UNIVERSITY OF GLASGOW

CONTRIBUTIONS TO THE IMMUNOLOGY AND EPIDEMIOLOGY OF CERTAIN INFECTIOUS DISEASES.

A Thesis

for the Degree of Doctor

of Medicine, presented

by

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"From these things the physician must proceed to investigate certain others in particular, so that, when he comes into a strange city he will understand the diseases there endemic, and the modifications of common maladies that there prevail".

(Hippocrates, Airs, Waters, and Places)

"Postremo observandum est quoties constitutio aliqua varias Epidemicorum species parit, singulas has species genere ab illis differre; quae cum idem plane nomen sortitantur, alia tamen constitutione generentur".

(Sydenham, Obs. Med., I, 2, (18))

"It is a misfortune which makes the theoretical consideration of our subject very difficult that so few observations have been so set out and described that they can be prudently used. There is in the whole field scarcely a proposition which cannot be supported and also refuted from the results of observation. This being so, some will warn us to abstain from theory until our experience is riper. I, however, believe that for the ripening of experience the light of an intelligent theory is required."

(Jakob Henle, Von den Miasmen und Kontagien).

"If the latent cause of epidemics cannot be discovered, the mode in which it operates may be investigated. The laws of its action may be determined by observation, as well as the circumstances in which epidemics arise, or by which they may be controlled."

(William Farr)

"When you can measure what you are speaking about and express it in numbers, you know something about it, but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind."

(Lord Kelvin)

PREFACE

The quotations on the preceding page give a concise picture of the problems which were before the writer from the commencement of the investigations which form the subject of this Thesis. The whole work is essentially a treatise on the reactions of communities to the introduction of new types of infections; and the result is a discussion, from experimental, historical, and bibliographical aspects, of a subject which is too often neglected even in these days of the advancement of epidemiology.

The first part of the Thesis is for all practical purposes a dissertation written with this end in view. It deals with the experimental work which the writer carried out on the immunology of diphtheria. In the introductory section it is shown that this work was carried out in the area in which the gravis strain of the C. diphtheriae first appeared, and in which it has been most prevalent. Practically the whole of these investigations were planned nearly three years ago; and with the exception of one item (which has since been dealt with) the writer was able to complete the whole of the scheme before he left Leeds to take up an appointment in London.

The second part of this Thesis deals with three diseases, influenza, cholera, and the encephalitis which is a rare complication of varicella. These three sections have been previously published; but they are included here because they illustrate, each in its own way, the reactions of communities to epidemic diseases of a new type. The writer originally intended to add an epilogue linking up these different observations, and showing their connections, from an

epidemiological standpoint, with the problems raised by the introduction of gravis-diphtheria into a community. It was found, however, that this discussion was leading the writer far beyond the bounds which he had set for the present work, and he therefore decided that this general discussion must be the basis for a future investigation.

Each of the three papers which constitute the second part of the Thesis is summarized individually. At the end of Part I will be found a very brief résumé of the experiments connected with diphtheria, but it will be obvious that only a few of the points which are discussed in the text could be included in this summary.

The Bibliography for the whole of the Thesis will be found at the end of the volume.

Note regarding publication. Section (h) of PartI was published in The Lancet (1935, i, 364 - 68), but all the other sections which make up Part I are quite new. Notes on the use of "one-shot" methods appeared in The British Medical Journal (1934, i, 1140) and The Lancet (1935,i, 137). Other papers on diphtheria appeared in The Lancet (1934,i,678) and The Medical Officer (1934,1i, 195, 205). Section (d) of Part I will be published unaltered in the forthcoming number of the Journal of Hygiene. Arrangements have not yet been made for the publication of the other sections of Part I.

The section on Influenza was published in the Journal of Hygiene (1934, xxxiv, 409). The section on Cholera was delivered before the Section of the History of Medicine of the Royal Society of Medicine, and was published in the Proceedings (1935, xxviii, 603). The third section of Part II is appearing in serial form in The British Journal of Children's Diseases, and the first two instalments have now been published.

C O N T E N T S

P A R T I.

S T U D I E S I N T H E I N C I D E N C E

A N D P R E V E N T I O N O F D I P H T H E R I A,

W I T H S P E C I A L R E F E R E N C E T O

"G R A V I S - I N F E C T E D" C O M M U N I T I E S.

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PART I.

SECTION (a)

Introduction: The recent behaviour of Diphtheria in Leeds and other Cities.

A peculiar feature of the behaviour of the infectious diseases in the twentieth century has been the increasing divergence of the characters of scarlet fever and diphtheria. Although Bretonneau first gave the name "diphthérite" in 1821 - if he was not the first actually to differentiate the two diseases- confusion of terminology existed for many years, and it was not until 1859 that scarlet fever was tabulated separately from diphtheria in the Annual Reports of the Registrar-General. These two diseases, although clinically closely related, have within recent years presented many contrasts when regarded from an epidemiological standpoint. Woods in her important studies on these two diseases (1928, 1933) showed that the diminution in the deaths from scarlet fever in England and Wales during the past thirty years was essentially due to lessened severity of the disease. On the other hand, though diphtheria had not diminished to anything like the same extent, the tendency of the mortality from this disease was to concentrate on early school age.

At the time when this paper of Woods (1928) appeared diphtheria was beginning to behave in a peculiar fashion in various parts of Europe. The first suggestion that all was not well was given by Finkelstein, Deicher, and Agulnick (1927), who in 1927 reported that in Berlin there was a significant increase in the

number of cases of diphtheria, and especially of cases of a severe type. They stated that there had been a steady rise in the case mortality of diphtheria in the Virchow Krankenhaus, from five per cent in 1924 to 26.7 per cent in the early months of 1927; that there was also a corresponding increase in the mortality for Alt Berlin as a whole. Von Bokay (1931) states that as early as 1926 he was of the impression that the type of diphtheria which he met with in Budapest was becoming more severe, and the events of the next few years, marked not only by an increase in the number of cases but also by increased frequency of severe angina, amply bore out this prediction. Severe epidemics were also reported from Prague by Feierabend and Schubert (1929); from Lille by Minet (1929); from Italy by Cayrel (1930); and from Roumania by Simic (1931).

In this country the first appearance of a type of diphtheria which did not respond to antitoxin appears to have been in Leeds, where Anderson was certainly noting such cases as early as 1930. By the use of a new tellurite medium McLeod and his colleagues were able to show that there were at least three distinct cultural forms of C.diphtheriae, and these they proposed to call the gravis, mitis, and intermediate strains. Anderson was able to demonstrate that in Leeds the C.diphtheriae gravis was associated with the severe toxic cases, and was responsible for nearly all the deaths. Mitis cases were much milder in degree, toxaemia was not in evidence, and difficulties occurred only when there was extension to the larynx. The intermediate types were at that time rare in Leeds, but it was suggested that they were associated with intermediate forms of the disease. (See Anderson et al. (1931) and Anderson, 1932).

Criticism of the work of these authors was made by Menton, Cooper and Fussell (1933), and by Menton, Cooper, Duke, and Fussell (1933) on the grounds that in Staffordshire the strains of the C.diphtheriae could not be classified into three definite types according to colony appearance and biochemical reactions; they were also unable to justify the term "gravis" in its original sense. Corroboration soon came, however, from certain other areas, notably Hull, Manchester and Glasgow. In Hull a severe type of diphtheria

had also appeared, and Leete had the strains examined by McLeod. It was found that in Hull nearly sixty per cent of all cases were due to the gravis strain, and that of forty toxic deaths, thirty-five were due to gravis and five to intermediate strains. In Manchester Robinson and Marshall (1934) showed that, apart from 1.5 per cent atypical strains, the strains of the C.diphtheriae could easily be separated into the three types of the Leeds workers, but that in that city the intermediate strain was at least the equal of the gravis strain in its capacity to produce severe infections. Robinson (1934) later showed that in Manchester the strains showed a high degree of type stability, even after animal passage. Carter (1933), working in Glasgow, showed that ninety nine per cent of 510 local strains could be separated into the three types by the method of the Leeds workers. In later papers Anderson, McLeod and their colleagues (1933a, 1933b) extended their observations on the correlation between types and clinical severity, on the stability of the types, and on the fermentation reactions.

It is not intended in this Introduction to discuss further the already extensive literature relating to these strains. Many of the later papers will be discussed in the appropriate place in this thesis. It should however be mentioned that the contentions of the Leeds workers have never been seriously challenged so far as the bacteriological aspect is concerned. Agreement regarding the clinical aspect depends largely on whether the gravis or intermediate strains are very frequent in the area under discussion. Apart from a few comments by Robinson and Marshall (1934) no paper dealing with the immunological aspect appeared until the publication of a paper which forms a portion of this thesis (Underwood, 1935b). It should also be mentioned that no areas present such favourable opportunities for the study of the behaviour of diphtheria due to the gravis strain as do Leeds and Hull. In Leeds especially this strain has been very prevalent for at least five years, and the sections which follow will show to what extent it has introduced difficulties in dealing with diphtheria which are incomparably greater than any other difficulty

since the introduction of antitoxin into this country in 1894.

The writer took up an official position in Leeds in January, 1932, and for nearly three years he was responsible for the control of this disease in the City. During the greater portion of this period he carried out the observations which will be detailed in later sections. It was early realised that the introduction of the gravis strain into Leeds was not merely a transient local phenomenon, but that there were indications that a definite epidemiological change had taken place, and that the results were in a way comparable to those which occur when a new or hitherto unexperienced disease appears in a locality. For some time an investigation has been proceeding on these lines, but the results are not yet sufficiently far advanced for publication. This introduction, though in no sense a complete epidemiological enquiry, is intended to give the lines upon which this investigation is proceeding. It will serve to indicate some of the difficulties associated with a "gravis-infected" area, and to explain the need for the investigations which are detailed in later sections of this thesis.

The Incidence of Diphtheria in England and Wales.

The notified cases of diphtheria which occurred in England and Wales from 1912 onwards are given in Table I, together with the case rates per 1,000 of the population. It will be seen that there has been no very remarkable increase in the incidence of the disease in recent years. Peaks of the curve of incidence occurred in 1920-21, and in 1930. There is, however, nothing here to indicate that the disease has taken on tendencies which are rather alarming.

TABLE I.

England and Wales: Diphtheria Incidence.

Year	Cases Notified	Case rate per 1,000 population.
1912	44,687	1.24
1913	50,850	1.39
1914	58,856	1.61
1915	53,549	1.52
1916	51,674	1.50
1917	43,274	1.28
1918	43,678	1.31
1919	53,843	1.50
1920	69,407	1.86
1921	66,452	1.76
1922	52,072	1.37
1923	39,953	1.05
1924	41,948	1.07
1925	47,684	1.23
1926	51,034	1.31
1927	51,973	1.33
1928	61,109	1.55
1929	62,739	1.59
1930	73,928	1.84
1931	50,208	1.26
1932	43,339	1.08
1933	47,388	1.18

Table II.

England and Wales: Diphtheria: Mortality

Year	Deaths	Death rate per million living
1890	5150	179
1891	5036	173
1892	6552	222
1893	9466	318
1894	8774	291
1895	7895	259
1896	8993	291
1897	7654	246
1898	7661	243
1899	9295	292
1900	9345	290
1901	8898	273
1902	7802	237
1903	6077	183
1904	5763	170
1905	5456	161
1906	6108	178
1907	5732	165
1908	5569	158
1909	5235	148
1910	4284	120
1911	3917	136
1912	4298	118
1913	4447	121
1914	5863	159
1915*	5865	165
1916*	5366	153
1917*	4477	130
1918*	4803	140
1919*	4888	137
1920*	5648	151
1921	4772	126
1922	4075	107
1923	2722	71
1924	2501	64
1925	2774	71
1926	2994	77
1927	2732	70
1928	3191	81
1929	3446	87
1930	3497	88
1931	2673	67
1932	2339	58
1933	2646	66

*The mortality for the years 1915-1920 relates to civilians only.

The figures for the deaths, which are given in Table II, are apparently even more satisfactory. After the preliminary antitoxin period, say from 1894 to 1900, the death rates declined from the region of 290 per million to a figure which varied from about 120 to 160 in the war years. Since 1921 there has been a very definite and hitherto unexplained fall in the death rate. In 1921 it was 126, in 1922 it was 107, and by 1923 the figure had fallen to 71. Since that date the highest death rate has been 88 (in 1930). Even in the last few years there has been a further fall, and the death rate for 1932 (viz. 58) is actually the lowest ever recorded from this disease. There is therefore nothing in these case or death rates to indicate that diphtheria in this country is not declining in incidence and severity - apart from moderate periodic epidemics - and that the efficacy of antitoxin is not being amply demonstrated by the gradual reduction in the death rates.

One of the peculiar features of the gravis strains is that they tend to be found only in larger urban areas, such as the great cities. This statement must be made with a certain amount of qualification, since comparatively few reports have appeared on the subject of the incidence of gravis infections in smaller areas. The population of the Registrar-General's 121 Great Towns is 20,850,000, which is less than half the total population of the country. When we consider that only a few of these "Great Towns" have infection to any degree with gravis strains, it will be evident that a consideration of rates for England and Wales will give little indication of the incidence and severity of diphtheria in those areas in which epidemics are most likely to be extensive and severe - viz., the great cities. It is therefore necessary to consider in some detail the rates for these cities.

Incidence of Diphtheria in certain cities.

It might be thought that statistics for diphtheria for the great cities would be easily obtained. Such is not, however, the case. In the first place, "fashion" in diagnosis plays a not-inconsiderable role. There is little doubt that the practice of taking

swabs in every case of sore throat, however slight, tends to magnify the number of notified cases of true diphtheria. This tendency is to a large extent counter-balanced by the fact that the notification is cancelled if the patient is removed to hospital - and in most large cities the majority of diphtheria patients are removed to an institution. In the second place, there is sometimes a remarkable discrepancy between different official statistics for the same city. In preparation for a larger epidemiological inquiry the writer began to collect statistics of the incidence of diphtheria in various areas, and it was soon evident that the figures which are given by the Registrar-General in his Annual Reports and Statistical Reviews often differed considerably from the figures given in the reports of the respective Medical Officers of Health. It may be objected that such a discrepancy is to be expected, and that it may be so small that it will be quite insufficient to affect any rates calculated therefrom. It is, however, easy to show that such objections carry little weight. The following are six percentages expressing the percentage differences between the Registrar-General's figures and the Medical Officers of Health's figures for six years taken at random in respect of four cities:- 20.7, 17.6, 3.0, 53.8, 1.1, 1.3. It will be obvious that for accurate work the Registrar-General's figures cannot be accepted, and that as a result of change of diagnosis in hospital, and possibly other factors, the Medical Officers of Health's figures must be taken as giving a truer indication of the incidence of diphtheria in any particular locality. Acting on this conclusion the writer collected statistics from the reports of various Medical Officers, and from these figures all the rates which are contained in the following tables were recalculated. It should also be mentioned that, apart from the difficulty of obtaining copies of the older reports, an unforseen obstacle was that the reports often did not give the information which was desired. In the earlier reports two figures are given for incidence - one being the Medical Officer of Health's official figure, and the other being

simply a statement of the number of notifications received during the year. This may sound trivial, but in practice it is often difficult to decide which figure gives the truer estimate of the incidence of the disease. It should also be mentioned that in every instance the cases of and deaths from membranous croup were added to the respective figures for diphtheria, so that the calculated rates in the following tables represent the incidence of cases and deaths for the two diseases taken together. The rates were calculated from the estimated mid-year population at each year. The statistics for six cities - Leeds, Hull, Manchester, London (Administrative County), Liverpool and Birmingham - are set out in Tables III to VIII inclusive.

Table III.

LEEDS; Diphtheria: Incidence and Mortality.

Year	Case rate per 1,000	Death rate per 1,000
1897	0.52	0.18
1898	2.17	0.56
1899	4.32	0.80
1900	2.95	0.60
1901	2.41	0.41
1902	1.53	0.22
1903	1.38	0.15
1904	0.84	0.12
1905	0.86	0.10
1906	1.54	0.19
1907	1.32	0.16
1908	1.43	0.11
1909	2.99	0.15
1910	2.14	0.16
1911	2.62	0.35
1912	1.58	0.21
1913	1.89	0.19
1914	1.53	0.13
1915	0.88	0.11
1916	0.95	0.09
1917	1.25	0.14
1918	1.27	0.11
1919	1.88	0.10
1920	1.97	0.14
1921	1.43	0.08
1922	1.01	0.06
1923	0.78	0.04
1924	0.61	0.06
1925	0.89	0.08
1926	0.79	0.05
1927	0.92	0.06
1928	1.34	0.04
1929	1.12	0.05
1930	2.08	0.11
1931	2.05	0.18
1932	1.83	0.10
1933	2.18	0.18
1934	4.62	0.33

TABLE IV

HULL: Diphtheria: Incidence and Mortality

Year	Case rate per 1,000	Death rate per 1,000
1893	0.50	0.10
1894	0.40	0.09
1895	0.48	0.12
1896	0.71	0.18
1897	0.87	0.08
1898	0.52	0.04
1899	0.67	0.10
1900	0.42	0.06
1901	1.96	0.16
1902	1.96	0.35
1903	0.98	0.30
1904	2.07	0.24
1905	2.71	0.28
1906	4.77	0.51
1907	2.61	0.24
1908	1.72	0.17
1909	1.61	0.22
1910	1.59	0.16
1911	1.23	0.08
1912	1.10	0.08
1913	1.46	0.12
1914	1.55	0.18
1915	2.48	0.22
1916	1.37	0.09
1917	1.47	0.11
1918	1.22	0.21
1919	1.68	0.12
1920	2.05	0.09
1921	2.23	0.12
1922	1.81	0.09
1923	1.42	0.08
1924	1.08	0.07
1925	1.68	0.09
1926	2.52	0.06
1927	2.52	0.11
1928	2.26	0.07
1929	2.84	0.12
1930	2.72	0.15
1931	3.63	0.30
1932	5.36	0.42
1933	4.74	0.29
1934	3.32	0.21

TABLE V.

MANCHESTER: Diphtheria: Incidence.

Year	Case rate per 1,000
1893	1.20
1894	0.98
1895	0.76
1896	0.45
1897	0.28
1898	0.36
1899	0.45
1900	0.61
1901	0.84
1902	0.77
1903	1.12
1904	0.85
1905	0.84
1906	0.86
1907	0.78
1908	0.84
1909	0.91
1910	0.70
1911	0.66
1912	0.65
1913	0.89
1914	1.01
1915	0.73
1916	0.83
1917	0.79
1918	0.69
1919	0.61
1920	1.24
1921	1.35
1922	1.08
1923	0.71
1924	0.75
1925	1.37
1926	1.50
1927	1.58
1928	1.35
1929	0.99
1930	1.10
1931	0.75
1932	1.15
1933	0.95
1934	

TABLE VI.

LONDON (Administrative County): Diphtheria: Incidence.

Year.	Case rate per 1,000
1894	2.57
1895	2.55
1896	3.11
1897	2.95
1898	2.63
1899	3.00
1900	2.66
1901	2.67
1902	2.34
1903	1.68
1904	1.55
1905	1.38
1906	1.70
1907	1.84
1908	1.67
1909	1.38
1910	1.21
1911	1.63
1912	1.57
1913	1.69
1914	2.02
1915	2.11
1916	2.06
1917	2.06
1918	1.84
1919	2.17
1920	3.04
1921	3.63
1922	3.38
1923	2.26
1924	2.33
1925	2.71
1926	2.94
1927	2.68
1928	2.73
1929	2.67
1930	3.06
1931	1.92
1932	1.86
1933	2.22

TABLE VII.

LIVERPOOL: Diphtheria: Incidence.

Year	Case rate per 1,000.
1894	0.65
1895	0.52
1896	0.81
1897	0.65
1898	0.83
1899	1.17
1900	1.04
1901	1.27
1902	1.55
1903	1.22
1904	1.47
1905	1.37
1906	1.23
1907	1.25
1908	1.36
1909	1.49
1910	1.50
1911	1.78
1912	1.48
1913	1.43
1914	1.79
1915	1.61
1916	1.44
1917	1.31
1918	1.67
1919	2.51
1920	2.12
1921	1.45
1922	1.16
1923	1.20
1924	1.32
1925	1.78
1926	1.82
1927	1.94
1928	2.20
1929	2.75
1930	4.57
1931	3.80
1932	3.84
1933	3.37

TABLE VIII.

BIRMINGHAM: Diphtheria: Incidence

Year	Case rate per 1,000.
1890	0.69
1891	0.48
1892	1.10
1893	0.79
1894	0.83
1895	1.50
1896	2.35
1897	1.41
1898	1.36
1899	1.40
1900	1.05
*1901	1.04
1902	1.44
1903	1.52
1904	1.15
1905	1.23
1906	1.46
1907	1.81
1908	1.49
1909	1.38
1910	1.28
1911	1.35
1912	0.95
1913	1.13
1914	1.84
1915	1.21
1916	1.07
1917	0.86
1918	1.02
1919	1.05
1920	1.93
1921	1.80
1922	1.39
1923	1.65
1924	1.97
1925	2.00
1926	1.88
1927	1.60
1928	1.59
1929	1.64
1930	1.73
1931	1.16
1932	0.61
1933	0.83

* The figures from 1901 onwards relate to Greater Birmingham.

Discussion of Trend of Incidence and Mortality.

The incidence of diphtheria at each year in six of the selected cities is plotted graphically in Figs. I to VI inclusive. (In the case of Hull it was quite impossible for the writer to obtain in London copies of some of the pre-War reports, and he is therefore much indebted to Dr. Nicholas Gebbie, the Medical Officer of Health, for supplying the missing figures).

From a rough inspection of the graphs it will be seen that the epidemic cycle is not the same in all cities. In Leeds, as the writer showed in a previous communication (1934b), the inter-epidemic period is 10 to 11 years, and this interval has been remarkably constant. In London the interval between the two major epidemics was 22 years; and the interval between minor epidemics seems to be about nine years. In Liverpool the interval is similar - about 8 to 11 years. On the other hand, both Manchester and Birmingham show a short inter-epidemic period - in the case of Manchester about six years, and in the case of Birmingham four to seven years - and usually six or seven. In Hull the minor epidemics are masked by the two outstanding peaks. Despite this, it would appear that in Hull minor epidemics tend to occur at intervals of seven to nine years.

The second point which emerges from a casual inspection of the graphs is that the introduction of the gravis strain into an area does not necessarily manifest itself by a marked increase in the incidence of the disease. As has been stated previously, the gravis strain was certainly prevalent in Leeds in 1930, yet the increased incidence in that year was not above what might have been expected. The effects of the new strain might possibly have shown themselves by an increase in the mortality rate; but when it was realised that a severe type of diphtheria was present in the community, measures were immediately taken to deal with the increasing severity. These measures - such as the giving of very large doses of antitoxin, in part intravenously - almost certainly modified the resulting death rate. In any case, the effects of the disease, though deferred,

were not prevented completely - as the enormous explosive outbreak of 1934 showed. We should therefore be cautious in thinking that, in a area such as Manchester where the introduction of the gravis and intermediate strains showed itself mainly by increased severity and not by increased incidence, a deferred epidemic will not occur after a lag period.

To the data in Tables III to VIII straight lines showing the trends have been fitted. It will be obvious that in several instances the fit is not very good, and it might be thought that logarithmic curves would give better results. Such a supposition is not entirely justified by results, since the writer has in some instances fitted logarithmic curves, and the fit was not altogether satisfactory. Apparently a series of more complex curves fitted to each graph would give a more rational explanation of the sequence of events. But even the simple trend line does bring out some important features, and the writer has accordingly decided to treat all the data in this manner for the purpose of this introduction.

The trend lines were fitted by an application of the well-known theorem of least squares. If an observed magnitude y depend on x in such a way that

$$y = a+bx$$

(which is the equation of a straight line), then the most probable values of a and b are such that the sum of the squares of the differences between any observation and the curve is a minimum. It can be shown that these conditions are fulfilled when

$$b = \frac{\Sigma y \Sigma x - n\Sigma(xy)}{(\Sigma x)^2 - n\Sigma(x^2)}$$

$$\text{and } a = \frac{\Sigma(xy)\Sigma x - \Sigma y.\,\Sigma(x^2)}{(\Sigma x)^2 - n\Sigma(x^2)}$$

where n is the number of observations, and x is the serial number of any individual observation y.

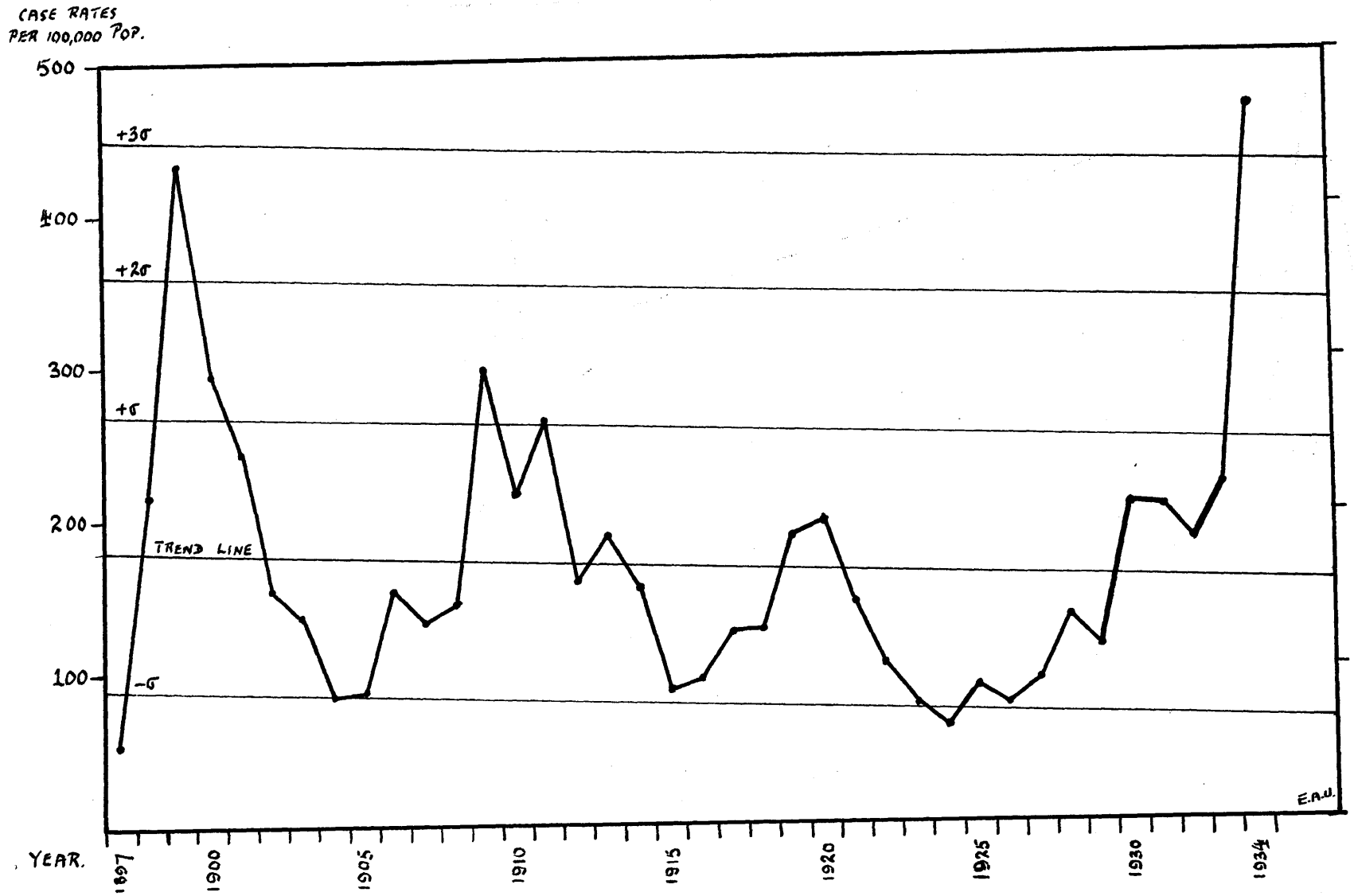

Fig. 1 : Leeds : Diphtheria : Case Rates and Trend.

$y = 179.74 - 0.598x.$

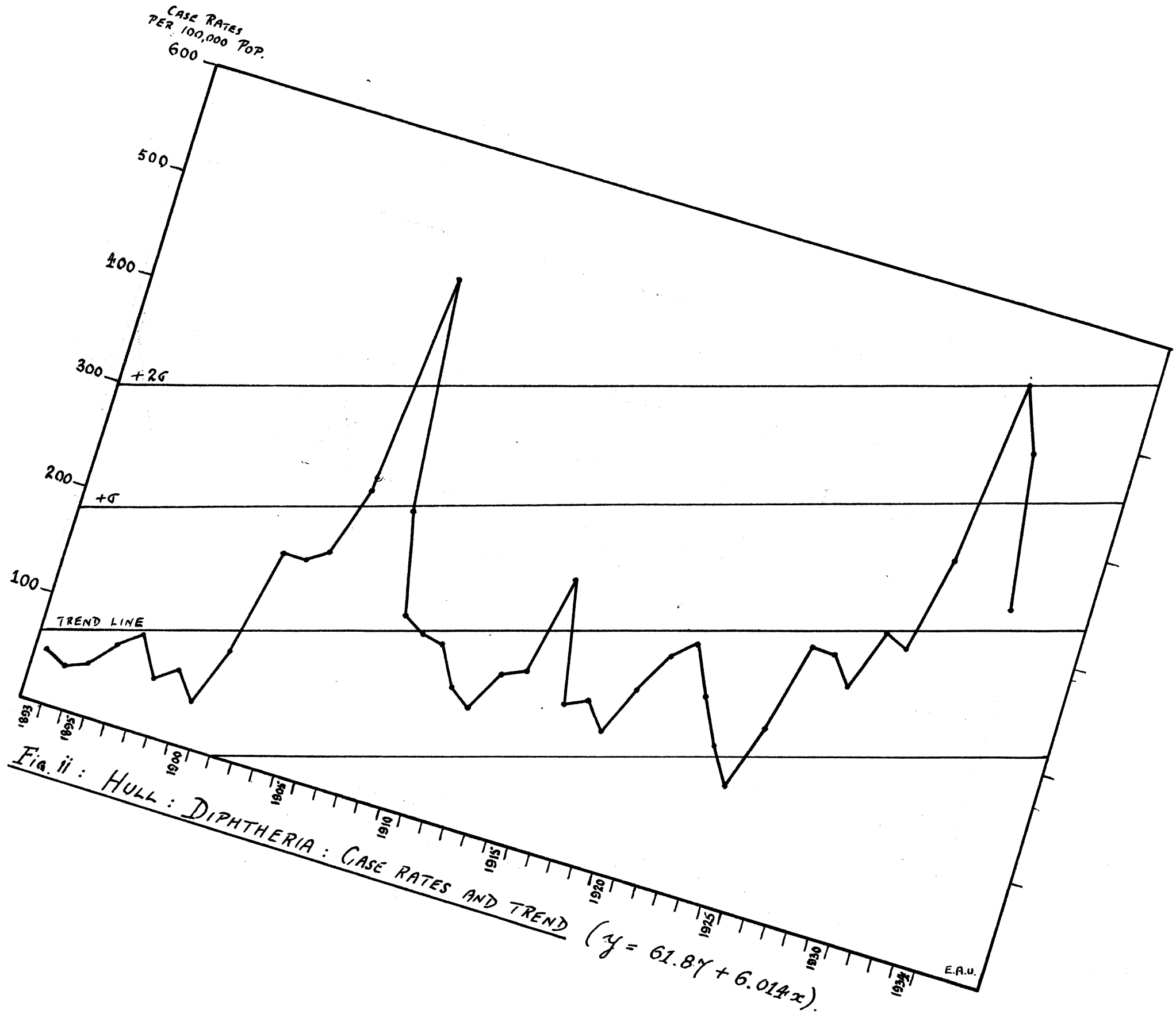

Fig. ii : HULL : DIPHTHERIA : CASE RATES AND TREND ($y = 61.87 + 6.014x$).

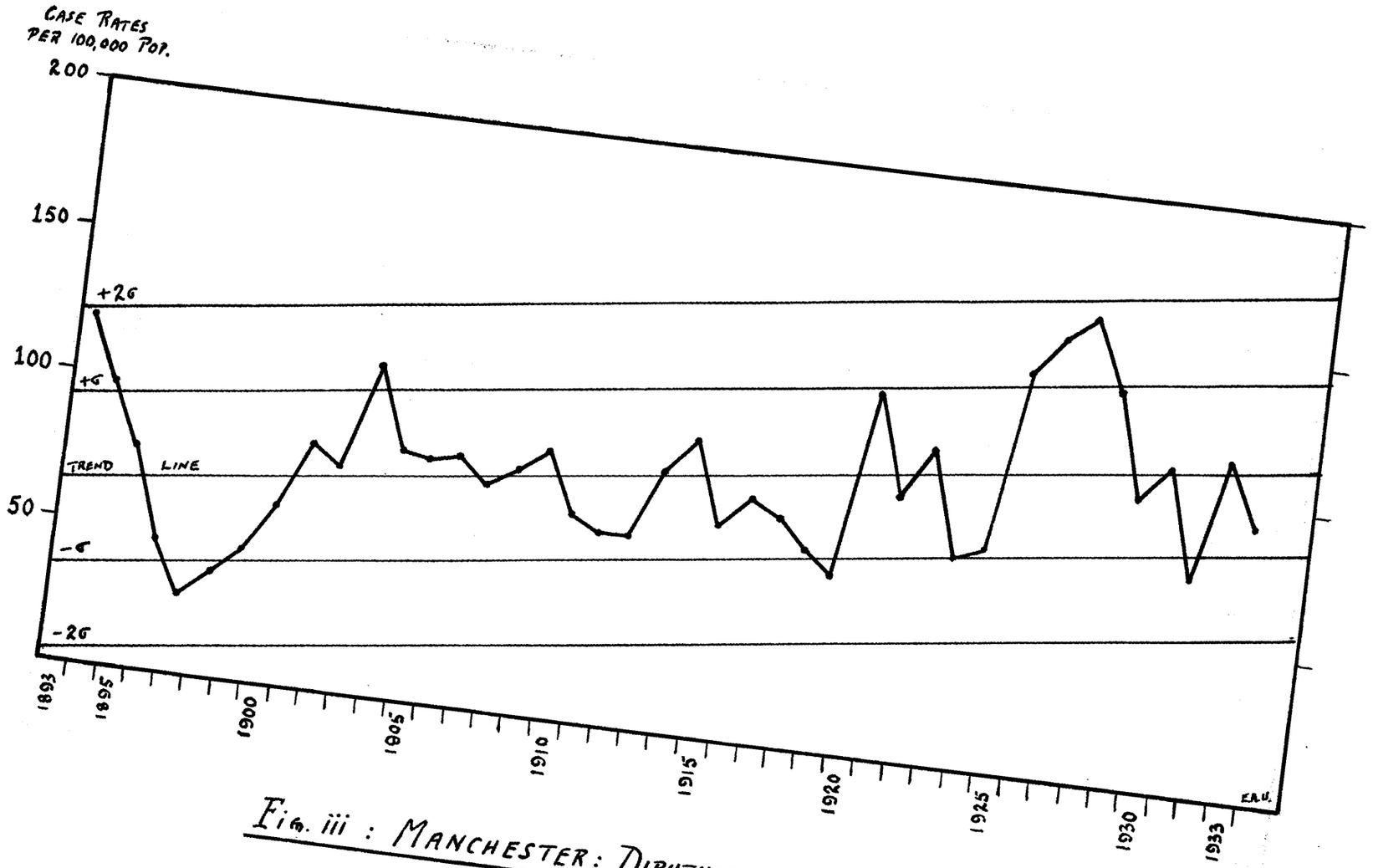

Fig. iii : MANCHESTER: DIPHTHERIA: CASE RATES AND TREND.

$y = 62.20 + 1.258x$.

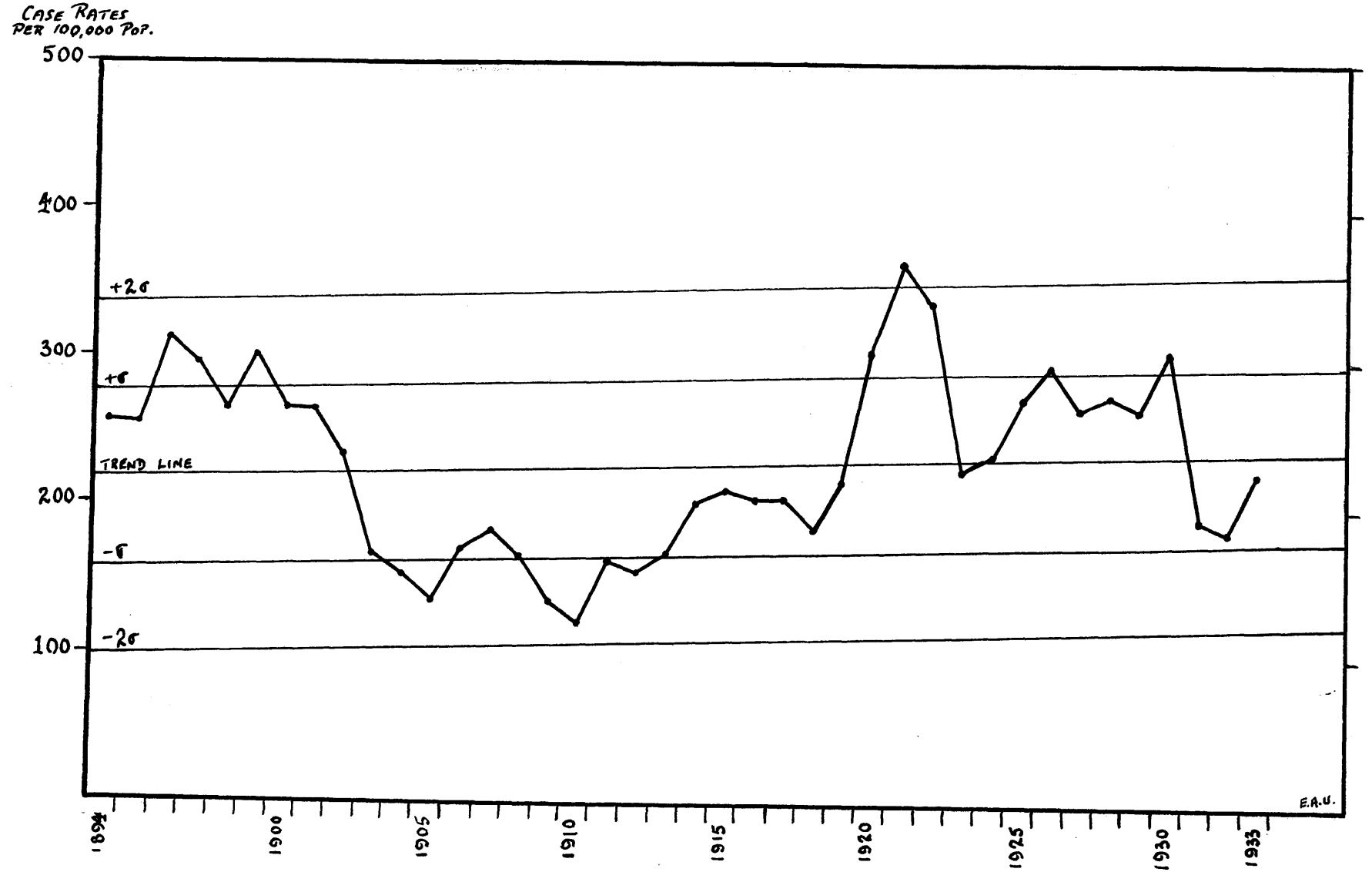

Fig. iv : LONDON (ADMINISTRATIVE COUNTY): DIPHTHERIA : CASE RATES AND TREND.

$y = 217.64 + 0.538x$

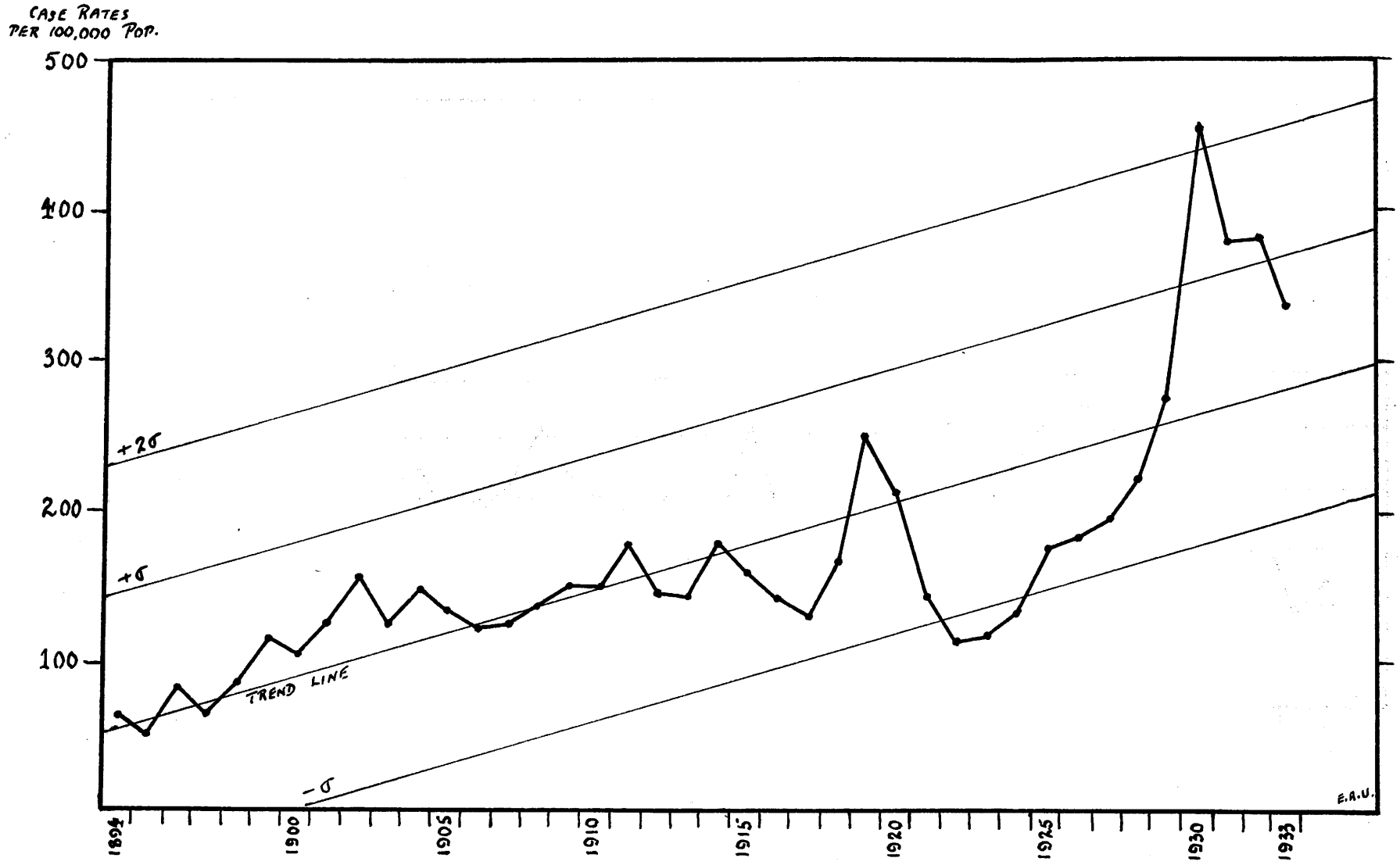

FIG. V: LIVERPOOL: DIPHTHERIA: CASE RATES AND TREND.

$y = 50.90 + 5.775x$

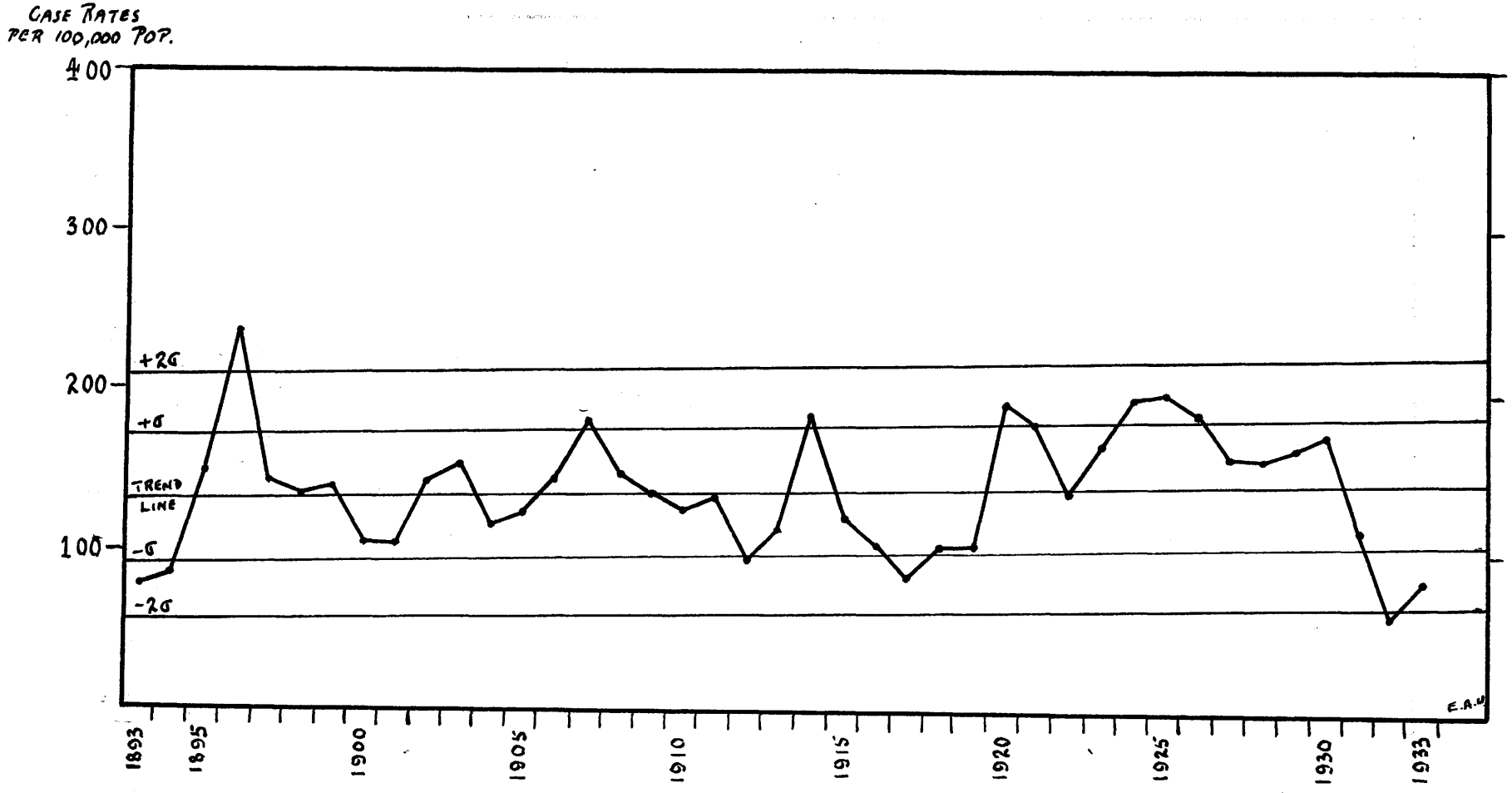

FIG. VI : BIRMINGHAM : DIPHTHERIA : CASE RATES AND TREND.

$y = 130.98 + 0.354x.$

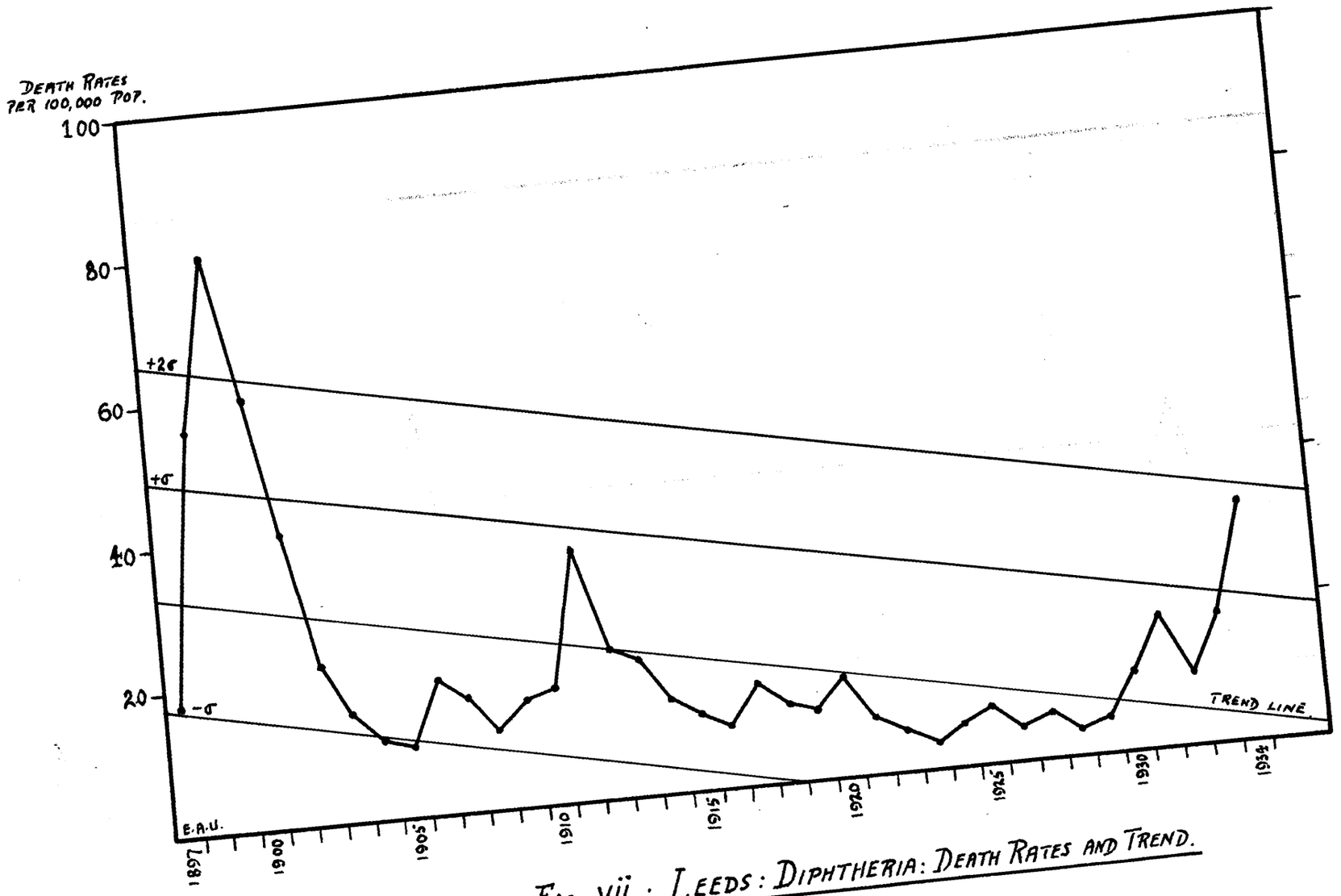

Fig. vii : Leeds : Diphtheria : Death Rates and Trend.

$y = 33.58 - 0.491x.$

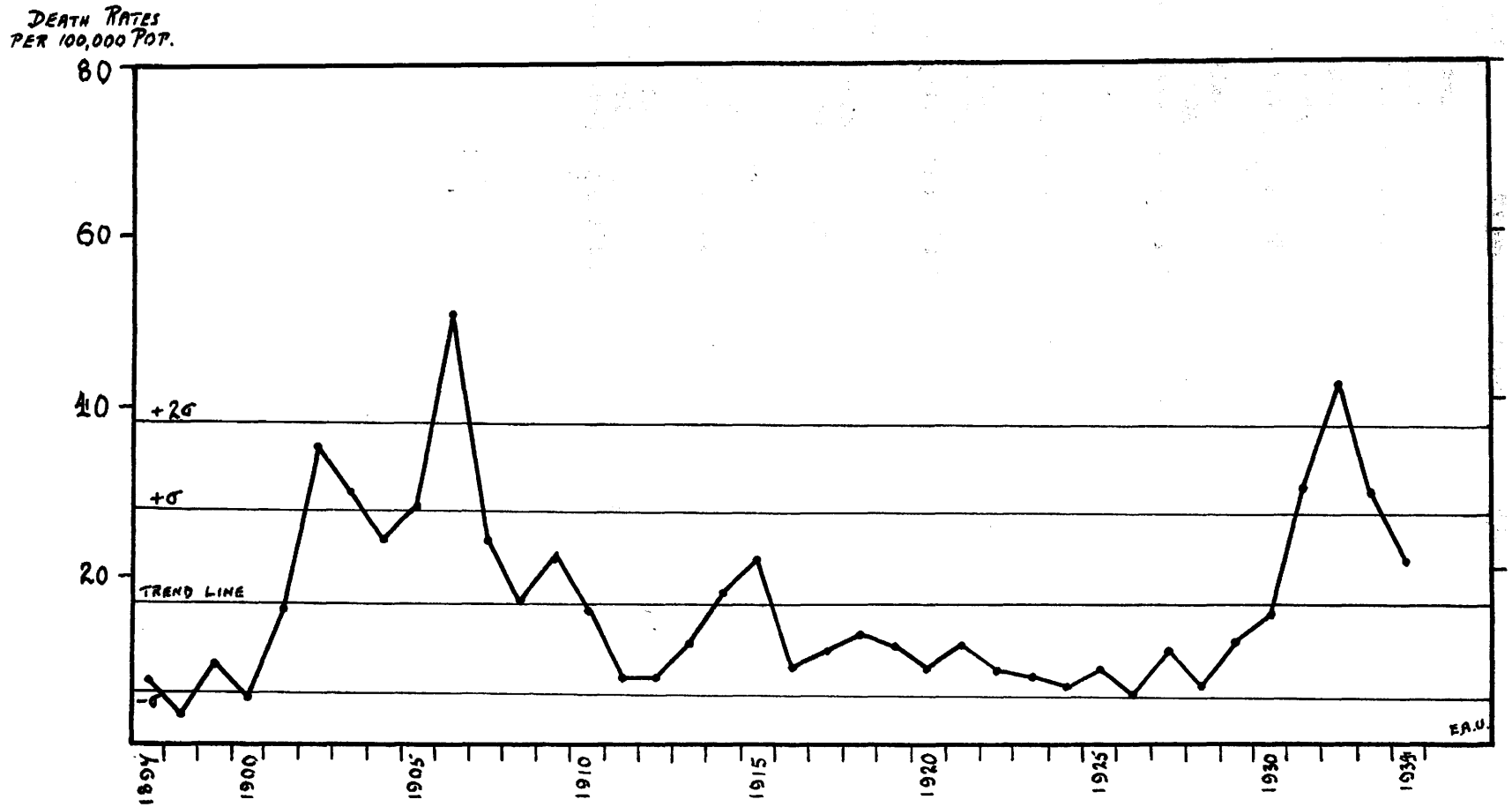

FIG. VIII : HULL: DIPHTHERIA: DEATH RATES AND TREND.

$y = 17.15 - 0.028x.$

TABLE IX.

DIPHTHERIA: Case Rates and Death Rates: Trends for Different Cities.

City	Trend equation	Means	S.D.
LEEDS: 1897-1934 (Case rate)	$y = 179.74 - 0.598x$	168.1 ± 9.886*	90.35 ± 6.990
LEEDS: 1897-1934 (Death rate)	$y = 33.58 - 0.791x$	18.2 ± 1.776	16.23 ± 1.256
HULL: 1893-1934 (Case rate)	$y = 61.87 + 6.014x$	191.2 ± 12.028	115.56 ± 8.504
HULL: 1897-1934 (Death rate)	$y = 17.15 - 0.028x$	16.6 ± 1.165	10.65 ± 0.824
MANCHESTER: 1893-1933 (Case rate)	$y = 62.20 + 1.258x$	88.6 ± 3.083	29.27 ± 2.179
LONDON: 1894-1933 (Case rate)	$y = 217.64 + 0.538x$	228.7 ± 6.330	59.35 ± 4.476
LIVERPOOL: 1894-1933 (Case rate)	$y = 50.90 + 5.775x$	159.3 ± 9.309	87.29 ± 6.583
BIRMINGHAM: 1893-1933 (Case rate)	$y = 130.98 + 0.354x$	138.4 ± 4.035	38.30 ± 2.853

* The ± values throughout this Thesis are probable, not standard, errors.

The death rates for Leeds and for Hull were treated in a similar manner, and the graphs with the fitted curves are shown in Figs. vii and viii. Table IX gives the equations, means, and standard deviations for the case rates and death rates for each of the cities. On each graph (where possible) lines have been drawn parallel to the trend line, and at a distance of 1, 2 and 3σ from it.

It will be seen that in the case of London no significant feature has occurred since the epidemic of 1921, and that in Birmingham there has been no increased incidence passing the limit of $+\sigma$ since 1925. In Leeds on the other hand the 1934 epidemic, when 2,239 cases of diphtheria were notified, giving a case rate of 4.62 per 1,000, is most interesting. This rate gives a deviation from the mean which is equal to almost 3.4 times the standard deviation. A table of the probability integral shows that such an event would occur by chance about once in 1400 years. In the graph for Liverpool it is seen that a large peak occurred in 1930. The magnitude of this peak is over-emphasised by the size of the preceding trough. Actually, the difference between the peak rate - 457 - and the trendvalue is 192.8, which is only 2.2 times the standard deviation. This value indicates that an epidemic of this magnitude might be expected to occur by chance about once in 35 years. If therefore we consider the two observations from the point of view of uniqueness, there is no comparison between the Liverpool epidemic of 1930 and the Leeds epidemic of 1934. In other words, any individual living in Liverpool might expect to pass at least once in his lifetime through an epidemic such as was experienced in 1930. The Leeds epidemic of 1934 was on the other hand an event which would practically never occur again unless as the result of the operation of some new or extraneous factor. In the case of the curve for incidence in Hull, the main features are probably considerably modified by the fact that a very large epidemic occurred in 1906. The deviation of the value for the 1932 Hull epidemic is 2.02 times the standard deviation, so that

these data give a probability of the occurrence of such an event once in about twenty one years.

Turning to the death rates (Figs VII and VIII) we find an interesting corollary. Accurate death rates were available for Hull since 1897. The death rate for the 1932 epidemic was 42 per 100,000 living, and the deviation - 25.93 - of this from the trend value is 2.4 times the standard deviation. Such an occurrence could only be expected on the basis of chance once in sixty years. The corresponding calculation for the death rate for the Leeds 1934 epidemic gives a probability of occurrence of once in thirteen years.

Summarising these results we see that in the two areas in which the gravis strain first appeared and has been most prevalent there is evidence that the resulting outbreaks, when considered in the one case from the point of view of incidence and in the other from the point of view of deaths, were of such magnitude as to be, in one case certainly and in the other possibly, beyond the probability of occurrence apart from the operation of some new factor. In London the gravis strain has not been in evidence, and so far as the writer knows it has not been reported from Birmingham. In Liverpool there has been no marked infection with this strain. On this evidence it would appear that in Leeds and Hull the change in type of the C.Diphtheriae was not merely an incidental happening which produced no significant effect on the herd; but was a distinct epidemiological phenomenon, comparable to a certain extent to the introduction of a new disease into these cities.

Effect of Age Incidence.

It may be objected that the age constitution of the populations of these cities must have altered very considerably during the last forty years. This objection is valid, and it must be admitted that standardised rates would have given more emphatic results. Newsholme dealt with this question over thirty five years ago (Newsholme 1898), and he concluded that in discussing the incidence or mortality of diphtheria in different areas, the errors introduced as a result of using non-standardised rates would be

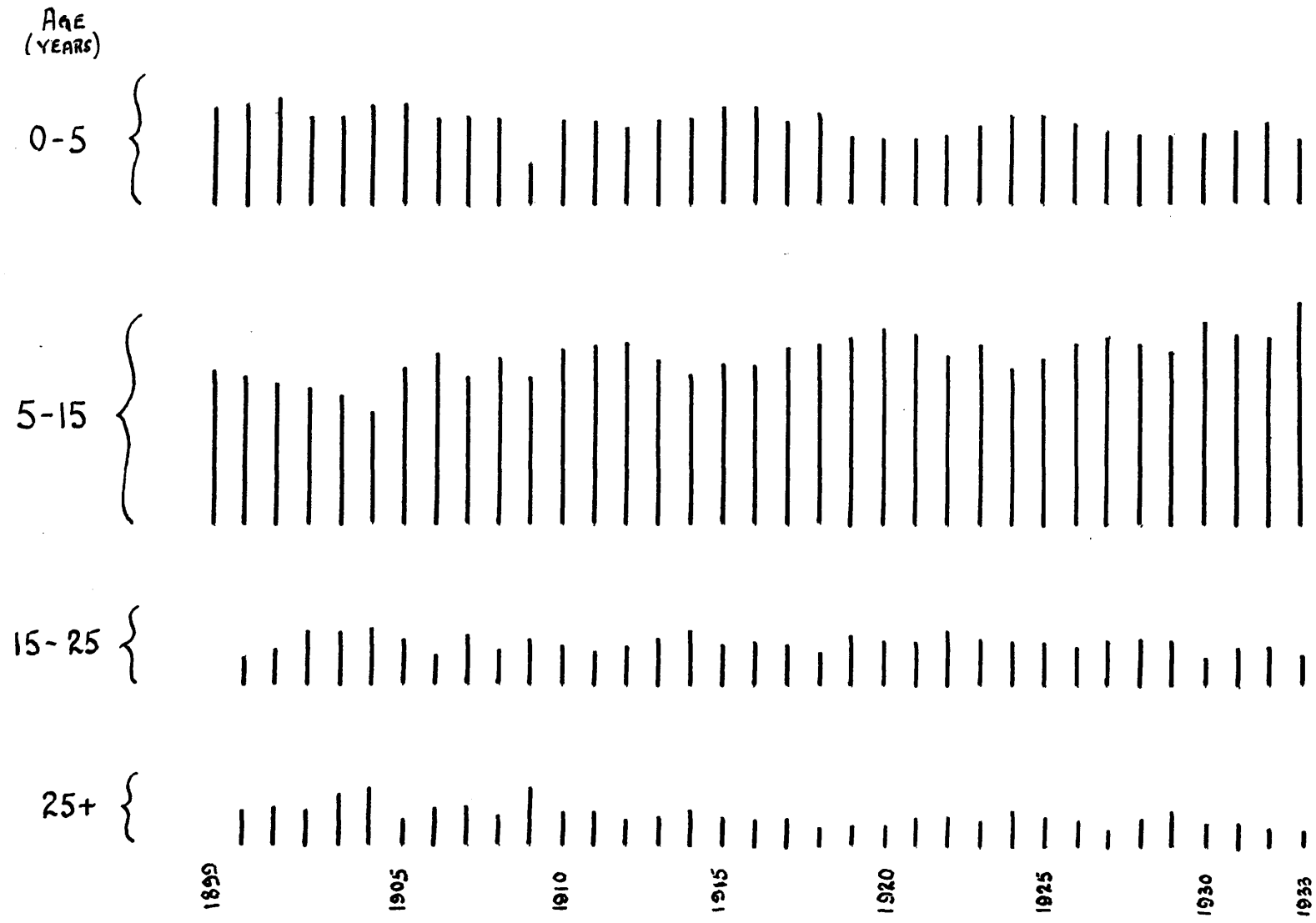

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Fig ix: Diphtheria in Leeds: Percentage age distribution of cases (1899-1933).

within very small limits. In the present investigation it has so far been impracticable to obtain the age distribution of the cases and deaths for all the cities investigated, and consequently correction for age has not been possible. An an indication, however, of the extent to which the age factor may invalidate the results, the following small inquiry into the effect of this factor in Leeds may be used.

Stevenson (1919) devised a graphic method which shows at a glance the main changes in the age grouping of cases of any particular disease year by year. Fig. ix shows the age incidence of diphtheria cases in Leeds from 1899 to 1933, charted according to this method. (The figures for 1934 are not available at the time of writing). It will be seen that, apart from long term smooth fluctuations, the incidence for the age groups 15 to 25 years and over 25 years has not altered very much in the last 35 years. The proportional incidence up to five years of age has been gradually decreasing throughout this long period, but the decrease has not been at all considerable. This decrease has been associated with a more or less corresponding increase in the incidence at ages 5 to 15 years - an increase which has also been gradual. It appears therefore that the non-standardisation of the rates has probably not introduced an error of any magnitude into the results which are given previously.

PART I.

SECTION (b).

THE SCHICK TEST IN A "GRAVIS-INFECTED" COMMUNITY: VARIATION OF NATURAL IMMUNITY WITH AGE, SEX, AND SEASON.

The Schick test, which was introduced by Schick and Michiels (1913) twenty two years ago, is more or less unique in immunological work, since it is the only accurate test which we have for gauging the immunity of an individual to a common infectious disease. The test, as is well known, is carried out by injecting intradermally 0.02 of a M.L.D. of diphtheria toxin, contained in 0.2 cc. of a suitable diluting medium. A positive reaction develops within twenty-four to forty-eight hours, and consists at first of a circular pinkish area. This area rapidly becomes of a deeper colour, and about the fifth day the skin becomes dry and hard; tiny fissures appear and desquamation, which may be either of a fine branny nature or much more definite and flaky, is found. A negative reaction shows no apparent erythema at the injection site, even after seven days. In many instances a positive reaction is quite definite within the first three days; but experience gained from thousands of Schick tests performed all over the world has shown that the most accurate results are obtained if the final reading is made on the seventh day.

The work of Dudley and others has demonstrated that the "immunizability" of a community - or the rapidity with which natural or artificial immunization is completed depends very largely upon the original immunity rate of the community. Consequently it follows that the description of any large scale experiment upon immunization and upon the use of different prophylactics should be preceded by a discussion of the natural immunity of the population upon which the experiment is performed. Despite this obvious

principle, it is surprising to find that many reports are quite worthless because they omit to give any of these particulars. In the case of Leeds it was especially necessary to have detailed knowledge of these factors, since the presence of the gravis strain suggested that the natural immunity rate would show considerable differences from the rates in other areas. The essential basis of the investigations which are detailed in this Thesis consisted therefore of 2,666 primary Schick tests which were performed and read personally by the writer. These tests were carried out between February 1933 and September 1934, and the population investigated was more or less homogenous, being made up of school and pre-school children of the working classes in Leeds. A few institutions were dealt with, but these are considered separately in other sections of this Thesis and the numbers of the inmates were so small in comparison with the total number of children tested that their inclusion could have little effect upon the general results of the investigation.

It should be mentioned here that this section is not a complete discussion of the Schick test and its implications. The literature of the test is very large. The writer has read a considerable amount of it, but in his opinion many of the papers are so incomplete that they connot be used for accurate statistical work. The best of the literature up to 1929 was adequately discussed by Dudley (1929), and that excellent review must stand as the last word on this subject up to the time of its publication. In a later work Dudley (1934) extended his discussion on a more specialised line. In this section of the Thesis the present writer deals only with those features of the test which appear to be peculiar to a "gravis-infected" area; he also deals with certain general features, such as the relationship between age, sex and season, which in the past have either been almost entirely neglected or inadequately dealt with. It is hoped that the statistical methods employed are as fine as any which could be applied to this type of data, and it would seem that the results obtained are much

more precise than any which have hitherto been published.

Technique.

The whole of the Schick test fluid and control fluid which was used was supplied to the writer by Dr R. A. O'Brien, Director of the Wellcome Physiological Research Laboratories, and all the fluids used were fresh. Agla all-glass syringes, each of 1 cc.capacity, and fine intradermic needles were used in every instance. Except in very special cases - such as deformities of one arm - the test injection was given in the left forearm, and the control injection in the right forearm. In each instance a Moloney test was also performed, and the results of these Moloney tests are dealt with in a separate section of this Thesis. Almost invariably the Moloney injection was done on the right forearm, proximal to the Schick control injection. All tests were read after forty eight hours, and also on the seventh day; in many instances the tests were also read at more frequent intervals. The results were recorded in two ways: (a) the diameter of each reaction was measured in millimetres by means of a steel rule: (b) each reaction was described and classified according to its appearance, colour, degree of hardness of the skin, and other features. The classification adopted depended largely on the writer's experience in reading individual tests, and hence it cannot be readily described in a few words. In general, it may be said that the following classification was used:- (a) Faint, "+" - any ordinary reaction up to 15 mm. in diameter, or a faint reaction up to 20 mm. (b) Moderate, "++" - an ordinary reaction from 20 to 25 mm. in diameter, or a bright reaction from 15 to 20 mm. (c) Marked, "+++" - any reaction which was bright and of diameter over 25 mm, or any exceptionally large reaction; extremely bright reactions under 25 mm. might also be included in this category. Special care was taken to give exact doses in each instance: this point in technique has been adequately discussed by Dudley (1929) who showed that 25 per cent over or under the correct dosage made no appreciable difference to the results. Care was also taken to give the injections intradermally. This

statement must of course be taken with a certain amount of reservation, since it is obvious that in some instances a portion of the dose may have unwittingly been given subcutaneously. It should be stated, however, that this possibility was no greater in the present series than in any carefully performed intradermal injection. In any event, there is some evidence that Schick fluid injected entirely subcutaneously gives results which are very similar to similar tests performed intradermally, and indeed Haidvogl (1926) recommended the subcutaneous in preference to the intradermal method, on the grounds that the resulting reactions were easier to read, and that pseudo-reactions were less frequent.

General description of results of tests.

It is not possible to discuss here detailed results of the writer's work on the Schick test in areas other than Leeds, but it is desirable to give some broad indication of the general difference between the results obtained in a "gravis-infected" area and those from other districts.

The writer was early impressed by the high frequency, not only of positive reactions, but of marked positive reactions in the Leeds children. Faint positive reactions were not at all frequent, and most positive reactions were of the type which is classified here as "moderate" - that is, of diameter up to 25 mm, or very bright reactions of diameter 20 to 25 mm. Marked positive reactions - that is, of diameter 25 to 35 mm, or over were comparatively common. In a large proportion of positives, and especially in moderate and marked positives, desquamation was marked. The writer also found that "giant" reactions were not at all infrequent. The most remarkable reaction was that of a positive result in a girl of thirteen years. The control arm showed a bluish area of about 10 mm. in diameter, which later became dusky. The test arm showed a very large area measuring about 80 mm by 70 mm. The central area measuring 40 mm. in diameter showed complete vesiculation even on the third day; this area was surrounded by the erythematous zone, 80 mm. in diameter. Outside the area mentioned

above there was a large ring of very faint erythema. On the seventh day there was a central area of desquamation 25 mm. in diameter, and swelling and pain were still present. The result was so contrary to any reaction which the writer had previously met with that he was inclined to suspect a protein reaction, and to assume that the girl was probably immune. Blood titration showed, however, that there was less than 1/1000 units of antitoxin in the serum, and that the Schick result was a true positive of an exceptionally strong degree.

The writer consequently formed the opinion early in the investigation that both the frequency of positive reactors was higher in Leeds than in many other areas, and also that the degree of reaction tended to be much stronger. Prolonged observation amply confirmed this view, and since the conclusion of the Leeds experiments the writer has had opportunities of carrying out and reading Schick tests in London. The degree of reaction in the Metropolis has certainly been lower than in Leeds, and in addition the natural immunity rate is definitely higher. Detailed results of some of these tests will be discussed later in this Thesis. In Leeds other peculiar features in connection with the Schick reaction soon emerged, and some of these will be discussed in this section.

A consideration of the figures for diphtheria incidence in Leeds during the previous few years, as given in the preceding sections of this Thesis, suggested at first that a considerable proportion of the child population at all ages would have been the subjects of "latent immunization", and that the natural immunity rate in Leeds would be rather high. The contrary proved to be the case. In a later section the proportions of positives in different groups of children are compared, and it is shown that on an average eighty-five per cent of all Leeds children are susceptible to diphtheria, as shown by a positive result to the Schick test.

This low degree of natural immunity - viz. 15 per cent - should be emphasized. It indicates that, despite the high incidence

of diphtheria in Leeds during the past few years, latent immunization as gauged by tests with ordinary Schick fluid, has not been proceeding to anything like the extent which modern views on the epidemiology of diphtheria would lead us to expect. It is instructive to compare this average figure of eighty-five per cent of children under fourteen years positive at the primary Schick test with the results obtained in some other areas. Accurate figures on these lines are difficult to obtain, and the following samples, though few, represent only those which are most likely to give a fairly accurate comparison. (The figures are taken largely from the works of Graham Forbes (1932a, 1932b). Birmingham - 1287 children of school age - 64 per cent positive. Cardiff - 7862 school children - 54 per cent positive. Hackney - 1442 children of all ages - 70 per cent positive. Wandsworth - 1978 children , mainly five to fifteen years - 74 per cent positive. In France, Ramon, Timbal, and Nélis (1933) reported that of 3298 children aged six to fourteen years, only 47.2 per cent were positive. In a recent paper Levin and Cary (1934) found that 86.0 per cent of 1016 children between the ages of one and fifteen years in Portland, U.S.A. were positive; 84 per cent of these children were between the ages of four and ten years. This series is the only one of which the present writer has knowledge which, for lowness of the natural immunity rate, is at all comparable with the Leeds series. This statement naturally applies only to figures collected from large urban centres.

It will be seen from later sections of this Thesis that there are still many questions in relation to diphtheria which are not understood, or concerning which the explanation at present held cannot be accepted. On the current view we would have to conclude that, if in any area diphtheria has been prevalent and the natural immunity rate nevertheless remains low, then the prevalent strain must be a poor immunizing agent. On this view we would have to accept the theory that, since the gravis strain had been prevalent in Leeds for several years, its immunizing powers must be low. The writer is not satisfied that

this is a complete explanation of the low natural immunity rate in Leeds, but for the present this explanation will be allowed to stand.

The effect of age on natural immunity.

It is an old observation - see Park and Zingher (1916) Zingher (1917) and the large series of Zingher (1923) - that the proportion of positives in a community is very high at the early stages of life, and decreases gradually till the percentage becomes small as adult life is reached. The exception to this statement is that during the first year of life, and especially during the first six months, a considerable number of infants show a negative Schick test as a result of immunity acquired from the mother.

The results which emerged from the writer's series of cases in Leeds were no exception to the general rule. Table X gives the percentage negative for each year of life from under 1 to 19 years. The results are plotted diagramatically in Figure X.

TABLE X.

Incidence of negative Schick tests at different ages.

Age	No. Negative	Percentage negative
Under 1	8	16.33
1	3	1.62
2	3	1.69
3	8	4.12
4	21	8.93
5	39	11.71
6	67	17.36
7	38	20.21
8	39	25.01
9	44	27.85
10	45	34.88
11	51	45.54
12	62	43.97
13	37	43.02
14	19	63.34
15-19	86	80.37

It is seen from the data presented that under the age of one year 16.33 per cent of children showed a negative Schick test, and that by the first year this figure had fallen to 1.62 per cent. The proportion of negative reactors remained more or less unchanged until the third year, when there was a slight increase. From this age onwards until the eleventh year there was a progressive and apparently continuous increase in the proportion of children who

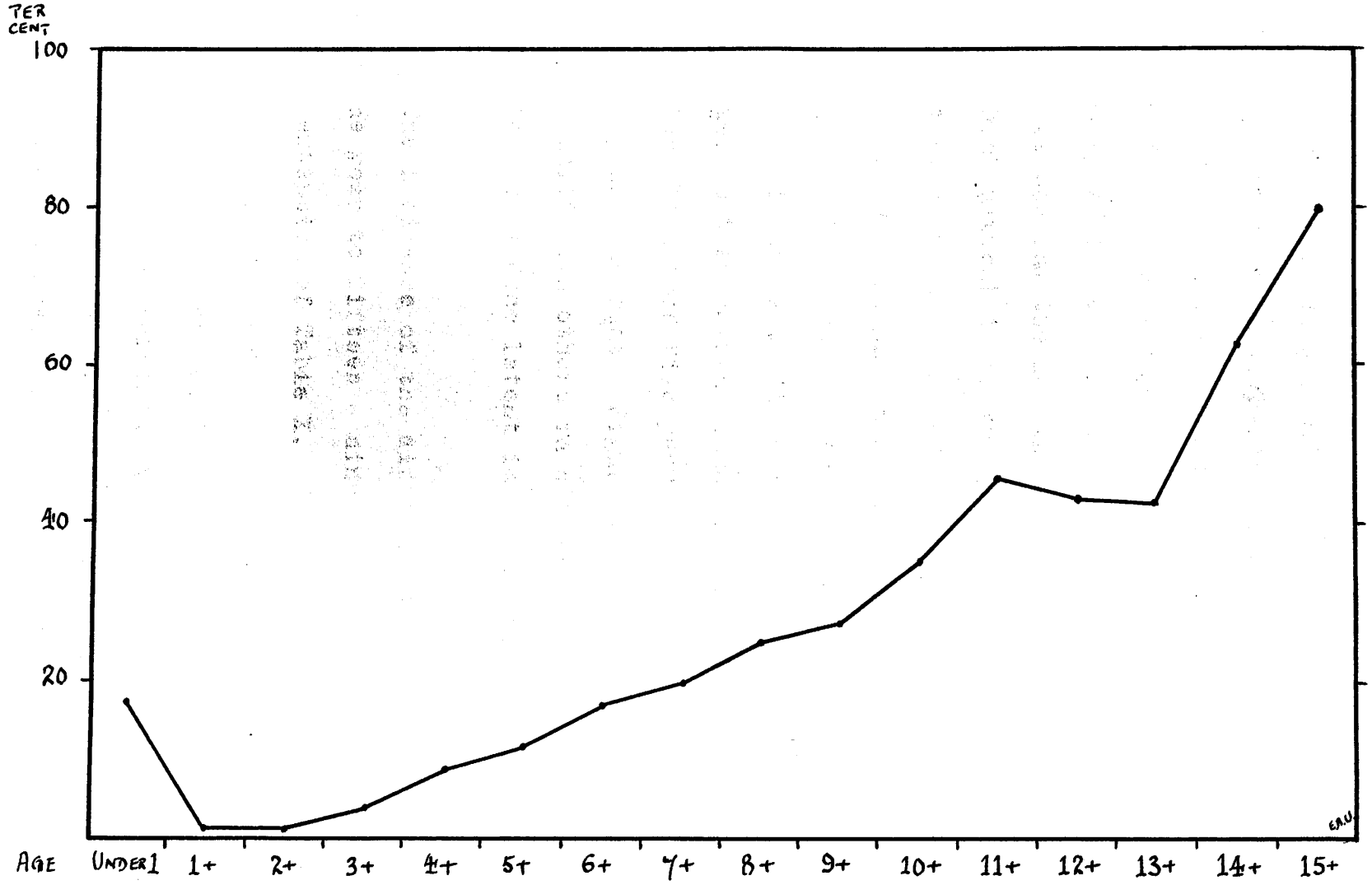


FIG. X: PER CENT OF TOTAL TESTED AT EACH AGE WHO SHOWED A NEGATIVE SCHICK TEST.

were Schick negative. Between the eleventh and the thirteenth years the percentage of Schick negatives fell slightly from 45.54 per cent to 43.02 per cent. This slight fall was succeeded by a marked rise to 80.37 per cent in individuals aged from fifteen to nineteen years.

More interesting results are obtained when these features are plotted on a logarithmic scale (Fig xi). It is seen that the loss of congenital Schick immunity during the first and second years is extremely rapid. A period of apparent quiescence is succeeded by a period of rapid increase of natural immunity between the second and the fourth years. This is definitely the period in which natural immunity is most rapidly acquired. After the age of four years the rate diminishes considerably, but continues more or less constant until early adult life. It might be thought that the early school period would be the time when the child would come most in contact with others who were harbouring the C.Diphtheriae, so that natural immunity would be most rapidly acquired after the age of five years. In Leeds, however, this was apparently not the case. Figure xi shows that the association of the young child with others in his home and elsewhere must have been sufficient to induce latent immunization at a very satisfactory rate.

The incidence of the different types of reactors at all ages from one year to fifteen - nineteen years is shown in Table XI, which is an extension of Table X. It is seen from this Table that the behaviour of the different types of positive reaction is not nearly so consistent as we would expect from a consideration of the gradual decrease of the percentages of positive reactors of all types from one year to the next. The table brings out the fact that, although this percentage of positives falls from 98.4 at one year to 19.6 in the age group 15-19 years, the decrease takes place almost entirely as a result of a progressive decrease in the proportion of moderate (++) reactors; the proportions of (+) and (+++) reactors remain almost unchanged throughout the whole of

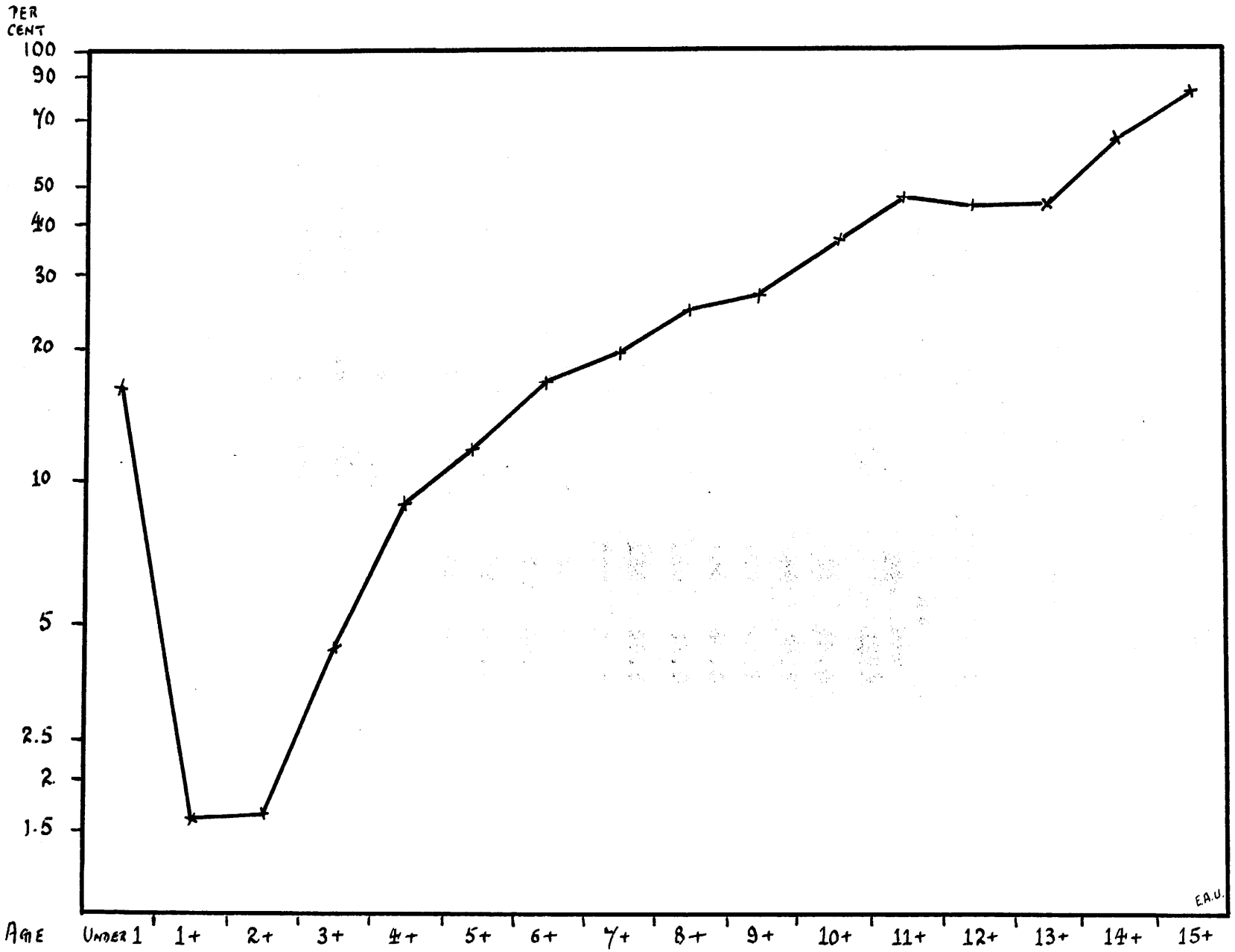


FIG. XI : LOGARITHMIC SCALE. PER CENT. OF TOTAL TESTED AT EACH AGE WHO SHOWED A NEGATIVE SCHICK TEST.

childhood and early adult life. This is of course a relative statement. Why such a relationship should hold is unknown. Possibly it suggests that the ++ reactor is the one who responds normally to the antigenic stimuli in an environment, becoming immune within a reasonable time if the stimulus is sufficiently strong; and that the + and +++ reactors represent the residue - made up largely of individuals who do not respond readily to such stimuli. Why such "Schick-fast" individuals should show mainly + or +++ reactions is difficult to understand.

TABLE XI

Incidence of Different Types of Schick Reaction at different ages.

Age in years	Negative No.	Negative Per cent	+ No.	+ Per cent	++ No.	++ Per cent	+++ No.	+++ Per cent	Total tested
Under 1	8	16.3	8	16.3	31	63.3	2	4.1	49
1+	3	1.6	16	8.6	140	75.7	26	14.1	185
2+	3	1.7	14	7.9	129	72.9	31	17.5	177
3+	8	4.1	15	7.7	132	68.0	39	20.1	194
4+	21	8.9	19	8.1	148	63.0	47	20.0	235
5+	39	11.7	35	10.5	188	56.5	71	21.3	333
6+	67	17.4	40	10.4	207	53.6	72	18.6	386
7+	38	20.2	21	11.2	90	47.9	39	20.7	188
8+	39	25.0	13	8.3	71	45.5	33	21.2	156
9+	44	27.8	14	8.9	79	50.0	21	13.3	158
10+	45	34.9	7	5.4	51	39.5	26	20.2	129
11+	51	45.5	6	5.4	45	40.2	10	8.9	112
12+	62	44.0	17	12.1	35	24.8	27	19.1	141
13+	37	43.0	14	16.3	16	18.6	19	22.1	86
14+	19	63.3	4	13.3	4	13.3	3	10.0	30
15 -19	86	80.4	3	2.8	10	9.3	8	7.5	107
Total	570	21.4	246	9.2	1376	51.6	474	17.8	2666

The relationship between sex, and the development of natural immunity.

It has long been known that female children tend to show a higher percentage of Schick positives than do male children. For example, Zingher (1917) found that, in an American institution, 4.1 per cent of the boys and 15.5 per cent of the girls had positive Schick reactions. Kelly, Stevens and Beattie (1925) reported on 6000 Schick tests performed on Californian children;

they showed that 60 per cent of 3532 females were positive, as against 50 per cent of 3082 males. The difference, viz, ten per cent, is considerable. In a series of 1200 children who were tested at Asbury Park, U.S.A. (see Dudley 1929) 74 per cent of females as against 62 per cent of males were positive. In Holland Bessemans found that 46 per cent of the girls and 41 per cent of the boys were positive reactors.

In the present series of Leeds children there were 1391 males and 1275 females. The group of individuals at ages 15 to 19 years was composed almost entirely of males in a large residential school; and the total population investigated did not embrace any similar series of females at these ages. In most of the following discussions the 105 males (of whom 20 were positive) at these ages were deducted from the total male population before any computation was carried out. Of the 1286 males 1026 were positive, which gives a proportion of 79.8±0.8 per cent. The corresponding figures for females were 1050 and 82.4±0.7 per cent. Hence in this series also the proportion of positive reactors was higher amongst females than in males. The difference is 2.6±1.1 per cent. It is small - in fact, much smaller than in the few other series in which the data were given arranged according to sex. Further, the observed difference between the two sexes, though more than twice, is less than three times its probable error, and is therefore of doubtful significance. The differences of from seven to ten per cent or more which were found in other localities were therefore not confirmed in Leeds.

Kinloch, Smith and Taylor (1927) discussed the incidence of a positive Schick reaction in 1448 Aberdeen children and young adults, and they showed that the incidence in males and females respectively at the different age groups was: (a) 0-5 years (total males and females, 63); males, 83.3 per cent; females 96.8 per cent: (b) 5-10 years (total males and females, 602); males, 81.5 per cent; females, 80.6 per cent; (c) 10-15 years (total males and females, 664); males, 65.1 per cent; females, 74.4 per cent; (d) 15-20 years (total males and females, 119); males, 68.4 per cent; females, 88.5

per cent. In this series it is seen that the preponderance of Schick positive reactions in females did not hold at all ages: in the very important group of children aged 5 - 10 years there was even a slight excess in the males. The following table shows the corresponding figures for the present writer's Leeds series. (Table xii).

TABLE XII.

Age Group	Total tested	MALES			FEMALES.		
		Total tested	Total positive	Per cent positive	Total tested	Total positive	Per cent positive
0-4	840	414	391	94.4±0.8	426	406	95.3±0.7
5-9	1221	606	480	79.2±1.1	615	514	83.6±1.0
9-14	498	266	155	58.3±2.0	232	129	59.6±2.2
15-19	107	105	20	19.0±2.6	2	1	--
	2666	1391	1046	75.2±0.8	1257	1050	82.4±0.7

As has been previously mentioned, in comparing the totals for males and females, it is desirable to exclude the 105 males aged 15-19 years, so that the total percentage for males becomes 79.8±0.8. It is seen from this table that the incidence of a ~~positiv~~ positive Schick test was practically idential in males and females, at all ages up to 14 years. The largest difference - 4.4 per cent - was in the group aged 5 - 9 years, and was very small.

The Seasonal variation in natural immunity.

Of all aspects of the Schick test, the possibility of a variation in the percentage of positives at different seasons has been least investigated. In fact it may be said that, of the three investigations which have been carried out on these lines, one was performed on hospital patients, and another dealt with only a comparatively small number of children. Only one investigation dealt with children in the general population and was of any magnitude. The premier observation was that of Harries (1927) who noticed that in convalescent scarlet fever patients in Birmingham the Schick immunity fell from 55 per cent negatives in February and March to 27 per cent negative at the end of the summer.

St. Tubiasz (1932) reported that about 50 per cent of a community near Warsaw were Schick immune in the winter of 1930-1931, but over 75 per cent were Schick negative in the late summer of 1930. The most detailed investigation was that of Nélis (1933) who reported the results of 3799 Schick tests which were carried out in Belgium during 1931, 1932 and 1933. Nélis found that the percentage of positive reactors was: in winter, 48; in summer 52; in spring, 51. His work did not therefore confirm the observations of Harries and St. Tubiasz.

All these investigations suffered from an important defect: they did not take fully into account the differences in natural immunity which are caused by age and sex respectively. Failure to discuss the age factor satisfactorily is obviously serious. In the present investigation the writer has examined the question fully, and it is hoped that as many as possible of such fallacies have been avoided.

The incidence of the different types of Schick reaction, in the Leeds series performed by the writer, for different ages, sexes and seasons ~~are~~ is set out in tables xiii to xvi inclusive. For the purpose of these tables the seasons were considered as follows: Spring - March, April, May; Summer - June, July, August: Autumn - September, October, November: Winter - December, January, February. The investigation embraced two Springs and two Summers, together with one Winter and four Autumn months. In order to eliminate the changes of Schick immunity which resulted from a high prevalence of diphtheria between, say, one Spring and the next, it would have been desirable to carry the investigation a stage further by re-classifying all the cases into six seasons instead of four. This will ultimately be done, but up to the time of writing it has not been found possible to find time for this laborious work. This criticism is probably not a very serious one. Inspection of the results of the primary Schick tests for each group (which will be found in the appendix to the paper on toxoid and alum toxoid) will show that there was no very marked variations as the months passed,

so that it is probable that any differences which are found in this inquiry must be attributed to seasonal factors, and not to the effect of latent immunization.

The percentage of positive reactors at each season, calculated for males and for females respectively, from the data in Tables XIII to XVI are set out in Table XVII. In the spring the actual number of males tested was 520, but this number included 105 males aged 14 to 19 years who were tested in a residential institution. The necessary deductions were therefore made as previously explained before this and similar percentages were calculated. The differences between these various results are set out in Table XVIII.

TABLE XIII.

Schick Tests by Sexes (2666) (a) Spring.

Age	Negative M.	Negative F.	Negative Total	+ M.F.	+ Total	++ M.F.	++ Total	+++ M.F.	+++ Total	M.F.	Total
Under 1	1	2	3	- -	-	3 6	9	- -	-	4 8	12
1	1	1	2	3 2	5	15 17	32	6 2	8	25 22	47
2	1	2	3	5 1	6	18 24	42	2 6	8	26 33	59
3	1	1	2	3 1	4	19 27	46	3 6	9	26 35	61
4	3	1	4	3 4	7	26 26	52	3 5	8	35 36	71
5	13	7	20	12 4	16	25 32	57	6 13	19	56 56	112
6	9	6	15	11 4	15	31 39	70	13 11	24	64 60	124
7	4	3	7	3 5	8	9 16	25	4 5	9	20 29	49
8	8	5	13	1 3	4	14 13	27	8 4	12	31 25	56
9	5	6	11	4 3	7	7 14	21	3 2	5	19 25	44
10	9	8	17	1 2	3	6 6	12	3 3	6	19 19	38
11	10	10	20	2 -	2	16 7	23	1 1	2	29 18	47
12	15	8	23	7 2	9	9 8	17	8 7	15	39 25	64
13	6	10	16	2 -	2	4 3	7	1 7	8	13 20	33
14	7	7	14	1 2	3	- 1	1	1 2	3	9 12	21
15-19	85	1	86	3 -	3	10 -	10	7 -	7	105 1	106
Totals	**178**	**78**	**256**	**41**	**55**	**94**	**212**	**239**	**451**	**69**	**74**	**143**	**520**	**424**	**944**

TABLE XIV.

Schick Tests by Sexes (2666) (b) Summer.

Age	Negative M.	Negative F.	Negative Total	+ M.	+ F.	+ Total	++ M.	++ F.	++ Total	+++ M.	+++ F.	+++ Total	Total M.	Total F.	Total Both
Under 1	-	2	2	3	4	7	5	6	11	-	1	1	8	13	21
1+	1	-	1	2	4	6	19	23	42	4	1	5	26	28	54
2+	-	-	-	3	1	4	24	12	36	6	4	10	33	17	50
3+	-	4	4	2	2	4	19	19	38	9	9	18	30	34	64
4+	8	2	10	1	2	3	21	32	53	8	9	17	38	45	83
5+	4	9	13	9	2	11	26	32	58	11	12	23	50	55	105
6+	14	15	29	6	7	13	29	39	68	14	15	29	63	76	139
7+	8	8	16	2	1	3	19	21	40	13	4	17	42	34	76
8+	13	9	22	4	3	7	11	12	23	7	8	15	35	32	67
9+	14	10	24	2	1	3	19	19	38	4	5	9	39	35	74
10+	9	9	18	-	1	1	11	12	23	4	2	6	24	24	48
11+	15	7	22	1	2	3	11	5	16	5	1	6	32	15	47
12+	17	10	27	3	2	5	9	6	15	5	1	6	34	19	53
13+	8	8	16	2	3	5	6	2	8	7	2	9	23	15	38
14+	-	2	2	1	-	1	2	1	3	-	-	-	3	3	6
15-19	-	-	-	-	-	-	-	-	-	-	1	1	-	1	1
	111	95	206	41	35	76	231	241	472	97	75	172	480	446	926

TABLE XV

Schick Tests by Sexes: (c) Autumn.

Age	Negative M.	Negative F.	Negative Total	+ M.	+ F.	+ Total	++ M.	++ F.	++ Total	+++ M.	+++ F.	+++ Total	Total M.	Total F.	Total Total
Under 1	1	-	1	-	-	-	2	2	4	1	-	1	4	2	6
1+	-	-	-	1	3	4	19	12	31	4	5	9	24	20	44
2+	-	-	-	-	-	-	13	10	23	2	4	6	15	14	29
3+	-	1	1	1	2	3	9	14	23	2	3	5	12	20	32
4+	2	1	3	5	1	6	9	12	21	6	5	11	22	19	41
5+	-	2	2	4	1	5	24	21	45	6	10	16	34	34	68
6+	4	7	11	5	3	9	22	22	44	6	6	12	38	38	76
7+	4	4	8	2	2	4	6	8	14	6	3	9	18	17	35
8+	3	-	3	1	-	1	2	4	6	2	3	5	8	7	15
9+	3	-	3	1	-	1	4	2	6	-	-	-	8	2	10
10+	2	3	5	3	-	3	3	1	4	2	-	2	10	4	14
11+	2	2	4	1	-	1	-	1	1	-	-	-	3	3	6
12+	4	3	7	-	1	1	-	2	2	1	1	2	5	7	12
13+	1	3	4	-	1	1	1	-	1	-	-	-	2	4	6
14+	1	1	2	-	-	-	-	-	-	-	-	-	1	1	2
15-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27	27	54	25	14	39	114	111	225	38	40	78	204	192	396

TABLE XVI.

Schick Tests by Sexes: (d) Winter.

Age	Negative M.	Negative F.	Negative Total	+ M.	+ F.	+ Total	++ M.	++ F.	++ Total	+++ M.	+++ F.	+++ Total	Total M.	Total F.	Both
Under 1	2	-	2	-	1	1	5	2	7	-	-	-	7	3	10
1+	-	-	-	-	1	1	20	15	35	1	3	4	21	19	40
2+	-	-	-	3	1	4	14	14	28	2	5	7	19	20	39
3+	-	1	1	2	2	4	13	12	25	1	6	7	16	21	37
4+	2	2	4	2	1	3	12	10	22	7	4	11	23	17	40
5+	3	1	4	2	1	3	14	14	28	7	6	13	26	22	48
6+	8	4	12	2	1	3	10	15	25	3	4	7	23	24	47
7+	6	1	7	2	4	6	5	6	11	2	2	4	15	13	28
8+	1	-	1	-	1	1	4	11	15	1	-	1	6	12	18
9+	2	4	6	1	2	3	6	8	14	2	5	7	11	19	30
10+	2	3	5	-	-	-	4	8	12	1	11	12	7	22	29
11+	1	4	5	-	-	-	2	3	5	2	-	2	5	7	12
12+	1	4	5	1	1	2	1	-	1	1	3	4	4	8	12
13+	-	1	1	3	3	6	-	-	-	-	2	2	3	6	9
14+	1	-	1	-	-	-	-	-	-	-	-	-	1	-	1
15-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	29	25	54	18	19	37	110	118	228	30	51	81	187	213	400

TABLE XVII.

		Males			Females		
		Total tested	No.+ (all types)	Per cent positive	Total tested	No.+ (all types)	Per cent positive
(i)	Spring*	415	322	77.6±1.4	424	346	81.6±1.3
(ii)	Summer	480	369	76.9±1.3	446	351	78.7±1.3
(iii)	Autumn	204	177	86.8±1.6	192	165	85.9+1.7
(iv)	Winter	187	158	84.5±1.8	213	188	88.3±1.5

*The figures for Spring given are after deduction of 105 males of whom 20 were positive (see before)

TABLE XVIII

	Males Difference	Females Difference
Spring and Summer	0.7±1.9	2.9±1.9
Spring and Autumn	9.2±2.1	4.3±2.2
Spring and Winter	6.9±2.3	6.7±2.0
Summer and Autumn	9.9±2.1	7.2±2.2
Summer and Autumn	7.6±2.2	9.6±2.0
Autumn and Winter	2.3±2.4	2.4±2.3

It will be seen that many of these differences, though not very marked, are appreciable, and that from a consideration of the probable errors they appear to be definitely significant. Further inspection of Table XVII will show that the percentages appear to be easily capable of grouping into pairs. For males, the figures for Spring and Summer are almost identical (difference = 0.7±1.9 per cent): and the figures for Autumn and Winter are also more or less identical (difference = 2.3±2.4 per cent). Combining these to form two groups we find that there were 8.5±1.5 per cent more positives among males in Autumn and Winter than in Spring and Summer.

For females, the difference between ~~the~~ Spring and Summer was 2.9±1.9 per cent; and between Autumn and Winter 2.4±2.3 per cent. Combining these we find that there were 7.1±1.4 per

cent more positives among females in Autumn and Winter than in Spring and Summer. These results are set out in Table XIX

TABLE XIX.

	Males.			Females		
	Total tested	Total positive	Per cent positive	Total tested	Total positive	Per cent positive
(a) Spring & Summer	895	691	77.2±0.95	870	697	80.1±0.91
(b) Autumn & Winter	391	335	85.7±1.2	405	353	87.2±1.1
Difference: (a) and (b) = 8.5±1.5				Difference (a) and (b) = 7.1±1.4		

The effect of seasonal incidence was therefore more or less identical in males and females respectively. Though the differences are not so marked as those reported by St.Tubiasz and by Harries, they are quite definite, and proceed in the same direction.

It seems probable that in the early Spring the process of natural immunization was hastened to such an extent that an additional eight per cent of children became negative. We must now enquire on what class of individual this effect was produced

Table XX shows the percentage distribution of the different types of positive reactors based on the total positives at each season, for both sexes and for males and females respectively. For both sexes the striking feature is the surprising uniformity of the percentages of ++ reactors in the four seasons. There is also considerable consistency in the + and +++ groups. When, however, we split the figures according to sex, we see that the uniformity of the ++ group is misleading. Actually, in the case of the males there was a progressive increase in the proportion of positives as spring advanced to winter: the difference between spring and winter was 7.6 per cent. In the case of the females, there was a progressive decrease in the proportion of positives as spring advanced to winter - the

TABLE XX

Incidence of different types of positive reaction at different seasons

Season	Total positives	Per cent. of total positives		
		+	++	+++
(a) Both sexes combined				
(i) Spring	668	13.2	64.1	19.8
(ii) Summer	720	10.6	65.6	23.9
(iii) Autumn	342	11.4	65.8	22.8
(iv) Winter	346	10.7	65.9	23.4
(b) Males only				
(i) Spring	342	17.8	62.0	20.2
(ii) Summer	369	11.1	62.5	26.3
(iii) Autumn	177	14.1	64.4	21.5
(iv) Winter	158	11.4	69.6	19.0
(c) Females only				
(i) Spring	346	9.5	69.1	21.4
(ii) Summer	351	10.0	68.7	21.4
(iii) Autumn	165	8.5	67.3	24.3
(iv) Winter	188	10.1	62.7	27.1

(The figures given for Spring for both sexes and for males are after deduction of 20 positive males ages 15-19 years (see text)).

difference here being 6.4 per cent. But, in the case of males there was an increase of 5.2 per cent., and in the case of females a decrease of 4.6 per cent. between autumn and winter. These changes occurred therefore for the most part during the late autumn or the winter months.

These differences are so small that it is difficult to draw any definite conclusions from them. There is, however, some ground for suggesting that late autumn and early winter are the periods when the most significant changes are liable to take place in natural immunity to diphtheria. This statement is, of course, purely tentative. A hard and fast conclusion could only be made on a result of figures for a series of years, and after due allowance has been made for increase in prevalence of the disease at these periods. When, however, we take into account the fact that the disease showed a constant high prevalence in Leeds during the period under consideration, there does appear to be some justification for stating that in Leeds such changes are most likely to take place at this particular season. A difficulty is that the change is in opposite directions in the two sexes.

The preliminary deductions from data such as these must be accepted with caution. But when we consider the fact that the degree of contact between children is obviously highest in winter, we may infer that the marked drop among males in the percentage of ++ reactors - that is, in the bulk of all positive reactors - which occurs between winter and spring, indicates a ready response on the part of the male to antigenic stimuli in the environment. Among females on the other hand the period of maximum antigenic stimulation is followed by a corresponding fall in the proportion of ++ reactors only after some months. There is a suggestion here that the difference may not be entirely artificial, but that the slower response of the female may be due to a true sex difference.

Association between Schick State and Age at Different Seasons.

It is common knowledge that the natural immunity of a child population varies directly with the ages of the individuals who compose it. But precise data on this subject are scarce. For example, a consideration of Table X and Figure x might lead us to believe that the association between Schick immunity and age is strong, but we are immediately confronted with the difficulty that "age" indicates not only the passage of years so far as the individual child is concerned, but also increased opportunities for contact with the C.diphtheriae. There is no doubt that the values at different ages vary considerably in different areas and possibly at different times; evidence on this point may easily be obtained by comparing Figure x of this Thesis with Figure vii on page 284 of the Medical Research Council's Monograph on "Diphtheria" (1923). It will be seen that in the Schick curve which is given in the Monograph, the percentage of negative reactors approaches its maximum very much more quickly, and is at all earlier ages at a very much higher level than in the Leeds curve. This comparison should emphasise the fact that the following observations and deductions do not necessarily apply to areas in which "gravis-infection" is not prevalent. To the best of the writer's knowledge the exact degree of association between Schick state and age, and especially the association for different sexes and for different seasons, has never been determined. An attempt to measure this association has been made in the following section, and it is believed that no investigation of a similar nature has ever been published. The statistical methods employed will be used again in other portions of this Thesis, and as some of them are by no means well known, it is desirable to commence with a brief explanatory note.

Note on Statistical Methods.

The ordinary methods for the calculation of the correlation coefficient "r" imply that the two variables are graduated. In practically all the problems which are dealt with

in Part I of this Thesis we have to consider variables which cannot be graduated, and in these cases Professor Karl Pearson's methods for contingency are applicable. Suppose that the whole population which is being investigated is arranged in columns according to the classes of one variate, and in rows according to the classes of the other variate, so that the whole population sampled is represented by the total M and is distributed in cells to form a contingency table. Now let m_{pq} be the frequency of the cell in the pth row and the qth column, and let the vertical marginal frequencies be given by $m_{:q}$ and the horizontal marginal frequencies by $m_{p:}$. Hence we have

$$m_{1q} + m_{2q} + m_{3q} + \dots\dots\dots + m_{pq} + \dots = m_{:q},$$

$$\text{and } m_{p1} + m_{p2} + m_{p3} + \dots\dots\dots + m_{pq} + \dots = m_{p:}.$$

Now, if the variates are independent, the expected frequency in the pq^{th} cell will be $\frac{m_{:q} m_{p:}}{M}$ and such values can be calculated for each cell.

From this the true value of mean square contingency (ϕ_t^2) can be calculated in exactly the same manner as that indicated for the calculation of the approximate value which is given below.

In most statistical phenomena, however, we do not know the sampled population except by investigation of the sample, and hence, as Karl Pearson has shown (Pearson, 1900) we must use for M, $m_{:q}$, and $m_{p:}$ the values which are known to us, viz, N, $n_{:q}$ and $n_{p:}$. The expected frequency for the cell in the pth row and qth column then becomes $\frac{n_{:q} n_{p:}}{N}$, and the contingency for that cell is $n_{pq} - \frac{n_{:q} n_{p:}}{N}$. The contribution of each cell to the value of χ^2 is obtained by dividing the square of this latter value by the expected frequency, so that

$$\chi^2 = S\left\{\left(n_{pq} - \frac{n_{:q} n_{p:}}{N}\right)^2 \Big/ \frac{n_{:q} n_{p:}}{N}\right\}$$

From the value of χ^2 the corresponding value of P, which indicates the probability that such a distribution as that actually found would occur on the assumption that it was due to

chance, is easily found if the degree of freedom, n, is known. In all the examples in this Thesis the value of P was found assuming that n was equivalent to Pearson's n' - 1 (see Fisher, 1935).

The value of ϕ_a^2, the approximate value of the mean square contingency which we are able to calculate from the sample of the population, is given by $\frac{\chi^2}{N}$, and the mean square contingency coefficient (C_2) is given by the equation

$$C_2 = \sqrt{\frac{\phi_a^2}{1 + \phi_a^2}}$$

The determination of the probable error of the contingency coefficient is more difficult. Pearson (1915) has shown that the probable error of ϕ_t^2 is more easy to calculate than that of ϕ_a^2 and is sufficiently accurate for all practical purposes. The probable error of the mean square contingency coefficient, as thus determined, is given by the expression

$$\frac{.67449}{\sqrt{N}} \left\{ \frac{\gamma_a^3/\phi_a^2 + 1 - \phi_a^2}{(1+\phi_a^2)^3} \right\}^{\frac{1}{2}}$$

where $$\gamma_a^3 = \frac{1}{N} S \left\{ \frac{\left(n_{pq} - \frac{n_{:q} n_{p:}}{N}\right)^2}{\frac{n_{:q} n_{p:}}{N}} \times \frac{n_{pq} - \frac{n_{:q} n_{p:}}{N}}{\frac{n_{:q} n_{p:}}{N}} \right\}$$

In all sections of this Thesis the probable errors of mean square contingency coefficients were calculated in this manner.

It should be mentioned that the values for C_2 could have been transformed into approximate values for the corresponding coefficients of correlation, of which the probable errors are easily found, but it was considered that more accurate results would be obtained by giving the coefficients in the form of mean square contingencies. The significance of differences between contingency coefficients was determined in the usual manner, as in the calculation of the probable errors of differences between means.

Schick State and Age: Sex Differences.

In Tables XXI and XXII are given the actual and expected

TABLE XXI.

Contingency Table: Schick State and Age; All Seasons: Males.

Schick State	Age Group					
	To 2+	3+ to 5+	6+ to 8+	9+ to 11+	12 and over	Totals
Positive +++	28 (35.66)	69 (61.90)	79 (61.07)	27 (34.65)	31 (40.71)	234
Positive ++	157 (101.7)	217 (176.4)	162 (174.1)	89 (98.78)	42 (116.0)	667
Positive +	20 (22.10)	46 (38.36)	40 (37.84)	16 (21.48)	23 (25.23)	145
Negative	7 (52.57)	36 (91.26)	82 (90.04)	74 (51.10)	146 (60.01)	345
Totals	212	368	363	206	242	1391

TABLE XXII.

Contingency Table: Schick State and Age: All Seasons; Females.

Schick State	Age Group					
	To 2+	3+ to 5+	6+ to 8+	9+ to 11+	12 and over	Totals
Positive +++	31 (37.46)	88 (74.17)	65 (69.08)	30 (36.33)	26 (22.97)	240
Positive ++	143 (110.66)	251 (219.10)	206 (204.08)	86 (107.32)	23 (67.84)	709
Positive +	18 (15.76)	23 (31.21)	34 (29.07)	11 (15.29)	15 (9.66)	101
Negative	7 (35.12)	32 (69.53)	62 (64.76)	66 (34.06)	58 (21.53)	225
Totals	199	394	367	193	122	1275

TABLE XXIII.

Contingency Table: Schick State and Age: Spring: Males.

Schick State	Age Group					Totals
	To 2+	3+ to 5+	6+ to 8+	9+ to 11+	12 and over	
Positive +++	8 (7.30)	12 (15.53)	25 (15.26)	7 (8.89)	17 (22.03)	69
Positive ++	36 (22.42)	70 (47.70)	54 (46.89)	29 (27.32)	23 (67.68)	212
Positive +	8 (6.45)	18 (13.73)	15 (13.49)	7 (7.86)	13 (19.47)	61
Negative	3 (18.83)	17 (40.05)	21 (39.37)	24 (22.94)	113 (56.82)	178
	55	117	115	67	166	520

TABLE XXIV.

Contingency Table: Schick State and Age: Summer: Males.

Schick State	Age Group					Totals
	To 2+	3+ to 5+	6+ to 8+	9+ to 11+	12 and over	
Positive +++	10 (13.54)	28 (23.85)	34 (28.29)	13 (19.20)	12 (12.13)	97
Positive ++	48 (32.24)	66 (56.79)	59 (67.38)	41 (45.72)	17 (28.88)	231
Positive +	8 (5.72)	12 (10.08)	12 (11.96)	3 (8.12)	6 (5.13)	41
Negative	1 (1.55)	12 (27.29)	35 (32.38)	38 (21.97)	25 (13.88)	111
Totals	67	118	140	95	60	480

TABLE XXV.

Contingency Table: Schick State and Age: Autumn: Males.

Schick State	Age Group To 2+	3+ to 5+	6+ to 8+	9+ and over	Totals
Positive +++	7 (8.01)	14 (12.67)	14 (11.92)	3 (5.40)	38
Positive ++	34 (24.03)	42 (38.00)	30 (35.77)	8 (16.21)	114
Positive +	1 (5.27)	10 (8.33)	9 (7.84)	5 (3.55)	25
Negative	1 (5.69)	2 (9.00)	11 (8.47)	13 (3.84)	27
Totals	43	68	64	29	204

TABLE XXVI.

Contingency Table: Schick State and Age: Winter: Males.

Schick State	Age Group To 2+	3+ to 5+	6+ to 8+	9+ and over	Totals
Positive +++	3 (7.54)	15 (10.43)	6 (7.06)	6 (4.97)	30
Positive ++	39 (27.65)	39 (38.24)	19 (25.88)	13 (18.24)	110
Positive +	3 (4.52)	6 (6.26)	4 (4.24)	5 (2.98)	18
Negative	2 (7.29)	5 (10.08)	15 (6.82)	7 (4.81)	29
Totals	47	65	44	31	187

TABLE XXVII.

Contingency Table: Schick State and Age: Spring: Females.

Schick State	Age Group To 2+	3+ to 5+	6+ to 8+	9+ to 11+	12 and over	Totals
Positive +++	8 (11.00)	24 (22.17)	20 (19.90)	6 (10.82)	16 (10.12)	74
Positive ++	47 (35.51)	85 (71.59)	68 (64.26)	27 (34.95)	12 (32.69)	239
Positive +	3 (4.90)	9 (9.88)	12 (8.87)	5 (3.83)	4 (4.51)	33
Negative	5 (11.59)	9 (23.36)	14 (20.97)	24 (11.41)	26 (10.67)	78
Totals	63	127	114	62	58	424

TABLE XXVIII.

Contingency Table: Schick State and Age: Summer: Females.

Schick State	Age Group To 2+	3+ to 5+	6 to 8+	9+to 11+	12 and over	Totals
Positive +++	6 (9.75)	30 (25.53)	27 (23.80)	8 (12.44)	4 (6.39)	75
Positive ++	41 (31.34)	83 (72.41)	72 (76.73)	36 (39.99)	9 (20.53)	241
Positive +	9 (4.55)	6 (10.52)	11 (11.14)	4 (5.81)	5 (2.98)	35
Negative	2 (12.35)	15 (28.54)	32 (30.25)	26 (15.76)	20 (8.09)	95
Totals	58	134	142	74	38	446

TABLE XXIX.

Contingency Table: Schick State and Age: Autumn: Females

Schick State	Age Group				Totals
	To 3+	4+ to 6+	7+ to 9+	10 and over	
Positive +++	12 (11.67)	21 (18.96)	6 (5.42)	1 (3.96)	40
Positive ++	38 (32.38)	55 (52.61)	14 (15.03)	4 (10.98)	111
Positive +	5 (4.08)	5 (6.64)	2 (1.90)	2 (1.39)	14
Negative	1 (7.88)	10 (12.80)	4 (3.66)	12 (2.67)	27
Totals	56	91	26	19	192

TABLE XXX

Contingency Table: Schick State and Age: Winter: Females.

Schick State	Age Group				Totals
	To 3+	4+ to 6+	7+ to 9+	10 and over	
Positive +++	16 (15.09)	14 (15.09)	7 (10.54)	16 (10.30	51
Positive ++	43 (34.90)	39 (34.90)	25 (24.38)	11 (25.82)	118
Positive +	5 (5.62)	5 (5.62)	7 (3.93)	4 (3.84)	19
Negative	1 (7.39)	7 (7.39)	5 (5.16)	12 (5.05)	25
Totals	65	65	44	43	213

frequencies for all males and for all females respectively arranged in 5 x 4 tables. From these tables the following values were calculated:-

	χ^2	n	P	C_2
Males	310.7600	12	<0.01	0.4273±.0372
Females	195.5123	12	<0.01	0.3646±.0174

The values of χ^2 and P indicate in each case that the distribution is not a random one. It is rather surprising that the values of C_2 for both sexes indicate only a moderate degree of association between age and the development of a negative Schick result. The association is in fact very much lower than one would have expected. The difference between the coefficients is 0.0627±.0411; that is, the difference is very small and is not significant.

Schick State and Age: Sex and Seasonal Variations.

The data were then treated in a more detailed manner, in order to investigate the possibility of any change in the strength of the association between Schick state and age at different seasons. Tables XXIII to XXX give the contingency tables from which the coefficients were calculated. In forming these contingency tables it was impossible to make the grouping the same in all cases, since this method would have left certain cells with zero frequency. In most cases it will be seen that the grouping is 5 x 4, and in only two instances was it necessary to make a slight alteration in the position of the divisions on the vertical scale. These alterations do not, of course, have any effect per se on the value of the coefficient. The actual results are set out in Table XXXI.

TABLE XXXI.

(a) Males.

Season	χ^2	n	P	C_2
i) Spring	152.8202	12	< 0.01	0.4766±.0188
ii) Summer	54.6468	12	< 0.01	0.3197±.0272
iii) Autumn	47.8181	9	< 0.01	0.4358±.0440
iv) Winter	32.2414	9	< 0.01	0.3839±.0410

(b) Females.

Season	χ^2	n	P	C_2
(i) Spring	82.9792	12	<0.01	0.4046±.0275
(ii) Summer	66.1155	12	<0.01	0.3593±.0280
(iii) Autumn	51.7478	9	<0.01	0.4608±.0558
(iv) Winter	32.5885	9	<0.01	0.3643±.0384

In this table the values of χ^2 and of P indicate that in each case the distribution is not random. From a casual inspection of the values of C_2 it would appear that the association for males is strongest in ~~the~~ Spring, and lowest in Summer. In the case of females, the association is again lowest in Summer, but it is apparently stronger in Autumn than in Spring. In order to appreciate the meaning of these results it is necessary to investigate the differences more fully.

In Table XXXII the differences between the values in Table XXXI are set out along with their probable errors. The probable errors show that none of the differences in the female group is significant. In the male group however, the difference between the coefficient for Spring and Summer is considerable, and is definitely significant, since it is more than five times its probable error. The differences between the coefficients for Spring and Winter and for Summer and Autumn respectively are also considerable, and each is probably significant, since it is more than twice its probable error.

TABLE XXXII.

Differences between seasonal values of C_2 in Table XXXI.

Males.		Females.	
(i) and (ii)	0.1569±.0331	(i) and (ii)	0.0453±.0393
(i) and (iii)	0.0408±.0479	(i) and (iii)	0.0562±.0622
(i) and (iv)	0.0927±.0451	(i) and (iv)	0.0403±.0472
(ii) " (iii)	0.1161±.0517	(ii) " (iii)	0.1015±.0624
(ii) " (iv)	0.0642±.0492	(ii) " (iv)	0.0050±.0475
(iii " (iv)	0.0519±.0601	(iii) "(iv)	0.0965±.0677

These differences are extremely interesting. In considering their meaning we must remember that, in the working class population of a city like Leeds from which these samples

were drawn, age is practically synonymous with length of exposure to the C.diphtheriae. We may assume therefore that, though the association between the development of a negative Schick reaction and exposure to the organism is practically the same in males and females respectively, variations in the degree of this association nevertheless takes place in males though not in females. Dudley (1929) discussed the evidence which was given by the incidence of pseudo-reactors in different communities, and he concluded that this evidence, though by no means definite, favoured the hypothesis that the potentiality of responding to antigenic stimuli from the diphtheria bacilli in the environment is greater in the average male than in the average female. The data which are given here lend considerable support to this hypothesis and even carry it a stage further. It will be noted that the greatest difference for males is between the coefficients for Spring and Summer and that the next greatest difference is between those for Summer and Autumn. Antigenic stimuli in the environment are liable to be most frequent in the Winter and early Spring, and hence we must assume that the increased liability of the male to become Schick negative in the Spring is in some way associated with these increased stimuli. Though contingency does not necessarily indicate causation, it is not necessary to neglect the suggestions which may arise from a consideration of contingency. There seems therefore to be some justification for concluding that the male has a capacity for increasing his power of responding to antigenic stimuli when it is most wanted. For some unknown reason the female does not appear to have this capacity. The evidence points to the fact, already suggested by Dudley, that susceptibility to diphtheria is a true hereditary sex-linked factor.

The Incidence and Significance of Protein Reactions.

During the course of the present investigation the writer was frequently at a loss to interpret certain Schick reactions. This was rather remarkable since difficulty had not previously been experienced in disintguishing pseudo-reactions from true Schick reactions. Dudley (1929) and others claimed that certainly in not more than 2 per cent of cases should it be impossible to interpret the Schick reaction, and it is evident that Dudley believes that an experienced observer would be able to give a more or less definite opinion on practically every test. It is admitted by many authors that the frequency of pseudo-reactions may vary in different places and at different times. For example, Dickinson (1922) noted that 76 per cent of 109 convalescent diphtheria patients had positive pseudo-reactions. Dudley (1929) also noted a very high incidence of pseudo-reactions at Greenwich just after a big epidemic of diphtheria in the School. The difficulty in Leeds, however, was not the frequency of anomalous reactions, for actually they were comparatively rare. The source of confusion was the type of reaction. In the past it has been customary to divide pseudo-reactions into ordinary pseudo ("pseudo-negative") and pseudo and positive ("combined")reactions. In the former the erythema affects only the control arm; in the latter it affects both arms, but the erythema on the test arm is more marked than thst on the control. In any case the protein reactions fade within two or three days, and by the seventh day a definite negative or positive Schick reaction can usually be read. In Dickinsons series 4.5 per cent of 222 pseudo-reactions were visible at the end of a week, and he does not appear to have had a great deal of difficulty in interpreting them. In the present series such pseudo-reactions were of course met with, but the reaction which will now be discussed was of a different type. In this reaction the erythema developed by the second day at the site of both the test and the control. These two areas of erythema were practically identical in appearance. Instead of the protein ~~reac-~~

reactions disappearing about the third or fourth day, however, the reactions on both arms continued to develop pari passu until by the seventh day the two were still almost identical.

Reactions of this type do not appear to have been previously described. In designating them it is obviously undesirable to refer to them as "pseudo" reactions, since this term has usually been applied to reactions which, while developing in both arms simultaneously, begin to show differences in their appearance after a few days, so that it should be possible to arrive in most cases at a definite decision by the seventh day. In the reactions which are here described the erythema develops simultaneously in both arms, and the further course is as given above. Further for the purposes of this paper it is proposed to designate these phenomena as "test-control" reactions, and for convenience "T.C.+" may be taken to indicate that the reaction thus described showed practically identical appearances at the test and control sites on the seventh day. A discussion of the titration results in these cases will be given in the next section, and it will be shown that if these reactions had been read as abnormally persistent pseudo-reactions, indicating that the individuals were immune, the inference would have been wrong in about fifty per cent of instances.

The incidence of true pseudo and true combined reactions certainly varies markedly with age; but it seems that in addition to these variations the incidence has been falling in recent years. In 1923 the frequency of pseudo-reactions was found by Dudley (1923) to be about 2.3 per cent in a series of boys aged 11 to 16 years. Kelly, Stevens, and Beattie (1925) in their Californian series found that the incidence of all types of pseudo-reactions was about 1.5 per cent. In most recent reports on the Schick test which the writer has read the incidence of pseudo-reactions has been so small that little mention has been made of the condition. In the present series the incidence of any type of pseudo-reaction was not more than 4 per cent. This decrease in the frequency

is probably to be attributed largely to improvements in the method of production of Schick test fluid. In young adults and adults, however, a considerable percentage may still occur. An example of this is given by the series of 559 adults in an institution, who were tested by Young, Bunney, Crooks, Cummings, and Forsbeck (1934). These authors found that pseudo-reactors constituted 13 per cent, and combined reactors 2 per cent of the total.

The incidence of true pseudo-reactions and positive test-control reactions among persons in the Leeds series who had positive Schick tests, is given in Table XXXIII. It will be seen that the frequency of both types of reaction is so low as to be almost negligible. The corresponding figures for the different types of pseudo-reactions and of test-control reactions in persons who had Schick tests performed after inoculation are given in Tables XXXIV(a) and XXXIV(b). It will be noted that the frequency of pseudo-reactions and of test-control reactions had increased very markedly - the pseudo-reactions by about eighteen times and the test-control reactions by about five times. Further, the incidence for the two types of reaction is practically identical. Von Gröer and Kassowitz (1919) were probably the first to suggest that pseudo-reactions are due to bacillary substances, and are really true allergic reactions. Dudley (1923, 1929) later showed that the occurence of a pseudo-reaction indicated fairly recent sensitization of the skin by the proteins of the C.diphtheriae. When we take into consideration the similar frequency (in the post-Schick tests) of the two reactions which are under discussion, there would seem to be some grounds for believing that the test-control phenomenon is also due to sensitization of the individual by the products of the C.diphtheriae, and possibly by its toxin derivatives.

Sex incidence of pseudo-reactors. It is a well known fact that pseudo and negative reactions are about three times as common as pseudo and positive reactions. In the present series the difference in the post-Schick tests was not quite so marked

TABLE XXXIII

Primary Schick Tests

Incidence of Pseudo-reactors and T.C. reactors

Individuals Observed.			Total Pseudo-Reactors			Total Test-Control Reactors		
Male	Female	Both Sexes.	Male	Female	Both Sexes	Male	Female	Both Sexes
1891	1275	2666	3	3	6	8	11	19
			Per cent. Pseudo.			Per cent. Test-control.		
			Male	Female	Both Sexes	Male	Female	Both Sexes
			0.22 ± 0.09	0.24 ± 0.09	0.23 ± 0.06	0.58 ± 0.14	0.86 ± 0.18	0.71 ± 0.11

TABLE XXXIV(a)

Post-Schick Tests: Incidence of Pseudo-reactions

	Individuals Observed			Total Ψ			Ψ +			Ψ −		
	M	F	Both Sexes	M	F	Both Sexes	M	F	Both Sexes	M	F	Both Sexes
Total Reactions	451	436	887	17	22	39	8	8	16	9	14	23
Repeat Tests in Same Individual	73	53	126	4	4	11	5	2	7	2	2	4
Individuals Observed	378	383	761	10	18	28	3	6	9	7	12	19
Percentages (for total individuals)				2.6 ± 0.55	4.7 ± 0.73	3.7 ± 0.46	0.8 ± 0.01	1.6 ± 0.43	1.2 ± 0.27	1.9 ± 0.47	3.1 ± 0.60	2.5 ± 0.38

TABLE XXXIV(b)

Post-Schick Tests: Incidence of Test-control Reactions

	Individuals Observed			Test-Control Reactions			Percentage of Test-Control Reactions		
	M	F	Both	M	F	Both	Male	Female	Both Sexes
Total Reactions	967	975	1942	34	35	69	3.5	3.5	3.6
Repeat Tests in Same Individual	84	61	145	5	4	9	6.0	6.6	6.2
Individuals Observed	883	914	1797	29	31	60	3.3 ± 0.41	3.4 ± 0.41	3.3 ± 0.28

since the respective percentages were 2.5 and 1.2. The difference is 1.3±.047 per cent, which is barely significant. The sex differences in certain large series of tests have been fairly marked, For example, Dudley (1929) showed that in the Californian series (Kelly, Stevens and Beattie, 1925) combined reactions are more frequent in males than in females, while the pseudo and negative reactions are equal in both sexes. In the present series of primary Schick tests the differences between the percentages for males and females respectively were : for pseudo and combined reactions 0.02±.13; for test-control reactions, 0.28±.23. In the post-Schick tests there is obviously no sex difference for the incidence of test-control reactors (see Table XXXIV(b)). In ordinary pseudo-reactions the differences between the percentages for males and females respectively are : for pseudo and combined reactions, 2.1±.92: for combined reactions, 0.08±43; for pseudo-reactions, 1.2±.76 per cent. None of these differences is definitely significant.

It is felt that this short discussion goes only part of the way towards the elucidation of the significance of protein reactions in a gravis-infected area, and that much more work will be required before a clear understanding is reached. The problem was, however, attacked from another angle and the conclusions which were reaached will be found in section (d) which deals with the Moloney test.

PART I

Section (c)

ON THE RELATIONSHIP BETWEEN THE SCHICK TEST AND THE DIPHTHERIA ANTITOXIN LEVEL OF THE BLOOD.

Since Römer (1909) first announced his method of determining the antitoxin content of blood serum frequent attempts have been made to ascertain whether there is an exact relationship between such findings and the results of Schick tests performed on the same individuals. The question is, however, one which is hedged about by many inherent difficulties, and it still eludes a definite solution. The actual presence of antitoxin in the blood of Schick negative individuals was first demonstrated by Karasawa and Schick (1910), using Römer's method, and they also showed that antitoxin was more often present in the blood of adults than of children. Three years later Michiels and Schick (1913) decided that a suitable antitoxin level for the Schick test was 1/30 unit - Schick positive cases having less than that amount, and schick negative cases more. Kolmer and Moshage (1915) confirmed these findings. In their work ten individuals who had positive Schick tests each showed less than 1/40 unit of antitoxin per c.cm. of blood; whereas twenty individuals with negative tests had each more than 1/20 unit, and some had as much as 10 units. Von Gröer and Kasowitz (1915) published a very important investigation dealing with the antitoxic content of the blood of infants, and they found that ~~eighty four~~ 84 per cent of these had 1/200 unit or more of antitoxin. In a further paper (1917) they showed that at least 81 per cent of normal adults had more than 1/200 units of antitoxin in their blood, and that of this percentage the majority had at least 1/50 unit. In 1919 these authors added still further to their investigations on the population of Vienna and reported the results

of titration of the blood of 1,062 normal individuals of all ages. The difficulties associated with the latter part of the War period in Austria prevented the investigators from making the investigation as complete, from the qualitative standpoint, as in their previous work, and they had to confine themselves to the estimation of the presence or absence in each case of 1/200 unit or more of circulating antitoxin. The curve which they published shows that the increase in the percentage of persons with this amount is inclined to advance by a series of approximately three steps to the high percentage of 84 in young adult life. The three steps are quite marked in the graph, and probably indicate certain definite stages in the lives of the individuals. These antitoxin estimations were accompanied by Schick tests on each individual, but a comparison betweem the two levels is somewhat vitiated by the fact that these authors had an unduly high percentage of pseudo-reactions, and also by the fact that in the larger part of the investigation titrations were carried out only within very broad limits. As a result of their work it is, however, possible to state that the curve showing the percentage of persons who have more than 1/200 unit of antitoxin in their blood agrees fairly closely with the curve showing the percentages of Schick negative reactors at different ages. From their earlier work it would further appear that these authors were of opinion that most immune persons have 1/50 unit of antitoxin in their blood, but that considerable immunity may also be given in some cases by amounts varying from 1/200 to 1/50 unit. In a later paper, Schick, von Gröer and Kassowitz (1924) definitely defined the Schick level of immunity as 1/200 unit. Although this large series of cases undoubtedly constitutes an important investigation, it should be noted that the results cannot be accepted without certain reservations. The criticism has been made that not only was the population peculiar in that it was suffering generally from the under-nutrition associated with the War, but the individuals were drawn from a specialised hospital class and many of them were actually residents in institutions. Similar criticisms may be laid against many such investigations, and it is doubtful whether we should regard these results

of von Gröer and Kassowitz as seriously invalidated. Beber (1921), from guinea-pig experiments, suggested that 1/20 unit was necessary to protect against one fatal dose in these animals; he also thought that 1/100 unit might give protection against less severe infections

In this country it has become customary to regard the Schick level of immunity as considerably higher than 1/200 unit. For example, O'Brien and his colleagues (1922) determined the antitoxin content of the blood of 66 individuals and they concluded that 1/30 unit of antitoxin was necessary to prevent the development of a positive Schick reaction. This standard was for long regarded as a useful average basis on which to work, but within the last few years it has come to be recognised that this level is much too high. For example, Hewlett (1930) states that immunity to diphtheria runs parallel with the presence of a small amount - 1/200 to 1/40 unit per c.cm. - of anxtitoxin in the blood. Topley (1933) is of opinion that less than 1/100 unit of antitoxin per c.cm. will render an individual immune to diphtheria. In his recent work at Greenwich Hospital School Dudley (1934) found that, of 89 boys who were naturally or artificially immune and on whom blood titration had been performed, only one had less than 1/100 unit of antitoxin but 20, or 22.5±3.0 per cent had 1/25 or less. From his observations Dudley suggests that at Greenwich the Schick level was nearer to 1/100 unit than to 1/30 unit, and it is to be noted that these investigations were carried out in a semi-closed community in which infection by C.diphtheriae gravis had taken place. O'Brien recently indicated that he was of opinion that individuals who have a titre of 1/250 unit have reasonable immunity, and that most cases with a titre of 1/100 should show negative Schick reactions.

Im America the tendency has been to retain the original standard, in the region of 1/30 unit of antitoxin per c.cm. For example, Moloney and Fraser (1927) titrated the blood serum of 133 individuals before immunization was carried out. Of 65 who showed positive Schick tests, all had less than 1/50 units. In

a series of 41 negative reactors - 24 of whom showed a positive Moloney test - the titration results were as follows: 33 had 1/10 unit or more; two had 1/15; two had 1/20; one had 1/25: two had 1/35; and one had less than 1/50. It is evident that in this series the Schick level was about 1/50. This conclusion from the early work of Moloney and Fraser is borne out by the later paper of Fitzgerald, Defries, Fraser, Moloney and McKinnon (1932), from which the inference may be drawn that in Toronto individuals who have less than 1/50 unit are regarded as positive Schick reactors, and that after immunization a titre of 1/25 unit is regarded as satisfactory. In a recent investigation Messeloff and Karsh (1932) found that 94 per cent of fifty one Schick negative children had at least 1/30 unit of circulating antitoxin. Flood (1930) reported less than 1/30 unit of antitoxin in a certain percentage of Schick negative persons. Klein (1934) on the other hand confirmed the results of Messeloff and Karsh. He titrated the blood of sixty-six Schick negative children, most of whom were artificial immunes, and found that sixty-five had a blood titre of over 1/30 unit per c.cm: the other case had between 1/75 and 1/30 unit. In native races the results seem to be more or less comparable to these. For example, Grasset, Perret - Gentil, and Friedman (1933) carried out a series of investigations on the natural immunity to diphtheria of South African Bantus. They showed that 7.56 per cent of 172 natives' sera had less than 1/50 unit of antitoxin. They assumed that 1/50 unit represented the level below which the individual will show a positive Schick reaction - the assumption being based largely on the fact that a similar percentage (viz. 8.29) of natives who were tested showed positive Schick reactions.

On the Continent such comparatively strict standards are being abandoned. For example, Meersseman, Friess, and Renard (1933) tested forty seven subjects with two strengths of Schick toxin: one fluid was of the normal strength prescribed by the Institut Pasteur, and the other was of double this strength. The

blood antitoxin results were correlated with the readings of these tests, and it was found that individuals who showed a negative reaction to the normal dilution, but a positive reaction to the double strength fluid, had an antitoxic content of less than 1/30 unit. Claus Jensen (1931a) carried out a series of investigations (a) on twenty nine natural immunes, and (b) on 141 artificial immunes (179 tests on 141 individuals). All the individuals investigated were Schick negative. In group (a) he found that 4 (14 per cent) had a titre of less than 1/2000, and in group (b) 9 (5 per cent) had titres of less than 1/200. Jensen says: "Il n'existe pas, comme on l'a admis généralement, un titre antitoxique fixe (0,03 ou 0,01 U.A.) - 'the Schick level' - pour lequel des individus passent du groupe 'négatif' au groupe 'positif' ou vice-versa, cette limite présente des variations individuelles considérables". In a previous paper Jensen (1931b) noted that an individual who had only 0.00007 units of antitoxin per c.cm, had a negative Schick reaction. Further, he showed (Jensen, 1931c) that 90 per cent of strong positive Schick reactors have less than 1/2000 unit of antitoxin in their blood. After immunization on the other hand positive reactors usually have anything up to 1/40 unit of antitoxin per c.cm of serum.

Description of the Investigation.

The aim of the present investigation was two-fold: (a) to gain some idea of the correspondence between the results of Schick tests and the antitoxin content of the blood in a community heavily infected with C.diphtheriae gravis: and (b) to investigate the actual antitoxin content of the blood in a number of cases in which it was difficult or impossible to form a definite opinion on the Schick state of the individual. The actual titration of the blood samples was carried out at the Wellcome Physiological Research Laboratories; for this service I am deeply indebted to Dr. R. A. O'Brien and Mr A. T. Clenny.

In every case - except those where the samples were taken in hospital - the blood was drawn on the seventh day after the performance of the Schick test, at the time when the latter was

read. It may be objected that this interval was perhaps sufficient to allow the process of secondary stimulation to come into play, and that therefore the antitoxin content might be in every case higher than it actually was when the Schick test was performed. There are, however, three good reasons for believing that this objection did not carry much weight: (a) in group A over 75 per cent and in group B over 50 per cent, of the tests were "primary" tests - that is, the blood was taken from, and the Schick tests were performed on, individuals who had not previously had any immunising injections. In the natural state we do not expect that a primary stimulus has previously functioned to any extent, and we therefore do not suppose that the small amount of toxin which is used for the Schick test will have any effect in raising the antitoxin level. Further, it is doubtful if this small amount of toxin could effect an appreciable increase in the antitoxin content in the short space of seven days. From Jensen's curves (Jensen 1933) it would appear that in his experiments the antitoxin level reached its peak only after about a fortnight or longer. (b) A second reason for believing that the interval does not vitiate the results in the present series of cases is that in the borderline cases the discrepancy is on the wrong side of the line of demarcation to favour such vitiation. This point will be discussed more fully later. (c) The most important evidence is given by a small investigation which the writer carried out, repeating the tests in the same individuals. This will be described later.

The present series of cases is divided into two groups: (i) those cases in which the reading of the Schick test was quite definite; and (ii) those cases in which it was difficult or impossible to read the Schick results.

Blood Content of Cases in which the Schick Result was Definite.

The main particulars regarding these cases are set out in Table XXXV. The information is summarised in Table XXXVI, which gives the percentage of cases showing a negative Schick test within each set of limits for each antitoxin content. It will be seen

TABLE XXXV.

ACCEPTED DEFINITE OR POSITIVE CASES.

Case No.	Age	Schick result	Moloney result	Antitoxin content of blood	P = Primary Schick S = Schick test after inoculation H = blood drawn in hospital
C1	6	++(25)	0	<1/1000	S
C2	6	+ (20)	-	<1/1000	S
C3	6	+ (15x20)	0	<1/1000	S
C4	6	++(30)	-	<1/1000	S
C5	6	++(30)	-	<1/1000	S
C6	6	+(17x20)	0	1/1000	S
C7	3	+(15x20)	0	1/1000	S
C9	6	+(20x10)	0	<1/1000	S
C10	6	+ (18)	0	<1/1000	S
C11	5	+ (20)	-	<1/1000	S
C12	5	+ (23)	0	<1/1000	S
C13	5	++(20x25)	0	<1/1000	S
C14	6	++(25x30)	0	<1/1000	S
C15	5	+ (25)	-	<1/1000	S
C16	6	0	0	1/10	S
C17	6	++(25)	Ft.+	<1/1000	S
C18	7	0	+D*	2	P
C19	9	0	+	1	S
C20	8	++(30)	+D	<1/1000	P
C22	13	0	+++	1/2	P
C23	11	0	+	1	P
C24	5	++(22)	+	<1/1000	P
C25	14	+(Ft:25)	+	<1/1000	P
C26	14	0	+++	1/2	P
C27	10	+(Ft.25)	+++	<1/1000	P
C28	5	0	0	1/10 - 1	P
C29	8	0	0	1/50	P
C30	7	+(Ft.)	+D	1/25	P
C31	11	0	++	1/10 - 1	P
C34	6	+(Ft.20)	-	<1/1000	S
C35	6	+(Ft.20)	-	<1/1000	S
C36	9	+(Et.10)	-	1/250	S
C37	10	0	++	1/25	P
C38	8	+(22)	0	1/100	P
C39	9	0	+++	1/25	P
C40	8	+(17)	++	< 1/1000	P
C41	11	0	+	1/5	P
C42	7	0	+D	1/10	P
C44	14	+(Ft.25)	+D	<1/1000	P
C45	13	0	+	1/5	P
C46	11	+ (25)	++	<1/1000	P
C47	9	0	+++	1	P
C48	11	0	+	2	P
C49	12	0	0	1/5	P
C50	9	0	0	1/2	P
C51	11	0	+	1/2	P
C52	12	0	+	2	P
C53	12	0	++	1	P
C54	11	0	+	2	P
C55	9	0	+	1	P
C57	8	0	+D	1/100	P
C58	10	0	+D	1/2	P
C59	11	0	++	2	P
C60	15	0	++	1	P
C62	11	0	0	>1	P

* see end of Table

TABLE XXXV (CONTD)

Case No.	Age	Schick result	Moloney result	Antitoxin content of Blood	P. = Primar S. = Schick
C64	10	0	+D	1/250	P
C65	13	++(20)	++	<1/1000	P
C66	10	+++(24)	++	<1/1000	P
C67	10	+(25)	+D	<1/1000	P
C68	13	++++(70)	+	<1/1000	P
C69	14	0	++	1	P
C70	15	0	++	1	P
C71	14	0	+	1/5	P
C72	14	0	++	1	P
C73	10	0	++	1	P
C74	13	0	+++	1/5	P
C75	8	0	0	1/25	P
C76	13	0	+	1	P
C77	13	0	++	1/5	P
C78	13	0	+	1/250	P
C79	3	0	0	1/250	P
C80	17	0	+D	1/5	P
C81	18	0	+D	1/5	P
C82	18	0	+D	1/5	P
C85	15	0	+D	1/5	P
C86	18	0	+D	1/2	P
C87	10	0	+	1/10	P
C88	11	0	0	1/5	P
C89	12	0	+	1/5	P
C90	12	0	+	1/250	P
C91	9	+(V.ft)	+++	1/250	P
C92	13	0	+++	1/5	P
C93	13	+(ft.35x 20	+	<1/1000	P
C95	7	+(20 with desquamation)	++	1/5	P
C96	14	0	+	2	P
C97	4	0	+	2	P
C99	5	0	++	>5	P
C100	7	0	++	2	P
C101	13	0	0	1/2	P
C102	11	0	+D	2	P
C103	11	0	++	1	P
C104	9	0	++	2	P
C108	12	0	-	2	S
C109	12	+(25)	++	1/2	P
C113	6	0	-	>5	H
C114	14	+(Ft.22)	+	<1/1000	P
C115	23	+(Ft.28)	++	<1/1000	P
C116	25	+(Ft.30)	+	<1/1000	P
C118	9	+(16)	-	1/500	H
C120	10	++(30)	+++	<1/1000	P
C123	12	+(25)	-	1/1000	S
C124	10	0	-	1/250	H
C125	10	0	-	1/25 - 1/10	H
C126	13	0	+++	5	P
C127	9	0	+D	1/2	P
C128	13	+(30x15)	0	<1/1000	P
C129	9	0	+	5	P
C130	6	0	+D	2	P
C131	3	++(27)	+D	<1/1000	P
C132	7	++(24 with desq.)	-	1/5	H
C133	10	+(28)	+	1/1000	P
C134	7	0	-	1/5	H
C135	6	0	-	1/50	H

TABLE XXXV (CONTD)

Case No.	Age	Schick result	Moloney result	Antitoxin content of blood	P - Primary S - Schick H -
C139	7	0	-	1/100	S
C140	8	0	-	5	S
C142	9	0	-	1/10	S
C143	9	0	-	1/100	S
C144	4	0	-	1/100	S

* For a description of this reaction see section (d) of this Thesis.

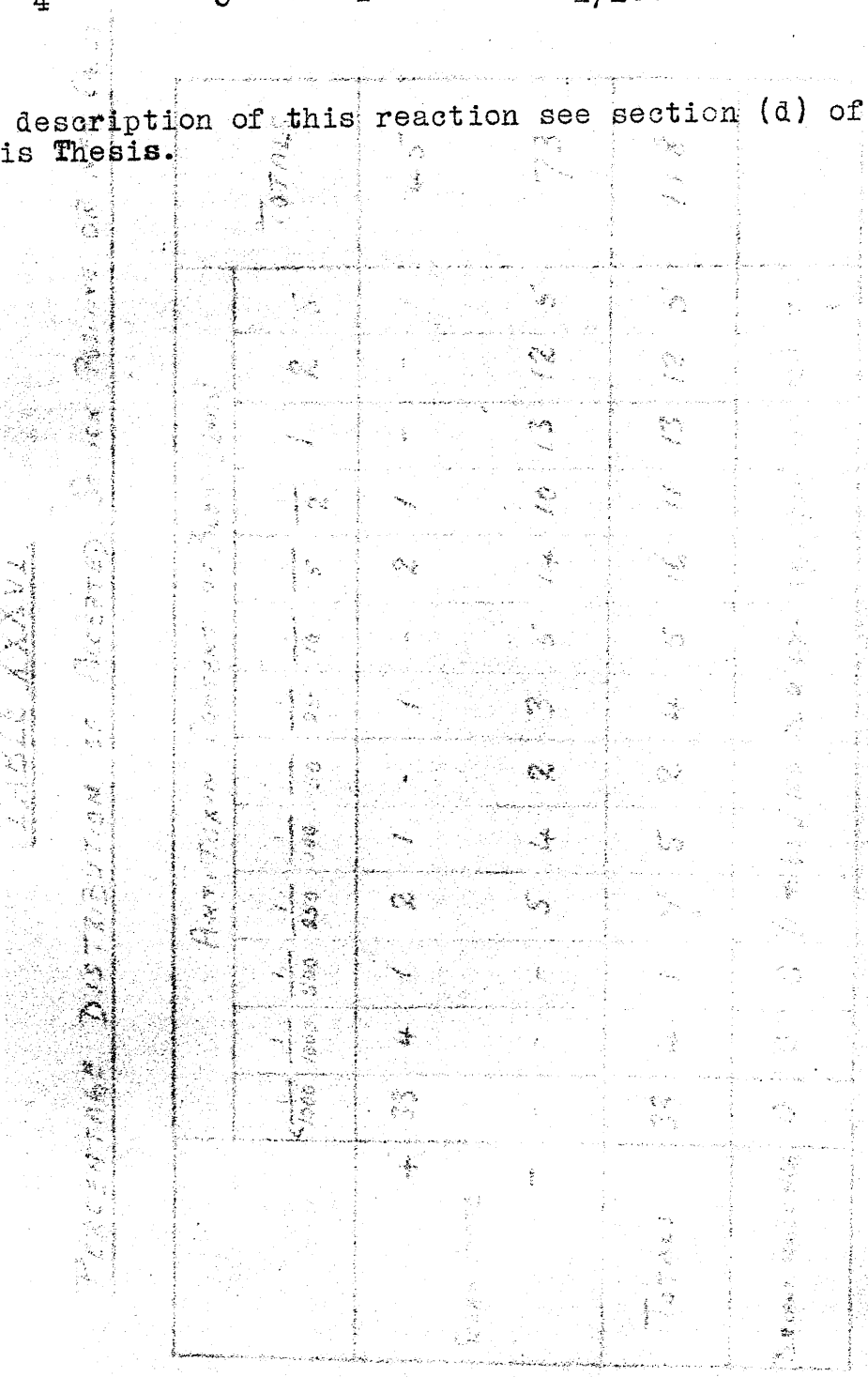


TABLE XXXVI.

PERCENTAGE DISTRIBUTION OF ACCEPTED SCHICK-POSITIVE OR NEG. CASES

	ANTITOXIN CONTENT OF BLOOD (UNITS)													TOTALS
	<1/1000	1/1000	1/500	1/250	1/100	1/50	1/25	1/10	1/5	1/2	1	2	5	
SCHICK STATE +	33	4	1	2	1	-	1	-	2	1	-	-	-	45
SCHICK STATE −	-	-	-	5	4	2	3	5	14	10	13	12	5	73
TOTALS	33	4	1	7	5	2	4	5	16	11	13	12	5	118
PER CENT. SCHICK NEG.	0	0	0	71.4	80.0	100	75.0	100	87.5	90.9	100	100	100	

that the cases fall broadly into two groups: (a) cases in which, for every titre the blood content agreed with the Schick test# results; (b) groups of cases, a proportion of which showed at certain titres discrepancies between the Schick test and the blood findings. Group (a) embraces all the cases in which the blood antitoxin was less than 1/250 unit per c.cm. (38 cases), and cases in which the blood content was over $\frac{1}{2}$ unit (30 cases). Group (b) lies between these two extremes and is made up of 50 cases. These will require further examination.

The cases which have 1/50 unit and 1/10 unit respectively may be left out of further consideration, since all cases in ~~which~~ these groups had an antitoxin content which accorded in each instance with the Schick result. Particulars of the other 43 cases are given in Table XXXVII.

TABLE XXXVII.

Blood content.		Cases	(P - primary Schick tests S - tested after inoculation)
1/250	Positive	2	(1P; 1S)
	Negative	5	(4P; one case bled when Schick performed)
1/100	Positive	1	(1P)
	Negative	4	(1P: 3S)
1/25	Positive	1	(1P)
	Negative	3	(3P)
1/5	Positive	2	(1P: one case bled when Schick performed)
	Negative	14	(13P: one case bled when Schick performed)
1/2	Positive	1	(1P*)
	Negative	10	(10P)

*see below.

The positive case which had ½ unit per c.cm. received 0.2 c.cm of toxoid when the Schick test was performed. Although there is some reason to doubt whether this amount of toxoid in all natural susceptible would affect the blood content to any marked extent, this case will not be further considered here. We are left with 42 cases, 35 of which were primary Schick tests, four were "secondary" Schick tests, and three were cases in which the blood was drawn at the time when the Schick text was performed. It is obvious that in the 1/250 and 1/100 unit groups we must pay particular attention to the negative reactors; on the other hand, in the 1/5 and 1/2 unit groups we must scrutinise especially the positive reactors.

It will be noted that in the 1/250 and 1/100 unit groups one of the negative reactors was bled at the time when the Schick test was performed and five of the other eight cases were primary Schick tests. Even if we allow for the influence of the Schick toxin in acting as a secondary stimulus the result would be that, by the time the blood was drawn the antitoxin content would be higher than the value actually represented by the Schick test. On this view the negative Schick tests in these two groups must have represented an antitoxin content of 1/100 at the highest. The conclusion must be that a negative Schick test is not infrequent in individuals who have not more than 1/100 unit of antitoxin in

their blood.

Considering now the groups with 1/5 or 1/2 unit of antitoxin per c.cm. we find three cases with positive reactions. It has already been agreed to exclude one of these (see 109). Of the other two positive cases, in one the blood was drawn in hospital at the time when the Schick test was performed; the resulting test was markedly positive with desquamation. The other case was a primary Schick and the reaction was quite definite with desquamation. These two cases are quite opposed to what is usually found, but on consideration it would seem that the evidence must be accepted and that therefore in a series of cases we may expect to find a few individuals who give similar anomalous results.

The difficulty about any investigation of this type is that the majority of the cases must always lie at one or other of the extremes of antitoxin content; hence, in order to obtain a satisfactory number of cases whose antitoxin content lay within the narrow limits - say, 1/100 to 1/10 unit per c.cm - in which we are especially interested, a very large series would have to be investigated. There has been nothing of this nature since the work of von Gröer and Kassowitz, and unfortunately in the bulk of their investigations the titration values were not sufficiently fine to elucidate the question at issue. This point has already been raised by Glenny (1931). In the present investigation, if we exclude case 109, we find that of 44 positive reactors, three (6.8 per cent) had antitoxin content of over 1/50 unit, and of 73 negative reactors nine (12.3 per cent) had an antitoxin content of less than 1/50 unit. It must therefore be concluded that the zone of demarcation which represents the Schick level of immunity, with the fluid at present used, is indeed a very broad one. The actual Schick level in any individual case may be towards either the upper or the lower limits of this zone, but the chances are roughly three to one in favour of the antitoxin content of a negative reactor being around the 1/100 unit limit.

Blood Content in Cases in which the Schick Reaction was Doubtful.

In the preceding section of this Thesis the incidence of pseudo-reactions and the difficulty in interpreting certain Schick test results was discussed. Further, an unfamiliar type of reaction, which the writer proposes to call a "positive test control reaction" ("T.C.+") was described. It was shown that these T.C. reactions are fairly frequent, and that they make it almost impossible to arrive at a decision regarding the actual result of the Schick test. The actual significance of these reactions was not considered then, but the question will be dealt with now.

Table XXXVIII gives particulars of 26 of these cases in which the results of blood titration are available. Of these fourteen were read on the second day as well as on the seventh day; the remainder were read only on the seventh day. It will be noted that in cases C 56, C 117, and C137 the control showed no reaction. These three cases were admitted to this table because the schick reaction was doubtful, and they could not therefore be placed in Table XXXV. In case C 117 the Moloney test was ++ at the time of the Schick test, and it is possible that the faint reaction to the Schick toxin may be due to the developement of a local allergy; this, however, does not explain why there was no reaction at the control site. In connection with Case C137 it should be noted that the Moloney test was definitely negative one month before the Schick test, which is referred to in the Table, was performed. Further, since the first Schick test - also carried out one month previously -was positive, it is evident that the individual developed a very high degree of immunity (5 antitoxic units) in this short period. The Moloney test was not repeated at the time of the second Schick test, and it is possible therefore that it may have been positive. If this were the case, the faint reaction on the test arm may again have been an expression of local allergy.

Of the remaining twenty three cases each of which

TABLE XXXVIII

Cases not accepted as definite positive or negative

CASE NO.	AGE	P = PRIMARY SCH. S = SECONDARY SCHICK.	SCHICK RESULT C = CONTROL T = TEST 2ND DAY	4TH DAY	MOLONEY RESULT	ANTITOXIN CONTENT OF BLOOD (A.T.U.)	PREVIOUS MOLONEY TEST WITH INTERVAL IN MONTHS (-)
C8	9	S	C = 30 mm. PINK. ELEVATED T = 30 mm. (SAME AS C)	C = 30 mm. DARK PINK. T = 25 mm. MOTTLED.	+	5	0 (2)
C21	9	S	—	C = ++ (30 mm.) T = + (28 mm. WITH DESQUAM.)	-	< 1/1000	0 (2)
C32	4	S	—	C = FAINT + (20) T = FAINT + (20) - MORE MKD. THAN CONTROL	-	< 1/1000	0 (2)
C33	11	S	—	C = ? FAINT + T = DUSKY STAIN (15 mm.).	-	< 1/1000	0 (2)
C43	8	P	C = ERYTH. (20) + INDURATION (5) T = ERYTH. (20) + INDURAT. (10)	C = DEF. STAIN (25) T = DEF. STAIN (30)	++	2	-
C56	13	P	C = 0 T = 0	C = 0 T = ? FAINT + (15) WITH FAINT DESQUAMAT.	+	2	-
C61	12	P	C = ++ (30) T = ++ (25 x 50)	C = DUSKY STAIN T = DUSKY STAIN	+++	1	-
C63	6	S	—	C = + (DIFF. (16)) T = + (PINK (20))	-	< 1/1000	0 (2)
C83	16	P	C = + (25) T = ? +	C = DEF. STAIN T = DEF. STAIN	+++	2	-
C84	16	P	C = + (30) T = + (30)	C = DEF. STAIN (20) T = DEF. STAIN (20)	+++	2	-
C94	4	S	—	C = + (20) T = + (22)	-	1/25.0	0 (3)
C98	10	P	C = FAINT + T = + (25)	C = FAINT RED T = FAINT RED	+	1/5.	-
C105	4	S	—	C = + (20) T = + (22)	-	1/50	0 (2)
C106	5	S	—	C = + (30) T = + (30)	-	< 1/1000	0 (2)

TABLE XXXVIII (contd.)

Case No.	Age	P=Primary Sch. S=Secondary Schick	Schick Result C=Control T=Test 2nd Day	7th Day	Moloney Result	Antitoxin Content of Blood (A.T.U.)	Previous Moloney Test with Interval in Months (-).
C107	8	S	—	C = +(30) with Desquam. T = +(30) with Desquam.	-	1/5	0(1)
C110	13	S	C = +(Faint: 25) T = +(Faint: 25)	C = +(Faint) T = +(Faint)	-	1	+(1)
C111	13	S	—	C = Dusky(25) T = Dusky(30)	-	< 1/1000	0(3)
C112	11	P	C = +(15 x 25) T = +(15 x 25)	C = Stain(20) T = Stain(20)	+++	> 5	-
C117	12	P	C = 0 T = ? Faint+	C = 0 T = Brownish Stain (25)	++	1/2	-
C119	10	P	C = Stain (20) T = +(35)	C = Stain T = Stain	+++	1	-
C121	13	P	C = +(25) T = +(50: Diff.)	C = Stain(15) T = Diff. Stain	+++	> 5	-
C122	13	P	C = +(40) T = +(50-60)	C = Stain (25) T = Stain (25)	+++	2	-
C136	9	S	—	C = Faint Stain T = Faint Stain	-	1/100	0(1)
C137	12	S	—	C = 0 T = ? Faint +	-	5	0(1)
C138	4	S	—	C = Faint + T = Faint +	-	1/500	0(1)
C141	7	S	—	C = Faint Red(12) T = Faint Red(12)	-	1/500	0(1)

presented more or less equivalent reactions on the control and test arms on the seventh day, twelve showed a blood antitoxin content of 1/5 unit or more (most cases being one unit or over); eight showed a content of 1/500 or less; and three gave 1/250, 1/100, and 1/50 respectively. In other words these cases may be divided roughly into two more or less equal groups - the blood results showing that the cases in one of these groups should have been positive, and in the other negative to the Schick tests. This means that if a typical seven day reaction of this type is met with, it is quite impossible to decide, apart from blood titration, whether the individual has or has not sufficient antitoxin in his blood. This disposes of the view that pseudo-reactions always disappear before the seventh day, and that Schick tests can therefore be easily read then. It will also be noted that two of these cases showed slight but definite desquamation. In Case C 21 the desquamation appeared only on the test arm, and the blood showed that the test should have been positive. In Case C 107 desquamation was noticed on both arms, and the blood showed that the patient was definitely immune.

These "test-control reactions" may be divided broadly into two types: (a) Those in which each reaction has on the second day more or less the appearance of a positive Schick reaction: that is, it exhibits definite erythema; on the seventh day the appearance and size of the reactions are not much altered, but the erythema may have become of a "fleshy" nature, or on the other hand it may be mottled. (b) On the second day the reactions may be as in (a), but by the seventh day they have both faded, and the test and control arms show a dusky staining which is always perfectly definite. In Table XXXIX the cases are divided into these two groups, and they are also arranged according to the type of Schick result which they should have shown - judged from the results of blood titration. The results of Moloney tests are also given. It will be apparent from this table that no conclusion as to the possible antitoxin content of the blood can be drawn

TABLE XXXIX.

Type of Schick Reaction on 7th Day	Antitoxin Content of Blood (Units)			Moloney Test Results	
	1/5 or more (i.e. Def. Negative)	1/500 or less (i.e. Def. Positive)	1/250 – 1/50	Positive	Negative
Control Reaction and Schick Reaction equal in size, and both showing definite or marked erythema.	[Cases: C8, C98, C107, C110] 4	[Cases: C21, C32, C63, C106, C138, C141] 6	[Cases: C94, C105] 2	[Cases: C8, C98, C110] 3	[Cases: C21, C32, C63, C94, C105, C106, C107, C138, C141] 9
Control Reaction and Schick Reaction equal in size, and both showing as "dusky" areas, or as "staining".	[Cases: C43, C61, C83, C84, C112, C119, C121, C122] 8	[Cases: C33, C111] 2	[Case: C136] 1	[Cases: C43, C61, C83, C84, C112, C119, C121, C122] 8	[Cases: C33, C111, C136] 3

from the appearance of these reactions either on the second or on the seventh day. It is suggestive, however, that all the cases which showed dusky reactions on the seventh day, and in which the antitoxin content was 1/5 unit or more, also showed positive Moloney reactions; Further, these were the only cases in group (b) which gave positive Moloney results. This may possibly be some guide to the interpretation of these puzzling reactions.

It will be noted that in cases C63 and C 107 the tests were repeated on the seventh day when the original reactions were read. The result in C 63 was a normal positive Schick reaction - which agreed with the antitoxic content of the blood. The result in C 107 was another positive test-control reaction. It would thus appear that these reactions may or may not recur, and it is possible that they have little relation to the actual antitoxin content of the blood. This suggestion bears out the conclusions drawn from Table XXXIX. These reactions are evidently not due to Schick toxin, which is thermo-labile - otherwise they would not appear at the control site. Again, it seems unlikely that they can be caused by the ordinary protein constituents which are usually held responsible for the occurrence of pseudo-reactions. It has for long been a fundamental principle in reading Schick tests that these pseudo-reactions disappear before the seventh day. There is a possibility, however, that these test-control reactions may be due to some other constituent of the reagent which is thermo-stable in nature and which is not identical with the ordinary proteins which cause the pseudo-reaction. When we take into account the frequency with which they appear in Moloney positive reactors, it seems possible that they may be of an allergic nature. The peculiar feature is that, if we admit that these reactions are allergic and affect both arms equally, in individuals who have less than, say, 1/250 unit of antitoxin the normal positive Schick reaction appears to be inhibited altogether, and does not become superimposed on the allergic manifestation.

Discussion.

It has been stated that in this investigation the blood samples were practically all drawn, not at the time when the test injection was made, but six days later. The only exception to this procedure was in the relapsed cases, which are specially referred to, and which have been dealt with in a separate investigation (Underwood, 1935b) and section (h) of this Thesis. The main reason for taking a particular blood sample was to determine whether the individual case was immune according to the result of the blood estimation. Hence it follows that a large number of the cases in this series presented some anomalous feature which made interpretation of the Schick reaction difficult. If the blood samples had been taken at the time when the Schick tests were performed, the only method of securing the investigation of a satisfactory number of such anomalous reactors would have been to take a very large number of blood samples. This course was obviously impracticable. In one sense, however, it was fortunate that the blood samples were not taken at the time when the Schick tests were performed, for the results offer a commentary upon the extent to which a small secondary stimulus will operate.

Glenny and Südmersen (1921) showed that in an experimental animal the injection of an antigen might produce a fairly rapid rise in the antitoxic content of the animal's serum, but this rise was succeeded by a rapid fall. A "secondary stimulus," however, in the form of a subsequent injection, even though it were much smaller quantitatively than the original stimulus, would be followed by a rapid rise of the antitoxin content, and this rise would be maintained over a considerable period. Glenny and Allen (1922) showed that a rabbit which had previously received a primary injection without the subsequent production of detectable antitoxin, produced 1 unit per c.cm. in seven days after the injection of a dose of toxin equivalent to that used in the Schick test. In summarizing his work on animals Glenny (1925) later suggested that in human communities the individuals

could be divided into five grades of relative immunity, the classification being based, not only on the Schick results but also on the individuals previous acquaintance with specific stimuli. In main Glenny's work was confirmed by O'Brien (1926) who showed that the amount of toxin used for the Schick test could, in certain individuals with a basal or active immunity, turn the scale between a positive and a negative Schick result. If Glenny's later views (Glenny, 1931) are studied it will be seen that in animals the minimum latent period before detectable antitoxin is produced by a primary stimulus is nine days, whereas after a secondary stimulus it is two days.

The details of the Tables should be studied in the light of these experimental considerations. From Table XXXV it will be seen that, of the 118 cases, 27 were "secondary" Schick test cases - that is, the Schick tests had been performed at least one month after a course of inoculations, and the blood was drawn six days after the performance of the Schick test. Yet in no less than twenty two of these twenty seven cases the antitoxin content of the blood was 1/100 unit or less per c.cm. The actual details of these twenty two cases are given in the following table (Table XL)

Table XL.

Antitoxin content (units per c.cm	< 1/1000	1/1000	1/500	1/250	1/100
Cases	15	3	-	1	3

The fact that such a large proportion of the cases had less than 1/500 unit of antitoxin per c.cm shows definitely that in many cases which are Schick tested at least a month after the completion of a course of inoculations the amount of toxin in the Schick injection is not sufficient to cause any rise whatever in the antitoxin content of the blood. It may be objected that four of the twenty seven cases gave an antitoxin content of 1/250 unit or over. But it will be readily seen that this objection carries no weight, since there is no evidence that, as a result of the

inoculations, the blood content had not risen to this level before the Schick test was performed, It would seem that these results suggest that in man the amount of toxin in the Schick test dose is not a sufficiently strong secondary stimulus to have an appreciable effect upon the antitoxin content of the blood six days after the performance of the Schick test. This consideration also lends weight to the previous argument that, in this series of cases the antitoxin results, in respect of individuals from whom the blood was withdrawn six days after the performance of a primary Schick test were not materially affected by the interval.

In most of the papers which are cited here the blood samples were not taken at the time of performance of the Schick tests . Quite recently it has been suggested that the amount of toxin in the Schick test fluid is sufficient to act as a secondary stimulus - especially in persons who have received prophylactic inoculations. Dudley (1934) specifically refers to this possibility and in a number of his cases the blood samples were drawn at the time when the Schick test was performed. Experimental work is evidently necessary to settle this question; but the writer does not know of any investigation in which blood samples were taken at the time of performance of the Schick tests, and repeated six days later on the same individuals when the tests were finally read. Table XLI gives the results of a small investigation on these lines, which was carried out by the writer on London children.

TABLE XLI - Results of repeated titrations in same individuals

Primary tests: Case No.	1	2	3
Antitoxic units (a) day Schick test performed	1/1000	1/5	1/5
(b) Six days later	1/1000	1/5	1/5

Schick tests after inoculation:

Case No.	4	5	6	7	8	9	10	11	12	13	14
(a)-as above	5	½	1/10	20	1/10	2	1/5	1/25	1/25	1/500	2
(b)-as above	5	½	1/5	10	1/10	2	1/5	1/25	1/25	1/500	2

This series is admittedly very small, but the taking of repeated samples at such short intervals presents difficulties. Such as it is, however, this experimental evidence bears out the theoretical

conclusions which were drawn before. It would seem that even in individuals who have received three doses of a prohpylactic three months previously the amount of toxin used in the Schick test is not a stimulus of sufficient magnitude to cause an appreciable rise in the antitoxin titre of the serum within the limits of six days which was observed throughout the whole of the present investigation.

A further interesting point in relation to Table XXXV is that, generally speaking there was no marked association between the amount of antitoxin in the blood and the strength of the Schick reaction. In guinea pigs Glenny and Waddington (1929) showed that there was a marked direct relationship between the number of Schick Test doses of toxin which was tolerated and the antitoxin content of the blood. At Greenwich Dudley (1934) found that exceptions to this rule were fairly frequent. In the present series the main features are given in Table XLII.

TABLE XLII.

Antitoxin content of blood.	Schick result			Total
	+++	++	+	
Individuals with < 1/1000	2	11	20	33
" with 1/1000 or 1/500	-	-	5	5
" " 1/250 or 1/100	-	-	3	3
" " >1/100	-	1	3	4
	2	12	31	45

In this table the figures for the +++ and ++ reactors are probably unduly low, since the tendency was to withdraw blood from faint positive reactors. Nevertheless, it would seem that the association between the two conditions was not very marked.

PART I.

SECTION (d)

THE DIPHTHERIA TOXOID-REACTION (MOLONEY) TEST: ITS APPLICATIONS AND SIGNIFICANCE.

The diphtheria toxoid-reaction test was introduced in 1927 by P. J. Moloney and C. J. Fraser for the purpose of detecting those individuals who would be likely to give undesirable reactions on the injection of toxoid for diphtheria prophylaxis (Moloney and Fraser, 1927). The test was soon used as a routine measure in Toronto, and Burke (1930) collected the results; a larger series was published by McKinnon and Ross (1933). Although the Moloney test has now been very extensively used in Canada, very little work has been done on it in this country. O'Brien and Parish (1932) described the results of testing 906 individuals, and decided that the Moloney test was equally effective in picking out persons who were likely to react unfavourably to the much stronger toxoids which they had used as it was in indicating those who would react to the weaker toxoids of the Canadian workers; and Parish (1933) later published a smaller series. Underwood (1934)(a) published the results of 905 tests and discussed their relation to the reactions which follow the injection of potent formol toxoids; he also described a delayed reaction to the Moloney test (+D reaction), which will be discussed in more detail later. The paper by McSweeney (1935) discusses certain features of 837 tests performed at Cardiff.

This section of the present work is based on 2666 Moloney tests performed before the injection of a prophylactic, and 595 tests carried out after the injection of toxoids. The whole of the work, including both the injections and the

subsequent readings of the reactions, was carried out by the writer, so that the fallacies which are sometimes associated with the different standards of reading adopted by individuals in groups of observers do not arise. It should be mentioned that all the tests were performed in Leeds, in the children of school and pre-school age whose Schick tests were discussed in section (b) of this Thesis.

Before proceeding to discuss the results a word should be said regarding nomenclature. In their original communication Dr. P. J. Moloney and Miss C. J. Fraser used diluted toxoid not only to detect sensitive individuals, but also as a substitute for the Schick control test. They said: "Previous work has shown that diluted toxoid is as effective as the heated control for determining pseudo reactors; in addition diluted toxoid serves to indicate those individuals who may react to a subcutaneous injection of toxoid." In Canada the test appears to have been known generally by the name of the "diphtheria toxoid-reaction test", and the name "Moloney test" seems to have been given first by O'Brien and Parish (1932). In a recent address Dr. O'Brien (1934) referred to the test as the "Moloney-Fraser reaction". As the test is now fairly well known in this country by the name of the "Moloney test" this name will be adopted in this Thesis. It should also be noted that Zoeller (1925) introduced a modification of the Schick test which consisted of the injection of a 1/100 dilution of Ramon's anatoxin at the same time as the Schick test was performed. Zoeller thought, however, that a positive result at this site was a transition stage between susceptibility to diphtheria and complete immunity. He also thought that such a positive reaction could again become negative, and accordingly complete immunity was represented by a Schick-negative combined with an anatoxin-negative reaction. In any case Zoeller does not seem to have recognised the important use of the test for the purpose of detecting toxoid-reactors, and hence the name "Moloney test" is justifiably retained.

Description of the Test

The test consists of the intradermal injection of diluted toxoid. The amount injected is usually 0.2 c.c. and the toxoid dilution 1:200. A positive reaction usually develops within 24 hours and is at its maximum in about 48 hours. The test can be read either after 24 or after 48 hours. In the series which will be described all the tests were read after 48 hours, though some were also read at 24 hours. The positive reaction is usually described as of three grades, +, ++, or +++. Fitzgerald, Moloney, Fraser and their colleagues (1932) describe the different types as follows: "1+ signifies an area of redness no greater than 1cm.; 2+ an area of redness greater than 1cm., but with little or no induration; 3+ indicates definite induration at the site of the injection." In the paper by McKinnon and Ross (1933) the reactions are classified as follows: + = redness of 1 cm. or less, with no induration; ++ = redness greater than 1 cm., with no induration; +++ = redness accompanied by induration. O'Brien and Parish (1932) gave the following description of the "definite" reactor (corresponding to +++): "'Definite' indicates a central zone of redness with a palpable thickening of the skin 15 to 35 mm. wide; this is surrounded by a flushed zone varying from an easily delimited area 40-120 mm. to a faint flush 10-15 cm. wide, merging into the colour of the surrounding skin." A "mild" reaction (corresponding to ++) they describe as "a faint red flush 25-100 mm. wide". In a later paper Parish (1933) gave the following description: "M+++ or 'definite' indicated a large zone of redness with palpable thickening of the skin; M++ or 'mild' was an area of bright hyperaemia of more than 10 mm. in diameter, with very slight induration; where induration was absent, a very faint flush irrespective of its diameter, or a bright reaction of less than 10 mm. was classified as M+ or 'faint'". The classification which the writer adopted was more or less that of Parish, but was

modified slightly as a result of preliminary work. All the tests described in this paper were classified according to the definitions which were given in a previous paper (Underwood, 1934a): "(1)'Definite reaction" (M+++): a large area of erythema up to 40 mm. or more in diameter, with a definite palpable area of induration in the centre; (2) 'mild reaction' (M++): an area of erythema of more than 10 mm. in diameter, with an area of slight induration; (3) 'faint reaction' (M+): a definite area of erythema of less than 10 mm. im diameter, without induration, or any area of a faint pink colour which showed no thickening, and which was not due to trauma." With the exception of the classification of McKinnon and Ross, these definitions are all more or less similar, but the one which is used in this paper probably allows of the most satisfactory classification of the reaction which consists of a definite area of erythema of about 15 mm. in diameter, but which shows no thickening. In actual practice all reactions fall naturally into one or other of these three groups, and reactions which are difficult ot classify are not often met with. The British systems of classification seem to be finer and more useful than that of McKinnon and Ross. For example, the cases classified in this paper as "++" or "+++" would all be classified in the latter category by these writers. Yet the distinction is easily made, and there is often an appreciable difference in the reactions of the two classes on the injection of toxoids. The writer is of opinion that it is better to have the finer division in the region of the strong positive reactors.

Distribution of positive reactors in the population

The Moloney state of 2666 children and young adults in Leeds before the inoculation of a prophylactic is given in Table XLIII, the individuals being grouped according to their Schick state and age. The essential features of this table, so far as age is concerned, are given in Table XLIV, from which Fig.xii was

TABLE XLIII

Schick and Moloney States of 2666 Individuals

Age Group	Moloney Result	Schick Result: Negative	Slight (+)	Moderate (++)	Marked (+++)	Totals	
Under 1	0	8	8	30	2	48	
	+D	-	-	-	-	-	
	+	-	-	1	-	1	
	++	-	-	-	-	-	
	+++	-	-	-	-	-	49
1	0	3	16	136	26	181	
	+D	-	-	-	-	-	
	+	-	-	4	-	4	
	++	-	-	-	-	-	
	+++	-	-	-	-	-	185
2	0	-	13	124	30	167	
	+D	-	-	2	-	2	
	+	2	1	2	1	6	
	++	1	-	1	-	2	
	+++	-	-	-	-	-	177
3	0	6	14	121	38	179	
	+D	-	-	1	-	1	
	+	1	-	6	1	8	
	++	1	1	4	-	6	
	+++	-	-	-	-	-	194
4	0	16	16	143	46	221	
	+D	-	-	-	-	-	
	+	4	1	5	-	10	
	++	1	-	-	1	2	
	+++	-	2	-	-	2	235
5	0	29	29	175	65	298	
	+D	1	-	3	1	5	
	+	5	5	8	3	21	
	++	4	1	1	2	8	
	+++	-	-	-	-	1	333
6	0	45	36	197	67	345	
	+D	5	-	1	-	6	
	+	9	-	5	4	18	
	++	6	4	4	1	15	
	+++	2	-	-	-	2	386
7	0	21	14	77	36	148	
	+D	3	1	-	1	5	
	+	7	4	5	1	17	
	++	5	11	7	1	14	
	+++	2	1	1	-	4	188

TABLE XLIII (contd.)

Age Group	Moloney Result	Schick Result: Negative	Slight (+)	Moderate (++)	Marked (+++)	Totals	
8	0	20	12	60	29	121	
	+D	3	-	-	1	4	
	+	9	-	4	2	15	
	++	5	1	6	1	13	
	+++	2	-	1	-	3	156
9	0	18	10	65	18	111	
	+D	1	1	1	-	3	
	+	7	1	9	1	18	
	++	14	-	2	1	17	
	+++	4	2	2	1	9	158
10	0	17	4	43	21	85	
	+D	2	0	1	-	3	
	+	13	2	3	-	18	
	++	7	-	2	3	12	
	+++	6	1	2	2	11	129
11	0	17	5	32	7	61	
	+D	1	-	1	-	2	
	+	12	-	5	2	19	
	++	14	1	5	1	21	
	+++	7	-	2	-	9	112
12	0	26	7	27	23	83	
	+D	2	1	1	-	4	
	+	17	6	4	3	30	
	++	9	3	3	-	15	
	+++	8	-	-	1	9	141
13	0	13	9	13	14	49	
	+D	-	1	-	1	2	
	+	8	1	2	3	14	
	++	9	1	1	-	11	
	+++	7	2	-	1	10	86
14	0	5	-	3	2	10	
	+D	1	1	-	1	3	
	+	2	2	-	-	4	
	++	8	1	1	-	10	
	+++	3	-	-	-	3	30
15-19	0	33	1	3	3	40	
	+D	5	-	-	-	5	
	+	19	2	2	1	24	
	++	19	-	5	3	27	
	+++	10	-	-	1	11	107
							2666

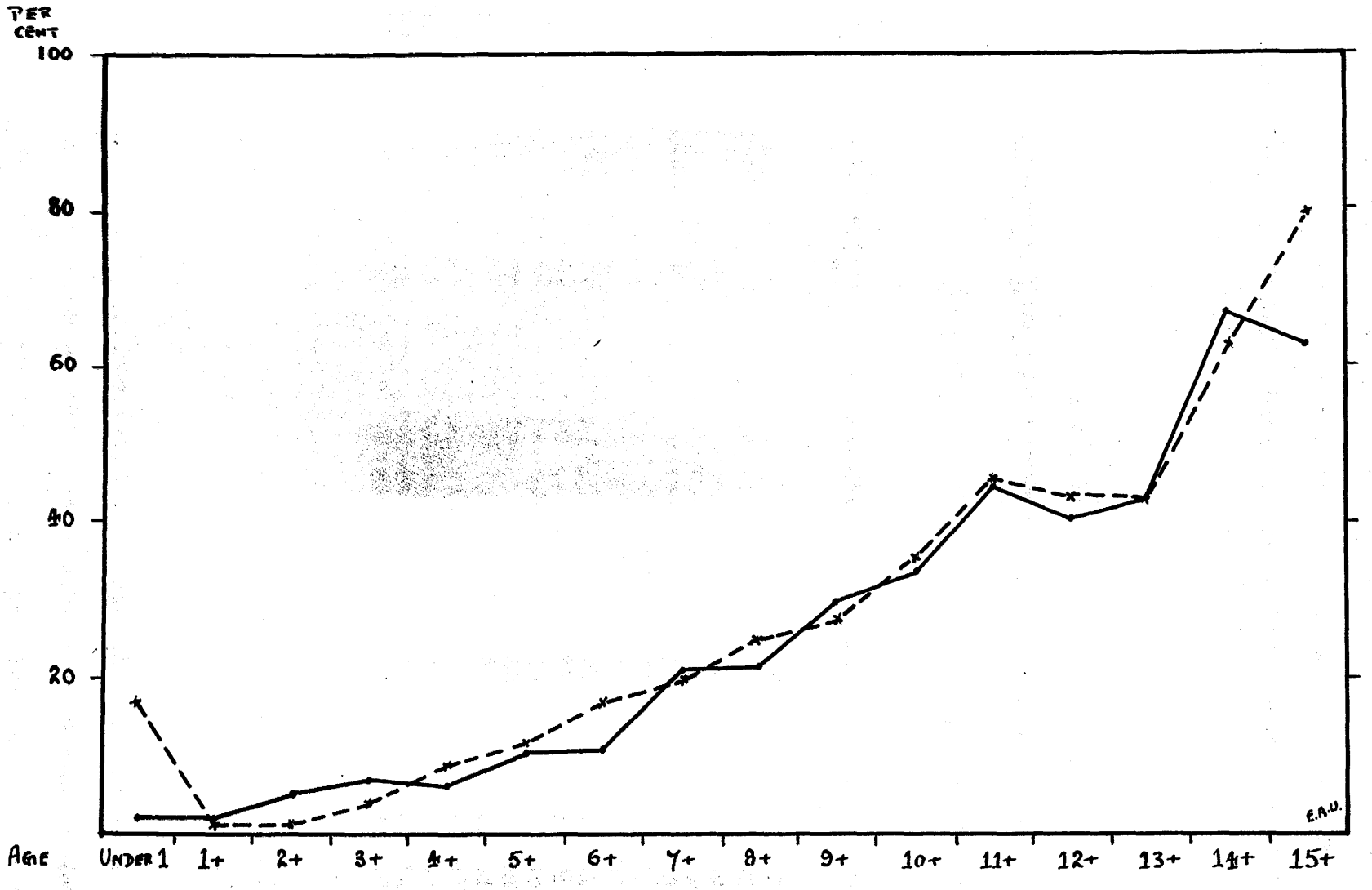


Fig. XII : Black line = per cent of total tested at each age who showed a positive Moloney test.
Dotted " = " " " " " " " " " " " " negative Schick "

drawn. It should be emphasised here that the following discussion applies to the community as a whole and not to individuals. The assumption that the individual always behaves in the same manner as the herd is liable to lead to certain fallacies in the interpretation of the Moloney test. These fallacies, and the significance of individual tests, will be discusses in detail later.

TABLE XLIV

Influence of age on positive Moloney incidence

Age	Moloney-positive			Primary Schick tests	
	Total tests	No. + (all types)	% positive	No. negative	% negative
Under 1	49	1	2.04	8	16.33
1+	185	4	2.16	3	1.62
2+	177	10	5.65	3	1.69
3+	194	15	7.73	8	4.12
4+	235	14	5.96	21	8.93
5+	333	35	10.51	39	11.71
6+	386	41	10.62	67	17.36
7+	188	40	21.28	38	20.21
8+	156	35	22.44	39	25.01
9+	158	47	29.74	44	27.85
10+	129	44	34.11	45	34.88
11+	112	51	45.54	51	45.54
12+	141	58	41.14	62	43.97
13+	86	37	43.02	37	43.02
14+	30	20	66.66	19	63.34
15-19	107	67	62.61	86	80.37

From the data presented in Table XLIV it is seen that the percentage of children who show a positive Moloney reaction increases more or less continuously as age advances. The table also shows that, as a positive Schick is most common in the early years of childhood, a positive Moloney test is on the contrary most often met with later in childhood or in adult life. It

should be emphasised here that the figures for the Schick tests at the different ages are given solely for reference purposes. The remarkable parallelism between the graphs for Moloney-positive reactors and for Schick-negative reactors, which is apparent in Fig. xii, is to a large extent fictitious, and the graphs cannot therefore be used for any other purpose than that of statingthe incidence of the two reactions at the different ages. The figure does not in itself express any fundamental relationship between the incidence of the respective conditions. This point is important, since it is often assumed that the reactions not only run a parallel course, but that in the later years of childhood a positive Moloney reaction is practically synonymous with a negative Schick reaction. Reference to Table XLIII will show that at the age of 12 years, of 62 negative Schick reactors, 26 showed a negative Moloney reaction; and of 79 positive Schick reactors, 22 showed a positive Moloney reaction. This feature may be found in a corresponding degree in the other age groups, and the early recognition of this fact was one of the starting points of the present investigation. This question will be dealt with more fully by other methods in a later section, and it is introduced here merely to emphasise the point that no conclusions can be drawn from the apparent similarity between the frequency of Moloney-positive and Schick-negative reactors, which is seen in the figure.

The incidence of positive Moloney reactors at different ages may be compared with the figures in the corresponding series of McKinnon and Ross. In the present series it may be said that, in children who had not attained their second year only about 2 per cent., and in those in the next three succeeding years not more than 7 per cent., showed any reaction at all to the Moloney test. Thereafter the percentages of positive Moloney reactors increased steadily as age advanced with the exception of a slight falling off between the age of 11 and 13 years, and in the group of 15 years and over. McKinnon and Ross

found that over 90 per cent. of children under 6 years of age gave negative reactions, and that only 46 per cent. of the 14-year old group gave negative readings. In the present series the corresponding percentages are 93.3 and 36.5 respectively. From these figures it would be possible to say something regarding the tendency of persons at different ages to develop reactions after the injection of prophylactic doses of toxoid, but it is advisable tocconsider this question in relation to the different types of Moloney reaction.

The results for the Moloney tests at different ages were plotted on a logarithmic scale (Fig. xiii). It is seen from the graph that the process of sensitisation starts very early in life, and, although the rate is slow during the first 18 months, it is definitely in progress during this period. During the first and second years of life, however, the rate increases very markedly.In fact, we may say that in these two years the rate of development of a positive Moloney reaction is higher than at any other period of the child's existence. Between the third and fourth years there is a temporary, though considerable, retardation; but from the fifth year onwards until the eleventh year there is a more or less continuous increase in the development of sensitivity to toxoid.

There is a considerable amount of evidence, much of which will be discussed in later sections, that the development of a positive Moloney test is due to the acquaintance of the individual with the proteins of the C. diphtheriae. McKinnon and Ross rightly emphasise the point that immunity may be obtained without sensitivity being developed at any time during the process. They also suggest that age per se may be a factor in the development of sensitivity. From an examination of Fig. xiii it would not appear that the results of the present investigation give much support to this suggestion. In certain individuals sensitisation evidently starts at a very early period of life, though the numbers who develop a positive reaction are

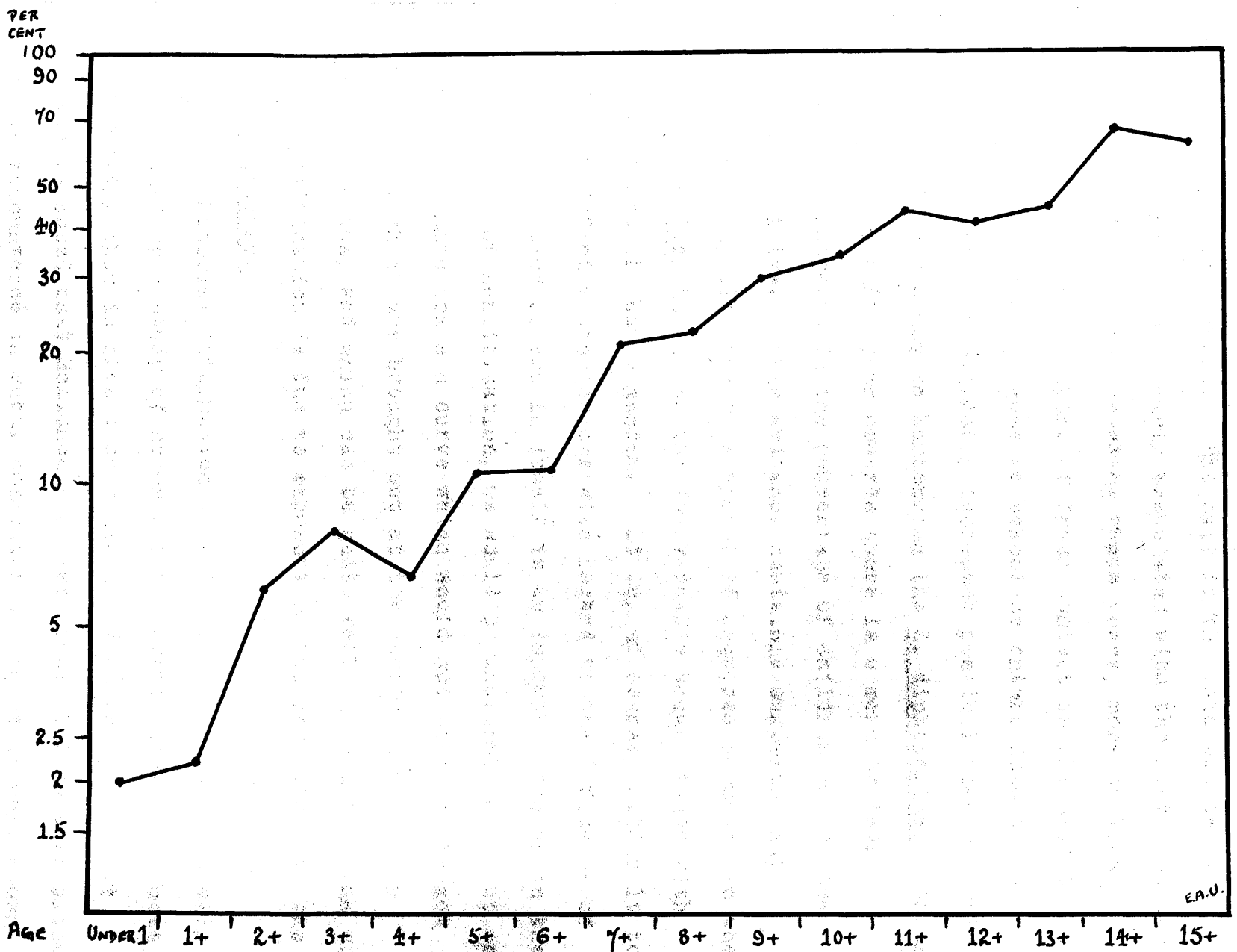


FIG. XIII: LOGARITHMIC SCALE. PER CENT OF TOTAL TESTED AT EACH AGE WHO SHOWED A POSITIVE MOLONEY REACTION.

very small. It is at this period that the individual is most cut off from contact with other children. But during the second year, when the opportunities for contact with the C. diphtheriae are greatly increased, the process of sensitisation becomes very marked. The fall in the rate between the third and fourth years is possibly associated with the fact that the child has, during the preceding three years, more or less exhausted the possibilities of fresh contact in the neighbourhood of his home. When he goes to school he enters into a new environment with considerably increased possibilities of contact with children who may be harbouring the C. diphtheriae. At any rate from the fifth year onwards there is a more or less continuous increase in the percentage of children who become Moloney positive. This evidence certainly suggests that sensitisation, like the development of acquired immunity to the C. diphtheriae, is due to the individual's acquaintance with the products of that organism. It is, of course, difficult to draw any definite conclusions with regard to the influence of age. If, however, age in itself is an important factor in the development of sensitisation, we would be inclined to expect on the logarithmic scale a curve which would not show the marked variations which are brought out at the earlier ages in the present series, and which can be quite well explained by the view that sensitisation is due to exposure of the individual to the C. diphtheriae.

Material information on this question could probably be obtained by a study of Moloney test results in virgin races, or, failing that in closed or semi-closed communities. It should be noted that McKinnon and Ross state that less sensitivity is encountered in rural communities, and it is well known that in these communities there is a higher ratio of Schick-positive individuals.

Degree of Reaction

Although these considerations are interesting from a scientific point of view, they have little bearing on the practical application of the test because the different grades of positive Moloney reaction do not indicate equal liability to the development of appreciable reactions on the inoculation of strong toxoids. In a previous paper (Underwood, 1934a) it was suggested from preliminary work that care was necessary when using strong toxoids, especially new and untried toxoids, in the case of an individual who showed any type of positive Moloney reaction. This point will be discussed more fully later. Meanwhile it may be said that with known toxoids individuals who show the +D or + reactions may be injected with the usual doses; great care must be exercised with ++ reactors, and +++ reactors should not be given toxoid at all. The statement is frequently made that infants and pre-school children can be inoculated with toxoid without a Moloney test, since they seldom or never show a ++ or +++ reaction (see McKinnon and Ross; Burke; also Lancet, 1935, Editorial, etc).

This point is brought out in Fig. xiv, which shows the percentage incidence of each type of Moloney reaction at each year of life. The figure also shows the combined incidence of ++ and +++ reactors at each year. It will be seen that all three grades of reaction increase in frequency as age advances. The +D and + reactions (combined grouping) are most frequent, and the +++ reaction least so. The graph for the ++ reaction is almost throughout in an intermediate position between the two extreme types. This statement does not apply to groups over the age of 13 years. After this age M++ reactions become much more frequent.

From the utilitarian point of view the reactions which are most important are the ++ and the +++. Any individual who gives one or the other of these reactions is definitely liable to develop some symptoms on the injection of toxoid in prophylactic doses. These reactions will not necessarily be inconvenient or

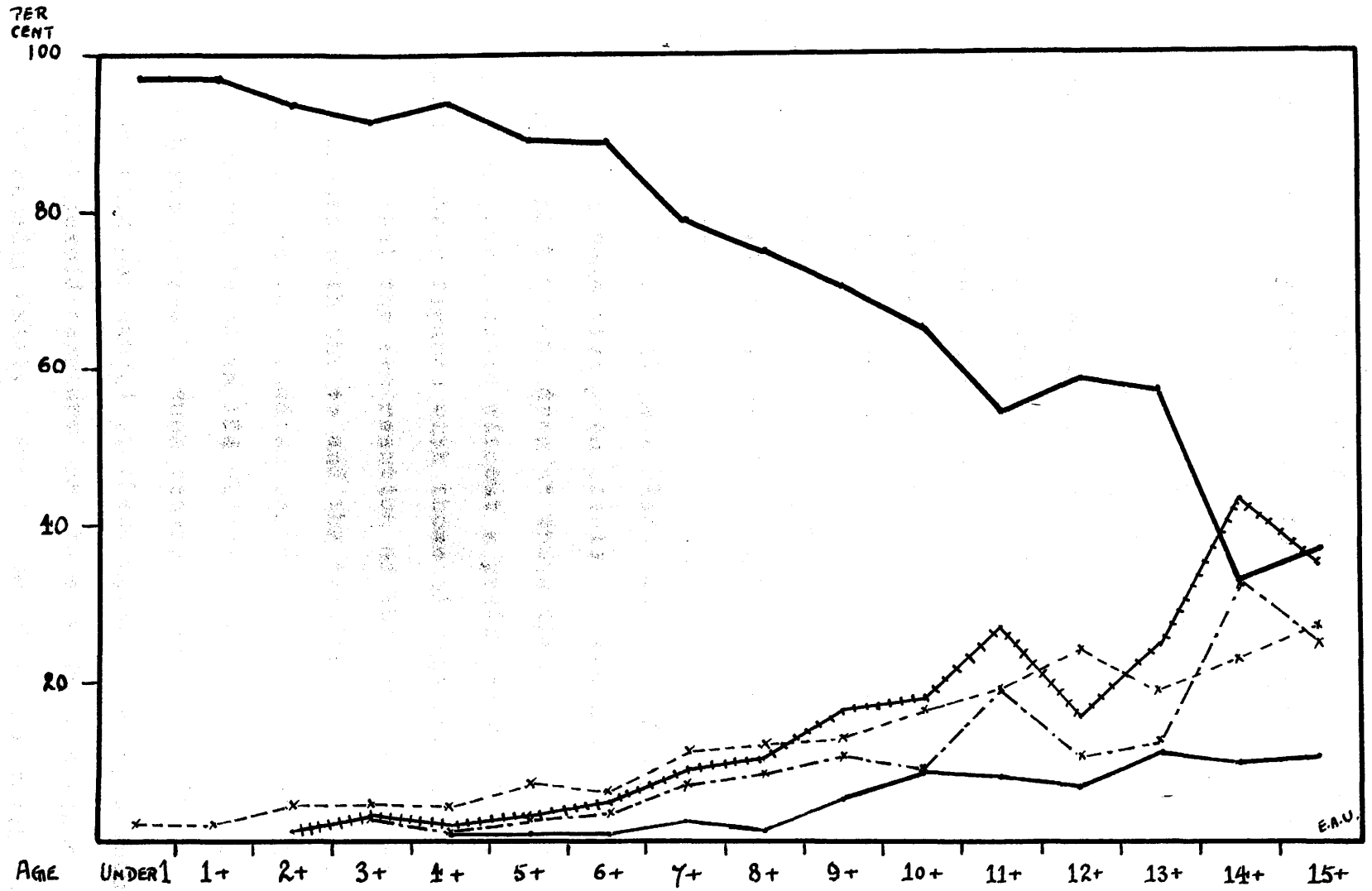


FIG. XIV : 2666 MOLONEY TESTS : PERCENTAGES AT EACH YEAR OF AGE.

——— = NEGATIVE. - - - - = +D AND + —·—·— = ++ ——— = +++ +++++++ = COMBINED PERCENTAGES (++ AND +++).

troublesome; they may consist merely of marked erythema at the injection site, On the other hand, a local reaction may be accompanied by a general reaction, and it is obviously important to decide which individuals are likely to give this. In this paper persons who are liable to show a reaction of some type on the injection of toxoid in prophylactic doses are called for convenience "potential toxoid reactors", and the term is only used in this special sense. The incidence of such "potential toxoid reactors" is given by the barred line in Fig. xiv; this line represents the combined percentages for ++ and +++ reactors. It will be seen that up to the age of 5 years the combined percentages of children who show such reactions do not exceed 3 per cent. at any age. At 6 years the incidence is 4.4 per cent.; at 7 years 9.6 per cent.; and thereafter the percentages increase rapidly. In this series of cases the maximum incidence of such "potential toxoid reactors" was reached at the fourteenth year, when 43.3 per cent. of the individuals at this age showed a ++ or +++ reaction.

McKinnon and Ross atate that in infancy and the pre-school group sensitivity to toxoid is practically a negligible quantity, and that children at these ages can, therefore, in general be given toxoid without a preliminary Moloney test. In comparing their figures with those of this series it should be remembered that the +++ reaction of the Toronto workers practically embraces both the ++ and the +++ types of reaction here described. In the Canadian series no reaction which showed any induration was found in 184 children under the age of 5 years. In the Leeds series 12 such reactors were met with in 840 children (1.4 per cent.) at these ages. Hence, although such "potential toxoid reactors" are rare in the pre-school group, they undoubtedly exist. The course of action which is to be adopted depends upon the individual who is actually giving the inoculations; but if it is particularly desired to exclude or treat specially all "potential toxoid reactors" then the Moloney

test must be employed for children over the age of 2 years.

Relation of Test to Symptoms on Injection of Prophylactics

These considerations are to a certain extent theoretical by reason of the fact that not all positive Moloney reactors do actually show undesirable reactions on the injection of toxoids in prophylactic doses. Most +++ reactors will do so, and many ++ reactors also show such symptoms with full doses of prophylactic. A more practical approach to the problem is arrived at by the consideration of those children who actually showed undesirable symptoms on the injection of prophylactics. In this section of the Thesis reactions will not be dealt with in detail, but the main results are set out in Table XLV. This table is based on 2041 persons who were inoculated. All of these had FT or alum toxoids, except 100 who had T.A.F. (In Table XLV "++" includes 2 persons and "+++" 4 persons who had only T.A.F.). In a later section the question of any reaction at all will be considered. The persons detailed in Table XLV are only those who showed significant reactions, which are defined here as follows: (1) any local reaction which was more than a mere erythema - e.g. swelling, tenderness, fullness, etc.; (2) general reactions - including slight vomiting, marked nausea, headache, or definite distaste for at least one meal. Of the 42 persons included in Table XLV 25 were seen by the writer on the day following the injection.

Table XLV shows quite clearly the value of the Moloney test, especially in children over the age of 5 years. It will be seen that only two children failed to give a positive response to the test. Neither of these was seen until some days after the inoculation, but from the description which was given of the condition of the children one probably had a definite toxoid reaction, and in the other case there was considerable doubt whether the injected toxoid was actually the cause of the condition. It is obvious that the most important reactions

TABLE XLV

Moloney state of Persons who showed Reactions after prophylactic doses of toxoid

Negative		+D		+		++		+++	
Age (yrs)	Persons	Age (yrs)	Persons	Age (yrs)	Persons	Age (yrs)	Persons	Age (yrs)	Persons
5	1	3	1	5	1	7	5	4	1
6	1	5	1	7	1	8	1	9	2
-	-	-	-	9	1	10	3	10	3
-	-	-	-	13	1	11	4	11	1
-	-	-	-	14	1	12	3	13	1
-	-	-	-	-	-	13	1	15	1
-	-	-	-	-	-	14	1	-	-
-	-	-	-	-	-	16	3	-	-
-	-	-	-	-	-	17	2	-	-
-	-	-	-	-	-	18	1	-	-
-	2	-	2	-	5	-	24	-	9

occurred in the ++ and the +++ groups. The total for the +++ is small when compared with that for the ++ group. But it should not be inferred from this that +++ persons were less likely to develop unpleasant symptoms than were ++ persons. This is far from being the case. The writer's experience is that the majority of +++ reactors will give such symptoms if injected with full doses of toxoids, and that many of them will behave in this way even after the injection of much smaller doses. The total of 9 for these +++ reactors is small because nearly all such reactors were dealt with specially(e.g. injection of T.A.F.) or were naturally immune, and were consequently not inoculated. This statement applies in less degree to the ++ reactors. The evidence from this section of the investigation points to the fact that children of school age should not be inoculated without a preliminary Moloney test.

Note on Statistical Methods

Although Pearson's coefficient of mean square contingency (C_2) is fairly well known, his coefficient of association (Q_5) in a fourfold table is not so widely used. If individuals are classified by the characters into A and not-A, B and not-B, then a tetrachoric table may be formed in which the cell frequencies are represented by (a), (b), (c), (d) in the position indicated in Table XLVII (Pearson, 1901). If the total frequency be N, then for the fourfold table

$$\chi^2 = \frac{N(ad-bc)^2}{(a+b)(c+d)(b+d)(a+c)}, \text{ and } Q_5 = \sin\frac{\pi}{2}\cdot\frac{1}{\sqrt{1+K^2}}, \text{ where}$$

$$K^2 = \frac{4abcdN^2}{(ad-bc)^2(a+d)(b+c)}.$$

In this section also the probable errors of the values of C_2 are derived from ϕ_t^2 and not from ϕ_a^2.

Association between the Schick-negative and the Moloney-positive states

A casual examination of Fig. xii might suggest that the practically simultaneous transition from Schick-positiveness to Schick-negativeness and from Moloney-negativeness to Moloney-positiveness, which is so marked in the community might apply to the individual as well. This suggestion has in effect been made, since certain workers have stated that the majority of strong positive Moloney reactors are immune and do not require inoculation. If this statement is true, then the Moloney test may to a certain extent be used as a substitute for the Schick-test.

It was thought desirable to investigate the association between these two conditions more closely, and for this purpose Pearson's methods for contingency were used. Table XLVI is a 4×4 table compiled from the main table (Table XLIII) and showing

the distribution of the 2666 persons according to their Schick state and their Moloney condition. For this table $\chi^2 = 504.76$; for $n = 9$ the value of P corresponsing to this value of χ^2 is less than 0.01, which shows that the distribution is not a chance one. The degree of association between the Schick-

TABLE XLVI

	Schick-positive				
	Marked (+++)	Mod. (++)	Slight (+)	Schick-negative	Totals
Moloney-negative	427	1249	194	277	2147
Moloney +(+D and +)	27	76	30	139	272
Moloney ++	14	42	14	103	173
Moloney +++	6	9	8	51	74
Totals	474	1376	246	570	2666

negative and Moloney-positive states was estimated by calculating Pearson's coefficient of mean square contingency (C_2). The value of C_2 is 0.399 $\pm$ .013, which would seem to indicate a moderate degree of association. While there is therfore a considerable degree of association between the two conditions, this is by no means perfect. It should be noted that, of a total of 74 +++ Moloney reactors, 15 showed a marked or moderate reaction to the Schick test (20.3 per cent.). Again, of a total of 173 individuals who gave a ++ Moloney reaction, 56 showed a marked or moderate Schick reaction (32.4 per cent). The tendency in the individual, as in the community, is for increasing toxoid sensitivity to develop *pari passu* with Schick immunity. The degree of association between the two conditions is brought out more clearly by a fourfold table (Table XLVII). In this table the +D and + reactions are included with the Moloney-negative reactions, since we are really interested in Schick-negativeness and Moloney reactions which indicate definite

TABLE XLVII

	Schick-positive (all degrees)	Schick-negative	Totals
Moloney-negative Moloney +D Moloney +	2003 (a)	416 (b)	2419
Moloney ++ Moloney +++	93 (c)	154 (d)	247
Totals	2096	570	2666

susceptibility to toxoid. For this table χ^2 = 271.81. For this value of χ^2 with n = 1, P is less than 0.01 - which indicates that the divergence in the table is not random. The amount of association was determined by calculating Q_5 (the coefficient of association), which is 0.632 ± .024. For the same table the value of r_p (Pearson's equiprobable coefficient) is 0.667, which is practically the same. These values confirm the previous findings.

Association between positive Moloney reactions and positive Schick-control reactions

It has already been stated that, in their original paper, Moloney and Fraser (1927) used the diluted toxoid which they employed in their test, not only to distinguish toxoid reactors, but also as a substitute for the heated control in the Schick test. Suggestions have been made on several occasions that the one test might be made to serve for both purposes. Burke (1930) admitted that in the Toronto campaign the number of Schick tests which were difficult or impossible to interpret by this procedure was increased; as a result it was finally decided to abandon this modified Schick test altogether and to employ only the Moloney test. This procedure was adopted on the assumption that most of the +++ Moloney reactors were already

immune - a suggestion which has already been dealt with. Dudley (1934) stated that those who give strong Moloney tests will generally give a pseudo-Schick as well; and in a recent editorial (Lancet, 1935) it was pointed out that "the occurrence of a pseudo-reaction, if a preliminary Schick test has been done, is evidence of probable sensitivity to bacterial protein", and the writer of this editorial recommends that if this sensitivity is shown to exist either by the presence of a pseudo-Schick or by a positive Moloney test, special methods should be adopted for immunization. The question is of importance, since if these statements are correct, one of these tests can evidently be discarded. The present writer has never been convinced that these two reactions are more or less interchangeable; and the accumulation of these personal records of Moloney reactions, practically all of which have been read at least twice after the test injections, has rendered possible a more detailed examination of the problem than has hitherto been attempted.

This is no place to discuss the rather complex features of pseudo-Schick reactions. Some notes on the peculiarities of these reactions have already been given in Section (b) of this Thesis. In the present Section a positive Schick-control test is taken to mean any reaction at all at the site of the injection of heated toxin - excluding, of course, reactions which are due to trauma. The whole of the individuals in the series were grouped according to the nature of the reactions to the Schick-control and Moloney tests, and the results are set out in Table XLVIII. In the following discussion +D and + reactions to the Moloney test are grouped together. For this table $\chi^2 = 309.31$; for $n = 3$ the P corresponding to this value is less than 0.01, which shows that the distribution is not a random one. The degree of association between the Moloney-positive state and the liability of the individual to show a reaction at the site of the Schick-control injection was estimated as before by calculating the value of the coefficient C_2. This value was found to be $0.322 \pm .033$.

TABLE XLVIII

All Schick tests: incidence of pseudo-reactors (all degrees)	Moloney results					
	Negative	+D	+	++	+++	Totals
Control test: no reaction	2119	45	216 (261)	153	48	2581
Control test: + reaction	28	-	11 (11)	20	26	85
Totals	2147	45	227 (272)	173	74	2666

Table XLVIII was then investigated by the fourfold method, making the dichotomy between Moloney-negative reactors on the one hand, and Moloney-positive reactors (all types) on the other. For the table thus formed the value of χ^2 was 126.84; for this value of χ^2 with $n = 1$, P was found to be less than 0.01, indicating that the distribution was not random. The value of the coefficient of association (Q_5) was 0.672 $\pm$ .022, and the equiprobable coefficient (r_p) was 0.679. After making the dichotomies at two other points in the main table, it was found that the resulting coefficients were not materially affected by the alteration. These coefficients are set out in Table XLIX.

Though these coefficients indicate a considerable or even a fairly high degree of positive association between positive Moloney and positive Schick-control reactions, this association is far from being perfect. When it is remembered that the object of this part of the investigation is to find whether either of these tests can be used as a substitute for the other, it will be obvious that the answer to this question must be in the negative. Substitution implies perfect or practically perfect association if the information gained from the tests used is to be of any value whatever, and this series of cases does not give

TABLE XLIX

Type of grouping	χ^2	P	C_2	Q_5	r_p
4x2 grouping	309.31	0.01	0.3224 ±.0329	-	-
(i) Fourfold: (a) M-negative (b) M+ (all types)	126.84	0.01	-	0.672±.022	0.679
(ii) Fourfold: (a) M-negative, M+D, M+ (b) M++,M+++	210.12	0.01	-	0.642±.023	0.720
(iii) Fourfold: (a) M-negative, M+D,M+,M++ (b) M+++	251.67	0.01	-	0.603±.025	-

much support to the contention which was expressed at the beginning of this section.

It may be objected that these are theoretical considerations and that possibly they have no marked connection with the practical question, whether a reaction at the site of the Schick-control test does actually indicate that the individual is liable to experience an appreciable reaction on the injection of toxoid, or alternatively whether or not most individuals who react to toxoid react also to the heated Schick-control toxin. To answer this question Table L was prepared. It gives the Schick-control reactions and Moloney reactions in every case in which any reaction was noted after the injection of prophylactics - toxoid, alum toxoids, or floccules. It will be noted that the number of cases is larger than that given in Table XLV. The reason for the increase is that in Table L all cases of reaction were included, the object being to determine the relationship between these tests and liability to react after toxoid in prophylactic doses. Almost one-half of the cases in Table L therefore experienced reactions which were so mild as to be unnoticed by the individual inoculated, and many of them

TABLE L

No. of cases showing reactions (all types)	+ Moloney (any degree)	+Schick-control	+Moloney and + Schick-control	Both tests negative	Total
(a)Cases not seen	8	1	1	4	14
(b)Cases seen	55	-	6	16	77
Total	63	1	7	20	91

consisted merely of an area of erythema measuring 25 mm. in diameter or more. The standard adopted was rather a severe one, and this probably explains the number of cases in the last group of line (b). It should be noted that in Table L the phrases "cases not seen" and "cases seen" do not apply to the actual reactors. In subgroup (b) every person who had toxoid in the particular groups of children was examined by the writer on the day following the injection; and in subgroup (a) every individual who was inoculated was not thus examined, whether he had a reaction or not, though many of the actual reactors were seen.

Comment on this table is unnecessary. It is obvious that the test with heated Schick toxin is valueless for the purpose of determining "potential toxoid reactors", and the conclusions arrived at from more theoretical considerations are amply confirmed.

Relation of Moloney result to antitoxin content of blood

The only investigation of any extent dealing with this question which has come to the writer's knowledge is that described in the original paper by Moloney and Fraser (1927). In the present investigation the blood sera of 108 persons upon whom the Moloney test was performed were titrated for diphtheria antitoxin. The complete results are given in Table LI. The serum from the blood samples was titrated to 100 per cent.

TABLE LI.

Moloney State	Antitoxin Content of Blood in Units per c.c.													Total Cases
	←1/1000	1/1000	1/500	1/250	1/100	1/50	1/25	1/10	1/5	1/2	1	2	5 or 5+	
M-negative	8	2	-	1	1	1	1	1	2	3	1	-	-	21
M+D(4 days)	4	-	-	1	1	-	1	2	4	3	-	3	-	19
M+(48 hours)	4	1	-	2	-	-	-	-	5	1	4	6	2	28
M++(" ")	5	-	-	-	-	-	1	-	2	3	4	4	1	23
M+++(" ")	2	-	-	1	-	-	1	-	2	2	3	3	3	14
Total Cases	26	3	-	5	2	1	4	3	15	12	15	16	6	108

differences. For example, 1/50 unit in this table signifies 1/50 unit or more of circulating antitoxin per cc., but not 1/25 unit. Titration to closer levels would not appear to be necessary. The majority of the samples were taken 6 days after the Schick and Moloney tests were performed; most of the samples were from persons who had not had any prophylactic treatment. Once again, mention should be made of the writer's indebtedness to Mr. A. T. Glenny for carrying out these titrations. From this main table two 4x3 tables were prepared. In each case the divisions on the horizontal axis were 1/500, 1/250-1/25, 1/10-1/2, 1 or 1+. On the vertical axis the divisions were: grouping (a) - (i)Moloney-negative, (ii) M+D and M+, (iii) M++ and M+++; grouping (b) - (i) Moloney-negative, (ii) M+D, (iii) M+, M++, M+++. The coefficients obtained were:

	χ^2	n	P	C_2
4x3 table: Grouping (a)	16.3647	6	0.0120	0.3628 ± .0484
4x3 table: Grouping (b)	21.3765	6	<0.01	0.4065 ± .0455

The question was then investigated by the fourfold method. In the following series the dichotomy on the vertical axis is in each case between Moloney-negative cases on the one hand and Moloney-positive cases (all types) on the other. The position of the dichotomy on the horizontal axis is given in Table LII. For each grouping the values of χ^2 and Q_5 denote practically the same degree of association. It should be noted that the values increase progressively as the dichotomy is shifted towards the higher values of antitoxin content. From these figures it cannot be said that there was in Leeds more than a considerable intensity of association between antitoxic content of the serum and a positive Moloney reaction.

TABLE LII

Type of grouping	χ^2	P	Q_5
(i) (a) Up to 1/100 unit; (b) 1/50 unit and +	6.6503	0.01	0.447±.039
(ii) (a) Up to 1/50 unit; (b) 1/25 unit and +	8.8463	0.01	0.506±.050
(iii) (a) Up to 1/25 unit; (b) 1/10 unit and +	9.1199	0.01	0.530±.055
(iv) (a) Up to 1/10 unit; (b) 1/5 unit and +	10.1688	0.01	0.568±.063

The Delayed (+D) Moloney Reaction

It has already been stated that this reaction was described by the writer in 1934. In the earlier portion of this series of cases seven of these delayed reactions were found, and the number has now been increased to a total of 45 cases in the present series. The frequency is therefore 1.69 ±0.17 per cent. In the delayed reaction there are no visible signs of erythema at the Moloney injection site when read at 24 or 48 hours; by the seventh day, however, a reaction which is

usually faint has manifested itself. The exact day of appearance is unknown and probably varies in different cases. It is possible, however, that the reaction does develop late - possibly about the fifth or sixth day. The positive delayed reaction practically always consists merely of an area of erythema. In the 45 cases met with a slight degree of induration was felt only once. On the injection of 1 c.c. of toxoid the child had malaise and distaste for food which lasted for about a day, but there was no local reaction; almost identical symptoms were met with after a/prophylactic (second) injection of toxoid. The reaction is sometimes of a bright red or a definite pink colour. More often it is faint red in appearance, and sometimes it consists merely of a blush, or even a dull stain. The edges are not infrequently difficult to delimit from the surrounding skin; but when this is possible it is seen that the erythematous area is nearly always circular. The diameter of the reaction varies. With such a small number of individuals an average value is not of much use; but it may be said that many of these delayed reactions measured from 8 to 14 mm. in diameter. The largest delayed reaction which the writer encountered was in a youth of 17 years; it measured 22 mm. in diameter (The criticism may be raised that a reaction of 8 mm. in diameter is not of much consequence, and may be due to causes other than the material injected. It should be noted, however, that Moloney and Fraser in their original paper (1927) gave instances of ordinary positive reactions which measured 6 mm. in diameter). The delayed reaction is therefore much less intense than an ordinary Moloney reaction. Further, this delayed reaction obviously develops spontaneously, and has no relation to the trauma of the needle prick. In this series there are a few data on the duration of these reactions, but it would appear that the time of the disappearance, like the time of the appearance, is variable. Certainly in some instances the reaction persists for a few days and then fades, leaving a faint stain. In those instances in which a stain alone was noted on the seventh day it may be that the actual erythema appeared a a day or two previously and then faded quickly.

In one instance the reaction consisted of a faint red area. The child, a boy of 9 years, was injected with toxoid and suffered no reaction. A month later a second Moloney test was performed; the resulting reaction was now definitely positive on the second day. On both occasions the Schick control test was negative.

The writer was originally led to describe the reaction because two of the seven cases which had been met with up to that time had shown reactions of some degree after the injection of toxoid in prophylactic doses (Underwood, 1934). In one of these instances the reaction was quite a sharp one. Since little was known in this country eighteen months ago regarding the reactions which an individual might experience on the injection of unconcentrated toxoids of high potency, this experience seemed to render it advisable to recommend greater care in the reading of the Moloney test. Although these two observations show that these delayed reactions may occasionally experience definite reactions, it would seem that the numbers who do so are not in excess of the Moloney-negative reactors who may likewise react - although actually taking into account the fact that the incidence in negative reactors was 2 in 2147 cases (or 0.1 per cent.) whereas the incidence in +D cases was 2 in 45 cases (or 4.4 per cent), the incidence in the latter was really very much greater. Even this latter percentage is too low, since only 24 of the 45 +D reactors were injected with toxoid - the remainder being either immune or having failed to attend at the time fixed. In these 24 cases, besides the two mentioned above who showed significant reactions, another seven manifested insignificant reactions such as redness at the injection site without other symptoms. It will be apparent from what has been written previously that the +D reaction may appear at any age over the first year of life, but generally the cases tend to be relatively more numerous in the later years of childhood.

The Moloney Test performed after the injection of Toxoid

In addition to the primary Moloney tests which have so far been dealt with, a further series of tests was performed on 562 individuals after the injection of toxoids in prophylactic doses; 33 of these were repeated for purposes of corroboration. In general these post-Moloney tests were performed 1 month after the injection of the prophylactic - or 5 weeks after the initial Moloney test. A Schick test was performed at the same time. Table LIII gives the results of these tests on the 562 individuals.

TABLE LIII

(a) Primary Moloney-negatives which remained M-negative	514
(b) + cases which remained + (approximately same degree)	12
(c) Cases originally faint + which became negative	10
(d) Cases originally neg. or faint + which became def. M+	26

The most significant feature which emerges from Table LIII is that primary negative Moloney reactors do not tend to develop a positive Moloney reaction on the injection of toxoid in prophylactic doses. Of the 26 cases classified under (d) 24 were originally Moloney-negative, and became Moloney-positive after inoculation. Some of these reactions were very faint, but a few were of moderate size though unaccompanied by induration. In addition to these 24 cases in this group, one case was originally Moloney-positive and after inoculation developed a more intense reaction: the other case was originally a +D reactor, and after inoculation showed a definite M+ reaction on the second day. Reference has already been made to this case. An interesting feature of the individuals in group (d) was the case of two brothers. They were originally tested on the same day and both gave negative reactions. When retested again after inoculation both manifested definite M+ reactions. Group (b) calls for little comment except to account for the small number of cases in this group. It should be remembered that most Moloney-positive reactors were dealt with by a special procedure and that they were not therfore due for testing along with the other members of the particular groups of children to which they belonged. Group (c) is more interesting. Two of the ten cases in this group were

definite M+ reactors who became Moloney-negative. Another seven gave originally a definite but very faint reaction. The tenth case was originally a +D reactor on the seventh day. On retesting the patient was negative on the second day, but a reading on the seventh day was unfortunately not made. It should be especially noted in connection with this subgroup of cases that none of them showed any induration. It is possible that the features noted in this group may have been due to the element of error; for example, a slight variation in the amount or strength of the injected fluid may have been responsible. On the other hand, there is a possibility that there may be a transition field between the negative and positive Moloney states, in which a certain amount of fluctuation may take place. At all events, once a definite positive Moloney state - with induration - is established, it seems to be permanent within the limits of this experiment.

O'Brien and Parish (1932) did not note any "sensitisation" by the injection of toxoid, and they amplified this definition by saying that none of the children who showed Moloney-negative reactions before immunization gave "mild" or "definite" reactions afterwards. On the whole the present series supports this view. It is obvious that "sensitisation", if it takes place, is comparatively rare. We must also bear in mind that transition from the Moloney-negative to the M++ or M+++ state is normally a gradual process. Hence we would expect that a certain number of children who had previously given a Moloney-negative reaction would on retesting, even without the injection of toxoid, give a faint positive reaction. From Table XLIV it is seen that in 15 years the percentage of positive Moloney reactors changes from 2 to 66 per cent - or roughly 64 per cent. Assuming that the rate of change over this period is constant - an assumption which is of course not strictly justified in the light of Fig.xiv - this gives an average rate of change of 4.3 per cent per annum for all ages in the population under 15 years. On this basis the 562 individuals who were retested should have shown about 24 whose

Moloney condition would have changed towards the positive end of the scale. It is impossible to decide whether or not the toxoid injections hastened the process in the 26 cases in which this actually happened. It may be that the toxoid caused a transition, which would naturally have taken about a year, to become effected in a few weeks.

Sex Incidence of Positive Moloney Reactors.

The possibility of a difference in the sex incidence of positive Moloney reactors does not appear to have been investigated previously. In the present series, of the 2666 individuals, 1391 were males and 1275 were females. The incidence of the different types of reaction in the sexes was as follows:

	+D	+	++	+++	Totals
Males	33	136	97	40	306
Females	12	91	76	34	213

Even when account is taken of the total preponderance of males, these figures appear to indicate that a positive reaction is much more frequent in the male. The group 15-19 years in Table XLIV however, was composed entirely of males in a large residential school (and the total population investigated did not embrace any similar series of females at these ages). If the positive cases at these ages are deducted from the different groups, and the total number of males in this school at these ages (105) is deducted from the total number of males, a more accurate comparison is obtained. The results, with the percentages for all males and females respectively for each type of reaction, are set out in Table LIV. (The percentages are shown in brackets).

TABLE LIV

	+D	+	++	+++	Totals
Males (1268)	28 (2.18)	112 (8.71)	70 (5.44)	29 (2.25)	239 (18.58)
Females (1275)	12 (0.94)	91 (7.14)	76 (5.96)	34 (2,67)	213 (16.71)

It is obvious from these results that the incidence of a positive Moloney reaction is practially identical in males and females.

The Moloney Test in Different Members of the same Family

The possible influence of fraternal relationships - and the close association which these imply - in hastening the development of positive Moloney reactions has not hitherto been investigated. Apart altogether from the congenital standpoint, we might expect that, if a number of brothers and sisters live together in a small house, and grow up there exposed to the unmeasured effects of submininal infections, the result might be that sensitivity might develop earlier than it would do if the children were members of separate families. It is difficult to get definite evidence on these questions.

In the present series of 2666 cases, 1430 individuals belonged to as many separate families, the remaining 1236 individuals were members of 503 different families, and in each of these families at least two members were tested. Table LV gives details of the number in each family and the number of these who gave a positive Moloney reaction. From this main table an abbreviated table was prepared, the divisions being: (a) on the horizontal scale, 2, 3, 4, 5 and over; and on the vertical scale, all negative, 1 positive, 2 or more positive. For this table $\chi^2 = 41.68$; for $n = 6$, the corresponding value of P is less than 0.01, which shows that the distribution is not random. The value of C_2 - viz. $0.277 \pm .030$ - indicates that the association between the number of children in a family and the number of these who will give positive Moloney reactions is very slight.

TABLE LV

	No. of children tested in family						
Moloney state	2	3	4	5	6	7	Totals
All negative	252	54	23	3	1	-	333
1 positive	66	32	12	1	-	-	111
2 positive	24	15	10	2	-	-	51
3 positive	-	3	3	1	-	-	7
4 positive	-	-	-	-	-	1	1
Totals	342	104	48	7	1	1	503

It has been pointed out that in a "family" investigation of this type it is impossible to exclude more than a few of the

factors which may presumably influence the results, Nevertheless, these figures do suggest that, when we meet with several members of a family who happen to give positive Moloney readings, we can assume that this is due not to the fact that they are all living together under one roof, but to the fact that they must generally be of different ages, and that therefore the older members have been longer exposed to the C.diphtheriae. This conclusion is further borne out by the observation that in the present series there are 15 pairs of twins; in two of these pairs one twin gave a positive reaction and the other a negative reaction; in the other thirteen pairs both members gave negative results. Certainly there are some grounds for considering that, in a large urban area in which diphtheria is prevalent, the chances of sensitisation of children owing to the fact that they are members of large families is not greater than it would be if they were members of families consisting of only one or two children, provided that they were still exposed to others of similar age outside their homes.

Discussion of the significance and applications of the Test.

A consideration of the mechanism of production of a positive Moloney test is an interesting speculation on the borderland of the study of allergy. It was recognised by the originators of the test that a positive result did not necessarily mean that the individual was immune, and the point was further amplified by McKinnon and Ross (1933). Further evidence on this question is presented in this paper in the section dealing with the relationship between a positive Moloney reaction and a positive reaction to the heated toxin of the Schick control. There is a possibility that a positive reaction may be an expression of the development of allergy due to exposure of the individual to proteins of different types. The writer has never been satisfied with this view, and in this paper a certain amount of indirect evidence is adduced which suggests that a positive Moloney test probably indicates previous contact with the C.diphtheriae. So far as our present knowledge extends, the analogy with protein allergic reactions is fairly close. Very suggestive are the facts that the skin responses are similar in the two conditions; that the degree of reaction for the

herd at least if not for the individual, increases in proportion to the length of contact with the allergen - that is, the J.diphtheriae; and that repeated tests are usually identical in type, with the exception of the borderland reactions already discussed. If we accept this theory, however, a peculiar feature emerges. True protein skin allergy is found only in a very small percentage of normal individuals. For example Bray (1931) quotes Baker's figures of ten positive and eleven doubtful reactions to various test proteins in 937 healthy children. On the other hand, a large percentage of allergics - for example, asthmatics - will give a positive skin reaction to test proteins, and in the particular case of the Moloney test it would seem that we must regard every individual in an urban community as a real or potential allergic for this particular protein which is contained in the Moloney test fluid.

Many other suggestive features emerge from a consideration of the test from this point of view. For example, Gibson and McGibbon (1932) working with a soluble intracellular products of haemolytic streptococci, showed that in a considerable proportion of scarlet fever patients an allergic skin reaction could be produced by the intradermal injection of this material in the second week of the disease or later. It is noteworthy that ~~in~~ all their patients who were over seven years of age developed marked allergy. These reactions were at their height in thirty six hours. Coburn (1931) had previously observed that cases of acute rheumatism were hypersensitive to the nucleo-proteins of haemolytic streptococci, and similar results were obtained by Collis (1931). If we assume that the Moloney reaction is in effect the result of hypersensitiveness to some protein derived from the diphtheria bacillus, other facts may be enlightened by the analogy. For example, Harley (1933) working with the sera of various animals described delayed reactions which were perhaps similar in type to the delayed Moloney reaction.

Much work still remains to be done on the interval between the injection of test proteins and the time of appearance of resulting reactions. It is therefore impossible to give any rational explanation of the delayed Moloney reaction. There seems to be

some evidence that it represents an intermediate stage between the Moloney-negative state and the development of a frank positive reaction. The infrequency of +D reactors and the evidence obtained from contingency coefficients discussed previously lend some support to this view. Obviously is is not merely a time factor which is involved. Dickinson (1922) saw six instances in 1091 positive Schick tests where the reaction did not appear until after the fourth day, and some work of the present writer confirms these observations. But, once this delayed Schick reaction has developed, it runs a normal course. The delayed Moloney reaction, on the contrary, is in effect a reaction which is so mild that it fails to appear at the usual time.

Some further features arise from a discussion of the view that a positive Moloney reaction is due largely to previous contact with the products of the C.diphtheriae, and is essentially an allergic phenonomem. It has already been stated in an earlier section that McKinnon and Ross thought that age might in itself be a factor in the development of the reaction; and it was suggested that the results of the present investigation did not give much support to their view. The data on which they base their opinion are worth considering more fully.

These authors divided their cases according to age and Moloney state respectively. They also gave a separate table which is entitled "Reaction test readings in 'Schick-negatives'". It seems probable from their paper that this table includes all the Schick-negative individuals in their main series, but it should be emphasised that this fact is not definitely stated. A portion of this table is produced here (Table LVI)with slight rearrangement.

Table LVI (Abridged from McKinnon and Ross)

Age.	No.Moloney-negative	Percentage negative	No.Moloney positive(all types)	Percentage positive	Total Schick Negative
0-1	5	100	-	-	5
1	6	100	-	-	6
2	14	100	-	-	14
3	13	100	-	-	13
4	11	78.8	3	21.4	14
5	58	93.6	4	6.5	62
* * *	* * *	* * *	* * *	* * *	* * *
14	231	52.7	207	47.3	438

79

McKinnon and Roos make the following comments on this table: "The fact that 33 Schick-negative children, 1-3 years of age, show no sensitivity is strong evidence that immunity may be obtained without senstivity developing at any time during the process. (The fact, too, that when using the diluted toxoid as a control in the Schick test one finds in the pre-school group approximately one sensitivity reaction for every five or six immune reactions, while from six years on the ratio changes till at 14 it is 1: 1, practically confirms this and suggests that age per se is a factor in the development of sensitivity.)" The argument of the authors is evidently along the following lines: In the early years of life certain individuals develop immunity as a result of contact with the C.diphtheriae, and yet no sensitization occurs; as age advances sensitisation becomes increasingly frequent, and the process proceeds at a relatively greater rate than the process of natural immunization. Hence it is possible that age in itself is a factor in the development. On further examination of the tables of McKinnon and Ross it was found that the total number of children tested at 0-1, 1, 2 and 3 years was 12, 30, 39, 55 respectively. In the present series the respective totals at these ages were 49, 185, 177, 194, and amongst the negative Schick reactors in these children five positive Moloney reactors were found (see Table XLIII). It might be suggested that the failure to find positive Moloney reactors in the Toronto series was due to the smallness of the samples at these ages. Further examination showed, however, that, whereas in the Toronto series no positive Moloney reactors were found in 38 Schick negative children under four years, the five positive Moloney reactors in the Leeds series were found among only 22 Schick negative children. Right through the different age groups marked differences were found in the percentages of children who were Schick negative in the Leeds and Toronto series respectively; but, since it was mentioned above that it is not certain that the Canadian Schick negative figures included all such cases in the main series, this question will not

TABLE LVII

Frequency of positive Moloney reactors (all degrees) in Schick-negatives, expressed as percentage of total Schick-negatives at each age.
(Delayed reactors are included as Moloney-negative in the Leeds series)

Age	LEEDS			TORONTO			DIFFERENCE
	Total Schick neg.	Moloney pos.	Per cent positive	Total Schick neg.	Moloney pos.	Per cent positive	
0-1	8	0	0	5	0	0	---
1	3	0	0	6	0	0	---
2	3	3	100	14	0	0	---
3	8	2	25.0±10.3	13	0	0	----
4	21	5	23.8 ± 6.3	14	3	21.4±7.4	2.4±9.7
5	39	9	23.1 ± 4.6	62	4	6.5±2.1	16.6±5.1
6	67	17	25.4 ± 3.6	439	70	15.9±1.2	9.5±3.8
7	38	14	36.8 ± 5.3	536	84	15.7±1.1	21.1±5.4
8	39	16	41.0 ± 5.3	762	173	22.7±1.0	18.3±5.4
9	44	25	56.8 ± 5.0	795	206	25.9±1.1	30.9±5.1
10	45	26	57.8 ± 5.0	824	237	28.8±1.1	29.0±5.1
11	51	33	64.7 ± 4.5	860	305	35.5±1.1	29.2±4.6
12	62	34	54.8 ± 4.3	862	337	39.1±1.1	15.7±4.4
13	37	24	64.9 ± 5.3	681	269	39.5±1.3	25.4±5.5
14	19	13	68.4 ± 7.2	438	207	47.3±1.6	21.1±7.4
15-19	86	48	55.8 ± 3.6	---	---	---	---

be considered further here. The percentages of Moloney - positive reactors in Schick negatives were in a different category, since the figures were tabulated in the original paper. These figures for the two cities are given along with the probable errors in Table LVII Since delayed reactions are not included in the Toronto series, the delayed reactors in Leeds are here considered as Moloney negatives.

It is seen from the table that at nearly every age over four years - when the percentages become significant - the percentage of Leeds Schick negative reactors who showed positive Moloney reactions was almost double the percentage for Toronto Schick negatives at corresponding ages. These differences might be due to at least three separate factors. Firstly, a different standard of reading may have been adopted. In considering this point it should be remembered that, although the classifications adopted were not strictly identical in the two series, the broad term "positive Moloney reactor" includes for both series all persons who showed any reaction at all at the injection site. The second possibility is that the Moloney test fluid used in Toronto may have differed in strength or quality or both from that used in this country. The writer has some evidence that the strength of the toxoid used does have an effect on the numbers of persons who give positive results, but the difficulty of excluding age and other factors prevents its presentation. Thirdly, the differences may be due to environmental or biological factors which cannot easily be assessed. It should be remembered, however, that during the time when these investigations were progressing in Leeds, the predominant type of C.diphtheriae in the city was the gravis strain, which in addition to causing a severe form of the disease also exerts its efforts in the direction of the breaking down of established immunity (Underwood, 1935b) and section (h) of this Thesis).

The percentages which are given in Table LVII were plotted on a logarithmic scale (Fig. xv). It is seen from the graphs that, although there were such marked differences in the actual values, the rates at which Schick negatives develop

Cranstoun,
Ember Lane,
ESHER,
Surrey.

28th October, 1935.

The Registrar,
The University,
GLASGOW.

Dear Sir,

On the 3rd. inst. I forwarded per registered post, addressed to the Dean of the Faculty of Medicine, at the Registrar's Office, a Thesis for the degree of M.D. I have not so far received an acknowledgment, and I should be glad to know whether the package was received safely.

It has just come to my notice that an earlier version of Fig. xv in Part I of the Thesis was erroneously copied and bound up - as, I think, page 140 of the complete Thesis. As the text refers to the later version, which I enclose herewith, some slight confusion may result. I should therefore be very grateful if you could have this drawing inserted at the appropriate place. I may mention that the particular section - Section (d) - in which this figure occurs will be published in the Journal of Hygiene in a few weeks, and if any further proof is required I should be pleased to forward a University of Cambridge Press proof, dated 29th July, 1935, in which the enclosed version of the figure is printed.

Yours faithfully,

E. Ashworth Underwood.

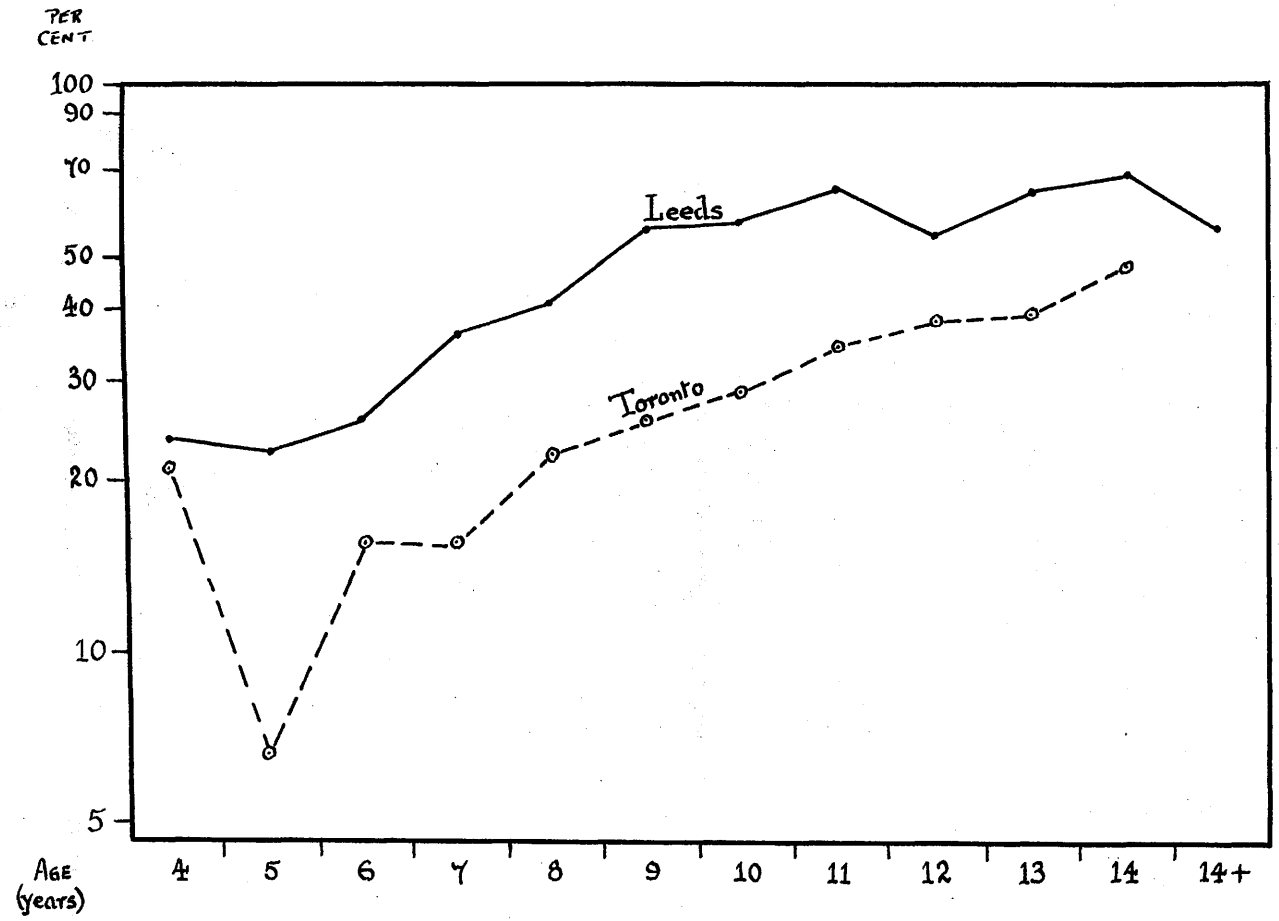


FIG. XV: FREQUENCY OF POSITIVE MOLONEY REACTORS (ALL DEGREES) IN SCHICK NEGATIVES, EXPRESSED AS PERCENTAGE OF TOTAL SCHICK NEGATIVE INDIVIDUALS AT EACH AGE (LOGARITHMIC SCALE). (a) LEEDS. (b) TORONTO.

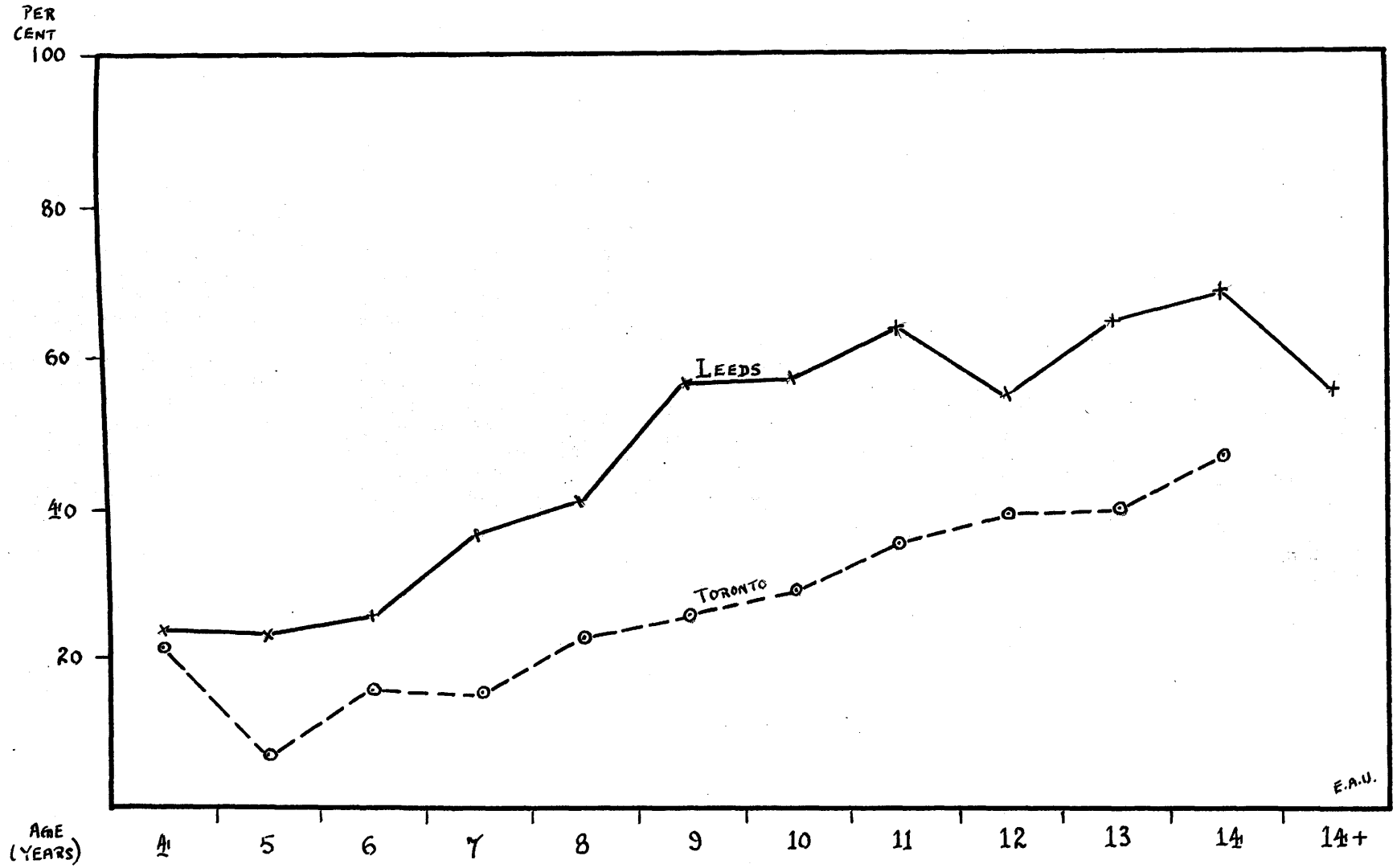


Fig. xv: Frequency of positive Moloney reactors (all degrees) in Schick negatives, expressed as percentage of total Schick negative individuals at each age. (a) Leeds. (b) Toronto.

sensitivity to toxoid as age advances is practically the same in the two cities. It has already been mentioned that in the different age groups there were marked differences in the percentages of children who were Schick negative in the Leeds and Toronto series respectively. Hence, this similarity in the rates of development of Moloney-positiveness in Schick-negatives is additional evidence that development of the two conditions, Schick-negativeness and Moloney-positiveness, is closely associated - possibly more so than would be the case if age in itself were an important factor in the development of the sensitivity.

Practical considerations.

The practical worker who is about to use undiluted toxoids of high potency will be faced with the question of whether it is possible or expedient to dispense with the Moloney test in view of the fact that by its inclusion three injections have to be given at the time when the primary Schick test is performed. The evidence of this paper is that the test is extremely useful from the practical point of view, and the writings of Burke and of McKinnon and Ross show to what extent it has been employed in Canada. The difficulty which faces workers in this country was evidently met with in Toronto also, for it will be remembered that the Schick-control injection was abandoned in that city. Hence, when toxoid is used as a prophylactic in this country there would seem to be a case for the retention of the test. Of the 2666 cases which are described here, 519 positive Moloney reactions of all types were met with; but in the same cases the number of pseudo-reactions to the Schick test was only 85. In a paper which is at present in preparation it will be shown that it is impossible to decide, apart from antitoxin titration of the blood serum, whether many of these pseudo-reactors are, or are not, immune. The writer is consequently of opinion that, if one test has to be omitted, that test should certainly be the Schick control and not the Moloney injection. It has been shown that one test cannot be used as a substitute for the other, and of the two the information given by the Moloney test is much the more important.

A word should be said regarding the importance of the Moloney test where alum preparations are used. Many of the persons dealt with in this section were injected with such preparations, and a previous communication (Underwood, 1935a) dealt with the significance of the test when used prior to the injection in prophylactic doses of the latest development of the alum group - viz, alum-precipitated toxoid (A.P.T.), and the question has been considered further in Section (g) of this Thesis. It is only necessary to say here that with this material Moloney-negative persons gave no trouble, whereas reactions of various types were met with in Moloney-positives. The writer is of opinion that this test is essential whenever such preparations are to be used in anything more than minimum prophylactic doses.

PART I.

SECTION(e)

ON THE USE OF TOXOID AND OF ALUM TOXOID IN MULTIPLE DOSES IN DIPHTHERIA PROPHYLAXIS.

Of all substances which have been used in the past twelve years as diphtheria prophylactics few are so interesting as toxoid and its alum modifications. Toxoid was first prepared by Ehrlich before the War, and Löwenstein in his work on diphtheria and tetanus toxoids showed that these could act as efficient antigens. Glenny and Südmersen (1921) succeeded in changing diphtheria toxin to toxoid completely by the agency of formaldehyde, and Glenny and Hopkins (1923) suggested its use for human immunization. The advantages of such a preparation are obvious; the toxoid contains no sera and consequently serum phenomena do not follow its use; and since the toxin has been more or less completely converted into toxoid by the aid of heat and formalin, there is no possibility of dissociation with the subsequent liberation of free toxin. Schroder and Park (1923) immunized a number of children with a modified toxin dilution, and in the same year Ramon (1923) used a completely modified toxin upon humans. This was the origin of "anatoxine", which has been extensively used in France and other Continental countries during the last twelve years.

It is no part of the purpose of this paper to discuss Ramon's claim for priority; this writer has set out his full case in numberous contributions to the literature (see for example (Ramon (1931a) and Ramon (1934a) and 1934b). These claims have been briefly though adequately dealt with by Okell (1931). It should however be mentioned at this point that for many years the toxoid which Ramon and his colleagues used was consistently of an

Lf

Lf value of about 10 units per ccm. Recently these workers have used toxoids of 20 and 30 units per ccm. (see Nicolle (1933), and Ramon, Nélis and Lacomble (1933). All the toxoids which were used in this investigation were of Lf value of at least 20 units per ccm. and the strongest toxoid employed had an antigenic value of 65 Lf units per ccm.

In Great Britain toxoid was used on a few nurses by Nash in 1924. Therafter there was a break until 1932 when Dudley (1932) published the results of an important investigation carried out on youths at Greenwich Hospital School. This investigation will be referred to later. O'Brien and Parish (1932) reported the use of potent unconcentrated formol toxoid in 248 Schick positive children; 95 per cent were negative within two to four weeks of the final injection. Parish (1933) later dealt with the use of this material for theimmunization of tuberculous children. Underwood (1934a) described the results obtained by using these potent formol toxoids in 348 Schick positive children, and found that 84 to 96 per cent were Schick negative within four weeks, and 90 to 100 per cent within eight weeks. Later papers dealing with toxoids will be discussed in the text. Various reports have emanated from other countries regarding the use of toxoid. Park and Zingher (1924) inoculated over 1,700 children with this material and Zingher (1925) later made a special study of 100 individuals. Roubinovitch, Loiseau and Lafaille (1924) studied 260 cases. In Canada Bloomberg and Fleming (1927) inoculated 278 children with Ramon's anatoxine, and in the United States Schwartz and Janney (1930) immunized 128 children with toxoid, and Ray (1931) made a suggestive study of 358 individuals. Further particulars regarding the history of toxoid will be found in the papers by Ray (1931) and by Kreitz (1932). Most of these toxoids have been of Lf value under 10 units.

The work of Glenny and his colleagues (Glenny and Barr (1931) and Glenny, Buttle and Stevens (1931)) showed that it was possible to precipitate the active factor of certain toxoids by

by means of alum, and that these properties possessed very high antigenic properties, with the additional advantage that the precipitate was absorbed slowly from the tissues. Glenny and his co-workers (1931) suggested from their experimental work in animals that, when an antigen is injected into the tissues, a condition of continual stimulation is produced "by lessening quantities of antigen acting on tissues whose power of response is rapidly increasing". They further suggested that the action of the alum was to retain the antigen in the tissues until these had acquired the power of responding rapidly to the stimulus. Saunders (1932) in Cork showed that alum toxoid (A.M.T.) was a very efficient immunizing agent when employed in three doses. A disadvantage in using it is the possibility of the occurrence of subcutaneous abscesses due to the retention of the alum in the tissues. In the series of 579 cases which Saunders described subcutaneous abscesses were met with four times. Apart from Dudley's work in the sami-closed community at Greenwich alum preparations were first used in single doses on the general population by the present writer (1934c), (1935a). This communication will deal with alum preparations when used in two doses, and a fuller discussion of the history of toxoid precipitants is given in section (f).

Scope of the Present Investigation.

This paper deals with children who were immunized in Leeds with potent formol toxoids and with alum toxoids during the period from March 1933 to September, 1934. The investigation includes those cases which were reported in a previous paper (Underwood, 1934) and other features of the immunity state have been discussed in other communications. The appendix contains details of the protocols which were used in this investigation. It should be stated that certain small groups had to be omitted from the inquiry owing to the fact that they were used for the preliminary testing out of new materials which necessitated the use of groups which were too small to give significant results.

SUB-SECTION A - POTENT FORMOL TOXOIDS.

Natural Immunity State.

It has been shown repeatedly that in general the higher the frequency of Schick susceptibles in the population the lower is the immunizability of that population. For example, Zingher (1922) found that when immunizing children from different schools with toxin antitoxin mixture, the schools with a low relative immunity rate were more difficult to immunize than those in which the rate was initially higher. This factor is of importance in considering the effect of various prophylactics on children drawn from different areas, since obviously a prophylactic which gives a satisfactory result in an area in which the immunizability is high may not do nearly so well in another area in which immunizability is low. Too much stress cannot be laid on this point since in the literature it is not uncommon to find criticisms of results which are made without any regard for the natural immunity rate of the samples upon which the investigations are carried out. Further, in a large urban area the immunity rate may vary according to the environment from which the various groups are drawn, and consequently undue credit may be given to a particular prophylactic.

The question as to what constitutes a reasonable degree of immunizability is complicated and cannot be dealt with in this paper. In section (b) of this Thesis will be found, however, a discussion of the natural immunity rate in Leeds and other areas. It was shown then that the natural immunity rate in Leeds is much lower than in most other areas.

The children in sub-section A of this paper can be divided broadly into two categories - those who had two or three doses of toxoid respectively. The percentages of positive reactors on initial testing of the groups which made up these two groups are given in Table LVIII.

Table LVIII.

Results of Primary Schick Tests.

3-dose Groups				2-dose Groups			
Group No.	Total tested	Total positive	Percent-positive	Group No.	Total tested	Total positive	Percent positive
1	25	20	80.0	9	34	29	85.3
2	23	18	78.3	11	51	43	84.3
3	85	68	80.0	13	60	49	81.7
4	22	21	95.5	15	54	48	88.9
5	29	26	89.7	16	45	34	75.6
6	207	174	84.0	17	45	36	80.0
7	31	29	93.6	18	40	35	87.5
8	33	29	87.9	19	93	87	93.5
10	63	60	95.3	20	76	66	86.8
12	62	54	87.1	21	91	82	90.1
14	39	28	71.8	22	54	46	85.2
				24	55	50	90.9
	619	527		25	52	45	86.5
				26	27	20	74.1
				27	53	44	83.0
					830	714	

3 doses for all groups, total positive - 85.1±0.97 per cent

2 doses for all groups, total positive - 86.0±0.81 per cent

The percentage of positive reactors in the 619 individuals was 85.1±0.97; for the two dose category, as a whole, the percentage of positives in 830 individuals was 86.0±0.81. It will be evident that none of the individual groups varied significantly from these figures, and that the child population of Leeds, despite the continued high incidence of diphtheria from 1931 onwards, was largely made up of children who were Schick-positive; and who therefore had a very low immunizability.

Time to produce effective immunity.

The earlier part of the present investigation was concerned not only with the establishment of a satisfactory dose, but also with the determination of a suitable interval between the last dose and the final Schick test. This interval is very important, especially when a community is beginning to experience a rise in diphtheria incidence. If the interval necessary for the production of a negative Schick test is a long one, then there will be little likelihood that an immunization campaign will be able to produce the desired results in the time which is available. Toxoid-antitoxin mixture

("T.A.M") was very largely used in this country until comparatively recently, when its place was partly taken by toxoid-antitoxin floccules (T.A.F). In certain areas the results obtained with "T.A.M" were satisfactory, but in Leeds it was found that this prophylactic required an unduly long interval before the establishment of immunity could be demonstrated. For example, in one group of 549 children upon whom a final Schick test was performed, about six months after the final injection of "T.A.M" 138 (25.1±1.25 per cent) showed a positive result. In one group of 105 children between the ages of five and seven years, 46 per cent were positive when tested seven months after the third injection. Such results could presumably be improved by the use of more powerful materials for immunization. The earlier group in the present series were final tested approximately nine weeks after the third immunizing injection. Table LIX gives the results obtained for these five groups.

TABLE LIX.

Group No.	Total inoculated and tested	No. of negative	Percent negative
1	17	16	94.1±3.9
2	18	18	100
3	56	56	100
4	20	18	90.0±4.5
5	20	19	95.0±3.3

Three of these groups were really tested after an interval of seven weeks (see Appendix). The assumption that the interval was nine weeks in every group errs on the safe side, since if the individuals had been tested again at nine weeks it is practically certain that a larger proportion would have been negative. The mean* for this series is 96.9±1.02 per cent. Particulars for six groups of children whose ages were more or less similar and who also had three doses but who were tested four weeks after the third dose, are given in Table LX.

*Note - In this paper the means for different groups are all weighted.

TABLE LX .

Group No.	Total inoculated and tested	No. negative	Percent negative.
6	144	121	84.0±2.1
7	25	24	96.0±2.6
8	26	24	92.3±3.5
10	53	41	77.4±3.9
12	46	37	80.4±4.0
14	20	15	75.0±6.5

The mean for this series is 83.1±1.43 per cent. The difference between these two means, viz, 13.8±1.76 per cent, is significant and of considerable magnitude. Broadly, this difference probably represents the increased production of immunity which took place during the four or five weeks which elapsed after the first month. (It will be seen later, however, that the materials and intervals of Table LX are different from ~~that~~ those of Table LIX, so that emphasis cannot be placed upon this increase in the ratio immunized). One point does, however, emerge from this consideration. The results obtained after an interval of four weeks were sufficiently satisfactory to warrant the adoption of this period as a standard for the remainder of the investigation.

Relative efficiency of three doses and of two doses of toxoid

Few investigations deal with this point in a satisfactory manner. In many the material employed is not the same in different groups of children, and in others the doses are not of the same size. In the present inquiry two comparable series were available, consisting of 145 and 140 children respectively. The material employed - a formol toxoid (B8337) of Lf 53 units per ccm. - was the same thoroughout.

Tables LXI and LXII give the detailed results and the means for three doses and for two doses respectively. In Table LXII the interval betwen the doses was four weeks, except in group 11 in which it was three weeks.

TABLE LXI.

B8337 (Lf 53 units) 3 doses (each 1 ccm.) at weekly intervals.

Group No.	No. inoculated and tested	Interval*	No. negative	Percent negative
8	26	4	24	92.3±3.5
10.	53	4	41	77.4±3.9
12	46	4	37	80.4±4.0
14	20	4	15	75.0±6.5

Mean = 80.7±2.21 per cent

*Interval in weeks between third dose and final Schick test.

TABLE LXII.

B8337 (Lf units 53) 2 doses (each 1 ccm.) at weekly intervals

Group No.	No. inoculated and tested	Interval*	No. negative	Percent negative
9	18	3	16	88.9±5.0
11	36	4	29	80.6±4.5
13	46	4	39	84.8±3.6
15	40	4	30	75.0±4.6

Mean = 81.4±2.22 per cent

*See note to Table LXI.

The difference between these means is 0.7±3.13 per cent, - which is obviously not significant, Hence there is a prima facie case for believing that the results obtained by the use of three doses is identical with that obtained with two doses. The result may possibly depend more upon the interval between doses and the size of dose than upon their actual number. But these figures do not tell us whether two doses wouldbe as satisfactory as three after, say, not one month but three or four months. In other words, would three doses be more successful than two doses, in immunizing the 20 per cent or so of children who still remained Schick positive after one month ? From the Tables which are given in a previous paper (Underwood,1935b) and section (h) of this thesis it will be seen that so far as any reliance can be placed on the small number of Schick positives who relapse, those children who had had two doses of FT were apparently no more liable to contract clinical diphtheria than were children who had had three doses.

These findings should be compared with those of other writers. For example, Harrison (1930) gives the results of immunization of children with toxoids which were of Lf 4 to 11 units per ccm. (The actual numbers of those who were negative are not given in the paper). These figures and the probable errors have therefore been calculated from the percentages, which are given.) Three doses of toxoid immunised 91.9±2.0 per cent of 86 children. Two doses of various toxoids immunised - after 119 days - 94.7±1.0 per cent of 318 children. The difference between these percentages is 2.8±2.2, which is not significant. Again, two doses of toxoid of Lf 4 units immunised 98.6±0.9 per cent of 72 children. The difference between this figure and 91.9±2.0 is 6.7±2.2 -which is not very significant. Hence it would appear that Harrisons figures do not substantiate the assumption that three doses are better or worse than two doses when a post-Schick test is performed about four months after the final injection. Monroe and Volk (1934) treated 461 children with New York City toxoid (8½ Lf units per ccm.) and they found that two doses each of 1 ccm. immunised 85.5 per cent after two months, and that three doses, each of 1 ccm., immunised 92.3 per cent in the same interval. It is probable that the difference was not significant; this is, however, only an assumption, since the actual numbers upon which the percentages were calculated are not given in their paper. On the other hand, after twelve months the percentages for the same two dose and three dose groups were 93.1 and 95.6 respectively. This does not suggest that three doses were any better than two doses even after this long interval.

Effect of total amount of Toxoids used for immunization.

It is often assumed that the efficiency of a prophylactic depends upon its "strength" - that is, upon its Lf value. For example, Dudley (1932) showed that strong toxoid was a much more efficient antigen than weak toxoid - Dudley's "weak" toxoid being a toxoid diluted 1 in 10. When the present investigation was planned over two years ago it was assumed that probably efficiency increased directly with increase in Lf value, and that probably

an ultimate optimum value ~~will~~ would be reached which would give efficient immunization. Throughout the investigation the groups were arranged to throw some light on this question. The available data will be divided into two sections - those groups which had three doses and two doses respectively.

The groups which had three doses and which were final tested four weeks after the third injection are set out in Table LXIII. In these groups the difference between the

TABLE LXIII

Three dose groups in order of efficiency

Group No.	Material	Total Lf Units	Per cent. negative
7	B248C	78	96.0 ± 2.6
8	B8337	160	92.3 ± 3.5
6	B8152* B263*	60	84.0 ± 2.1
12	B8337	160	80.4 ± 4.0
10	B8337	160	77.4 ± 3.9
14	B8337	160	75.0 ± 6.5

* equivalent batches of material

highest and lowest B8337 groups is 17.3 ± 7.4. This is not definitely significant, so that the weighted mean of these groups should represent the efficiency of B8337. The mean is 80.7±2.2 per cent. The difference between this mean percentage and the percentages for groups 7 and 6 are 15.3 ± 3.4 and 3.3 ± 3.0. The former is significant, the latter is not. In both the results produced by three doses of toxoid of moderate Lf value were better than those produced by a very strong toxoid.

In the same way the groups which had two doses are set out in order of efficiency in Table LXIV. (Groups 26 and 27 were of a special nature, and were omitted; they will be discussed later. Summing the results in the table

TABLE LXIV.

Two dose groups in order of efficiency

Group No.	Material	Total Lf units	Number injected	Percent negative
9	B8337	110	18	88.9±5.0
21	B8553	110	52	88.5±3.0
25	B8553	110	37	86.5±3.8
17	B8553	110	27	85.2±4.6
13	B8337	110	46	84.8±3.6
11	B8337	110	36	80.6±4.5
16	B8553	110	25	80.0±5.4
20	B8553	110	54	79.6±3.7
19	B8553	110	67	76.1±3.5
15	B8337	110	40	75.0±4.6
24	B8553	110	48	75.0±4.2
18	B8553	110	22	63.6±6.9
22	B8605	130	31	58.1±6.0

for those groups which had one hundred and ten units - i.e., all the groups except No. 22 - we find that the mean is 80.3±1.2 per cent. The difference between this mean and the percentage for the single group which had 130 units is 22.2±6.1 per cent, which is just significant. It should be noted that this difference is considerable, and that the groups which had the smaller total Lf units gave better results than the group which had 130 units.

These conclusions which are to be drawn from this section and from the preceding one appear to be that, so far as the results one month after the final injection are concerned, it does not matter whether we use two doses or three, nor does it matter how many total Lf units are given, provided that a minimum total dosage is injected. In this investigation the minimum total dosage was between 60 and 80 units - but it is possible that a smaller dose might have been equally effective. With other prophylactics the results might of course have been different.

It is interesting to compare these results with those obtained by others. Monroe and Volk (1934) used a concentrated toxoid of 30 Lf units per ccm. on 229 children, the dose being a single injection of 1 ccm. After two months 68.7 per cent, and after twelve months 90.5 per cent were negative. On the other hand, with a single 1 ccm. injection of New York toxoid(Lf 8½) only 40 per cent were negative at the end of two months, and 57 per cent at the end of a year. The result with a Michigan toxoid

of Lf 7½ were very similar. Hence these writers have given some evidence that the antigenic value does have some effect on the efficiency of a toxoid so far as a single injection is concerned. But it should be borne in mind that a total dosage of about 8 units is possibly not sufficient to provide a minimum stimulus required for effective immunization. Ramon and his colleagues have carried out several investigations on these lines - though it must be said that none of the toxoids used in his investigations appears to have been of really high value; the Lf value of the strongest toxoid used was 30 units. Ramon, Timbal, and Nélis (1932) used toxoid of 16 Lf, 7 Lf and 2 Lf respectively on groups of children. The writer has calculated the percentages and probable errors from the figures given by these authors. The difference between the means for the 16 Lf and 7 Lf groups was 14.4±2.2 per cent; and between the 7 Lf and 2 Lf groups was 71.0±5.2 per cent. More extensive figures were given by the same authors in a later paper (1933). The toxoids used were apparently the same, and the respective differences were 12.9±2.1 per cent and 66.3±6.6 per cent. In other words Ramon's work tends to show that the strength of the toxoid does affect the results with toxoids up to 16 Lf. In the present invesitgation no toxoid had an Lf value below 20. Hence there would seem to be some ground for suggesting that the total dosage of toxoid must be of a certain minimum value if optimum results are to be obtained, but over the minimum value the strength does not appear to exercise any marked effect.

The findings from animal experiments more or less bear out these conclusions. For example, Asakava (1933), working with Ramon, carried out experiments on 18 rabbits with toxoids of 7, 12.5, 20 and 30 Lf units respectively. The course of immunisation was followed by antitoxin titration of the pooled sera from the different groups, andby intracutaneous reactions on individual animals. From the tables which Asakava gives there appears to be a slight balance in favour of the toxoid of 30 Lf units, but

but this is by no means definite. The 7 Lf toxoid gave almost as good results as the toxoid of 20 Lf. The experiments may of course be criticised from the point of view of the smallness of the samples. Despite the findings which are set out in the paper Asakava concludes rather paradoxically that all the information which his experiments furnished could have been supplied equally well by the flocculation result, that is, by Ramon's Lf value. Schmidt (1933) used toxoids of 16-17, 8.5, 4.5, and 0.8 Lf units respectively on guinea pigs; fifty animals were put up at each test. Schmidt concluded that qualitative differences between toxoids played an important part in determining their antigenicity, though in the absence of qualitative differences the Lf value gave a good idea of the potency of a particular toxoid. Povitzky (1932) also showed that in guinea pigs the degree of immunization increases with the strength of the toxoid, but here again the strongest toxoid used had an Lf value of only 11.6 units.

It will be obvious that this question has not yet been definitely settled. The matter is of some impprtance, since in the manufacture of an alum toxoid it is desirable that the single dose should contain sufficient Lf units to effect rapid immunization. A cursory review of the literature of the question will show that the investigations dealt with in most papers refer to toxoids of Lf value below 20 units - the exception being the work of Ramon and his colleagues who used toxoids up to 30 units in value. In the present investigation the weakest toxoid used had an Lf value of 20 units; most of the toxoids were of Lf 53 units, and the strongest toxoid had an Lf value of 65. These facts suggest that when toxoids are used in two or three doses, each of 1ccm. the antigenic value is of importance up to 20 to 30 Lf units, but that after this limiting value has been reached nothing is to be gained by using an even stronger toxoid.

Intervals between doses.

It is often stated that a toxoid given in three doses at intervals of two weeks between the doses is much more effective than the same toxoid given in three doses at weekly intervals. This investigation was designed to test this and similar statements Tables LXV and LXVI show the results obtained with two different toxoids, each of Lf 53 units per ccm., when used in two doses at intervals of four and three weeks respectively. The difference between the mean for four weeks and three weeks for B8337 is 1.2±5.2 per cent, and the corresponding differencefor B8553 is 6.1±3.0 per cent. Hence there was no significant difference between the results obtained with intervals of four weeks and of three weeks respectively with either batch of material used.

TABLE LXV.

B8337 (Lf 53 units): Two doses, each 1 ccm.

4 weeks interval.				3 weeks interval			
Group No.	Total injected	No.of negative	Per cent negative	Group No.	Total injected	No. Negative	Percent Negative
9	18	16	88.9±5.0	11	36	29	80.6±4.5
13	46	39	84.8±3.6				
15	40	30	75.0±4.6				
Mean= 81.8±2.6							

TABLE LXVI.

B8553 (Lf 53 units): Two doses, each 1 ccm.

4 weeks interval				3 weeks interval			
Group No.	Total injected	No.of Negative	Percent negative	Group No.	Total injected	No. negative	Percent Negative
20	54	43	79.6±3.7	16	25	20	80.0±5.4
21	52	46	88.5±3.0	17	27	23	85.2±4.6
Mean = 84.0±2.4				18	22	14	63.6±6.9
				19	67	51	76.1±3.5
				24	48	36	75.0±4.2
				25	37	32	86.5±3.8
				Mean = 77.9±1.9			

In examining the groups which had three doses, groups 1 to 6 inclusive were not available since these children had their post-Schick test at nine weeks. Table LXVII gives the results obtained with intervals of two weeks and one week respectively between each dose. It will be noted from the details given in the Appendix that groups 6 and 7 were treated with different though equivalent toxoids; groups 8, 10, 12 and 14 were all treated with the same toxoid (B8337). The difference between the two means in Table LXVII

TABLE LXVII.

Three doses, each 1 ccm.

Two weeks interval				One weeks interval			
Group No.	Total injected	No. negative	Percent negative	Group No.	Total injected	No. negative	Percent negative
6	144	121	84.0±2.1	8	26	24	92.3±3.5
7	25	24	96.0±2.6	10	53	41	77.4±3.9
	Mean = 85.8±1.8			12	46	37	80.4±4.0
				14	20	15	75.0±6.5
				Mean = 80.7±2.2			

is 5.1±2.9 per cent. Hence, with the particular toxoids used, it apparently did not matter whether the intervals between the doses were of one week or of two weeks. [6] Ide and Kato (1933) used Ramon's toxoid on 3579 children. The first series received toxoid at intervals of three weeks between the first and second doses, and two weeks between the second and third. After four months 73 per cent were negative. In the second series the three doses were given at weekly intervals, and after four months 74.6 per cent were negative. In that investigation alteration of the interval had therefore no effect upon the efficacy of the toxoid. Harrison (1932) inoculated 318 children with two 1 ccm. doses of toxoid with an interval of thirty days between the doses; 94.0±0.9 per cent were negative. Another series of 72 children received the same doses with an interval of 42 days between; 98.6±0.9 per cent were negative. There was apparently some evidence that the increased interval tended to produce a slight improvement in the immunizing effect; since the difference between these

results is 4.6±1.3, which is just significant.

Summing up this section we may conclude that, contrary to what is often stated, weekly intervals between doses where three doses are given, and intervals of three weeks between doses where two doses are given will give quite satisfactory results.

Effect of site of Injection.

This factor is seldom considered, and indeed seldom mentioned, in papers on this subject. Most workers inject the prophylactic subcutaneously in the arm; a few employ the leg. Claus Jensen recommends that the injection should be given subcutaneously in the scapular region, in order to avoid irritation and a possible reaction as a result of muscular movement (see Leach, Claus Jensen and Pöch, 1935). The whole question is of importance only so far as the individual's liability to develop reactions is concerned, and it will be considered from that angle in another communication. In this section it is however desirable to consider whether the site and mode of injection have any bearing upon the results obtained with any particular toxoid.

For ordinary work the writer has a slight preference for intramuscular injection in the lateral aspect of the thigh. If the needle is entered normal to the skin and with a rapid movement, there is practically no pain as a result of the injection; further, the large muscles retain the fluid easily and do not permit any transient swelling. The writer was also under the impression that the slight red area which is liable to follow a subscutaneous injection was much less marked if the intramuscular method was used. This feature will be discussed elsewhere. In the present series intramuscular injection in the thigh was employed for all groups up to No. 23 in the appendix. As a start was then about to be made with alum preparations and as it was thought desirable to inject these subcutaneously, an experiment was carried out to decide whether the results of the injections at the two sites would be comparable.

Groups 24 and 25 were formed by splitting a large group of 107 children. On preliminary Schick testing these

groups were found to have approximately the same degree of natural immunity. The children in both groups came from the same types of houses and the splitting of the original group was done without any selection of individuals. The children in both groups were injected on the same day on each of the two occasions on which inoculations were given. The final tests were also carried out on the same day. Group 24 received the inoculations in the lateral aspect of the thigh. Group 25 in the left deltoid region.

The result of Schick tests performed four weeks after the last inoculations were: Group 24 (thigh) - 74.0±4.2 per cent negative. Group 25(deltoid) - 86.5±3.8 per cent negative The difference between these two results is 11.5±5.7 per cent, which is not significant. Hence, so far as the degree of immunity which is effected in four weeks is concerned, the site and mode of injection appear to have no effect.

Effect of age upon Immunizability.

Comparatively few investigations have dealt with the effect of the age of the person inoculated on the facility with which he can be immunised. Indeed, despite the large number of reports on the use of prophylactics which have been published in recent years, very few show the data in a form which permits of any investigation of this question. Although definite statements on the effect of age upon immunizability are seldom made, the opinion seems to be fairly generally held that immunizability increases as the child grows older. Even if this theory is true, it does not necessarily follow, of course, that age in itself is the factor which is responsible. Length of exposure to organisms a factor which would naturally be associated with age would be much more likely to have an effect. For example, Dudley (1932) says that "the degree of Schick immunity of a group is ....an index of the 'immunizability" of the positive Schick reactors of that group". Since the Schick immunity is a function of age(after the first year) it follows that immunizability should also be

directly proportional to the age of the Schick positive reactor.

In considering this question it is obviously desirable to work on material which is more or less homogeneous - that is, the essential relationship between age and immunizability should, if it is present, not be obscured by such extraneous factors as gross alteration of technique or variations in the material used. In the present series there were available for investigation on these lines 16 groups, consisting of 617 children. These groups were divided into four subgroups as follows (Table LXVIII):

TABLE LXVIII

Sub-groups	Group Nos.	No, in Group	Material	Lf per ccm.	No. of doses	Interval between doses(wks)
(a)	8,10,12,14	145	B8337	53	3	1
(b)	9,11,13,15	140	B8337n	53	2	4*
(c)	16,17,18,19, 24,25	226	B8553	53	2	3
(d)	20,21	106	B8553	53	2	4

*One group - No. 11 - had an interval of 3 weeks between doses. It is included in this group in view of the conclusions which were reached in connection with Table LXV.

The age distribution of the individuals in these groups is given in Table LXIX. In the first place, these figures were arranged according to four broad age groups, and the percentages negative at these ages were calculated . The results are shown in Table LXX. This table also shows the probable errors of the two largest differences between percentages for each of the groups (a), (b), (c), (d). It will be seen from the table that there are apparently no significant differences between the percentages of Schick positives at different ages who became immunized. In none of the groups do the differences equal three times their probable errors, and only in group (b) do they exceed twice their probable errors.

TABLE LXIX.

Schick Group	State	Age (Years) 0-	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	Totals	Total - & +
(a)	Neg.	3	20	9	10	10	25	16	11	3	2	6	1	1	-	-	117	
	Pos.	-	7	5	2	2	4	3	2	-	1	-	1	-	1	-	28	145
(b)	Neg.	1	10	10	9	10	25	28	5	7	1	3	-	4	1	-	114	
	Pos.	-	1	1	5	5	7	4	2	-	1	-	-	-	-	-	26	140
(c)	Neg.	4	25	15	16	20	17	14	7	10	11	22	7	4	2	2	176	
	Pos.	-	6	5	7	5	6	5	4	1	6	3	1	-	1	-	50	226
(d)	Neg.	1	19	13	7	8	15	9	7	6	5	1	3	2	5	-	89	
	Pos.	-	2	1	3	2	1	5	-	-	-	1	1	1	1	-	17	106

TABLE LXX

AGE.	GROUP AND MATERIAL											
	(a) B8334			(b) B8334			(c) B8553			(d) B8553		
	TOTAL	NO. NEG.	PER CENT. NEGATIVE.	TOTAL	NO. NEG.	PER CENT. NEGATIVE	TOTAL	NO. NEG.	PER CENT. NEGATIVE.	TOTAL	NO. NEG.	PER CENT. NEGATIVE
UNDER 2	30	23	76.7±5.2	12	11	91.7±5.4	35	29	82.9±4.3	12	10	83.3±7.3
2 AND UNDER 5	38	29	76.3±4.7	40	29	72.5±4.8	68	51	75.0±3.5	34	28	82.4±4.4
5 AND UNDER 7	48	41	85.4±3.4	64	53	82.8±1.5	42	31	73.8±4.6	28	22	78.6±5.2
7 AND OVER	29	24	82.8±4.7	24	21	87.5±4.6	81	65	80.2±3.0	32	29	90.7±3.5
DIFFERENCES	(a) 85.4 − 76.3 = 9.1 ± 5.8 (b) 85.4 − 76.7 = 8.7 ± 6.2			(a) 91.7 − 72.5 = 19.2 ± 7.2 (b) 87.5 − 72.5 = 15.0 ± 6.7			(a) 82.9 − 73.8 = 9.1 ± 6.3 (b) 80.2 − 75.0 = 5.2 ± 4.6			(a) 90.7 − 78.6 = 12.1 ± 6.3 (b) 90.7 − 82.4 = 8.3 ± 5.6		

This evidence suggests that age does not play as important a part in determining immunizability as is commonly assumed. This method is, however, somewhat crude. The calculation of a correlation coefficient is impracticable, since although one of the variables is graduated, the other is what Pearson has termed "categoric" - that is, capable of being graduated only into two alternative groups. An elegant and little known method of estimating the correlation in such a distribution is by calculation of Pearsons "bi-serial r" (Pearson 1909). This coefficient not only makes allowance for the fact that one of the variables is continous, but it also determines whether the correlation is positive or negative. The values of bi-serial r for the four groups are given in Table LXXI. (The method of calculation of the value of bi-serial r is not easily given in a few words, and the reader is therefore referred to Pearson's papers).

TABLE LXXI

Correlation between age and immunizability. Value of bi-serial r

Group (a)	$r = .0805$
" (b)	$r = .1097$
" (c)	$r = .0572$
" (d)	$r = .0180$

From these results we conclude that there is a slight, though sensible, correlation between age and immunizability, and that in a group of Schick positive persons the proportion of positives after immunization decreases with age, in other words there is a sensible tendency for immunizability to be increased as a Schick positive person become older. (These results apply only up to 14 years).

Harrison (1930) gives, arranged according to age groups, the percentages of negative reactors after immunization in (a) 355 Schick-positive white children inoculated with toxin-antitoxin mixture; (b) 475 schick positive white children inoculated with toxoid; and (c) 386 Schick positive negro children inoculated with toxin-antitoxin mixture. Unfortunately Harrison's first age group

embraces all children under 6 yesr of age. From the table whibh he gives the present writer calculated the correlation between age and immunizability. The results are shown in Table LXXII.

TABLE LXXII

Age and immunizability. Values of bi-serial r calculated from Harrison's data.

Group (a):	white children	(T.A.T.)	r = +.0498
" (b):	" "	(toxoid)	r = -.1914
" (c):	negro "	(T.A.T.)	r = +.0661

The coefficients indicate that there was a slight though sensible correlation in all groups between age and immunizability; and that whereas in the toxoid group increasing age was associated with increase of immunizability, in the groups treated with toxin-antitoxin mixture increasing age was associated with a decrease of immunizability.

The results for toxoid therefore confirm the results of the present investigation. It seems curious that the correlation should be of opposite sign for both groups treated with toxin-antitoxin mixture, and if the association were of higher degree one would feel inclined to enquire whether the type of prophylactic would explain the discrepancy.

SUB-SECTION B :- ALUM TOXOIDS

This section deals with the use of alum preparations - toxoid with added alum and alum-precipitated toxoid - in two doses. Full particulars of the nine groups of children will be found in the Appendix.

Natural Immunity State

The natural immunity rate of these groups is given in Table LXXIII. The mean rate for Schick-positives - viz. 81.8±1.51 per cent - is slightly lower than the rates for the three dose and

TABLE LXXIII

Group No.	Total tested	Total positive	Per cent. positive
A1	17	9	52.9
A4	39	37	94.9
A6	42	34	81.0
A7	40	39	97.5
A8	18	13	72.2
A9	26	24	92.3
A10	27	17	62.9
A11	54	42	77.8
A17	33	27	81.8
	296	242	

Mean for all groups = 81.8 ± 1.51.

two dose groups in Table LVIII. The differences - 3.3±1.10 and 4.2±1.26 - are not definitely significant, and the original immunity of these alum groups was much the same as in the case of the toxoid groups. Individual groups showed somewhat wide variations because many of them were drawn from residential schools or homes for children; many of tem were older and had already become immune.

A toxoid with added alum was first used in two doses on a series of seven girls in a residential institution (Group A1). Four weeks after the second dose all were negative. Full details of the other groups which were treated with two doses of alum preparations are given in the Appendix. These do not include certain very small groups of children which were used for the preliminary testing of new supplies of alum toxoids, and in which the final testing was done at irregular intervals. Particulars of groups which were treated by means of s single injection are dealt with in another Section of this Thesis, and are therfore omitted from the Appendix.

Generally, the technique in inoculating children with alum toxoid was the same as in the case of formol toxoids. A Moloney test was performed on each child, and positive reactors

were excluded. The non-reactors were then inoculated in the left deltoid region. In all the groups the second dose was 1ccm.; the first dose varied in size up to 1 ccm., according to the nature of the investigation. It will be seen from the Appendix that the results, with the exception of those for Group A11, in which a special technique with a very small first dose was used, were excellent. The average percentage of children who were immune when tested from three to five weeks after the second injection was 84.5.

General Efficiency of Toxoid with added Alum in two doses

All except one of the groups of children in Section B of the Appendix were inoculated with batch B8725 - which was a toxoid of approximately 30 lf units per ccm. with added alum. (The single exception, group A17, was treated with a very potent alum-precipitated toxoid, B9017, which gave excellent results when used in a single dose (Underwood, 1935a, and Section (f) of this Thesis)). Hence this toxoid with added alum fulfilled the criterion already established that the material should be of Lf value of at least 20 units per ccm.; but it should be noted that the total Lf units which were injected were in many instances considerably less than the amount which was given in toxoid groups - even in those groups which received the smallest total Lf units of toxoid. Too much stress must not be laid on this factor, since the efficiency of an alum toxoid does not depend largely on the flocculation value of the toxoid from which it is made. It will be seen from the Appendix that the results for two doses of alum compared very favourably with those which were obtained with full doses of toxoid. For example, the mean percentage for groups A4, A7 and A9, which received two doses of alum toxoid, each of 1 ccm., is 84.9 $\pm$ 2.6. The mean of the two dose toxoid groups which are set out in Table LXIV is 78.9 $\pm$ 1.2. The difference between these percentages is 6.0 $\pm$ 2.9 - which is not definitely significant.

Effect of interval between doses and fractional initial dose

Particulars regarding the three groups which had two doses of B8725, each of 1 ccm., are given in Table LXXIV. The only variable factor was the interval between the doses. The differences are : between A4 and A7 - 1.6 ± 6.4; between A7 and A9 - 13.4 ± 5.9; between A4 and A9 - 11.8 ± 5.5.

TABLE LXXIV

2 doses of B8725, each 1 ccm.

Group No.	Total injected	Interval between doses (weeks)	No. negative	Per cent. negative
A4	35	4	29	82.9 ± 4.3
A7	32	3	26	81.3 ± 4.7
A9	19	2	18	94.7 ± 3.5

Mean = 84.9 ± 2.6

There is therfore no significant difference between the three percentages in Table LXXIV. The groups had almost identical herd immunity to start with, and all were given the same material inthe same doses. Hence this evidence suggests that these results are not dependent upon the interval between the two doses - that is , provided that the interval is not less than the minimum of two weeks which has been employed in this particular section of the investigation.

It will now be advisable to examine the effect of reducing the size of the first dose - a procedure which is sometimes desirable when alum preparations which are more or less untried are being used. Table LXXV gives particulars concerning those groups in which the first dose varied from 0.2 to 0.5 ccm. The difference between the means for Tables LXXIV and LXXV is 9.6 ± 4.0 per cent. Hence, with this alum preparation two doses, consisting of a small initial dose followed by a dose of 1 ccm., gave almost as good results as two doses, each of 1 ccm.

TABLE LXXV

2 doses - a fractional dose followed by 1 ccm.

Group No.	Total injected	Doses (ccm.)	Interval between doses (weeks)	No. negative	Per cent. negative
A1	7	.3: 1	4	7	100
A6	31	.5: 1	3	26	83.9 ± 4.5
A8	11	.3: 1	2	10	90.9 ± 5.9
A10	15	.5: 1	1	11	73.3 ± 7.7
A11	33	.2: 1	1	19	57.6 ± 5.8

Mean = 75.3 ± 3.0 per cent.

It was shown above that the interval between the doses does not appear to have much effect, provided that the mimimum interval is two weeks. But the mean 75.3 ± 3.0 per cent. is derived from a series two groups (A10, A11) of which had doses at intervals of one week. If we split Table LXXV we find that the mean for groups A1, A6 and A8 is 87.8 ± 3.2 per cent.; whereas the mean for groups A10 and A11 is 62.5 ± 4.7 per cent. The difference is therefore 25.3 ± 5.7 per cent. (It should be noted that the first post-Schick test which was performed on group A11 was nine weeks after the second dose. If the group had been tested earlier, the percentage would almost certainly have been lower than 57. This would have made the difference even more suggestive). The difference between the two groups is definitely significant, and it suggests that if the interval between the two doses is less tha two weeks, there will be a definite reduction in the efficiency of alum preparations when the first dose is a small one.

Groups A10 and A11 require further discussion. If a small dose is to be used as a primary stimulus to start with, there will obviously be a lower limit to this dose, so far as efficiency is concerned. Groups A6, A1, and A8 show that 0.5 ccm. and 0.3 ccm. are quite satisfactory as primary doses provided that the next dose is not given until after an interval of two weeks. When this interval is reduced, the question

becomes more difficult. Group A10 shows that with an interval of one week between doses, a first dose of 0.5 ccm. gives quite a satisfactory result (73 per cent. negative in three weeks). On the other hand with a first dose of 0.2 ccm. (Group A11) only 57 per cent. were negative after nine weeks. This is definitely not satisfactory, and the poor result is apparently due to the smallness of the first dose. When the results of the second terminal Schick test (see Appendix) on these two groups are examined , however, it is seen that, while the percentage negative for A10 had increased from 73 to 86 (or by about 13 per cent.) in 13 weeks, the percentage for A11 had increased by 21 per cent. (from 57 to 78) in only five weeks. This suggests that even a very small first dose of an alum preparation, when followed after one week by a dose of 1 ccm., will give satisfactory results within three or four weeks.

This section should be qualified by the following statements: (a) The results should be read in conjunction with the results for this particular batch of material (B8725) when used in a single dose (Underwood, 1934c). (b) This material (B8725) was the first alum preparation to be used, and it was not nearly as efficient as the later preparation (A.P.T.). The results are, however, discussed, since A.P.T. was used later mostly in single doses.

The "priming dose": its use and effectiveness

In Section (d) of this Thesis the writer discussed the uses of the Moloney test. The opinion was there expressed that the test is definitely of value in deciding which individuals are liable to develop unpleasant reactions on the injection of toxoids in prophylactic doses. The performance of this test, together with a primary Schick test, involves the individual in a number of visits to the clinic, and it would obviously be advantageous if some of these visits could be dispensed with.

The suggestion was made by Chesney (1934) that if a

small dose of the actual toxoid which is to be used prophylactically be injected subcutaneously when the Schick test is read, erythema at the injection site will indicate that the individual is toxoid-sensitive, and that a larger dose should not be given. If there is no reaction to this test injection - or, as Chesney calls it, "detector dose" - then the subject is not toxoid-sensitive, and full doses may be given after a few days. The writer tried this method independently before Chesney's paper appeared, but he was unable to satisfy himself that a small subcutaneous injection of toxoid would indeed give the desired information. An investigation was therefore carried out on a number of children, each of whom was tested for toxoid-sensitivity by the Moloney test. When this test was read a subcutaneous injection of a small dose - 0.2 or 0.3 ccm. - of toxoid or alum toxoid was given, and each child returned after 24 hours, and sometimes also after 48 hours, for examination of the injection site. Detailed notes were kept of the area of erythema or induration which resulted.

Discussion of the results is rather complicated by the fact that a considerable proportion of children show a slight area of redness at the site of injection of a small dose of toxoid, or especially of alum toxoid. The majority of these children show no unpleasant symptoms whatever on the injection of, say, 1 ccm. of toxoid or of alum toxoid. Furthermore many Moloney positive reactors showed no erythema at the site of the test injection. Some indication of the strength of the evidence may be obtained from the statement that, among the definite positive (++ or +++) Moloney reactors in the groups which were specially observed for this purpose, eight showed no reaction whatever at the site of the test injection of toxoid (0.2 or 0.3 ccm.), and eleven showed slight erythema. Even in themselves these figures do not suggest that the subcutaneous injection of toxoid may be used as a substitute for the intradermal injection of a weak dilution of toxoid, as used in the Moloney test. Further,

these figures take no account of the numerous Moloney negative reactors who showed some erythema at the site of the test injection of toxoid. The explanation of the discrepancy is possibly due to the different site of the injection of toxoid or its dilution in the two tests; toxoid injected into the deep subcutaneous tissues may not come sufficiently into contact with the epidermis to cause a typical reaction.

It will be evident from the discussion of the results in previous sections that 0.2 or 0.3 ccm. of toxoid is sufficient to act as a tolerably satisfactory primary stimulus. The suggestion now arose that, if this "priming dose" - as it may be called for convenience - could be injected at the time when the Schick and Moloney tests were first performed, the second injection could be given a week later when the Schick test was finally read. Such a procedure would obviously save at least one visit to the clinic. A difficulty was the possibility that the injection of a small dose of toxoid might interfere with the interpretation of the primary Schick test. To test this point the children who made up group 26 were Schick and Moloney tested. The tests were read 48 hours later, and finally on the seventh day. On this day 0.2 ccm. of toxoid (B8605) was given subcutaneously in the left deltoid region, and the Schick test was repeated. Six days later all the tests were read again. The results were available for 20 individuals. In each case the two Schick tests agreed surprisingly well - that is, when the seventh day reading of the first test was compared with the seventh day reading of the second. The second test was as frequently a little brighter as it was a little fainter when compared with the first test. In size the two reactions were practically always identical, and in no instance would the second Schick test not easily have been recognised as positive if the first test was positive. The conclusion was therefore drawn that a small "priming dose" of toxoid, if given at the time when the preliminary Schick test was performed, would not interfere

with the reading of that test.

In all, four groups of children were immunized by using this method of a "priming dose". These groups (Nos. 26, 27, All, and Al7) are the only groups in the Appendix which have not so far been discussed. It will be seen that the natural immunity rate in these four groups ranged from 74.1 to 83.0 per cent. Groups 26 and 27 were treated with formol toxoid, group All with toxoid to which alum had been added, and group Al7 with alum-precipitated toxoid. In the first three groups the "priming dose" was 0.2 ccm., and in group Al7 it was 0.3 ccm. In each case the interval between the two doses was one week - since the second dose was given on the day on which the primary Schick test was read. Table LXXVI gives the results obtained in each group.

TABLE LXXVI

Results obtained by using a "priming dose".

Group No.	Material used	Lf per ccm.	Interval between doses (weeks)	Total injected	No. negative	Per cent. negative
26	Toxoid: B8605	65	1	19	11	57.9±7.6
27	Toxoid: B8553	53	1	39	27	69.2±5.0
AII	Toxoid with added alum		1	33	19	57.6±5.8*
Al7	Alum-precipitated toxoid		1	24	20	83.3±5.1

* Result of further test 13½ weeks after second injection - 78.8 ± 4.8 per cent.

It will be seen that the result for group 26 is unsatisfactory. Toxoid B8605, which had the highest antigenic value of any material used in these investigations, was specially chosen for this reason to test the theory of the "priming dose" - although at the time when this was done sufficient time had not elapsed to obtain the results of final Schick tests after its use in more orthodox doses. From what has been written already

it will be evident that, despite its high Lf value B8605 was not nearly such a good antigen, when tested on the Leeds population, as were B8337 and B8553. The second group - No. 27 - was treated with B8553, and the results were very promising. The first alum batch which was used - i.e. B8725 - very surprisingly gave a poor result as judged by the first post-Schick test. It has already been pointed out, however, that in a further period of five weeks the percentage of negatives in AII had increased by 21 per cent., which, considering the low immunizability of Leeds children, is not unsatisfactory. The last batch to be used - B9017 - was definitely very satisfactory, 83.3 ± 5.1 per cent. of the individuals being negative within three weeks of the last injection. This percentage should be compared with that of 83.6 ± 2.0, which was the mean of the results for five groups of children inoculated with a single dose of this material (see Section (f) of this Thesis and Underwood, 1935a). In the case of the single dose groups the final test was performed one month after the injection, whereas in the case of the "priming dose" group the post-Schick test was performed three weeks after the second injection. It will be seen that with this material 80 to 85 per cent. of individuals should be negative within one month of the date of the first injection.

It is regrettable that the departure of the writer from Leeds prevented further experiments on the effectiveness of this "priming dose" on a population which presents so many peculiar features so far as its response to the C. diphtheriae and its products is concerned. There is, in the writer's opinion, quite wrongly, a certain amount of prejudice against the use of prophylactics in single doses, and the employment of this method adds only one extra visit to the number which is necessary for the single dose method, and in the short time

required all the information which can reasonably be considered as necessary may be obtained. With a prophylactic which is as effective as B9017 there is no reason why the method should not be developed to give very satisfactory results.

APPENDIX (a) — TOXOID GROUPS.

GROUP NO.	PRIMARY SCHICK			TOTAL NO. INJECTED AND FINAL TESTED (FIRST TEST) AFTER FULL COURSE	MATERIAL AND BATCH NO.	DOSES (AMOUNTS AND DATES)	NO. OF DOSES (TO FIRST FINAL TEST)	INTERVALS BETWEEN DOSES (DAYS)	TOTAL LF UNITS GIVEN.	FIRST FINAL SCHICK TEST.			
	TOTAL TESTED AND NO. +[-]	PER CENT. POSITIVE	DATE OF TEST							INTERVAL SINCE LAST DOSE (WKS)	DATE OF TEST	NO. NEG.	PER CENT. NEGATIVE.
1	25 [20]	80.0 ± 5.4	29 : 3 : 33	17	FT/P240B	(c.c.) 0.2 - 5 : 4 : 33 0.7 - 21 : 4 : 33 1.4 - 3 : 5 : 33	3	16 : 13	55	9	5 : 7 : 33	16	94.1 ± 3.9
2	23 [18]	78.3 ± 5.8	2 : 5 : 33	18	FT/P240B P244	0.7 - 8 : 5 : 33 1.1 - 17 : 5 : 33 1.2 - 29 : 5 : 33	3	9 : 12	72	7	18 : 7 : 33	18	100
3	85 [68]	80.0 ± 2.9	10 : 5 : 33	56	FT/P240B P244	3×1 c.c. 19 : 5 : 33 1 : 6 : 33 14 : 6 : 33	3	13 : 13	72	9	14 : 8 : 33	56	100
4	22 [21]	95.5 ± 3.0	22 : 5 : 33	20	FT/P244 P248C P815·2	3×1 c.c. 28 : 5 : 33 12 : 6 : 33 26 : 6 : 33	3	15 : 14	70	7	14 : 8 : 33	18	90.0 ± 4.5
5	29 [26]	89.7 ± 3.8	25 : 5 : 33	20	FT/P244 P248C	3×1 c.c. 31 : 5 : 33 13 : 6 : 33 28 : 6 : 33	3	14 : 15	76	7	15 : 8 : 33	19	95.0 ± 3.3
6	207 [174]	84.0 ± 1.7	19 : 6 : 33	144	FT/B815·2	3×1 c.c. 27 : 6 : 33 11 : 7 : 33 25 : 7 : 33	3	14 : 14	60	4	22 : 8 : 33	121	84.0 ± 2.1
7	31 [29]	93.6 ± 3.0	15 : 6 : 33	25	FT/P248C	3×1 c.c. 22 : 6 : 33 6 : 7 : 33 19 : 7 : 33	3	14 : 13	78	4	16 : 8 : 33	24	96.0 ± 2.6
8	33 [29]	87.9 ± 3.8	18 : 10 : 33	26	FT/B8337	3×1 c.c. 24 : 10 : 33 1 : 11 : 33 8 : 11 : 33	3	8 : 7	160	4	6 : 12 : 33	24	92.3 ± 3.5
9	34 [29]	85.3 ± 4.1	18 : 10 : 33	18	FT/B8337	2×1 c.c. 24 : 10 : 33 22 : 11 : 33	2	28	110	3	14 : 12 : 33	16	88.9 ± 5.0
10.	63 [60]	95.3 ± 1.8	22 : 11 : 33	53	FT/B8337	3×1 c.c. 29 : 11 : 33 6 : 12 : 33 13 : 12 : 33	3	7 : 7	160	4	10 : Ṫ : 34	41	77.4 ± 3.9
11	51 [43]	84.3 ± 3.4	22 : 11 : 33	36	FT/B8337	2×1 c.c. 29 : 11 : 33 20 : 12 : 33	2	21	110	4	17 : Ṫ : 34	29	80.6 ± 4.5
12	62 [54]	87.1 ± 2.9	28 : 11 : 33	46	FT/B8337	3×1 c.c. 4 : 12 : 33 12 : 12 : 33 19 : 12 : 33	3	8 : 7	160	4	16 : Ṫ : 34	37	80.4 ± 4.0
13	60 [49]	81.7 ± 3.4	28 : 11 : 33	46	FT/B8337	2×1 c.c. 12 : 12 : 33 10 : Ṫ : 34	2	29	110	4	7 : 2 : 34	39	84.8 ± 3.6
14	39 [28]	71.8 ± 4.9	27 : 11 : 33	20	FT/B8337	3×1 c.c. 2 : 12 : 33 9 : 12 : 33 16 : 12 : 33	3	7 : 7	160	4	12 : Ṫ : 34	15	75.0 ± 6.5
15	54 [48]	88.9 ± 2.9	27 : 11 : 33	40	FT/B8337	2×1 c.c. 2 : 12 : 33 30 : 12 : 33	2	28	110	4	27 : Ṫ : 34	30	75.0 ± 4.6

APPENDIX (a) - contd. —— TOXOID GROUPS.

GROUP No.	PRIMARY SCHICK			TOTAL No. INJECTED AND FINAL TESTED (FIRST TEST) AFTER FULL COURSE	MATERIAL AND BATCH No.	DOSES (AMOUNTS AND DATES).	No. OF DOSES (TO FIRST FINAL TEST)	INTERVALS BETWEEN DOSES (DAYS)	TOTAL LF UNITS GIVEN.	FIRST FINAL SCHICK TEST			
	TOTAL TESTED AND No. + [-]	PERCENT. POSITIVE	DATE OF TEST							INTERVAL SINCE LAST DOSE (WKS)	DATE OF TEST	No. NEG.	PER CENT NEGATIVE.
16	45 [34]	75.6 ± 4.3	13 : 12 : 33	25	FT/B8553	2×1 cc. { 21 : 12 : 33 13 : Ṫ : 34	2	23	110	4	13 : 2 : 34	20	80.0 ± 5.4
17	45 [36]	80.0 ± 4.0	14 : 12 : 33	27	FT/B8553	2×1 cc. { 21 : 12 : 33 13 : Ṫ : 34	2	23	110	4	13 : 2 : 34	23	85.2 ± 4.6
18	40 [35]	87.5 ± 3.5	14 : 12 : 33	22	FT/B8553	2×1 cc. { 21 : 12 : 33 13 : Ṫ : 34	2	23	110	4	13 : 2 : 34	14	63.6 ± 6.9
19	93 [87]	93.5 ± 1.7	18 : Ṫ : 34	67	FT/B8553	2×1 cc. { 25 : Ṫ : 34 16 : 2 : 34	2	22	110	4	16 : 3 : 34	51	76.1 ± 3.5
20	76 [66]	86.8 ± 2.6	18 : Ṫ : 34	54	FT/B8553	2×1 cc. { 26 : Ṫ : 34 22 : 2 : 34	2	27	110	4	23 : 3 : 34	43	79.6 ± 3.7
21	91 [82]	90.1 ± 2.1	13 : 2 : 34	52	FT/B8553	2×1 cc. { 21 : 2 : 34 23 : 3 : 34	2	30	110	4	19 : 4 : 34	46	88.5 ± 3.0
22	54 [46]	85.2 ± 3.3	17 : 3 : 34	31	FT/B8605	2×1 cc. { 27 : 3 : 34 18 : 4 : 34	2	22	130	4	16 : 5 : 34	18	58.1 ± 6.0
23	87 [73]	83.9 ± 2.7	4 : 4 : 34	64	TAF/P301	3×1 cc. { 10 : 4 : 34 24 : 4 : 34 8 : 5 : 34	3	14 : 14	—	4	6 : 6 : 34	40	62.5 ± 4.1
24	55 [50]	90.9 ± 2.6	5 : 4 : 34	48	FT/B8553 (in leg)	2×1 cc. { 12 : 4 : 34 3 : 5 : 34	2	21	110	4	31 : 5 : 34	36	75.0 ± 4.2
25	52 [45]	86.5 ± 3.2	5 : 4 : 34	37	FT/B8553 (in arm)	2×1 cc. { 12 : 4 : 34 3 : 5 : 34	2	21	110	4	31 : 5 : 34	32	86.5 ± 3.8
26	24 [20]	74.1 ± 5.7	5 : 5 : 34	19	FT/B8605	0.2 cc. - 11 : 5 : 34 1.1 cc. - 17 : 5 : 34	2	7	82	3	7 : 6 : 34	11	57.9 ± 7.6
27	53 [44]	83.0 ± 3.5	11 : 6 : 34	39	FT/B8553	0.2 cc. - 11 : 6 : 34 1.05 cc. - 16 : 6 : 34	2	6	64	8	16 : 8 : 34	27	69.2 ± 5.0

APPENDIX (b) - ALUM PREPARATIONS : MULTIPLE DOSES.

GROUP No.	PRIMARY SCHICK			TOTAL NO. INJECTED AND FINAL TESTED (FIRST TEST) AFTER FULL COURSE.	MATERIAL AND BATCH No.	DOSES (AMOUNTS AND DATES).
	TOTAL TESTED & No + [-]	PER CENT. POSITIVE	DATE OF TEST.			
A1	17 [9]	52.9 ± 8.2	5 : 3 : 34	7	ALFT/B8725	0.3 cc. – 12:3:34 1 cc. – 10:4:34
A4	39 [37]	94.9 ± 2.4	25 : 4 : 34	35	ALFT/B8725	1 cc. – 2:5:34 1 cc. – 28:5:34
A6	42 [34]	81.0 ± 4.1	5 : 5 : 34	31	ALFT/B8725	0.5 cc. – 11:5:34 1 cc. – 30:5:34
A7	40 [39]	97.5 ± 1.7	2 : 5 : 34	32	ALFT/B8725	1 cc. – 9:5:34 1 cc. – 29:5:34
A8	18 [13]	72.2 ± 9.0	9 : 5 : 34	11	ALFT/B8725	0.3 cc. – 15:5:34 1 cc. – 30:5:34
A9	26 [24]	92.3 ± 3.5	12 : 5 : 34	19	ALFT/B8725	1 cc. – 15:5:34 1 cc. – 1:6:34
A10	27 [17]	62.9 ± 6.3	16 : 5 : 34	15	ALFT/B8725	0.5 cc. – 25:5:34 1 cc. – 1:6:34
A11	54 [42]	77.8 ± 3.8	11 : 6 : 34	33	ALFT/B8725	0.2 cc. – 11:6:34 1 cc. – 16:6:34
A17	33 [27]	81.8 ± 4.5	25 : 8 : 34	24	APT/B9017	0.3 cc. – 25:8:34 1 cc. – 1:9:34

No. OF DOSES (TO FIRST FINAL TEST)	INTERVALS BETWEEN DOSES (DAYS)	FIRST FINAL SCHICK TEST			
		INTERVAL SINCE LAST DOSE (WKS.)	DATE OF TEST	No. NEG.	PER CENT. NEGATIVE.
2	29	4	4:5:34	7	100
2	26	3	16:6:34	29	82.9 ± 4.3
2	19	4	26:6:34	26	83.9 ± 4.5
2	20	4	26:6:34	26	81.3 ± 4.7
2	15	4	28:6:34	10	90.9 ± 5.9
2	14	5	9:7:34	18	94.7 ± 3.5
2	7	3	25:6:34	11	73.3 ± 7.7 (1)
2	6	9	16:8:34	19	57.6 ± 5.8 (2)
2	7	3	20:9:34	20	83.3 ± 5.1

(1) SECOND POST-SCHICK ON 20:9:34:- TOTAL NEG = 13 (86.7 ± 5.9)

(2) " " " " " " " = 26 (78.8 ± 4.8).

PART I.

SECTION (f).

IMMUNIZATION BY MEANS OF ALUM PREPARATIONS IN SINGLE DOSES.

Those who have had experience of the practical aspect of diphtheria immunization will realise that the history of this subject is a record of attempts on the part of administrators and laboratory workers to humour the patience of parents and their children. The experimental work which is detailed in previous sections will show that repeated visits on the part of the children at least were necessary if the desired information were to be obtained from the investigation. But quite apart from experimental work, the ordinary routine practice of immunization demands frequent visits to the clinic. There is no doubt that those who are prepared to make these visits will ultimately reap their reward in the form of a more or less assured immunity to diphtheria. But though the attendances at the Leeds clinics were excellent, this result was obtained to a large extent as a result of propaganda which was carried out by the writer. At a later date it was due more to fear - the greatest of all propagandists. Every one who has had at any time any connection with an immunization clinic must have felt there was a need for some method of reducing the number of attendances which are normally required. The introduction of a method of precipitating toxoid by alum suggested that there was a possibility that a prophylactic would be evolved which would immunize with one dose. No attempts were made in this country to supply the need - although Great Britain was for all practical purposes the country of origin of alum toxoid - but nevertheless the wish was in evidence. In 1929, however, Dudley (1929), who had used alum toxoids on small numbers of boys at the Greenwich Hospital School, wrote: "At present there is little hope of being able to produce an effective herd immunity to diphtheria with only one injection -

the ideal prophylaxis for which the public health administrator at present sighs in vain." Though no one can question Dudley's great contributions to the epidemiology of diphtheria, there is little doubt that this statement -as he himself admits (1934) - was largely responsible for the delay in the adoption of the one-shot method in in this country.

Following upon his experiments with alum toxoid when used in two doses, the writer determined to test the possibility of making practical use of single injections, and the earlier experiments which are discussed in this section represent the first occasion on which the one-shot method was practised in Great Britain.

History of the Precipitation of Toxoids.

It is generally recognised that the efficiency of a diphtheria prophylactic depends not only upon the antigenic value of the toxin employed, but also upon the rapidity with which the antigen is absorbed from the site of injection. One dose of a prophylactic which is rapidly absorbed and eliminated leads only to a small increase in antitoxic titre of the serum, and to produce a sufficiently high immunity repeated doses are necessary. Consequently, high value formol toxoids, which have been shown in the preceding section to be exceedingly effective when given in the usual multiple doses, are unsuitable for use as a single-dose prophylactic because the rate of absorption is too great to provide a continuous stimulus over a period of time. For example, Tron (1934) used a single dose of unconcentrated formol toxoid, equivalent to 50-100 Lf units given at one injection, on a series of 110 children. One month after the injection 49.09 per cent were Schick negative, and two months after the injection only 57.2 per cent were negative. These results are more or less in accord with those of White and Schlageter (1934) who found 49.2 per cent of children, inoculated with a single dose of toxoid, Schick negative in two months and 82 per cent in six months. Quite recently it has come to be realised that these intervals are much too long for any practical scheme of immunization.

It is no new observation that certain bacterial toxins can be precipitated from culture filtrates by the addition of various salts. As early as 1899 Roux and Yersin (1899) demonstrated that salts of calcium and aluminium when added to diphtheria broth form precipitates which retain smaller or larger amounts of the antigen. Glenny, Pope, Waddington and Wallace (1926) used alum for precipitating diphtheria toxoid, and noted the increased antigenic efficiency shown by the precipitate. Wallace (1927) carried out further experiments on the precipitation of toxoid by alum, and introduced the use of Rochelle salt as a solvent for the precipitate. Hosoya and Miyata (1928) used zinc chloride and uranyl acetate successively for precipitation and purifying tetanus toxin. Salts of zinc have also been used for the same purpose by Ohyama (1931), who used as well the hydroxides and phosphates of the alkaline earths. Abt (1928) added calcium chloride and potassium phosphate to diphtheria toxin to precipitate calcium phosphate which carried down the toxin with it; he reconstituted his precipitated toxin by solution in sodium citrate. Gross (1929) was able to remove the diphtheria toxin from culture filtrates by absorption on magnesium hydroxide suspension, and recovered the absorbed toxin by treatment with carbon dioxide or ammonium phosphate. Schmidt (1931) did important work on the preparation of high value toxoids and he used tapioca in addition to alum as a precipitant. Glenny and Barr (1931) showed that such precipitates possessed very high antigenic properties with the additional advantage that the precipitate was absorbed slowly from the tissues. They also showed that as the amount of alum is increased, the amount of toxoid and non-specific substances precipitated increases at first and then decreases, but that this decrease is far greater for non-specific than for specific matter, so that precipitates formed with large amounts of alum are relatively purer.

Precipitants which have been used are by no means confined to the substances mentioned. It has been shown that diphtheria toxoid can be precipitated from solution by salts of most metals which have insoluble hydroxides, by some phosphates, and by various gelatinous

precipitates. Llewellyn Smith (1932) carried out a very extensive laboratory investigation into the methods of precipitation of various toxoids with special reference to the antigenic efficiency of the resulting precipitates when used in experimental animals. A perusal of her paper will show that among others the following substances are able to effect satisfactory precipitation of toxoids: potassium alum, sodium aluminate, colloidal aluminium hydroxide, colloidal ferric hydroxide, magnesium hydroxide, cerous nitrate and sulphate, zinc sulphate, zirconium oxychloride, thorium acetate, uranyl acetate, calcium phosphate, and tungstic acid. Of all these substances alum was apparently the best, though aluminium cream, zirconyl chloride and calcium phosphate also give excellent results. It was found that toxoid precipitated by various precipitants and at various hydrion concentrations is ~~five~~ fifty to ~~fifty~~ times as pure as the original toxoid, and that the yields of specific toxoids are in most cases from 70 to 95 per cent. Toxoid precipitated first by acid and subsequently by a metallic salt apparently retains its original high purity and at the same time acquires an antigenic efficiency as high as that of crude toxoids precipitated by metallic salts. This method is valuable in that it secures an antigen which is as free as possible from non-specific nitrogenous material. The latter is almost certainly the cause of most of the reactions which are considered in detail in the sections of this Thesis which deal with reactions and with the Moloney Test.

The immunizing properties of Diphtheria Toxoid Precipitates.

Glenny, Pope, Waddington and Wallace (1926) found that an emulsion of the alum precipitate from diphtheria toxoid was a better immunizing agent than the original toxoid, and they suggested that this increased efficiency is due to the retention of the toxoid by the alum and to its delayed absorption and elimination in the body. About the same time Ramon (1926) found that the addition of tapioca to toxoid increased its antigenic response. Glenny, Buttle and Stevens (1931) suggested from their experimental work in animals that, when an antigen is injected into the tissues a condition of

continual stimulation is produced "by lessening quantities of antigen acting on tissues whose power of response is rapidly increasing." This enhanced antigenic response may be due to two factors. Firstly, it may be due to retention of the toxoid by the precipitate so that it is only slowly liberated into the system and acts as its own secondary stimulus (Glenny, Pope, Waddington and Wallace, 1926). Secondly, it may due to local irritation caused by the precipitate at the site of injection (Glenny & Waddington, 1928). It is probable that both these factors are in part responsible. Glenny, Buttle and Stevens (1931) were able to demonstrate toxoid at the injection site three days after injection when alum precipitates were used, whereas with controlled toxoid only a trace was left after this time. On the other hand the fact that such substances as turpentine and toluol also cause an increased response is evidence for the irritation hypothesis.

Alum preparations were early used in the hyper-immunization of horses for the production of diphtheria antitoxin. At the Wellcome Laboratories good results are apparently being obtained by this method, but in certain quarters in America the results do not seem to have been so favourable. As these experiences have a certain bearing on the use of alum in the human, one instance will be mentioned here. Leonard and Varley (1932) found in ~~the~~ three groups of horses that the antigenic effectiveness of immunization with alum treated toxoid and toxin was about three times as great as that of toxoid and toxin without the addition of alum. But a cumulative toxic effect was observed in the horses which received alum preparations so that two thirds of them gradually died with symptoms of alum poisoning. These authors are, however, careful to state that the extremely small amount of alum which would be required in human immunization would be unlikely to produce such toxic effects.

The use of Treated Toxoids in human Immunization.

Glenny (1930) appears to have been the first to suggest the use of alum preparations for the immunization of human subjects, but the method seems to have been first subjected to a practical

test by Park and Schroeder (1932) in 1930. From the latter paper it would appear that in the small series of cases which were thus treated alum toxoid gave definitely better results than toxoid or toxin-antitoxin-mixture though the results with the latter were incomparably better than those which were previously given by the writer for Leeds. In a short time these preparations came to be used in multiple doses on small groups of American children. Claus Jensen (1931) was probably the first to use such preparations in single doses. He inoculated 21 children, each child receiving 1 ccm. of formol toxoid concentrated by alum. The resulting preparation had an Lf value of 150 units per ccm. One month after the injection twenty of the children had become Schick negative. Graham, Murphree, and Gill (1933) used an alum-precipitated formol toxoid, which was prepared after the method of Glenny by Havens and Wells (1933). A single inoculation was given to 185 children who were all Schick positive. When tested two to six months later 171 (92.4 per cent) of the children had become Schick negative. Straus (1933) used toxoid made up to a special lanoline mixture with sterile hydrous wool fat. Straus injected 98 pre-school children and five nurses, and 102 of the 103 subjects were negative after two months. In the children there were practically no reactions, but the reactions in the adults were quite severe.

In 1932 Saunders (1932) used alum toxoids in three doses, on 436 children in Cork, Ireland. In a later communication he dealt with the reactions following their use (1933).

In this country alum toxoid was used up to 1934 only by Dudley (1929) in Greenwich Hospital schoolboys. In 1934 and early in 1935 there appeared the two short communications of the present writer upon which this section is based. The investigation can be divided into two parts. The first results deal with the use of a batch of toxoid with added alum (ALFT B8725). Although this material gave suggestive results, it should be realised that the addition of the alum did not alter the composition of the toxoid very materially, since there was no actual precipitation.

The second part of the investigation deals with the use of a very effective alum-precipitated toxoid (APT B9017). In the interval between the use of these two preparations, while the writer was preparing to leave Leeds he inoculated about 150 children with a single dose of another toxoid with added alum. The efficiency of this batch was of course quite unknown, and the short time available did not permit of delay until the results of final Schick tests on preliminary groups were known. On final testing it was found that the immunizing power of this particular material was extremely low, so that it was quite unsuitable for use in single doses. As a result this material was wasted so far as results of single doses are concerned; but the cases were available for the study of the features which are dealt with in other sections. Since the publication of these articles two further communications have appeared. Jensen has continued his use of the Willstätter preparation of alum hydroxide $Al(OH)_3$, which was prepared for him by Schmidt (1932). Jensen has apparently immunised about 6,000 Danish children by this method, but these results are so far unpublished. His most recent paper is a joint one describing the immunization of 553 children in a small Austrian town in which diphtheria had been particularly prevalent. (Leach, Jensen and Pöch, 1935). The results were gauged not by Schick testing but by antitoxin estimation, carried out by the well known method of Jensen (Jensen, 1933). The results of immunization appeared to have been excellent. From America there has just appeared a study of the one-shot method by Volk (1935), who was able to render about 65 per cent of 722 children negative in four weeks by means of alum precipitated toxoids.

The efficiency of toxoid with added alum.

The results of immunizing four small groups with ALFT (B8725) are set out in Table LXXVII. The first two groups were followed up in a school. It was found in each group that, though the percentages negative on testing one month after the single dose were low - 47 and 43 per cent respectively - these percentages increased fairly rapidly after a short interval.

TABLE LXXVII.

SINGLE DOSES OF ALFT

Group	No. injected and tested	First final Schick			2nd final Schick			3rd final Schick		
		*Interval	No. negative	Percent negative	*Interval	No. negative	Percent negative	*Interval	No negative	Percent negative
1A1	17	4	8	47.1±8.2	6	12	70.6±7.5	21	14	82.4±6.2
1A2	21	4	9	42.9±7.3	20	14	66.6±6.9	-	-	-
1A3	20	4	17	85.0±5.4	-	-	-	-	-	-
1A4	20	10	8	40.0±7.4	-	-	-	-	-	-

*Interval in weeks between dose of prophylactic and performance of final Schick test.

In group 1A1 a re-test was carried out two weeks after the first post-Schick and the percentage immune had increased from 47 to 71 - i.e., a 50 per cent increase. When this group was tested again after a lapse of three months the percentage of immunes had risen by only a further 12 per cent. The second group - 1A2 - on the other hand did not respond so well. At the first post-Schick test the percentage of immunes was similar to that of group 1A1, but after a lapse of four months it had increased by only 23 per cent to 67. The children who made up these two groups were very similar as regards age and social status, and there wasno apparent reason why the two groups should have responded in different ways.

The third group which was treated consisted entirely of youths aged 14 to 19 years, who were inmates of a large residential school. So far as the general result is concerned, it must be said that 85 per cent negative with a single dose of prophylactic was excellent. At first sight it might be thought that this result is contradictory to the findings for the association between ~~the~~ age and immunizability which were discussed in the last section of this Thesis. The contradiction is, however, only apparent, for it will be remembered that the previous discussion dealt only with children below the age of 15 years, and it seems probable on general principles that an investigation which deals with adults might give different results. Further, the twenty positive individuals in group 1A4 were the only positives in the 99 individuals who were given a primary Schick test - i.e., the immunity rate was 80 per cent. This figure is markedly different from the rates for all other groups of children, and it would appear that the excellent result obtained was associated with the high degree of herd immunity rather than with the increased age.

The fourth group of children who were treated with this material gave the poorest results of all - viz, 41 per cent negative after ten weeks. Again, there was no obvious reason why this result should have been so low, the ages and social conditions of the children were very similar to those of the first two groups treated.

It is easy to be critical after the event. These figures are presented here, not because of their inherent merits or defects, but because they form a milestone, to the writer at least, in the search for an efficient one-shot prophylactic. Indeed, at the time when the earlier results were obtained the writer was considerably encouraged, and he was then of opinion that a good alum- precipitated toxoid would give satisfactory results. After a short experimental period Dr. O'Brien kindly supplied him with the material which will be discussed in the remainder of this section.

Before finally taking leave of toxoid with added alum, it will be as well to discuss briefly its advantages and defects. This material certainly has in it the possibilities of alum-precipitated toxoid, but there is no comparison between the efficiency of the two materials In two doses toxoid with added alum does not appear to be any better than ordinary formol toxoid; but the writer is of opinion that it is definitely better when used in a single dose. The figures in TableLXXVII show that its action is slow but progressive. One advantage in using it in preference to ordinary toxoid would possibly be that those children who defaulted after the first injection would have a good chance of becoming immune, though in about 50 per cent of cases this would happen only after the lapse of a few months. When it is remembered that these products were supplied to the writer by the Laboratory of origin of Alum-precipitated toxoid, it may be wondered why this material was not used in the first instance. The answer may be given in a single word - reactions. The writer is strongly of opinion that in carrying out immunization work on the general population, it is desirable, even essential, to proceed with extreme caution. Other toxoids had never previously been used on the general population of this country, and this batch of toxoid with added alum certainly served, if it did nothing else, to give the writer valuable experience of the behaviour of such preparations.

The efficiency of Alum-precipitated Toxoid (A.P.T)

The particular material used was an alum-precipitated toxoid (B9017) of high antigenic value. This material had been treated according to a method worked out by G. C. Pope which removed a large proportion of inactive material, and was expected to produce much less reaction thanprevious preparations.

The material for the investigation consisted of 228 children, divided into five groups, which are here labelled 1A5 to 1A9. The ages of the children ranged from 1 year to 14 years, but the majority were between 1 and 5 years of age. Particulars of the Schick-state of these children are given in Table LXXVIII.

TABLE LXXVIII

Group	No.tested	Number positive	Percent Positive
1A5	47	35	74.5±4.3
1A6	53	37	69.8±4.3
1A7	36	33	91.7±3.1
1A8	48	35	72.9±4.3
1A9	44	39	88.6±3.5
Totals	228	179	78.5±1.8

Each child received preliminary Schick and Moloney tests, and the Schick positive, Moloney negative reactors were each given 1 cc. of A.P.T (Batch B9017) subcutaneously in the left deltoid region. Of the cases so treated it was practicable to perform a final Schick test on 152 four weeks after the injection. The results in this paper are therefore based on these 152 cases. Table LXXIX summarises these results.

TABLE LXXIX.

Group	No.final tested within 1 month after single dose	No.negative after 1 month	Per cent negative.
1A5	29	22	75.9±5.4
1A6	25	24	96.0±2.6
1A7	28	23	82.1±4.9
1A8	33	26	78.8±4.8
1A9	37	32	86.5±3.8
Totals	152	127	83.6±2.0

Since the probable errors not only for the collected results but also for the results of each individual group show that these figures are highly significant, it would seem that the investigation has yielded a very promising line of enquiry. It is true that the figures for individual groups show some variation, and the probable errors of certain differences suggest that these differences are probably significant. Hence too much stress should not be placed on the figures for individual groups. Apart from this, however, the results as they appear in Table LXXIX require further examination.

(a) In the first place, there is a possibility thatthe high percentage of negative reactors on retesting may have been due to the inclusion of an unusually large number of children at one particular age. A rough idea of the results as related to the age distribution is given in Table LXXX. (Note: In Tables LXXX and LXXXI only the 152 children included in column 2 of Table LXXIX are dealt with).

TABLE LXXX

Age Group	Under 1	-2	-5	-7	-10	10+
Total tested	11	27	57	18	20	19
Results of tests-						
(a) Positive	2	2	9	2	4	6
(b) Negative	9	25	48	16	16	13
Per cent. negative	82	93	84	89	80	68

These figures indicate in a broad way that up to the end of ten years there was no marked difference in the immunizing effect of the prophylactic. Children over ten years were apparently rather more difficult to immunize by this method. At all events the figure 83.6±2.0 must be taken as representative of the average percentage of children in any age-group who would, in the Leeds district, be made immune within one month of the administration of a single dose of a prophylactic.

(b) In the second place, the figures in Table LXXIX give no indication of the state of immunity of the 152 Schick

positive subjects before inoculation. It might be objected that possibly many of the children had a considerable degree of immunity to start with (i.e., the positive Schick reaction would be slight in degree), and that therefore a small stimulus would be sufficient to induce effective immunity and to change the slight positive into a negative Schick reaction. This point is dealt with in Table LXXXI which gives the initial Schick state and the effect of inoculating the 152 cases.

TABLE LXXXI

Primary Schick Type	Number and per cent	Final Schick (1 month after inoc.) No.positive	No.negative	Per cent neg.1 month after inoculation.
Slight	16 (10.5)	1	15	93.8
Moderate	97 (63.8)	15	82	84.5
Marked	39 (25.7)	9	30	76.9
Total	152	25	127	-

As would be expected there is a difference in the ease with which the three groups can be immunized, but the difficulty is obviously progressive, and the difference in the percentages for "slight" and for "Marked" groups respectively is not as great as might have been anticipated. The outstanding points which emerge from this table are that the average percentage of children Schick negative after one month (viz. 83.6±2.0) is a representative figure for "Moderate" and for "Marked" primary Schick reactors; and further, that almost eighty per cent of "marked" Schick reactors can thus be rapidly immunized by this method.

It should be mentioned that the writer followed his usual practice of reading as "positive" all Schick reactions in which there was the slightest suspicion of erythema. As a consequence of this procedure one case is included as "positive" in the above figures for the final Schick tests, although the result of blood titration showed that the patient was immune at the time when the Schick test was carried out.

It should be remembered in assessing any results of

~~Single~~

single injections that a Schick test performed six weeks after the injection is equivalent, so far as the total time is concerned, to a similar test performed four weeks after the second injection where there is an interval of two weeks between the injections. Consequently these A.P.T. groups are equivalent to toxoid groups tested two weeks after the second dose (interval of two weeks between doses). Regarded in this light these A.P.T. results are most satisfactory.

It should be mentioned that only one communication dealing with one-shot immunization in this country has appeared since the publication of the writer's data. The paper referred to(Murphy,1935) deals with the immunization of school and pre-school children, but only a little over one third of the inoculated children were re-tested, and this operation was not carried out until some months after the inoculation. The material used emanated from a laboratory other than that which supplied the writer with his material.

The future of the single-dose method is still to be decided, and it is yet too early to be dogmatic. There would seem to be grounds for believing, however, that, given patience and care on the part of the field worker very satisfactory results will ultimately be achieved, - with a consequent saving not only of expense but also of the time of parents and children.

PART I.

SECTION (g)

A STUDY OF THE REACTIONS FOLLOWING FORMOL TOXOID AND ALUM TOXOIDS.

In several published papers and in various parts of this Thesis the writer has referred to the reactions following formol and alum toxoids, but these references have been very brief. It is well known that the fear lest such reactions would develop delayed the use of formol toxoid in this country, and that this fear is even yet militating against the use of alum toxoids. So far no detailed study of the writer's cases has been presented with reference to subsequent reactions, and this exposition will now be attempted here. It should be mentioned that the writer kept very full notes of all reported reactions, and in most cases he examined the reactors as early as possible. Further, it will also be shortly apparent that, of the 1968 individuals who received prophylactics, over 36 per cent were examined personally by the writer on the day following the first injection, so that very detailed notes regarding the incidence of reactions after the first injection are available.

From a perusal of the section which deals with the Moloney test it will be obvious that reactions after the powerful prophylactics which were used in these investigations are of various degrees of severity. Many reactions are so mild as to be quite unnoticed by the individual, while in other instances, especially in positive Moloney reactions, severe local and general reactions may occur. It should be pointed out that all degrees of reaction indicate some tendency on the part of the individual to react abnormally to the stimulus of a prophylactic. Hence, although for practical purposes severe reactions are more important owing to

their dramatic effects, from the point of view of the research worker the mild reaction is equally interesting.

Definitions. For the purpose of this section reactions are divided into two types, (a) local reactions, and (b) general reactions. Local reactions are defined as any reaction at all at the injection site which was more than a mere erythema; such reactions included swelling of a general nature, tenderness, and "fulness". Stiffness of the arm, unless it was accompanied by other features, was not included as a local reaction, since such a condition is quite often traumatic, and is not due to the material injected. General reactions include any systemic disturbance which is sufficiently severe to interfere with the normal course of the individual's daily routine or habits. General reactions include vomiting, marked nausea, marked headache, or definite distaste for food.

In some of the tables which follow it will be seen that certain local and general features are tabulated separately, and that the resulting totals do not always agree with the total number of local and general reactors. The explanation of this discrepancy is that isolated symptoms do not necessarily constitute a reaction. These individual features were noted at the time when the examination was made, but a definite note was also made as to whether the individual was or was not suffering from a local or general reaction.

No attempt will be made here to discuss fully the literature of reactions following toxoid or alum toxoid. The former is already considerable, and the latter is increasing gradually. It will be observed that the definition of what constitutes a reaction must vary according to the judgment of the observer, and the whole question is further complicated when it is remembered that reports on diphtheria immunization work very often emanate from groups of observers, the individuals of which may consciously or unconsciously have different views regarding such a definition. A perusal of a few of Ramon's papers will show that

in general he treats reactions very casually, and it is the practice in France to immunize tuberculous children with anatoxine. Certain of the smaller groups of children which are discussed in this Thesis were inmates of a children's sanatorium, and even though extreme care was used and the Moloney test employed, the writer found that local reactions were by no means infrequent, and that a slight degree of pyrexia was not uncommon. Despite Ramon's assurances to the contrary Aubertin and Boudou (1932) assert that a flare up of tuberculous foci after immunization with anatoxine has occurred. Within recent years attempts have been made, especially in America, to present reliable information on the incidence of reactors.

Dudley (1932) was especially favourably situated for the observation of reactions in the 408 boys upon whom he reported. He found that weak toxoid produced 10±1.8 per cent of local reactions, over one-third of which were accompanied by mild constitutional disturbances. Stronger toxoid caused 15±2.5 per cent of reactions. A very interesting feature about Dudley's work is that he used Ramon's anatoxine on 51 subjects and he found that 45±4.7 per cent were followed by a local reaction of more than 3 ccm. in diameter, and that on an average these reactions were more intense than those following the use of English toxoid. To the best of the present writer's knowledge Dudley is the only British observer who has reported upon the use of French toxoid, and his high proportion of reactors offers an interesting commentary upon a rather neglected aspect of Ramon's work. In fairness to Ramon it should be said that he does not employ any toxoid reaction test, and that he recommends the use of anatoxine in the earlier years of childhood. Dudley's subjects were of course just at the age when reactions would be most likely to develop.

Saunders (1933) made a very full study of reactions following alum toxoid and he found that four abscesses occurred in a series of 579 cases. McGinnes, Stebbins and Hart (1934) and Monroe and Volk (1934) made studies of the reactions following

toxoid with and without alum. These results will be referred to later. Tomcsik (1932) compared Pasteur Institute anatoxine, Budapest toxoid, and samples of English toxoid and concluded that such materials prepared in different ways were very similar in their tendency to give rise to undesirable reactions. It is very frequently stated that toxoid may be safely given to very young children, and in this connection it is of interest to note that Greengard (1931) inoculated 145 children aged four days to two years, sixty per cent of whom were under eight months of age. In this series he had two reactions of a constitutional nature. In the present writer's series the significant reactors were practically always in older children. Pöch and Leach (1934) inoculated nearly 5000 children, aged two to eight years, with toxoid, and they found that 4.8 per cent gave local and general reactions.

Incidence of reactions after injection of Toxoid and Alum Toxoid.

In the present series of 1941 cases who received FT or alum preparations 36 showed significant reactions, which gives a percentage of 1.9±0.21. In 100 individuals who had T.A.F. there were six significant reactors, which gives an incidence of 6.0±1.60 per cent. It should be mentioned that this is not a fair statement of the position with regard to T.A.F., but it shows how easy it is to misinterpret figures which are published regarding the incidence of reactions. Apart from a special group of children T.A.F. was only used in Moloney positive cases, who would almost certainly have given more severe reactions with toxoid. A more accurate comparison is obtained from this special group of 73 Schick positive children who were all inoculated with T.A.F., and who were observed by the writer on the day following the injections. In these there was one significant reactor - an M +++ case - so that the true incidence of reactions in unselected individuals treated with T.A.F. is 1.4±0.93 per cent. Again the comparison is not at all accurate, since the percentage of 1.9 for toxoid and alum excludes all the definite Moloney positive reactors who would have been most likely to give unpleasant symptoms on inoculation with toxoid or alum toxoid.

Age and Sex Differences in the Incidence of Reactors.

For the purposes of this paper the whole of the individuals who received toxoids or alum toxoids may be divided into two main categories, viz. unobserved and observed groups. In the unobserved groups, which consist of 1173 individuals, the children or their parents were asked about the appearance of reactions, and when such were reported the subjects were usually examined. The observed groups consist of 768 individuals, each of whom was examined by the writer on the day following at least the first injection, so that all types of reactions in this large series are accurately known.

(a) Unobserved Groups. Particulars regarding the unobserved groups are given in Tables LXXXII and LXXXIII. For all ages and both sexes (1078 individuals), 14 insignificant reactions (1.3±0.23 per cent) and six significant reactions (0.6±0.16 per cent) were reported after immunization with toxoid. When these figures are divided for sexes, it is found that the incidence of insignificant reactions in males and females respectively was 1.5±0.36 per cent and 1.1±0.30 per cent. The difference is 0.4±0.47 per cent. The corresponding figures for significant reactions are: males, 0.4±0.19 per cent; females 0.7±0.24 per cent. The difference is 0.3±0.31 per cent. There is therefore no sex difference in the incidence of such reactions. When the corresponding alum groups are considered, it is found that four out of ninety five individuals gave significant reactions. This gives a percentage of 4.2±1.39.

The difference in the incidence of significant reactions after toxoid and alum respectively (for all ages and sexes) is 3.6±1.40 per cent. There is a possibility that some significance must be attached to this figure, and hence there does ~~not~~ appear to be a tendency, so far as the evidence of these unobserved groups is concerned, for alum preparations to produce a slightly higher proportion of significant reactors than ~~does~~ ordinary toxoid

(b) Observed Groups. The particulars regarding the observed groups are given in Tables LXXXIV and LXXXV. Considering first the individuals who received formol toxoid it will be seen that the

TABLE LXXXII

Reactions in Unobserved Groups injected with FT

Age-group	Total receiving FT	No. of reactors			Per cent. reactors		
		No reaction	Insig.	Sig.	No reaction	Insig.	Sig.
(a) Males:-							
0-3	147	145	1	1	98.6±0.65	0.7±0.46	0.7±0.46
4-7	310	307	2	1	99.1±0.36	0.6±0.30	0.3±0.66
8-11	58	55	3	-	94.8±1.97	5.2±1.97	---
12 & +	12	10	2	-	83.3±7.27	16.7±7.27	---
All ages	527	517	8	2	98.1±0.40	1.5±0.36	0.4±0.19
(b) Females:-							
0-3	149	147	2	-	98.7±0.63	1.3±0.63	---
4-7	325	317	4	4	97.6±0.57	1.2±0.41	1.2±0.41
8-11	65	65	-	-	100	---	---
12 & +	12	12	-	-	100	---	---
All ages	551	541	6	4	98.2±0.38	1.1±0.30	0.7±0.24
(c) Both sexes, all ages:-							
	1078	1058	14	6	98.1±0.28	1.3±0.23	0.6±0.16

TABLE LXXXIII

Reactions in Unobserved Alum Groups

All ages	Total receiving alum toxoid	No. of reactors			Per cent. reactors		
		No reaction	Insig.	Sig.	No reaction	Insig.	Sig.
Males	48	45	1	2	93.4±2.42	2.2±1.43	4.4±2.0
Females	47	45	-	2	95.6±2.02	---	4.4±2.02
Both sexes	95	90	1	4	94.7±1.55	1.1±0.72	4.2±1.39

TABLE LXXXIV

OBSERVED GROUPS OF FT.

	Age Group	Total receiving FT	General reaction	Observed local erythema -10.	10-20.	20+	Induration	Total designated reactors
(a) Frequencies.								
Both sexes	0-3	40	-	6	-	1	1	-
	4-7	67	-	8	7	7	1	9
	8-11	42	1	6	3	2	2	6
	12 and over	17	-	2	1	5	5	3
	Total	166	1	22	11	15	9	18
All ages by sexes.	Males	77	-	11	3	7	6	9
	Females	89	1	11	8	8	3	9
(b) Percentages								
Both sexes	0-3	40	-	15.0	-	2.5±1.67	2.5±1.67	-
	4-7	67	-	11.9	10.4	10.4±2.58	1.5±1.00	13.4±2.81
	8-11	42	2.4	14.3	7.2	4.8±2.22	4.8±2.22	14.3±3.64
	12 and over	17	-	11.8	5.9	29.4±7.46	29.4±7.46	17.7±6.24
	Total	166	0.6	13.3	6.6	9.0±1.50	5.4±1.18	10.8±1.63
All ages by sexes	Males	77	-	14.3	3.9	9.1±3.21	7.8±3.06	11.7±2.47
	Females	89	1.1	12.4	9.0	9.0±3.05	3.4±1.30	10.1±2.16

TABLE LXXXV

OBSERVED GROUPS OF ALUM: PERCENTAGES

	Age Group	Total receiving Alum	Reactions: Local only	Reactions: General only	Reactions: Local and General	General: Malaise	General: Nausea	General: Vomiting	Erythema over 20 mm.	Induration	Total designated reactors
(a) **Males**											
(i) Frequencies	0-3	96	-	-	-	-	-	-	5	2	-
	4-7	75	3	1	-	1	-	1	3	2	4
	8-11	82	7	1	6	5	2	2	13	8	14
	12 and over	71	1	5	9	14	4	2	9	8	15
		324	11	7	15	20	6	5	30	20	33
(ii) Percentages	0-3		-	-	-	-	-	-	5.2±.153	2.1±0.98	
	4-7		4.0	1.3	-	1.3	-	1.3	4.0±1.53	2.7±1.26	5.3±1.75
	8-11		8.5	1.2	7.3	6.1	2.4	2.4	15.9±2.72	9.8±2.25	17.1±2.80
	12 and over		1.4	7.0	12.7	19.7	5.6	2.8	12.7±3.95	11.3±2.53	21.1±3.27
			3.4	2.2	4.6	6.2	1.9	1.5	9.3±1.09	6.2±0.90	10.2±1.13
(b) **Females**											
(i) Frequencies	0-3	96	6	-	1	1	-	-	11	5	7
	4-7	84	2	2	1	1	2	2	2	3	5
	8-11	65	4	1	1	2	1	2	6	3	6
	12 and over	34	6	-	1	1	-	-	7	6	7
		278	18	5	4	5	3	4	26	17	25
(ii) Percentages	0-3		6.3	-	1.1	1.1	-	-	11.6±2.22	5.3±1.55	7.4±1.81
	4-7		2.4	2.4	1.2	1.2	2.4	2.4	2.4±1.13	3.6±1.37	6.0±1175
	8-11		6.2	1.5	1.5	3.1	1.5	3.1	9.2±2.42	4.6±1.75	9.2±2.42
	12 and over		17.6	-	2.9	2.9	-	-	20.6±4.68	17.6±4.40	20.6±4.40
			6.5	1.1	1.4	1.8	1.1	1.4	9.4±1.18	6.1±0.97	9.0±1.16
(c) **Both sexes (all ages)**											
(i) Frequencies		602	29	10	19	25	9	9	56	37	58
(ii) Percentages			4.8	1.7	3.2	4.2	1.5	1.5	9.3±0.80	6.1±0.66	9.6±0.81

incidence of designated reactors in the 166 observed individuals was 10.8±1.63 per cent. This figure is of course very much higher than the incidence of significant reactors in the unobserved groups. The term "designated reaction" includes any condition, whether noticed by the subject or not, which might reasonably have been considered to indicate on his part a tendency to develop unpleasant reactions. It therefore includes erythema of an area over 20 to 25 mm. in diameter, and every type of constitutional disturbance, including slight restlessness, and even mild degrees of malaise. The incidence of such designated reactions in males and females was 11.7 and 10.1 respectively; the difference - 1.6±3.28 - is very small and is not significant. Considering the incidence of areas of erythema of over 20 mm. in diameter, we see that there is considerable fluctuation between the ages of 0 and 11 years, but that over the age of 11 years there is a very marked increase in the frequency of this condition. Induration showed the same features, since over the age of 11 years the incidence was 29.4 per cent. All these features are shown to be remarkably similar in frequency in the two sexes. The only outstanding difference is that for induration, betweenmmales and females, but the difference, viz, 4.4±2.44 per cent - is not significant. From Table LXXXIV it will also be seen that the incidence of definite general reactions was very low - viz 0.6 per cent.

Turning now to the large group of 602 individuals who make up the observed groups treated with alum preparations (TableLXXXV), it will be seen that for both sexes the incidence of designated reactors was 9.6±0.81 per cent. The difference between this and the corresponding figure for formol toxoid is 1.2±1.82 per cent. There is therefore no difference between the incidence of designated reactions following toxoid and alum toxoid respectively In the alum groups the incidence of these reactions for males and females respectively was 10.2 and 9.0 per cent, so that again the sex incidence is identical. Reference to Table LXXXV will show that the frequencies of erythema over 20 mm. in diameter and of induration were also identical in the two sexes, and a comparison

of the figures given with the corresponding figures for toxoid in Table LXXXIV will show that the incidences were again identical for this degree of erythema and for induration.

Considering now the variations in the frequency of those conditions at the various ages, it is seen that there is a gradual increase in the frequency of reactors as age advances, and that the earlier age groups do not show any very marked differences. There is, however, a rather outstanding sex difference in these conditions. It will be seen that so far as males are concerned the main increase in frequency of reactions occurs between the 4 - 7 age group and the 8- 11 age group. In females, on the other hand, the marked increase is not found until a later year - viz - between the 8 - 11 age group and the group of 12 years and over.

When we are considering the incidence of general reactions and the various symptoms associated therewith we are on less definite ground, since the frequencies given depend upon the statements and sensations of many individuals, and not upon the judgment of a single observer. It will be seen, however, that the frequency of general reactions in these alum groups was 1.7 per cent for both sexes, a value which is not markedly different from that found for the observed groups of FT. In the alum groups the frequency of local and general reactions was naturally somewhat higher - viz. 3.2 per cent. The infrequency of the observations makes it difficult to consider individual symptoms in detail, but it will be noted that in both sexes malaise and headache were about twice as common as nausea or vomiting. The percentage of 19.7 for malaise in males of twelve years and over is considerably higher than any of the other frequencies found, and is probably partly accounted for by the fact that it includes a number of youths ages from 15 to 19 years who, as has been mentioned in other sections of this Thesis, were rather liable - as was also shown by Moloney tests - to have slight degrees of indisposition after the injection of alum toxoid.

It will be noted that the inclusion of these large numbers of observed individuals has given results which are much more precise than the vague and indefinite statements which are usually made

regarding the incidence of reactions. There is essentially no sex difference, and the statement of Ramon, Timbal and Nélis (1933) that reactions are more frequent in girls than in boys is therefore not confirmed for Leeds. A further interesting observation arising out of these results is found when we compare them with frequencies of reactors in certain American investigations. Monroe and Volk (1934), who relied for their information solely on written reports from parents, found that about 5 per cent of children gave moderate local reactions after inoculation with several types of ordinary or alum toxoids. In the present series the incidence is somewhat higher. On the other hand they found that with the toxoid usually employed (except concentrated toxoid) up to eight per cent of children showed a moderate or severe general reaction - a figure which is several times that found for the present series of observed toxoid and alum cases. Again, McGinnes, Stebbins and Hart (1934) give particulars of the reactions found in 353 individuals who received alum-precipitated toxoid. From tables which they give it would appear that about 22 per cent had a local reaction only, about 17 a general reaction only, and about 30per cent both local and systemic reactions of all types. It is of interest to note that there is no mention that a preliminary toxoid reaction test was employed. These figures are so much higher than the corresponding figures in the present invesitgation that it is difficult to avoid the conclusion that the alum-precipitated toxoid which was used by these writers was much more liable to give reactions than the materials employed in the present series.

Abscess formation. In the series of alum treated cases which are reported in this Thesis there was no example of abscess formation. This complication is certainly the most serious which may occur after the injection of alum preparations.

It should be mentioned that the injected site after a dose of an alum toxoid is peculiar in that the dose can be felt in the subcutaneous tissues for some time. After a day or so the feeling is as if a small pea had become embedded in the tissues.

The writer found that this small nodule was present in at least two thirds of his cases. Such a phenomenon is to be expected, and it should not be classified as a reaction. The nodule is quite painless and unless a slight reaction develops, the overlying skin shows no erythema.

In concluding this section it should be mentioned that the close similarity between the frequencies of different types of reaction in different groups is an index of the consistency of the standard of reading which was adopted. These standards are subject to the influence of so many external factors that the writer fully expected that, when results came to be examined, the discrepancies would be considerable.

It is also necessary to point out finally that a Moloney test was performed on each individual. Hence, the figures which are given in this section certainly do not give a true indication of the number of reactions which would occur if a large group of unselected Schick-positive individuals were injected with toxoid or its alum derivatives.

PART I.

SECTION (h)

SCHICK IMMUNITY AND DIPHTHERIA INFECTION.

The preceding sections of this Thesis have dealt with the measures which the writer adopted in dealing with many of the difficulties which were caused through the high degree of infection of the community with the gravis strain of C.diphtheriae. We shall now consider another equally important property of this strain - a property which, until the publication of the investigation which forms the subject of this section, was practically unsuspected.

The increase in the practice of preventive inoculation against diphtheria in recent years, and the occasional occurrence of cases of diphtheria in persons who have been "immunized" has led to a scientific interest in the relationship between the Schick level of immunity and the level which is necessary to prevent the onset of this disease. Although such cases are reported from time to time, comparatively few papers of any importance have been published, since in most cases a negative response to the Schick test has not been obtained before the onset of the disease.

In 1929 O'Brien stated that in rare instances cases of "mild diphtheria" had been seen in patients who were known to be negative to the Schick test, and that these cases were characterized by the formation of only a small amount of membrane, a slight rise of temperature and an absence of complications. O'Brien, Okell ,and Parish (1929) stated that in an immunized Schick-negative population of 20,000 persons they had been able to trace the occurrence of 18 definite cases of diphtheria in a period of six years. They divided these cases into two groups: (a) relapsed Schicks, (ei), persons who were Schick-positive, but actively

immune, and able to respond rapidly to the secondary stimulus of the disease; (b) patients who gave a negative response to the Schick test within 48 hours of the onset of clinical diphtheria. These authors state that in 17 out of the 18 cases the disease was very mild, and the mildness was ascribed to the capacity of those who had once been Schick negative to respond rapidly by the production of circulating antitoxin. Neale (1928) reported the occurrence of diphtheria in two nurses, each of whom had been immunised and in whom subsequent negative Schick tests had been obtained. Neale also refers to the mildness of the attacks; but in one case there was definite paralysis of the ocular muscles and the palate, and there was apparently also some diaphragmatic weakness. Saunders (1933) recently reviewed the cases of diphtheria which had occurred in "immunized" persons in Cork during a period of approximately four years. Of a total of 78 such cases it was shown that the majority could be explained away, either on the grounds of incorrect diagnosis, the non-completion of the course of inoculations, or the shortness of the latent period between the completion of treatment and the onset of the disease; there remained, however, two cases which had been inoculated and in each of which a negative Schick test had been obtained, and in which diphtheria developed. In both of these cases the disease was moderately severe, but the response to treatment was rapid. It is noteworthy that in only seven of Saunders's inoculated cases was a negative final Schick test obtained. Nash (1933) stated that no case of diphtheria had occurred among 2019 children who had become Schick negative after inoculation.

It is of interest to compare these findings with those obtained in other countries.

Park (1926) in America stated that he had never seen an undoubted clinical case of diphtheria in a child who had recently had a negative Schick reaction after a test performed by an expert. The number of Schick negative persons upon which this statement is based is not stated, but the very extensive

experience of this author is well known. Bauer (1931) discussed the figures for Philadelphia, and he showed that, out of 96,252 children in whom negative final Schick tests had been obtained six months or more after inoculation, only 21 cases of clinical diphtheria occurred, Bauer is apparently inclined to think that these cases were really Schick-positive; he says that "this extremely small number of cases among immunes would represent the human element of error in the interpretation of Schick reactions." In Munich Pfaundler (1931) reported the occurrence of diphtheria in only 18 out of 2485 children who had been inoculated. Tomcsik (1932) stated that the number of children who have been inoculated in Hungary is 250,000. During 1930 the morbidity from diphtheria among the inoculated was only one-tenth of that among the non-inoculated children, and the majority of cases of diphtheria which occurred among the inoculated children were mild. Meersseman, Friess, and Renard (1933) mentioned that they noted that diphtheria occurred in a proportion of 1 per cent in several thousands Schick-negative subjects.

It is obvious from these statements that the occurrence of such cases of diphtheria in inoculated persons, though by no means rare, is not common. Most of these papers, however, have little bearing on the point at issue, since there was no definite statement as to whether the inoculated persons had been shown to be "immune" by means of the Schick test. An important investigation of the type which we are seeking is that of Dudley (1934), who reports 23 cases which were notified as diphtheria at Greenwich Hospital School, and each of which had either previously been naturally Schick negative, or had been inoculated. From a study of Dudley's particulars it is evident that only 1 of the 18 cases described in detail was an example of clinical diphtheria in a person who had at one time had a negative Schick reaction; and in this case the reaction was weak positive two days after the onset of the disease. It is also evident that in most of Dudley's 23 cases the diphtheritic nature of the illness was not obvious. This fact is worth considering along with his statement that "all

practical experience shows that cases with negative Schick tests do not give recognisable diphtheria"

Investigation of cases in Leeds.

The present investigation was carried out in an attempt to elucidate the relationship between the type of infecting organism and the breakdown in immunity to diphtheria. Artifical immunization was begun in Leeds in 1928, and from that date until February, 1933, T.A.M was used as a routine prophylactic. Although 2004 cases were dealt with in this period, negative final Schick tests were available in only 794 of these cases. From February, 1933, until September, 1934, in the course of work on various prophylactics which is dealt with in preceding sections of this Thesis, the writer obtained 1403 negative final Schick tests, and 564 cases were known to be naturally immune because the preliminary Schick tests in these cases were negative. The population under observation therefore consists of 2761 persons. Too much emphasis however cannot be placed on this figure, because some cases in which no negative final Schick tests had been obtained in the routine fashion were admitted when it was found at the omset of the disease that they were apparently immune (see Tables).

The day of disease, i.e., since the first onset of symptoms - on which the blood was withdrawn and the Schick tests were performed is given in parenthesis after each case, as follows: Case 1(second; Case 7 (fourth), Case 9 (second) Case 15 (first), Case 16 (second); Case 17 (second); Case 19 (first); Case 20 (third). The serum from the blood samples was titrated to 100 per cent differences - e.g 1/50 unit in the table signified 1/50 unit or more of circulating antitoxin per c.cm, and not 1/25 unit. Titration to closer levels would not appear to be necessary.

Particulars of these cases of diphtheria which occurred in the population under observation are set out in Tables **LXXXVI** and **LXXXVII.** It should be mentioned that other cases of diphtheria were notified in inoculated persons, but in none of these had a negative Schick result been obtained, and in most of these cases the ~~the disease~~

disease supervened shortly after the date of the final injection. These cases are not relevant to the present inquiry, and they will not be further discussed.

From Table LXXXVI it is seen that in the first 18 cases a definite negative Schick test was obtained. In cases 6 and 9 these were primary negative Schick tests - i.e., these two children were natural immunes, and were therefore not inoculated. The other 16 cases were naturally susceptible, and had been rendered artifically immune. Cases 19 and 20 have been separated from the others in the Tables, because in neither of these cases was a negative Schick obtained in routine fashion after inoculation. Case 19 was "immunized" by a practitioner, and a final Schick test was not carried out. Case 20 gave a very faint positive result at examination on 13/2/34. The required desiderata were fulfilled in both these cases, however, by the production of definite evidence of immunity - according to the accepted standards - at the onset of the disease.

From the particulars in Tables LXXXVI and LXXXVII it may be concluded that all these 20 cases had been definitely immune according to accepted standards, at some time before, or actually at the onset of disease. Table LXXXVII shows the result of infection of these cases by the C.diphtheriae. It will be seen that 16 out of the 20 cases were definitely diagnosed as "clinical diphtheria" and the other four cases were of a sub-clinical type, one being tonsillitis with a positive swab. Thirteen of the 16 definite cases required serum in doses varying from 8000 to 100,000 units (Cases 1,2,4,6,8,9,11,12,13,14,16,18,20)

It may be objected that in certain of these 16 cases a relapse of the immune state may have taken place. Such an argument might apply to Cases 4, 8, 12, 13, 14. Parish and Okell (1928) showed that 28, or 2.9 per cent of 973 children who had been Schick negative relapsed to the positive state within a period of from one to seven years. Further, this relapse of the Schick state was almost five times more liable to occur in artifical immunes than it was in natural immunes.

Nélis (1934) re-tested 162 children at various long intervals after they had given negative Schick results subsequent to artificial immunization. He showed that 97 per cent were still negative, but his groups are too small to be of much value. A larger series comes from Australia (see Melbourne 1932) where in 1931, 93 per cent of those who had received the full course of injections in 1927 were found to be still negative. In the present series, however, if for the sake of argument we assume that in 5 of these 13 cases a relapse had taken place, we are still left with 8 cases (Cases 1,2,6,9,11 16,18,20) in which the Schick negative state was known to obtain at the time of infection, and in which the resulting disease was so severe that in every case except one 16,000 units or more of antiserum were considered necessary for treatment. It is therefore difficult to escape the conclusion that in the Leeds area the immunity state, as indicated by a Schick test performed with the fluid which is at present adopted as standard, is not always sufficiently powerful to prevent the development of definite clinical diphtheria on infection by the C. diphtheriae.

Rôle of the Gravis strain.

To explain this phenomenon of the occurrence of clinical diphtheria in "immune" persons there are two possibilities: (a) the immunity level of the affected persons may have been lower than is generally found in so called "immune" subjects. (b) the infecting organisms may have been of such a type, or so virulent, that the accepted level of immunity did not afford complete protection. These possibilities will be discussed separately.

(a). The immunity state in these eight cases was shown by negative responses to Schick tests performed with the standard fluid supplied by Messrs Burroughs Wellcome & Co. This fluid has been used all over the world and has always given excellent results. Further, in four of the eight cases immunity of a degree sufficient to prevent the onset of diphtheria was shown to exist by the demonstration of the blood of immune bodies in the accepted titre (see later).

(b) On the other hand, it is evident that in 19 out of the 20 cases

in the Tables the organism was of a particularly virulent strain. In each of these the bacilli were of the gravis type which Anderson et al. (1931, 1933) showed was responsible for the most severe cases of diphtheria in Leeds. O'Brien Okell and Parish (1929) stated that "a Schick negative person is provided with sufficient diphtheria antitoxin to deal with any ordinary infection, but one can conceive that it might not be sufficient if the amount of toxin produced by the invading micro-organism is appreciably greater than the normal". So far no qualitative difference has been discovered between the toxins liberated by the gravis and the Park-Williams strains of C.diphtheriae (Parish Whatley, and O'Brien 1932), but McLeod is of opinion that it is possible that the organisms differ in their invasive power.

In view of the importance of these results, it will be as well to examine the possible fallacies. Dudley (1929) states that, before it can be assumed that typical membranous diphtheria can originate in an individual who has a Schick negative reaction, three criteria must be satisfied: (a) there must be no doubt regarding the clinical diagnosis; (b) a real toxigenic strain of C.diphtheriae must be isolated from the throat; (c) the Schick test must have been negative before the onset of symptoms, and the toxin used for testing must have been beyond all suspicion. These possible objections will be answered seriatim.

(a) The diagnosis in each of these 20 cases was made personally by Dr. J. S. Anderson, whose experience in this work is well known. It can be assumed that in all the cases which are labelled as "Mild", "moderate" or "severe" in Table LXXXVII the patients were suffering from clinical diphtheria.

(b) The organism was not tested for virulence in any of these cases. But in 19 out of the 20 the gravis strain was determined by Prof. J. W. McLeod, whose experience is that all gravis strains are virulent. Dudley's experience at Greenwich (Dudley 1934) was the same.

(c) In Cases 3, 5, 6, 7, 9, 12, 13, 14, 15, 16, 17, 20 the final

Schick results recorded in TableLXXXVI were obtained by the writer using Schick fluid which had recently arrived from the Wellcome laboratories. The same batches of fluid were used to test other individuals, and in no case was there any reason to doubt the efficacy of the material. The Schick tests in Cases 1 and 2 were performed by Dr.Anderson with similar material. The tests recorded in the last column of TableLXXXVII were also performed by Dr. Anderson; in each case the test fluid was injected at least one hour before serum was administered, and in those cases in which antitoxin titration was carried out the blood was drawn at the time the test was performed. It is therefore reasonable to assume that the cases listed as "mild", "moderate" or "severe" were undoubtedly cases of diphtheria occurring in persons who had had negative Schick reactions.

These results are on lines similar to those of Robinson and Marshall (1934) who noted 12 cases of diphtheria in Schick negative reactors. In their series one case was "severe", three were "moderately severe", and the other eight were "mild". In eight of their cases the organism was of the gravis strain, and in four it was of the intermediate strain. It is worthy of note that, although the intermediate strain was ten times more common in Manchester than the gravis, three of the four cases which were "severe" or "moderately severe" were due to the gravis strain.

It is an old established claim that diphtheria in inoculated individuals is always mild. This series of cases does not bear out this statement completely, since, for example, Case 13 was "severe", and five other cases were of "moderate" severity. When account is taken, however, of the high incidence of severe cases in Leeds, it may be assumed that the inoculations had some influence on the course of the disease. Even more important is the comparative absence of complications. Two cases developed albuminuria, but apart from this the progress was rapid, and no complications were met with in the other cases.

Relation to antitoxic content of Blood.

It is regrettable that antitoxin titration of the blood was not carried out in more of these cases at the onset of the disease. There was sometimes, however, an appreciable interval between the actual notification of the case and the realisation that inoculations had been given at some time previously. The results which are available are interesting. It is seen from Table LXXXVII that the antitoxic content varied between 1/250 unit and 5 units per ccm, and that in the cases which have been adcepted as definite clinical diphtheria the limits were 1/250 unit and 1/5 unit per c.cm. From Table LXXXVII it cannot be said that the severity of the disease - as judged by the amount of serum required - was in all cases inversely proportional to the antitoxic content of the patient's blood at the onset.

The minimum quantity of antitoxin which the blood must contain in order that complete protection may be established has never been definitely ascertained; there is in fact a possibility that other factors may have an effect, and that this minimum quantity of antitoxin cannot be ascertained with exactitude.

In section (c) of this Thesis an attempt was made to correlate the Schick state of the individual with his blood antitoxin content. Owing to the inherent difficulties of this subject, it cannot be said that the investigation was successful in establishing a hard and fast line of demarcation between the antitoxin content of the Schick positive and Schick negative individuals. Nevertheless it would seem that with the materials in use on the population of Great Britain the most satisfactory level of immunity is about 1/100 unit. On this basis five out of the seven definite cases in this series in which such results are available should have been definitely immune; the other two should have had a reasonable degree of immunity, which would probably have been broken down only by a very virulent infection.

Relative incidence in "susceptibles" and in "immunized" persons.

In this section the words "susceptible" and "immunized" are used in the accepted sense of the terms. That is, an "immunized" person is one who has been inoculated against diphtheria and in whom a subsequent Schick test with standard Schick test toxin has given a negative result. A "susceptible" person is one who has given a positive result to a Schick test performed with this material. In a discussion of this type it is impossible to eliminate all fallacies, but in the following an attempt has been made to make the results as accurate as the available data will permit.

From the 1931 census figures it was calculated that 22.044 per cent of the total population of Leeds were under 15 years of age. The estimated population of the city for 1933 was 485,000, and a calculation from this figure shows that, on the ratio of the 1931 census figures, there were 106,913 children under 15 in the city in 1933. The writer personally performed 2559 preliminary Schick tests on Leeds children under 15 years of age, and 81.09 per cent of these gave positive results. Applying this percentage to the 1933 figures, we can estimate broadly that in the years 1933-34 there were approximately 86,696 susceptible children in Leeds. But 794 of these had been rendered immune between 1928 and February 1933, and a further 1403 between February 1933, and September, 1934. The final estimate is that there were 84,499 susceptible children under 15 years in the city in 1933-34. In 1933 there were 934 cases of diphtheria in children under 15 years - which gives a case rate of 11.05 per 1000 susceptible children under 15.

In the selected population of immunes it is advisable to omit those which were natural immunes. The reason for this is that, as has been stated, although Cases 6 and 9 were the only two cases of diphtheria in natural immunes which came to the writer's knowledge, it is not certain that there may not have been one or two other cases of this type. We are left therefore with 12 definite cases of diphtheria (aged under 15 years) which occurred

in 794 plus 1403 - i.e., 2197 artifical immunes under 15 years between September, 1933, and September, 1934. This gives a case rate of 5.46 per 1000 immune children under 15 years. It is thus seen that in Leeds during 1933-34 susceptible children were more than twice as liable to develop clinical diphtheria as were children who had been rendered artifically immune. These results are of considerable importance, especially in the light of findings in other areas.

The incidence of definite diphtheria in the Schick-negative population described by O'Brien, Okell and Parish (1929) was 0.9 per 1000. In Cork Saunders' (1933) two cases of diphtheria in negative Schick reactors occurred in a population of 2398 such negative reactors, which is equivalent to an incidence of 0.83 per 1000. In Munich (Pfaudler, 1931) the incidence in a non-inoculated population of 39,582 children - mainly 2 to 7 years of age - was 32.0 per 1000. In 2485 children of equivalent age who had been inoculated - but who had not necessarily given negative final Schick reactions - 18 cases occurred. This gives an incidence of 7.4 per 1000, which is roughly a quarter of the incidence in the non-inoculated. Tomcsik (1932) states that in Hungary the incidence of diphtheria in the inoculated was only one-tenth of that in the non-inoculated. Bezançon and Dreyfus (1932), working on Paris school and pre-school children, reported that between 1928 and 1931 3,837 individuals had been inoculated with three injections. In 1930 there were 43.12 cases per 1000 in the uninoculated, and only 6.24 per 1000 inoculated. In 1931 the rate for the uninoculated was 42.99, and for the inoculated it was 1.82. Hence the ratio of the incidence in uninoculated to that in inoculated fell from 7 to 1 in 1930 to approximately 25 to 1 in 1931. The incidence of diphtheria in an inoculated population depends, of course, upon the degree of efficiency of the immunization. For example, Sdrodowski (1934) showed that 158,000 children were inoculated against diphtheria in Leningrad in 1931 and 1932. More than 50 pr cent were followed up; the incidence of diphtheria in those who had three injections was 12.8 per 10,000;

among those who had only one injection it was 49.0 per 10,000.

Further examples of the relative incidence of diphtheria in inoculated and in non-inoculated populations respectively are set out in Table LXXXVIII. It should be noted that in only certain of these cases did the inoculated population consist entirely of known Schick immunes. From this Table it will be seen that in most areas the non-inoculated were at least six times more liable to contract diphtheria than were the inoculated, and that in only three instances (with the exception of Leeds) did the ratio fall to approximately 4. In Leeds, on the other hand, the non-inoculated were only twice as likely to contract the disease as were those who had been Schick tested and found immune. It is evident that in Leeds some factor is at work which renders a breakdown of immunity much more liable to occur.

Discussion.

The argument of this paper is that the only constant factor which can be adduced to explain the occurrence of this high incidence of cases of clinical diphtheria amongst inoculated persons in Leeds is the incidence of the gravis strain of C. diphtheriae in the community. During the last few years few areas have suffered more from diphtheria than Leeds (see Underwood, 1934b), and in 1934 the incidence of the disease continued to increase. Most of the cases were due to the gravis strain of the organism. The present investigation suggests that, when this strain of the organism is very common in a community, a breakdown of immunity will result not infrequently, and that, when this occurs, the chances are that the infection of the individual cases will be by the gravis strain.

It should be noted that cases 6 and 9 were the only cases which came to the notice of the writer of persons who were Schick tested and found naturally immune, and who later developed clinical diphtheria. Although a special enquiry was made on this

point, no other cases were traced. It is difficult to give the actual numbers of natural and artificial immunes in the city over this extended period, because for one thing, the numbers were subject to constant fluctuation, especially in the direction of increase. By the end of September, 1934, however, it is estimated that there were in Leeds about 570 known natural immunes, and about 2200 children who had been artifically immunized. This series therefore gives some support to the contention that artificial immunes are likely to relapse more easily than natural immunes, though the difference ~~for~~ between the numbers ~~of~~ for the two groups is not very marked. The investigation also suggests that this breakdown in immunity may take place at any time, since the figures in column 9 of Table LXXXVI show so much variation that no reliable inference can be drawn. As a corollary it seems likely that the actual cause of the breakdown may usually lie more outside than inside the individual's body, i.e., it is the function of the germ rather than of the host. One feature, however, suggests that the possibility of an alteration in the host cannot be lightly discarded. It is seen that, although immunization had been carried ~~out~~ on in Leeds since 1928, the first case of diphtheria in an inoculated person did not occur until 1933; thereafter the majority of the cases occurred in the middle of the months of June and July 1934.

The possibility of the alteration of the resistance of a host at different times has never been adequately dealt with. It should be noted, however, that Meerssoman and Renard (1934) obtained a certain amount of evidence to show that influenza lowers immunity to diphtheria, and they suggest that this possibly explains the occurrence of diphtheria in Schick negatives. In this connection the reader will find in Part II of this Thesis a full discussion of a considerable epidemic of influenza which occurred in Leeds in the winter of 1932 and the spring of 1933. Despite this the majority of the instances of the breakdown of immunity occurred in 1934.

The evidence is, however, in favour of the view that these cases were due to the continued presence in the community of the gravis strain of C. diphtheriae. During the summer of 1934 the incidence of diphtheria in Leeds was very high, and all members of the community, inoculated and non-inoculated alike, would be brought much into contact with this strain. It is generally assumed that the gravis strain produces its severe effect owing to its great invasiveness. Povitzky, Eisner, and Jackson (1933) have, on the other hand, suggested from their experiments that the gravis effects are due to the rapidity with which this strain produces its toxin. On this assumption, there would be, in gravis-infected cases, little time for the body to respond to the primary stimulus of the infection. It would seem that the blood titration results in this series of cases lend some support to this view. The only case in which there was what might be called a very high level of immunity (Case 15) was suffering merely from a chance infection superimposed on a sore throat. In the other cases the antitoxic level was probably not high enough to cause a rapid response, not only to the primary stimulus, but also to the continued rapid production of toxin by the gravis strain of organisms.

The evidence can be interpreted as a plea for increased immunization in communities which are infected with the gravis strain of C.diphtheriae. During the year 1933 the number of cases of diphtheria which were notified in, Leeds was 1057, and the deaths numbered 88. This gives a case-mortality rate of 8.2 per cent. During the first ten weeks of 1934 the case-mortality rate rose to 9.5 per cent - a figure which is higher than that for any other area in England and Wales. When we compare this figure with that for the Schick negative population, viz 0 per cent - it is evident that, although the effect of immunization on the incidence of the disease may not have been as great as we might have expected, the influence on the course of the disease was so marked as to be ~~in itself~~

TABLE LXXXVI.

CASE NO.	AGE.	SEX.	PROPHYLACTIC USED.	NO. & SIZE OF DOSES. c.cm.	DATE OF LAST INOCULATION.	DATE OF FINAL SCHICK	RESULT OF FINAL SCHICK	INTERVAL IN MONTHS BETWEEN FINAL SCHICK AND ONSET.
1	ADULT	M	TAF	3×1	21 : 10 : 32	2 : 2 : 33	NEG.	3
2	ADULT	F	TAF	3×1	29 : 10 : 32	5 : 1 : 33	"	1½
3	5	M	FT	3×1	19 : 7 : 33	22 : 8 : 33	"	1
4	7	M	TAM	3×1	1 : 3 : 32	5 : 11 : 32	"	12
5	4	F	FT	2×1	1 : 6 : 33	14 : 8 : 33	"	3
6	4	M	—	—	—	28 : 11 : 33 (PRIMARY)	"	1½
7	6	M	FT	2×1	10 : 1 : 34	7 : 2 : 34	"	0
8	9	F	TAM	3×1	7 : 5 : 32	28 : 1 : 33	"	14
9	8	F	—	—	—	17 : 3 : 34 (PRIMARY)	"	0
10	5	M	TAM	3×1	EARLY 1932	10 : 9 : 32	"	21
11	6	F	TAM	3×1	4 : 6 : 32	2 : 10 : 32	"	20
12	6	F	FT	3×1	25 : 7 : 33	22 : 8 : 33	"	10
13	6	F	FT	3×1	25 : 7 : 33	22 : 8 : 33	"	10
14	3	F	FT	3×1	25 : 7 : 33	22 : 8 : 33	"	10
15	6	F	FT	3×1	25 : 7 : 33	15 : 11 : 33	"	8
16	6	M	FT	3×1	26 : 6 : 33	14 : 8 : 33	"	11
17	4	F	FT	2×1	30 : 12 : 33	13 : 3 : 34	"	4
18	6	M	TAM	3×1	7 : 5 : 32	17 : 12 : 32	"	20
19	8	F	TAM	3×1	18 : 2 : 34	—	—	—
20	10	F	FT	2×1	13 : 1 : 34	13 : 2 : 34	FAINT +	—

TABLE LXXXVII

Case No.	Date of Onset	Clinical Type	Complic-ations	Amount of Serum	Strain of Organsm	Titrat. (AU)	Schick test on adm.
1	15/5/33	Mod.Fau*	Nil	70,000	G+	1/250 -1/100	Neg.
2	22/2/33	" " *	"	20,000	G	--	"
3	21/9/33	Bact.case	"	Nil	G	--	"
4	1/11/33	Mild fau.*	"	8,000	G	--	---
5	15/11/33	Sub. clin.	"	Nil	G	--	Neg.
6	16/1/34	Mild fau.*	"	16,000	G	--	---
7	13/2/34	" " *	"	Nil	G	1/10	Neg.
8	26/3/34	" " *	"	8,000	G	--	---
9	27/3/34	" " *	"	8,000	G	1/50	---
10	8/6/34	Sub. clin.	"	Nil	G	--	Neg.
11	20/6/34	Mild fau.*	"	16,000	Nt.	--	"
12	22/6/34	" " *	Sl.alb.	16,000	G	--	---
13	22/6/34	Severe " *	Alb.	100,000	G	--	---
14	5/7/34	Mod. " *	Nil	20,000	G	--	---
15	7/7/34	Sub. clin.	"	Nil	G	> 5	Neg.
16	14/7/34	Mod. fau.*	"	16,000	G	1/250	"
17	18/7/34	Mild fau.*	"	Nil	G	1/25 -1/10	"
18	23/8/34	Mod. fau.*	"	20,000	G	--	"
19	9/9/34	Mild fau.*	Nil	Nil	G	1/5	"
20	11/9/34	Mod. fau.*	"	20,000	G	1/50	"

Bact. = bacteriological. Fau. = faucial. Sl. alb. = slight albuminuria. Nt. = not typed. * = Bie's (Copenhagen) classification (Bie, 1922). +G = *gravis* strain.

TABLE LXXVIII.

Relative Incidence of Diphtheria in Inoculated and in Non-inoculated Populations.

Author and locality	Inoculated			Non-Inoculated.			
	Total	Cases of Diphtheria	(a)Case-rate per 1000	Total Controls	Cases of Diphtheria	(b)Case-rate per 1000	Ratio b/a.
1. Poulain, 1932 Loire, France	11,771	47	4.0	8646	131	15.1	3.8
2. Doyer, 1931 Hilversum, Holland	1,900	2	1.1	10800	87	8.1	7.4
3. Ibid, Holland	5,450	3	0.6	18750	78	4.2	7.0
4. Sparrow, 1931 Warsaw, Poland	5,000	15	3.0	295800	3400	11.4	3.8
5. Isabolinski et.al 1931 Smolensk, Russia	4,185	6	1.4	19000	285	15.0	10.7
6. Park, 1922 New York U.S.A.	90,000	14	0.16	90000	56	0.6	4.0
7. Kinloch, 1927 Aberdeen	8,188	23	2.8	33300	612	18.4	6.6
8. Rae* Aberdeen	7,264	44	6.0	23600	918	38.9	6.5
9. Adams, Ontario	11,000	17	1.6	9000	103	11.4	7.1
10. Underwood, Leeds	2,197	12	5.5	84862	934	11.1	2.0

*Quoted by Forbes 1932.

in itself practically an argument for immunization. This influence not only manifested itself in the complete prevention of fatal results; it also undoubtedly modified the disease and prevented those complications which so so liable to follow infection with the gravis strain.

It should be mentioned that, the publication of the material which is contained in this section was the first evidence which had appeared on this question of the relationship between anti-diphtherial immunity - as shown by Schick tests and blood titrations - and a particularly invasive strain of C.diphtheriae. This paper was soon followed by independent corroboration of the writer's views. Parish and Wright (1935) made similar observations, though the population under review was in their case much smaller and was not homogeneous. Marshall and Robinson (1935) have at the time of writing published a paper which extends their work in Manchester, and they report the death from diphtheria of an immune patient - as shown by a negative Schick test, but not by antitoxin estimation which was not carried out. In their case the infecting strain was also gravis.

A fairly obvious suggestion arising out of the present section of this Thesis is that the Schick test fluid which is now in use is not suffciently strong. The writer frequently considered whether it would not be wise to use a stronger fluid, but he was always of opinion that, despite the possibility of partial failure in the use of ordinary Schick fluid, it was desirable to retain it, rather than to set up a new standard for all future Schick tests. His ideas on this subject are set out in the concluding section of Part I. Now, however, Parish and Wright (1935) have tentatively suggested that there should be some alteration in the strength of the Schick fluid, and Chesney (1935) has used such stronger fluids in a few four-fold Schick tests.

After due consideration the present writer feels that science, like art, is long, whereas the existence of gravis strains in epidemic proportions is a matter for discussion. Let us therefore retain the Schick fluid which has given such good results in the past, and from which all statistics for the test are derived.

PART I.

SECTION (i)

EXPERIMENTS WITH SCHICK TEST FLUIDS DERIVED FROM GRAVIS STRAINS.

The Schick test experiments which have been described in the preceding sections of this Thesis were all carried out with ordinary Schick test fluid derived from the standard Park Williams strain, and the results should therefore be comparable with similar results from most areas in the civilised world. Quite near the beginning of these investigations, however, it occurred to the writer that there might be a difference in the rate at which individuals become immune to ordinary strains and to gravis strains respectively of the C.diphtheriae. The preceding section of this Thesis has given fairly conclusive evidence that an individual who is definitely immune to diphtheria, as judged by the tests which are so far known to us, may yet be susceptible to infection with the gravis strain. For these reasons it seemed desirable to investigate the susceptibility of the population to gravis infection.

So long ago as the early spring of 1934 the writer approached Professor J. W. McLeod and requested him to prepare Schick test fluid from gravis strains. Professor McLeod kindly proceeded with this work, but he was confronted with the difficulty that these strains are not good toxin producers, and unfortunately no satisfactory fluid had been produced by the time that the writer left Leeds. Since then Mr. Glenny, the Immunologist to the Wellcome Physiological Research Laboratories, has succeeded in producing a sufficient quantity of this material and the writer has used it on 349 London children. At the same time my former colleague, Dr. J. S. Anderson, co-operated in the study by using

this fluid on 158 children who were admitted to the Leeds City Hospital suffering from scarlet fever or diphtheria. These results will be published in a joint investigation. In this Thesis a brief description will be given of the London results, and only the salient features of the Leeds series will be used for comparative purposes.

Technique.

In every case the control tests were performed on the right arm, and the Schick tests on the left arm. Gravis fluids were injected proximally and ordinary fluids distally. The distance which separated the two tests on any arm varied from 6 to 16 mm. depending on the age of the child. The tests were read on the second day and also on the seventh day. Accurate measurements were taken of the size of the different types of erythema, and full notes were made of the appearance of the reactions.

Degree of Natural Immunity.

As judged by the ordinary Schick test result 130 of the 349 individuals were negative, and 219 positive. The percentages were therefore: negative, 37.2; positive 62.7. The ages of the children were very similar to those in the much larger Leeds series, which has been dealt with in preceding sections of this Thesis. It will be noted that the percentage of positives - viz. 63 - is over 20 per cent lower than the average for the different Leeds groups, and these results therefore emphasize the extraordinary low rate of natural immunity in Leeds. In the small London series which is at present under discussion the percentages of +, ++, and +++ Schick reactors were 30.4, 29.8, and 2.6 of the whole sample. These figures again emphasize the fact that the slight positive reactor is much more common in London and marked positive reactors are more frequent in Leeds.

Results of the Tests.

The main results of the tests are set out in Table LXXXIX. These results do not include the final group, which will be dealt with separately. From these results it will be evident that 91.2 per cent of the tests agreed so far as the final interpretation was concerned, but that in a further 6.6 per cent the gravis test was definitely positive while the ordinary test was negative.

TABLE LXXXIX.

Results of gravis Schick tests performed in London.

Group	Total tested	Ordinary and Gravis Schick results* S+G+	S-G-	S-G+	Doubtful reaction	Pseudo-reactions Sψ Gψ	Gψ	Sψ
L1	36	27	5	4	-	-	-	-
L2	33	22	6	4	1	1	-	-
L3	70	41	22	6	1	1	-	-
L4	49	31	16	-	2	2	1	-
L5	44	20	21	3	-	-	-	-
L6	43	22	16	2	3	-	1	-
L7	16	8	8	-	-	-	-	-
L8	13	8	4	1	-	-	-	-
L9	28	24	2	2	-	-	-	-
Total	332	203	100	22	7	4	2	-
Percentage		61.1 ±1.80	30.1 ±1.70	6.6 ±0.92	2.1±0.53	1.2± 0.40	0.6± 0.29	-

* Abbreviations used in table: S = ordinary Schick result. G = Gravis Schick result. Sψ = pseudo-reaction at ordinary Schick sites. Gψ = pseudo reactions at gravis Schick sites.

Although from the numerical point of view this discrepancy is not very marked, nearly every case showed a difference in the degree of reaction of the two tests. In the case of marked positive reactors the tests were very similar, but in moderate reactors the gravis test was usually a few millimetres broader and much brighter than the ordinary test. Desquamation, which was often coarse, was usually present in these moderate and/marked positive gravis tests, whereas in the case of the ordinary tests desquamation was usually much less in degree and was more often of a powdery nature. Although this description can be applied to most of the tests there were quite a number of instances in which the ordinary Schick test, although classified in the Table as

positive, was very faint and consisted merely of a small pale pink area; whereas in these cases the gravis tests were obviously markedly positive.

Although these results seem to indicate that there is a definite difference in the degree of reaction to the two test fluids, before making a decision it should be remembered that the possibility of differences being due to non-specific protein effects must be taken into account. Since gravis strains are poor producers of toxin in vitro, this toxin will therefore require very little dilution in order that 0.02 M.L.D. should be contained in 0.2cc. of the diluting fluid. Consequently the gravis schick test fluid which was used must have had a higher nitrogen content than the ordinary fluid. To overcome this difficulty A.T.Glenny prepared a batch of gravis test and control fluids, which were made up in such a way that the nitrogen content was strictly comparable with that of the ordinary Schick fluid which was used. Up to the present the writer has only been able to use these special fluids on seventeen individuals. Of these 12 were positive to both ordinary Schick and gravis tests; four were negative to both tests; and in one instance the ordinary test was negative, but the gravis test showed a pseudo-reaction which interfered with the reading of the test. In this latter instance it is probable that the gravis result was of the nature of a test-control reaction, since the results of the titration of blood, which was drawn at the time of performance of the Schick test, showed that the individual had 1/5 of a unit of antitoxin. Blood titration in a further two cases of this group of children gave no unusual results.

It is unfortunate that this last group of children was so small and that it happened to include no individual who showed a marked discrepancy in the results of the two tests. Further light on the problem may, however, be obtained from a consideration of the psuedo-reactions in the 349 individuals in the groups which are included in Table LXXXIX. Four individuals - 1.2 per cent - showed pseudo-reactions at the sites of both the ordinary Schick

and gravis Schick tests, and two individuals - 0.6 per cent - showed a pseudo-reaction at the site of gravis and gravis control injections only. In the 17 individuals who were tested with the special material mentioned above, there was one pseudo-reaction at the site of the gravis inoculations. If the differences between the result of ordinary and gravis Schick tests in the main series of Table LXXXIX are due to the higher nitrogen content of the gravis fluids, then we would expect this additional protein material to give increased reactions not only at the site of the test injections but also at the control sites. From a consideration of the figures for thepseudo-reactions it does not seem that this actually occurs, and consequently there appear to be some grounds for suggesting that any differences which have been noted between the gravis and ordinary test readings must be due to differences in the response to the specific toxins.

Tests after Inoculation.

The individuals who are discussed in this section were inoculated with three doses of T.A.F., and were re-tested by the writer approximately three months after the last injection. So far the results of gravis and ordinary Schick tests are available for 70 children. Of these 61 were definitely negative to both tests; four were positive to both tests; two were definitely negative to the ordinary test and positive to the gravis test; two were definitely negative to the ordinary test but doubtfully positive to the gravis test; and in one instance the gravis test was negative, but there was at the ordinary test site a reaction which might have been interpreted as a very faint positive.

Comparison with the Leeds series.

The full implications of Anderson's Leeds series and the writer's London series have not yet been fully worked out. Preliminary investigation shows, however, that whereas in London the percentage of children who gave a negative ordinary Schick but a positive gravis Schick was 6.6±0.92, the corresponding figure for Leeds was only 2.5±0.84. (It may be mentioned that Dr.

Anderson

Anderson and the writer agreed to read and record the tests in the same manner). The difference between these two percentages is 4.1±1.25. The difference is therefore statistically significant. Whether or not any importance can be placed on this small difference further investigation must show. In the meantime the results can be regarded with some interest. The *gravis* strain has been endemic in Leeds for so long that we would certainly expect that most individuals, if they become immune to diphtheria at all, will also develop an immunity to this strain. In London, on the other hand, the *gravis* strain has not been nearly so prevalent, so that we might expect, if there is any immunological difference between the two strains, that there would be a greater proportion of individuals in the community who are immune to the ordinary strain but not to *gravis*. This difference has now actually been demonstrated, but only to a slight degree. If the results are not as definite as we might require them to be if they are to demonstrate that the difference is an important one, they are at least sufficiently suggestive to warrant the pursuit of this line of inquiry.

GENERAL SUMMARY OF PART I

In this summary it is possible to mention only a few of the points which have been discussed in the preceding sections.

From a discussion of the case rates and death rates for England and Wales and for certain cities, it has been shown that the rates for England and Wales as a whole do not express the gravity of the situation in certain localities. The introduction of the *gravis* strain of *C. diphtheriae* into Leeds and Hull was possibly a distinct epidemiological occurrence. The resulting epidemics were of such magnitude of of such severity as to be beyond the probability of occurrence apart from the operation of some new factor; and some evidence is given that this new factor was the *gravis* strain of the organism.

Peculiarities of the Schick test reactions in a *gravis*-infected area are discussed. It is shown that in Leeds there was only a slightly higher percentage of positives among females than among males.

The seasonal incidence of positive reactions is discussed for the two sexes, and it is shown that the percentages are higher in autumn and winter thanin spring and summer, and the differences are more or less equal in the two sexes.

The degree of association between Schick state and age is discussed statistically in some detail, and it is shown that the two sexes differ on some fundamental points. The evidence which is adduced suggests that susceptibility to diphtheria is a true hereditary sex-linked factor.

Protein reactions are discussed, and one such reaction which is different from ordinary pseudo-reactions is described.

A study of the relationship between the Schick test result and the antitoxin content of the blood showed that there was no definite line of demarcation between Schick positive and Schick negative persons. The dividing zone is very broad; and the actual Schick level in any individual case may be towards either the upper or the lower limits of this zone. The chances are, however, roughly three to one in favour of the antitoxin content of a negative reactor being around the 1/100 unit limit.

In a small series of cases blood for antitoxin titration was taken at the time when the Schick test was performed, and a further sample was obtained six days later. The results suggested that the amount of toxin which is given in the Schick test dose is not sufficient to act as a primary or secondary stimulus in the human.

The studies on the Moloney test were based on 2666 tests performed before the injection of prophylactics and 595 tests carried out after the injection of toxoids in prophylactic doses. Methods of reading the test reactions are described.

Certain individuals develop sensitivity to toxoid at a very early stage of their existence - even during the first 18 months of life. In the herd the periods of most rapid development of sensitivity to toxoid are between the first and second years, between the fourth and the seventh years, and just before the age of fourteen years,

For practical purposes the important reactions are those which are designated ++ and +++. Up to the age of 5 years the combined percentages of children who showed these ++ and +++ reactions did not exceed 3 per cent at any age. At 7 years the incidence was 9.6 per cent. and therafter there was a rapid increase until the maximum (43.3 per cent) was reached at 14 years. Not every individual who shows such ++ or +++ results will develop unpleasant reactions on the injection of

toxoids in prophylactic doses. But an investigation into the age and Moloney state of 2041 persons who received prophylactics demonstrated quite definitely that children who showed general and local reactions after inoculation were nearly always over 5 years of age, and most of them (79 per cent) showed ++ or +++ results with the Moloney test. Hence the test should be used whenever it is intended to inoculate children of school age with prophylactic doses of toxoid or its alum preparations.

The assumption that strong positive Moloney reactors are usually immune is unjustified. Of 74 individuals who showed a +++ Moloney reaction 20.3 per cent., and of 173 individuals who showed a ++ Moloney reaction 32.4 per cent. were definitely non-immune as judged by the Schick test. The coefficient of association (Q_5) between the two conditions was 0.632.

The relationship between positive pseudo-Schick reactions and positive Moloney reactions is examined statistically, and it is shown that the association is not sufficiently high to warrant the substitution of one test for the other. Confirmation of this conclusion was obtained from an examination of the pseudo-Schick and Moloney states of all individuals who showed any degree of local or general reaction after the injection of prophylactic doses of toxoids or other prophylactics.

The degree of association between the antitoxic content of the blood serum and the positive Moloney state was investigated in 108 cases. The coefficient (Q_5) was found to be in the region of 0.5.

The delayed Moloney reaction (+D) is described and its significance is discussed. The frequency of +D reactions is 1.69 ± 0.17 per cent.

In general, a definite Moloney-positive state appears to be permanent. The injection of toxoid in prophylactic doses does not tend to render the injected individual sensitive to toxoid. Exceptions to this rule may be due to the acceleration of a normal process

of a normal process.

The incidence of positive Moloney reactions is practically identical in males and females.

The association between the number of children in a family and the number of those who will give positive Moloney reactions is very slight. The effect of an urban environment appears to be more important than the actual family environment in determining the development of sensitivity to toxoid.

The relationship between protein skin tests and the Moloney reaction is discussed. Toxoid sensitivity is probably an allergic condition which is due to previous contact with the products of the C. diphtheriae. There is little evidence that age is in itself a factor in the development of sensitivity.

In a large investigation on the use of potent formol toxoids and alum toxoids it was shown that effective batches of material should give a satisfactory percentage of negatives within a month after the last injection.

When tested at this interval most toxoids are as effective when given in two doses as they are in three. But it is probable that the three dose method would be more effective if the tests were performed after several months.

Similarly, it does not seem to matter how many total Lf units are given, provided that a minimum total dosage is injected.

The minimum effective interval between doses appears to be one week. Weekly intervals between doses where three doses are given, and intervals of three weeks between doses where two doses are given, will give quite satisfactory results.

The site of injection of toxoid - i.e. arm or leg - has no effect upon the efficiency of the toxoid.

A study of the correlation between age and immunizability

showed that, for children up to 14 years of age, there is a sensible tendency for immunizability to be increased as a Schick-positive person becomes older. The coefficient is usually less than 0.1.

The effect of size of dose and of interval between doses is discussed for alum toxoids. A method of immunizing by using a "priming dose" is described.

The writer's work on immunization by the "one-shot" method is discussed in detail. The most effective material which was used was a batch of alum-precipitated toxoid (A.P.T.). In 152 cases 83.6 ± 2.0 became immune within one month after a single injection of this material.

The paper on reactions discusses age, sex, and other factors which may have some effect upon the incidence of unpleasant symptoms after the injection of toxoids, but the evidence is not suitable for presentation in summary form.

The investigation on the breakdown of Schick immunity deals with the notified cases of diphtheria which occurred in a Schick-negative population of 2761 persons in Leeds. Twenty cases of diphtheria were notified, and it was found that 16 of these were definite clinical cases of diphtheria. Thirteen of these cases required serum in doses varying from 8,000 to 100,000 units.

Eight of the 13 serum-treated cases gave a negative response to the Schick test at the time of onset of the illness.

In 19 out of 20 cases the infecting organism was of the gravis strain. It is concluded that this strain was the cause of the breakdown in the immunity of these cases.

Blood titration at the onset was carried out in 8 of these cases. According to the accepted standards for the antitoxin level of immune subjects, 5 of the 7 definite cases in which this was done should have been definitely immune; the other two cases should have had a reasonable degree of immunity.

It is calculated that in Leeds in 1933-34 the case-rate for diphtheria (in cases under 15 years) was 11.1 per 1000 susceptible children under 15. In the same period the case-rate for diphtheria in children rendered artificially immune was 5.46 per 1000 immune children under 15 years. Non-inoculated children were therefore more than twice as liable to develop diphtheria in Leeds as were children who had been "immunized" according to accepted standards.

The case-mortality rate from diphtheria in Leeds rose to 9.5 per cent in the first three months of 1934. In the 20 cases with which this paper deals no deaths occurred. It is concluded that, although natural and artificial immunity is much more likely to be broken down in a community in which gravis infections predominate, the resulting disease is much milder than it would otherwise have been. Although one of the 13 cases which required serum was "severe", and five others were of "moderate severity", the only complication which occurred was albuminuria,

In an investigation which the writer carried out in London, and in which Schick test toxins derived from gravis strains were employed, it was shown that the discrepancy between gravis Schick and ordinary Schick results was nearly 7 per cent. The full implications of these results have not yet been worked out.

P A R T I I

S T U D I E S I N

I N F L U E N Z A, C H O L E R A,

A N D V A R I C E L L A.

PART II.

SECTION (a)

THE EPIDEMIOLOGY OF AN INFLUENZA OUTBREAK IN LEEDS.

Although influenza caused many more deaths in England and Wales during the seven years 1926-32 than did scarlet fever, diphtheria, measles and whooping-cough taken together, comparatively few accounts of the clinical and epidemiological features of influenza outbreaks have been published. This is probably explained by the fact that influenza is not a notifiable disease; consequently, reliable figures are difficult to obtain, and investigations are robbed of much of their interest. During the outbreak of influenza which affected Leeds in the winter of 1932-3, the writer was able, through the courtesy of the heads of many large commercial firms and official departments, to obtain information regarding the extent to which their respective establishments were affected. These particulars served to augment the data which were otherwise available, and made possible the following account.

The outbreak evidently started early in December, 1932, since in the second week of this month there was a significant increase in the notifications of influenzal pneumonia. Thereafter, although it was evident from other information that influenza was very prevalent in the city, there was a slight lag until the first week of January 1933, when the notifications of influenzal pneumonia reached eleven, and in the following week they reached their peak figure of thirty-two. The number of deaths registered as due to influenzal pneumonia continued to rise until the weeks ending January 21st and 28th, during each of which forty-eight deaths from this condition were registered. Since it was probable that in each

of these cases some days had elapsed between the actual onset of the disease and the fatal termination, it seemed likely that the peak of the epidemic in Leeds was reached during the week ending January 14th. The notifications of influenzal pneumonia decreased fairly rapidly after the peak period, and by the beginning of March 1933, cases were very few.

Investigation of a selected population.

During the decline of the epidemic arrangements were made with the heads of large firms and corporation departments, and these gentlemen kindly supplied figures showing the number of new cases of influenza among their respective employees, which had been notified to them week by week. Owing to lack of reliable records the figures from certain firms and departments had to be neglected. The remaining figures were subjected to as thorough an examination as was possible in the circumstances, and every effort was made to ensure that the cases had actually been certified as influenza by medical practitioners. In many instances consent was given for an official of the Health Department, acting under the writer's supervision, to examine the sickness returns of different establishments. As a result of this selection it was possible to give weekly figures for the incidence of influenza in a selected population of 17,195 persons during the period from the week ending December 3rd, 1932, to the week ending February 25th, 1933. This selected population was made up of twenty groups. Table I gives the total number of persons in each group, together with the total number of cases and deaths in each during the period under investigation, and certain other particulars.

In this investigation it was unfortunately impossible to obtain the age and sex of each patient, so that the age and sex distribution of the 2772 cases of influenza is unknown. It was not thought necessary, therefore, to give details regarding the sex distribution of each group of the selected population, since this

would have entailed a further special enquiry and additional inconvenience to the firms and departments concerned. The information in column 2 of Table I is of no importance so far as the investigation is concerned: the estimates in this column serve merely to give a broad indication of the sex constitution of the selected population and is added as a matter of interest.

At this point it may be briefly stated that only five deaths were reported in this selected population. This is equivalent to a death-rate of 0.29 per 1000, and to a case-mortality rate of 0.18 per cent. The case-rate for the selected population as a whole was 16.1 per cent.

Table I shows that the different groups can be combined into four main categories: (a) factory workers (groups A, D, E); (b) shop workers (groups B, C); (c) clerical workers and teachers (groups G, H(b), I, R, S, T(a), T(b); (d) outdoor workers (groups H(a), K, M, N, P). (In groups E, J, L, O and Q the data could not be subdivided to fit into this grouping; these groups have therefore been omitted from this classification). If the incidence of influenza in these four main categories of workers is now investigated separately, some interesting features emerge. Table II summarises the essential points.

Table I. *Incidence of influenza in selected population.*

Designation of firm or department	*Approx. sex constitution	Total employees	Total cases	Total deaths	Average duration in days	No. off duty over 7 days	Case-rate per 100 of selected population
Business firms:							
A (factory)	♀	2296	294	—	14·1	203 (69·1 %)	12·8±0·5
B (departmental store)	♀	1250	225	—	9	123 (54·6 %)	18·0±0·7
C	♀	450	87	—	7	30 (34·5 %)	19·3±1·3
D (printers)"	♂>♀	500	50	—	15	34 (68·0 %)	10·0±0·9
E (wholesale chemists)	♀>♂	162	23	—	9	16 (69·6 %)	14·2±1·9
F (sweet factory)	♀++	400	59	—	18	59 (100 %)	14·7±1·1
G (bank)	♂=♀	162	66	—	7	15 (22·7 %)	40·7±2·6
Official departments:							
H (*a*) Outdoor	♂	217	71	—	28	71 (100 %)	32·7±2·2
(*b*) Clerical	♂>♀	45	12	—	10	9 (75·0 %)	26·7±4·4
I Clerical	♂>♀	338	101	2	6·6	39 (38·6 %)	29·8±1·7
†J Clerical and executive	♂	199	107	—	6	31 (29·0 %)	53·7±2·4
K Mainly outdoor	♂	716	158	1	24·8	156 (98·7 %)	22·1±3·3
L Outdoor and works	♂	1091	156	—	19·5	137 (87·8 %)	14·3±0·7
M Mainly outdoor	♂	182	31	—	19	27 (87·1 %)	17·0±1·9
N "	♂	3423	538	2	17	538 (100 %)	15·7±0·4
O Outdoor and works	♂	1360	139	—	16·6	139 (100 %)	10·2±0·1
P Outdoor	♂	315	62	—	21	56 (90·3 %)	19·7±1·5
Q Clerical and nurses	♂=♀	965	135	—	11	96 (71·1 %)	14·0±0·9
R Clerical	♀>♂	112	24	—	10	10 (41·6 %)	21·4±2·6
S Clerical	♀>♂	341	46	—	13	32 (69·5 %)	13·5±1·2
Schools (Staffs):							
T (*a*) Elementary	♀=75 % ♂=25 %	2175	330	—	10·5	182 (55·2 %)	15·2±0·5
(*b*) Secondary	♂=♀	496	58	—	9·5	32 (55·2 %)	11·7±1·0
	Totals	17,195	2772	5			16·1±0·2

* Where only one sex is stated it is understood that either (*a*) the particular population consists entirely of this sex, or (*b*) that the other sex is very much in a minority.

† There is some evidence that in this group many cases of colds were included along with cases of influenza.

TABLE II.

Type of work	Total persons at risk	Cases of influenza	Case-rate per cent
(i)Factory workers (groups A,D,F)	3196	403	12.6 ± 0.4
(ii)Shop-attendants(groups B,C)	1700	312	18.4 ± 0.6
(iii)Clerks,etc. and teachers (groups G,H(b),I,R,S,T(a),T(b)	3669	637	17.4 ± 0.4
(iv)Outdoor workers (groups H(a), K,M,N,P)	4853	860	17.7 ± 0.4

The difference in the last column between categories (ii) and (iii) is 1.0 ± 0.7; between categories (ii) and (iv) it is 0.7 ± 0.7; and between (iii) and (iv), 0.3 ± 0.6. None of these differences is significant. On the other hand, there is a significant difference in the case-rate in category (i) from that in the other three categories. This is shown as follows:

Difference in case-rate between categories (i) and (ii) 5.8 ± 0.7

" " " " " " (i) " (iii) 4.8 ± 0.6

" " " " " " (i) " (iv) 5.1 ± 0.6

It would seem, therefore, that in this selected population the workers in factories were less liable to contract influenza than were assistants in large shops, outdoor workers of various types, clerks and office workers, and school teachers. The data are insufficient to offer any explanation of these differences, but it may be mentioned that the three factories are among the best of their respective types in the city. Further, it is of interest to note that the employees in all three factories (A, D, F) had frequently had talks by members of the Health Department on the subject of general hygiene, with special reference to colds and influenza; and also, that these were the only groups of employees in the selected population which had ever had such propaganda, at least within recent years. At the present time, when so much emphasis is placed on personal attention in the prophylaxis of influenza, such observations are not without interest.

Course of the epidemic in the selected population.

The total cases returned for each week from each establishment are set out in Table III. It will be seen that, while many of the groups showed a regular increase of cases to a maximum as the epidemic continued, others did not. In some of these the outbreak appeared to start abruptly. This phenomenon was probably due to the fact that the earlier cases were not reported to the respective authorities, or were reported as other conditions. On the other hand, there is a possibility that many of the workers in these groups were infected outside their place of business, so that the appearance of a number of cases was the first indication of the presence of the disease.

Table III. *Weekly distribution of cases of influenza in selected population.*

Designation of firm or department (see Table I)	Week ending Dec. 3	Dec. 10	Dec. 17	Dec. 24	Dec. 31	Jan. 7	Jan. 14	Jan. 21	Jan. 28	Feb. 4	Feb. 11	Feb. 18	Feb. 25	Totals	Duration of outbreak in weeks
A	6	3	5	8	43	75	68	31	29	15	9	2	—	294	12
B	4	1	3	8	16	48	75	24	16	16	8	4	2	225	13
C	—	—	10	3	4	20	15	13	10	6	4	—	2	87	11
D	3	—	—	1	1	6	23	7	4	2	3	—	—	50	11
E	—	1	—	—	1	5	3	3	4	3	2	1	—	23	11
F	—	—	—	—	3	15	23	11	3	2	1	—	1	59	9
G	1	5	1	1	4	9	15	12	8	3	2	4	1	66	13
H (*a*) and (*b*)	2	—	—	—	2	11	11	13	23	15	4	1	1	83	13
I	1	4	4	4	14	22	21	11	6	9	1	—	4	101	13
J	5	2	10	2	8	10	21	19	15	6	2	4	3	107	13
K	3	2	1	2	11	36	27	28	18	18	5	5	2	158	13
L	3	—	1	3	16	41	36	34	10	7	1	1	3	156	13
M	—	—	—	—	—	6	8	8	5	2	1	—	1	31	8
N	4	12	6	7	6	96	153	97	62	47	19	20	9	538	13
O	2	1	1	2	2	23	36	40	15	9	4	1	3	139	13
P	1	1	—	—	1	6	22	12	13	3	2	—	1	62	13
Q	2	2	4	2	5	28	29	38	10	8	1	4	2	135	13
R	—	—	—	—	—	2	5	4	1	4	4	2	2	24	8
S	—	—	—	—	—	7	17	10	4	5	3	—	—	46	6
T (*a*) and (*b*)	—	1	1	—	1	1	61	145	85	48	27	14	4	388	12
Totals	37	35	47	43	138	467	669	560	341	228	103	63	41	2772	

Mean duration = 11·55 ± 0·29 weeks. σ = 1·92 weeks.

When the cases are summed for each week, however, an orderly sequence is obtained, which shows the course of events week by week for the selected population as a whole. Plotted out as a graph (Fig.1) these totals form a curve of the typical epidemic type. There is no suggestion from this curve that the outbreak was other than a solitary one, showing a single peak. The observed distribution approximates closely enough to a smooth curve to suggest that the course of events in the selectedpopulation gives a fair idea of the course of events in the population as a whole. Fig. 2 shows the same figures plotted on an arithlog scale. The graph suggests that the change from ascent to descent was not nearly so abrupt as Fig 1 indicates, and that it was possibly effected during the week ending January 14th, which was the peak week for the selected population. It is also seen from Fig.2 that, with the exception of the first four weeks, the rate of ascent and descent of the curve was surprisingly smooth.

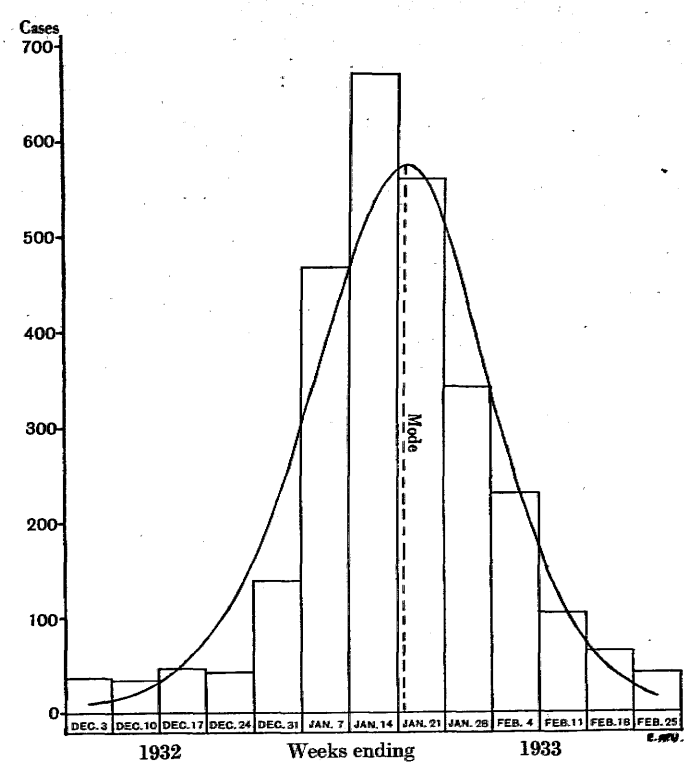


Fig. 1. Incidence of influenza in selected population (Arithmetic scale) Leeds, 1932–33. Type IV.

$$y = 552\left(1+\frac{x^2}{33{\cdot}358}\right)^{-5{\cdot}389} e^{-1{\cdot}048\tan^{-1}\frac{x}{5{\cdot}776}}.$$

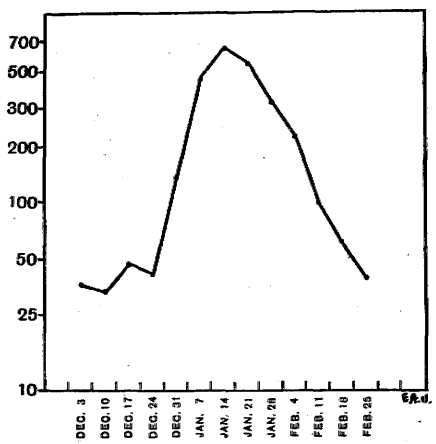


Fig. 2. Incidence of influenza in selected population (Logarithmic scale).

An attempt was made to fit a curve to the data. The value of the criterion, k_2, being 0.0138, i.e., >0 <1, indicated that the appropriate curve was a Pearson's Type IV for which the equation is:

$$y = y_0\left(1+\frac{x^2}{a^2}\right)^{-m} e^{-\nu \tan^{-1}\frac{x}{a}}$$

Inserting the values obtained from the date, the equation becomes

$$y = 552\left(1+\frac{x^2}{33.358}\right)^{-5.389} e^{-1.048 \tan^{-1}\frac{x}{5.776}}.$$

Table IV gives the observed and calculated frequencies week by week:

Table IV

Week ending	Actual cases.	Cases as calculated from curve	Week ending	Actual cases.	Cases as calculated from curve
Dec.3	37	9.2	Jan.21	560	562.0
10	35	21.4	28	341	430.9
17	47	49.4	Feb.4	228	248.2
24	43	109.8	11	103	114.1
31	138	222.7	18	63	45.2
Jan.7	467	387.3	25	41	16.6
14	669	537.9			

It will be readily seen that that curve does not fit the observed distribution very well. The discrepancy is found especially towards the peak, and is considerable. The occurrence of two epidemic waves in the city might explain the higher peak of the curve of observed frequencies, but when we take into consideration the smoothness of the logarithmic curve, this explanation does not seem at all likely. On the other hand, examination of Table III shows that the notification of cases from certain establishments started only in the weeks ending December 31st and January 7th. This suggests that a number of cases occurred during the first four weeks of the outbreak which were never reported as influenza to the heads of the respective establishments .

In the last column in Table III is given in weeks the approximate duration of the outbreak in each establishment. Owing to the fact that cases of influenza probably occurred before the initial limiting date of the investigation and that they continued

to occur in small numbers in the city after the final limiting date, there is a possibility that these estimates sometimes fall slightly short of the actual duration. It is noteworthy, however, that the general weekly death-rate for the city as a whole rose above the average for this period over the preceding ten years only during the week ending December 24th. If a period of two weeks is allowed for lag between onset of disease and deaths of patients, it is seen that the commencement of the chosen period is sufficiently early.

In the selected establishments the duration of the outbreak ranged from six to thirteen weeks (S.D.= 1.92 weeks). This gives a mean duration of 11.55 $\pm$ 0.29 weeks.

Course of the outbreak in the city as a whole.

Influenza in the city as a whole followed closely the course which has been described in the selected population. Table V gives the main statistics for respiratory diseases in the city during the period from the week ending November 5th to the week ending April 1st. The gross weekly death-rates from all causes for the ten years 1922-33, over that portion of each year which corresponded to the period from December 3rd, 1932, to March 11th, 1933, are set out in Table VI. In addition, the actual number of deaths from all causes registered each week are given in detail for the years 1928-9, and 1923-4, since these were "influenza years". The mean weekly death-rate for this period during these ten years was 17.10.

From Table V it is seen that the general death-rate first exceeded this ten-year average during the week ending December 24th. During the week ending December 3rd, the notifications of acute primary penumonia showed an increase, and in the following week (ending December 10th) there was a distinct increase in the notifications of influenzal pneumonia. It is evident that during this period the "influenzal constitution" was becoming manifest.

Table V

City of Leeds	1932, week ending November 5	12	19	26	December 3	10	17	24	31	1933, week ending January 7	14	21	28	February 4	11	18	25	March 4	11	18	25	April 1
General death-rate	13·2	12·3	10·8	15·3	14·8	16·1	15·6	17·6	16·8	19·7	22·9	31·7	31·6	24·4	21·6	16·2	14·3	16·1	16·8	15·6	13·1	13·3
Notifications of:																						
(*a*) Influenzal pneumonia	1	—	1	—	1	6	3	3	3	11	32	31	26	23	6	9	—	3	1	1	1	3
(*b*) Acute primary pneumonia	7	11	14	11	20	34	39	39	22	41	75	43	52	43	29	4	7	12	18	12	12	22
Deaths from:																						
(*a*) Influenza and influenzal pneumonia	1	2	—	—	1	3	2	2	6	14	33	48	48	31	13	9	2	3	5	2	1	2
(*b*) Lobar pneumonia	8	3	6	5	3	3	—	7	2	7	16	11	9	9	3	1	4	—	3	7	1	2
(*c*) Broncho-pneumonia	4	3	2	5	8	5	9	11	13	16	15	26	28	13	10	6	6	3	10	1	3	2
(*d*) Other forms of pneumonia (excluding influenzal)	—	1	1	—	2	4	—	1	3	1	6	3	2	1	1	2	5	1	—	2	1	—
(*e*) Bronchitis	8	3	8	5	9	8	12	8	13	18	19	33	38	27	20	10	9	6	10	7	6	7

Table VI. *Gross weekly death-rates (city of Leeds) from all causes for certain weeks in* 1932–33, *and corresponding weeks for previous years.*

Mean weekly death-rate for period 1922–3 to 1931–2 (10 years)—17·10.

Period		Dec. 3	Dec. 10	Dec. 17	Dec. 24	Dec. 31	Jan. 7	Jan. 14	Jan. 21	Jan. 28	Feb. 4	Feb. 11	Feb. 18	Feb. 25	Mar. 4	Mar. 11	
1932–3	Rate	14·8	16·1	15·6	17·6	16·8	19·7	22·9	31·7	31·6	24·4	21·6	16·2	14·3	16·1	16·8	19·75
1931–2	Rate	11·9	15·3	14·9	16·3	15·8	16·3	16·3	15·3	13·5	17·5	16·2	14·3	17·8	15·9	18·0	15·70
1930–1	Rate	14·6	13·3	15·6	15·9	16·0	17·8	16·2	17·0	16·2	16·7	18·2	18·2	19·2	18·0	23·1	17·14
1929–30	Rate	14·7	11·1	14·1	12·9	11·6	14·4	16·6	14·2	15·0	11·6	14·0	14·4	12·2	16·1	17·8	14·05
1928–9	Deaths	119	123	136	169	167	174	185	164	142	177	223	328	625	539	374	3645
	Rate	12·9	13·3	14·7	18·3	18·1	19·1	20·3	18·0	15·5	19·4	24·4	35·9	68·4	59·0	41·0	26·44
1927–8	Rate	14·1	13·9	13·9	16·5	16·6	17·5	16·1	14·4	14·3	13·7	13·3	16·7	15·8	16·4	13·8	15·06
1926–7	Rate	14·2	17·0	13·2	12·9	15·8	14·8	16·2	15·5	16·0	17·7	20·1	22·5	20·6	23·0	21·8	17·43
1925–6	Rate	18·7	19·8	17·3	15·7	19·6	16·7	14·3	15·6	15·3	18·4	13·8	19·1	14·8	13·9	17·1	16·60
1924–5	Rate	12·7	11·4	14·1	12·0	12·9	15·5	14·3	14·6	14·1	19·6	15·5	16·3	16·7	16·8	14·3	14·72
1923–4	Deaths	110	142	127	118	146	128	133	180	145	140	154	175	217	270	346	2531
	Rate	12·3	15·9	14·2	13·2	16·3	14·2	14·8	20·0	16·1	15·5	17·1	19·4	24·1	30·0	38·4	18·80
1922–3	Rate	11·9	13·2	15·5	13·1	13·8	14·3	15·2	13·1	13·2	14·8	15·0	17·6	15·4	17·0	15·3	14·56

The death-rate showed no very marked changes until the beginning of January, when there was an evident upward tendency, the peak being reached during the week ending January 21st. These features fit in with the general tendency shown in the selected population, except that, as is to be expected, the death curve shows a slight time lag. Early in February the general death-rate fell rapidly below the ten-year average.

Sir George Newman (1920) stated that an increased incidence of pneumonia and bronchitis not infrequently precedes an increased incidence of influenza, and that "the true curve of influenza mortality can apparently be constructed by combining with it the simultaneous waves of penumonia and broncho-pneumonia." In Leeds the deaths from influenzal pneumonia reached their maximum (forty-eight in each week) during the weeks ending January 21st and 28th. In each of these two weeks the deaths from broncho-pneumonia and from bronchitis reached their maxima, which were considerably above the normal range. On the other hand, the deaths from lobar pneumonia showed no very marked increase. Pari passu with these increased deaths, the notifications of acute primary pneumonia reached their maximum (seventy-five) in the second week preceding the occurrence of the maximum number of deaths from broncho-pneumonia, from bronchitis and from influenza and influenzal pneumonia respectively - that is, during the week ending January 14th. Van Loghem (1928) showed that the curve for influenzal pneumonia followed broadly that from other respiratory affections. It would seem, nevertheless, that the influenza virus, directly or indirectly, was responsible for a large number of these deaths which were registered as "broncho-pneumonia" or as "bronchitis".

Another interesting point which is seen in Table V is that, while the deaths from all respiratory diseases under consideration had reached minimal figures by April 1st, the notifications of acute primary pneumonia, which had fallen to very low figures during the second half of February, had again shown a rise, and by April 1st had reached significant figures. Whether this was

simply a trailer in the wake of the influenza epidemic, a "sympathetic rise" of an entirely distinct condition; or whether it was due to the continued notification of non-fatal cases of influenzal pneumonia, cannot be determined.

The city of Glasgow suffered from a similar outbreak of influenza about the same period, and for this the main statistics have been given by Dr. A. S. M. Macgregor (1933). From his figures it is evident that the infection became epidemic in Glasgow probably during the last week in November - that is, practically simultaneously with the Leeds outbreak. The peak of the Glasgow epidemic evidently occurred during the week ending December 24th, when 117 notifications of influenzal pneumonia were received: the peak death-rate for this condition occurred in the following week. In Leeds, on the other hand, the peak of the epidemic did not occur until about January 21st - that is, approximately four weeks after the Glasgow peak. Although the figures are given only up to January 28th, they suggest that the Glasgow epidemic had practically terminated by the time that the Leeds epidemic had developed its maximum force.

Pearl (1924), in his studies on the 1918-19 influenza epidemic as it affected different American cities, devised a series of six epidemicity indices ($I_1 \ldots I_6$) which are of value in comparing the explosiveness of outbreaks in different localities. For our present purpose the index I_6 is most suitable. The expression is:

$$I_6 = \frac{P-M'}{T'}$$

where P denotes the maximum peak mortality observed during the duration of the epidemic; and T' is the number of weeks which elapsed between (a) the date when the mortality curve first passed outside the range of fluctuation exhibited by the curve between a week preceding the epidemic rise by about six or eight weeks, and the end of the week immediately preceding the epidemic rise of the curve, and (b) the week in which the mortality curve attained its first epidemic peak. M' is the mean death-rate in the period noted under (a) above.

In the present outbreak the available figures for Glasgow and Leeds respectively are as follows:

November				December					January			
5.	12.	19.	26	3.	10.	17.	24.	31.	7.	14.	21.	28
12.4	14.9	15.2	14.9	14.6	18.2	20.0	28.7	27.3	19.4	18.4	18.0	18.0
13.2	12.3	10.8	15.3	14.8	16.1	15.6	17.6	16.8	19.7	22.9	31.7	31.6

In both the Glasgow and the Leeds outbreaks it has already been stated that the commencement can in each case be taken as the beginning of the week ending November 26th. The figures available for the calculation of M' are rather few. With the available data, however, the indices give:

$$I_6 \text{ (Glasgow)} = \frac{28.7 - 14.2}{5} = 2.9$$

$$I_6 \text{ (Leeds)} = \frac{31.7 - 12.1}{8} = 2.4$$

It is thus seen that despite the much longer rise of the Leeds outbreak, in epidemicity it did not fall far short of the Glasgow outbreak.

Age and sex distribution of deaths.

Of the 228 patients who died in the city between the beginning of the week ending November 5th, 1932, and the end of the week ending April 1st, 1933, 226 were residents in the city; the other two were non-residents who died in city institutions. The age and sex distribution of the 226 cases is as follows:

Ages	Under 1	1-	2-	5-	10-	15-	20-	25-	30-	35-	45-	50-	55-	60-	65-	75-85-	TOTALS
Males	4	3	2	-	-	2	2	2	3	12	9	12	14	7	17	18 3	110
Females	1	1	2	2	1	-	3	2	2	19	11	9	7	8	21	24 3	116
TOTALS	5	4	4	2	1	2	5	4	5	31	20	21	21	15	38	42 6	226

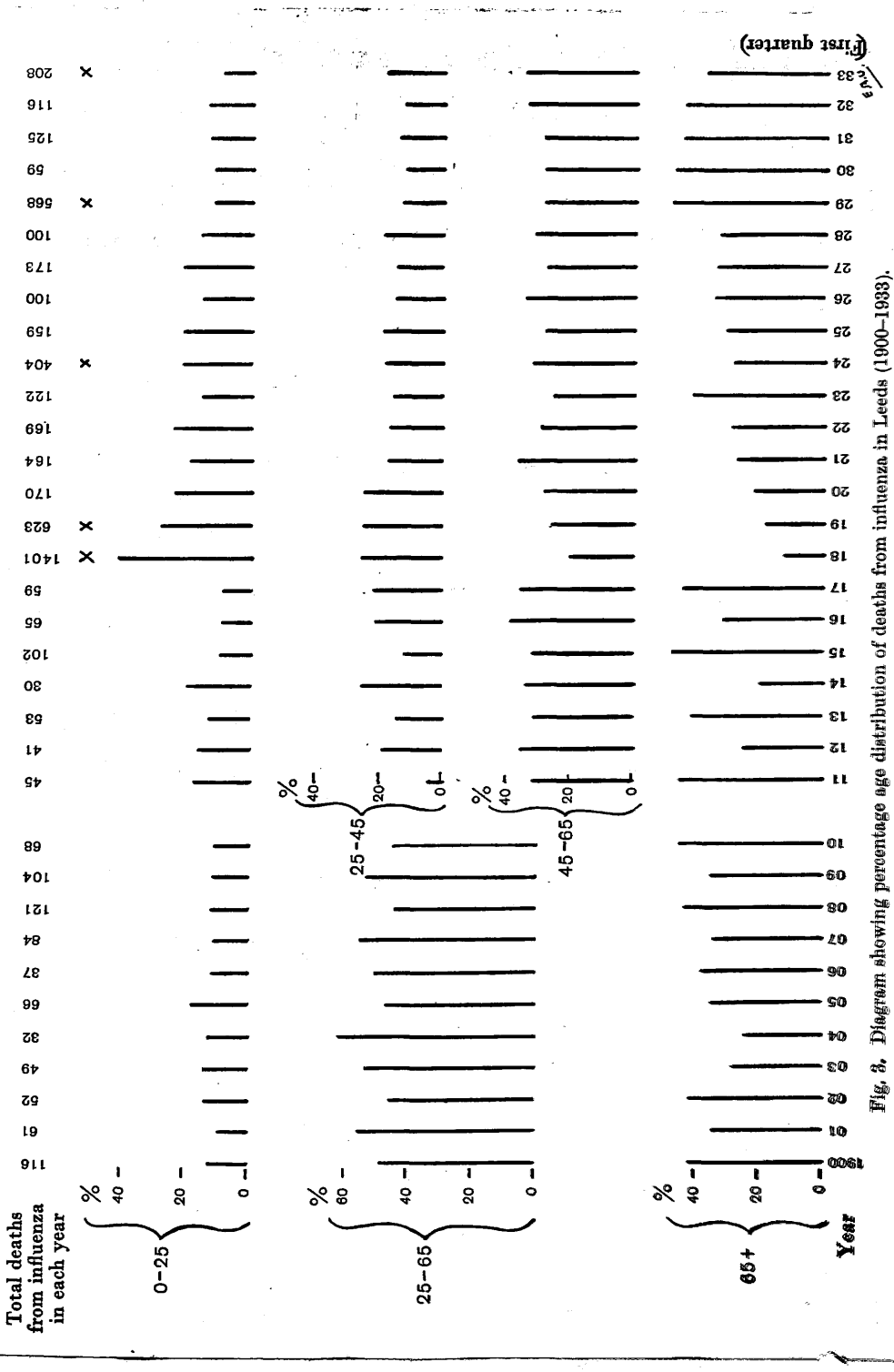


Fig. 3. Diagram showing percentage age distribution of deaths from influenza in Leeds (1900–1933).

From the information supplied by the death returns it seems probable that there were no marked differences in the sex distribution of the disease.

Since the pandemic of 1918 the age incidence of influenza in different outbreaks has been a feature of considerable importance. The late Dr. T. H. C. Stevenson (1919) showed that in London the age incidence of influenza had been more or less constant from 1890 until 1918. During the July outbreak of 1918 there was an abrupt change, the emphasis falling heavily on young adults, and persons over 45 years being relatively little affected. This peculiarity was even more marked in the October outbreak of 1918. It was suggested in the Ministry of Health report on the pandemic that the toll taken at the young adult ages of life was without any known West European or North American precedent (Ministry of Health Report, 1920, p.40).

The experience of Leeds since 1900 is shown in figure 3. The method used is that employed by Stevenson to demonstrate the age incidence in the London outbreak from 1890 to 1918. The peculiar age incidence of the 1918-19 outbreaks is evident. An interesting feature is, however, that whereas the transition to the 1918-19 grouping was abrupt, the return to the status quo has been gradual. These features are most clearly seen in the age groups embracing the extremes of life - 0-25 years, and 65 years and over. The figures at the top of each column give the total number of deaths on which each set of percentages was calculated. The years 1918, 1919, 1924, 1929 and 1933 are marked X, since in these years influenza was evidently epidemic. (As the majority of the deaths from influenza during the epidemic which is under consideration occurred in the first quarter of 1933, for the purpose of discussing mortality figures this year has been considered to be the epidemic year in preference to 1932). From the diagram it would appear that the normal balance of ages affected was reached in the 1929 epidemic. It is also seen from the diagram that these epidemic years fit in with the theory of

the gradual return to the status quo better than do some of the inter-epidemic years. This feature may be due to the small number of deaths in some of these inter-epidemic years, or, on the other hand, some unknown factor may be responsible. At all events, there seems to be some justification for the assumption that the 1918-19 epidemic was not an isolated peak, entirely unrelated to the main waves of epidemic influenza which preceded and followed it. Granted that its onset was catastrophic and precipitated by influences which are not yet understood; in its wake there remained changes in the "influenzal constitution" which as far as the age incidence is concerned, in round figures were effective in the city of Leeds for a period of ten years.

Distribution of deaths in the city.

To obtain some idea of the mode of spread of the epidemic in the city as a whole the information furnished by the death certificates has had to be used, since no definite statement regarding morbidity was possible. This information is summarised in line 4 of Table V.

Each death from influenza which occurred in the city was plotted according to sex on a time-ward diagram. (The resulting diagram did not warrant reproduction). It shows that generally the earliest deaths occurred in those wards of the city in which the mortality was afterwards highest. A number of deaths occurred in every ward, so that the infection certainly affected the city as a whole. It is noteworthy, however, that in these wards in which the mortality was greatest, the first deaths occurred earlier than in the other wards.

Table VII gies the density of population (persons per acre) in the twenty six wards of the city, which are arranged in descending order of density. (The density is given to the nearest whole number, and in calculating these densities allowance was made for the various parks and open spaces in each ward). The Table also gives the actual number of deaths from influenza in each ward from the week ending November 5th, 1932 to the week ending April 1st, 1933;

these figures are also shown as death-rates per 10,000 persons living in each ward.

From the table it is evident that there was no direct association between the incidence of death as a result of influenza and the density of the population in the ward. An important objection is that we are here dealing, not with actual cases, which were undoubtedly numerous, but with deaths, which were so few as not to warrant any satisfactory deduction being made from such evidence. Nevertheless, it may perhaps be said that the absence of any apparent association between density of population and death-rate from influenza bears out the negative results which were obtained for the 1918-19 epidemic. As a result of a minute analysis of the available data the conclusion was drawn that it was impossible that domestic overcrowding could be deemed a principal factor in the spread of epidemic influenza.

More interesting results are obtained when the ward death-rates are incorporated on a ward map (Fig.5). It is seen that the wards with the heaviest death-rates are arranged in an inner ring round the centre of the city. Surrounding this inner ring is an intermediate zone, and on the outskirts of the city lie the wards in which the deaths were fewest. It might be thought from the map that ward 6 does not conform to this arrangement. It will be seen, however, that the innermost point of the boundary of this ward lies very near the centre of the city, and that the outermost boundary forms parts of the boundary of the city itself. In this large area that part which is nearest to the centre of the city is very congested; the outer part is made up entirely of a residential area. In actual fact, however, only one of the ten deaths which were registered as having taken place in this ward occurred in what might be termed a "congested area".

The map (Fig.5) also shows that ward 1, which forms the hub of the city, and ward 23, which forms part of the inner ring, both showed only a few deaths. No significant deduction can, of course, be made from the study of deaths alone; yet it is strange

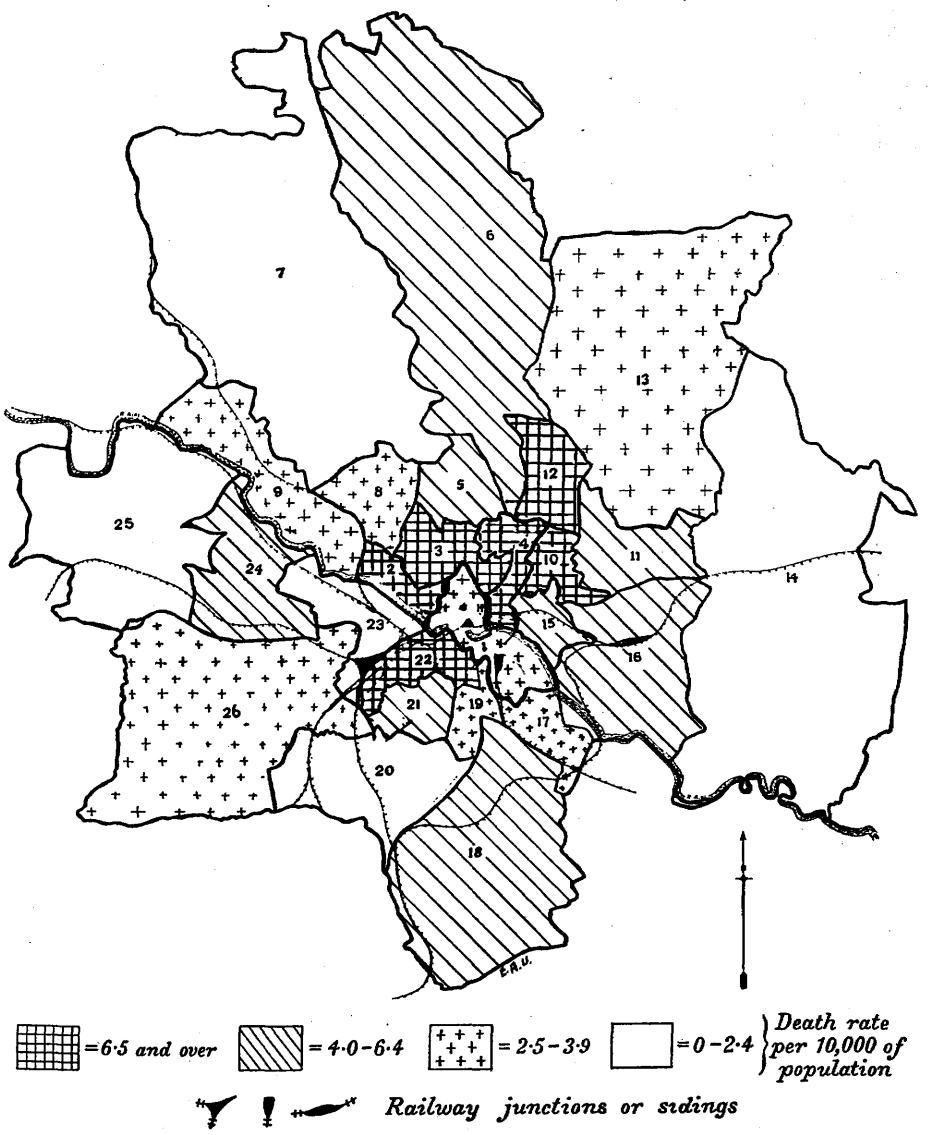


Fig. 4. Map showing death-rates from influenza in different wards of the city of Leeds.

that these two wards showed so few deaths in comparison with the heavily affected wards which lie adjacent. The few deaths in ward 1 may perhaps be explained by the fact that this ward contains the business quarter of the city, and that, while there is a resident population of 15,778 persons, many of these are housed in fairly modern flats over business premises. The case of ward 23 is more difficult to explain away. It should be mentioned, however, that the north boundary of this ward is constituted by the River Aire. The main railway lines run close to the south boundary of the ward, and extensive railway sidings are situated on a large portion of the actual ward boundary. Stocks (Stocks and Karn, 1932) has shown that whooping-cough in London has been known to respect natural and artifical boundaries of this type; and it may be that with influenza, despite the traditional rapidity of spread and the posting effect, some similar but unknown factors are at work. It is perhaps significant that the first death in ward 23 occurred on January 12th, by which time practically every other ward in the city had suffered several deaths.

From a consideration of the map and the time-ward diagram it would appear that the influenza outbreak showed its effects earlier and more severely in the centre of the city, and that from there it extended outwards with decreasing "potential" towards the outskirts.

It is interesting to note that the figures in Table VII suggest no apparent association between the number of deaths from influenza and the average number of persons per room in each ward.

TABLE VII.

WARD No.	15	19	10	2	4	5	3	17	21	22	12	23	8	11	1	24	9	16	20	25	18	26	13	7	6	14
DENSITY OF POPULAT.	96	95	87	84	69	60	54	51	51	49	42	38	35	32	29	21	19	16	14	9	9	7	5	4	3	3
PERSONS PER ROOM.	1.3	0.92	1.20	1.03	1.00	0.90	0.74	1.14	0.92	1.21	0.67	0.98	0.64	0.77	1.14	0.82	0.84	1.00	0.76	0.86	0.98	0.89	0.59	0.67	0.63	0.76
ACTUAL DEATHS.	11	6	18	17	14	10	15	7	8	13	14	3	6	11	5	7	5	11	3	2	9	7	5	4	10	3
DEATH RATE PER 10,000.	4.5	3.3	7.8	8.7	6.6	5.3	6.5	3.8	5.8	7.1	7.1	1.5	3.0	5.6	3.2	4.1	2.6	5.1	2.0	1.1	4.5	3.8	3.3	2.2	6.4	2.1

Association between respiratory tuberculosis and influenza.

Many writers have called attention to the close association between influenza and other acute respiratory affections, but the possible connection between influenza and tuberculosis is less frequently commented on. Table V gives the weekly deaths from lobar pneumonia, broncho-pneumonia, and from chronic bronchitis during the period of the influenza epidemic. In each case there is an increase in the number of deaths from the respective conditions, and this increase, and the subsequent decline, proceed parri passu with the increase and decrease in the deaths from influenza and influenzal pneumonia. Records of various outbreaks, including the London epidemic of 1918-19, show that there is an increase in the number of deaths from phthisis during an influenza epidemic, and that this increase tends to reach its maximum about the time of the peak period of the influenza outbreak. It is mentioned in the Ministry of Health Report (1920, p.26) that such records show that "the infection of influenza, if not specially attracted by the phthisical, is at least particularly deadly to them".

The position with regard to tuberculosis of the respiratory tract in Leeds is shown in Table VIII.

Table VIII.
No. of registered deaths from tuberculosis of respiratory system

Year	1st quarter	2nd quarter	3rd quarter	4th quarter
1926	158	125	86	108
1927	154	126	97	80
1928	125	136	75	117
1929	180	130	102	96
1930	135	110	85	102
1931	130	103	100	106
1932	109	109	75	93
1933	129	107	77	99
Mean deaths in each quarter (8 years)	140	118	85	100

The influenza epidemic of 1929 reached its height during February and March of that year, and by the end of March it had practically died down. It is seen from the table that not only were the deaths from tuberculosis of the respiratory system

distinctly above the average during the first quarter of 1929, but that they were actually the greatest registered in any quarter of the years 1926-33. On the other hand, the number of deaths registered as due to respiratory tuberculosis during the fourth quarter of 1932 and the first quarter of 1933 were distinctly below the average. These statements seem to hold even when the natural decrease in the death rate from pulmonary tuberculosis is taken into account. This unusual feature is difficult to explain. Taking into account the very low case-mortality rate in the selected population, it is possible that the particular type of influenza which was prevalent was of a non-killing variety and of a mild nature which was not prone to lead to a fatal flare-up of an already existing tuberculous condition. If this be the case, then the actual number of deaths registered as due to influenza in the city must have represented a very extensive epidemic of this mild form of the disease.

Meteorological conditions.

The association between weather conditions and the incidence of respiratory infections has been examined on several occasions. During the 1919 influenza epidemic a report issued from the Direction d'Hygiène suggested that in Paris a sharp fall in temperature coincided with a rise in the curve of mortality, and that these periods were associated with a period of drought. This feature was examined in detail in the Ministry of Health Report, and it was shown that, when the death-rates in the three waves were plotted along with accumulated day-degrees of temperature, the onset of each wave of the epidemic was coincident with a rapid fall of temperature. It was further shown that, when the correlation between the pneumonia death-rate and the temperature during the previous week was worked out over a number of years, there was a significant increase in the correlation between these two variables during the 1918-19 epidemic. Dr. Matthew Young (1924), in a very extensive analysis, concluded that there was a definite inverse relationship

between the prevailing temperature and the mortality from respiratory diseases in children under five years of age. It is not intended in this communication to discuss the effects of meteorological conditions; but since any report of an outbreak of repiratory disease would be incomplete without some description of these factors, the conditions which prevailed during the period of the outbreak will be briefly described.

The prevailing meteorological conditions during the period of the epidemic are shown graphically in Fig. 5, together with the notifications of influenzal pneumonia and primary pneumonia taken together, and the incidence of influenza in the selected population. Exception may be taken to the inclusion of cases of primary pneumonia with cases of influenzal pneumonia, especially in view of the fact that Macgregor (1933 a) has shown that there are grounds for considering lobar pneumonia to be a disease which is itself primarily associated with meteorological conditions. In favour of the method adopted in the figure, however, is the fact that during the epidemic there was an increase in cases of primary pneumonia at a time when this disease is not usually very prevalent in the city, and that the increase was associated quantitatively with the increase of influenzal penumonia. In the light of these facts it would seem that a true idea of the incidence of pneumonia, arising directly from the prevalence of influenza in the city, is obtained by combining the deaths from the two forms of pneumonia.

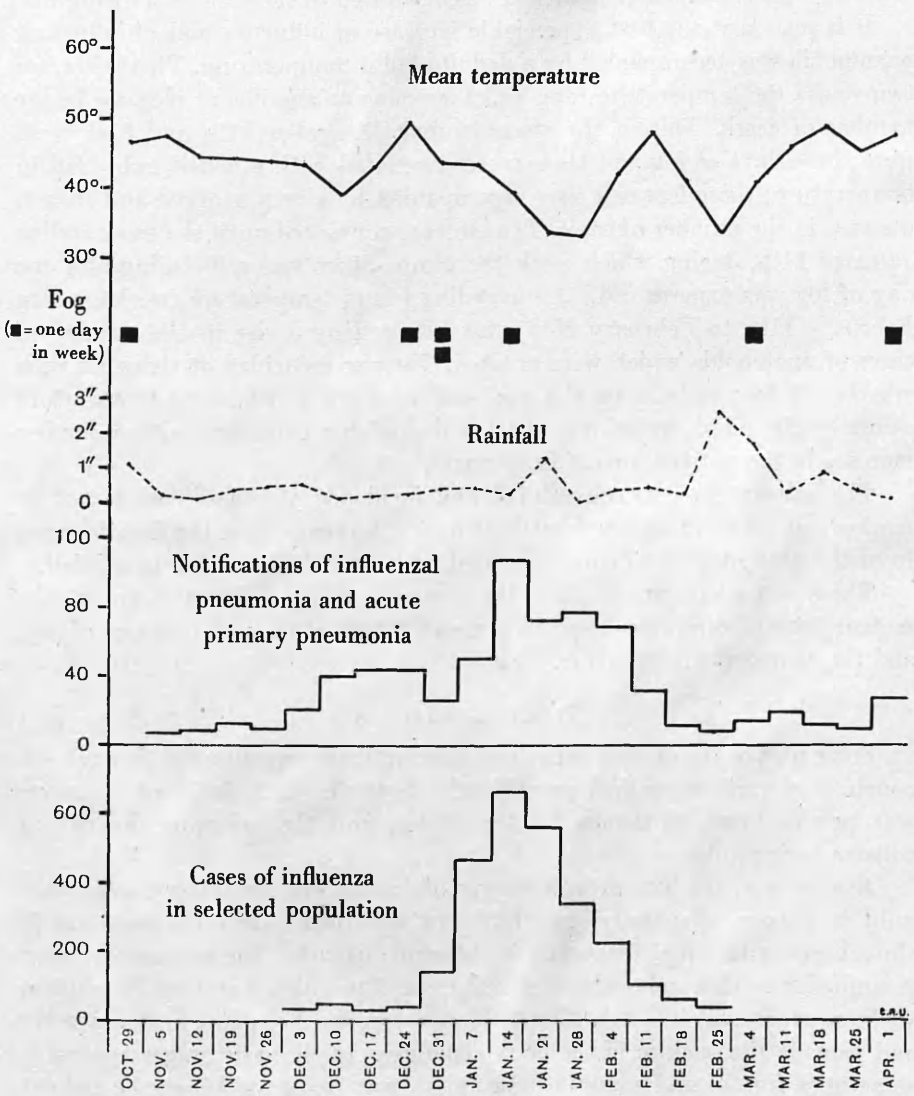


Fig. 5. Diagram showing meteorological conditions in relation to influenza.

A curve based on influenzal pneumonia must necessarily show some lag so far as influenza itself is concerned, and probably a truer idea of the state of affairs is obtained by using the figures for influenza prevalence in the selected population. These figures are incorporated in the form of a histogram.

It is seen that the first appreciable increase of influenza and of influenzal penumonia was accompanied by a definite fall in temperature. Thereafter, for two weeks, the temperature rose, and there was no significant increase in the number of cases. During the weeks ending December 24th and 31st there were three days of fog and these were associated with a considerable fall in temperature; these features were accompanied by a very marked and sudden increase in the number of cases. The increase continued until the week ending January 14th, during which week the temperature was still falling, and one day of fog was experienced. A succeeding fall of temperature (weeks ending February 11th to February 25th) was followed by a rise in the number of cases of pneumonia which were notified. The rise coincided at the start with one day of fog; and during the week ending April 1st, when the temperature showed only slight variations, another day of fog coincided with a further increase in the notifications of pneumonia.

The association between rainfall and incidence of the disease is not so marked. It seems to be worth while to notice, however, that the first decrease from the peak of the epidemic coincided with a marked increase in rainfall.

These remarks suggest that there was some association between the meteorological conditions, especially mean temperature and presence of fog, and the course of the epidemic.

Clinical features.

Some idea of the clinical aspects of the outbreak was obtained through the courtesy of various medical practitioners in the city. A series of questions was put to these gentlemen by the writer, and the following description collates their replies.

Respiratory type. The prevalent type of disease was respiratory and rather mild in nature. Generally, two forms of respiratory disorder were found, though not with equal frequency in different districts. The commonest form resembled the classical picture of influenza. The onset was usually sudden, without prodromal features. The patients complained of depression, shivering and pains in the back or in the body generally. In the early stages headache, sometimes frontal and sometimes occipital, was frequent. When the patient was first seen by the practitioner the temperature was high - often 102 or 103°F. These acute features lasted two to five days; thereafter, the temperature fell suddenly and profuse sweating brought relief from the symptoms. In many cases the disappearance of acute symptoms was followed by debility or depression which lasted for about a week.

The other form of the respiratory type appears to have been mainly tracheitis. The onset was again sudden, and an important feature was sternal pain. The throat was often raw and injected, and sputum was sometimes present. In certain districts of the city this seems to have been the prevalent form of the disease.

In both forms physical signs were few. If present, they were mainly of the nature of bronchial signs, such as moist rales, which were found at the lung bases only at the start of the disease.

Gastric type. Gastric cases were infrequent. Only one of the practitioners who was consulted had seen more than three cases of this type of the disease in his practice during the whole course of the epidemic, and several had not seen any gastric cases. On the whole it seems that the gastric type tended to appear more towards the end of the outbreak. The condition simulated acute gastritis, with or without diarrhoea, and a common symptom was pain in the epigastrium just below the xiphisternum. Abdominal tenderness was usually absent. In a few cases diarrhoea was apparently practically the only symptom.

Complications. Apart from depression or prostration, which was noticed above to have been quite common, complications and sequelae were not frequent, and were mainly of two types:

(a) Influenzal pneumonia. None of the practitioners who were consulted had seen more than a few cases of this condition, so that the total notified cases (discussed previously) must have represented a widespread incidence of influenza. The clinical features of the influenzal pneumonia presented nothing unusual.

(b) Acute otitis media. This complication is recognised as being fairly frequent after influenza. In the present outbreak it was apparently much commoner than pneumonia. It affected both adults and children, but the complication was more frequent among children. Paracentesis was frequently not required.

(c) Other complications. Some practitioners had cases of cardiac complications - such as tachycardia, palpitation, "D.A.H." following the acute attack. In other districts throat conditions supervened, and in one district at least acute skin conditions - such as seborrhoeic eczema - were not infrequent after, if they were not actually complications of, attacks of influenza.

It is of interest to compare the salient clinical features of the Leeds outbreak with the symptoms found elsewhere. In the Glasgow outbreak (Macgregor, 1933) the disease was apparently very similar to that seen in Leeds. The gastric type was not common, and the illness usually began with a sharp onset, followed by pyrexia with general and joint pains, which lasted a few days. Dr.Macgregor notes that the acute symptoms were apt to be followed by cough and general debility. On the other hand, Marriott (1933) described forty-seven cases of influenza in the staff of the Middlesex Hospital, London. Of these seven (15 per cent) were gastric cases. The respiratory cases very frequently complained of sternal pain, and the fauces were sometimes inflamed. Chest signs were few. The Leeds outbreak therefore seems to have combined the clinical features of both the London and the Glasgow outbreaks.

Multiple cases in households. Most of the practitioners

consulted had seen several examples of this occurrence. Commonly the infection appeared to be from husband to wife, or vice versa. These cases followed each other at intervals which varied from two to four days. In one remarkable instance a mother developed the disease first, then two daughters and a son were attacked successively; all of these persons developed influenzal pneumonia.

Duration of incapacity for work.

In respect of the duration of incapacity the replies of the various practitioners could be divided roughly into two groups. Those practitioners whose clientèle consisted largely of clerical, administrative, and mental workers, stated that their patients had generally returned to their duties after an absence of about a week or ten days. On the other hand, practitioners who attended many persons of the artisan class stated that their patients were generally off work for more than ten days: the period was often a fortnight and sometimes three weeks.

This question was also considered from the point of view of the selected population. Particulars of two classes were furnished by the heads of the respective establishments: (a) the number of persons under their control who, owing to influenza, were off work for more than seven days; (b) the mean period of absence for all the employees which their returns had shown as having suffered from influenza. These particulars are set out in columns 6 and 7 of Table I.

For the purpose of comparing the duration of incapacity in the different types of workers, the same groups of establishments were taken as previously (see Table II). The mean period of absence for each group was calculated from the total cases in each establishment and the average period of absence for these cases. The results are given in Table IX.

Table IX.

Type of work	Mean duration of absence in days.
(i) Factory workers (groups A,D,F)	14.8
(ii)Shop workers,(groups B,C)	8.4
(iii)Clerks,etc.,and teachers (groups G,H(b),I,R,S. T(a), T(b))	9.6
(iv) Outdoor workers (groups H(a),K,M,N,P)	19.7

This enquiry therefore bears out the tentative statements made by individual doctors that brain workers and persons in the middle classes were off duty for a shorter time than were members of the artisan and poorer classes. From the above table it is seen that the difference is considerable. This difference may possibly be associated with the better care andz attention which members of the middle classes presumably receive, so that they recover more quickly. Another alternative is that the members of the artisan class feel that when they return to work they will be called upon to undertake heavy duties, often in the open air, without any preliminary period in which to become thoroughly well. A third alternative, and one which must definitely be given consideration, is that the members of the artisan class feel little responsibility towards their work and their employers, and that they therefore do not feel called upon to return to duty until their doctors take the initiative, and sign them up as fit. This alternative probably does not apply to all members or all groups of members of the artisan class. But, that the possibility is worth consideration is shown by the fact that certain practitioners are inclined to hold this view. As one doctor, whose practice embraces a large working-class clientèle, expressed it - "They will stay off work as long as I will let them". At any rate it would appear that a measure of the severity of any influenza outbreak which was based on the duration of incapacity for work of only one social section of the community would probably not be of much value.

Summary and conclusions.

1. This paper deals with the epidemiology of an outbreak of influenza which started in Leeds late in November, 1932 and continued until the end of February 1933.

2. Returns of the actual cases which occurred each week in certain official departments and large firms made possible the investigation of a selected population. This consisted of 17,195 persons, and among its members there were 2772 cases of influenza with five deaths. The case-rate was therefore 16.1 ± 0.2 per 100 of the selected population. It is shown that in this population factory workers were less liable to contract influenza than were assistants in large shops, outdoor workers, clerks and office workers, and school teachers; and it is suggested that propaganda relating to respiratory infections may have had some effect in lessening the incidence of the disease among the workers in these factories. The mean duration of the outbreak in the selected establishments was 11.55 ± 0.29 weeks

3. The total cases which occurred each week in the selected population were plotted as a graph; the resulting curve was of the typical epidemic type. A Pearson's Type IV curve was fitted to these data. The available evidence points to the fact that the outbreak consisted of a single wave.

4. From the data for the city as a whole it is shown that the Leeds outbreak took approximately twice as long to reach its peak as did the Glasgow epidemic, though both outbreaks started practically simultaneously. Nevertheless, in actual epidemicity, the Leeds outbreak did not fall far short of that which occurred inGlasgow.

5. An investigation of the age distribution of deaths from influenza in Leeds since 1900 shows that, though the 1918 epidemic produced a sudden change in the age distribution, in that there was an unprecedented toll of young adult life, the return to the status quo was not sudden, as is often assumed, but was effected gradually over a period of about ten years.

6. It is shown from an investigation of influenza deaths in different parts of the city that there was no direct association between these deaths and the density of the population in the wards in which they occurred. Neither was there any association between

the number of influenza deaths and the average number of persons per room in each ward. From a study of the distribution of the deaths in time and space it appeared that, with certain exceptions discussed in the paper, the outbreak started in the centre of the city and extended outwards from the centre with decreasing potential.

7. An investigation of the meteorological conditions suggested that there was a definite relationship between a fall of temperature, especially if accompanied by fog, and an increase in the incidence of influenza and of pneumonia.

8. The clinical features of the outbreak are discussed fully in the text. The disease was essentially of the respiratory type. Two forms of this were in evidence, (a) a form resembling the classical picture of influenza, and (b) a form in which the predominant feature was tracheitis. The gastric type of influenza was not common. Complications were not very frequent, but the commonest were influenzal pneumonia and acute otitis media. The duration of incapacity for work varied according to the social status of the individuals. Brain workers and persons in the middle classes were generally off duty for about nine days. On the other hand, the duration of incapacity in artisans and factory workers was usually about a fortnight or three weeks.

9. From the evidence which is adduced in this paper as a whole, including a consideration of the tuberculosis statistics of the city, it would seem that, though the outbreak of influenza in Leeds during the winter of 1932-3 was of a mild and non-killing type, infection was widespread, and the total incapacity must have resulted in a considerable economic loss to the community.

P A R T II.

SECTION (b)

THE HISTORY OF THE 1832 CHOLERA EPIDEMIC IN YORKSHIRE.

The history of Asiatic cholera in this country is interesting, not only because the disease was entirely unknown to British practitioners before the "thirties" of last century, but also because of the light which the first outbreak threw on the glaring social and sanitary abuses which existed a hundred years ago. It is true that the profession had been warned that the disease was epidemic in the East, but only those practitioners who had had Indian experience could form any conception of the possible ravages of cholera. On the social side, the epidemic in Britain was directly responsible for the reporting of conditions which had hitherto been regarded as the unalterable lot of the poor - conditions which were regarded by most of the rich as belonging to a world with which their own contact was of the slightest.

Asiatic cholera became epidemic in the East in 1817, and the disease continued to reap a terrible toll of life for many years. It first appeared in this country at Sunderland in August 1831. The approaching outbreak was not unexpected; on June 21st, 1831, a Board of Health was gazetted in London in anticipation of the event, and it was suggested that local Boards of Health should also be set up. An excellent account of the working of such a Local Board was given by Shapter (1), and the writer previously commented upon the administrative difficulties which beset the path of these boards (2).

In the annals of the 1832 cholera epidemic in this country attention is not usually focussed on the ravages of the

disease in Yorkshire. Yet in this county there were 1,960 deaths (3). This neglect is not surprising, for some of the published descriptions of the individual outbreaks are very rare. Leeds especially has suffered from omission at the hands of the historian. Yet the township of Leeds was severely affected. The total deaths in Leeds were almost half of the deaths for the whole county, and this total was approximately equal to the deaths in Manchester and in Plymouth, and it was exceeded only by the deaths in London, Liverpool and Newcastle.

It is probable that the first death from cholera in Yorkshire occurred at Doncaster on January 7th, 1832 . Branston* (4) states that two sailors arrived at the town on the evening of January 6th. They had been to Stockton, Hull, Leeds, and Wakefield. They stayed overnight at a lodging house and next morning one of them was seized with symptoms which were very suspicious of Asiatic cholera: he died within a few hours. No outbreak followed this incident. There is no doubt, however, that in Doncaster the occurrence caused considerable perturbation. The writer previously commented (5) on a popular pamphlet which was published in that town and which was dated January 21st, 1832. The pamphlet is entitled "Cholera morbus. Precautions, Preventives and Remedies, compiled by a Clergyman for the use of his Parishioners". In the main, the advice followed the official instructions which were issued from time to time by the Central Board of Health.

In Yorkshire generally various cases were reported during February and March; these were later shown not to be cases of true cholera. In the first week of April, however, the disease broke out at Goole, and almost simultaneously at Hull. On April 6th a man in Hull developed suspicious symptoms, but he recovered (6). On April 13th a man died in a south-western suburb of Hull, and both

*In the literature of the period this name occurs both as Branston and "Branson", cf. references (4) and (9). The person referred to was the Chairman of the Doncaster Medical Board of Health

these cases were marked "cholera" by the Central Board of Health, which considered the port infected as from April 6th. It is probable, however, that the first authentic death from cholera occurred on April 13th. Later on it was discovered that five days before a man had been helped ashore from a steam-packet which had come from London, and this man died on April 15th. Subsequent enquiries showed that he had been admitted to St. Olave's Cholera Hospital in London on March 29th, suffering from a severe attack of the disease and that he was discharged "convalescent" on April 6th. It is tempting to assume that this man was the means whereby Asiatic cholera was introduced into the port of Hull. From Alderson's (6) description it would appear that the disease did not produce a great number of deaths until June when it broke out again with renewed virulence in a poor north-western suburb.

From the descriptions in the literature it is evident that the disease became epidemic throughout Yorkshire about the end of May or the beginning of June 1832. The disease appeared at Leeds on May 28th (7); at York on June 2nd (8), and at Doncaster on the same day (9); at Cawood, near Selby on June 5th (10). A death which was probably due to cholera, was that of an immigrant into Sheffield on June 14th. It is fairly certain, however, that the Sheffield epidemic did not commence until July 8th. At Wakefield it affected the House of Correction on June 23rd, and in a mild type it persisted there for a long period (12). While the epidemic lasted it caused the deaths of 1,960 persons in the county. The most important places which were affected were: Leeds 702 deaths; Sheffield 402; Hull 300; York 185; Wakefield 62 deaths. Bradford, Goole, Selby, Rotherham, Whitby and Doncaster had each fewer than forty deaths. Where so many districts were affected, it is obviously impracticable to deal with all the foci of disease. In this paper, therefore, emphasis will be placed mainly on the outbreaks at Leeds, Hull, and York. These outbreaks were all fairly fully described, and, in addition, the reports from Leeds are especially valuable in throwing light on the sanitary conditions which obtained at the period. The Sheffield outbreak will not be dealt with

in detail, since Stokes (11), in a book published in 1921, made a very valuable collection of the contemporary writings which deal with this outbreak. These writings were mainly official reports and extracts from the lay press. Although they do not add considerably to our knowledge of the medical or epidemiological aspects of cholera, they are very valuable in that they describe fully the administrative procedures carried out in these days.

The essential information concerning the Leeds outbreak is contained in a report by Dr. Robert Baker, a District Surgeon to the Leeds Board of Health (7). The report is dated January 3rd, 1833.

The actual introduction of Asiatic cholera into Leeds was preceded by a general tendency to bowel complaints, and violent diarrhoea was not at all uncommon. This "diarrhoeal tendency" was often referred to about this time. For example, John Parkin (13) writing in 1841, says:

> "This phenomenon was particularly observed during the prevalence of the epidemic cholera; for not only was the severe form of the malady preceded for many days by slight attacks of diarrhoea, but a variety of anomalous symptoms, indicative of derangement in the digestive organs, was observed to prevail for many weeks before the epidemic manifested itself in its most severe form. To this particular affection the term "cholerine" was applied by the French".

Shapter (1) also describes this milder form of disease under the term "cholerine", and he indicates that it was common in Exeter at the time of the cholera epidemic. Shapter says that it was characterized by

> "tinglings of the surface, weakness and numbness of the limbs, slight spasms, feelings of langour and depression, often accompanied by a flatulent dyspepsia or diarrhoea, in which the discharges were occasionally of a light colour".

Shapter did not decide whether this condition was a mild form of true cholera, or whether it was an "accidental accompaniment of the period". He does note, however, that persons who had experienced these milder symptoms did not appear to be exempt from true cholera. No extended description is given by Baker of this condition in Leeds, but it probably resembled Shapter's "cholerine" very closely.

In 1832 the town of Leeds had a population of 76,000 persons, and it was surrounded by a number of small townships which have since been incorporated into the modern city. The first case of true cholera occurred in a child, aged two years, who lived in a small dirty cul-de-sac called the Blue Bell Fold. It would appear from the report that the date of this occurrence was either May 26th or 28th, 1832 - more probably the latter date. The child became ill in the morning, and died at 5 p.m. on the same day. On the following day, May 29th, a play-fellow, who lived a few doors away, was attacked and died in about twelve hours. Baker says that although it was known that cholera was then present at Selby and Goole, no connection could be traced between these towns and the first case. He is, however, evidently unwilling to admit contagion as a cause of the outbreak. Speaking of the direct contact which was proved between the first and second cases in Leeds, he says:-

> "This would have looked something like contagion, had it not happened that in the course of the week three cases occurred in individuals at a considerable distance from the Blue Bell Fold, where there had been no connection with the former families"

The greater part of the report is an incrimination of unpaved streets and yards as causes of the disease. There is, however, a valuable appendix, in which particulars are given which enable us to trace the course of the disease in the community. Cases continued to occur in different parts of the town, and by the end of the first fortnight in June widely separated districts had been affected. A reference to the "cholera map" which is attached to the report shows that the incidenne was certainly highest in the north and north-east quarters of the town, that is, in those parts which are still densely

populated. Those parts of the town which lay south of the River Aire were comparatively little affected. The distribution of the epidemic in time is given in the following table:-

	Cases.	Deaths.
May 28th	2	1
June	164	42
July	427	187
August	668	273
September	334	123
October	216	73
November (up to 12th) ...	6	3
Total	1,817	702

The epidemic reached its height on August 16th, on which day there were fifty nine new cases and twenty-one deaths. With a water-borne epidemic of this type we would have expected multiple cases in households to be very common. In point of fact, this does not seem to have been the case, since Baker gives the following particulars: In 53 familes two persons were attacked in the same house at the same time; in seven families three persons; in three, four persons; in one, five, and in one, seven persons.

The age and sex distribution of the cases and deaths is given in an appendix under the curious title of "A Calculation on the Probabilities of Human Existence in Persons attacked by Cholera, in every age from six months to ninety years, both inclusive". From this table the following abbreviated statement of the age and sex distribution of the 1,817 cases has been compiled:-

Leeds Cholera (1832)

Age	-5	-10	-20	-30	-40	-50	-60	-70	70+	Age un-known	Total
Cases	151	139	193	215	307	310	179	125	77	121	1817
Deaths	76	45	51	60	89	104	100	80	56	41	702
Case mortality percentage	50	32	27	28	29	34	56	64	73	-	38.7

It is evident from the Report that considerable care was taken to make the returns, as reasonably complete as could be expected at that time. There is, therefore, some justification for accepting the conclusion that the case mortality rate for the Leeds epidemic

was somewhat higher than the rate (33 per cent) which was generally experienced at that period. We see also that the disease was very fatal in the early years of life. During maturity the chances of recovery were more favourable, but the case mortality rate again increased rapidly and progressively after the age of 50 years.

It is interesting to compare the incidence of the disease in Leeds with its progress in York. The outbreak in the latter city was well described by Needham (8). It has already been stated that cholera broke out in York on June 2nd, 1832. The epidemic was not unexpected, and, by order of the officials of the City, precautions were taken to prevent the spread of the disease from Goole, Hull and Leeds. The poor were fed, and in very needy cases their rooms were whitewashed at the public expense. Needham says that the epidemic appeared just at the end of York races, and the disease

> "was beyond all doubt introduced by some of the ragged and beggarly 'gentlefolk' who had come to be present at our 'festivity', and to profit as largely as they could by the folly and vices of other people"

Many of the earliest cases were in vagrants, who were known to have come from Selby, Hull, and Leeds.

The earlier history of the York outbreak presents an interesting exercise in epidemiological speculation. (i) Thomas Hughes, aged 21 years, who lived in a street near the River Ouse, had been employed on May 28th in ferrying across the water persons of all kinds from Selby, Hull, and Leeds. On June 2nd he was stricken with cholera; he recovered. (ii) On June 7th, his brother William was attacked; (iii) On the 8th, John Hughes, the father of these two young men developed cholera. (iv) When Thomas Hughes was first taken ill he called at the house of his uncle, James Kendle, who resided on the opposite side of the river. On June 12th, Kendle was attacked, and (v) on the following day his daughter was also affected. (vi) On June 5th, i.e., three days after the definite onset of the disease in the first case - John Graves who resided in the same Court as the Hughes family, was affected. Needham notes

that he used the privy into which the evacuations of the Hughes' patients were cast. (vii) On June 12th, the wife of John Graves was affected. And so on. In the light of this evidence it is difficult to see how the question of some common factor could have been avoided. The disease now progressed fairly rapidly at York, but from the daily returns which Needham gives it would seem that many cases must have escaped unnoticed. The peak of the epidemic appears to have been reached about July 1st and 2nd, on which two days forty-four cases were recorded, and seventeen deaths occurred. The disease did not finally disappear until the middle of October. During the whole outbreak 450 cases were considered to be cholera, and 185 deaths occurred.

From a table which is given the following summary of the age and sex in 185 fatal cases has been compiled:-

York Cholera (1832)

Age	-5	-10	-20	-30	-40	-50	-60	-70	70+	Un-known	To-tal
Male	7	5	8	7	9	15	23	15	15	(12)	104
Female	3	4	1	17	14	8	8	7	7		69
Total	10	9	9	24	23	23	31	22	22	(12)	173

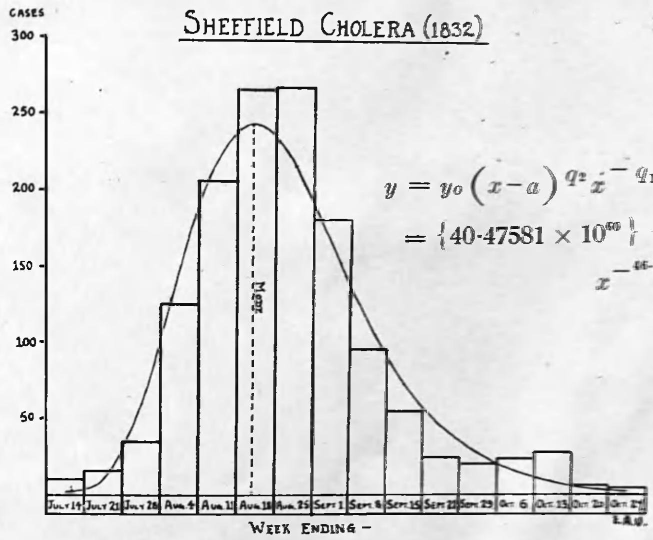
The outstanding feature of this table is the preponderance of male deaths over the age of 40 years. On the figures which Needham gives, the case mortality was 41 per cent - and is thus considerably greater than the case mortality for Leeds. It has been mentioned, however, that in York many of the cases were probably not reported.

The writer was interested to see how far the available data for these epidemics could be treated on modern lines. It has been mentioned that the figures for the Sheffield epidemic, as given in the reprints of reports which are collected in Stokes' book (11) are apparently complete. The cases, week by week, are shown in the form of a histogram in Fig.I. The writer found that it was possible to fit a Pearson's Type VI curve to these data, and the actual frequencies week by week, with the corresponding theoretical frequencies, are given in the following table:-

Week ending	Actual cases	Cases as calculated from the curve
July 14th	10	0.2
21st	15	8.7
28th	36	57.3
August 4th	124	145.8
11th	204	220.0
18th	266	241.6
25th	267	215.3
Sept. 1st	180	166.3
8th	94	117.0
15th	56	73.5
22nd	24	46.3
29th	19	27.4
Octr. 6th	23	15.8
13th	28	8.9
20th	6	4.9
27th	3	2.7

The theoretical frequencies are plotted as a smooth curve in the figure and it will be seen that the fit is fairly good, considering the age of the data.

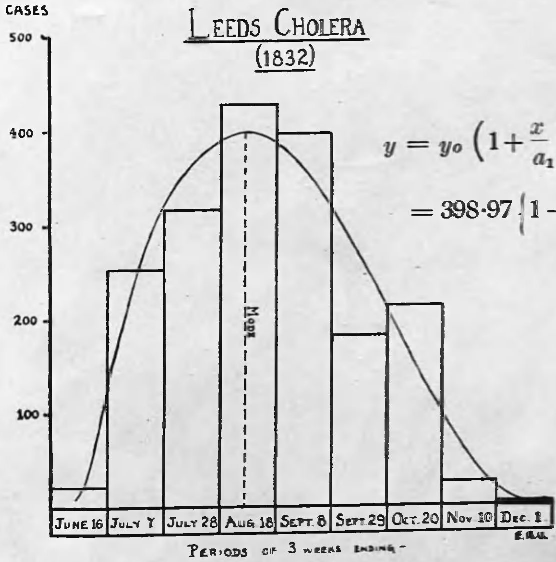
It was more difficult to treat the Leeds data in a similar fashion, because detailed figures were not given for the first four weeks. By applying the frequency which occurred week by week during the first five weeks in Sheffield to the Leeds data, an approximate frequency was obtained for the start of the Leeds epidemic. When the total figures were dealt with in three-weekly periods it was found that a reasonably good curve was obtained. A Pearson's Type I curve was found to fit these data best, and the following table shows the actual and theoretical frequencies:-



$$y = y_0 \left(x - a \right)^{q_2} x^{-q_1} \left[\text{Type VI} \right]$$

$$= \left\{ 40 \cdot 47581 \times 10^{60} \right\} \left\{ x - 21 \cdot 26304 \right\}^{10 \cdot 56366} x^{-46 \cdot 96128}$$

FIG. 1.



$$y = y_0 \left(1 + \frac{x}{a_1} \right)^{m} \left(1 - \frac{x}{a_2} \right)^{m_2} \left[\text{Type 1} \right]$$

$$= 398 \cdot 97 \left\{ 1 + \frac{x}{3 \cdot 00322} \right\}^{1 \cdot 093141} \left\{ 1 - \frac{x}{4 \cdot 95059} \right\}^{1 \cdot 801962}$$

FIG. 2.

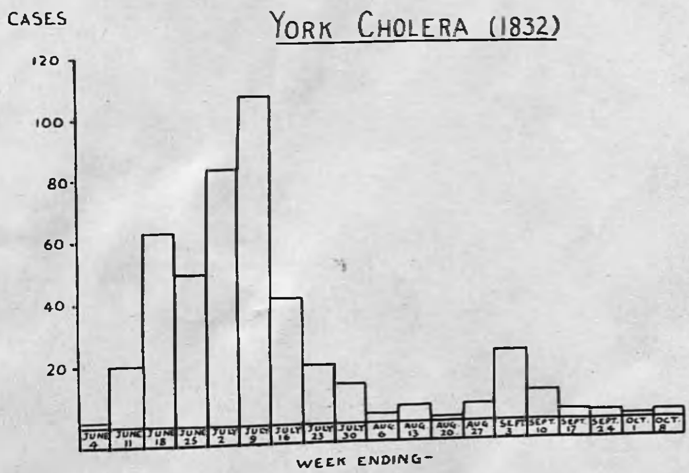


FIG. 3.

Period ending	Actual cases	Cases as calculated from the curve.
June 16th	21	11.3
July 7th	253	230.9
" 28th	318	361.2
Aug. 18th	428	398.9
Sept. 8th	399	359.5
" 29th	182	268.9
Octr.20th	216	152.9
November 10th	27	47.1
December 1st	2	-

These results are set out in Fig.II. It will be seen that a good curve is again obtained.

The actual cases for the York outbreak are shown in the form of a histogram in Fig.III. The distribution was so irregular that no attempt was made to fit a suitable curve.

These data suggest that the figures which are given in the records for the epidemics at Leeds and at Sheffield are probably quite accurate, despite the fact that they were compiled over a hundred years ago. Further, when we compare the two theoretical curves, for Leeds and for Sheffield respectively, we see that the Sheffield epidemic differs in important respects from the Leeds epidemic. At Sheffield the rise to the peak - that is, the mode - was rapid and was effected in less than five and a half weeks; but thereafter the disease continued to prevail, and it was a further ten weeks before the infection subsided. In Leeds, on the other hand, the epidemic took ten weeks and a half to reach its peak, and, comparatively speaking, the decline was somewhat more rapid; in actual fact the decline took about sixteen weeks. Generally we may say that the Sheffield curve was much more typical of cholera than was the Leeds curve.

Clinical Features.

The clinical features of Asiatic cholera in Leeds are not dealt with in Baker's Report. This is somewhat unfortunate, because in the year 1832 - probably before the actual cholera outbreak - a short treatise on this subject was published in Leeds by Charles Turner Thackrah (1795-1833) (14). This author was a pupil of Sir Astley Cooper, and in the same year he published one of the first treatises on industrial dust diseasss. Thackrah discusses the main

types of cholera, and it is evident that, in his description of the Asiatic type, he is drawing, not on his own experience, but on the descriptions of Indian surgeons. The main interest of his work for us is that he gives an excellent description of an outbreak of "cholera" which affected Leeds and the surrounding townships in 1825. The outbreak is briefly referred to by Creighton. Thackrah made a house-to-house survey in certain districts for the purpose of ascertaining the extent of the infection, and he was accordingly in a position to describe the disease comprehensively. The outbreak commenced in May 1825, but cases were not numerous until August. The disease prevailed until December. The symptomatology was striking:

"purging and vomiting, cramp, coldness of the extremities, and prostration of strength"

The disease was frequently severe, and the stools often resembled "gruel or barley-water". The point of interest is that Thackrah argues that this type of cholera which he saw in 1825 was identical with the Asiatic cholera which visited the town seven years later. His argument is interesting. After giving a full description of some severe fatal cases which he say, he continues:

"No two cases can in any country, or of any disease, be expected to have precisely the same symptoms. But I contend that the signs which are considered to characterize the Indian Cholera are found in a marked and decided degree in the cases just stated. Thus we have the peculiar character of the evacuations - the sudden and great prostration of strength - the extraordinary reduction of pulse - the shrunk and purple countenance - the loss of voice - the purple, or pale, contracted state of the extremities - and death sometimes in a few hours. I conceive, therefore, that no fair reasoner can refuse to admit the identity of the disease. A physician sent down by government to ascertain the character of the Cholera in the north of England has repeatedly declared that he could distinguish the Indian from the English only by the prevalence of the former - the number, not nature - of the cases

giving the distinctive character of the Indian. Cholera in England has certainly not produced as great a mortality as Cholera in India. Neither has inflammation of the liver; yet no-one considers Indian Hepatitis as distinct in nature from English."

In his treatise Thackrah mentions 339 cases of "cholera" which he had seen, or about which he had information. Only six of these were fatal. A comparison of these figures with the case mortality of nearly thirty nine per cent which the true cholera showed when it did reach Leeds, does not lead us to doubt the historical assertion that Asiatic cholera was not seen in this country before 1831. There is little doubt that Thackrah's outbreak was one of "cholera nostras".

To return to the 1832 epidemic. We may infer the clinical findings in Leeds from other writings of the period. The best which the writer has encountered are those of Shapter (1) for Exeter. An epidemic which was geographically closer to Leeds was that atHull, and Alderson's description may be used (6). It has been stated that the outbreak started in Hull during the second week of April, 1832, and there were 300 deaths before it subsided. Alderson lays most stress on the barley-water discharges as a characteristic symptom. He says:

"So great in some cases is this discharge, that it is not unusual to find the apartment completely inundated with watery fluid,which has been passed from the bowels.....through the bed of the patient".

He described the vomiting as being of a peculiar type -

"sudden without retching, and ejected in large quantities, as if pumped from the stomach"

At Hull the cramps do not appear to have been so marked as they were in some other localities. He speaks of

"The dark purple hue of the face and extremities, and corrugation of the skin of the hands and fingers"

In the state of true collapse he says

"the eyes have been much depressed, and the voice almost lost; what remains consists of a peculiar whisper, with, in some cases, a shrill syllable now and then involuntarily escaping"

Alderson gives a minute description of the different types of evacuation. In the consecutive fever of the Hull cases he says that hiccup was a prominent feature, and it apparently occurred most frequently in fatal cases.

Needham (8) says that at York diarrhoea was a very general premonitory symptom. In some cases it was of very short duration, but in many it lasted a week or more before the typical features presented themselves. The collapse stage at York never lasted more than forty-eight hours; before the end of this period the patient either died or showed symptoms of reaction. Delirium was quite frequent. Needham also states that the most rapid cases of collapse were those in which the stomach manifested the greatest irritability. The consecutive fever he had known to continue for eight days.

Pathology.

Contemporary reports of the pathology of the disease are not very interesting. Most of them emphasize the essential features.. For example, Bell (15) gives a good description of the gorged appearance of the veins. He says that the internal coat of the stomach, and sometimes of the duodenum,

"has very generally a peculiar appearance; there are vascular patches, not of inflammation, but as if vessels had burst, and blood had been extravasated between the coats. These spots, though no doubt commonly a congeries of congested veins, do certainly sometimes arise from extravasation. Patches are also described as having the character of inflammation, generally near the pylorus; which are by some considered the result of local irritation, produced by portions of the remedies prescribed having adhered to these parts of the stomach; and they must be taken as a proof that reaction had commenced before death"

This description applies to cases which Bell saw in India. Alderson (6) at Hull described how

> "the mucuous glands were seen in groups much enlarged, and here and there occur patches of dark brown spots, indicating the presence of diseased mucuous follicles, with the appearance of a deposit of the red particles of the blood beneath the membrane"

The interesting part about Alderson's book is that he gives two excellent coloured illustrations of these appearances. From a contemporary review of the book (16) it would appear that he was possibly the first to illustrate these findings in this way.

Treatment.

Baker's Report to the Leeds Board does not deal with treatment, but, in a short note to the Central Board he summarized the methods which he adopted (17). From this note it would seem that Baker tried most of the accepted or suggested methods of treatment. Brandy and ammonia were very commonly recommended (see Shapter (1) for Exeter), but Baker is more reticent. He says that they are useful up to a point and then detrimental; he adds that

> "so difficult is it to know when to stop, that I have relinquished all but capsicum"

This latter remedy, incidentally, is seldom mentioned in contemporary reports. A very common method of treatment at that period was mercurials, especially in the form of large doses of calomel (for example 20 grains every few hours). Baker had no good results from this method. Transfusion was also in vogue in some places, but he experienced fatal results. The cold water method was first introduced by Shute of Gloucester (18), and consisted of giving the patient large quantities of cold water to drink. One patient was actually forced to drink seventeen gallons! Baker tried this on two occasions, both of which were successful, and he saw it tried on five others, four of which recovered. Treatment by means of the injection of saline fluids into the veins was first practised in

cholera by Latta of Leith (19), and in all contemporary reports there is much discussion of the efficacy of the method. Baker reports that he had no success by this means (17). He also says

> "I have tried injecting saline matter and spirit into the veins, from 3 pints to 14 - in no case successfully permanently".

Contemporary confirmation of Baker's findings is given by Richard Birtwhistle (2), who was deputed by the Skipton Board of Health to visit Leeds and York and see how the cases were treated. Birtwhistle says that the injection of saline solution into the veins was extensively tried in Leeds, but that the method was not found to "answer the sanguine expectations which its first announcement created". He also comments that some recommended abstraction of blood from one arm at the same time that fluid was injected into the other! This "roundabout and swings" conception shows the fundamental errors of the physiology of the period. Birtwhistle's conclusion was that saline injections gave much temporary relief in all cases, but in none did permanent benefit result, and the method was ultimately abandoned in Leeds. In general, the chief methods employed seem to have been those described by Shapter, viz. keeping the patient warm, especially by tins filled with hot water; stimulants such as ammonia and camphor; and the frequent exhibition of calomel (for example, scruple doses every hour). Casson and Horton (21) reported on the treatment of 109 cases in the cholera hospital at Holbeck - which is now part of Leeds. Their treatment resembled that of Baker, but at the commencement of the disease they gave opium, and they employed large doses of calomel for collapse.

The treatment adopted at York was fully described by Needham (8) and by Anderson (22). Needham's methods may be summarized as follows: (i) First stage: for diarrhoea, opium (1 gr. to 1½ gr. repeated). Bleeding for headache. If evacuations thin, calomel. Friction, warm applications, mustard emetics for oppression in the chest. (ii) Second stage: Calomel every half

every half hour, with opium. External heat. Mustard emetics in early stage of collapse. Turpentine enemata had been used, but were seldom efficacious. Bleeding was not advised at this stage. (iii) Third stage: a dose of calomel daily. Bleeding may be necessary, "but we do not think that large bleedings can generally be borne", and free application of leeches in generally much safer. Needham comments on the saline treatment recommended by Stevens, that is, the exhibition of non-purgative neutral salts. He did not find the method efficacious; he says that

"in many cases it has either increased the purging or failed to control it"

Anderson's views agree on the whole with those of Needham, but there is a discrepancy regarding intravenous injection of salines. Needham says that thirty cases were injected in York with four recoveries. Anderson says that this method was used in twenty-three cases, of which only five died. He adds that

"if it were no other use than to give a short time to persons who had not made their wills, &c., that alone would amply repay all the trouble in such circumstances"

At Hull the treatment seems to have been more or less on established lines (6). Alderson used early venesection, followed by calomel and rhubarb in the first stage. With reference to bleeding he says that the operation can be performed even when the stage of collapse is impending, provided that there is a good pulse and the evacuations are not profuse. For the stage of collapse itself large mustard plasters or poultices were most successful. For profuse diarrhoea he recommended a lead and opium suppository, and he also tried rectal injections of nitrate of silver with laudanum. He was no blind supporter of the cold water method. The saline treatment he had tried, but with so little success that he was inclined to attribute any which he had to the vis medicatrix naturae. About this time Ayre of Hull was writing frequently to the medical press about the advantages of minute doses of calomel in cholera (23),(24). Ayre said that he had treated 191 cases by this method, and of these only 34 died.

It was, perhaps, these statements which called forth the following indictment in Alderson's book:

"Mercury and opium ever have been, in all obscure diseases, the sheet anchor of the quack; and on the first appearance of cholera, when the medical world were alike unacquainted with its nature and treatment, there was a great disposition to employ them, and particularly mercury, that most severe as well as efficacious remedy. It appears at present that ample experience has brought the practice much into disrepute in Cholera, and although both calomel and opium still continue to be given in all doses, and at intervals throughout the disease, there are few advocates found for their indiscriminate use"

Favell (25) at Sheffield also tried out Ayre's method, and was not impressed, as we can infer from his question

"If calomel cures cholera at Hull, how is it that it does not at Sheffield?"

Favell, on the other hand, favoured stimulants and friction of the limbs with flannels dipped in strong liniments. He was an adherent of galvanism, which produced in most cases, he says, most decided and immediate improvement.

This is no place to raise anew the stormy history of venesection. Nearly all the medical writings of the period abound with arguments for or against its use, but most authors admit that in cholera it must be employed at least in the early stages of the disease, and nearly all, without exception, are unwilling to oppose the cult of the lancet. In this connection a few sentences from a pamphlet by Dickson (26) an Indian army officer who wrote on cholera in 1829 are interesting. He says

"Having seen the analogy subsisting betwixt the disease called Cholera and that which takes place in a person who has lost much blood, can we for a moment hesitate as to the proper treatment to be pursued? . . . Many practitioners bleed, blister and stimulate in a breath. Is this either sensible or scientific? If one of these practitioners were called to the bed of a patient

who had suffered from haemorrhage or had been poisoned by metallic vapours, he would (if he were not previously informed of the cause) draw out his lancet under the idea that the disease was cholera. This is not prescribing for symptoms but for a name. Ask him why he bleeds in cholera, he will tell you that it is to relieve cerebral congestion, or to unload the gorged lungs, or to subdue spasms. . . . The lancet then, while it robs the muscles of that diminished energy which constitutes spasms weakens the powers of the respiratory muscles also - and hastens asphyxia. The blood in such a case is the life, and without blood a muscle cannot contract nor a gland secrete. Many die of cholera who from the first have never suffered from spasm. In the last stage of the disease there is no spasmodic action. The internal parts are palsied and the external strength is laid prostrate. If it be boasted that bleeding has been followed by success, I answer, in those cases that have recovered after venesection, the irritating cause has not been so great, the atmospheric poison has not been so deeply inhaled, and the records of our science bear witness to a successful result in other dangerous diseases where the practice has been confessedly erroneous. If it be proved (which it has been by Magendie) that the action of poisons is favoured by bleeding and retarded by an artificial plethora why should the lancet be resorted to in a disease which has its origin indubitably in an atmospheric poison? The word poison is a relative term. It means anything in any shape destructive to the nervous energy constituting animal or vegetable life. Misled by the relations of authors and the encomiums they have passed on the lancet, I gave venesection a trial to an extent which I can never sufficiently regret. Instead of depriving the patient of his blood, I should have been more successful had I transfused blood into his almost empty veins."

These sensible views appear to have passed unnoticed; they certainly were not ventilated or practised in Yorkshire.

Contemporary views on Aetiology.

The remarks of Baker on aetiology are obscured by the views of the miasmatic theory of infectious diseases which were current in his day. Some of these arguments must have caused the partisans of the contagion theory considerable difficulty. For example, Baker points out that the disease was most prevalent in the low-lying districts of Leeds, especially in those parts where

"from a want of local cleanliness and ventilation, a malignant state of the atmosphere was likely to obtain"

As has been pointed out, multiple cases in households were not infrequent in these districts. But, says Baker

"if these distinct attacks upon members of the same family depended entirely upon contagion, how happens it that we so nearly find cases recorded of two persons being attacked in the same habitation situated higher where the miasm does not exist, and where proper attention is paid to cleanliness and ventilation?"

It is obvious that Baker had no idea that the poison might be taken into the system in the drinking water. For example, he mentions that the disease broke out in the first storey of a large building, which had several flats for different families.

"The matter ejected from the patients was thrown down the sink into a sump hole, over which there were persons residing who did not take the disease till this time. Here then were two sources of epidemy - the sump hole and the ejected matter. Under this roof there were 6 cases, 3 deaths"

Thackrah quotes Chapman as stating that

"The grass cutters of his party were found to suffer severely from Cholera, while the rest of the party suffered little. Out of eighteen of these people, five died in three weeks, and as many more were attacked. They were resorting daily to a putrid standing pool, for the purpose of washing their grass. They had themselves connected the attacks with this pool, and of their own accord deserted it, after which no further cases occurred"

Despite many such suggestive incidents, the only writer of the period

who seems to have suspected the waterborne theory of cholera was John Parkin (27). Yet the solution was evidently not far distant, as is evidenced by Baker's statement that

"the cause of an epidemic disease may depend upon the egesta here and there scattered from patients labouring under such complaints, with as much if not more probability than actual contact"

Social Conditions and Reform.

The major portion of Baker's thesis is devoted to the part which filthy domestic conditions and unpaved, uncleansed and unsewered streets played in the spread of cholera. Before approaching this section, it will be as well to review what previous steps had been taken to secure a reasonable degree of cleanliness in the town. In 1790 a local Act (30 Geo.3,chap.68) was passed:

"for better supplying the Town and Neighbourhood of Leeds, in the county of York, with water; and for more effectually cleansing the Streets and other Places .... and removing and preventing Nuisances, Annoyances, Encroachments, and Obstructions therein" (28)

In 1809 an amending Act was passed (49 Geo.3,chap.122) and in 1824 and Act (5 Geo.4,chap.124) with much wider powers was placed on the Statute Book. Despite this legislation, conditions in Leeds, as described by Baker, were truly appalling. He shows that about half of the population of the town of Leeds itself (that is, excluding the surrounding townships) lived in streets which were sewered drained paved and cleansed. In this half of the town only 245 cases of cholera occurred. On the other hand, in that half of the town in which there was neither common sewerage nor cleansing 1,203 cases occurred. According to Baker, the populations of each of these halves were apprximately equal. In three parallel streets, housing about 386 persons, the sanitary conveniences consisted of two smallprivies, which were in such a state as to be totally unusable. Offensive matter was allowed to accumulate in the unpaved streets. From the privies in Boot and Shoe Yard seventy

cart-loads of filth - the accumulation of thirty years - were removed by order of the Commissioners. In many streets there was a sump hole - long stagnant - under the flags of every house. Baker's conclusion is that

"if our streets are not properly cleansed, we can hardly expect much cleanliness in either the dwellings, the habits, or the manners of the people"

The report was presented to the Local Board of Health on January 21st, 1833, and that body's third resolution was to the effect that

"As the facts communicated in the said report are applicable to all large towns, this Board is of opinion that a General Act of Parliament for sewering, drainage, cleansing, and paving, would prove a public benefit"

We would expect that the people of Leeds, no less than the population of Britain as a whole, once roused to a white heat of indignation by such enormities, and by the train of death left by the epidemic, would have demanded energetic measures for the "cleansing and purifying" of populous centres. The sequel will be found in a Statistical Report (29) issued in October 1839 by the new Leeds Corporation - which had been formed as a result of the passing of the Municipal Corporations Act in 1835. This report was the outcome of investigations which extended from November 1838 to October 1839. The writer has summarized some of the information given in this excellent report, and from this it would appear that there were 586 streets in the town. Of these 247 were classified as "good", 108 as "middling", 135 as "bad", and 96 as "very bad". The authors of this report point out, however, that the designation "good" must be taken with a considerable amount of reservation, since, of the 247 streets which were classified thus, only 70 belonged to the town and were cleansed by scavengers. The old difficulty regarding the shortage of privies had not been remedied. In three adjoining streets, containing a population of 452 persons, there were only two such conveniences, neither of which was fit for use. The streets had become so full of ashes, filth and refuse of every description that their surfaces were far above their original level

The conveniences which did exist were usually situated under archways, and were consequently used by every passer-by.

"Uncleansed because it is nobody's business, and everybody's perquisite, they become offensive in the highest degree"

The Boot and Shoe Yard to which reference has already been made, now contained 340 inhabitants (ten to every house). There were only three privies, and one of these had not been cleansed since the cholera. There was no water within a quarter of a mile. In the North-East Ward - in which cholera had been rampant - there were several horrible places which were utterly impassable for filth of the most offensive description. Some important streets were wholly without sewers; many cellar dwellings were never dry; and on the surface of the public way there was frequently a permanent collection of soil and water "a perpetual nuisance to the eye, and a perpetual fever to the whole body"

As an echo of the conclusions of the 1832 Board of Health, the authors of the present report summed up by saying:

> "One thing is certain, that the greater part of the town is in a most filthy condition, which demands an immediate remedy; a remedy which does not seem attainable under any local Act now existing; but calls for an especial enactment, which is doubtless required, not only for Leeds, but, more or less for every town in the Empire".

It was not until twenty-seven years later, on the appointment of the first Medical Officer of Health for Leeds in 1866, that the necessary sanitary reforms were initiated.

History provides a salutary check to overweening sanguineness. We may point to our modern cities and say, Thus far, and thus, we have travelled in the last hundred years. Yet it should be remembered that in such matters progress should be measured not in terms of actual accomplishments, but rather in relation to those needs which the expert opinion of the time indicates as necessary. There had been abundant evidence before 1832 that the Augean stables of our cities would require a great cleansing, yet seven years after the catastrophe

we find that in Leeds progress had been non-existent. In the last few years there has been ample evidence that both medical and engineering experts are setting up new standards of communal life. The future will show whether the community as a whole is educating itself to these standards.

Copies of a number of the publications mentioned in this section are in the possession of the writer. For the loan of certain other publications he is indebted to Mr. F. J. Boardman, Chief Librarian, Rotherham Public Library, to Mr. R. J. Gordon, Chief Librarian, Leeds City Libraries, and to Mr. J. P. Lamb, Chief Librarian, Sheffield Corporation Libraries. Other volumes were obtained at the British Museum and in the library of the Royal Society of Medicine.

PART II.

SECTION (c).

THE NEUROLOGICAL COMPLICATIONS OF VARICELLA.

A CLINICAL AND EPIDEMIOLOGICAL STUDY.

One of the most remarkable features of the infectious diseases in the post War epoch has been the emergence of the nervous system into a position of importance. Attention was focussed on this fact by the outbreaks of cerebro-spinal fever and of encephalitis lethargica in the last years of the war, and a few years later widespread outbreaks of acute anterior poliomyelitis coincided more or less in time with the appearance of numerous cases of post-vaccinal encephalitis in this country. On the Continent encephalitis following the commoner acute infections was not unknown, and among these a number of cases of encephalomyelitis following varicella were reported. This condition has always been rare in Britain, and although the literature has been reviewed on several occasions on the Continent, no paper purporting to serve this function has appeared in English journals in recent years. The main purpose of this paper is to examine afresh the question of the etiology of these conditions, to discuss their features from clinical and epidemiological standpoints, and incidentally to add a further personal case.

Historical. The first report of a condition which was almost certainly encephalitis following varicella was by Marfan (6). In 1914 Miller and Davidson (28) reported the first English case of acute cerebral tremor following varicella and reviewed the literature, and later Winnicott and Gibbs (43) described a case of polio-encephalitis. Glanzmann (46) reported three cases, and in discussing the etiology of the first condition he first suggested the possibility of an anaphylactic phenomenon as the cause of such complications. This suggestion has since received much consideration by foreign writers. Two further English cases were

described by Rake (56) and by Cohen (57) in 1929, and from Glasgow Graham (67) reported two cases in 1930. Ludo van Bogaert of Antwerp made a very extensive historical investigation of a fatal case in 1930 (68), and in the following year Zimmerman and Yannet (79) made a similar study of a fatal American case.

Investigation of Cases previously described. Investigation of the literature has revealed 119 cases which have been previously described, together with twenty three cases which are often quoted as examples of this condition, but which, for reasons which will be explained forthwith, have been discarded. This list of accepted cases is much longer than that found in any other paper, and it is probable that it contains most of the cases of these conditions which have been reported up to the end of 1933. The list of accepted cases will be found in the Appendix.

Reported cases due to other conditions.

As instances of chorea following varicella many writers quote eight cases of Mackenzie (104), but these cases showed concurrent scarlet fever or measles and are therefore inadmissible. Tezner (105) quotes the case of Binswanger and Berger (106) as an example of a non-characteristic encephalitis, but admits that an influenzal condition preceded the varicella; from the discussion in their paper it is evident that Binswanger and Berger consider the influenza to have been the main etiological factor. In the cases of Désandré (107), Lenoble and Thiélemans (108), Mitchell and Fletcher (109), and Pierret and Pruvost (110), the meningitis which was present was of a suppurative type; amd in that described by Potter (111) the meningeal condition was evidently due to tuberculosis. Gordon (112) described a case, which is not infrequently quoted as an example of a post-encephalitic syndrome following varicella. The patient, a boy of six years, developed right facial paralysis, loss of speech and hearing, and right hemiplegia, five weeks after the onset of varicella. Since, however, acute haemorrhagic nephritis was diagnosed three weeks before the onset of hemiplegia, it is probable that the condition was more allied to uraemia. In a case

of Stuchlik (113) measles was followed by varicella and a few days later polyneuritis appeared. In the case of Winnicott (114) measles was present at about the same time as the varicella. Similarly in the case of meningo-encephalitis which was described by Zimmermann and Cochran (115) the authors consider that the condition was a post-vaccinal encephalitis, with the shortest known incubation period, viz, one day, and that the varicella was merely a concomitant factor. Gillot, Sarrouy, and Dupuy d'Uby (116) described a case of cerebral encephalitis which occurred four days after the appearance of a varicellous eruption; it was later shown that the condition was syphilitic. Some authors - e.g. Wilson and Ford (45) - exclude the case of peripheral paralysis described by Allaire (19), but, since recent work has suggested that such complications can follow the acute infectious diseases after a considerable interval, it has been included here in Group 4. The case of palpebral gangrene (Rolleston (117)) following varicella, which led to ectropion, has evidently no connection with this subject. Gordon and Rolleston (118) described the case of a boy of three years who developed a bullous and gangrenous varicella, which terminated fatally. Lumbar puncture after the child was admitted to hospital yielded a clear fluid under slight pressure. This case was not described by these authors as that of a nervous complication of varicella, and it seems that the increased pressure of the cerebro-spinal fluid was merely incidental. This condition is not infrequently met with in the acute fevers, and it is doubtful whether it is of much significance. The two cases of tuberculous meningitis following varicella, which were reported by Castro Sophia (119) are evidently not admissible.

Report of a Case.

The patient was a girl of eight years and eleven months. Two years before the present illness she had had whooping cough, and this was followed shortly afterwards by German measles. Vaccination had been performed in infancy, and a definite scar was present on the arm. The family history showed nothing which was relevant to the present investigation.

History of the illness. During the week ending 20th February, 1932, the child had some malaise and slight sickness. On 24th February a few spots on the skin surface were noticed by the child's mother. Dr. A.L.C. Harrop attended the patient. The spots developed rapidly into typical chicken-pox papules, which were numerous on the face, scalp, trunk and back, but discrete on the arms and legs. The temperature was not above 99°F, and the course during the next few days was that of a typical mild varicella.

On 28th February the child had some frontal headache which persisted for two days. About the same time she commenced to have vomiting, which was not accompanied by nausea, but was rather of the cerebral type. This feature persisted for about forty-eight hours, and during this time the act recurred at frequent intervals. On the 29th February she had slight dizziness, and on the following day the mother noticed that she was "wobbly on her legs", and that walking was difficult. The mother also noticed that she was talking with a slight drawl and more slowly than usual.

Examination by Dr. Harrop at the time of the onset of the cerebral condition showed that there was a marked incoordination in the arms and legs. Tremor was not present. Both eyes showed slight paralysis of the external rectus muscles. There was no difficulty in accommodating; the pupils reacted normally to light; ~~ther~~ and ptosis and nystagmus were both absent. Speech was described by Dr. Harrop as being very slow. There seemed to be some difficulty in getting the words out, and there was also apparently some difficulty in deciding what she wanted to say. The speech was not of the scanning type. Apart from slowness no other abnormality was noticed in the mental condition. No paralysis of any muscle was elicited. The knee jerks were present and active, the plantar responses were flexor, and there was no ankle clonus. Muscle tenderness was not elicited. The patient did not have any disturbances of sensation in the limbs, and in this connection the usual tests failed to elicit any abnormality. There was no disturbance of bladder or bowel. The temperature during the early part of the cerebral condition did not rise about 99°F.

Examination of the patient by the writer on the 15th March showed an apparently healthy and well-nourished girl with no gross evidence of anaemia. The throat was clear. Examination of the heart and lungs was negative. On the abdomen, chest, and lower part of the back there were fairly numerous scars of chicken-pox, and a few scars were also present on the legs. The pupils were medium and **equal** and they reacted to light and on accommodation. There was no strabismus, nystagmus or ptosis. The tongue was protuded in the midline and showed no tremor. No evidence of facial paralysis was elicited. The abdominal reflexes were present on both sides; they were slightly increased in intensity. The knee jerks were present; both plantar responses were flexor, and there was no clonus at knee or ankle. Examination of the power of individual muscles in the arms and legs revealed no definite abnormality, apart from a slight generalised weakness. There was no rigidity of the limb muscles. Inco-ordination of the arms and legs was definite and quite marked. When the patient was asked to perform various tests, hypermetria was apparent, and there was difficulty in performing voluntary actions. On attempting to stand or walk she showed definite ataxia, and Romberg's sign was easily elicited. When she tried to walk she tended to put the heels down first. Tremor or spasms of the muscles were not elicited. There was no nuchal rigidity and Kernig's sign was absent. The speech was definitely slow, and had a slight "sing-song" accent. The memory was apparently not affected, since she remembered past events and the onset of the illness quite well. The temperature was 98.7°F. The pulse was regular, and the rate was eighty-two per minute. The Wassermann reaction performed on the blood serum gave a negative result. At the time of my first visit there was no justification for lumbar puncture, and this operation was therefore not carried out.

Course of the illness.

After the first examination the patient was seen at frequent intervals for some time, and since then the progress has been followed up. By the 30th March inco-ordination was disappearing,

but it was still present in the hands and legs. She was able to write and thread a needle fairly well. About the middle of April, 1932 she had an attack of nausea and headache which lasted for about a day. When she was examined on the 22nd April inco-ordination was absent in the legs and arms. The condition was apparently clearing up without any sequelae; but about the middle of May she began to have difficulty in sleeping at night, and during the day she was rather fretful. Difficulty in falling asleep at night persisted for about three months, and her mother stated that some degree of general nervousness was present - a condition which had never been noticed before her illness. After August, 1932 these features gradually became less and less marked.

CLINICAL FEATURES.

Classification. Broadly speaking the neurological complications of varicella may be classified in two ways: (a) according to the evident symptoms and signs: (b) according to the supposed pathological basis for these symptoms and signs. In any classification the first difficulty is that few of the cases are "pure" - except possibly some cases of the cerebellar syndrome - and most cases show diverse symptoms and signs, though those pointing to the affection of one particular part of the nervous system may predominate. If a classification according to the predominant symptoms is adopted, the classes which have to be introduced tend to become rather numerous. On the other hand, a purely pathological classification cannot be founded upon any scientific basis, since very few autopsies have been performed on these cases. Russell Brain (120) classified these conditions into ten groups according to the predominant symptoms. In another paper (121) he gave a useful classification of the infective encephalopathies as a whole:-

(1) exclusively or mainly meningeal: acute or fulminating onset, with temperature often 105° or 106°. Headache, vomiting, delirium, irritability or photophobia, cervical rigidity, presence of Kernig's sign, cutaneous hyperaesthesia, and diminished tendon reflexes.

(2)

(2) both meningeal and cerebral or spinal: acute or fulminating onset with temperature often 105° or 106°. Convulsions, coma, and delirium, rapidly passing to unconsciousness. Aphasia, hemiplegia, diplegia, papilloedema, ocular palsies, and signs of cerebellar defect may be present.

(3) cerebral or spinal only: cerebral symptoms, without meningeal, usually occur in cases of less acute onset.

Other writers who have suggested various different classifications are:- de Toni (36); Wilson and Ford (45); Miller and Davidson (28); Eckstein (122); Borra (77); Van Bogaert (68) divides these conditions, on a pathological basis, into (a) neuromyelitis; (b) encephalomyelitis; and (c) meningitis. Glanzmann (46) gives a very useful classification of the conditions found in different cases, and Tezner (105) increased Glanzmann's ten groups to sixteen. The classification of Dudevant (83) is very complete, but it is too rigid to allow of overlapping of symptoms. The methods suggested by Bérode (90) and by Miget (123) resemble each other, and are logical and complete.

The classification adopted in this paper, which is based on those of Bérode and of Miget, is as follows:-

Group P	- Nervous prodromal features; description not sufficient for more exact classification.	
Group 1	- Meningoencephalitis	
Group 2	- Encephalitis ...	(a) cerebral encephalitis (lethargic forms, etc.)
		(b) acute cerebral tremor
		(c) cerebellar syndrome
		(d) choreo-athetotic forms ("stria tites")
		(e) non-characteristic encephalitic forms.
Group 3	- Myelitis	(a) typical (ascending or transverse).
		(b) anterior poliomyelitis.
		(c) multiple sclerosis.
Group 4	- Neuritis and polyneuritis	
Group 5	- Ocular manifestations.	
Group 6	- Other conditions (two cases)	

This classification is sufficiently complete to cover all reported

cases, and although it is not based on purely pathological features, it gives some indication of the division of the cases into groups in accordance with the four main sections of the nervous system - meninges, brain proper, spinal cord, or peripheral nerves - which may be involved.

Group P - Nervous prodromal features. The earliest examples which I have been able to discover of definite nervous conditions occurring before the appearance of the rash in cases of varicella are the two cases described by Kassowitz (1) in 1873. In his first case, a girl of five years, the patient was seized with generalised convulsions and vomiting; there was later deep stupor and twitching of the face muscles. On the following day the first signs of a chicken-pox eruption appeared. In the second case of Kassowitz (female, seven years) there was pain in the left upper jaw and violent irritability. Two days later the varicella eruption appeared. In 1875 Hunter (2) described a case of convulsions in a boy of three years, occurring on the day before the appearance of the rash; and in discussing the case Hunter said that the nervous condition was "due apparently to incipient cerebral meningitis". Similar cases were also described by Dumas (3), Tham (4), Augier (8), and Cerf (18). Some writers dismiss these cases as conditions which might arise during the prodromal period of any infectious disease. In the last few years, however, cases have been described in which these prodromal features were later followed, not only by the rash, but also by symptoms pointing to some definite organic change in the nervous system. Indeed, Henner (101) (102) claims that the nervous condition may precede the eruption by eighteen days, or may follow it after as long an interval as three months. It seems to be desirable, therefore, to include in a special group the cases which are mentioned above.

In all the cases mentioned the predominant feature was convulsions, usually of a violent type, and each attack lasted from two or three minutes (Kassowitz) to thirty minutes (Hunter). The

convulsions were usually generalised, but in several cases clonic spasms of the limbs, sometimes unilateral, occurred. The temperature was usually elevated; the highest noted being that of over 104°F recorded by Hunter and by Cerf. In all cases in which the further progress was mentioned, it was normal after the onset of the eruption.

Group 1 - Meningoencephalitis. This group of cases could be quite well classified as "meningitis" if it were not for the fact that symptoms and signs pointing to involvement of other systems in addition to the meninges are nearly always present. Apart from this the typical features of the condition are usually meningeal; in fact, of the sixteen cases in this group, eleven were at first diagnosed as tuberculous meningitis, and another case resembled meningococcal meningitis so strongly that antimeningococcal serum was continued despite negative findings in the cerebro-spinal fluid (Goussis (37)). Clinically the condition usually commences with severe headache (eight cases), vomiting (ten cases), and some disturbance of the sensorium (ten cases) - either stupor, a confusional state, or later definite coma. Convulsions or epileptiform seizures were noticed by Mya (5); Laignel-Lavastine, Miget and Constantinesco (73); Bérode (90); and by Gorini (91). Thereafter the illness progresses rapidly until a frankly meningeal picture appears. Nuchal rigidity was noticed in nine cases, and Kernig's sign was present in nine cases. Several writers, for example Goussis, Bérode, and also Hallé and Arondel (100) mention the marked retraction of the neck, and even opisthotonos and gun-hammer position, which were present. Other signs of meningitis are not so common. Brudzinski's signs are noted by de Toni (36); Glanzmann (Case b (46)): Bonabo (51); and by Gorini (91); but the scaphoid abdomen, which is so typical of tuberculosis meningitis, was present in five cases. Signs of involvement of other systems are sometimes found; for example, the tendon reflexes were affected in six cases, and an extensor plantar response was present in two (de Toni (36); Gorini (91)). In the case of Glanzmann (case b (46)) and of Rupilius (95) ataxia developed - probably indicating some cerebellar involvement; and some degree of paresis

of the limbs was found in four cases. Ocular changes are uncommon. Strabismus, inequality of pupils, and ptosis were each found in two instances. In these conditions it is evident that there is sometimes a vaso-motor complex, since dermographism was noted in several instances. Cutaneous hyperaesthesia and muscular hypertonia both occur but are uncommon. Trismus was found only once (Bérode (90)). Dysphagia was noted by Koplik (23) and also by Guinon and Halbron (29). In the later instance it was due to paralysis of the right half of the palate and was associated with nasal voice and functional asymmetry of the lips. The authors say that these features recalled nasal diphtheria. Hydrocephalus, which is not uncommonly found in the later stages of meningitis, is definitely rare in this condition. It was noted only in the two cases of Koplik (23) and in that of Mya (5); and in the latter instance the condition is not of much importance, since it was associated with a congenital encephalocele. In the case of de Toni (36) total left ophthalmoplegia developed and persisted for over four months. Huisman's (25) case of sudden blindness, which was in its essential features a meningo- encephalitis, will be referred to later. Hallé and Arondel (100) noted a marked respiratory disorder which consisted of irregular respiration with expiratory pauses, but with no definite Cheyne-Stokes rhythm.

Glanzmann's second case (Case b(46)) is interesting in that, although meningo-encephalitis developed definitely on the tenth day after the appearance of the eruption, slight signs of this condition had been present on the day before the rash came out. Further, in the case of Hallé and Arondel (100) the typical signs of meningitis, with gun-hammer position, retraction of the abdomen and nuchal rigidity, appeared three days before the development of the rash. It is evident therefore that the dividing line between this group of cases and the prodromal group is rather fine.

In general it may be said that most cases present typical features of meningitis at the onset; later signs of involvement of other systems may be present, but the meningeal signs still predominate. Diagnosis in these cases is liable to be

difficult and rests finally upon a negative basis. The main points are the absence of organisms (on culture or by animal inoculation) from the cerebral-spinal fluid; and the presence of typical varicella associated fairly closely in time with the appearance of the nervous symptoms. Bérode (90) and others have, however, used a complement fixation test with varicella crusts as antigens.

Group 2 - Encephalitis. (a) Cerebral encephalitis.

This group consists of thirteen cases - some of which are by no means typical. The earliest example of the condition which I have been able to find is that of Morichau-Beauchant (22). The case was that of a girl of four months who developed, almost concurrently with the onset of varicella, vomiting and convulsions; the child rapidly sank into coma with dilated pupils, and she died on the following morning. The case of Debré, Lévy-Solal, Netter and Longchampt (39)is much better known. The patient, a married woman in the eighth month of pregnancy, showed mental dulness at the onset of a varicella eruption. Within twenty-four hours she had passed into profound coma, with stertorous breathing, stiffness of the muscles of the face and neck, and trismus. The patient died twenty-four hours after the onset. The classic case of Zimmerman and Yannet (79) will be referred to later.

As might be expected the symptoms and signs in this condition are very diverse. The commonest feature is a dulling of the intellect, in various degrees up to complete coma. Mental dulness or lethargy occurred in six cases (Debré, Lévy-Solal, Netter and Longchampt (39); Boenheim (52); Bernheim (59); Reimold and Schädrich (Case a (64)); Dagnelie and Dubois (89); Corda (Case c (103)). This sometimes passed into complete coma, which was found in five cases. Convulsions and vomiting were each found on four occasions. Eye signs were frequent. Strabismus was found in five cases (Boenheim (52); Vermeylen, van Bogaert, and Vervaeck (69); Borra (Case b (77)); Dagnelie and Dubois (89); Corda (Case c (103)), and the pupils were dilated in five cases. Ptosis was found once

(Lucksch (94); and nystagmus was present only in the case of Vermeylen, van Bogaert and Vervaeck (69). This latter case was also unusual in that it was the only one which showed ankle clonus. An extensor plantar response, a feature which was present in this case, was also present in Corda's third case (Case c (103)). Violent agitation, hallucinations, and a contrary mental attitude, reminiscent of encephalitis lethargica, were only found in the case of Vermeylen, van Bogaert and Vervaeck (69). Bonheim's case (52) showed the mask-features and muscular rigidity which are also suggestive of encephalitis lethargica. The following conditions were found only once: Vertigo (Fry (92)): hemiparesis (Fry (92): exaggeration of painful and thermal sensations (Borra, Case b (77)): paresis of both lower extremities (Lucksch (94)): clonic movements of head and limbs, dermographism, and meningeal signs (Corda, Case c (103)). Contrary to what might be expected, headache is uncommon; it is reported only twice (Bernheim (59); Vermeylen, van Bogaert, and Vervaeck (69)). Facial paralysis and ataxia each occurred twice, and epileptic seizures of Jacksonian type once (Babonneix (80)). Slight meningeal symptoms may be present - as was found in two cases, and speech defects are very uncommon.

Vermeylen, van Bogaert, and Vervaeck (69) note that in their patient, a girl of fourteen years, two definite conditions were found: (a) a state of mental depression related to the menstrual periods; and (b) a condition recalling the psychical form of encephalitis lethargica in the child. The case of Lucksch (94) is also interesting, in that a recrudescence of the neurological condition occurred after two months.

(b) Acute cerebral tremor. The honour of having first described this interesting condition is usually given to Zappert, who reported the tremor of the left leg in a child, following an attack of pneumonia. In June, 1909 Zappert (124) published a complete review of such conditions. Miller (125), however, also described a very similar condition in 1909, and in 1914, along with Davidson (28), he reported an example of such a tremor following varicella. The

earliest reported case following varicella is that of Forest (24), in which a ten months' old boy developed a tremor of the whole body fourteen days after the onset of the eruption. The tremor resembled that of paralysis agitans, and continued almost without interruption, even during sleep. The tendon reflexes were apparently increased, and the only important constitutional symptom was vomiting. Two days after the onset the tremor became intermittent, with one or other extremity affected in turn. In the later stages the tremor was present only when the child became excited.

Zappert (124) states that this condition is a disease of early childhood, occurring about the sixth to the eighteenth month, and boys are more frequently affected than girls. The tremor shows a sudden onset, is increased on movement, and tends to be less during sleep. Meningeal signs are absent, and the reflexes are slightly increased or normal. The plantar response is always flexor. According to Zappert (124) the condition may manifest itself in the legs not as a tremor but as ataxia.

I have been able to trace six examples of this interesting condition occurring as a complication of varicella. The tremor itself is the most outstanding feature. Miller and Davidson (28) describe the condition as a slow rhythmic movement, which prevented the patient, a boy of two and a half years, from standing without support; the movements of the limbs, particularly of the arms, were slow and stiff. In the cases which I have investigated the tremor involved the whole body at the onset only once (Forest (24)). In L. Rossi's case (99) the tremor appears to have affected most of the body, but was specially localised in the head; it was intensified by crying and by voluntary movements. In Mühlenkamp's case (93) it affected the arms, legs, and head at the start, but the whole body was later involved; in this case it was of a Parkinsonian type, and was increased by voluntary action. The head only was involved in the case of Greppi (85). The tongue was affected twice, and Glanzmann (46) described the condition in this organ as being one of "regular spasms". In only one case (Forest (24)) was

the condition noted as having been present also during sleep. The duration of the tremor is variable. In each of four cases it lasted over three weeks, and in the others the duration was shorter - for example, in Mühlenkamp's case eight days (93).

Apart from the tremor the most constant feature was spasm of muscles, which occurred in three cases. In Glanzmann's case (Case a (46)) this feature was so pronounced as to produce a definite opisthotonos. The tendon reflexes were increased in two cases (Forest (24); Glanzmann, Case b (46)). Vomiting occurred only once (Forest (24)), and marked excitability once (Greppi (85)). Mühlenkamp's case appears to have been associated with some cerebellar involvement, since he noticed, in addition to the tremor, strabismus, staggering gait, and slight hesitation in speech. In the case of L. Rossi (99) there was some ataxia at the onset, but the eyes and speech were not affected.

No definite information is available regarding the site of the lesion in these cases. Wieland (126) and also Miller and Davidson (28) suggested that the condition is due to a lesion in the rubro-spino-cerebellar tract, but Glanzmann (46) is inclined to regard the condition as due to an amyostatic or extra-pyramidal complex. Greppi (85) favours the first theory. Cornil and Kissel (76) on the other hand think that acute cerebral tremor is a "forme fruste" of acute cerebrellar ataxia.

(c) Cerebellar ataxia. Although the first case of the condition was reported by Caccia (17) in 1904, it was not until 1925 that two further cases were described by Galli (38). In all I have been able to find twenty nine cases of the condition in addition to my own. This syndrome constitutes the most common nervous complication of varicella. The case of Chavany and Chaignot (86) and the case now described both showed a particularly pure cerebellar syndrome; but in most other cases there were, in addition to the cerebrellar features, symptoms and signs pointing to involvement of other parts of the nervous system.

Generally speaking, the cerebrellar syndrome after varicella is marked by ataxia, vomiting, speech changes, nystagmus, vertigo, and tremor. Ataxia was noted in practically every case, and vomiting occurred in nineteen. In Galli's first case (Case a (38)) there was also constant retching. Some alteration of the voice was reported in fourteen cases. In most of these the condition could be described as bradyphasia, or monotonous speech; but in two cases - Cornil and Kissel (76); Borremans (97) - the speech was definitely of the scanning variety. Nystagmus, which occurred in twelve cases, varied in type, but the horizontal variety was most common. Contrary to expectations, vertigo was not a very common feature; it occurred in only thirteen out of the thirty cases in this group. Other features which are typically associated with cerebrellar trouble were not so common. Romberg's sign was positive in six cases; it was very marked in the first case of Tramer (75). Adiadokokinesis occurred in four cases (Runge (50); Bertoye and Garcin (58); Henner (102); Corda (case b (103)). It was also noted to be slightly present in the cases of Puig (82) and of Borra (Case a (77)). Propulsion and retropulsion were noted by Bertoye and Garcin (58); latero-pulsion was reported by Cornil and Kissel (76); and a combination of the two types by Kramer (34). Inco-ordination of the hands and legs was described in five cases, and was especially marked in that of Nyssen and Vervaeck (74), and in the present case. Asynergism of muscles occurred thrice. Dysmetria or hypermetria was found in four cases, and astasia in two (Poinso (96); Rendu (60)). A tremor of some part of the body was quite common. If affected the whole body in six cases, and the limbs alone in nine cases; on eight occasions it was of the intention type and was more marked on active movement. In certain cases the patient adopted a wide base in walking. Pyramidal symptoms were not very common. Ankle clonus was found on five occasions and an extensor plantar response was present in six cases. If the tendon reflexes were affected, it was usually in the

direction of an exaggeration (eight cases); a diminution of these reflexes occurred only twice (in the second case of Borra (Case b (77)) the knee jerks were absent). These features were more or less different from what one might expect from the condition of the muscles. Hypertonia of one or more limbs was noticed in only four cases - Caccia (17); Reimold and Schädrich (case b (64)); Graham (case b (67)); Poinso (96) - whereas hypotonia occurred in eight cases. Graham (67) noticed loss of abdominal reflexes (case a). Eye signs were definitely rare. In the cases of Kramer (34) and Corda (Case b (103)) a widening of one pupil occurred. In the second case of Reimold and Schädrich (64) there was a convergent strabismus. The fatal case of van Bogaert (case b(68)) was the only one to show definite meningeal symptoms (muchal rigidity and a positive Kernig's sign); it also showed choreic movements and patellar clonus. Corda (103) mentions that his second case showed slight meningeal signs later in the illness, and this late onset was also seen by Rendu (60). The most important symptoms of the condition, apart from ataxia, are headache, which occurred in ten cases, and lethargy, which was found in five. An interesting condition which was seen in the cases of Rendu (60) and of Chavany and Chaignot (86) was bradycardia; in both cases the pulse rate was reduced to about 50 perminute. In the second case of Corda (103) this feature occurred later in the illness. Other rare conditions, each of which was found in only one case, were dermographism, epistaxis, and scaphoid abdomen. The first case of Borra (77) showed oscillatory movements of the limbs which allied it with the choreo-athetotic group; and the cases of Meunier (53) and Corda (Case b (103)) showed similar movements. An epileptic seizure occured at the onset in the case of Borremans (97).

The average duration of the condition in those cases in which this is stated was thirty one days. Most cases centre around the period from fifteen to thirty days, and the longest duration was about three months.

The case of Chavany and Chaignot (86) is particularly interesting in that the cerebellar syndrome was very pure. The

main features of this case were vertigo, incessant vomiting, marked prostration, marked bradycardia, asynergism, slight adiadokokinesis, and speech changes which were described as resembling those of Friedreich's ataxia; all the tests for deficient tonus were positive. It is noteworthy that ataxia is not mentioned as having been present. The case of Puig (82) and the present case are also remarkable on account of the purity of the cerebellar syndrome.

It will be evident thatacute cerebellar ataxia following varicella is a condition which is characterised by a sudden onset with prostration, loss of muscular tone, vomiting, ataxia, and other pure cerebellar symptoms. In addition, in certain cases features of involvement of other tracts, such as the pyramidal system, are present. Despite the picture of extreme gravity which the cases present, they tend to clear up in a comparatively short time without leaving any sequelae. The only fatal case was that of van Bogaert (Case b (68)), and as has been pointed out, this case was associated with a meningeal condition. In the case of Crépin (72) there was some diminution of intellectual faculties for about six months after the cure of the ataxia.

(d) Choreo-athetotic forms. The premier case of this description was that described by Menko (11) of Amsterdam as "choreiforme Bewegungen" in 1899. The patient showed contortion of the facial muscles and a tremor of the head and upper extremities. Menko himself considered that the condition might be a "forme fruste" of chorea minor accurring after varicella. I have been able to trace eight examples of this condition. A particularly good description is that of the case of Sendrail, which was first reported by Dudevant (83) in her thesis, and which was later published separately in a joint paper by these authors (84). The symptoms and signs in this group are so diverse that they are difficult to analyse. The most constant feature was movement of choreo-athetotic type, which occurred in six cases. Menko's case (11) showed in addition to the tremor of the head and upper extremities a definite sucking movement of the tongue. The cases of Netter (13) and of Gautier and Monedjikova (87)

both showed tremor of an undefined type. Convulsions were present twice and lethargy or a sub-comatose state on three occasions. The case of Babonneix, Adeline, and Colombo (54) was noteworthy in that it showed at the onset Jacksonian convulsions followed by hemiplegia; this was succeeded by involuntary movements of great amplitude, violent, incoherent, incessant, affecting the whole of the musculature and completely recalling chorea. The right arm showed athetotic movements with a decerebrate attitude. There was also complete loss of speech and intermittent strabismus. The case of Wilson and Ford (Case a (45)) showed vertigo, vomiting, and ataxia on the seventh day after the onset of varicella; this was later followed by horizontal nystagmus, twitching of the muscles and choreiform movements of the arms; the knee and ankle jerks were increased, but the arm reflexes were diminished. Some degree of hypertonia seems to be fairly common, since it was noted in three cases - but in the case of Dudevant (83) meningeal features were also present. An extensor plantar response occurred in two cases, and ankle clonus in one. An increase in tendon reflexes also seems to be fairly common. It is interesting to note that some degree of dyspnoea was noted both by Babonneix, Adeline, and Colombo (54), and by Dudevant (83); in the latter case the condition was described as being one of periods of dyspnoea followed by periods of increased respiration, without the regular rhythm of Cheyne-Stokes respiration. Symptoms such as headache, vertigo, and vomiting are definitely uncommon.

The case of Dudevant (83) is interesting in that a good description is given of a sudden change from a meningeal condition to one of athetosis. When this occurred the lower half of the body became paralysed, though the upper half was in a state of constant motion. Two types of movement were found; one involving the musculature as a whole and consisting of movements of great amplitude; the other a true athetotic movement which affected the hands. The case of Fushiki (Case b (65)) showed a primary condition of meningo-encephalitis, which appeared just before the

onset of the eruption. This condition was cured about the forty-ninth day, but ten days later the child returned with clonic spasms.

(e) Non-characteristic encephalitic forms. In 1912 Osler (26) mentioned a case of infantile hemiplegia following varicella, and a typical case of similar type was reported by Sterling (27) in 1913. Winnicott and Gibbs (43) described a case of paralysis of the left arm of a child of three years; the onset was sudden on the ninth day after the appearance of the eruption. The stiffness of the left arm was apparently due to a voluntary muscle contraction, but the left leg showed true spasticity with an extensor plantar response in the left foot; the right leg was later similarly involved. Complete recovery resulted. From Spain Lavallén (78) described a condition of tonic spasms of long duration, with a paresis of the left side of the body and resistance of the left arm to passive movement; the left leg was also paretic. Recovery was rapid. In Giuffrè's case (88),a girl of four years showed nervous disturbances on the fifth day after the appearance of a varicella eruption; the main features were bradykinesia, hypotonia, various motor disturbances, and alteration of the reflexes; recovery was complete in twenty days.

Group 3 - Myelitis. (a) Transverse or ascending.

This group consists of eleven cases, and included in it are cases of typical asecending or transverse myelitis, together with conditions which were mostly myelitic in nature, but in which signs pointing to the involvement of other systems - for example, the cerebellum - were also present. In 1915 Wharton Smith (30) described a case in a boy of seven years; the patient showed definite paralysis of both legs and the left arm, with loss of sensation over these parts and extreme tenderness on handling. Examination showed slight atrophy of the affected muscles with marked increase of knee jerks and bilateral ankle clonus. The plantar reflex was later extensor. The case of Krabbe (41) was also typically a transverse myelitis with hyperaesthesia and

analgesia of the trunk below a transverse line through the nipples; the paralysis was flaccid with absent tendon reflexes and entensor plantar responses. The case of Waldman (42) is interesting in several ways. The patient, a woman of thirty-two years, showed a very sudden onset of the condition. Vomiting was accompanied by persistent and painful hiccough, and there was sharp pain round the chest and the back of the neck, with tingling in the hands and forearms. Examination showed complete anaesthesia of the entire body extending roughly up to about the level of the third ribs. A few days after the onset there was a zone of hyperaesthesia on the neck, and in this area there was distinct localised sweating; associated with this there was a slight ptosis of the left eyelid (Horner's syndrome). The patient died in coma five days after the onset. The case of Crouzon and Liège (55) was described by them as a neuritis, but other features suggest that the condition was really a myelitis. The main features were a painful quadriplegia, with sphincter disturbances; all deep reflexes of the limbs were abolished and diverse sensory disturbances were associated with adiadokokinesis and a staggering gait which suggested some cerebellar involvement. This case is quite unusual in that three years after the onset marked signs of nervous involvement were still present. The English cases of Rake (56) and of Cohen (57) are by no means typical. The case of Rake showed a spastic paralysis of the left side of the body with increased tendon reflexes and an extensor plantar response; the bladder was markedly distended. There was later definite weakness of the upper arm muscles on the right side, the right pectoral muscles, and the right intercostals. The case of Cohen (57) showed persistent nuchal rigidity and a marked "negativism". The two cases of van Bogaert (cases a and d (68)) also showed marked meningeal symptoms. The case of Heller (61) on the other hand was more or less typical of transverse myelitis.

Where conditions are so diverse it is difficult to make any suggestive analysis of symptoms. Paralysis - usually of the

legs - occurred in most cases, and loss or alteration of sensation over the affected part occurred in five. Pain in these parts was also a common symptom (six cases) and involvement of the bladder was also found on six occasions. Increase of tendon reflexes was found approximately as frequently as their absence, and the abdominal reflexes were absent in four cases. Though an extensor plantar response was quite common, ankle clonus was found only twice (Wharton Smith (30); Heller (61)). Vomiting occurred in three cases. Eye signs were noted only in the following instances: Krabbe (41) - dilatation of one pupil; Heller (61) - immobility of one pupil; Waldman (42) - contracted pupils; Rake (56) - convergent strabismus. Other uncommon features have been sufficiently discussed in the description of cases above.

(b) Poliomyelitis. Eight cases have been discovered which might reasonably be included in this group. The premier observation was that of Marfan (6). In 1893 this author described the case of a boy of nine months who, on about the fifth day of an eruption of varicella, developed a flaccid paralysis of the left arm which had not cleared up at the time of writing. Other features were present which might possibly have been claimed as causes of the condition. But thirty nine years later Marfan (127) again referred to this case and concluded that the varicella had been the cause of the paralysis. In the case of G. Rossi (15) a boy of eleven months had been restless for about twenty days; he then developed fever which persisted for two days; on the third day paresis of the right arm was manifest, and twenty-four hours later the child showed a typical eruption of varicella. Recovery was rapid and complete. It is noteworthy that this child had been vaccinated fifteen days before the development of malaise; although there is an element of doubt, it is more rational to conclude that the varicella and not the vaccination was the primary cause of the condition.

In some of the cases reported the description of signs and symptoms is meagre. Of the eight cases, one or both arms were

affected in four; one leg in one case - Corda (Case a (103)); both legs in two cases; and both arms and both legs in one case - Babonneix (Case b (80)). In every case in which the type of paralysis was mentioned it was flaccid. Abolition of the reflexes in the affected parts was the rule. In two cases - Tramer (Case b (75)); Babonneix (Case b (80)) - there was tremor in the arms. Both these cases also showed some disturbance of speech, and Tramer's second case exhibited in addition a marked flaccidity of the trunk and neck muscles. In van Bogaert's example (Case d (68)) photophobia and nuchal rigidity were also present. The case of Nucci (81) is somewhat remarkable, in that, in addition to the flaccid paralysis of the right arm, there was also present swelling of the right knee and ankle joints. The author discusses the differential diagnosis of the condition. Slight horizontal nystagmus was also present in this case.

(c) Multiple sclerosis. Nearly all authors quote the case of Bouvy (10). The patient, a boy of three years, showed weakness of the legs with marked exaggeration of the knee jerks, intention tremor, nystagmus, strabismus, and left facial paralysis. Double optic neuritis was present, and the speech was definitely of the "scanning" variety. This condition developed slowly after varicella. We have seen above that in these conditions of post-varicellous encephalitis intention tremor, nystagmus, and strabismus are by no means uncommon. Further, the fact that only one case has been described of this condition rather discredits this interpretation of the pathological condition. Miller and Davidson (28) quote Batten's views on the rarity of multiple sclerosis in children, and on the similarlity between it and cerebellar encephalitis. They suggest that this case of Bouvy's was an example of the latter condition.

Group 4 - Neuritis. The symptons and signs in the six cases in this group do not differ very much from those in ordinary cases of pheripheral neuritis. The case of Gay (7) was typical of the condition affecting the legs, as also was the case of Gaucher and Mirallié (16). In the case of Allaire (19) the condition affected

affected the left arm; the only interesting feature is that regurgitation through the nose also occurred, appearing first about a month after the onset of the varicella; this feature was suggestive of the presence of diphtheritic paralysis. Camus and Sézary (20) described the onset of flaccid paralysis three days after the eruption of varicella in a girl of eleven years. The condition remained stationary for five years; the arms then became affected. The authors suggest that the condition was an interstitial myositis. In the case of Fasella (62) the symptoms developed in the legs six days after the onset of varicella. The trouble cleared quickly, but by the twelfth day after the onset the arms werealso affected. Naito (14) in 1900 observed a transient paralysis of the facial nerve after varicella, but no further particulars are available about this case.

Pain occurred in four cases. It is noticeable that in Fasella's instance (62) the pain flitted from place to place. Slight increase in the tendon reflexes was noted only in the case of Fasella (62). Wasting of the affected muscles was common. Anaesthesia does not appear to have been frequent, except in the case of Gay (7), in which pins could be stuck into the patient's feet.

Group 5 - Ocular Manifestations. In 1898 Marfan (9) described the case of a girl aged twenty-two months, who developed a few days after the onset of varicella bilateral ptosis due to paralysis of both the levator palpebrae superioris muscles. There was also bilateral strabismus and paralysis of all the muscles acting on the eye-balls, except the external recti. Marfan discussed the pathology and concluded that the condition was of nuclear origin. Rolleston (117) later mentioned Marfan's further report that the condition had improved considerably apart from some sluggishness in the superior levator muscles.

In 1908 Chavernac (21) reported the case of a boy of eleven years who, a few days after the onset of varicella, developed sudden failure of vision, which was progressive. Examination

showed an increase in the diameter of the optic disc, with some fine haemorrhage at the edges; the veins were tortuous and dilated. Considerable improvement resulted. Paton (32) described a neuro-retinitis in a boy of fourteen years. The condition developed suddenly on the third day of the illness. Examination some months later showed pallor of the left disc with blurring of the edges and irregular spotting towards the macula. In the case of Ratner (40) total blindness suddenly occurred nine days after the onset of varicella and persisted for six days. Ophthalmological examination showed pallor of the left disc with dilated veins; in the right eye there was optic neuritis with marked swelling. Ratner considers that such cases of blindness in children following the acute infections can be divided into two large groups: (a) cases due to pressure from blocking of the foramen of Magendie; (b) cases due to direct retrobulbar neuritis of toxic origin. The case of Bouvy (10) also showed optic neuritis. In a recent interesting paper Mayerhofer and Breitenfeld (98) describe the case of a boy who sickened nineteen days after the onset of varicella with a condition which was reminiscent of lethargic encephalitis accompanied by hydrocephalus; as a result of this a bilateral choked disc appeared and complete blindness ensued. The authors performed Förster's operation and a complete cure resulted. The case of Huismans (25) has already been referred to. The condition was at first a meningitis, but on the fifth day after the onset of the eruption sudden blindness developed. This was found to be due to thrombosis of the central vein of the retina.

Ophthalmoplegia interna was described by Fuchs (31) in a female of twenty years. Ten days after the appearance of the eruption the pupil ceased to react and became wide. Eight months later accommodation was possible but the pupil was still wider than normal. A similar case of bilateral paralysis of the sphincter iridis muscles was described by Butler (70) in a girl of fifteen years. Six weeks later the eyes had not become completely normal. The patient of de Toni (36) was left with total unilateral

ophthalmoplegia as a sequelae. Babonneix, in the discussion following the paper by Grouzon and Liège (55), stated that he had seen inequality of the pupils in the course of varicella.

Group 6 - Other conditions. The case of Sachs (12) is unusual in that a mental condition was practically the only evidence of cerebral involvement. During an attack of varicella the patient, a boy of fifteen months, developed convulsions and loss of the power to use words; he soon fell into a state of complete mental alienation. Eight years later the physical condition was normal but the idiocy continued. In 1929 Tono (66) mentioned a case of encephalitis following varicella in a girl of five years, but no clinical features were given.

Infrequent conditions. The spleen was found enlarged in four cases - de Toni (36); Mya (5); Gorini (91); Corda (Case a (103)). The liver was enlarged in two cases - Conrad (63); Zimmermann and Yannet (79). The second case of Borra (77) showed enlargement of the cervical glands and of a right mammary gland. In the first case of Corda (Case a (103)) there was definite enlargement of the glands in the neck, axillae and inguinal regions.

Pyrexia. The temperature during the period of the cerebral complication was mentioned in thirty-nine cases. In eight of these cases no pyrexia was recorded at any time during the period. Of the remaining thirty one cases pyrexia about 100°F was noted at some period of the cerebral complications in all except four (Dagnelie and Dubois (89); Kramer (34); Corda (Case b (103)); and the present case). Of the twelve fatal cases the degree of pyrexia is mentioned in nine. Of these the lowest temperature recorded, in the case of Dagnelie and Dubois (89) was 99.5°F. In the other eight cases the temperature was high, usually 102° to 104°F. The highest temperature mentioned in any case was found in that of Zimmermann and Yannet (79), viz, 107°F.

Laboratory Investigation.

The results obtained by lumbar puncture are available in fifty-eight cases. To the naked eye all the fluids were clear,

except two which showed xanthochromia (de Toni (36); Debré, Lévy-Solal, Netter, and Longchampt (39)). Of forty-two fluids in which the degree of pressure was mentioned, there was no increase in twenty, and a slight increase in ten; the other twelve showed a marked increase in pressure. The cell content was usually normal (thirty-one cases) or showed a slight increase (twenty cases). In the remaining four in which this feature is mentioned there was a marked increase in the cell content (eighty or more cells per ccm). In the cases in which the cell content was increased there was a preponderance of lymphocytes or mononuclears in all except two, which showed a preponderance of polymorphonuclears (Goussis (37); Gorini (91)). The protein content was normal, or showed only a slight increase, in thirty-one out of forty fluids; in the remaining nine there was a marked increase. The figures with reference to glucose and chloride contents were not so definite. In a considerable proportion of cases the glucose was normal, and in the remainder an increase was rather commoner than a decrease. The same might be said of the chloride content, but in this case the estimations were infrequent. The Wassermann reaction was negative in all the fluids on which this examination was made - viz., twenty-two. Of seven cases in which Lange's colloidal gold test was performed, the curve was normal or nearly normal in four; it was of the meningeal type in two; and in another case it tended to be of the type associated with encephalitis lethargica.

Relations between the occurrence of nervous complications and type of varicella.

Table I gives an idea of the relation between the type of varicella and the accurrence of these nervous complications in those cases in which the information was available.

Table I.

Type of Varicella	Group P.	1.	2.	3.	4.	5.	Total Cases
Severe	-	2	9	4	1	2	18
Moderate	4	2	17	7	2	-	32
Benign	1	7	16	4	1	-	29
Total	5	11	42	15	4	2	79

It is seen that moderate and benign cases were of equal frequency and severe cases were considerably less frequent. Nevertheless, when we take into account the fact that severe cases of varicella are not nearly so common as milder types, it would seem that these nervous complications are really more liable to occur in patients who have a severe attack of varicella. This is especially the case where the complications are of the nature of myelitis. It is of interest to note that Eckstein (122) mentions that within recent years there has been no increase in the severity of chicken-pox itself - even in those cases which develop nervous complications.

Prognosis.

Of the hundred and twenty cases which are listed in the Appendix, information regarding the further course is available for one hundred and nine. Of these twelve died, eighty recovered completely, fifteen showed recovery with sequelae of some type, and two cases - Bouvy (10); Sachs (12) - did not recover. In the fatal cases death took place at any time from eighteen hours - Morichau-Beauchant (22) - to seventeen days - van Bogaert (Case b (68)) - after the onset of the nervous complication. In those cases which had not completely recovered at the time of reporting, a wide range of sequelae was left. Some of these were of no great consequence, such as lack of concentration - Fry (92), and widening of an affected pupil - Fuchs (31). On the other hand, many of the sequelae were of great importance, such as total ophthalmoplegia - de Toni (36) - and definite ataxia - Crouzon and Liège (55). Complete recovery was commonest in the cerebellar group (2c), and least common in the groups embracing myelitis and eye conditions.

Pathology.

A detailed description of the pathology of the condition is foreign to the purpose of this paper. Nevertheless, a brief review of the pathological appearances is material to the argument.

Of the hundred and twenty cases in the Appendix, a post-mortem examination was performed in six - Mya (5); van Bogaert (case

b (68)); Laignel-Lavastine, Miget, and Constantinesco (73); Zimmerman and Yannet (79); Dagnelie and Dubois (89); Lucksch (94). These cases are not all equally important from the point of view of the study of varicellous encephalo-myelitis, but it will be desirable to review briefly the findings.

(a) Mya (5). It has already been stated that this patient, a male of one year, suffered from a meningo-encephalocele, and the specific findings are therefore to a certain extent vitiated. The autopsy showed a loss of the bony substance of the cranium, through which the meninges and brain matter herniated. On the internal surface of the dura mater there was an exudate of coagulated blood. The condition was described as a pachymeningitis haemorrhagica.

(b) Van Bogaert (Case b (68)). The patient, a girl of twelve years, developed sore throat and elevated temperature on 3rd April, 1929. These conditions persisted until 11th April, when a typical severe attack of chicken-pox developed. Within two days the patient showed symptoms of meningeal involvement, which were later succeeded by marked cerebellar ataxia with mania and visual hallucinations. She died in the night of 29-30th April. The post-mortem examination showed extreme congestion of the brain, especially in the white substance of the cerebrum and cerebellum, where there were circumscribed inflammatory foci. The microscopical examination showed some perivascular infiltration, with nodules of glial proliferation centred round the vessels. In the cerebellar cortex the cells of Purkinje had lost their dendrites; they were glassy and opaque in appearance, and the tigroid substance was dispersed as a fine dust throughout the cell body. Van Bogaert concluded that these changes were probably degenerative in nature.

(c) Laignel-Lavastine, Miget, and Constantinesco (73). At autopsy the gross changes were congestion of the dura with thickening of the meninges generally. The superficial vessels of the cortex were dilated. Microscopically the surface of the pia showed a thick layer of leucocytes and of altered monocytes. A deeper layer showed much thickening and oedema with numerous distended capillaries filled with blood, and for the most part

surrounded by a perivascular cuff, due either to thickening of the neuroglial network surrounding the vessel or to accumulation of inflammatory cells. There was some chromatolysis of the neurons of the superficial layer of the cortex, with distension of the pericellular spaces.

(d) Zimmerman and Yannet (79). The patient, a girl of thirteen months, developed four days after typical varicella a condition which was classified above as encephalo-myelitis of the cerebral type. Death took place on the following day. The post-mortem examination showed lesions only in the central nervous system. Microscopically the ganglion cells of the whole brain and spinal cord showed degenerative lesions, which varied in intensity but not in kind from place to place. The cytoplasm of these cells showed large translucent vacuoles. There were focal haemorrhages around the cortical vessels. In the white matter of the parietal and occipital lobes the vessels showed perivascular cellular cuffs, and the latter were surrounded by demyelinated zones. Fat granule cells were frequent in certain areas of the white matter, and also in the leptomeninges.

(e) Dagnelie and Dubois (89). The patient, a girl of eight months, developed extreme irritability fifteen days after the onset of varicella; coma ensued and death took place within twenty-four hours. Microscopical examination of the brain showed excessive congestion of all the white matter, with dilatation and engorgement of the small capillaries. In certain areas there was a perivenous demyelinization without alteration of axons. The nuclei of the base showed much congestion; most of the vessels were here surrounded by granular substance, but no demyelinization was demonstrable by the Spielmeyer process.

(f) Lucksch (94). The patient was a boy of fourteen months who, four days after the onset of varicella, developed left ptosis and paresis of the left pupil. There was also paresis of the right side of the face and of both lower extremities. After thirteen days the symptoms suddenly disappeared, but two months after the first

onset there was a recrudescence, with a purulent discharge from the left ear. The patient died about a week later. The opinion was expressed at post-mortem examination that there was no connection between these two attacks. Microscopical examination of the central nervous system showed, especially in the white substance, numerous distended vessels packed with blood.

Van Bogaert (128) later made a more complete examination of his fatal case, with special reference to its relationship with multiple sclerosis and the encephalomyelitis following other infectious diseases. He noted that a considerable number of foci showing demyelinization were visible to the naked eye. These foci varied in size, and they looked like small oil drops. They were often centred round vessels; but this feature was not so characteristic as is the case in the encephalomyelitis following measles and vaccination. His case was similar to that of Zimmerman and Yannet (78) in that both showed perivascular changes in the white substance, with infiltration of phagocytic cells; a marked increase of granular cells in the meninges; and cortical and subcortical changes in the cells without gross alteration of the structure of the brain substance. On the other hand, van Bogeart's case showed certain features which that of Zimmerman and Yannet lacked, viz, proliferation of glial cells in a manner reminiscent of multiple sclerosis; and a certain amount of inflammatory material around the vessels. Van Bogaert concludes that his case of post-varicellous encephalitis showed features which rendered it more closely akin to multiple sclerosis than to the encephalo-myelopathies of measles and vaccinia.

From these findings it will be apparent that there is no definitely typical pathological feature of varicellous encephalitis. In the case of post-vaccinal encephalitis Greenfield (129) states that, though perivascular cuffing is very common, the typical feature of the condition is perivascular demyelinization. In the six cases discussed this feature was by no means so constant and marked as to be considered typical of the condition.

There is an inclination on the part of many writers to include chicken-pox among the diseases in which the typical features of the post-infectious encephalo-myelopathies are found. For example, Rivers (130) includes varicella in the group of diseases in which the post-infectious encephalitis is characterised by perivascular demyelinization. There is little doubt that the post-mortem findings are more or less identical in the encephalo-myelitis following vaccinia, smallpox, and measles. This condition has very generally been described as acute disseminated encephalomyelitis, the name which was given by Westphal (131) in 1872. Recently this condition was extensively studied by Marsden and Hurst (132) in smallpox, and they placed so much emphasis upon the feature of demyelinization as to propose the new term "acute perivascular myelinoclasis" for the condition. In 1928 Turnbull (133) mentioned that he had not seen any satisfactory report in the literature of a post-mortem examination in encephalomyelitis after varicella. Greenfield (134) admits that in this condition there is no satisfactory evidence that the pathological picture is the same as in encephalomyelitis following certain other infectious diseases. Wohlwill (135) states quite definitely that it is wrong to assume that the pathological findings are the same in varicellous as in post-vaccinal encephalitis. Zimmerman and Yannet (79) concluded that their case showed features which were characteristic both of the encephalitis following vaccination and of that following measles and the non-specific encephalitis described by Low (136). Lucksch (94) was of opinion that the histological findings were entirely due to the pneumonia and otitis which were present in his case, and that no typical findings of encephalitis, such as those described by van Bogaert (68) were present. Lucksch therefore argues that, since it is very improbable that severe pathological conditions, such as have been described by others, would resolve within the period of two months which in his case elapsed between the attack of encephalitis and the occurrence of the pneumonia, otitis and death, these pathological features are therefore not

unconditionally necessary for the occurrence of clinical manifestations of encephalitis, and that they probably only occur in those cases which lead to a fatal termination. Van Bogaert (137) is inclined to agree with Lucksch that it is in the grave cases that the typical features are more likely to develop. He suggests that each virus has a predilection for certain regions of the nervous system - a statement which receives much support from the frequency of cerebellar complications in varicella.

Epidemiology.

In a strict sense the heading for this section cannot be justified, since the use of the word "epidemiology" assumes that the condition which is under discussion is a separate entity, that it is communicable by direct or indirect spread from one person to another, and that it has affected a considerable number of persons within a reasonable period of time. It will be seen from what follows that these desiderata are not fulfilled. There is at present no definite proof that the meningoencephalitis or the associated nervous conditions which occur in close time relationship to the onset of varicella constitute a separate disease. Even if this is ultimately admitted, evidence of communicability is singularly lacking; and again, if we admit that the word "epidemic" is a relative term, there is no suggestion that there has never been an outbreak of a magnitude sufficient to warrant its use. The term "epidemiology" is, however, a convenient one to describe, in connection with these nervous conditions, certain features which are not purely clinical in nature. In this sense it has been so used in various official reports, and it has accordingly been retained here.

It should also be noted that the following discussion applies to all the cases which are listed in the Appendix. The suggestion may be made that Groups P, 4, 5, and 6 may not be strictly comparable with Groups 1, 2, and 3. There is, however, no evidence to the contrary, and the inclusion of all cases makes the investigation more complete, and does not materially

affect the results. Further, since the majority of the cases discussed were true cases of encephalitis or meningo-encephalitis, the term "encephalitis" will be frequently used in the general sense ~~of~~ in the discussion.

(a) Sex. Of the 120 cases referred to in this paper, the sex was given in 112. Of these sixty-nine were males and forty-three females. The sex incidence in the different groups is set out in Table II. It is seen that the preponderance of the male sex exists in practically every group, but is particularly marked

TABLE II.

Group	P.	1.	2.	3.	4.	5.	6.	Total
Males	3	12	34	11	4	4	1	69
Females	3	4	22	9	1	3	1	43
Total	6	16	56	20	5	7	2	112

in the group which consists of the true cases of encephalitis. This sex incidence may be compared with that of post-vaccinal encephalitis: of sixty-two cases of this condition forty were females and twenty-two males (138).

(b) Age. The age was given in 114 of the 120 cases. The age incidence for the different groups is given in Table III.

TABLE III

Group.	Under 1	1-	2-	3-	4-	5-	10-	15-	20+	Total
P	-	1	1	2	2	2	-	-	-	6
1	1	1	1	1	2	7	-	2	1	16
2	5	6	4	10	12	16	3	1	1	58
3	2	2	1	5	-	7	-	-	3	20
4	-	-	1	-	1	2	1	-	-	5
5	-	-	1	-	1	-	3	1	1	7
6	-	1	-	-	-	1	-	-	-	2
Total	8	11	9	18	16	35	7	4	6	114

It will be seen that ninety-seven of the cases, or eighty-five per cent, occurred in children under ten years of age. Some writers laid stress on this point with regard to the etiology of the condition, but it seems more reasonable to assume that this frequency is associated solely with the greater frequency of chickenpox at the younger ages of life. For example, the age grouping of 9,486 cases of chickenpox which were notified in Leeds

during the five years 1927 to 1931 inclusive is given in Table IV. (139) (Figures for the age groups 5 - 10 years were not available).

TABLE IV.

Year	Under 1	1-	5-	15-	25-	45-	65-	All ages
1927	10	125	177	6	3	-	-	321
1928	89	615	987	22	4	-	-	1,717
1929	112	846	1,535	40	12	-	-	2,545
1930	111	937	1,664	41	14	1	-	2,768
1931	90	725	1,278	30	10	2	-	2,135
Total	412	3,248	5,641	139	43	3	-	9,486
Per cent	4.3	34.2	59.5	1.5	0.5	0.03		

From these tables it will be seen that the virus, whatever it may be, is probably as likely to affect the nervous system in patients over fifteen years as in patients under that age.

(c) Date of occurrence. The approximate date of occurrence of each case was available in 118 of the 120 cases. In tabulating these features the actual date of occurrence, as given in the original paper, was used whenever possible. When this date was not given, the year of publication of paper was taken as the year of occurrence. Any discrepancies which occurred are probably not very appreciable. The information obtained is shown in a chart (Figure I). It will be seen that the incidence of this complication assumed what might be termed "epidemic proportions" between the years 1921 and 1925, and reached its peak in the following quinquennium. The actual figures for the years 1921 to 1933 are given in Table V.

TABLE V.

Year	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933
Cases	1	-	-	1	12	3	9	12	14	13	6	10	2

Where the cases are so few and scattered over so many years, it is impossible to base any considered conclusions upon the evidence of time incidence; but as a matter of interest, whenever possible, the month of occurrence of each case was also noted. These particulars are available for seventy-six cases, and the results are given in Table VI.

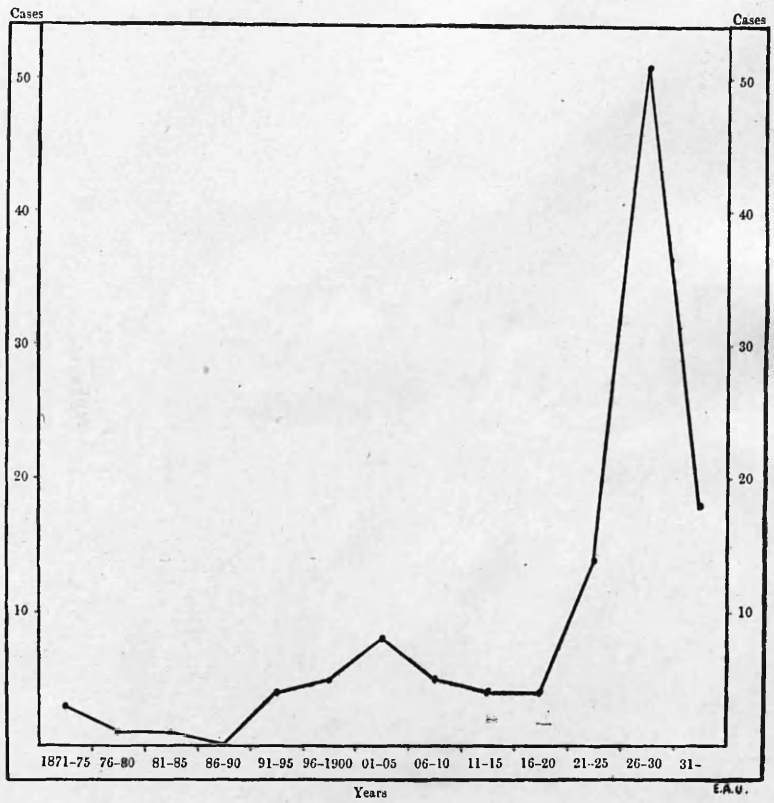


FIG. 1.—Incidence in 5-year periods of encephalomyelitis and other neurological conditions associated with varicella.

TABLE VI.

Month	Jan.	Feb.	Mar.	Apl.	May.	June.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Cases	4	14	8	6	11	5	3	3	2	2	12	6

To conclude this section it may be said that the season of the year would not seem to play any part in the occurrence of this complication per se. On the other hand, the year of occurrence is so important that it will be discussed in a later section.

(d) Country of origin. The countries of origin of 119 of the 120 cases are given in Table VII. It will be seen that France and Belgium account for more than one third of all reported cases. The incidence was also high in Italy and comparatively high in the United States of America and in Germany. The British cases are rather misleading. Of these eleven British cases (which figure includes the present case) four were reported before 1926. When the number

TABLE VII.

Austria	5	Corfu	1
Great Britain ...	11	Denmark	1
France	32	Switzerland ...	4
Sweden	1	Poland	1
Italy	19	South America ...	1
Holland	1	Belgium	9
United States of Amer.	13	Canada	1
Germany	9	Bohemia	3
Japan	3	Croatia	1
Czecho-Slovakia...	2	Spain	1
		Total	119

of cases reported in France, Belgium and Italy, since that date, are taken into consideration, this incidence must be considered low.

(e) Multiple cases in households. The reports have been carefully scrutinised with a view to the study of instances where more than one case of nervous complications occurred in single households. Only one definite instance of this was discovered, namely, in the two cases described by Galli (38). These two cases, a brother and a sister, were of a family of nine children, eight of whom had varicella more or less at the same time. The boy developed the nervous condition first, and two days later the girl showed similar symptoms. In both cases the condition was of the nature of cerebellar ataxia. This low incidence of multiple cases in households is especially interesting in view of the statement of

Marfan (40) that he had frequently seen small epidemics of nervous conditions in children who lived near, or in association with, actual cases of chicken-pox.

(f) Previous medical history. A complete description of the previous illnesses reported in these cases will not be attempted here. It is sufficient to note that, of twenty-seven cases in which the writer found full reports of the previous history, ten of the cases had suffered from some more or less organic condition, such as rickets, convulsions, anaemia, or backwardness. In two of these cases (Borremans (97); Crouzon and Liège (55)) there was a definite history of a neuropathic tendency; and in the case of Bouvy (10), a child of three years, there had been slight facial paralysis after an attack of measles a year before. It would appear that there is some probability that the virus of the condition is disposed to attack nervous systems which are unstable or which have previously shown some clinical evidence of involvement.

(g) "Incubation" period. Of all the epidemiological aspects of the problem this is the most important from the point of view of pathogenesis. The available reports were examined with a view to finding the "incubation period" - or the exact interval between the appearance of the eruption and the development of nervous symptoms. These features were available in 105 of the 120 cases listed in the Appendix. This interval might be either positive or negative. The cases have been arranged on the assumption that the interval is "positive" when the nervous complications develop after the appearance of the eruption, and "negative" when they develop before. This interval, stated in days, varied from 0 (that is, the nervous symptoms appeared practically concurrently with the onset of the eruption) to 90. The long "incubation period" of ninety days shown by the cases of Henner (102) and Marfan (9) is quite unusual. Incidentally, it should be mentioned here that in a large proportion of the cases which occurred before 1900, the "incubation periods" were short and of negative sign. This possibly suggests that the old clinicians were interested merely in those features which were typically associated with the onset of the disease and the appearance

of the rash. As a corollary, it seems possible that some cases with longer "incubation periods" may not have been considered as due to varicella, and may not have been reported.

Post-vaccinal encephalitis differs from all forms of post-infectious encephalitis in that the precise date of the introduction of the virus can be ascertained. In the post-infectious cases, although a fairly accurate guess at the approximate date of introduction of the virus can usually be made from the date of the appearance of the eruption, nevertheless there are grounds for believing that the actual symptoms of the disease may not appear for some considerable time, and that during this period certain hitherto unexplained phenomena connected with the immunological responses of the body may occur. The "incubation periods" were tabulated in a manner similar to thatadopted by the Rolleston Commission on Vaccination (138). When the sums of cases were plotted out against the time intervals, it was found that the resulting curve approximated fairly closely to the logistic curve of population growth. This suggests that there is probably a close relationship between the occurrence of chicken-pox and the development of such nervous complications.

The average "incubation periods" for each group are set out in Table VIII.

TABLE VIII.

Group	P	1	2a	2b	2c	2d	2e	3a	3b	4	5
Average "incubation period" (days)	-1.5	+6.9	+4.7	+8.9	+9.1*	+7.4	+7.3	+8.2	+3.0	+15.6	+23.5

* If Henner's two extreme cases are excluded, this figure becomes +6.8, which gives a truer conception

Owing to the small number of cases in most of the groups, and the wide deviations from the mean, this table does not give a good idea of the actual "incubation periods". Figure 2 gives a better idea of the main features. It is seen that the onset of a nervous complication more than three days before or twenty days after the appearance of the chicken-pox eruption is quite exceptional. The bulk of the cases occur between the fourth and the tenth day after the appearance of the eruption.

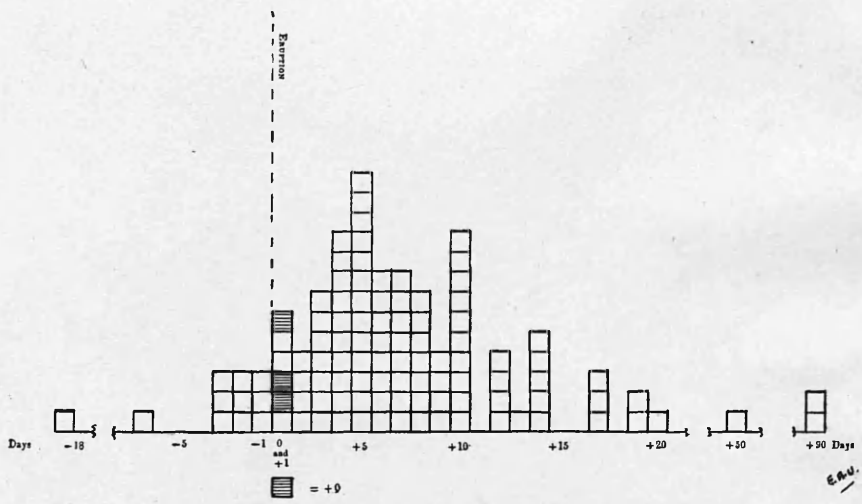


FIG. 2.—Diagram showing "incubation periods" of 107 cases of encephalomyelitis and other neurological conditions associated with varicella.

Theories with regard to Aetiology.

There are five main possibilities which might explain the occurrence of this condition. (a) The occurrence of the encephalitis following the varicella may be purely fortuitous. (b) The nervous symptoms may be due to the virus of some known disease. (c) The nervous symptoms may be due to some unknown virus. (d) The condition might be due to the virus of the primary disease, in this case varicella, or to toxins of the virus (e) The nervous symptoms may be essentially caused by a change in the allergic state of the nervous system. It should be mentioned that it is impossible to discuss the pathogenesis of encephalomyelitis following varicella apart from that associated with other primary conditions. The above causes will now be briefly considered.

(a) Possibility of a fortuitous occurrence. If the theory of fortuity is to carry any weight, then it should be possible to show that cases of acute nervous disorders occurred in the same district and at the same time as these cases of varicellous encephalomyelitis; and we should be able to show also that there was no definite association in time between the onset of the eruption and the occurrence of the nervous condition. So far as the first desideratum is concerned, it would also be necessary to show that there is a condition which is clinically and pathologically indistinguishable from varicellous encephalomyelitis, but which is not associated with varicella as a primary disease. Such a condition possibly exists. Strümpell (142) described in 1885 an encephalitis which did not appear to be associated with a primary condition. Stooss (143) described in 1926 a simple encephalitis, and in 1929 Brain and Hunter (144) reported six cases of acute meningo-encephalitis in children, one of which was fatal; in this report there is no mention that any of the children had recently had any infectious disease. Brown and Symmers (145) in 1925 reported cases of acute serous encephalitis in childhood. On the other hand in the papers dealing with varicellous encephalitis the writer has read there was only one mention of the occurrence, about the same time as the

illness of the case described, of such conditions in children who were not suffering from one of the infectious diseases. This exception was the statement made by Marfan (140) and it will be shown later that it can be explained apart from fortuity. In fact most of the evidence points in the other direction. The concurrent existence of nervous features in the brother and sister described by Galli (38), both of whom developed chicken-pox practically at the same time, is suggestive of the direct association between the two conditions. So far as the second desideratum is concerned, it has already been mentioned that the question is similar to that for vaccinal encephalitis; it was discussed in the section which deals with the "incubation period".

(b) Theory of the virus of a known disease. Some authors suggest that these conditions are essentially a typical form of acute poliomyelitis which develop in association with varicella. From a clinical point of view the good prognosis and the fact that subsequent paralysis was seldom noticed is sufficient to discount this view. In recent years a few authors, for example Babonneix, have been inclined to hold that these were really atypical cases of encephalitis lethargica. Again, the clinical findings are sufficient to negative this supposition - viz., the fact that somnolence is rare after the acute phase of the condition, the entire absence of sequelae such as are characteristic of the juvenile type of lethargic encephalitis, and the sudden and often fulminating course with rapid evolution. Other viruses have been incriminated, but the evidence in their favour is slender.

(c) Theory of an unknown virus. The suggestion that the encephalitis following vaccination and certain infectious diseases is due to an unknown virus which is activated by the primary condition has many supporters, especially in this country. It would appear that Bastiaanse (146) and Perdrau (147) were among the first to make this suggestion. In discussing the pathology and pathogenesis of the encephalomyelitis of measles, Greenfield (129) points out that the earliest case of this condition reported in

this country was that described by Mr. James Lucas (148), a surgeon to the Leeds General Infirmary, in 1790. In this case of measles paraplegia a similar attack had come on previously after smallpox, and Greenfield says that this case of itself affords strong support for the view that the condition was due to another virus which was activated by the exanthemata. It seems to the writer that the case may be equally well argued from the other direction; the patient developed the nervous complications, not because she had been so unfortunate as to have picked up on two separate occasions a hypothetical virus, which certainly rarely produces definite lesions, but because some unknown factor had altered the response of her nervous tissues, so that they reacted abnormally to the viruses of two separate conditions. This instance therefore rather seems to favour the the view of van Bogaert (see later). On the other hand, Levaditi and Nicolau (149) have shown experimentally that the inoculation of a neurotropic virus can activate another virus which may be present, but latent, in the experimental animal. Important evidence in favour of the fact that post-vaccinal encephalitis is due to some unknown organism has been furnished by Reisch (150), who reported eight cases of the condition with two deaths among 233 vaccinated persons in a Tyrolese valley; of more than 300 children in the same area who had not been recently vaccinated, a comparable number showed similar symptoms. The most important evidence against this theory would seem to be furnished by the comparative clinical findings and the relative prognosis in different types of post-infectious encephalitis We have already seen that cerebellar conditions are very frequent in the varicellous type. Greenfield (151) notes that in post-vaccinal and other types of these encephalitic conditions trismus is common; in the present series of cases it was noted in only three instances (Dudevant (83); Debré, Lévy-Solal, etc (39); Bérode (90)). So far as prognosis is concerned Ford (152) shows that in measles encephalitis the mortality rate is ten per cent, and complete recovery occurs in only twenty five per cent. For

post-vaccinal encephalitis Rolleston has estimated the mortality at thirty to forty per cent (153). For the present series of 120 cases of post-varicellous encephalitis the mortality is 11.2 per cent. Further, Brain and Strauss (154) point out that if vaccinia and measles encephalitis were both due to the same virus, vaccination would need to be a much stronger predisposing cause, since the mortality is so much higher. To sum up, it is very difficult to explain on this theory why the same virus should act in such different ways when associated with different primary diseases.

(d) Theory of the virus of the primary disease. This theory has been supported by a number of writers on the Continent, for example, Bertoye and Garcin (58); Ingelrans (155); Cornil and Kissel (76); Laignel-Lavastine (73); Marfan (127); but it has so far not found a great deal of favour in this country. For post-vaccinal encephalitis McIntosh is inclined to hold that the vaccina virus is responsible. So far as varicella is concerned certain objections have been pointed out, such as the variability of the intervals between the development of the eruption and the onset of the nervous symptoms. But reference to Figure 2 will show that these "incubation periods" generally do not exhibit differences which are much more marked than the "incubation periods" of many infectious diseases. Another objection is that even in vaccinia it is seldom possible to recover the virus from the brains of the patients who have died. But Levaditi (157) has shown that many of these viruses are really auto-sterilisable. Further, encephalitis has been produced in monkeys by Eckstein (159), who injected vaccine virus by the sub-arachnoid route. The experimental work of Pincherle and Negri (160) suggests that there is a constitutional nervous factor which predisposes to a development of neurotropism on the part of the virus of varicella. Reference should here again be made to the fact that Marfan (140) has seen small epidemics of nervous conditions in the neighbourhood of children who were suffering from varicella, and similar conditions have been mentioned by Babonneix. Varicella without eruption is not unknown and is perhaps commoner

than is usually held (Nicolle (161)). It seems reasonable to assume with van Bogaert (162) that the nervous condition may be the central equivalent of the exanthem in certain cases.

Related to this theory is the suggestion that the condition may be due to the toxins of the primary virus. This was suggested by Perdrau for vaccinal encephalitis, and Dagnelie, Dubois, Fonteyne, and their collaborators (158) point out that the theory would apply rather to diseases such as diphtheria and scarlet fever which are non-septicaemic in nature. In any case this suggestion does not explain the source of the toxins.

(e) The anaphylactic theory. This theory was first enunciated in 1927 by Glanzmann (46), who described cases of encephalitis following varicella, smallpox and vaccination. Glanzmann thought that the complications were local anaphylactic phenomena due to the sensitization of the nervous system to the actual virus of the disease. In support of his theory he instanced the usual narrow limits of the "incubation period", and he pointed out that there was considerable evidence that tuberculosis leads to a non-specific sensitization, which may dispose to an allergic reaction of the nervous system when the patient contracts another infection. He claimed that the lesion was not specific but could be produced by different antigens, and he quoted Sahli's view that the anaphylactic reaction leads to a speedy destruction of the virus of the primary condition.

The most doughty exponent of this anaphylactic theory is Ludo van Bogaert, who with his colleagues has probably done more work than anyone else on these conditions. Van Bogaert's views are rather difficult to explain concisely, since they depend upon interpretations of allergic phenomena which are somewhat foreign to the current views in this country. Interested students should certainly read his original papers on the subject. Van Bogaert interprets the skin eruption in these infections as an expression of the interaction between antigens and antibodies, which takes place especially in ectodermal tissues (158), (162). Normally

this reaction takes place only in the cutaneous tissues. The central nervous system, the other important ectodermal structure, is protected by the "barrière-hémato-encéphalique" - or the B.B.B. (blood brain barrier), a system whose importance has recently been emphasised in this country in the writings of Friedemann and Elkeles (163). Any type of inflammation of the brain is supposed to upset this barrier, so that the nervous tissue absorbs antigens and becomes sensitized; a subsequent infection causes an allergic reaction in the nervous system, and the more hypersensitive the brain tissue, the earlier would appear the nervous complication, which is essentially an anaphylactic phenomenon. Van Bogeart claims that this theory explains, on the grounds of marked hypersensitivity, those rare cases in which the allergic response in the nervous system, with its co-incident neurological features, precedes the allergic response in the cutaneous tissues, which are made manifest by an eruption. He thinks (137) that there is a biological balance between the exanthem and the encephalomyelitis, and that this neuro-cutaneous alteration of sensitivity is perhaps a manifestation of a general law, and that the "encephalitic accident" occurs in the period of hyperallergy through which the individual passes before reaching the state of immunity. Van Bogaert claims (137) that this theory explains the occurrence of all these types of post-infectious encephalomyelitis, since, though they all depend upon a common type of reaction, each individual virus - i.e. of the primary disease - acting as an antigen, imprints upon the resulting clinical picture features which are pecular to itself. It explainsalso the differences in the reaction periods in the encephalo-myelopathies due to different primary conditions.

Certain criticisms have been raised against this theory. It is stated that, if the encephalitis is truly an anaphylactic phenomenon, depending on the action of the primary virus in the nervous system, the nervous symptoms should appear after a more or less constant interval following the onset of the eruption. The reply to this objection is that, if the interval between the onset

of the varicella and that of the nervous complications varies, there is some evidence that these complications almost always appear at the time when the eruption is disappearing. The other objection is that the nervous complications show considerable polymorphism; if they were due to the association of two constant factors - viz., the virus of the primary disease and the neuro-allergy of the individual - then they should be more constant in type. This objection does not seem very valid, since we have seen that varicellous encephomyelitis shows a special tendency to be cerebellar in type, and other forms of the post-infectious encephalo-myelopathies show individual peculiarities. This polymorphism, as is noted by Bérode (90), is really in support of the theory that the virus of the primary disease is responsible; if one specific virus were the cause of all the post-infectious encephalo myelopathies, then we should expect it to have special affinities for special parts of the nervous system - whatever the primary disease.

General discussion on pathogenesis.

The evidence which has been adduced so far is that post-varicellous encephalitis is a condition which supervenes usually between the second and the tenth day after the appearance of the eruption. Clinically, it shows the main features of other types of post-infectious encephalitis, but cerebellar forms are much commoner in the varicellous type. Pathologically, it has been shown that the disease is not identical with these other conditions. It would appear, therefore, that the condition, though closely related to these other encephalo-myelopathies, must differ from them etiologically in some essential feature. If we discard the theory of fortuity, this feature might be either the infecting agent, the response of the individual, or both factors combined. Applying the principal of Ockham's razor - Entia non sunt multiplicanda praeter necessitatem - there remains for discussion two possibilities - a peculiarity in the causal virus, be it new or old, or a peculiarity in the individual. Though we may dismiss the theory that the virus

of a known disease is the cause, definite arguments have certainly not been adduced to contradict those partisans who hold that such conditions are due to a hitherto unknown virus. The writer is not inclined to accept this theory, and further arguments will be brought against it later. If we set this theory aside in the meantime, we are left with two possibilities; a change in the behaviour of the virus of the primary disease, or an alteration in the reaction of the infected individual. As has been pointed out, van Bogaert holds the latter view, and his attitude is perhaps best summed up in a quotation from a letter to Lucksch (94). Van Bogaert says: Je suis comme vous convaincu, que la réaction allergique du cerveau des enfants a changé depuis ces dernières années et que ce ne sont pas les virus, qui sont devenus neurotropes, mais les individus, qui sont devenus neuro-allergiques! ".

The epidemiology of this condition has been discussed in this paper because it appears to the writer that it affords important evidence in favour of the neurotropic theory of the germ as against the neuro-allergic theory of the individual. Any inquiry of this type must discuss, or assume knowledge of, the theories of Sydenham and his modern followers. Sydenham's conception of an "Epidemic constitution", and of a stationary fever which impressed its character on all other fevers which effloresced under its influence, is bearing rich fruit in these post-war years of troubled epidemiology. As Greenwood (164) says of Sydenham: "What was fruitful and just was his conception of an epidemic succession which if present in the minds of his predecessors, was never so forcibly and even magnificently expressed as by him". The year 1918, marked by one of the most terible pandemics in history, introduced an epoch which was particularly suitable for an examination of Sydenham's doctrines. The theories of Hamer (see for example (165), (166), and especially (167)) and Crookshank (see (168), (169)) are well known. Crookshank's view may be summed up in a quotation from his writings (170); "Epidemic meningo-encephalomyelitis represents an intensive and specialised reaction

that has the same epidemiological relation to pandemic influenza as have the prevalences and epidemics of "septic" pneumonia, of gastro-intestinal illness, and of other maladies described as occurring before and after the wide diffusions generally referred to as pandemic influenza. Owing to the relative infrequency of epidemic encephalomyelitis and its marked variation in type, historical investigation is necessary in order that contemporary occurrences be viewed in a correct perspective." There is much evidence that, once a particular "constitution" manifests itself, its effects last for a considerable time. For example, Stevenson (171) demonstrated that the 1918-19 pandemic of influenza, as it affected London, differed from all the other London epidemics since 1890, in that there was a marked toll on young adult life, and in that the later ages, which had previously suffered severely, were in that pandemic comparatively little affected. The writer has recently shown that these features were equally marked in Leeds during the 1918-19 pandemic, and also that influenza in that city did not return to its customary type, with the more common age-grouping, until after a period of about ten years. Further, the return to the status quo was gradually effected during this period (Underwood (172)). Whether or not we accept Crookshank's theory in toto, with all its implications, we can hardly escape from the conclusion of MacNalty (173) that, though each of the main epidemic diseases of the central nervous system is an independent entity, nevertheless the resemblances between them indicate that they belong to the same family tree; the study of one disease may make clear the epidemic behaviour of the others. We may even carry this dictum further and say that the study of the epidemic behaviour of certain of these diseases whose origins are known may throw some light on those in which they are unknown.

The incidence of influenza and of the main epidemic diseases of the nervous system since 1870 is set out in Figure 3. (In compiling this chart the data for influenza were taken from (174); (175) and (176); those for cerebro-spinal fever, poliomyelitis, and for epidemic encephalitis from (175) and (176); those for post-

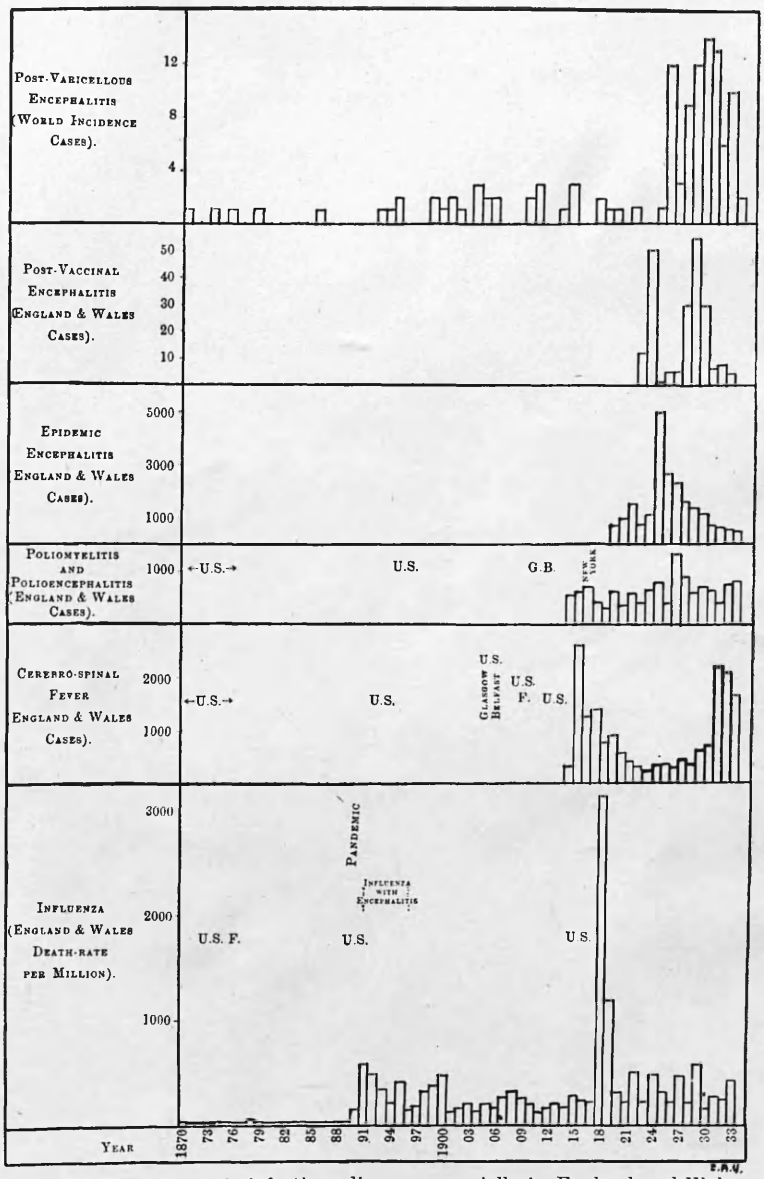


FIG. 3.—Incidence of certain infectious diseases, especially in England and Wales, with indications regarding world prevalence. (In this figure the abbreviations for countries are the same as those used in the Appendix).

vaccinal encephalitis from (175) and (177); and those for post-varicellous encephalitis from the present investigation. The notes regarding world-incidence of certain of these diseases were compiled from information in (169), (173), and (174). The salient feature in Figure 3 is the great peak of the 1918 influenza pandemic. This peak is associated in time with an increased incidence of all the other diseases discussed, and within a few years each showed a peak. The only disease which might be considered to be an exception is cerebro-spinal fever. In this case the peak occurred in 1915, but during the years from 1916 to 1919 the incidence was far above normal. Further, it is recognised by many authors that outbreaks of cerebro-spinal fever not infrequently occur before influenza epidemics. It is noticeable also that post-vaccinal encephalitis was first noted, apart from isolated earlier instances not shown on the chart, in the year 1922. It will be evident from this figure that in 1925 post-varicellous encephalitis suddenly became "epidemic" (if the word can be applied to a disease which is so rare), and during the next seven years it continued to prevail to a hitherto unknown degree.

The possibility of fortuity and causation by the virus of a known disease have been sufficiently considered in a previous section. From the epidemiological standpoint we are left with three theories for consideration. It should be said at the outset that Figure 3 gives considerable evidence to discredit those who state that the increase in the number of cases of post-varicellous encephalitis is due to greater assiduity on the part of the clinicians who report cases. It is seen that post-vaccinal encephalitis, which was first noticed in 1922, was very prevalent in 1923; and the year 1924 was the peak year of the epidemic of encephalitis lethargica. Clinicians would therefore be quick to notice and report any unusual cerebral conditions. Yet in the year 1924 only one case of post-varicellous encephalitis was reported. Although twelve cases of the condition occurred in 1925, in the following year, during which poliomyelitis reached its peak, only three cases of the condition

occurred. Further, van Bogaert (162) points out that there is no mention of such conditions in the works of the older writers such as Gowers, Leyden and Trousseau. It would appear therefore that there are no grounds for supposing that any marked importance can be ascribed to this supposition.

By themselves the data set out in Figure 3 lend considerable support to the suggestion that an unknown virus is the cause of post-varicellous and of other types of post-infectious encephalitis. On this assumption this unknown virus must have become active, or capable of activation so far as the nervous system is concerned, about the year 1925. But it is surely unreasonable to suppose that an active virus of this nature could exist without causing a very much larger number of cases. The only virus which has possibly been known to play such a role is that of epidemic encephalitis, and the history of the years 1919 to 1929 shows how we might expect such a virus to behave - causing an epidemic with a peak of considerable magnitude. On the other hand, in the case of post-varicellous encephalitis the incidence was more or less constant betwen the years1925 and 1932. From the geographical standpoint also (see Table VII) it would seem that the incidence is most marked in those countries in which there are large and highly civilised populations. There are practically no infectious diseases, except those such as tetanus and anthrax which are non-contagious in the broad sense, which are not endemic in some part of the world or which do not from time to time become epidemic, in the true sense of the word. When we take into consideration the rarity, not only of post-varicellous encephalitis, but of nervous complications following all the infectious diseases, it cannot be said that these conditions have ever assumed epidemic proportions. When we add to this evidence the fact that the hypothetical virus has never been isolated, that the clinical type of encephalitis varies according to the primary infectious condition, and that the pathological features have not been shown to be identical in all these different conditions, there appear to be consider-

able grounds for concluding that an unknown virus is not responsible.

We are left therefore with two alternatives. These conditions are due either to some change in the infecting organism or to an alteration in the reaction of the host. These two alternatives have been discussed before from certain aspects. If a "constitution", using the word in the sense of Hamer and of Crookshank, is admitted, then the increase of post-infectious encephalitis is a trailer in the wake of the great influenza epidemic of 1918. Figure 3 also suggests that cases of post-varicellous encephalitis have tended to occur in the past especially in the years following pandemics of influenza or notable epidemics of cerebro-spinal fever of poliomyelitis. As is to be expected the influence of the "constitution" of 1918 has been most far reaching, and has been associated with more cases than any previous years produced. Whether this "constitution" has affected mainly the virus or the host is a matter of opinion. It seems more reasonable to conclude, however, that the main action has been upon the virus. Cerebro-spinal fever is one example of an infectious disease which shows marked variation in virulence, not only within short periods of time but also in different localities at the same time (Underwood (178)). The *gravis* type of *C. diphtheriae*, described only four years ago by Anderson, McLeod and their colleagues (179), is already tending to disappear in certain areas. There is therefore ample evidence that the effects produced by micro-organisms may alter rapidly from time to time. On the other hand, evidence of a sudden change, so far as their reaction to micro-organisms is concerned, in the individuals who compose a nation, is much more difficult to obtain. Van Bogaert (137) himself admits that the rarity of these conditions is against his theory. From the geographical standpoint also it is interesting to note that, whereas post-vaccinal encephalitis has been, comparatively speaking, very common in Holland, only one case of post-varicellous encephalitis has been reported from that country.

The prevalence of post-vaccinal encephalitis would make the Dutch physicians eager to report any cases of encephalitis following other conditions. It may be assumed therefore that post-varicellous encephalitis is rare in Holland. But that population evidently contains a considerable number of individuals who, on van Bogaert's theory, should have nervous systems which are sensitized to the action of viruses. If this sensitization were the only factor concerned, then we would expect a certain number of cases of post-varicellous encephalitis to occur in Holland.

Perhaps the most suggestive evidence which can be used to support the theory that the virus of chicken-pox is the cause of the condition has been put forward by von Balogh and by Wohlwill. In 1922 von Balogh (180) examined 212 spinal ganglia taken from five cases of varicella. Within the capsules of a considerable number of these ganglia he found injected arteries and marked perivascular infiltration of leucocytes; in the fibrous capsule he found marked hyperaemia and recent haemorrhage, which he considered to be due to thrombosis in the small vessels in the capsule. Wohlwill (135) confirmed this finding in four cases, and recently he investigated another fatal case of chicken-pox which showed no clinical evidence of nervous complications before death. In the white and grey matter of the cerebrum, cerebellum, and spinal cord he found around certain of the vessels, cells which he described as young histiocytes. The process involved capillaries and small veins, seldom larger veins, and never arteries. If these findings are corroborated they should do much to elucidate the neurotropic action of the virus of varicella, and to demonstrate that, even in cases which never show any nervous features, this virus is able to produce definite pathological changes in nerve tissues.

The evidence which has been adduced in this paper suggests that, though the time is not ripe for dogmatism, there are considerable grounds for accepting the conclusion of Zinsser (181). As a result of his experiments on Cebus olivaceus monkeys he

concluded as follows: "It would seem to us most rational on the basis of our own experience and the general experience of others to assume for the present that the clinical and pathological injuries grouped together as encephalitis might be due to the development of neurotropism by a number of different filtrable agents, and that the similarity in pathology is due to an analogous reaction on the part of the tissues to various members of a single group of closely related agents."

Summary and Conclusions.

1. The case is described of a girl of eight years and eleven months who developed, five days after the onset of varicella, nervous symptoms which were diagnosed as being due to encephalitis. The features were those of the "cerebellar syndrome" Complete recovery resulted.

2. The literature relating to nervous symptoms associated with varicella is examined afresh; in most cases the original papers were read. One hundred and twenty cases of the condition (including the case described in this paper) are accepted. Twenty three cases which are often quoted as examples of the condition are discarded for reasons given in the text.

3. Various systems of classification are discussed. The one adopted in this paper is new, but is based largely on the systems of Bérode and of Miget. The clinical features of the different groups are discussed in detail in the text. It is seen that cases of true encephalitis constitute exactly half of all the reported cases. In these cases of encephalitis, cerebellar forms were very frequent. This cerebellar condition is most typical of varicella encephalitis, which thus tends to be differentiated from post-vaccinal and other forms of post-infectious encephalitis.

4. Information regarding the ultimate result is given in one hundred and seven cases. Of these twelve died. The prognosis is therefore generally good. But sixteen other cases showed sequelae of various types.

5. The pathology of six cases, in which post-mortem

investigations were made, is discussed. It is shown that there is no typical picture of the condition, and that it is probable that the findings are different from those found in post-vaccinal and post-infectious encephalo-myelopathies generally.

6. The sex incidence in one hundred and ten cases was: males, sixty-seven; females, forty-three. Age does not appear to play any role in determining the onset of the complication - apart from the fact that varicella is itself more common in the early years of life. Most cases have been reported from France and Italy; but the incidence has also been high in the United States, Belgium, and Germany. Four of the eleven British cases were reported before1926. Only one definite instance was discovered of two cases of the condition occurring in a single household.

7. The exact interval in days between the appearance of the eruption and the development of nervous symptoms was available in one hundred and five cases. These symptoms may develop either before or after the appearance of the eruption, so that the interval - "incubation period" - may be negative or positive. Extreme cases have occurred; but in general it may be said that the onset of a nervous complication more than three days before or twenty days after the appearance of the eruption is quite exceptional. In most cases the onset is between the fourth and the tenth day after the appearance of the rash.

8. Most of the cases occurred between the years 1925 and 1932. The "peak year" was 1929, since when there has been a distinct decline.

9. Five possible theories respecting the aetiology are discussed. Of these, the most important are: the condition may be due to (a) an unknown virus which is activated by the primary disease; (b) a change in the infecting agent, which becomes neurotropic; or (c) a change in the infected individual, who for some unknown reason becomes neuro-allergic. Reasons are given in the text for discarding the first of these theories. The third theory was advanced by Glanzmann and has been extended by van Bogaert

In the absence of experimental and bacteriological proof there is little to choose between (b) and (c), and it is possible that both factors may play their part. In this paper, however, it is shown that the evidence which may be deduced from the recent behaviour of those infectious diseases which affect the nervous system suggests that the encephalitis which follows varicella - and by analogy the other nervous complications - is essentially due to the development of neurotropism by the virus of varicella itself

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Postcript. Since this paper was written reports of a few recent cases have appeared. These have been added at the end of the Appendix, but it was impracticable to incorporate the findings in the paper as a whole.

(a) Kimura (182) reports a case of serous meningitis as a complication of varicella in a girl of four years. The condition manifested itself on the fifth day after the onset of the primary condition. Lethargy, repeated vomiting, headache, paresis of the legs, but no increase of temperature. The pulse was slow, breathing irregular; knee jerks absent on both sides; considerable ataxia but no nuchal rigidity. Kernig's sign absent. The patient recovered without sequelae within twenty days.

(b) Rupilius (183) reports two further cases. Case(i): Girl of three years. Seventeen days after the onset of varicella she showed weakness, nasal speech and loss of appetite. A flaccid paralysis of both legs and of the shoulder muscles rapidly developed; considerable nuchal rigidity with slight Kernig. Recovery within two months. Case (ii): Boy, eleven months. Onset eight days after the appearance of the rash; tremor of hands and feet; hypertonia of leg muscles, with mechanical hyperexcitability of the right peroneal nerve and both femoral nerves. The child showed signs of blindness. No meningeal reaction. Recovery complete within two months.

(c) Eckstein (184) mentions very briefly three cases which have apparently not been reported previously. Case (i):

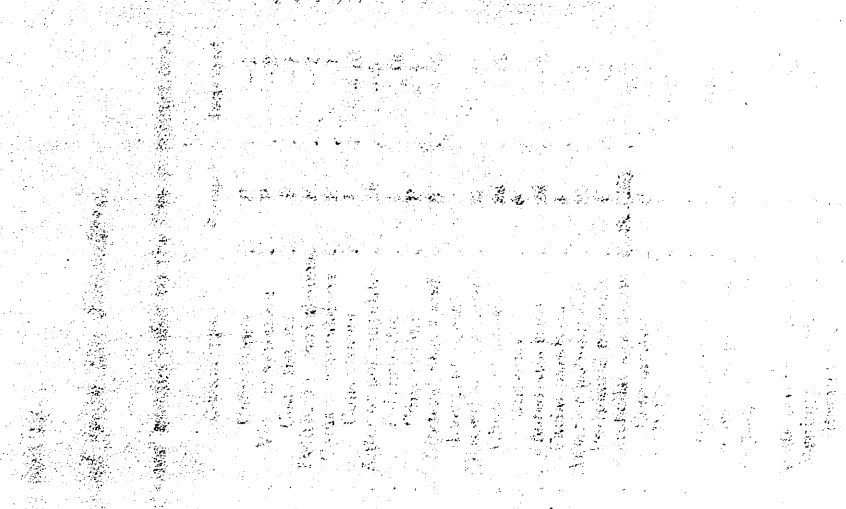
girl of four years. On fifth day after appearance of rash she showed ataxic symptoms. Case (ii): boy of six years: On sixth day showed ataxic and paretic signs in arms. Case (iii): boy of six years who showed meningitis on the seventh day after the appearance of the rash (increase of pressure in fluid, slight increase of cells, fluid sterile).

(d) Lelong, Moussoir, and Lefranc (185) report two cases of varicella which were complicated by meningo-encephalitis. (This report was not available at the time of writing).

(e) Bullowa and Wishik (186) carried out an investigation on the frequency of complications in 2,534 varicella cases who were admitted to hospital in the five years 1929 to 1933 They mention that five cases of encephalitis were met with, two of which were fatal. Detailed reports will be published later by these authors.

Eckstein's paper (184) is very important, since it deals with the author's experimental work with the contents of the pustules of varicella and of herpes zoster cases respectively. These materials were injected intracerebrally into Macacus rhesus monkeys, and several animals sickened after seven to seventeen days from the time of injection. The symptoms were mainly cerebral. Histological examination of the nervous systems of those animals which died or were killed showed features - increase of blood-vessels, perivascular infiltration, presence of fat granule cells, changes in the axis cylinders, and degeneration of the pyramidal tracts - which were not incompatible with a diagnosis of encephalitis. Control experiments were negative. From his experiments Eckstein concludes that the same virus may produce a herpes zoster or a varicella encephalitis according to circumstances, and that there is no uniform aetiology for the different types of post-infectious encephalitis. There is therefore some justification for hoping that this problem will ultimately be solved by further experimental work. Meanwhile it is gratifying that the conclusions which have been reached in this paper after a journey

along the devious path of epidemiology, tend to be confirmed by the more direct evidence which is afforded by Eckstein's initial experimental work.



Appendix.

List of Cases Collected from the Literature.

(The figures in brackets after the Authors' names refer to the list of references.)

Case No.	Year of publication.	Author's name.	Sex.	Age.	Clinical type.	Group.*	Interval.†	Result.‡	Country of origin.§
1	1873	Kassowitz (1) (*a*)	F.	5	Convulsions	P	−2	C	A.
2	1873	Kassowitz (1) (*b*)	F.	7	Violent agitation	P	−2	C	A.
3	1875	Hunter (2)	M.	3	Convulsions	P	−1	C	G.B.
4	1878	Dumas (3)	..	..	Delirium : convulsions.	P	−2	C	F.
5	1885	Tham (4)	F.	3½	Coma : convulsions	P	−1	C	Sd.
6	1893	Mya (5)	M.	1	Convulsions	1	+10	D	I.
7	1893	Marfan (6)	M.	9/12	Anterior poliomyelitis	3*b*	+5	Inc. C	F.
8	1894	Gay (7)	M.	2½	Polyneuritis	4	+14	Inc. C	G.B.
9	1894	Augier (8)	M	1	Convulsions	P	−½	C	F.
10	1898	Marfan (9)	F.	2	Bilateral ptosis and divergent squint	5	+90	Inc. C	F.
11	1898	Bouvy (10)	M.	3	Disseminated sclerosis	3*c*	?	Not C	F.
12	1899	Menko (11)	F.	4	Choreo-athetosis	2*d*	+12	C	H.
13	1900	Sachs (12)	M.	1 3/12	Insanity	0	..	Not C	U.S.
14	1900	Netter, H. (13)	M.	4	Choreo-athetosis	2*d*	+12	C	G.
15	1900	Naito (14)	..	..	Facial paralysis	4	..	C	J.
16	1903	Rossi, G. (15)	M.	1	Anterior poliomyelitis	3*b*	−3	C	I.
17	1904	Gaucher and Mirallié (16)	M.	4	Peripheral neuritis	4	+5	C	F.
18	1904	Caccia (17)	M.	3	Cerebellar syndrome	2*c* (2*b*)	+5	C	I.
19	1904	Cerf (18)	M.	2	Convulsions	P	..	..	F.
20	1905	Allaire (19)	M.	8	Polyneuritis	4	About +50	C	F.
21	1907	Camus and Sézary (20)	F.	11	,,	4	+3	C	F.
22	1908	Chavernac (21)	M.	11	Optic neuritis	5	Gradual	Inc. C	F.
23	1909	Morichau-Beauchant (22)	F.	4/12	Cerebral encephalitis	2*a*	+½	D	F.
24	1910	Koplik (23) (*a*)	M.	7	Meningo-encephalitis	1	?+10	C	U.S.
25	1910	Koplik (23) (*b*)	M.	9	,,	1	?+14	C	U.S.
26	1910	Forest (24)	M.	10/12	Acute cerebral tremor	2*b*	+14	C	G.
27	1911	Huismans (25)	F.	28	Meningo-encephalitis	1 (5)	+2	Inc. C	G.
28	1912	Osler (26)	..	..	Hemiplegia	2*e*	..	..	U.S.

29	1913	Sterling (27)	..	2	Hemiparesis	2*e*	?+7	..	P.
30	1914	Miller and Davidson (28)	M.	$2\frac{1}{2}$	Acute cerebral tremor	2*b*	+5	C	G.B.
31	1914	Guinon and Halbron (29)	M.	4	Meningeal syndrome	1	+0	Inc. C	F.
32	1915	Wharton Smith (30)	M.	7	Myelitis	3*a*	+14	Inc. C	U.S.
33	1917	Fuchs (31)	F.	20	Ophthalmoplegia	5	+10	Inc. C	A.
34	1917	Paton (32)	M.	14	Neuro-retinitis	5	+3	Inc. C	G.B.
35	1919	Holt and Howland (33)	M.	7	Transverse myelitis	3*a*	..	..	U.S.
36	1920	Kramer (34)	M.	4	Cerebellar ataxia	2*c* (2*b*)	+3	C	Sz.
37	1921	Rocaz and Lartigue (35)	..	..	Choreo-athetosis	2*d*	+(?)	..	F.
38	1924	De Toni (36)	F.	$3\frac{1}{2}$	Meningitis	1 (3)	+20	Inc. C	I.
39	1925	Goussis (37)	F.	7	„	1	?+9 to 11	C	Co.
40	1925	Galli (38) (*a*)	M.	5	Cerebellar ataxia	2*c*	+5	C	I.
41	1925	Galli (38) (*b*)	F.	2	„ „	2*c*	+7	C	I.
42	1925	Debré, Lévy-Solal, Netter and Longchampt (39)	F.	23	Cerebral encephalitis	2*a*	+0	D	F.
43	1925	Ratner (40)	M.	$4\frac{1}{2}$	Transitory blindness	5	+9	C	U.S.
44	1925	Krabbe (41)	M.	8	Myelitis	3*a*	+10	C	D.
45	1925	Waldman (42)	F.	32	Ascending myelitis	3*a*	+10	D	U.S.
46	1926	Winnicott and Gibbs (43)	F.	$2\frac{10}{12}$	Polio-encephalitis	2*e*	+9	C	G.B.
47	1926	Pruvost (44)	M.	4	Meningo-encephalitis	1	+4	C	F.
48	1927	Wilson and Ford (45)(*a*)	M.	9	Choreo-athetosis	2*d*	+7	C	U.S.
49	1927	Wilson and Ford (45)(*b*)	M.	3	Myelitis	3*a*	+7	C	U.S.
50	1927	Glanzmann (46) (*a*)	..	$\frac{9}{12}$	Acute cerebral tremor	2*b*	+17	C	Sz.
51	1927	Glanzmann (46) (*b*)	M.	5	Meningitis	1	+8	C	Sz.
52	1927	Glanzmann (46) (*c*)	M.	3	Cerebellar ataxia	2*c*	+8	C	Sz.
53	1927	Porta (47)	M.	3	„ „	2*c*	+4	C	I.
54	1927	Werner (48)	..	..	„ „	2*c*	..	..	..
55	1927	Reimold (49)	M.	4	„ „	2*c*	+10	C	G.
56	1927	Runge (50)	M.	6	„ „	2*c*	+3	C	G.
57	1927	Bonaba (51)	M.	9	Meningo-encephalitis	1	+6	C	S.A.
58	1927	Boenheim (52)	F.	3	Cerebral encephalitis	2*a*	+8	C	G.
59	1928	Meunier (53)	M.	3	Cerebellar ataxia	2*c*	+8	C	B.
60	1928	Babonneix, Adeline and Colombe (54)	F.	$1\frac{10}{12}$	Choreo-athetosis	2*d*	$+1\frac{1}{2}$	Inc. C	F.
61	1928	Crouzon and Liège (55)	F.	28	Encephalomyelitis	3*a*	+6	Inc. C	F.
62	1929	Rake (56)	M.	$2\frac{1}{2}$	„	3*a*	+17	Inc. C	G.B.
63	1929	Cohen (57)	F.	6	„	3*a*	+8	..	G.B.
64	1929	Bertoye and Garcin (58)	F.	$3\frac{1}{2}$	Cerebellar ataxia	2*c*	+12	C	F.
65	1929	Bernheim (59)	M.	$7\frac{1}{2}$	Cerebral encephalitis	2*a*	+3	C	F.

APPENDIX—*continued.*

Case No.	Year of publication.	Author's name.	Sex.	Age.	Clinical type.	Group.*	Interval.†	Result.‡	Country of origin.§
66	1929	Rendu (60)	F.	6½	Cerebellar ataxia	2*c*	+(?)	C	F.
67	1929	Heller (61)	M.	34	Myelitis	3*a*	+9	C	U.S.
68	1929	Fasella (62)	M.	6	Polyneuritis	4	+6	C	I.
69	1929	Conrad (63)	M.	4	Cerebelalr atacia	2*a*	+8	C	U.S.
70	1929	Reimold and Schädrich (64) (*a*)	..	10½	Cerebral encephalitis	2*a*	+1	D	G.
71	1929	Reimold and Schädrich (64) (*b*)	F.	3	Cerebellar ataxia	2*c*	+3	C	G.
72	1929	Fushiki (65) (*a*)	F.	5	Anterior poliomyelitis	3*b*	−7	C	J.
73	1929	Fushiki (65) (*b*)	F.	1 9/12	Choreo-athetosis	2*d*	+2	Inc. C	J.
74	1929	Tono (66)	F.	5	..	0	..	..	I.
75	1930	Graham (67) (*a*)	M.	3	Cerebellar ataxia	2*c*	?+7 to 10	C	G.B.
76	1930	Graham (67) (*b*)	M.	6	,, ,,	2*c*	+19	C	G.B.
77	1930	van Bogaert (68) (*a*)	F.	3½	Meningomyelitis	3*a*	+3	C	B.
78	1930	van Bogaert (68) (*b*)	F.	12	Cerebellar ataxia	2*c*	+2	D	B.
79	1930	van Bogaert (68) (*c*)	F.	7	Poliomyelitis	3*b*	+1	..	B.
80	1930	van Bogaert (68) (*d*)	M.	3½	Meningomyelitis	3*a*	+4	C	B.
81	1930	Vermeylen, van Bogaert and Verraeck (69)	F.	14½	Meningo-encephalitis	2*a* (2*c*)	+0	C	B.
82	1930	Butler (70)	F.	15	Paralysis of iris	5	?+10	C	G.B.
83	1930	Neumann (71)	M.	5	Meningo-encephalitis	1	+5	..	A.
84	1930	Crépin (72)	M.	4½	Cerebellar ataxia	2*c*	+6	C	F.
85	1930	Laignel-Lavastine, Miget and Constantinesco (73)	M.	18	Meningo-encephalitis	1	+10	D	F.
86	1930	Nyssen and Vervaeck(74)	M.	8	Cerebellar ataxia	2*c*	+4	C	B.
87	1930	Tramer (75) (*a*)	M.	7	,, ,,	2*c*	+5	C	C.S.
88	1930	Tramer (75) (*b*)	F.	3	Anterior poliomyelitis	3*b*	+5	C	C.S.
89	1930	Cornil and Kissel (76)	M.	4	Cerebellar ataxia	2*c*	+7	C	F.
90	1930	Borra (77) (*a*)	M.	4 10/12	,, ,,	2*c*	+3	C	I.
91	1930	Borra (77) (*b*)	F.	8	Cerebral encephalitis	2*a*	?+10	C	I.
92	1930	Lavallén (78)	M.	6	Non-charac. enceph.	2*e*	+4	C	Sp.
93	1931	Zimmerman and Yannet	F.	1 1/12	Cerebral encephalitis	2*a*	+4	D	U.S.
94	1931	Babonneix (80) (*a*)	M.	..	,, ,,	2*a*	?+5 to 7	C	F.
95	1931	Babonneix (80) (*b*)	F.	7	Anterior poliomyelitis	3*b*	+(?)	..	D.
96	1931	Nucci (81)	M.	10/12	,, ,,	3*b*	+13	C	I.

97	1931	Puig (82)	M.	8	Cerebellar ataxia	2*c*	+8	C	F.
96	1931	Dudevant (and Sendrail) (83), (84)	M.	7½	Choreo-athetosis	2*d*	+10 to 15	C	F.
99	1931	Greppi (85)	M.	1 1/12	Acute cerebral tremor	2*b*	+5	C	I.
100	1931	Chavany and Chaignot (86)	F.	16	Cerebellar ataxia	2*c*	+4	C	F.
101	1931	Gautier and Monedjikova (87)	M.	4	Choreo-athetosis	2*d*	?+5 to 10	C	F.
102	1931	Giuffrè (88)	F.	4	Non-charac. enceph.	2*e*	+4	C	I.
103	1932	Dagnelie and Dubois (89)	F.	7 5/12	Cerebral encephalitis	2*a*	+14	D	B.
104	1932	Bérode (90)	M.	19	Meningo-encephalitis	1	+7	C	F.
105	1932	Gorini (91)	F.	6 1/12	,,	1	+5	C	I.
106	1932	Fry (92)	F.	10	Cerebral encephalitis	2*a*	+3 to 6	Inc. C	C.
107	1932	Mühlenkamp (93)	M.	3 9/12	Acute cerebral tremor	2*b* (2*c*)	+5	C	G.
108	1932	Lucksch (94)	M.	1 7/12	Cerebral encephalitis	2*a*	+4	D	Bo.
109	1932	Rupilius (95)	M.	7	Meningo-encephalitis	1	+4	D	A.
110	1932	Poinso (96)	M.	4	Cerebellar ataxia	2*c*	..	C	F.
111	1933	Borremans (97)	M.	8	,, ,,	2*c*	+7	C	B.
112	1933	Mayerhofer and Breitenfeld (98)	M.	14	Choked disc	5	+19	C	Ct.
113	1933	Rossi, L. (99)	F.	1 4/12	Acute cerebral tremor	2*b*	?+7	C	I.
114	1933	Hallé and Arondel (100)	M.	2 6/12	Meningitis	1	−3	C	F.
115	1933	Henner (101) (*a*)	M.	7	Cerebellar ataxia	2*c*	−18	C	Bo.
116	1933	Henner (102) (*b*)	M.	7	,, ,,	2*c*	+90 (approx.)	Inc. C	Bo.
117	1934	Corda (103) (*a*)	F.	1 8/12	Poliomyelitis	3*b*	+17	C	I.
118	1934	Corda (103) (*b*)	F.	3	Cerebellar ataxia	2*c*	+6	C	I.
119	1934	Corda (103) (*c*)	M.	4	Cerebral encephalitis	2*a*	+6	D	I.
120	..	Underwood	F.	8 1/12	Cerebellar ataxia	2*c*	+5	C	G.B.

* When two groups are shown, that in brackets is of secondary importance.

† "Incubation period", or interval between appearance of eruption and onset of cerebral symptoms. − = Onset before eruption. + = Onset after eruption. +0 = Onset concurrent with appearance of eruption.

‡ D = Died. C = Cured. Inc. C = Sequelæ present. Not C = No improvement.

§ A., Austria. G.B., Great Britain. F., France. Sd., Sweden. I., Italy. H., Holland. U.S., United States of America. G., Germany. J., Japan. C.S., Czecho-Slovakia. Co., Corfu. D., Denmark. Sz., Switzerland. P., Poland. S.A., South America. B., Belgium. C., Canada. Bo., Bohemia. Ct., Croatia. Sp., Spain.

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The lists of papers and books which are given are of course by no means exhaustive, but they form a sufficiently elaborate framework, around which other studies may be constructed. In Part I and the first two sections of Part II only those works are quoted which are directly referred to in the text. Hence many important papers which were consulted but which had no direct bearing upon the issues were purposely omitted.

The study on varicella is rather on different lines. The writer set out to make an exhaustive search of the literature, and it is believed that the list was complete at the time of writing. The nature of the study also made it desirable that as many as possible of the papers should be read completely in the original, and those papers which could not be obtained for this purpose are specially indicated.

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