

**Antibody status to influenza A/Singapore/1/57(H2N2)
in Finland during a period of outbreaks caused by H3N2
and H1N1 subtype viruses**

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SUMMARY

The incidence of haemagglutination inhibition (HI) antibody (titre ≥ 12) to influenza A/Singapore/1/57(H2N2) in sera collected from a Finnish population in the summer of 1981 was 58%. Subjects born after 1968 were essentially seronegative, and a comparable low HI antibody status was also recorded among the elderly, the lowest being in people born during the period 1901-10. A small increase in antibody titre to the H2N2 virus was observed in the different age groups after infections with the H3N2, but not the H1N1, subtype influenza A viruses. The heterotypic response, which could be due to HI or NA antibodies, was restricted almost exclusively to subjects already exhibiting this antibody in acute phase sera. Moreover, the anamnestic boosting was not as strong as that described in earlier studies from samples collected at the beginning of the present era of H3N2 viruses. At population level, the HI antibody status to H2N2 was about the same at the beginning and end of the follow-up period which covered eight epidemic seasons. The results are discussed with respect to the doctrine of 'original antigenic sin' and to the threat of re-emergence of the H2N2 viruses.

INTRODUCTION

Recycling of influenza A virus subtypes has been one theory for the origin of pandemic influenza viruses, since sero-epidemiological evidence (reviewed by Webster, 1972) was gathered on the circulation of virus strains related to H2N2 subtype viruses in man prior to (about 1890-1918) the pandemic of 1957. In 1977, virological confirmation of recycling was obtained when the H1N1 subtype viruses reappeared after an interval of 20 years. Moreover, in serological, biochemical and genetic analyses (Palese & Young, 1983) the 1977 virus was shown not to be a recombinant between human and animal influenza viruses, as had been expected from the re-emergence of H2N2 viruses in 1957 and H3N2 viruses in 1969, but practically identical to the H1N1 strains prevalent in 1950. The way in which the H1N1 virus was stored for about 27 years without significant genetic variation is still obscure, although several explanations have been put forward (Webster, Laver & Air, 1983).

Among the three influenza-A virus subtypes pandemic in man this century, there

is one, H2N2, which is not epidemic in human populations today. Its era came to an end with the pandemic of H3N2 viruses in 1968. Any re-emergence of the H2N2 viruses will depend on factors such as the immunity of communities. Haemagglutination inhibition (HI) antibody, in contrast to antibodies against internal components of the virus, is correlated with protection against infection (reviewed by Al-Khayatt, Jennings & Potter, 1984), but the protective titre varies greatly with factors such as the virus and the age of the host (Pyhälä & Aho, 1975, 1981; Fox *et al.* 1982). In order to evaluate the immune status and its possible changes in the Finnish population, the level of HI antibody to A/Singapore/1/57(H2N2), the virus responsible for the pandemic of 1957, was monitored during the present era of H3N2 and H1N1 subtype viruses.

MATERIAL AND METHODS

Serum collections

Four collections of sera were tested for HI antibody to influenza A/Singapore/1/57(H2N2), the first collection also being tested for A/HongKong/1/68(H3N2) antibody.

(1) Serum specimens from a total of 400 subjects, 50 in each of eight age groups (see Fig. 1), were taken in June–August 1981 after the influenza A(H1N1) outbreak of the 1980/1 season. The subjects were patients in general hospitals in different parts of the country. The sera, taken during the acute phase of various infectious diseases, were first sent to the Virological Department of the National Public Health Institute, Helsinki, for routine antibody testing. The sera were stored at 4 °C for a few months and then at –20 °C until tested in the spring of 1982.

(2) Paired sera were taken in the winter of 1979/80 from 69 patients and in the winter of 1982/3 from 52 patients who had contracted an influenza-like disease. The acute sera were taken not later than the fifth day after the onset of illness and the convalescent sera on the 12–20th day. In each of the patients an influenza-A infection with H3N2 subtype viruses was verified serologically by HI with a panel of antigens, thus excluding H1N1 subtype viruses as aetiological agents. In some cases throat washings were available and the H3N2 aetiology was confirmed by isolation of the virus.

The results are for different age groups, initially grouped for the 1979/80 samples on the following basis: the number of patients born during the era of H2N2 viruses, between 1957 and 1965, came to 20 altogether; the sera of older patients were divided into two equal groups, with 19 and 20 subjects in each; the group of subjects born after the era of H2N2 viruses contained as many as were available. The sera were stored at –20 °C until tested, the 1979/80 sample in the autumn of 1982 and the 1982/3 sample in the summer and autumn of 1984.

(3) The subjects and sera were as in the second collection, but this time infections with H1N1 subtype viruses were accepted. Twenty-two of the patients were infected in the winter of 1980/1 and 31 in the winter of 1983/4. The outbreaks mainly affected children and young adults, which is reflected in the age distribution of the patients studied (Table 1). The sera from the winter of 1980/1 were tested in the autumn of 1982 and the sera from the winter of 1983/4 in the summer and autumn of 1984. The sera were stored at –20 °C.

Table 1. HI antibody to influenza A/Singapore/1/57(H2N2) in patients infected with H3N2 (the 1979/80 and 1982/3 seasons) and H1N1 (the 1980/1 and 1983/4 seasons) subtype viruses

Birth date	Rate of seropositive subjects*	Rate of ≥ 2 -fold increase in H2N2 antibody among:		Rate of ≥ 4 -fold increase in H2N2 antibody among:	
		Seropositive subjects*	Seronegative subjects†	Seropositive subjects*	Seronegative subjects†
H3N2 virus					
1895-26	11/32 (34%)	1/11	2/21	1/11	2/21‡
1927-56	29/40 (73%)	7/29	0/11	0/29	0/11
1957-65§	13/39 (33%)	4/13	0/26	1/13	0/26
1969-	0/10	0/0	0/10	0/0	0/10
Total		12/53 (23%)	2/68 (3%)	2/53 (4%)	2/68 (3%)
H1N1 virus					
1950-6	5/6 (83%)	0/5	0/1		
1957-65	11/39 (28%)	0/11	0/28		
1969-	0/8	0/0	0/8		
Total		0/16	0/37		

* H2N2 antibody to a titre of ≥ 12 in acute phase.

† H2N2 antibody to a titre of < 12 in acute phase.

‡ The two subjects born in 1895 and 1908.

§ Twenty-six of the patients were conscripts.

|| Eighteen of the patients were conscripts.

(4) Samples of serum specimens were collected in May in each of the five years 1973, 1975, 1977, 1979 and 1981 and stored at -20°C . The subjects and sera were as in the first collection, and the influenza A outbreaks were practically over when the specimens were taken. Only sera from patients born before 1958 were preserved and were thus available for the tests. From these specimens a total of 995 acute-phase sera were selected. Each of the 5 years was represented by 197-201 sera grouped by age of the subjects. There were 48-51 subjects in each of the age groups. The tests were carried out in the autumn of 1982 and the winter of 1983.

Screening of antibodies

The influenza A strains, Singapore/1/56(H2N2) and HongKong/1/68(H3N2), serving as antigens in the HI tests, were cultivated in embryonated eggs and used as infected allantoic fluids. The HI tests were conducted according to the principles of Robinson & Dowdle (1969). Sera were pretreated with cholera filtrate (Philips-Duphar B.V., Holland) at a dilution of 1 in 6 for 18 h at 37°C and subsequently heated for 1 h at 56°C to remove non-specific inhibitors. Four haemagglutinating units of virus were used as antigen. Titres were expressed as the reciprocal of the serum dilution giving 50% haemagglutination. All experiments included reference antisera.

The set of sera tested simultaneously contained as far as possible the same number of specimens from all age groups when each of the four collections was studied. In tests of the fourth collection, each of the five years was always represented by an equal number of sera. The paired sera of the second and third collections were, of course, studied simultaneously.

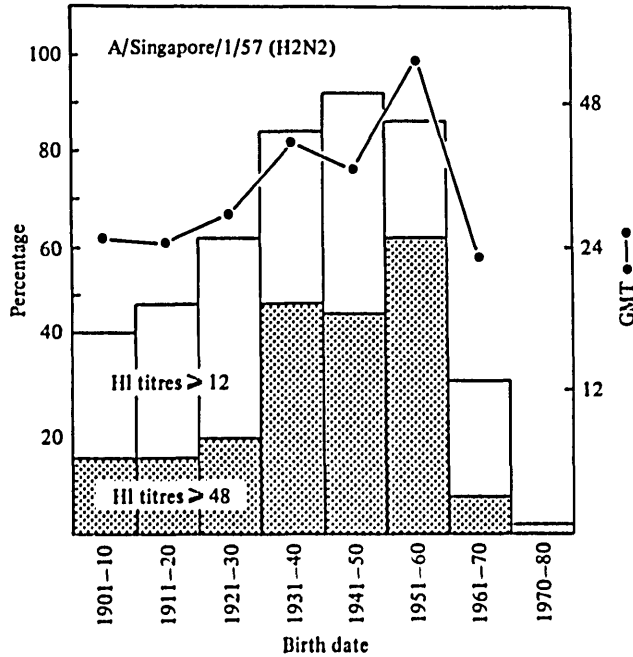


Fig. 1. HI antibody to influenza A/Singapore/1/57(H2N2) in summer 1981.

Influenza A activity during the follow-up period

Influenza A activity due to H3N2 subtype viruses was recorded in Finland every winter during the follow-up period from 1973 to 1981. The circulation of H1N1 subtype viruses in the community was recorded three times: in the winters of 1977/8, 1978/9 and 1980/1. The results of the present study indicate that outbreaks of H3N2 subtype viruses, as opposed to H1N1, may have an effect on antibody status to H2N2 subtype viruses. Thus, only the H3N2 outbreaks are discussed here.

As indicated by the H3N2 viruses isolated from throat washings in our laboratory, variants closely related to the following serotypes were circulating: 1972/3: England/42/72; 1973/4 and 1974/5: Finland/1/74 and Finland/22/74, respectively (related but not identical to A/Port Chalmers/1/73); 1975/6, 1976/7: Victoria/3/75; 1977/8: Victoria/3/75, Texas/1/77; 1978/9: Texas/1/77; 1979/80: Texas/1/77, Bangkok/1/79; 1980/1: Belgium/2/81.

In the winter of 1971/2 a sero-epidemiological follow-up survey was started to evaluate infection rates of influenza outbreaks (Pyhälä & Aho, 1975). Since then the survey has been repeated every winter throughout the follow-up period of the present study. Pre-epidemic and post-epidemic serum specimens were collected from the same subjects (Rh-negative pregnant women) each season and tested for HI antibodies to the epidemic and some other influenza-A viruses. Fig. 3 was compiled using data on the H3N2 infections detected in these surveys.

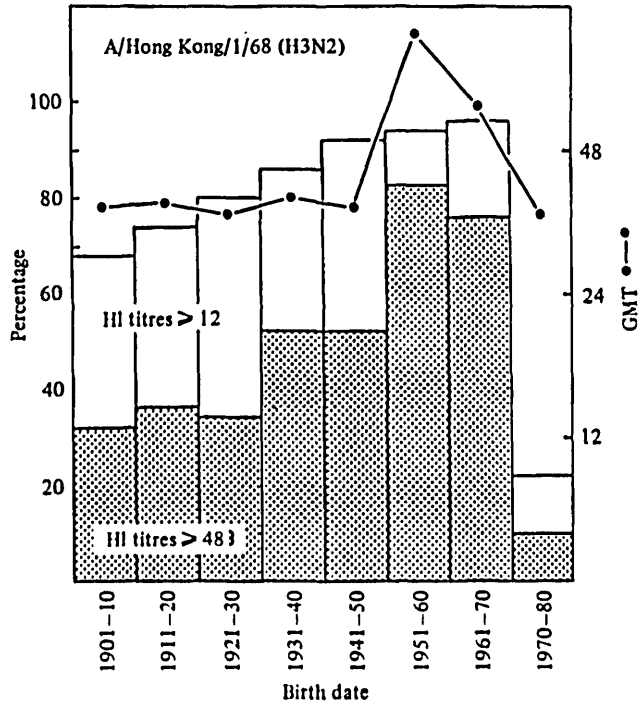


Fig. 2. HI antibody to influenza A/HongKong/1/68(H3N2) in summer 1981.

RESULTS

Fig. 1 shows the prevalence of HI antibody to influenza A/Singapore/1/57(H2N2) in different age groups in sera collected in summer 1981. A high prevalence was recorded in subjects born during the period 1931-60; among older and younger subjects the incidence of antibody gradually decreased. There was only one seropositive case (a titre of 24) among subjects born after 1970 (in fact after 1968). This distribution shows that the proportions of citizens in Finland possessing a titre of ≥ 12 or ≥ 48 in 1981 is of the order of 58% and 29%, respectively. Corresponding proportions with antibody to influenza A/HongKong/1/68(H3N2) (Fig. 2) were 77 and 50%, respectively. A high prevalence of this latter antibody was also recorded among the young subjects born in 1961-70, which contrasts with the prevalence of H2N2 antibody in this age group.

Four of the patients infected with H3N2 and H1N1 subtype viruses (collections 2 and 3) exhibited a twofold decrease in antibody titre to the H2N2 virus when acute and convalescent phase sera were compared. All four cases concerned infections with H3N2 viruses; two of the patients were from the 1895-1926 age group and two from that of 1927-56. The increases in antibody titre to the H2N2 virus are shown in Table 1, and the following points are emphasized:

(1) In H3N2 infections twofold increases in titre were frequent among subjects exhibiting H2N2 antibody in the acute phase (6/28, 21% in 1979/80 and 6/25, 24% in 1982/3). An increase could be detected only twice among seronegative

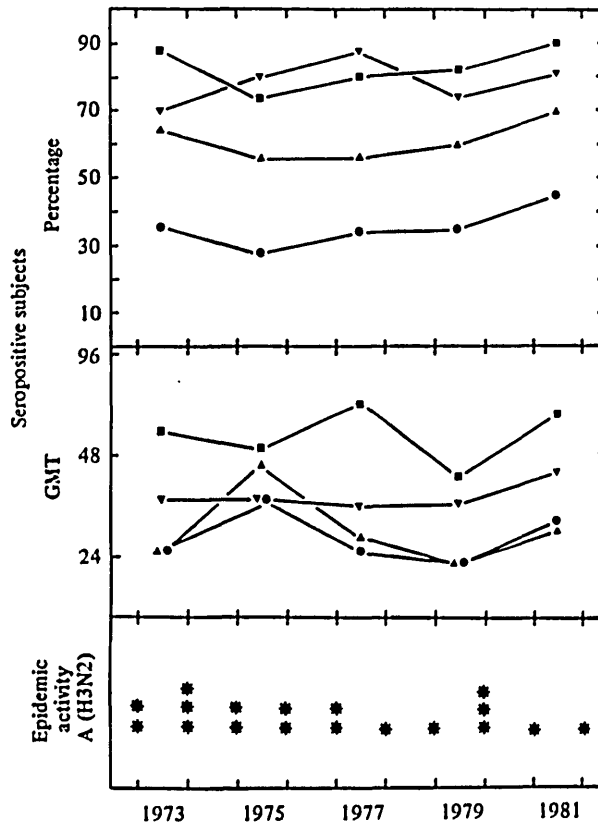


Fig. 3. Changes in the status of HI antibody to A/Singapore/1/57(H2N2) in 1973-81. Age groups by birth date: ●, <math><1026</math>; ▲, 1927-36; ▼, 1937-46; ■, 1947-56. Symbols for the epidemic activity of the H3N2 outbreaks indicate serological infection frequencies (see Material and Methods) as follows: one asterisk $\geq 10\%$, two asterisks 11-20%, three asterisks 21-30%.

subjects, both in the oldest age group. (2) In H1N1 infections there was no sign of development of antibody to the H2N2 virus.

The prevalences of antibody to the H2N2 virus in the five post-epidemic sets of sera collected every two years between 1973 and 1981 are shown in Fig. 3. Only minor changes were noted from year to year in the proportion of seropositive subjects in each of the four age groups, and a similar antibody status was recorded at the beginning and at the end of the study period. The differences in the proportion of seropositive subjects between the age groups were similar to those in the 1981 collection. The changes in geometric mean titres of the seropositive subjects were not parallel in the different age groups but as a general rule the titres decreased during the low epidemic activity of H3N2 viruses after collection of the 1975 sample and increased in the 1981 sample collected after a more severe outbreak in winter 1979/80.

DISCUSSION

The H1N1 outbreak in 1977 showed that the re-emergence and world-wide spread of an influenza subtype can occur in circumstances in which a considerable proportion of the human population is protected by immunity due to previous contact with this virus. A prevalence of 35% of seropositive subjects (HI antibody titres ≥ 12) against A/HongKong/117/77(H1N1) was recorded in Finland before the outbreak of 1977 (Pyhälä, 1978). In the present study on antibody status to the H2N2 subtype virus (A/Singapore/1/57) in 1981, the proportion of subjects exhibiting antibody was still higher, 58%. This proportion will decrease, however, as the number of people born after 1967 increases in the community. In contrast, in the age groups comprising subjects born before 1957 and followed up from 1973 to 1981, there was no evidence of conversion from seropositive to seronegative contributing to this decrease. In fact, in a number of patients exhibiting H2N2 antibody in their acute phase sera, recent infections with H3N2, but not H1N1 viruses, were shown to be capable of boosting to some extent the heterotypic H2N2 antibody. A reservation in that antibody to N2 may have contributed to this apparent heterotypic response to HA. Studies with reassortant viruses will need to be carried out to evaluate further any role of anti-neuraminidase antibody.

During the early outbreaks of H1N1 viruses in the present era it became evident that in spite of the relatively low prevalence of pre-epidemic HI antibody in sera, old people usually escaped infection (Pyhälä, 1979). During subsequent outbreaks, serological (Chakraverty, Cunningham & Pereira, 1982; Pyhälä & Aho, 1982) but rarely clinical (Mathur *et al.* 1981) infections were described. In the present study, the status of H2N2 antibody was poor in old people. In a majority of subjects in this age group the H2N2 immunity has been established late in life (1957–67), which may indicate a poorer protection than against H1N1 viruses in 1977.

The titre of antibody measurable with H2N2 viruses was shown to increase frequently in infections with H3N2 viruses during the first outbreaks after their appearance in 1968 and similar anamnestic heterotypic responses were repeatedly detected after vaccination with H3N2 viruses (Marine, Workman & Webster, 1969; Tauraso *et al.* 1969; Marine & Thomas, 1979). These responses could also be demonstrated in HI tests employing recombinant strains (H2N1) as antigens (Masurel & Marine, 1973; Dowdle *et al.* 1972), which indicates that the responses were mediated by the viral haemagglutinin. Heterotypic responses of H2N2 antibodies were still frequent during the H3N2 outbreaks in 1976 and 1977 (Pyhälä, 1978). In the present study the apparent boosting of the heterotypic antibodies in infections with H3N2 viruses was less. Only two of the H3N2 patients lacking a detectable level of HI antibody to H2N2 virus in their acute phase sera responded with an H2N2 antibody rise; both were born at the turn of the century, at a time for which there is serological evidence of the circulation of H2N2 viruses in human communities (Mulder & Masurel, 1958).

The specificity of the antibody produced in recent infections with H3N2 viruses and measured in the present study using the H2N2 virus has not been solved. The results are, however, consistent with the doctrine of 'original antigenic sin' (Francis, Davenport & Hennessy, 1953; Masurel & Heijtkink, 1983). Infections with H3N2 viruses have been shown previously to be capable of boosting antibody to

H2N2 viruses more efficiently than antibody to H1N1 viruses (Marine, Workman & Webster, 1969; Morita, Suto & Ishida, 1972; Pyhälä, 1978; Marine & Thomas, 1979). On the other hand, an anamnestic response to H1N1 antibody was regularly evoked by H3N2 and H2N2 viruses experimentally in rats (Angelova & Shvartsman, 1982). Consistent with the idea of two original antigenic sins in man (Marine & Thomas, 1979), the present study showed that H3N2 infections provoke anamnestic response of H2N2 antibody more efficiently than do H1N1 infections.

In the spring of 1980 some H2N2 viruses were isolated from children in Leningrad (Galitarov *et al.* 1980, quoted by Slepushkin *et al.* 1984). However, no evidence of circulation of H2N2 viruses in Moscow was obtained in a sero-epidemiological survey (Slepushkin *et al.* 1984). Similar findings were obtained in the present study in Finland, in which only one low titre of antibody (24) to the H2N2 virus was found in sera collected during or after 1981 from 66 young subjects born after 1968.

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REFERENCES

- AL-KHAYATT, R., JENNINGS, R. & POTTER, C. W. (1984). Interpretation of responses and protective levels of antibody against attenuated influenza A viruses using single radial hemolysis. *Journal of Hygiene* **92**, 301–312.
- ANGELOVA, L. A. & SHVARTSMAN, Y. S. (1982). Original antigenic sin to influenza in rats. *Immunology* **46**, 183–188.
- CHAKRAVERTY, P., CUNNINGHAM, P. & PEREIRA, M. S. (1982). The return of the historic influenza A H1N1 virus and its impact on the population of the United Kingdom. *Journal of Hygiene* **89**, 80–100.
- DOWDLE, W. R., MARINE, W. M., COLEMAN, M. & KNEZ, V. (1972). Haemagglutinin relationships of Hong Kong (H3) and Asian (H2) influenza strains delineated by antigen-specific recombinants. *Journal of General Virology* **16**, 127–134.
- FOX, J. P., COONEY, M. K., HALL, C. E. & FOY, H. M. (1982). Influenzavirus infections in Seattle families, 1975–1979. II. Pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. *American Journal of Epidemiology* **116**, 228–242.
- FRANCIS, T., JR., DAVENPORT, F. M. & HENNESY, A. V. (1953). A serological recapitulation of human infection with different strains of influenza virus. *Transactions of the Association of American Physicians* **66**, 231–239.
- MARINE, W. M. & THOMAS, J. E. (1979). Antigenic memory to influenza A viruses in man determined by monovalent vaccines. *Postgraduate Medical Journal* **55**, 98–108.
- MARINE, W. M., WORKMAN, W. M. & WEBSTER, R. G. (1969). Immunological interrelationships of Hong Kong, Asian and Equi-2 influenza viruses in man. *Bulletin of the World Health Organization* **41**, 475–482.
- MASUREL, N. & HEIJTINK, R. A. (1983). Recycling of H1N1 influenza A virus in man – a haemagglutinin antibody study. *Journal of Hygiene* **90**, 397–402.
- MASUREL, N. & MARINE, W. M. (1973). Recycling of Asian and Hong Kong influenza A virus hemagglutinins in man. *American Journal of Epidemiology* **97**, 44–49.
- MATHUR, U., BENTLEY, D. W., HALL, C. B., ROTH, F. K. & DOUGLAS, R. G., JR. (1981). Influenza A/Brazil/78(H1N1) infection in the elderly. *American Review of Respiratory Disease* **123**, 633–635.

- MORITA, M., SUTO, T. & ISHIDA, N. (1972). Antigenic memory in man in response to sequential infections with influenza A virus. *Journal of Infectious Diseases* **126**, 61–68.
- MULDER, J. & MASUREL, N. (1958). Pre-epidemic antibody against 1957 strain of Asiatic influenza in serum of older people living in the Netherlands. *Lancet* **i**, 810.
- PALESE, P. & YOUNG, J. F. (1983). Molecular epidemiology of influenza virus. In *Genetics of Influenza Viruses* (ed. P. Palese and D. W. Kingsbury), pp. 321–336. Wien: Springer-Verlag.
- PYHÄLÄ, R. (1978). Antibodies to influenza A viruses of the H1N1 subtype: age-dependency and effect of recent infections with H3N2 viruses. *FEMS Microbiology Letters* **3**, 165–167.
- PYHÄLÄ, R. (1979). H1N1 influenza and old people. *FEMS Microbiology Letters* **6**, 175–176.
- PYHÄLÄ, R. & AHO, K. (1975). Serum HI antibody and protection against influenza: a follow-up survey at community level of three epidemics caused by different H3N2 variants. *International Journal of Epidemiology* **4**, 127–129.
- PYHÄLÄ, R. & AHO, K. (1981). Seroepidemiology of H1N1 influenza: the infection and re-infection rate in winter 1978–79. *Journal of Hygiene* **86**, 27–33.
- PYHÄLÄ, R. & AHO, K. (1982). Seroepidemiology of H1N1 influenza: the range of the affected population is widening. *Medical Biology* **60**, 328–332.
- ROBINSON, R. Q. & DOWDLE, W. R. (1969). Influenza viruses. In *Diagnostic Procedures for Viral and Rickettsial Infections* (ed. E. H. Lennette and H. K. Schmidt), pp. 426–427. New York: American Public Health Association.
- SLEPUSHKIN, A. N., RITOVA, V. V., FEKLISOVA, L. V., FEDOROVA, G. I., OBROSOVA-SEROVA, N. P., SAFONOVA, E. S., KUPRYASHINA, L. M., MOLIBOG, E. V., SLEPUSHKIN, V. A. & ZHDANOV, V. M. (1984). Results of a two-year study of humoral immunity to influenza A and B viruses in children under the age of 14 years in Moscow and its suburbs. *Bulletin of the World Health Organization* **62**, 75–82.
- TAURASO, N. M., GLECKMAN, R., PEDREIRA, F. A., SABBAB, J., YAHWAK, R. & MADOFF, M. A. (1969). Effect of dosage and route of inoculation upon antigenicity of inactivated influenza virus vaccine (Hong Kong strain) in man. *Bulletin of the World Health Organization* **41**, 507–516.
- WEBSTER, R. G. (1972). On the origin of pandemic influenza viruses. *Current Topics in Microbiology and Immunology* **59**, 75–105.
- WEBSTER, R. G., LAVER, W. G. & AIR, G. M. (1983). Antigenic variation among type A influenza viruses. In *Genetics of Influenza Viruses* (ed. P. Palese and D. W. Kingsbury), pp. 127–168. Wien: Springer-Verlag.