60 % of adults, and that antibodies are usually low at birth, rise during early adult life and fall slightly during old age. It has further been noted that following the 1937 epidemic a greater proportion of high titres was found in the population than at the time immediately preceding the epidemic, and that in the case of convalescent sera the titres were usually much higher than those obtained during inter-epidemic periods. Fairbrother & Martin (1938) found, however, that this high level was not usually maintained for any length of time, but that in the majority of cases the titres returned to the normal range in 6–9 months.

During the non-epidemic period, 1937–9, the proportion of high titres in the population has gradually declined until at the end of the period the distribution of titres was becoming similar to that immediately preceding the 1937 epidemic. It has been shown by previous workers that an attack of true epidemic influenza is almost invariably followed by a rise in complement fixing antibody titre, and that the antibody titre is an approximate guide to an individual's immunity to the virus of epidemic influenza. It appears, therefore, that while infection by the influenza virus produced an appreciable rise in antibody titre of the general population during the 1937 epidemic, in the period following the epidemic, infections by the influenza virus were considerably rarer. Interpreting the results in terms of the probable immunity of the population it suggests that following the 1937 epidemic the population as a whole acquired an increased immunity, and that as a result of the relative absence of infections by the influenza virus during the succeeding two years the immunity of the population has since then been gradually declining and approaching the degree of susceptibility which preceded the 1937 epidemic.

When the individual titres of a group of some 267 students were considered it was found that nearly all remained remarkably constant during the period under investigation. A few who had high titres at the beginning of the investigation showed a gradual fall in titre, and very few showed small spontaneous

rises. At the end of the period there were very few high titres amongst the group of students. The observations on individual titres therefore bear out the conclusions already arrived at as a result of the study of the titres of sections of the general population, and indicate that, in non-epidemic times, the incidence of infection by the influenza virus must be very low. Occasional sudden rises in titre do, however, occur, often in people who have not experienced recently any respiratory ailments, and suggest that the virus may not be entirely absent from the community.

The relationship of fluctuations in complement fixing antibody titre to various respiratory tract ailments suffered by the students has been investigated with the object of determining whether the influenza virus was responsible for any of the milder upper respiratory tract infections, or whether the virus was found only as the etiological agent of typical influenza attacks during epidemic times. No evidence could be found that any of the commoner upper respiratory tract infections were associated etiologically with the influenza virus. Even in cases which were diagnosed clinically as sporadic cases of influenza, and which closely resembled in clinical features the true epidemic disease, serological evidence that the influenza virus was involved was almost invariably absent, and of a small number of these cases in which attempts were made actually to isolate the virus the results were negative. Occasional variations in titre were as frequent in healthy individuals as in those who had experienced respiratory ailments.

The more important findings of this investigation are therefore in agreement. The virus of epidemic influenza is undoubtedly the cause of periodic widespread influenza epidemics, but in non-epidemic times little disease is caused by the virus. Carriers and a few subclinical or atypical cases may exist, but the vast majority of upper respiratory tract infections in non-epidemic times are due to other causes. Some evidence of the periodicity of disease due to the influenza virus is obtained from an examination of the antibody titres of samples of sera from the general population; immediately after an epidemic there is a sudden rise in titre of the population and this is followed by a gradual decline in titre which appears to continue until the next epidemic occurs. When the titres of individuals are considered few fluctuations are found, and this again suggests the almost complete absence of both typical and atypical cases of epidemic influenza in non-epidemic times. Low titres remain remarkably constant. High titres such as are frequently found after an epidemic, many of which are due to actual infection by the virus, usually undergo a gradual decline, though a few may remain constant. Since in the majority of individual cases the antibody titre is an approximate guide to a person's immunity, it appears that one factor in the periodicity of influenza epidemics may be the periodic rise in titre of the population associated with the epidemic, and the slow fall in titre which occurs during the subsequent inter-epidemic period.

SUMMARY

An investigation has been described in which the complement fixing antibody titres of selected groups of the population have been correlated with the occurrence of epidemic and sporadic influenza and of other cases of respiratory disease which it was thought might be due to the virus of epidemic influenza.

During two non-epidemic seasons the influenza virus could not be isolated from cases of apparent influenza neither could any serological evidence be obtained of the existence of any form of respiratory disease caused by the virus.

With rare exceptions individual antibody titres either remain constant, or if they are high show a tendency to fall during non-epidemic times, and periodic investigations of sample groups each consisting of several hundred sera show a wave-like fluctuation in the titres of the population consisting of a sudden rise followed by a gradual fall, the former being an accompaniment of an epidemic. Such a wave-like fluctuation appears to be an indication of variations in the immunity of the population, and to be responsible in part, at least, for the occurrence of large influenza epidemics.

I wish to acknowledge the help received from all who took part in this investigation, and in particular to thank the students of the University Halls of Residence in Manchester, the numerous doctors who helped by supplying particulars of cases of influenza and to Dr R. W. Fairbrother who has given advice and maintained a constant interest throughout the investigation.

REFERENCES

ANDREWES, C. H., LAIDLAW, P. P. & SMITH, W. (1935). *Brit. J. exp. Path.* **16**, 566.
FAIRBROTHER, R. W. & HOYLE, L. (1937). *J. Path. Bact.* **44**, 213.
FAIRBROTHER, R. W. & MARTIN, A. E. (1938). *Lancet*, **1**, 718.
FRANCIS, T., junr. & MAGILL, T. P. (1936). *J. exp. Med.* **63**, 655.
—— —— (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 132.
HOYLE, L. & FAIRBROTHER, R. W. (1937*a*). *Brit. med. J.* **1**, 655.
—— —— (1937*b*). *J. Hyg., Camb.*, **37**, 512.
MAGILL, T. P. & FRANCIS, T. junr. (1938). *Brit. J. exp. Path.* **19**, 273.
SMITH, W. & ANDREWES, C. H. (1938). *Brit. J. exp. Path.* **19**, 293.
SMITH, W., ANDREWES, C. H. & LAIDLAW, P. P. (1933). *Lancet*, **2**, 66.
STUART-HARRIS, C. H. (1937). *Brit. med. J.* **2**, 516.
STUART-HARRIS, C. H. and others (1938). *Spec. Rep. Ser., Med. Res. Coun., Lond.*, no. 228.

(*MS. received for publication* 26. IX. 39.—Ed.)