EPIDEMIC INFLUENZA IN CANADA

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PREFACE

This work was carried out in the Virus Section of the Connaught Medical Research Laboratories, University of Toronto, under the direction of Prof. C. E. van Rooyen, M.D., D.Sc., M.R.C.P. (Lond.). Acknowledgements are due to Dr. W. R. Feasby, M.D., Superintendent of the Western Hospital, to the Nurses of that hospital and their Director of Health Education, to the Physicians of the Hospital for Sick Children, Toronto, to Dr. L. E. Elkerton, M.D., D.P.H., of the Division of Laboratories, Ontario Department of Health, and to the R.C.A.F. who kindly made available pathological and serological specimens for this investigation.

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PART I

HISTORICAL SURVEY AND INTRODUCTION

The earliest recorded epidemic of influenza in the Western Hemisphere was in the year 1627 according to Hirsh (1883). The history of epidemic influenza prior to that date can be traced with a fair degree of epidemiological certainty as far back as 1173 when the disease was epidemic in England, Germany and Italy. Although numerous epidemics and even pandemic influenza were observed in the intervening time between the 12th and late 19th centuries, the pandemic of 1889 was the first to fall in the bacteriological era which required that strict fulfillment of Koch's postulates for the establishment of the microbial aetiology of a disease. Pfeiffer observed the extraordinary prevalence of a small gram-negative bacillus in the sputum of patients who succumbed to the illness but it was not until 1892 that he managed to characterize the micro-organism by growing it upon artificial media and thus attempt to fulfil Koch's postulates. Sir Frederick Andrewes (1920) refers to the publication of Pfeiffer's work in 1893 and comments that although the bacterium had been isolated in pure culture and inoculated into

several species of animals including monkeys the experiments failed to reproduce the disease.

Since then and until the 1918 pandemic as many as four epidemics of influenza occurred with frequent isolations of Pfeiffer's B. Influenzae. However, it was not always found to be present and other bacteria were noted, some of them as frequently as Pfeiffer's bacillus. The case against this organism was considerably weakened by the experience of most investigators of the summer wave of influenza in 1918. Nevertheless, it was isolated during the second wave of influenza from a high proportion of patients by numerous workers and many remained convinced of its primary role in this disease.

Others remained doubtful. Pfeiffer himself obtained only 51.6 per cent positive isolations from 217 patients, and on an average of approximately 20,000 cases examined by various bacteriologists just over 60 per cent were negative for Pfeiffer's bacillus. These figures were presented by Townsend (1933) who quotes Donaldson. The conclusions drawn in the Ministry of Health Report (1920) were

that the bacillus was not the proven primary aetiological agent of the 1918 pandemic, and that the evidence for a filter-passing virus was suggestive. The high fatality rate was considered due to complications following secondary infections in which Pfeiffer's bacillus and the haemolytic streptococcus played a predominant part.

It was reported that the pandemic reached Canada in September 1918 perhaps by way of the New England States. Although its exact place of entry is difficult to determine it swept rapidly over the whole Dominion sparing neither Indian trappers in the Far North nor Eskimoes in remote Labrador. In Ontario, a province at that time of 2,500,000 people, there were almost 2,000 deaths from influenza in 5 months. In Toronto at the peak of the epidemic about 150 deaths were occurring every day.

Attempts to transmit influenza to human volunteers in America by Wahl et al (1919) were reported. Cultures of Pfeiffer's bacillus recently isolated from fatal cases were used; however, these experiments were upon a small scale and were carried out during the

epidemic. A larger scale experiment was performed many years later on similar lines by Smorodintseff et al (1936,a) and he failed to transmit the true diseases. During the 1937 epidemic Hoyle and Fairbrother (1937) found H. influenzae in only 1 out of 28 cases. This is notable for the outbreak was of almost pandemic proportions. Andrewes (1937) commented that the isolation of the bacillus was of too irregular occurrence to consider it to be a cause. Hare (1937) surveying recent advances in the study of influenza states that the position of H. influenzae was doubtful in 1918 and has since then become even more so. As late as 1945 Stuart-Harris (1945) writing of pneumonic complications of influenza A virus affirms that the position of Pfeiffer's bacillus is not at all clear. Shope (1944) whose discovery of the viral aetiology of swine influenza in 1931 gave encouragement to those supporting a viral aetiology of human influenza, warns against a tendency of modern research workers to ignore the Pfeiffer bacillus, a trend which he feels might be regretted in any future pandemic.

The isolation of a ferret-pathogenic virus from cases of epidemic influenza by Smith, Andrewes, and Laidlaw (1933) furnished the necessary piece of evidence required to establish the viral etiology of this condition.

They showed that the disease produced in ferrets by filtered human throat washings was transmissible by bacteriologically sterile filtrates. The mucous membrane covering the turbinates of the infected animals when ground up and filtered proved infective for fresh animals. Moreover the ferrets could acquire the disease naturally by contact amongst themselves. They failed to produce the disease with material obtained from non-influenzal cases. Finally they established that the serum from convalescent patients and recovered ferrets neutralized the virus which caused the ferret infection.

The following year the virus was isolated by Francis (1934) from specimens of sputum received from Puerto Rico. These specimens had been treated with 50% glycerine. A strain of virus (PR5) isolated on repeated passage in ferrets and inoculated intranasally under ether

anaesthesia produced pulmonary consolidation in the sixth passage animal. Clinical disease did not follow subcutaneous inoculation of the filtrates. He succeeded in adapting this strain to mice and reported that the pathological findings in the lung were similar to pulmonary lesions produced by other virus infections. At the same time Andrewes, et al., (1934) had succeeded in adapting both the human and the swine influenza virus to mice, and had demonstrated that the action of the virus could be neutralised by specific antiserum.

In June, 1935 still further confirmatory evidence of the viral aetiology of epidemic influenza was obtained by Burnet (1935) in Melbourne, Australia. Neutralization tests with mouse adapted virus from this epidemic and immune horse serum obtained from the Hampstead workers established its serological relationship to the English WS strain isolated in 1933. Thus in the space of two and a half years ferret pathogenic virus had been isolated from human cases of epidemic influenza occurring in widely separated areas of

the Northern Hemisphere and, in addition, from patients in the Southern Hemisphere.

Meanwhile, further strains of the virus had been isolated in Britain; Andrewes et al (1935) reported the recovery of 5 strains in 1933, 1 strain in 1934, and 8 strains in 1935, a total of 14 strains. In the United States virus isolations were reported from places as far apart as Philadelphia and Alaska. In 1936 Smorodintseff et al (1936,b) reported isolating strains in Russia.

The first isolation of the virus in Canada was made in the winter of 1936-37 by Hare and Yen (1938) in Toronto, Ontario. They described the epidemic in the Northern Hemisphere as starting first in Northern Germany, occurring one month later in Britain and the eastern seaboard of The United States from whence it spread rapidly to the Pacific coast. The Province of Ontario, situated in the interior of the eastern part of Canada, did not suffer until late January and early February; the Canadian eastern seaboard was not affected until still later. Thus the epidemic seemed to arise in Europe, sweep

across the United States to the Pacific only to return to the Atlantic Coast via Canada.

After the 1918-19 pandemic, epidemics of influenza in Canada were reported but they were localised. For example, in May 1919 the Department of Indian Affairs' Annual Report (1920) noted a sharp outbreak affecting 125 Indians in the Yukon resulting in 11 deaths; a year later in March 1920, there was an outbreak at a school in Carcross involving 36 people and causing one death. Again in July 1927, a severe epidemic swept the basin of the Mackenzie river and was recorded in the Annual Report of the Department for Indian Affairs (1928). Spread was very rapid and it is reported that a whole settlement was stricken at once so that there was not a sufficient number of healthy persons to care for the sick or bury the dead. This was undoubtedly an epidemic of influenza spreading from Fort Smith to the Great Slave Lake and Aklavik. The significance of epidemics in the Far North must not be underrated as outbreaks of this kind have been known to herald the occurrence of much larger

and more serious epidemics of continental or global extent. Burnet and Clark (1942) noted that the great pandemic of 1889 was preceded by small outbreaks in "the North Circumpolar region", and they mention Athabasca in Canada. Furthermore, these outbreaks occurred in the summer months. Much more recently similar findings have been reported of epidemics arising in remote areas. In Sardinia Magrassi reported late summer outbreaks of influenza which preceded the epidemic of 1948-49, Andrewes(1950). Again, the 1950-51 epidemic was preceded by a summer outbreak in Sweden, Svedmyr et al(1951).

By 1937 a certain amount of confidence had been gained in the investigation of epidemic influenza,and physicians were beginning to consider the possibility of defining the disease clinically in such a manner that the illnesses caused by influenza virus might be more readily distinguished from other symptom-complexes covered by the umbrella diagnosis of influenza. Such confidence as pointed out by Stuart-Harris (1940) was rudely shattered by the experience of the 1938-39 epidemic. Reports from different sources, Stuart-Harris

et al (1940) and Horsfall et al (1940), repeated the same tale; and although ferret pathogenic virus was isolated from some of the cases, others clinically indistinguishable yielded no virus, Stuart-Harris (1945). The virus strains which were recovered proved poorly infective for mice and ferrets. In contrast to this Taylor and Dreguss (1941) found that the strains which they isolated in Central Europe at this time proved easily adaptable to ferrets although about 60 per cent of the animals gave no febrile response.

The recognition that epidemic influenza remained aetiologically complex, and that the virus isolated in 1933 was not wholly responsible for the disease lead to the publication of an article upon the nomenclature of the disease by Horsfall et al., and Andrewes et al (1940). Henceforth, viruses closely related to the strain isolated in 1933 were to be known as Influenza A virus, and different viruses causing this disease would be designated as Influenza B,C, etc.

The identification of a new type followed swiftly. Francis

(1940) reported the isolation in ferrets of a strain immunologically distinct from Influenza A virus. The epidemic which he was investigating occurred amongst children in a convalescent home. Although four patients were investigated he was successful in isolating the virus from only one of them, a patient named Lee. The strain was to become well-known as the standard type B (Lee) strain. This new type was adapted with difficulty to mice after ten passages in ferrets.

Francis (1940) showed that, amongst patients involved in the epidemic occurring in N. Carolina at that time, antibody rises were observed only towards the B type. He found the infection difficult to detect in ferrets, and the diagnosis by animal inoculation had to be established by demonstrating that the animals developed antibodies to the Lee strain. In addition he was able to make a retrospective diagnosis of Type B influenza in cases which had occurred in the winter of 1935-36 by demonstrating rises in antibody titres of the acute and convalescent sera which had been stored at

-70° C since that time. Shortly afterwards Eaton and Beck (1941) isolated another strain of Type B virus (Montgomery) which they found to be slightly different antigenically. This strain was obtained from one of the cases in the California outbreak of January 1940 when 8,000 mild cases of influenza were reported. An epidemic of Type B influenza in Canada was reported by Hare et al. (1943) preceding a wave of Type A.

The discovery of Influenza B virus was a great step forward in elucidating the character of the influenza syndrome, for until 1940 all the strains of influenza virus isolated had proved at least to be related immunologically to one another.

The antigenic relationship of Type A strains had been investigated by means of mouse neutralization tests and mouse protection tests. In the earlier investigations when the Hampstead immune horse serum had been used no difference had been detected between the WS strain and the Melbourne strain by Burnet (1935). Francis (1935) too had failed to differentiate between the WS, PR5, PR8, and Phila-

delphia strains. However, the first indication of strain differences was noted in a publication by Magill and Francis (1936) in which they used rabbit antisera to differentiate between the PR8 and Philadelphia Strains.

More extensive investigations, employing separate strains from representative areas, were made with ferret immune sera by Smith and Andrewes (1938) who detected four different major antigenic components in 28 strains analysed. In the United States Magill and Francis (1938) conducted similar investigations but employed strains of virus which they had adapted to growth in a minced chick embryo tissue culture medium, Francis and Magill (1935). They differed also from the British workers by using rabbit immune serum. The results of these investigations indicated that certain strains of Influenza A virus were more specific than others.

Prior to 1940, isolations of influenza virus had been made almost entirely by intranasal inoculation of the ferret, and attempts to infect other animals directly although occassionally successful

generally failed. Francis and Magill (1937) managed to infect mice with throat washings concentrated by centrifugation at 14,000 rpm. and made serial passages in these animals. Virus infective for ferrets was obtained after three "passages by faith" in mice, and lesions were demonstrated in the lungs of the fourth passage mice. An indirect method of diagnosis, made by taking samples of blood from ferrets before inoculating them intranasally with throat washings and comparing the serum-neutralizing antibody level in this specimen of serum with the level obtained two weeks after the inoculation, was advocated by Francis et al (1937). Taylor (1940) succeeded with the method but substituted the hamster for the ferret. This animal proved much easier to handle and was less susceptible to cross-infection. Andrewes et al (1935) had established earlier that a diagnosis of influenza A infection could be made by examining the patient's serum for increases in specific neutralising antibodies and Smith (1936) had introduced the complement-fixation test for the same purpose.

In 1940 a new method of influenza virus isolation was devised

by Burnet (1940). He introduced the intra-amniotic technique of inoculation of fertile hen eggs. This was not the first time that influenza virus had been grown in the fertile hen's egg - that was accomplished by Wilson Smith (1935) - but it was the method which proved to be the most sensitive for the isolation of virus from human material (Hirst 1945).

At this time several workers were experimenting with methods of cultivating the influenza virus in the hens egg and Nigg et al. (1940) reported the value of infected chorio-allantoic, amniotic and yolk-sac membranes as sources of virus for complement-fixation tests. The outstanding discovery which rivetted attention upon chick embryo cultivation was the detection, independently, by Hirst (1941) and McLelland and Hare (1941) of the chicken red blood cell agglutination phenomenon.

It was shown that allantoic fluid infected with influenza virus was responsible for a haemagglutination effect which was directly associated with the virus particle. This could be demonstr-

ated with egg-adapted strains of influenza A (PR8) or influenza B (Lee). The method rendered the detection of virus in infected eggs much easier as previously workers had depended upon microscopical examination of tracheal smears taken from the embryo. A further application of the technique was the detection of antibodies toward influenza virus, their presence having been shown to inhibit haemagglutination.

These discoveries gave a tremendous impetus to research upon the influenza virus which now proceeded along two main paths of investigation; firstly, the immediate application of the haemagglutination phenomenon to the detection of virus and its antibody in human subjects and to the production of concentrated vaccines; and secondly, the more fundamental and academic approach. The examination of the phenomenon itself.

Both lines of research were extremely fruitful. The former gave us diagnostic methods which could be proceeded with in any moderately well equipped laboratory which did not have the facilities

required for the handling and quarantine of ferrets, and obviated the necessity of using costly animal neutralization tests. Furthermore, as previously mentioned, suitable complement-fixing antigens could be prepared from egg material. Wiener et al. (1946) made use of infected allantoic fluid to demonstrate the fact that two components were involved in the complement-fixation reaction; a soluble small non-infective component which was type specific, and a larger more easily sedimentable particle which proved to be infective and was in fact the virus particle itself. Knight (1946) used infected allantoic fluid as a source of material for his chemical analysis of the influenza virus.

Hirst (1942,a) described in more detail the sequence of events which followed the mixing of a virus suspension with chicken erythrocytes at 37° C. Initially the virus was adsorbed to the red cells which became agglutinated, but after a time he found that the virus was released from the cells and appeared once more in the supernatant fluid. The freed virus proved to be infective and was capable

of agglutinating fresh cells but the treated cells were inert and could not be reagglutinated by fresh virus. It appeared that the virus was functioning as an enzyme, the action of which altered the surface of the erythrocyte and destroyed the 'receptor sites' which were responsible for the specific adsorbtion. Later Hirst (1943,a) showed that the same sequence of events, adsorption of virus followed by elution and destruction of receptor sites, could be observed to follow the application of virus to the epithelium of the excised lung of a ferret.

When the haemagglutinating properties of influenza viruses were first described the ability of specific immune serum to inhibit the reaction was particularly noted by McLelland and Hare (1941). Since then the existence of a host of non-specific inhibitors has been brought to light. The first of these was described by Francis (1947). He showed that a substance was present in normal human serum which inhibited the haemagglutinating power of heated Lee virus to a considerable degree. Unheated virus was only slightly affected.

The heating of the virus at 55° C. did not destroy the haemagglutinating property of the virus but, as shown by Briody (1948), it was no longer eluted from the red cell. Viruses which have been treated to render them sensitive to non-specific inhibitor have been referred to by Stone (1949) as being in the "indicator state".

As Burnet (1951,a) records it was realised gradually that the inhibitor was mucoprotein in nature and numerous mucoproteins and mucopolysaccharides were found to act as non-specific inhibitors. Various sources of inhibitor have been described varying from ovarian cyst mucoid and purified blood group O substance which were described by Burnet (1948,a) to the urinary inhibitor of Tamm and Horsfall (1950). The presence of inhibitor substance was noted in allantoic fluid by Svedmyr (1948), in various tissues by Friedwald et al. (1947), and in the stromata of human erythrocytes by de Burgh et al. (1948). Egg white was shown to be a source of it by Lanni and Beard (1948) whilst its presence in this complex substance was traced to the ovomucin fraction by Gottschalk and

Lind (1949).

The problem of the nature of the cell receptor and its relationship to these inhibitors was probed by Burnet (1948,b) and his team of workers with the aid of an enzyme which had been obtained from cultures of V. cholerae. This enzyme, discovered by Burnet et al. (1946), was found to destroy the receptor sites upon the erythrocytes and on that account has been termed receptor destroying enzyme, or more briefly R.D.E. As this same enzyme was found to attack Francis's inhibitor and other mucoids it was concluded that there must be a mucin of similar type on the surface of the erythrocytes. This acted as substrate for the action of R.D.E. and virus enzyme. The Francis inhibitor besides being destroyed by R.D.E. has been shown to be heat-labile, properties which distinguish it from another inhibitor found in certain normal animal sera and referred to recently by Sampaio (1952) as Chu inhibitor.

With the help of all this accumulation of recent know-

ledge research workers were able to investigate influenza epidemics more easily and more widely; and, with the adaptation of the newer techniques to vaccine production, they were in a better position to improve and study the immunising power of the influenza vaccine.

The early vaccine experiments were carried out with active tissue culture virus and formalin-killed virus prepared from infected mouse lung. Then experiments by Francis (1939) with mice suggested that a higher concentration of virus was required to give an adequate antibody response.

This was achieved in 1942 when more concentrated vaccines were prepared by Hirst et al. (1942), with the aid of high speed centrifugation. They determined, moreover, that those with the higher concentration proved superior in stimulating protective antibodies. Other methods of concentration were described such as the method of freezing and thawing of allantoic fluid. This resulted in the adsorption of the virus onto a precipitate formed during the process and subsequently

it was eluted off into a smaller volume of fluid. The method was described by Hare et al (1942) and concentrations varying from two-fold to twenty-fold were attained.

Another method of concentration utilised the embryonic chicken red cell. The red cell eluate vaccine of Francis and Salk (1942), was prepared by adsorption of virus onto red cells and then eluting it off into one tenth of the original volume of fluid. It was adopted for a large-scale vaccination programme which was carried out by the United States Army Epidemiological Board's Commission upon Influenza in the winter of 1942-43. As influenza did not occur during that period they performed an experiment upon vaccinated and control groups with artifically induced influenza. The results of spraying individuals with Influenza A virus were reported by Francis et al (1945). A smaller percentage of the vaccinated people showed febrile reactions of 100° F or more as compared with the control group. Individuals with a prevaccination level of 1:256 or more proved less susceptible; only 14 per cent of

them responding with a febrile reaction, whereas 49 per cent of those with titres of 1:128 or less had a febrile response. Whereas 32 per cent of individuals vaccinated 4½ months previously reacted with a febrile response of 100° F or over, only 14 per cent of individuals vaccinated two weeks previously did so. Salk et al (1945a) reported similar findings with groups exposed artificially to Influenza Type B infection, although better results were demonstrated with the group vaccinated 4½ months previously.

The following winter, still using formalin-killed virus obtained from allantoic fluid and concentrated by the red cell eluate method, an adequately controlled vaccination programme was carried out with the help of the U.S. Army Student Training Programme Units in 9 universities. This gave overall results which seemed to demonstrate the efficacy of the vaccine. The vaccine was polyvalent and contained the PR_8 strain of Type A influenza virus along with a very recently isolated strain of Type A called Weiss which had been recovered earlier in the year by

Salk et al (1944). The Lee strain of Type B virus was the third component. In 6,211 controls the incidence was 7.11 per cent whereas in the vaccinated group of 6,263 it was 2.22 per cent - a ratio of 3.2 to 1 in favour of the vaccinated group.

The next large scale epidemic during which the same type of vaccine was employed occurred in the autumn of 1945. In this epidemic, due to Type B virus, even more satisfactory results were recorded; however the groups involved were not as rigidly controlled as in the 1943 experiments for, instead of alternate individuals receiving either vaccine or a suitable control fluid, the groups compared consisted of army and navy students. The army students received vaccine, the navy students did not. Although they attended the same college they went to different classes and were housed in different dormitories and contact occurred only socially. Hirst et al (1947) reported 132 cases in the control group of 1050 naval students, and only 3 cases amongst 550 army students. Similar trials at other centers were reported by Francis

et al (1946) and Friedman (1946) with equally successful results. As Hirst points out, these experiments were of a different nature to the 1943 trials in that a programme of mass vaccination was carried through and the incidence in a completely vaccinated group was compared with the incidence in a comparable but wholly unprotected group. The vaccination of alternate individuals in a community tends to lessen the exposure to infection in the whole group and may lower the attack rate in the unvaccinated.

The results found in vaccinated and control groups exposed to the influenza epidemic of the early months of 1947 were in sharp contrast to the foregoing. Reports by Francis et al (1947) and Smadel (1947) indicated no protection despite the fact that high antibody levels existed in the vaccinated groups for the known types. The strains of Influenza A recovered from different areas proved to be similar to each other but were only distantly related to the A strains incorporated in the vaccine (PR8 and Weiss).

The position with regard to the value of vaccination in

influenza up to the year 1947 has been fully and admirably summarised by Blake (1948); since then work has been continued upon methods of improving influenza virus vaccines by modifying the methods of preparation and extending the antigenic coverage of the formula.

The vaccines concentrated by absorption on and elution from red blood cells which had been shown to be satisfactory as prophylactic agents in 1943 and 1945 were discarded in favour of a preparation, obtained by high speed centrifugation, which gave less severe reactions. Stanley (1947) who introduced the method in 1945 records that this method was adopted by the U.S. Army at that time and made available to civilians in 1947. Cox et al. (1945) introduced a chemical method of concentration by precipitation of the virus with methanol in the cold and subsequently centrifuging and resuspending the material.

Following the experience of 1947, the National Institutes of Health in Washington introduced the strain isolated at Fort Monmouth into the vaccine. This strain became the standard repre-

sentative in the American continent of what was referred to as the A-prime subtype of type A influenza virus. Although the A-prime virus seemed to strike the American Continent suddenly in 1947 a very similar strain had been reported in 1945-46 in Australia by Anderson (1947). The new strain apparently reached the American Continent via the western Seaboard and spread from two western army installations to the civilian population, Baetjer (1948). Loosli et al (1948), recording the failure of the old-formula vaccines (PR8, Weiss, Lee) to protect despite good titres reported that the epidemic spread from California to Colorado, thence to New Jersey and Chicago, where the military population was affected first.

Despite occasional isolations of Type A virus resembling the old strains, Lépine et al (1949), van Rooyen et al (1949), Nagler et al (1949), almost all the A outbreaks reported and certainly all the epidemics reported since that date have been caused by the A prime subtype. The homogeniety of the strains isolated in the 1948-49 in Europe has been commented upon by Chu et al (1950).

It is clear from the foregoing that the laboratory studies of influenza epidemics have assumed great importance from the point of view of determining the nature of clinical influenza and the prevention of pandemics as serious as the 1918-19 disaster. The present study was made with the object of following the trend of influenza in Canada and with the purpose of relating that experience to events of a similar nature in other parts of the world. Stuart-Harris (1945) in describing epidemiological experiences with epidemic influenza over a period of 10 years speaks of the British Isles and the American Continent forming an epidemiological unit. The present studies of the 1950-51 epidemic in Canada would seem to support that contention.

PART II

HAEMAGGLUTINATION-INHIBITION ANTIBODY TITRES IN TORONTO PRIOR TO THE 1950-51 INFLUENZA EPIDEMIC

MATERIALS AND METHODS

Nature and Source of the Antisera Tested: - The influenza epidemic struck Ontario during the second week of February 1951. During the period July 1950 to the end of January 1951 thirty-eight sera were collected from children under two years of age in the surgical wards of the Hospital for Sick Children in Toronto. A further thirty-six sera were collected during the period of the epidemic from February 1951 to April 1951. In the last week of January 1951 serum was obtained from forty-nine student nurses in the Toronto Western Hospital and from seventy-five members of the clerical and technical staff of the Provincial Laboratory, Toronto. All sera were stored in the frozen state at -70°C. Inactivation at a temperature of 56°C for 1/2 hour was carried out before testing and after the sera had thawed.

The virus Suspensions:- Pools of freshly harvested infected allantoic fluid were stored at -70°C and drawn upon for preparation of suspensions of the four strains of virus used in the

investigation. The passage history of these strains, PR_8 (Type A), FM1, and FW_{50} (Type A-prime) and Lee (Type B), is recorded in Part IV page (91). Fresh vials of virus were selected for each days work. The fluid was allowed to melt at 37°C and was kept at that temperature for three quarters of an hour to permit the elution of virus from any precipitate which might have formed. The fluid was then diluted 1:8 and titrated.

Titration of Virus. Titrations were performed upon plastic plates similar to that advocated by Salk (1948). Doubling dilutions of virus were made in 0.5 cc volumes and for the diluent phosphate buffered saline (pH7) was used. An equal volume of a 0.5% suspension of chicken erythrocytes was added to each dilution. Titres were recorded as the final dilution after the addition of the erythrocyte suspension and the end-point was determined by reading the pattern of the sedimented cells after one hour at room temperature. The highest dilution showing an even distribution of cells on the bottom of the depression without concentration of the cells

into a button at the centre was taken as the end-point. The dilution of virus which would contain four haemagglutinating doses of virus was determined from this.

The Erythrocyte Suspension. Blood was taken by heart puncture or from the wing-vein of individual roosters of about 6 lbs weight. The blood was mixed with a 2 per cent sterile solution of sodium citrate and immediately centrifuged. The deposited cells were washed three times in buffered saline and the final separation of cells from fluid by centrifugation was made in a 15 cc graduated centrifuge tube in order that the packed cells might be spun to a constant volume. A 0.5 per cent suspension of erythrocytes was made up in buffered saline and a small amount of saline was added to the remainder of the packed cells which were stored and kept for further use for a period of one week. The suspension was tested for stability by adding 0.5 cc of the suspension to an equal volume of saline in the plastic plate. A suitable cell suspension began to show 'button formation' in half an hour and had completely

sedimented in one hours time.

The Haemagglutination Inhibition Test. For brevity this test is referred to in the literature as the HAl test, and this abbreviation is used hereafter.

A volume of 0.25 cc of virus suspension containing approximately 4 haemagglutinating units was added to equal volumes of doubling dilutions of the patient's serum; to this mixture 0.5 cc of a 0.5 per cent suspension of erythrocytes was added. Readings were made after 45 minutes at which time the end-point, the highest dilution showing a fully formed button, was easily defined.

Controls. A check was made upon the virus concentration by making a titration of the virus suspension along with the HAl test. Occasionally the virus suspension proved too strong or too weak in which case the days results would be correlated with other days findings by a titration of the virus suspension with its homologous virus antiserum

Methods of Testing with R.D.E.:- The dried RDE was

reconstituted in a 10 cc. volume and diluted 1:6 in calcium acetate buffer saline, pH6.0, to which 1000 units per cc of penicillin and streptomycin had been added. A volume of 0.2 cc of the patient's serum was mixed with 0.6 cc of diluted R.D.E. and incubated in a screw-cap vial at 37°C overnight. A further volume of 0.2 cc of the same serum was incubated with 0.6 cc of acetate buffer treated with the same concentration of antibiotic. The latter constituted the control and was handled and titrated exactly as the R.D.E. treated specimen. Both specimens were then heated at 56-58°C for an hour and tested for haemagglutination-inhibition titre. The method of titration was as detailed already except that 2 per cent citrate saline was substituted for the ordinary buffered saline.

RESULTS.

The haemagglutination-inhibition levels obtained with these one hundred and ninety-eight sera are presented in Table 1. Four influenza virus strains were employed for this investigation namely the PR8 (A), FM1 and FW50 (A-prime), and Lee (B) strains.

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As all the tests could not be completed at one time the results obtained upon different days had to be correlated. Correlation was made possible by titrating the four antigens with standard sera upon each occasion the tests were performed.

TABLE 1

DISTRIBUTION OF HAl TITRES OF SERA FROM 198 INDIVIDUALS

GROUP INVESTIGATED	TOTAL NUMBER IN GROUP	STRAIN OF VIRUS	DISTRIBUTION OF SERUM TITRE* LEVELS				
			32 or less.	64	128	256	512 or more.
PROVINCIAL LABORATORY	75	PR_8	29	17	16	6	7
		FM_1	1	3	21	31	19
		FW_{50}	73	2	0	0	0
		Lee	50	15	4	3	3
TORONTO WESTERN NURSES	49	PR_8	13	5	10	9	12
		FM_1	9	13	12	9	6
		FW_{50}	45	3	1	0	0
		Lee	9	9	12	7	12
CHILDREN UNDER 2 YRS. (Pre-epidemic)	38	PR_8	35	2	0	0	1
		FM_1	0	1	7	17	13
		FW_{50}	36	2	0	0	0
		Lee	36	0	0	2	0
CHILDREN UNDER 2 YRS. (During Epidemic)	36	PR_8	35	0	1	0	0
		FM_1	1	4	18	6	7
		FW_{50}	33	1	1	1	0
		Lee	24	10	1	0	1

* Reciprocal of final titre - after addition of all reagents - quoted in this and subsequent tables.

Reference to Table 2, which shows the HA1 titres of acute phase sera from seventy-one patients diagnosed during the epidemic as suffering from clinical influenza, reveals the fact that a titre of 1/128 or more to the FW50 strain was obtained in only five patients. Infection with an A-prime influenza virus closely related to the FW50 strain was confirmed later in fifty-seven of these patients.

TABLE 2

HA1 TITRES OF SERA FROM 71 PATIENTS IN ACUTE STAGE OF INFLUENZA ATTACK

STRAIN OF VIRUS	DISTRIBUTION OF SERUM TITRE LEVELS				
	32 or less.	64	128	256	512 or more.
PR_8	28	17	13	10	3
FM_1	3	16	15	17	18
FW_{50}	60	6	3	1	1
Lee	39	10	9	9	3

With this fact in mind Table 3 was compiled from the foregoing tables by recording the proportion of individuals in the different groups exhibiting a titre of less than 1/128.

TABLE 3

PROPORTION OF INDIVIDUALS WITH HA1 TITRE OF LESS THAN 1/128

STRAIN OF VIRUS	DIFFERENT GROUP INVESTIGATED							
	PROVINCIAL LABORATORY		WESTERN NURSES		CHILDREN		ACUTE STAGE INFLUENZA	
PR_8	46/75	61%	18/49	37%	72/74	97%	45/71	63%
FM_1	4/75	5%	21/49	43%	6/74	8%	21/71	30%
FW_{50}	45/75	100%	48/49	98%	72/74	97%	66/71	93%
Lee	65/75	87%	18/49	37%	70/74	95%	49/71	69%

The following points are illustrated by the above table-:

1. The extremely high proportion of individuals, in every group examined, lacking significant antibody levels to the A-prime strain FW50.

2. The obviously different picture obtained with the A-prime strain FM1.

3. The high proportion of individuals in all groups examined (with the exception of the nurses group) with low HA1 titres to the Lee strain of influenza Type B virus.

4. A lesser but still high percentage of adults showing low HA1 titres to Type A PR8 strain.

5. The almost complete lack of significant HA1 titres in children under two years of age. In this respect the FM1 strain differs from the other three strains as only 8 per cent of the children's group had titres lower that 1/128.

As the latter finding did not seem to represent a fair assessment of the amount of antibody to the FM1 strain of influenza virus present in children of this age it was decided to investigate the nature of the inhibiting substance present. Therefore, a proportion of these high-titred sera were tested before and after treatment with the cholera vibrio enzyme, R.D.E.

For this purpose a supply of purified and concentrated R.D.E. was obtained from Sir Frank Burnet. The results obtained with this reagent, presented in Table 4, indicate clearly the nature of the inhibiting substance. The FM1 strain of influenza virus used in this investigation was obviously particularly susceptible to the non-specific inhibitor destroyed by R.D.E. A control influenzal serum was not appreciably altered by similar treatment.

TABLE 4

EFFECT OF R.D.E. UPON TITRES OF NORMAL CHILDREN'S SERA FOR FM1 VIRUS

SERUM #	HAl TITRES NOT TREATED WITH R.D.E.	HAl TITRES AFTER R.D.E. TREATMENT
N1	4069	<16
N10	1024	<16
N13	1024	<16
N14	1024	16
N22	1024	<16
N37	128	<32
N39	1024	<16
N42	512	<16
N46	1024	<16
N50	512	<16
N51	2048	<16
N52	2048	16
N53	2048	<16
N54	2048	<16
N56	2048	<16
N63	1024	16
N64	2048	32
Control Serum	512	128

It is noted also in Table 3 that there was a distinct difference between the Provincial Laboratory and the Nurses groups with regard to their Type B HAl titres. Eighty-seven percent of the Laboratory group had low or insignificant titres towards the Lee strain, whereas the corresponding figure for the Nurses Group was thirty-seven

percent. As one of the main differences between these two groups was the age distribution - the nurses forming a homogeneous age group of 18-30 yrs whilst ages in the Laboratory group ranged from 20 to over 50 years - the results obtained with the Provincial Laboratory Group have been split into two groups, a twenty to thirty age group and an over thirty age group. Only five members of the latter group were over fifty. These further groupings have been recorded in Table 5. Figures for the children's group, excluding those individuals of four months of age or less who might still have maternal antibody, were included for comparison in Table 5.

TABLE 5

PROPORTION OF INDIVIDUALS WITH HAl TITRE OF LESS THAN 1/128 ARRANGED ACCORDING TO AGE.

STRAIN OF VIRUS	DIFFERENT GROUPS INVESTIGATED							
	CHILDREN 4-24 MONTHS		NURSES 18-30 YRS		PROV. LAB. 20-30 YRS		PROV. LAB. OVER 30 YRS	
PR8	48/48	100%	18/49	37%	13/34	38%	33/41	80%
FM_1	2/48	4%	21/49	43%	1/34	3%	3/41	7%
FW_{50}	46/48	96%	48/49	98%	34/34	100%	41/41	100%
Lee	48/48	100%	18/49	37%	31/34	91%	34/41	83%

The difference noted in Table 3 with the Lee strain was still apparent with either age group of the Provincial Laboratory personnel. On the other hand, with the PR8 strain, the percentages recorded for the nurses group and the corresponding twenty to thirty age group of the laboratory personnel are almost identical.

DISCUSSION

In interpreting and discussing these results one must keep in mind that the HAl levels recorded may not be due entirely to influenzal antibody but may be influenced by the presence of non-specific inhibitors. These normally inhibit heated virus but significant inhibition of strains in the unheated state has been noted by Burnet (1951,b). The sensitiveness of the unheated FM1 strain was plainly seen in the results obtained with the infants sera: however, if these results are studied further (Table 1) it will be noted that whilst high levels were found with the FM1 strain this was not so with the strains (PR8, FW50, and Lee) tested with the same sera.

On this account

titres obtained with these strains have greater significance. In either case low titres, irrespective of their specificity, can only indicate a degree of susceptibility to the disease, Hirst et al (1945) and Salke et al (1945, a), and the results show conclusively that antibody toward the FW50 A-prime strain is lacking in all the groups of individuals investigated. This is true also for the sera obtained from infants under two years of age not only with the FW50 strain but with all the other three strains tested.

Ignoring for the present the results obtained with the FM1 strain, it is noticeable how with the Type A PR8 strain the majority of the antibody levels are low in infants, find a higher level in the twenty to thirty age group and fall off once more in the older age group. This is in accord with the findings of Francis and Magill (1936) who titrated the neutralising antibody of different age groups and Martin (1940) who obtained similar results with complement-fixing antibody.

Whilst a similar trend is noted with the Lee (Type B) virus the lack of significant antibody levels in the twenty to thirty age group of the laboratory personnel might indicate a somewhat different situation. It may be as Type B virus tends to occur in localised outbreaks that quite a high proportion of young adults have low antibody levels to that type. In which case the higher proportion of elevated titres as found in the corresponding age-group of nurses might be taken as an indication of recent exposure to Type B virus.

High HAI titres with the FM1 strain were obtained in over 50% of all the groups of individuals investigated, but as already noted the significance of this is not very great. On the other hand it is noticeable that the only other group of individuals which has an appreciable number of sera with low titres to the FM1 strain is the "acute phase" group, made up of people who eventually succumbed to an A-prime infection.

SUMMARY AND CONCLUSIONS

As a result of this investigation in the Toronto area the following conclusions were drawn. That prior to the epidemic-

1. Insignificant HAl levels to the PR8, FM1, FW50 and Lee strains were present in the children over four months and under two years.

2. Insignificant HAl levels to the FW50 A-prime strain existed in the 198 normal sera investigated. This included a study of people ranging in age from infancy to between fifty and sixty years of age.

3. That, with the exception of the nurses group, at least 60 percent of each group of sera investigated had low titres to the PR8 strain.

4. That the twenty to thirty age group had the smallest percentage of low titre sera (37 per cent) with the PR8 strain. This applied equally to the nurses group and that part of the personnel of the Provincial Laboratory of similar age.

5. That, with the exception of the nurse group at least 69 percent

of every group investigated had low titres to the Lee strain.

6. A significally smaller proportion of nurses had low titres to the Lee strain suggesting that there may have been recent exposure to Type B infection in this group.

7. The FM1 strain used proved particularly sensitive to non-specific inhibition, and therefore results obtained with it are difficult to interpret.

PART III

THE 1950-51 INFLUENZA EPIDEMIC IN ONTARIO, CANADA.

ACCOUNT OF THE EPIDEMIC

The earliest reports of influenza in Canada came from Grand Falls in Newfoundland about the third week in January. Subsequently and very rapidly cases were reported from every province in Canada. At the same time influenza was spreading rapidly in the United States of America. It was significant that the peak of the epidemic was reached on February 12 in areas in Canada and the United States situated thousands of miles apart.

At this time throat washing and blood specimens were received from widely separated parts of Southern Ontario, a Province as large as the British Isles and with a population of approx. 4.6 million. In addition to examining these specimens the writer had the opportunity to observe the clinical nature of the disease as it appeared in two separate communities. The individuals in these groups were all young adults living a community life. The first group consisted of personnel in an R.C.A.F. camp and the second of student nurses some of whom had been vaccinated prior to the outbreak.

The Epidemic in an Unprotected Community

Outbreak at an R.C.A.F.Camp:- On February 5 a sudden increase in the number of cases of upper respiratory disease was noticed at sick parade, moreover, these cases exhibited many of the features which are commonly associated with influenza. The sudden increase is well illustrated graphically in Fig. 1, which shows the numbers attending sick parade during the months of January and February.

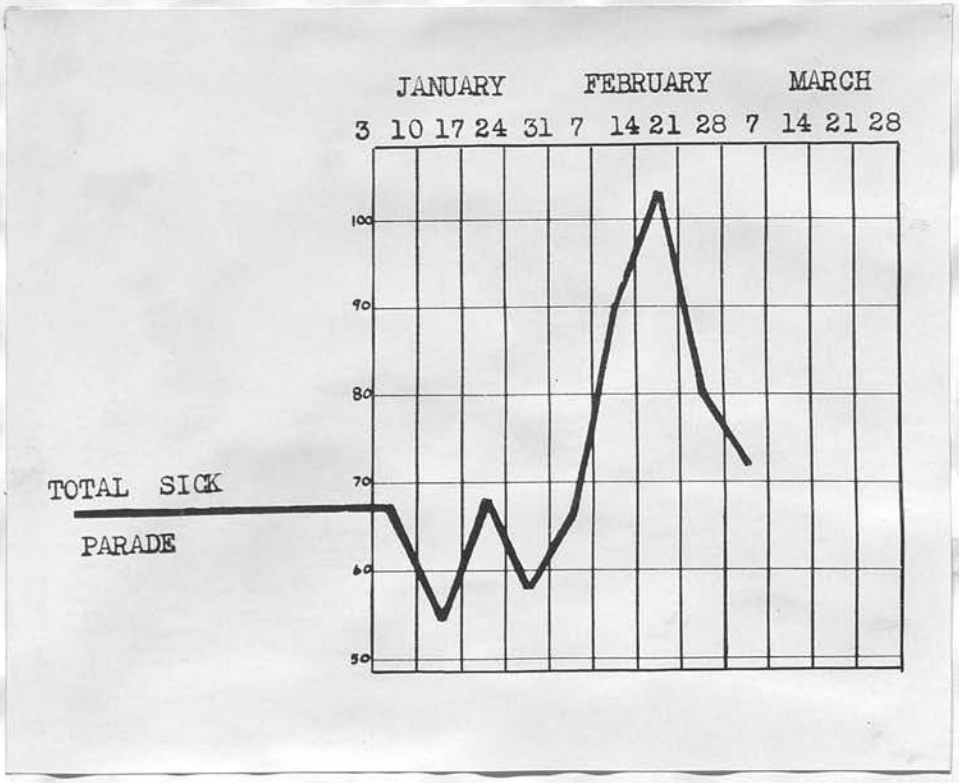

Figure 1.
Sick parade attendance.

The single sharp peak indicates the occurrence of an epidemic disease. During the peak of the epidemic 90 per cent of the men reporting sick had clinical influenza. The incidence of the outbreak was 247 cases out of approximately 800 personnel, or 31 per cent and of these 76 or 31 per cent were sufficiently ill to require hospitalization. The numbers admitted to hospital during January and February are illustrated in Fig. 2, and figure 3 shows a record of station strength for that period.

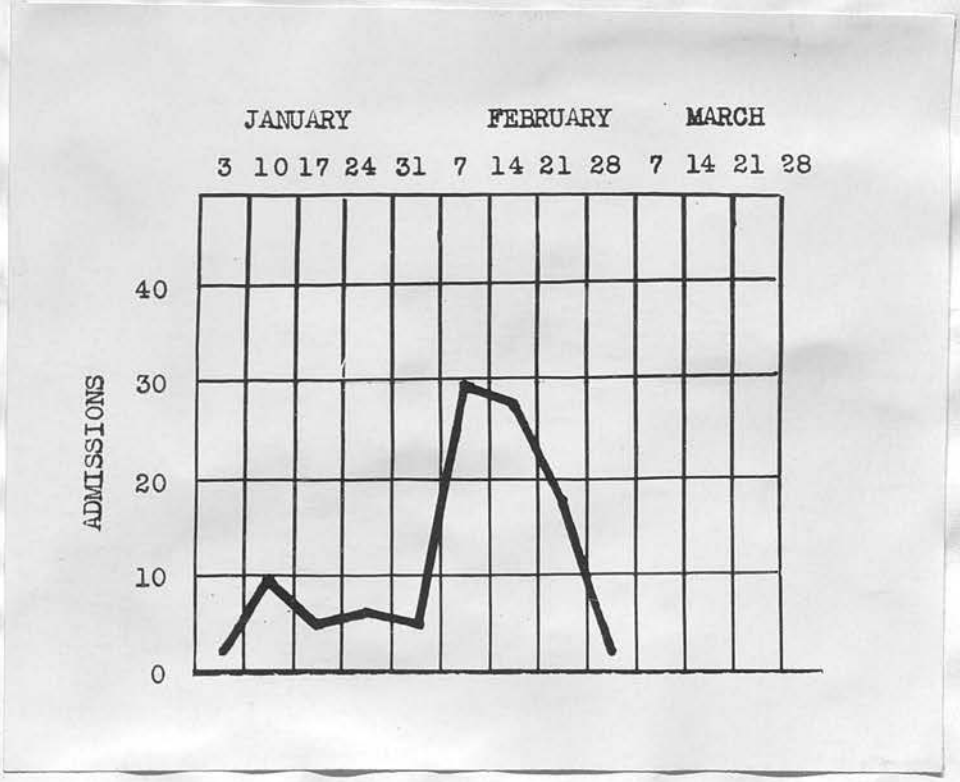

Figure 2.
Hospital Admissions.

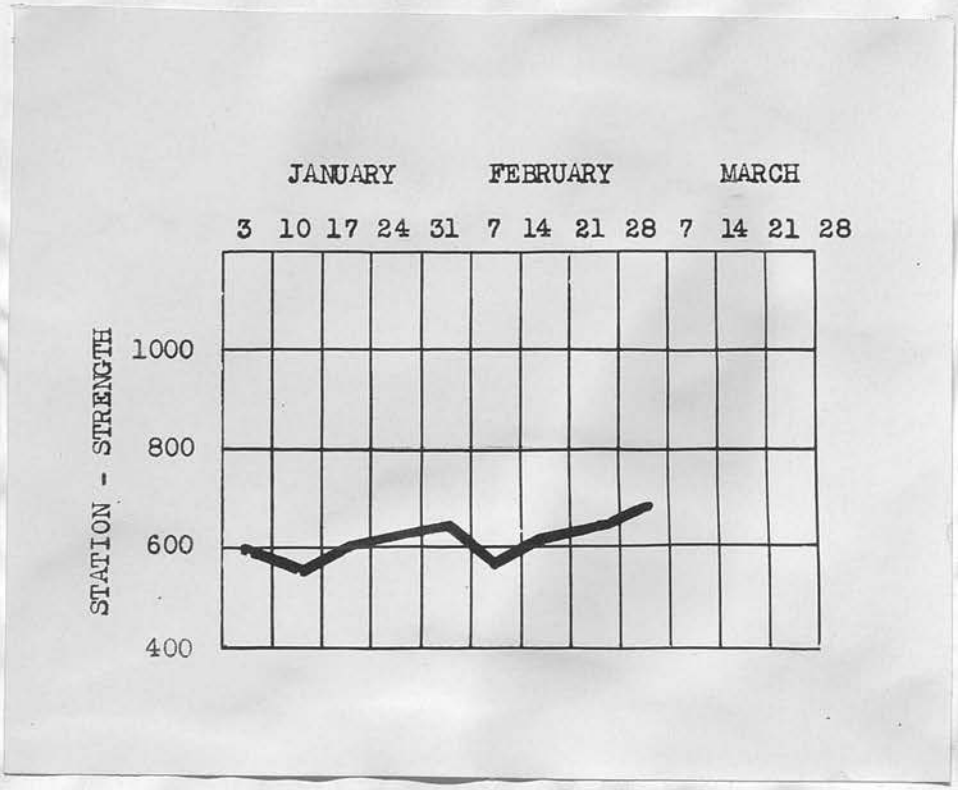

Figure 3.
Station Strength

Following the outbreak amongst the personnel there was a secondary wave of infection noted amongst the dependents living at the camp. Out of 700 dependents there were approximately 200 cases, the peak number of cases in this secondary wave numbered about 75.

The age groups affected in this secondary wave consisted of adults (23 yrs to 40 yrs), school children (5 yrs to 14 yrs), and

children of pre-school age. The general impression was that the pre-school age group was the least involved, although cases did occur amongst them. The whole outbreak lasted three weeks.

The onset of the outbreak followed closely the arrival at Camp Borden on February 3, of a fresh draft of over 100 men from Aylmer station where they had been in training. Subsequent enquiries revealed that the influenza outbreak at Aylmer started the same week as the Camp Borden outbreak. If the original cases were imported from Aylmer, spread must have been very rapid as cases of laboratory confirmed influenza were demonstrated at Camp Borden as early as February 7 amongst men belonging to several different drafts. Such a rapid spread is common experience with epidemic influenza. It is interesting to note that the admitting clerk to the MI room was one of these earlier victims and he was followed by the mess orderly. Approximately 10 per cent of men, in a later draft from Aylmer, who arrived at Camp Borden on February 15, contracted clinical influenza. This was despite the institution of

strict quarantine at Aylmer and for five days subsequently at Camp Borden because of an outbreak of scarlet fever at the former place. There was no evidence of contact with any personnel recently arrived from U.K.

The Signs and Symptoms of those Cases Diagnosed as Clinically Suffering from Influenza:- The symptoms of this illness were, generally speaking, those of an acute upper respiratory infection associated with a marked constitutional reaction. The febrile response varied between 100 and 105° F. with a maximum temperature of 102 or 103° F. in the majority of cases. The fever was associated with a feeling of chilliness and on the average lasted two to four days; resolution was by lysis. A daily temperature rise about noon and an evening fall in temperature appeared to be a fairly constant feature of the illness. Headache, fronto-parietal in distribution, was the commonest symptom and in almost all cases there was generalized muscular aching of the neck, back and limbs, which lasted for one to two days. The most persistent symptom which

occurred in 80 per cent of cases was a non-productive cough which lasted from two to four weeks and was initially associated with considerable substernal pain. In about 75 per cent of cases there was increased lacrimation sometimes with and sometimes without photophobia lasting for two to three days. Although a definite pharyngitis was found upon direct observation, in almost all cases the patient's other aches and pains overshadowed this and very often he did not complain about his throat. Short attacks of dizziness were complained of by some patients, generally those with higher temperatures or greater degree of malaise. Rhinitis was not a prominent symptom but was present in 50 per cent of cases.

There was a relative absence of physical signs and apart from the ill appearance of the patient, the flushed skin, the mild pharyngitis and often a conjunctival hyperaemia, there was little to observe. No respiratory complications were encountered. Treatment was symptomatic and only 18 of the 76 patients hospitalized received any antibiotic treatment. Twelve were given procaine

penicillin (300,000 units) and crystalline penicillin (100,000 units) twice daily and six others received aureomycin 2.0 gm. daily in divided doses. The illness lasted from four to seven days but patients felt poorly for about two weeks after and many complained of a persistent cough.

Clinical Features of Cases Confirmed by Laboratory Examination:- The commonest and earliest presenting symptoms were headache and a troublesome irritative cough followed in 12 to 24 hours by very marked malaise accompanied in some cases by sore throat, in others by dizziness and conjunctival irritation. Retro-sternal pain was a feature in about 25 per cent of the cases and in a similar number the onset of illness was preceded or accompanied by a head cold. In this particular group of cases the highest white blood count at the onset of symptoms was 10,500 per c.mm. and in the majority of cases was between 7,000 and 8,000 per c.mm. All but one of these cases had recovered in five days and there were no relapses. In one case recovery was delayed until the eighth day.

The Epidemic in a Partially Protected Community

Outbreak in a Nurses Residence:- There were 190 student nurses undergoing training in the Toronto Western Hospital at this time. In addition there were 34 graduate nurses. As already indicated a group of these student nurses, 50 in number, had been bled prior to the onset of the epidemic. The results obtained from these bleedings indicated that the student nurses had, when contrasted with other groups, lower titres to the standard FM1 virus (see Table 3) and in common with other groups very low titres against the FW50 A-prime strain. As the student nurses formed an ideal group for a vaccination study and as we had the pre-epidemic antibody levels for 50 members of the School we decided to vaccinate these individuals. The remaining 140 student nurses living and working under the same conditions served as an adequate control group.

The only vaccine available in Canada at that time had the following formula: 25 per cent A (PR8), 25 per cent A-prime (FM1),

50 per cent (Lee). The United States had vaccinated their armed forces with a vaccine which had a higher proportion of A-prime antigen, and incorporated the FW50 strain: 22 2/9 per cent A (PR8), 22 2/9 per cent A' (FM1), 22 2/9 per cent A' (FW50), 33 3/9 per cent B (Lee). Through the courtesy of Dr. Cox, Lederle, a gift of sufficient vaccine for this experiment was made to us. As soon as the vaccine was received it was arranged to vaccinate the group, and on the 12th February they were injected with a single dose of 1 cc of the vaccine. Any individuals with histories of allergy or of sensitivity to egg protein were not vaccinated. A total of 40 nurses received vaccine.

Reaction to vaccine:- The immediate reactions to the inoculation were a sore, throbbing arm in almost every case for 8-24 hours. About 10 per cent experienced general malaise for 24 hours.

From the time of the pre-epidemic bleeding on the 24th January these nurses were under close observation. The writer in addition to visiting the cases in the acute phase of their illness

had full access to their case histories. The course of the epidemic amongst the vaccinated and control groups is shown in Table 6. Altogether there were 8 cases of upper respiratory infection in the vaccinated group 4 of which were definitely proved by isolation of the virus to be due to infection with an A-prime influenza strain. In the control group there were 21 cases of which 6 were proven A-prime infections.

Table 6

Course of Epidemic Amongst Student Nurses

Calendar Period	Vaccinated Group Total 40	Unvaccinated Group Total 150	Remarks
February 1st week	0	0	Date of vaccination was 12th February
February 2nd week	1	5	
February 3rd week	5	9	
February 4th week	1	0	
March 1st week	1	4	Period when vaccine should be effective
March 2nd week	0	2	
March 3rd week	0	1	

The clinical findings in these cases were essentially similar to those of the Camp Borden outbreak and are summarised in

Table 7

Summary of Illness in 21 Unvaccinated Nurses

Details of Illness		Individual Cases																							Total
Onset	Sudden	1	1	0	1	1	1	1	0	0	1	1	1	0	1	0	1	1	0	0	0	0			12
	Gradual	0	0	1	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	1	1	1			9
Symptoms	Malaise	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1			20
	Aches	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0			20
	Cough	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	1	1	1			16
	Sore throat	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1			16
	Headache	1	0	1	0	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1			15
	Sneezing	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1			7
	Chest pain	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0			5
Signs (Local)	Throat	0	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	1	1	0	0	1			13
	Nose	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0			3
Signs (General)	Temperature 1 to 3 dys	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			18
	Temperature over 3 dys	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			3
	Temperature above 102° F.	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0			4
Acute Illness	One week	0	1	0	0	1	1	1	1	1	0	1	1	1	0	1	0	1	0	0	1	0			12
	1-2 weeks	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1	0	1			9
Residual Symptoms	After 2 weeks	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	1	0	0	1	1	0			11
Treatment	Aspirin	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1			19
	Antibiotic	P	P	PA	A	0	P	P	P	P	PA	P	A	P	PA	P	P	P	0	P	0	0			17

P = Penicillin
A = Aureomycin

Tables 7 and 8. Although it is realised that the numbers involved are too small to be of statistical significance several points are noteworthy.

1. The higher temperatures occurred in the unvaccinated group.
2. The illness was prolonged into the second week in the unvaccinated group and eleven members of this group still had residual symptoms two weeks after returning to duty.

Table 8

Summary of Illness in 8 Vaccinated Nurses

Details of Illness		Individual Cases								Total
Onset	Sudden	0	0	1	0	1	1	0	1	4
	Gradual	1	1	0	1	0	0	1	0	4
Symptoms	Malaise	1	1	1	1	1	1	1	0	7
	Aches	1	1	1	1	1	1	1	0	7
	Cough	1	1	1	1	1	0	0	1	6
	Sore throat	0	1	0	1	1	1	0	1	5
	Headache	1	1	0	1	0	1	1	0	5
	Sneezing	0	0	1	0	0	0	0	0	1
	Chest Pain	0	0	0	0	0	0	0	1	1
Signs (Local)	Throat	0	1	0	1	1	0	0	0	3
	Nose	0	1	0	1	0	0	0	0	2
Signs (General)	Temperature 1 to 3 days	1	1	1	1	1	1	1	0	7
	Temperature over 3 days	0	0	0	0	0	0	0	1	1
	Temperature above 102° F.	0	0	0	0	0	0	0	0	none
Acute Illness	1 week or less	1	1	1	1	1	1	1	1	8
	1-2 weeks	0	0	0	0	0	0	0	0	none
Residual Symptoms	After 2 weeks	0	0	0	0	0	0	0	0	none
Treatment	Aspirin	1	1	1	1	1	1	1	1	8
	Antibiotic	0	0	P	P	P	P	0	P	5

P = Penicillin

In the words of the physician attending the cases and commenting upon the course of the illness in the vaccinated group:-

"there is no doubt that their illness was less severe and less prolonged. The initial temperatures were approximately two degrees lower on the average, the presenting symptoms were much less severe, the acute illness lasted two days as opposed to four or five days. The period in bed averaged about five days as compared with eight or nine days and the convalescence was brief compared with the fourteen to twenty-one days required by the unvaccinated group."

LABORATORY INVESTIGATIONS

Materials and Methods

Throat washings:- These were obtained by requesting patients to gargle with 10 to 15 ml. of saline-broth mixture. The gargles were immediately frozen with dry ice and transported to the laboratory in vacuum flasks where they were stored at -70° C. until such time as embryonated hens' eggs of a suitable age were available for inocualation. Seven of the specimens were transmitted to the laboratory in a container cooled with ordinary ice and these were 2½ hours in transit. In this latter group, virus was isolated from six of the seven specimens.

The washings were treated with streptomycin and penicillin (1,000 units/cc) for a period of 20 minutes prior to inoculation into the amniotic sac of 13-day old embryos. In each case four egg passages, two amniotic passages followed by two allantoic passages, were made. Eight eggs were used for each passage.

Egg inoculations:- Inoculations were carried out in a

specially constructed wooden cabinet fitted with an adjustable glass front. After each batch of inoculations the cabinet was steam-sterilised before proceeding with another specimen. For amniotic inoculation strong embryos were selected by candling and 0.2 ml of the antibiotic-treated gargle fluid was introduced into the amniotic sac; successful inoculations were performed by painting a small area over the air-sac with 4 per cent iodine in alcohol boring a tiny hole through the shell immediately over the top of the air-sac with a filed off dental explorer, and injecting the fluid with a $1\frac{1}{2}$ inch No. 22 gauge needle. The hole over the air-sac was sealed with melted paraffin wax and the inoculated eggs were incubated in an egg-incubator regulated to a temperature of between 35 and 36° C.

The amniotic fluid was harvested from all living eggs after 72 hours incubation. The fluid was pooled, antibiotic was added and a fresh batch of eggs were inoculated amniotically on the same day with the freshly harvested material. The amniotic fluid was obtained by sterilising the shell over the air-sac with

iodine, breaking and removing that part of the shell with sterile forceps, tearing the shell and chorio-allantoic membranes with sterile forceps and tipping out the allantoic fluid into a petri dish. The embryo was gently coaxed over the edge of the broken shell by tipping it up, and then the amniotic sac hung down like a bag and was tapped with a sterile Pasteur pipette fitted with a rubber test. A volume of between 0.5 to 1.5 cc was obtained from each egg.

The second passage amniotic fluids were not harvested as a pool. Fluid from each egg was tested for haemagglutination and sterility. The fluids which proved to be sterile and gave a positive haemagglutination "Spot test" were pooled and inoculated into the allantoic cavity of a further batch of eggs. The "Spot tests" were made by putting approximately 0.1 cc of amniotic fluid into a depression in the plastic plate and then adding to that 0.9 cc of 0.25 per cent suspension of chick red cells; the results were read after one hour. Similar tests were made with guinea-pig cells although this was not always possible owing to difficulty in

obtaining a good stable suspension of these cells and sickness amongst the stock.

Allantoic inoculations were conducted in a similar fashion but with 10-11 day old embryos and using a No.23 gauge needle 5/8 inch in length. The eggs were incubated at 35-36° C. for 48 hours and after candling the live embryos were chilled overnight at 4° C and harvested the following morning. The fluid was obtained after sterilising a small area at the side of the egg just below the line of the air-sac. With the egg supported in a metal ring and the air-sac upwards a Pasteur pipette was introduced into the allantoic cavity through a small hole bored in the sterilised area; about 4-6 cc of fluid was obtained. A second hole was bored to allow the access of air as the fluid was being withdrawn. The allantoic fluids were tested for sterility and haemagglutination in the same manner as used with the amniotic fluids. Positive fluids were pooled and passed once more by the allantoic route.

The Patient's Serum:- Samples of blood were taken within

the first two days of illness and 14 days later during convalescence. These were tested for influenzal antibody using the haemagglutination inhibition test. Three standard strains A (PR8), A-prime (FM1), and B (Lee) were used and a fourth strain FW50 was also included to represent a recently isolated A-prime strain. Titrations were made upon plastic plates similar to the type recommended by Salk (1948). Doubling dilutions of serum were mixed with equal volumes of a fixed concentration of virus (4 haemagglutination units). To this mixture an equal volume 0.5 cc of 0.5% suspension of chicken red blood cells was added. Readings were made after 45 minutes. Appropriate serum, virus and saline controls were included.

Serological Typing of Strains:- Initially specific immune rabbit antiserum was used; this was discarded at an early stage in favour of rooster antiserum. The rabbit antiserum was prepared by the intravenous injection of 1 cc of infected allantoic fluid at weekly intervals for 3-4 weeks. The blood was withdrawn by heart puncture and the separated serum was stored frozen at -70° C.

The serum was inactivated at 56° C. for ½ hour before it was used.

Specific rooster antisera were prepared by the combined intravenous and intraperitoneal inoculation of birds of 6 lb. weight with 5 cc of allantoic fluid by each route. The serum was harvested, stored, and inactivated in the same manner as the rabbit antiserum. Birds were tested before immunisation with the FM1 strain to detect any with high non-specific HA1 levels. The FM1 strain was chosen for this purpose because it had been shown to be a sensitive indicator (see Part 11 page 45).

The unknown strain was typed by the haemagglutination-inhibition technique employing four haemagglutinating units of the virus and titrating in the presence of the specific immune serum. The methods of titration were identical with those used in HA1 tests already described (see Part 11 page 33).

Virus Isolations and Serological Typing

A total number of thirty-nine isolations was made. The details of the second, third and fourth egg passages are given in

Table 9 together with the chicken cell agglutination (C.C.A.) titre attained by the fourth egg passage. As described in the section under Methods, infection of the egg was detected by a haemagglutination "Spot-test". Because of the chance that the virus might be completely in the "O" phase the second passage amniotic fluid was tested when possible with both guinea-pig and chicken erythrocytes. The third and fourth passages were made in the allantoic cavity and on that account only chicken red cells were used to test the allantoic fluid harvested.

In most isolations the proportion of eggs infected increased with each passage fairly gradually. In some cases the transference from amniotic to allantoic passage (second to third egg passage) resulted in a sharp drop in the number of eggs infected but good adaptation was obtained upon further allantoic passage. Three strains had five egg passages before becoming properly adapted.

The majority of strains attained a CCA titre of 1/128 after four egg passages and were sufficiently well adapted for use in cross-typing tests.

Table 9

Results of 39 Virus Isolations by Egg Passage

Case #	Proportion of Eggs Infected as determined by Spot Test				C.C.A. Titre after 4th egg passage	
	2nd passage Guinea-pig	2nd passage Fowl	3rd passage Fowl	4th passage Fowl		
SR2	—	3/4	7/7	7/7	64	
Mi12	—	4/8	5/5	5/5	32	
Mi13	0/5	2/5	4/5	6/7	64	
AF2	2/5	2/5	0/8	8/8	128	
AF3	0/8	0/8	7/7	7/7	256	
AF4	0/8	0/8	8/8	7/8	128	
AF6	2/6	2/6	4/8	7/8	32	
AF8	—	—	6/6	7/7	128	
AF9	0/6	4/6	0/7	6/7	64	
AF10	0/7	6/7	8/8	6/7	64	
AF13	—	—	6/6	6/7	128	
AF14	—	—	4/4	6/6	128	
CW4	—	—	6/7	4/4	256	
CW5	—	—	0/7	7/7	128	
CW7	—	—	4/7	7/7	256	
CW8	—	—	4/7	7/7	256	
CW9	3/6	3/6	7/7	6/6	128	
CW10	—	—	7/7	7/7	128	
CW12	—	—	6/7	7/7	128	
CW13	2/7	2/7	7/7	7/7	128	
CW14	—	—	7/7	7/7	256	
CW15	1/8	2/8	6/7	7/7	<128	(64 5th passage)
CW16	—	—	7/7	7/7	512	
CW17	—	—	5/7	7/7	128	
TR1	2/8	2/8	7/7	7/7	128	
TR2	0/8	0/8	0/7	1/7	<128	(64 5th passage.)
TR5	0/3	3/7	7/7	5/5	128	
TR8	3/6	5/6	7/7	7/7	128	
TR10	0/7	0/7	2/7	6/7	512	
TAF1	1/8	2/8	7/7	6/6	64	
TAF3	0/7	0/7	6/6	3/7	128	
TAF4	0/7	1/7	7/7	2/7	128	
TAF6	1/7	1/7	7/7	7/7	128	
SU1	0/7	0/7	3/6	7/7	128	
SC55	4/6	4/6	6/6	9/9	<128	(128 5th passage.)
ES30	—	1/8	7/7	5/7	128	
GI1	—	0/7	5/6	4/4	—	
GI2	—	0/8	7/7	4/5	32	
GI5	—	0/5	6/6	7/7	<128	

Some strains did not reach such a high titre at that stage and had to be carried further. It was found difficult to maintain such a large number of strains and two were lost before they could be typed at all.

Results of Serological Typing:- The first 4 strains were typed with specific rabbit antisera and the results of these tests are shown in Table 10.

Table 10

Serological Typing with Rabbit Antisera

Virus Strains	HA1 Titres with Rabbit Antisera		
	PR8	FM1	Lee
PR8	256	<32	<32
FM1	<32	1024	<32
Lee	<16	<16	512
SA2	32	256	64
Mil2	64	512	64
Mil3	128	512	128
AF6	64	512	64

Although the tests indicated that the strains were A-prime in character the differentiation was not sharp enough on account of cross-reactions found with unrelated types, particularly Type B (Lee). The non-specific nature of the titres obtained with the Lee antiserum and the new strains was confirmed by testing the

latter with normal rabbit serum. Difficulties of this nature were not encountered when rooster antiserum was used. The typing of strains was continued with the rooster antisera (Table 11) and the A-prime character of all the strains tested is apparent.

Table 11
Serological Typing with Rooster Antisera

Virus Strains	HA1 Titres with Rooster Antisera PR8	FM1	FW50	Lee
PR8	4096	<32	<32	<32
FM1	64	4096	512	<32
FW50	<32	32	256	<32
Lee	<32	<32	<32	512
AF2	<32	512	256	<32
AF3	<32	1024	128	<32
AF4	<32	1024	128	<32
AF8	<32	256	512	<32
AF9	<32	2048	256	<32
AF10	<32	1024	256	<32
AF13	<32	256	128	<32
AF14	<32	256	128	<32
CW4	<32	512	256	<32
CW5	<32	512	256	<32
CW7	<32	512	256	32
CW8	<32	512	256	32
CW9	<32	4096	512	<32
CW10	<32	2048	256	<32
CW12	<32	256	128	<32
CW13	<32	2048	256	<32
CW14	<32	256	256	<32
CW15	<32	1024	128	<32
CW16	<32	512	256	<32
CW17	<32	512	256	<32
TR1	32	512	256	<32
TR2	<32	256	128	<32
TR5	<32	512	128	<32
TR8	<32	1024	128	<32
TR10	<32	512	64	<32
TAF1	<32	1024	256	<32
TAF3	<32	512	64	<32
TAF4	<32	512	128	<32
TAF6	<32	1024	128	<32
SU1	32	512	256	<32
SC55	<32	256	256	<32
ES30	<32	256	64	<32
GI2	<32	1024	128	<32

The Serological Diagnosis

Acute and convalescent sera were obtained from a total of 66 patients diagnosed clinically as suffering from epidemic influenza. Five of these patients had been vaccinated shortly before their illness and on that account are excluded from Tables 12 and 13, which show the results of the laboratory investigation. In Table 12 are presented the fold-rises in antibody titre as found in patients from whom virus was isolated. In Table 13 are shown the corresponding rises in titre obtained from patients in whom virus was either not detected or not looked for. A serologically positive diagnosis - a four-fold rise in titre was recorded in 79 per cent of the cases with the combination of A and A' antigens used.

If we consider only the 50 cases upon whom both virological and serological examinations were made it is noted that 52 per cent were diagnosed by virus isolation in the egg embryo alone and 74 per cent were diagnosed by serological methods. This demonstrates the superiority of the serological method of diagnosis.

Table 12

Serological Results for Patients from Whom Virus was Isolated

Patient's #	Virus Isolated	Fold-rise in HAl Titre PR8	FM1	FW50	Lee
Mi12	A-prime	8	16	16	0
Mi13	A-prime	0	2	4	2
AF2	A-prime	8	0	0	0
AF3	A-prime	8	16	8	0
AF4	A-prime	4	8	16	0
AF6	A-prime	8	4	4	0
AF9	A-prime	2	16	32	2
AF10	A-prime	2	8	4	0
AF13	A-prime	128	16	128	2
AF14	A-prime	8	8	8	0
CW4	A-prime	4	4	8	0
CW5	A-prime	4	0	0	2
CW7	A-prime	4	4	16	0
CW10	A-prime	512	2	4	0
CW13	A-prime	8	8	8	0
CW14	A-prime	8	8	2	16
CW15	A-prime	2	2	2	0
TR1	A-prime	8	4	16	0
TR2	A-prime	0	0	2	0
TR5	A-prime	8	0	8	0
TR10	A-prime	0	0	4	0
TAF1	A-prime	8	4	2	0
TAF3	A-prime	2	2	0	0
TAF6	A-prime	4	4	4	0
ES30	A-prime	2	4	2	0
GI1	Not typed	0	0	0	0
GI2	A-prime	0	2	0	0
GI15	Not typed	2	8	16	0

0 = Less than 2-fold rise

Table 13

Serological Results for Patients from Whom Virus was not Recovered

Patient's #	Fold-rise in HAl Titre			
	PR8	FM1	FW50	Lee
Mil 1	2	8	4	0
AF1	4	8	8	0
AF5	4	2	8	0
AF7	8	8	4	0
AF11	128	32	64	0
AF12	8	4	4	0
CW1	4	4	2	0
CW3	4	0	4	0
CW6	4	4	2	0
CW11	0	0	0	4
CW18	16	32	16	0
TR3	0	0	0	0
TR4	2	0	0	0
TR6	0	0	0	8
TR9	8	16	4	0
TR13	8	0	2	0
TAF7	0	0	0	0
ES12	0	16	16	2
ES21	4	2	4	0
ES52	0	0	2	0
ES75	4	2	4	0
ES96	2	16	4	2
ES111	16	16	4	2
GI6	2	4	8	0
GI7	4	4	4	2
GI8	2	0	0	0
GI10	0	0	2	0
GI12	32	32	8	0
GI13	32	16	0	0
GI14	32	16	8	0
GI17	8	64	16	0
CO10	4	2	0	0
CO11	8	0	2	0
CO13	2	0	2	0

0 = Less than 2-fold rise

An Analysis of the Serological Diagnosis:- Observations made upon the fold-rise in haemagglutination-inhibition titre with the four strains PR8, FM1, FW50, and Lee are shown in Table 14 for the 26 cases in which an A-prime influenza virus was isolated. It is interesting to note that the PR8 Type A strain compared favourable with the A-prime strain as a diagnostic antigen in these A-prime infections. Indeed as seen previously in Table 12 there are two cases in which the PR8 antigen was the only one to detect a rise in antibody titre.

Table 14

Rise of HA1 Titre in 26 cases from which A-prime Virus was Isolated

Fold-rise in HA1 Titre	Type A PR8	Type A-Prime FM1	Type A-Prime FW50	Type B Lee
Less than 2	4	5	5	21
2	5	5	4	4
4	5	7	6	0
8	10	5	5	0
Greater than 8	2	4	6	1

The use of a single A or A-prime antigen would have resulted in the diagnosis of 16 or 17 of the 26 cases. This is more clearly shown in Table 15 which also illustrates the advantage of using more than one antigen for the diagnosis of Type A influenal infection. Best results were obtained when using all three antigens, but a combination of the older PR8 Type A strain with the more recently isolated A-prime strain FW50 gave results which were almost as good.

Table 15

Comparison of Strains in Diagnosis of 26 cases of A-prime Infection

Antigens	Number of cases with rise of 4-fold or greater
PR8 alone	17
FM1 alone	16
FW50 alone	17
PR8 + FM1	20
PR8 + FW50	22
FM1 + FW50	21
PR8 + FM1 + FW50	23

A diagnostically significant rise in titre to influenza A and B was obtained in one patient from whom we isolated only

A-prime influenza virus. (Table 12). This patient was a nurse and the severity of her illness was such that the physician-in-charge decided to move her from the nurses residence to the main hospital. In two other patients from whom no virus was isolated (Table 13) a significant rise was obtained only with Type B virus. Thus in the 60 or so cases examined serologically only 3 showed evidence of a Type B infection that is about 5 per cent.

The Serological Response in the Vaccinated:- Thirty of the nurses who had been vaccinated just prior to the outbreak of the epidemic were bled four weeks after inoculation. The fold-rise in HA1 titre is recorded in Table 16 and the geometric mean-fold rise has been calculated for each antigen. The interpretation of these figures is difficult owing to the fact that some of these nurses must have been exposed to subclinical infection with A-prime virus.

Table 16

Influenza Vaccination Experiment - Fold Rise in HA1 Titre 4 Weeks after Vaccination

Number	Type A (PR8)	A-prime Subgroup (FM1)	(FW50)	Type B (LEE)
W7	4	8	16	4
W10	2	0	2	2
W11	4	4	2	8
W12	8	4	0	16
W13	16	4	4	0
W14	4	2	8	0
W17	4	2	4	2
W18	64	32	16	2
W19	8	4	4	8
W20	2	2	0	16
W22	4	2	2	8
W23	16	16	16	8
W28	4	4	16	32
W29	4	4	4	8
W30	2	4	4	4
W32	2	4	4	4
W33	2	2	0	0
W34	2	2	0	2
W36	4	0	4	64
W37	8	32	8	8
W38	16	16	32	128
W40	2	4	8	2
W41	128	8	4	8
W42	4	4	8	8
W43	2	2	0	2
W44	4	16	8	2
W45	4	4	4	2
W46	4	4	0	4
W49	2	16	8	16
W50	4	8	2	8
Mean-fold rise	4.6	3.7	3.8	5.4

It is possible that W.23 had such an infection as she shared a room with a known case and reported a head cold at that time. Another nurse W.7 was off with a suspected pneumonic condition and of the remainder only three reported untoward symptoms - W.37 a head cold, W.42 was off one day with a sore throat and intestinal upset and W.45 was off for one day. The remaining 25 nurses remained completely healthy during the four weeks following the inoculation.

The mean-fold rise with PR8 and Lee strains was noted to be greater than that obtained with the A-prime strains. Failure to stimulate a rise in antibody titre was most commonly observed with the FW50 strain, whereas an antibody rise to the PR8 strain was detected in every one of the thirty nurses.

DISCUSSION

The serological typing of 37 of the strains isolated in Ontario during the 1950-51 epidemic showed that they belonged to the A-prime group. A more detailed analysis of the relationship of these strains to one another and to strains isolated concurrently

in the U.K. and Sweden follows in the next section. However it is of interest to compare the severity of the illness as seen clinically here in Ontario with other A-prime outbreaks occurring at the same period.

As recorded in most of the cases seen the acute phase lasted three to five days and in the twenty to thirty age group complications were rare, although convalescence often accompanied by a dry irritative cough, lasted about two to three weeks. No first-hand experience was gained of the illness as it presented in the older age group but the Dominion Bureau of Statistics recorded that approximately 80 per cent of the deaths from influenza occurred in people of 55 years and over. In the very young, under two years of age, the writer found only two cases of pneumonia, one of them fatal, which could be attributed to influenzal infection, and at that time all cases of virus pneumonia in infants were being referred to him from the Hospital for Sick Children in Toronto.

Collins and Lehmann (1951) recorded that the epidemic

of 1951 was the twentieth outbreak of influenza and pneumonia in the United States since the 1918-19 pandemic. Apparently the influenza and pneumonia mortality rate has decreased since 1937 from about 100 per 100,000 population to only 34 per 100,000 in 1950 and in this respect the 1950-51 epidemic in the United States was considered small. The epidemic as described by Svedmyr and von Magnus (1951) in Scandanavia was very similar; typical mild influenza lasting two to five days, with complications relatively uncommon. In this part of the world deaths were infrequent and only 18 were recorded during the one and a half months which covered the peak of the epidemic. These deaths were in old persons.

In Britain, Harrison (1951) writes that the epidemic in the north-west was the most severe in morbidity and mortality since the 1918-19 epidemic; he records how in one hospital out of 80 patients admitted with influenzal pneumonia 20 died, all of whom were over 50 years of age. Isaacs and Andrewes (1951) record that in Liverpool alone there were 216 deaths from influenza in one single

week approximating to the maximum figure of 230 per week found in the 1918 pandemic. The overall picture, which was presented by Freyche and Klimt (1951), showed that the influenza epidemics of 1950-51 as described in various parts of the world were mild. They remark upon the fact that throughout the world during the past four years the A-prime viruses have been the major causal agents of epidemic influenza.

All of the numerous strains of virus recovered in the present investigation were of the A-prime type and although it was not possible to determine whether they were in the "O" phase when isolated, as described by Burnet and Bull (1943), it was noted that they became adapted only gradually to growth in the egg and that even after four passages the titres were low. As will be seen in the next section representative strains of the 1950-51 viruses showed no pathogenicity towards mice. The value of rooster antisera which was first used in the typing of influenza strains by Hudson et al (1943) was confirmed, and the difficulty of using untreated

rabbit antiserum was observed.

In commenting upon the serological diagnosis the high efficiency of the PR8 antigen in the detection of infections caused by A-prime influenza virus is of interest. Appelby et al (1951) noted the well-marked antigenic capacity of the PR8 strain and commented upon how it excelled the A-prime strains in this respect. Meiklejohn et al (1952) observed the same fact. It is possible that the quantities of PR8 antigen present in the current A-prime virus although small are as effective as the specific A-prime components because of greater antigenicity; moreover the response to this antigen is likely to be of an anamnestic type. The PR8 antigen might not be as useful for first infections with an A-prime strain in children, where no anamnestic reaction can be called into play. The combination of the PR8 strain and the more recently isolated A-prime strain FW50 gave diagnostic results which compared favourably with those of Nagler et al (1951) who employed the freshly isolated strain. The advantages of using a freshly isolated

strain has been commented upon previously by Rickard et al (1945) and by Salk et al (1945,b).

The diagnostically significant rise in HAl titre to influenza A and B obtained with one of the patients raises the question as to whether such a finding denotes a double infection or merely a broadening of the antigenic response as found in the partially immune human as distinct from the non-immune animal. In support of the possible occurrence of a double infection it may be noted that in two patients there was serological evidence of a Type B infection without any signs of A-prime, indicating that type B infections were occurring. Furthermore a consideration of the figures obtained in this investigation for all age-groups shows that high titres for Type B (1/256 and over) were found in about 15 per cent of the population sampled. A similar proportion of the acute phase sera had high titres for Influenza Type B virus. If one accepts these raised titres as evidence of recent infection then it is reasonable to suppose that during the A-prime epidemic a few people must have

been exposed to both types of virus simultaneously. The nurse who was suspected of having a double infection had a severer illness than any of the other nurses. It is interesting to note that Freyche and Klimt (1951) report the simultaneous occurrence of influenza types A-prime and B in Florence during 1951 and that in Switzerland the A-prime epidemic was followed by a Type B epidemic whereas in Yugoslavia a Type B epidemic preceeded the A-prime outbreak.

In considering the results of the vaccination experiment it was unfortunate that the day of inoculation, the 12th of February, corresponded with the peak incidence of cases in Canada. However, the majority of cases in Toronto occurred slightly later than this and the members of the United States Commission upon Influenza (1944) have reported some success in vaccination during the early part of an epidemic. In assessing the efficiency of the vaccine to give complete protection it is necessary to dismiss all cases occurring within fourteen days of the date of inoculation. There was only one case of upper respiratory infection in the vaccinated group compared

with six in the control group after this time interval. This represented an incidence of 1:33 and 1:19 respectively. However the figures are small.

It is noteworthy that the incidence of upper respiratory infection amongst the Western Nurses was only 15.3 per cent compared with an incidence of 31 per cent at the R.C.A.F. Camp. It is not safe however to attribute such differences to the efficacy of vaccine for many writers, for example Horsfall et al (1941) and Salk et al (1945,b) have shown that morbidity rates often exhibit wide variability. Indeed enquiries at two other Toronto hospitals revealed a morbidity rate for respiratory infections amongst the nurses of 14.3 per cent and 19.6 per cent respectively during the epidemic. None of these nurses had been vaccinated.

There was however clinical evidence that the disease was milder in the vaccinated attacked during the first wave of infection in February. It is possible that the absence of a secondary wave of influenza amongst the vaccinated group was due to vaccination.

SUMMARY AND CONCLUSIONS

A study of clinical and laboratory material available during the 1950-51 epidemic in Ontario, Canada, lead to the following conclusions.

1. An outbreak at an R.C.A.F. Station at Camp Borden, Ontario, which involved 31 per cent of the personnel was mild in nature and no severe complications were encountered. Convalescence usually of 2-3 weeks duration, after an acute phase lasting 2-5 days, was accompanied often by a persistent cough.

2. The clinical features of the epidemic as it affected the Nurses residence of the Toronto Western Hospital were similar in nature. The incidence there was 15.3 per cent.

3. The vaccination of about one fifth of a group of 190 nurses did not result in any appreciable reduction in incidence of the disease in inoculated and uninoculated nurses. This could be explained by the fact that all but one of the cases in the vaccinated group occurred within fourteen days of inoculation.

4. Reactions to the vaccine were mild.

5. Lower temperatures, a shorter illness, and a quicker convalescence was noted in cases of the disease which occurred in vaccinated individuals.

6. During the epidemic 37 strains of influenza virus were isolated and identified as belonging to the A-prime subgroup.

7. Diagnosis by isolation of virus was made in 52 per cent of cases and by HAl tests in 74 per cent.

8. The value of the classical PR8 Type A strain as a diagnostic antigen in HAl tests during an epidemic caused by an A-prime strain was noted. Best results were obtained by using the PR8 strain along with an A-prime strain.

9. There was serological evidence of Type B infection in three cases. In two of these there was no evidence of an A-prime infection but in the remaining case A-prime virus was isolated and a serological response to A antigens was also found suggesting the possibility of a double infection.

10. The antigenc value of the four different strains PR8, FM1, FW50

and Lee was assessed by bleeding the vaccinated nurses one month after inoculation.

11. The superiority of the PR8 (A) and Lee (B) strains over the A-prime strains FM1, and FW50 as antigenic agents was observed.

PART IV

THE SEROLOGICAL PATTERN OF STRAINS OF INFLUENZA ISOLATED IN CANADA 1948-51

PART IV

THE SEROLOGICAL PATTERN OF STRAINS OF INFLUENZA ISOLATED IN CANADA 1948-51

During the winter of 1948-49 many strains of influenza virus were recovered from different provinces throughout Canada, and in addition to these, several strains were obtained from an outbreak amongst Eskimoes which aroused considerable interest. A full description of the isolation of these strains has been given by van Rooyen et al (1949) and Nagler et al (1949). The antigenic analysis presented here includes studies made with some of the strains recovered in 1949 and thirty-eight others isolated during the 1950-51 epidemic in Ontario.

The Ontario strains came from widely separated areas, one from Sarnia, one from Sudbury, four from Trenton, and nineteen from the Toronto area, ten of which were obtained from nurses participating in the vaccine trial. Twelve were recovered from Camp Borden as described by Dempster et al (1952) and an additional strain was provided from Cornwall; thus they were obtained from all parts of the more heavily populated

districts of Southern Ontario.

These strains have been compared one with another and with standard A and A-prime viruses by the haemagglutination inhibition technique first described by Hirst (1942,b). In addition, the relationship between some of the 1949 and 1951 viruses has been examined and for this purpose also a Swedish and an English strain from the 1950-51 epidemic have been included. None of the strains examined belonged to Type B.

MATERIALS AND METHODS

Virus Strains: The Lee and FM1 strains used in this investigation were obtained in 1950 from Dr. Morris Schaeffer of the United States Communicable Disease Centre, Alabama. The Lee strain had undergone several ferret passages, about 50 mouse passages and between 20-30 egg passages. The FM1 strain had had 10 egg passages followed by 7 mouse passages

after which it had been through a further 5-10 egg passages. The PR8 strain had been maintained in this laboratory for several years and had had several ferret passages, about 8 mouse passages and 10-20 egg passages. The FW50 (Cuppett) strain was obtained from Dr. Hilleman of the U.S. Army Medical School, Washington and had been isolated and passed three times in eggs.

Twelve Canadian Arctic strains isolated in 1949 from the Cambridge Bay outbreak were used; in addition attempts were made to pass 10 strains of virus which had been isolated from different parts of Canada during the early part of 1949 and which had been stored at -70°C since that time. Of the latter only 3 strains were recovered and used in this investigation. All the 1949 Canadian strains had been isolated in eggs with 2-5 subsequent allantoic passages.

The isolation of the 1951 Canadian strains with the exception of the Cornwall strain from different parts of Southern

Ontario has already been described (Pt.III). The Cornwall strain was isolated by Dr. Labzoffsky at the Provincial Laboratories, Toronto. The Canadian strains had been isolated and passed only in eggs, having received two amniotic passages and not more than four allantoic passages. A list of all the strains used in this investigation is shown in Table 17.

TABLE 17

LIST OF STRAINS EMPLOYED IN THIS INVESTIGATION

STRAIN DESIGNATION	ORIGIN	DATE OF ISOLATION	WORKER
PR_8	Puerto Rico	1934	Francis
Lee	New York	1940	"
FM1	Forth Monmouth	1947	Army Med. C.
NewI	Newmarket, Ont.	1949	van Rooyen and McClelland
SJ2	St. John, N. B.	1949	"
Ed2	Edmonton, Alta.	1949	"
CA (12 strains	Canadian Arctic Cambridge Bay, N.W.T.	1949	"
FW50	Fort F. Warren	1950	Army Med. C.
Sweden3	Sweden	1950	Svedmyr
London2134	London	1951	Andrewes
CW (11 strains)	Toronto, Ont. Western Hosp. Nurses	1951	Dempster and Buchner
AF (8 strains)	Camp Borden, Ont.	1951	"
Su1	Sudbury, Ont.	1951	"
SA2	Sarnia, Ont.	1951	"
TAF1	Toronto, Ont., R.C.A.F.	1951	"
TR5	Trenton, Ont., R.C.A.F.	1951	"
SC55	Toronto, Ont., H,S.C.	1951	"
CO16	Cornwall, Ont.	1951	Labzoffsky

Specific Antisera:- The twelve Canadian Arctic strains were investigated solely with rabbit antisera prepared by intravenous injection of 1 cc of infected allantoic fluid at weekly intervals for 3-4 weeks. The harvested sera were absorbed with Lee virus prior to testing the cross-reactions with members of the A and A prime groups. To further reduce non-specific reactions the rabbit antisera were heated at 65°C for 1/2 hour. The technique followed was similar to that of Tamm et al. (1950). It was not considered necessary to treat the rooster sera in this manner.

Specific rooster antisera were prepared by the combined intravenous and intraperitoneal inoculation of birds of 6 lbs weight with 5 cc of allantoic fluid by each route. The serum was obtained by heart puncture after 10 day interval. Where there had been a large number of strains isolated from a particular area as for example Toronto, an antiserum was prepared against only one of them. In this way antisera were prepared to strains from the different areas involved.

The HAl tests were carried out on plastic plates similar to that advocated by Salk (1948). The plates were made from leucite and constructed to the following specifications (overall size: 32.5 cm x 25 cm x 1.6 cm; eight rows of twelve depressions - diam. 2 cm and depth 1.4 cm).

A volume of 0.25 cc of virus suspension containing approximately 4 haemagglutinating units, was added to equal volumes of doubling dilutions of antiserum; to this mixture 0.5 cc of 0.5% suspension of chicken red blood cells was added. Readings were made after 45 minutes.

To minimize possible variation due to different sources of chicken erythrocytes, blood was taken from individual fowls which could be subsequently identified. In the whole investigation a total of 11 fowls were used for the supply of blood and of these 2 proved to be unsuitable and were discarded because of auto-agglutination. Phosphate buffered saline (pH 7) was used as a diluting and suspending reagent.

It was thought possible that lack of sharpness of the endpoint upon the plastic plate might have been due to electrostatic charge on the plate influencing the sedimentation pattern. This possibility was investigated by carrying out parallel titrations upon a plastic plate and one made from a material known to have little or no surface charge (porcelain). The poreclain plate was moulded to the same dimensions as the plastic one. The titrations revealed no significant difference and the plastic plate was adopted for use.

Readings. In virus titrations the last dilution showing an even distribution of cells over the bottom of the depression was taken as the endpoint. On some occasions, the distribution was not uniform and the determination of the exact endpoint was a matter of experience and judgement obtained by recording varying degrees of partial sedimentation of the cells.

In serum inhibition titrations the endpoint was

usually very sharp and was taken as the highest dilution in which a sharply outlined button was observed. Titres were recorded as final dilutions after addition of all reagents.

Controls. Apart from the usual saline control a check of the virus concentration used in the inhibition tests was made. On some occasions the virus control indicated that the concentration used in the test was too weak or too strong and then the results obtained had to be correlated with other days work by means of the homologous serum titer. This serum control was included when necessary.

Cleaning and disinfection of plastic plates. The routine procedure for glassware could not be used. After a test was completed the deposited cells were shaken off the bottom of the depressions and the whole plate was emptied into and finally immersed, for a short time, in a tank of 2-3% phenolor. (If left too long in this solution the plastic tends to soften.) The phenolor was washed off with distilled water and the plates were put into weak glass-cleaning

solution overnight, rinsed in tap water, followed by distilled water, dried, and finally cleaned with ether. Satisfactory cleansing of the plastic plates was found to be the greatest drawback to their use.

Pathogenicity tests were made with groups of 4 Swiss mice of 15-20 gm weight. Two to three drops of freshly harvested infected allantoic fluid were administered intranasally to the lightly etherized animals. Any animal sickening within one week was killed and examined for lung lesions; after the seven days all the animals were killed and examined likewise.

RESULTS

Antigenic Analysis with Rabbit Antisera

Twelve strains isolated in 1949 from the Cambridge Bay outbreak were available for comparison with one another. The antigenic analysis of these strains has been carried out with rabbit antisera prepared against each one of the strains.

The antisera were absorbed first with Lee virus and

afterwards subjected to heating at 65°C to minimize non-specific effects. As indicated in Table 18, CA2 and eight other strains behaved in a similar fashion, whereas three others showed differences. The similar strains, represented by CA2 in the table, have the characters of typical A (PR8) strains. Two of the others CA7 and CA11 behave like A strains but have A prime components, in their antisera. The remaining strain CA12 is very similar to the A prime strain FM1 and has no PR8 component in its antiserum.

It would appear that the twelve Canadian Arctic strains are not identical with one another and that although eleven of them resemble A strains most closely, one is more like an A prime.

TABLE 18

SEROLOGICAL ANALYSIS OF CANADIAN ARCTIC STRAINS

VIRUS	RECIPROCAL OF HA1 TITRES WITH RABBIT ANTISERA					
	CA2	CA7	CA11	CA12	PR8	FM1
CA2*	1024	8192	2048	<16	512	<16
CA7	2048	16384	4096	<16	1024	<128
CA11	512	8192	2048	<16	512	<64
CA12	<128	256	2048	256	64	256
PR8	512	8192	512	<16	256	<16
FM1	<16	128	1024	256	<16	2048

*Eight other strains and their homologous antisera behaved like CA2.

Antigenic Analysis with Rooster Antisera:- As it would not have been possible to prepare antisera to all the strains isolated, representative strains were selected from each area or community for immunising roosters. The strains from each community were then tested against their corresponding antiserum. No differences were detected amongst the eleven strains isolated from the Nurses Residence when they were tested with the antiserum for the representative strain and with antisera prepared against strains from other areas. On this account when the representative strain (CW14) for the nurses group died out during the investigations it was considered possible to substitute for it another, CW15, which came from the same community. As reported by Dempster et al (1952) no differences were noted between the eight strains obtained from patients at Camp Borden.

Comparison of strains isolated in different parts of Ontario with Swedish (1950) and London (1951) strains and the three standard strains (PR8, FM1, FW50). The Ontario

strains came from Toronto Western Hospital (CW15), Toronto Hospital for Sick Children (Sc55), the RCAF station in Toronto (TAF1), Camp Borden (AF9), Cornwall (C016), Sudbury (Sul), the Air Force Station at Trenton (TR5), and lastly from Sarnia (SA2). Thus they were obtained from widely separated parts of Southern Ontario. As will be seen from Table 19 they differ little from one another in HA1 tests and resemble closely the London and Swedish strains. The recently isolated Ontario strains can be seen to be in a position intermediate between the FM1 and FW50 A-prime strains. Apart from C016 and Sweden3, the 1950-51 strains show no cross-relationship with PR8 and the former are inhibited by PR8 antiserum only to a very slight extent.

TABLE 19

COMPARISON OF STRAINS ISOLATED IN 1950-51

VIRUS	RECIPROCAL OF HA1 TITRES WITH ROOSTER ANTISERA										
	STANDARD STRAINS			SWEDEN 1950	UK 1951	CANADA (ONTARIO) 1951					
	PR8	FM1	FW50	SWEDEN3	LONDON2134	CW14	AF9	SA2	TR5	SC55	CO16
PR8	4096	<32	<32	<32	32	<32	<32	<32	<32	<32	32
FM1	64	4096	512	4096	1024	256	512	512	1024	2048	1024
FW50	<32	32	256	512	512	128	256	128	128	256	256
Sweden3	32	512	128	2048	2048	512	512	512	256	512	256
London2134	<32	256	256	1024	1024	512	512	512	256	512	512
CW15	<32	256	256	2048	2048	256	512	512	256	1024	1024
AF9	<32	512	256	2048	2048	512	512	512	256	1024	1024
SA2	<32	512	128	4096	2048	512	512	512	512	1024	2048
Tr5	<32	512	256	1024	8192	1024	512	512	1024	4096	2048
SC55	<32	512	256	-	2048	512	256	256	512	2048	2048
CO16	64	1024	1024	4096	2048	1024	1024	2048	1024	4096	4096
Sul	<32	256	256	2048	2048	256	512	1024	256	-	1024
TAF1	<32	256	256	2048	2048	256	512	512	256	-	1024

The strain C016 is inhibited at titres sometimes four-fold higher than that of the homologous strain. This characteristic has been described by Hirst (1943, b) as an avidity factor of certain strains for antibody. Strains of this nature have also been described by van der Veen and Mulder (1950) in serological analyses with ferret antisera. They called them R strains. A further interesting point is the weaker antigenic potency of the recently isolated Ontario strains. This may be associated with less frequent allantoic passages, as the roosters were immunised with second allantoic passage material. The antiserum prepared to the Sudbury strain was of too low titre for use.

The serological analysis of strains isolated in 1949.

It is clearly shown in Table 20 that the Newmarket strain is identical to the standard A (PR8) and does not behave like the 1950-51 strains, which are A-prime. (See Table 19). While there is a cross-relationship between the 1949 Saint John (SJ2) and 1950-51

strains it can be seen from the low inhibition titer of SJ2 virus with FM1 antiserum that the former is more nearly related to the FW50 member of the A-prime subgroup, whereas the latter are intermediate between FM1 and FW50. The SJ2 strain differs too in its lack of reaction with the antiserum prepared to the strain SA2 isolated from the Sarnia district of Ontario in 1951. The Edmonton strain (Ed2) is peculiar for although all the A-prime strains are inhibited by its antiserum the reciprocal relationship does not apply.

TABLE 20

SEROLOGICAL ANALYSIS OF THE 1949 STRAINS

VIRUS	RECIPROCAL OF HA1 TITRES WITH ROOSTER ANTISERA								
	STANDARD STRAINS			1948-49 STRAINS			1950-51 STRAINS		
	PR8	FM1	FW50	NEW1	ED2	SJ2	SWEDEN3	SA2	TR5
PR8	4096	-	-	8192	32	32	-	-	-
FM1	-	4096	-	128	1024	4096	-	-	-
FW50	-	-	256	<32	128	1024	-	-	-
NEW1	4096	<32	<32	4096	32	<32	<32	<32	<32
Ed2	<32	<32	<32	<32	256	<32	<32	<32	<32
SJ2	<32	64	512	64	256	2048	512	<32	128
Sweden3	-	-	-	64	256	512	2048	-	-
SA2	-	-	-	64	256	512	-	512	-
TR5	-	-	-	128	512	1024	-	-	1024

TABLE 21

CORRELATION AND INTERPRETATION OF HA1 TESTS USING ROOSTER ANTISERA

VIRUS	YEAR OF ISOLATION	HA1 TITRES* WITH ROOSTER ANTISERA							
		PR8 T=4096	NEW1 T=4096	FML T=4096	SWEDEN3 T=2048	TR5 T=1024	FW50 T=256	SJ2 T=2048	Ed2 T=256
PR8	1934	T	2T	0	0	0	0	T/64	T/8
New-market1	1949	T	T	0	0	0	0	0	T/8
FM1	1947	T/64	T/32	T	2T	T	2T	2T	4T
Sweden3	1950	T/128	T/64	T/8	T	T/4	T/2	T/4	T
TR5	1951	0	T/32	T/8	T/2	T	T	T/2	2T
FW50	1950	0	0	T/128	T/4	T/8	T	T/2	T/2
SJ2	1949	0	T/64	T/64	T/4	T/8	2T	T	T
Ed2	1949	0	0	0	0	0	0	0	T

*T= titre with homologous virus;
other titres expressed in terms of T

In order to bring these results together in an interpretive form, Table 21 has been prepared to show the relationship between the standard strains, the three differing 1949 strains, and the similar London, Swedish and Ontario 1950-51 strains.

This table on the preceding page illustrates the great differences between the typical A strains (PR8 and Newmarket) and the other strains which are A-prime in character. The intermediate position of the Ontario 1951 strains, represented by TR5, between the two standard A-prime strains FM1 and FW50 is well seen.

Tests for Pathogenicity Towards Mice

Four of the recently isolated 1950-51 strains have been tested in this manner; they include two strains isolated in Toronto, one from a student nurse (CW15) and another from an infant (SC55); the third strain was from Sarnia, Ontario (SA2), and the fourth strain was from Sweden

(Sweden3). The tests were negative after two passages.

Two of the 1949 strains were similarly tested. The Newmarket strain was pathogenic upon first passage, the Saint John strain (SJ2) was not so, even after second passage.

DISCUSSION

The changing serological pattern of strains of influenza virus as interpreted from the haemagglutination-inhibition technique has been the subject of several publications during recent years (Bozzo, 1952; Chu et al., 1950; Hilleman et al. 1950, 1952; Tamm et al., 1950; van der Veen and Mulder, 1950). Gradual transitions amongst antigenic types have been noted but the question as to how these variations arise has not been adequately answered. It has become apparent, however, that strains belonging to the A-prime subgroup have become the dominant and prevalent agents, replacing the classical type A influenza virus encountered in earlier years.

The haemagglutination-inhibition technique has

been employed exclusively in this work as a serological method of differentiating strains of influenza virus isolated in this laboratory during the years 1948-51; therefore in discussing the results it is essential that full appreciation be given to the degree of emphasis which may be laid upon differences as revealed by this method. It should be stressed that the haemagglutination-inhibition system is a very complex one and easily disturbed by many non-specific factors.

At the commencement of the investigation rabbit antiserum was employed and was treated to reduce non-specific reactions; however rooster antiserum was employed for most of the investigation owing to its relative freedom from non-specific inhibitors, Hilleman (1952). Troubled by variations in the results of titrations of virus pools, and on some occasions by discrepancies between virus control tests of a suspension and the titer of the pool from which it was obtained, the possible

effect of the electrostatic charge on the plastic plate was investigated. It was finally considered, when the bharge theory was dissipated, that the variation was due to the non-homogenous nature of the virus contained in the pool. Hirst (1943, b) describes a similar finding. In the great majority of HA1 titrations the results were reproducible with no more than the accepted 2-fold variation. Where results were not reproducible, the geometric mean of several titrations was determined and the dilution nearest to that value was accepted. In analysing the results then, the minor differences should be neglected and attention paid only to well-marked distinctions.

It is only to be expected that strains isolated from one community should be almost identical but other reports, Francis et al. (1950), and Minuse and Davenport (1951), Kilbourne et al. (1951) have shown that more than one virus may be present at one time in a localized outbreak. There were no major differences displayed by any of the 1951 strains compared in this work, in spite of the fact that they were isolated in

widely separated parts of the world. On the other hand the three 1949 Canadian strains examined show evident diversity, and differences were observed amongst the twelve Arctic strains investigated with rabbit antisera.

The analysis of the twelve Arctic strains reveals the fact that both A and A-prime strains were isolated from that single localized outbreak. Our findings confirm the work of Hilleman et al. (1950) who used the strains isolated by Nagler et al. (1949) from the same patients and indeed from the same original pathological specimens. It would appear that the absorbed rabbit antisera gave results very similar to those obtained by Hilleman who used rooster antisera. In addition to these findings there were indications from the present work that there may have been both A and A-prime strains in the individual specimens obtained from some of the patients. The presence of the strong A-prime components in the antiserum

to CA11, and to a lesser extent in the antiserum to CA7, could be explained by the presence of an A-prime strain which had been overgrown by the A strain upon further passage.

The isolation of a PR8 type A virus during 1949 in Canada is of interest since during 1949 yet another type A WS virus was also isolated at Ankara, Turkey as recorded by Magill and Jotz (1952).

The mouse pathogenicity tests confirmed the fact that the Newmarket strain behaved like the Saskatchewan and Arctic strains isolated during the same year by van Rooyen and McClelland (1949). The other five strains tested were non-pathogenic, and the interest in them lies more in the serological analysis. The Saint John strain is very closely related to the Cuppett FW50 strain and this is probably the first strain of FW50 isolated in Canada.

The lack of reciprocal serological relationship exhibited by the Edmonton strain may be due to some peculiarity

of the chicken antisera. In this connection it may be noted that Shope (1939) observed a lack of reciprocity in virus neutralization tests between swine influenza strains when tested with immune rabbit sera whereas immune pig sera revealed no such differences.

SUMMARY AND CONCLUSIONS

1. There is no significant antigenic difference detectable with the haemagglutination-inhibition technique, between a strain isolated in Sweden in June 1950, another obtained from London in the 1950-51 epidemic, and several representative strains isolated from different parts of Ontario in February 1951. The strains definitely belong to the A-prime subgroup and show relationship to both the FM1 and the FW50 strains.

2. The three 1949 strains examined with rooster antisera differ from each other and from the 1951 strains. The Newmarket strain behaves like a PR8 strain and is mouse pathogenic. The Saint John strain is very like the Cuppett (FW50) A-prime strain. The Edmonton strain is distinctive in its lack of

reciprocal HA1 reactions but its antiserum has the properties of an A-prime antiserum.

3. An analysis with rabbit antisera of twelve strains obtained from the Cambridge Bay outbreak in 1949 shows that both A and A-prime strains were isolated from that epidemic, and there are indications that they may have co-existed in the early allantoic passage material from two of the patients.

PART V

GENERAL SUMMARY AND CONCLUSIONS

An extensive study has been made of the 1950-51 influenza epidemic as it affected the Province of Ontario in Canada. Details of the haemagglutination-inhibition antibody titres found amongst individuals of different age-groups prior to the onset of the epidemic have been presented. The antibody levels were determined for both Types A and B influenza virus and for two antigenic variants of Type A known as A-prime strains. The epidemic was followed both clinically and in the laboratory and the findings have been described. The opportunity occurred to observe outbreaks in a completely unprotected community and in a community partially protected by vaccination. The vaccine used contained all the strains employed in the earlier study of normal antibody titres. Altogether thirty-nine isolations of virus were made from all corners of Southern Ontario; thirty-seven of these strains were typed and identified as belonging to the A-prime subgroup.

The 1951 strains recovered were compared serologically by the red cell agglutination method with each other and with two other strains isolated from different parts of the world in 1950 and 1951. The first of these was isolated by Svedmyr in 1950 and the second by Andrewes in London during the early part of 1951. Further comparisons were made with three strains which had been isolated by van Rooyen and McClelland from different provinces of Canada in 1949. The serological analyses were performed with antisera prepared from the rooster. A parallel study was made of the inter-relationship of twelve strains isolated by van Rooyen and others from the Cambridge Bay outbreak in an Eskimo community in 1949. The latter study was conducted with absorbed rabbit antisera.

The antibody titrations of nearly two hundred normal human sera demonstrated the almost complete absence of antibody in infants sera to any of the strains tested. Much higher levels were attained in the sera from young adults and lower titres were observed in the older age group. This is in agreement with

results obtained by earlier workers upon the antibody content of sera from different age-groups. Two further points were established. The vast majority of individuals irrespective of age had minimal titres for the A-prime strain known as FW50; and significantly higher titres for the Type B virus were found in a group of fifty student nurses.

Titres observed with the A-prime strain FM1 proved to be unreliable owing to the abnormal sensitiveness to Francis' inhibitor of the strain used. This sensitivity became apparent when it was observed that abnormally high titres toward the FM1 strain were being recorded for the majority of normal infants' sera. The non-specific nature of these high titering sera was established by demonstrating that the inhibitory substance in the serum was destroyed by receptor destroying enzyme under conditions which did not materially affect specific influenzal antibody.

The epidemic as observed in young adults was mild and the incidence varied from about 15 per cent to 30 per cent in different outbreaks. The term mild is used implying an acute illness of relatively short duration without complications; it is not a description that the patient himself might have used as the majority of them complained of the usual post-influenzal fatigue which lasted one or two weeks after the febrile period had subsided and was often accompanied by an irritating persistent cough. It is perhaps noteworthy that none of the patients who had received vaccine and subsequently developed the disease suffered from these after-effects.

The vaccine was administered to a group of nurses whose antibody serum levels had been determined before the onset of the epidemic. These young women had in common with the other people investigated low titres towards the FW50 strain and in this group there was a higher proportion of low-titer sera for the FM1 strain. Thus they formed an excellent

test group, for the epidemic strain was later identified as having serological characteristics which placed it in an intermediate position between these two A-prime strains. Unfortunately the vaccination programme was barely completed when the influenza outbreak commenced in the Nurses Residence. This is undoubtedly one of the reasons why there was not an obvious reduction in incidence. Nevertheless there was definite clincial evidence of reduction in the severity of the disease as found in the vaccinated.

Virus of the A-prime type was isolated from just over half of the washings investigated and a diagnosis of influenza A infection was made in more than three-quarters of the cases by examination of acute and convalescent specimens of the patients' sera by the red cell agglutination method. The early use of rabbit antisera for the typing of the strains isolated was dropped in favour of rooster antisera on account of the high non-specific inhibiting titres encountered with the former.

A serological diagnosis of Type B infection was made in about 3 percent of cases; however type B virus was not recovered from any patient. One of the student nurses, who was more severely ill than the others, from whom A-prime virus was recovered had significant antibody rises to both Types A and B. This finding is in agreement with the earlier observation that higher than normal Type B antibody levels were encountered in the nurses group before the onset of the A-prime epidemic. It has already been suggested that the higher levels indicated recent exposure to Type B virus within the group.

The serological analysis of the strains isolated in the epidemic years of 1948-49 and 1950-51 established the diversity of those isolated during the former winter. This was in direct contrast to the uniformity of the 1950-51 strains. In this connection it might be well to remember that the 1948-49 strains were recovered from places thousands of miles apart in

Canadian Provinces whilst in 1950-51 the large number of strains isolated came from only the Province of Ontario. In spite of this no major difference was detected between the 1951 Canadian strains and the Swedish and London strains of the same period. Some controversy has arisen over the meaning of the Type A strains isolated in 1949. It is an established fact that in recent years the majority of A influenza virus strains isolated have been A-prime in character. This would not seem to banish the possibility of the occurrence of Type A strains in more remote areas; however this is a question which time and further experience in these areas will settle.

The present investigation has shown that a strain isolated in Saint John in 1949 was very similar in nature to the FW50 variant of the A-prime group. This was the strain included by the United States Army in the vaccine which they used for their troops in 1950-51. Yet another strain isolated in Canada in 1949 although A-prime in type proved to be highly specific.

It was not surprising to discover that all the Ontario strains isolated in 1951 were closely related to the FW50 strain, for the pre-epidemic antibody levels of normal individuals in this area were extremely low for this particular strain, a finding which could be taken to indicate susceptibility towards it;

As a result of these investigations the following conclusions have been made.

1. There were insignificant amounts of antibody towards a recently isolated A-prime strain, FW50, in the 198 sera tested by the haemagglutination-inhibition method. These sera were withdrawn from individuals representing age-groups from infancy up to over fifty years of age.

2. The distribution of antibody levels observed amongst individuals of different age-groups observed by the above method was similar to findings by previous workers who used virus neutralization and complement fixation techniques.

3. On the whole antibody levels before the epidemic to Type A and Type B influenza virus were low.

4. A larger proportion of higher titered sera towards Type B virus was observed in the Western Nurses group. This suggested possible recent infection and during the epidemic one case of influenza in this group gave a diagnostic rise in the titre with Type B virus as well as Type A. The A-prime virus alone was recovered from this patient.

5. The epidemic as it affected young adults studied in two different communities was mild although post-influenzal fatigue and persistent cough lasted for about two weeks after recovery from the acute illness.

6. About one fifth of the total number of one of the communities was vaccinated a day or two before the first cases began to appear. This resulted in no obvious reduction of incidence but a shorter illness and absence of residual symptoms was noted in the vaccinated.

7. The antigenic superiority of the A (PR8) and B (Lee) components of the vaccine were noted although 45 percent of the inoculated material contained the A-prime strains FM1 and FW50.

8. The epidemic was caused by an A-prime influenza virus. This was established by isolation and typing of thirty-seven strains from widely separated areas of Ontario.

9. A very small number of Type B infections occurred at the same time and upon one occasion simultaneous infection of the same patient was suspected. The Type B infections were diagnosed by serological investigation of the patient's serum.

10. The serological relationship, as established by the red cell agglutination method with the use of specific rooster antisera, between the A-prime strains isolated during this epidemic and two other strains isolated in Sweden and England during 1950-51 was extremely close.

11. Some strains isolated in 1949 were markedly different and resembled the typical type A(PR8) virus. Others were

A-prime in character but showed certain differences from the 1950-51 strain.

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