mination of the end-point of the whole blood clotting time. Using a graph or formula, the results may be interpreted in terms of equivalent whole blood clotting time. As performed in this way, a whole blood clotting time of 20 minutes may be determined in about 2½ minutes, of 30 minutes in about 3½ minutes, and of 45 minutes in about 5 minutes.

Résumé On a mis au point une méthode qui permet d'évaluer rapidement, au chevet du malade ou dans la salle même d'hémodialyse, les résultats de l'héparinothérapie. Cette épreuve appelée à remplacer le temps de coagulation de sang total consiste en fait en une détermination du temps partiel de thromboplastine. On prélève du sang total directement sur le malade ou dans le tube du dialyseur et on le dépose dans un tube de verre contenant des réactifs pré-étalonnés. Il est inutile de se servir d'anticoagulant et de recalcifiant. La phase terminale s'observe quand on incline le tube et est aussi facile à établir que la phase terminale du temps de coagulation du sang total. Les résultats s'interprètent à l'aide d'une courbe-étalon ou d'une formule standard et sont exprimés en équivalent du temps de coagulation du sang total. Avec cette méthode, un temps de coagulation de sang total de 20 minutes s'effectue en 2½ minutes environ, un temps de 30 minutes en 3½ minutes et un temps de 45 minutes prend environ 5 minutes.

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Influenza in British Columbia, 1966-1968

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ALTHOUGH epidemic influenza due to type B virus appeared in the eastern United States in January 1966, type A2 influenza was first noted in British Columbia and California, where concurrent epidemics due to both types occurred during February and March of that year.[1] In common with the United States, eastern Canada experienced type B influenza in 1966.[2] The situation was reversed in 1968, when type A2 virus infections predominated in eastern Canada[3] and the U.S.A.[4] and type B infections on the west coast. The present study describes the virological investigation of two epidemics of influenza which occurred in British Columbia during the winters of 1966 and 1968.

MATERIALS AND METHODS

Medical officers of health and private practitioners throughout the province of British Columbia obtained throat swabs and paired sera from patients with an influenza-like illness who were seen in schools, at university and in private practice. Throat swabs were leeched out in 3 ml. of nutrient broth contained in a 10-ml. screw-cap vial, or were placed intact in the vial and held at −20° C. for shipping. Alternate procedures were also adopted following collection of blood samples. Either sera were separated from clots by centrifugation on the day of collection, and subsequently shipped frozen to Vancouver, or samples of whole clotted blood were shipped unrefrigerated to Vancouver where sera were separated. All specimens were held at −20° C. until tested. Throat swab specimens were treated with 25 units of penicillin G potassium, 12 μg. streptomycin sulfate and 100 units nystatin per ml., and centrifuged at 8000 r.p.m. for 30 minutes at 4° C. to deposit bacteria. Supernatants, in 0.2-ml. volumes, were inoculated into each of four tissue-culture tubes containing 1.5 ml. of maintenance medium CMRL HB-597. The tissues employed were primary rhesus monkey kidney, secondary frozen rhesus monkey kidney* and primary cynomolgus monkey kidney. Following incubation at 35° C. on roller drums, cytopathic effects were observed five to seven days after inoculation of specimens containing virus. Hemadsorption was observed in virus-

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*Supplied by Connaught Medical Research Laboratories where it was prepared from primary cultures of rhesus monkey kidney cells shown to be free of adventitious myxoviruses.

positive cultures when 0.2 ml. of a 0.4% suspension of guinea-pig erythrocytes was added. The hemagglutination (HA) titre of the supernatant fluids was regularly between 1:16 and 1:128.

Supernatants were also inoculated amniotically into each of four chick embryos. After incubation at 33° C. for three days, amniotic and allantoic fluids were removed, pooled and examined for their content of hemagglutinin. Regularly two to four passages by the allantoic route of inoculation were required before fresh isolates showed HA titres of 1:32 or greater. Isolates were identified by the hemagglutination-inhibition (HI) test using 4 agglutinating doses of the virus and rooster antisera to the following influenza antigens: B/Lee/40, B/Great Lakes/54, B/Taiwan/62, B/Singapore/64 and A2/Canada/9/66.*

Paired sera from each patient were assayed for antibodies to the soluble antigens of influenza A/PR8 and influenza B/Lee by complement-fixation (CF) tests. These tests were conducted in Microtiter† plates[5] using 4 units of complement and 2 units of hemolysin. All sera were heated at 56° C. for 30 minutes before being used in the tests. The initial dilutions were 1:2.

In all cases where either a four-fold rise or an elevated CF titre occurred, the heated sera were treated with 25% kaolin[6] and tested by HI using 4 units of the following influenza antigens: A/PR8/34, A1/FM1/47, A2/Singapore/57, A2/Japan/62, A2/Taiwan/64, A2/Canada/66, B/Lee/40, B/Great Lakes/54 and B/Singapore/64. The initial serum dilution was 1:20.

RESULTS

Epidemiological Observations During 1966

Abnormally high rates of absenteeism from Greater Vancouver elementary schools beginning in the first week of February 1966 provided the first warning of the influenza outbreak of that year. Within one to two weeks students and teachers throughout this school system were afflicted, with rates of absenteeism averaging 20% to 40%. Relatively few reports of major influenza outbreaks were received from rural areas of this province. Patients complained of headache, sore throat, cough, myalgia, dizziness, photophobia, nausea and vomiting, and presented with fever, conjunctivitis and a characteristic malar flush.[7] This illness was mild when first observed in elementary school children; symptoms were more debilitating and of longer duration when older students and adults became affected.

Epidemiological Observations During 1968

The outbreak of 1968 involved mainly communities in southeastern British Columbia remote from the Vancouver metropolitan area which, by contrast, was attacked more heavily during 1966. Using absenteeism from schools as evidence of an outbreak of influenza,[8] the normal 7% was exceeded early in February in the East Kootenay Health Unit where one school recorded an incidence of 56% absenteeism on February 21. Schools in the North Okanagan Health Unit recorded maximum absenteeism of 27% on March 5, returning to normal by March 11. Schools in a third health unit (West Kootenay) exceeded the normal absenteeism rate from March 11 to April 1; the peak incidence of 26% was observed on March 20. The illness during 1968 was generally milder than in 1966.

Laboratory Results, 1966

During the epidemic of 1966, nine strains of influenza A2 virus were isolated; five of the six virus excretors from whom paired sera were received showed a four-fold or greater increment of antibody. Of the remaining 51 patients from whom paired sera were received, 16 of 51 examined by HI and 2 of 10 examined by CF showed a four-fold rise of influenza A antibody (Table I). Although no strains of influenza B virus were isolated, 5 of 55 paired sera showed a diagnostic rise of HI antibody to B/Great Lakes/54 virus, and 3 of 15 showed a similar rise of CF antibody to influenza B antigen. Tissues obtained post mortem from 12 patients in whom influenza was considered to be a contributing cause of death were examined for the presence of virus. Lung tissue from one of these patients yielded influenza A2 virus. Microscopic sections of trachea from this patient and three others revealed a necrotizing tracheitis which has been observed in patients who died from influenza.[9]

The first three strains of virus isolated were forwarded to the World Health Organization In-

TABLE I.—VIRUS ISOLATIONS AND SEROLOGICAL RESPONSES IN 57 CLINICAL CASES OF INFLUENZA DURING 1966

Influenza A2					*Influenza B*			
		*Rising antibody titre**				*Rising antibody titre**		
Virus isolation		*No.*	*%*	*Type of response*	*Virus isolation*	*No.*	*%*	*Type of response*
V+	6.....	5/6	83	1 HI only 2 CF only 2 HI and CF	V+ 0	0	0	0
V—	21.....	6/21	28	4 HI only 2 CF only 0 HI and CF	V— 27	3/27	11	0 HI only 0 CF only 3 HI and CF
NT	30.....12/30		40	12 HI CF not done	NT 30	2/30	7	2 HI CF not done

*: A four-fold or greater increase in homotypic antibody.
V+: Virus isolated.
V—: Virus not isolated.
NT: Not tested; specimens for virus isolation not received.
HI: Sera containing hemagglutinin-inhibiting antibodies.
CF: Sera containing complement-fixing antibodies.

*Provided by the Department of National Health and Welfare, Ottawa, Ontario.
†Cooke Engineering Co., Alexandra, Virginia, U.S.A.

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ternational Influenza Center for the Americas, Atlanta, Georgia. A close resemblance to the A2/Albany/3/65 strain was confirmed. These strains were designated A2/Canada/1/66 through A2/Canada/3/66.

Laboratory Results, 1968

During the epidemic of 1968, a single strain of influenza A2 virus antigenically closely resembling A2/Canada/66 was isolated, and paired sera from this patient showed a homotypic antibody conversion in both HI and CF tests. Of the remaining patients from whom paired sera were received 7 of 67 examined by HI and 6 of 62 examined by CF showed a four-fold rise of influenza A2 antibody.

TABLE II.—VIRUS ISOLATIONS AND SEROLOGICAL RESPONSES IN 68 CLINICAL CASES OF INFLUENZA DURING 1968

Influenza A2				*Influenza B*			
	*Rising antibody titre**				*Rising antibody titre**		
Virus isolation	*No.*	*%*	*Type of response*	*Virus isolation*	*No.*	*%*	*Type of response*
V+ 1.....	1/1	100	0 HI only 0 CF only 1 HI and CF	V+ 9	9/9	100	1 HI only 3 CF only 5 HI and CF
V— 45.....	6/45	13	2 HI only 1 CF only 3 HI and CF	V— 37	26/37	70	3 HI only 12 CF only 11 HI and CF
NT 22.....	4/22	18	2 HI only 2 CF only 0 HI and CF	NT 22	16/22	72	2 HI only 7 CF only 7 HI and CF

*: A four-fold or greater increase in homotypic antibody.
V+: Virus isolated.
V—: Virus not isolated.
NT: Not tested; specimens for virus isolation not received.
HI: Sera containing hemagglutinin-inhibiting antibodies.
CF: Sera containing complement-fixing antibodies.

Strains of influenza B virus antigenically closely related to B/Singapore/64 were isolated from 10 patients; sera received from 6 of 9 virus excretors showed homotypic antibody increments when examined by HI, as did all 8 pairs examined by CF. Of the remaining patients from whom paired sera were received, 23 of 59 examined by HI and 37 of 55 examined by CF showed a four-fold rise of influenza B antibody. No postmortem specimens were received (Table II).

Regional details of laboratory confirmed cases of influenza in 1968 are shown in Table III. Of 31 patients examined from the East Kootenay Health Unit, evidence of infection with influenza A2 virus was obtained in one subject, and with influenza B virus in 18 patients, while an additional 4 persons exhibited serological responses suggestive of mixed infection due to both of these agents. Strains of influenza B virus were recovered from 8 subjects in the North Okanagan Health Unit, while 15 of 20 patients demonstrated serological evidence of infection

TABLE III.—REGIONAL DISTRIBUTION OF 68 LABORATORY-CONFIRMED CASES OF INFLUENZA, DURING 1968

	Influenza type			*Total positive*	
Health unit	*A2*	*B*	*A2 + B*	*No.*	*%*
East Kootenay.	1	18	4	23/31	74
North Okanagan...	1	12	2	15/20	75
West Kootenay	0	12	1	13/13	100
Vancouver.....	1	1	1	3/4	75
Total.....	3	43	8	54/68	77

with one or both of these viruses. In the West Kootenay Health Unit, 12 of 13 patients were shown to be infected with influenza B virus, while evidence of infection due to both influenza A2 and B viruses was discovered in the remaining subject; strains of influenza B virus were isolated from 2 patients. Three of 4 Vancouver patients showed evidence of influenza virus infection. The only strain of influenza A2 virus isolated in 1968 was recovered from one of these Vancouver cases.

DISCUSSION

Isolation of influenza type A2 and type B strains from patients with clinical attacks of influenza together with the detection of serological responses to both these agents in respective patients demonstrates clearly that the two serotypes were involved etiologically in the outbreak of influenza in British Columbia during the first quarter of 1968. The dominance of influenza B virus strains in 1968 contrasts sharply with the high prevalence of influenza A2 isolates in an epidemic during the same period of 1966. Furthermore, the high incidence of clinical influenza of mild severity in rural areas during 1968 differs widely from the predominantly urban prevalence of clinically severe influenza in 1966.

In the period 1946 through 1963, epidemics due to influenza type B virus occurred in Canada in 1946, 1955 and 1959, while the influenza A family of viruses was responsible for epidemics in 1951, 1953, 1957, 1959 and 1963.[10] Although 1962 and 1964 were not recorded as epidemic years in Canada, influenza type B virus[11] and type A2 virus[12] were isolated from Toronto children with acute laryngotracheobronchitis during these years. Concurrent epidemics due to type A2 and type B viruses occurred in this country in 1966 and 1968, as was the case in 1959. Although both virus types were responsible for clinical illness in the British Columbia epidemic of 1966, type A2 virus was dominant in western Canada, type B virus in eastern Canada.[2] In 1968, although type B virus was pre-

valent in British Columbia, eastern Canada[3] together with eastern U.S.A.[4] experienced a sharp outbreak of influenza A2.

The strains of influenza B virus and the strain of influenza A2 isolated in 1968 proliferated readily in primary or secondary tissue cultures of monkey kidney cells, producing a characteristic cytopathic effect.[13] In contrast, the A2 virus strains of 1966 and 1968 were difficult to isolate in chick embryo and formed hemagglutinins poorly even after many passages. This difficulty was also encountered during the isolation of influenza A2 strains during the pandemic of 1957.[14]

Summary Epidemics of influenza occurring in British Columbia in the first quarter of 1966 and 1968 were shown by virus isolations and serological responses to be caused by influenza A2 and B strains. Influenza A2 and B viruses were involved in both epidemics but influenza A2 strains were predominant in 1966 while influenza B strains were predominant in 1968. The high incidence of clinically severe influenza which occurred mostly in the urban areas in 1966 contrasts sharply with the high incidence of clinically mild influenza which occurred mostly in the rural areas in 1968. Representative strains of the 1966 isolates have been designated A2/Canada/1/66 through A2/Canada/3/66 and are antigenically similar to A2/Albany/3/65. The B virus strains of 1968 were antigenically closely related to B/Singapore/64, while the A2 strain of 1968 resembled A2/Canada/1/66.

Résumé Les épidémies de grippe survenues en Colombie Britannique durant le premier trimestre de 1966 et celui de 1968 ont été causées, à en juger par l'isolement des virus et les réactions sérologiques, par des souches d'influenza A_2 et B. Les deux virus A_2 et B de la grippe étaient impliqués dans les deux épidémies, mais il y avait prédominance des souches A_2 en 1966 et des souches B en 1968. L'épidémie de grippe qui avait sévi en 1966 surtout dans les régions urbaines avait revêtu une forme sévère, tandis que l'épidémie de grippe de 1968 avait surtout atteint les régions rurales et revêtait une forme bénigne dans une forte proportion de cas. Les souches typiques isolées en 1966 ont été désignées de A_2/Canada/1/66 à A_2/Canada/3/66 et, sur le plan antigénique étaient similaires à A_2/Albany/3/65. Les souches des virus B de 1968 étaient, au point de vue antigénique, très voisines de B/Singapour/64, tandis que la souche A_2 de 1968 ressemblait à A_2/Canada/1/66.

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