THE INCIDENCE OF HAEMAGGLUTINATION INHIBITING ANTIBODIES FOR INFLUENZA A AND B VIRUS STRAINS IN SERA OF CHILDREN AND INFANTS

by

J. VAN DER VEEN

(Received November 21, 1950).

Almost all influenza A outbreaks since 1947 have been associated with the new type of A virus known as A-prime. This holds true for individual influenza infections in 1947 and the more extensive 1949-epidemic of influenza in Holland (Van der Veen and Mulder (6)). During the Canadian Arctic epidemic of 1949, however, influenza A virus related to the former PR8 strain (1934 U.S.A.), was isolated (Van Rooyen e.a. (5)). In France there have been recorded recoveries of strains of both sub-groups (A and A-prime) from the epidemic of 1949 (Lépine e.a. (2)). Little is known about the former incidence of influenza B infections in Holland and the serological characteristics of the causative virus strains.

Further information about the type of occurring virus strains may be obtained from an investigation of the incidence of antibodies for different influenza virus strains in sera of children and infants.

The present paper reports the results of antibody titrations of sera, collected from young children in Holland.

Sera.

Blood samples of eighty normal children were taken in Amsterdam, during the summer of 1949 and kindly sent to us by Dr M. G. Stronk (Binnen Gasthuis, Amsterdam). Moreover, sera of thirty-seven whooping cough patients were obtained in 1950 through the courtesy of Dr J. E. Minkenhof (Wilhelmina Gasthuis, Amsterdam) and another twenty-one sera of normal children were collected in Amsterdam during the spring of 1950. As the
Haemagglutination inhibiting antibodies for influenza A and B virus.

The age of the children varied from 0 to 5 years, their sera could not contain antibodies resulting from clinical or subclinical influenza A or B infections before 1945. The children had never been vaccinated against influenza. The sera were stored without preservative at $-20^\circ$ C.

In order to obtain clear results the non-specific inhibitors in the sera were eliminated by treating them with a crude filtrate of V. cholerae (Burnet and Stone (1), Van der Veen and Mulder (6)). The capacity of the cholera filtrate to destroy the non-specific inhibitors completely in serum-dilutions of 1 to 12 was checked with normal ferret and rabbit sera.

**Technique.**

The following strains were used:

- **Influenza A**: PR8 (1934 U.S.A.), passage formula: F_{199}M_{589}E_{91} and F_{92};
- **Influenza A-prime**: FM1 (1947 U.S.A.), passage formula: E_{M_{8}}E_{78};
- **Influenza B**: Lee (1940 U.S.A.), passage formula: F_{M_{137}E_{139}M_{18}E_{2}} and B (1950 Ned), passage formula: E_{11}.

Passage of each strain was routinely made by the allantoic route in 11-day-old chick embryos. The eggs were incubated at $35^\circ$ C. for 2 days. Positive allantoic fluids were pooled and used as antigens in the haemagglutination inhibition tests.

The antibody titrations were performed by a slightly modified form of the Hirst-Salk technique, *viz.* a micromethod, using tiles of porcelain with concavities (Mulder and Goslings (3); Van der Veen and Mulder (6)). A series of twofold serum dilutions was made in saline and next mixed with half a volume (two drops) of virus suspension. After interaction for half an hour at $2^\circ$ C. two drops of a 2 per cent chicken red cell suspension were added, the final concentration of red cells being 0.5 per cent. Readings were made after the tiles had remained undisturbed for half an hour at $2^\circ$ C. The titres were expressed as the reciprocal of the highest final

---

1) This strain was isolated in Holland during an outbreak of influenza B in April, 1950 and obtained through the courtesy of Prof. Mulder, Leyden. In preliminary experiments (Mulder (personal communication)), the strain proved to be antigenically identical to 7 other B strains isolated in the same period, and to a strain of B-virus recovered in 1949 in Leyden. As this group of strains deviated very much from the classical B-strain, Lee (1940 U.S.A.), the incidence of antibodies for one of them, B (1950 Ned), was investigated by us.