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Type A influenza (H2N2) viruses isolated in Leningrad in 1980

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SUMMARY

In April–May 1980, two independent outbreaks of influenza-like illness occurred in Leningrad among children's-home children aged from 3 months to 2 years (of 68 children under observation, 50 became ill) and among boarding-school pupils aged 15–17 years (of 50 pupils under observation, 13 became ill).

A total of five influenza A virus strains were derived from one clinically healthy and three affected children of the children's home. Similar viruses were obtained from one affected boarding-school pupil and from an infected woman aged 24 years (a sporadic case within a household). On the basis of laboratory findings, all these seven strains were identified as influenza A H2N2 subtype strains.

Six of the affected children showed significant seroconversion only to H2 haemagglutinin from February to May 1980. Type A influenza H2N2 virus was isolated from three persons, including the sporadic case, who also showed significant seroconversion to H2 haemagglutinin. H2N2 influenza A virus was isolated on two occasions, at a 7-day interval, from the girl N. Ju.

Laboratory findings obtained from the study of the viruses isolated using up-to-date immunological and molecular-biochemical techniques enable us to conclude the following. The A/Leningrad/80 isolates belong to H2N2 sero-subtype. The viruses isolated are similar but not identical to the A/Singapore/1/57 reference strain in details of polypeptide and gene composition.

INTRODUCTION

In 1979, Hilleman summarized the information on the so-called antigenic anachronisms of the isolation of influenza A(H_{SW}1N1), A(H0N1) and A(H1N1) viruses. Thus, A(H_{SW}1N1) viruses were isolated occasionally in the United States of America in 1976; A(H0N1) viruses were derived from Eskimo persons and from French children in 1949, and from Canadian children in 1950. As for A(H1N1) virus anachronisms, three isolates were obtained between 1936 and 1941, and since 1977 they have been isolated throughout the world. This list does not exhaust all cases of the isolation of antigenic anachronisms of influenza A viruses. Thus, a total of 19 strains of H0N1 influenza A viruses were isolated in Alma-Ata, U.S.S.R., from October 1964 to January 1965 (Mikhailov, Zhumatov & Cheretenko, 1965), and further isolations were obtained later in Kiev, U.S.S.R. (Frolov, Shcherbinskaya

& Rybalko, 1978). H1N1 influenza A virus strains were isolated in Bulgaria in 1974 (Nikolova & Kotseva, 1979), in 1961–2 – in Tambov and Moscow, U.S.S.R. (Klimov & Ghendon, 1981). The infection with H1N1 virus of a young English soldier in 1960 appears to have been valid (Isaacs, Hart & Law, 1962, cited by Kilbourne, 1975). In 1979, an H3N2 strain which was similar but not identical to A/Aichi/I/68 virus (Moore *et al.* 1981) was obtained from an Australian boy with acute respiratory disease (ARD).

The present paper deals with cases of influenza apparently caused by A(H2N2) viruses, most of which occurred among young children and teenagers in Leningrad in the spring of 1980. The cases were confirmed by virological and serological methods. Thus we show evidence of biological activity of the H2N2 subtype outside the period of its prevalence. This has been demonstrated serologically only once, in Amazonian Indians in 1970 (Napiorkowski & Black, 1974).

MATERIALS AND METHODS

Between September 1979 and July 1980 a total of 68 children's-home children aged from 3 months to 3 years and 200 boarding-school pupils aged 16–18 years were under continuous clinical and epidemiological studies. A sample of blood was obtained from everybody at an early stage of the study and then at 2–3 month intervals. Nose and throat swabs were taken from some patients with ARD and from their contacts for virological and immunofluorescent investigations. In addition, 15 children from the children's home were chosen to be examined for virus isolation. A total of 10–15 of them were under examination twice weekly during the study period.

In May–June 1980, clinical and epidemiological studies of 58 families with ARD were undertaken. The paired sera and nose and throat swabs were obtained from individuals when they had acute respiratory or febrile illnesses. All paired sera were tested for complement-fixing antibodies against influenza A and B viruses, respiratory syncytial (RS) virus, adenovirus and *Mycoplasma pneumoniae*.

The HI test was performed on the sera. The viruses used in the test were A(H1N1), A(H2N2), A(H3N2) and influenza B.

Blood specimens and isolated viruses were obtained according to the W.H.O. circular Vir/81.4 entitled 'Guidelines for evaluating the authenticity of unusual influenza virus isolates' (*Voprosy Virusologii* (1981), no. 4, 506–507).

Thus, specimens for virus isolation were collected from patients in various parts of Leningrad by four members of the Epidemiology Laboratory of the Research Institute. Three other members of the Etiology Laboratory were involved in the isolation of viruses. Re-isolation of viruses from the specimens, which were kept in a refrigerator, was carried out in the Laboratory of Molecular Biology.

All members of the Research Institute involved in the study were healthy and had not been inoculated with live influenza vaccines. The Etiology Laboratory is separated physically from the other laboratories. The laboratories of the Research Institute had not cultivated type A influenza H2N2 virus during the three months preceding the time of virus isolation. Therefore, the primary isolation of H2N2 viruses and their re-isolation were done under conditions when the possibility of laboratory pick-ups was as far as possible excluded.

Sera were heated at 56 °C for 30 min before using them in the HI test to remove thermolabile inhibitors. In addition in the HI test inhibitor-resistant viruses were used.

For immunochemical characterization of antibodies, sera were treated with routine solution of merkamine (0·15 M), which is a weak reducing agent analogous to β-mercapthoethanol. All convalescent sera were divided into two equal parts (untreated (control) and treated with merkamine) and were examined simultaneously in the HI test without preliminary heating. All sera studied had been diluted 1 in 5 with physiological saline and absorbed by sedimented chicken erythrocytes. Control sera were diluted 1 in 10 with physiological saline and the other sera were mixed with equal volumes of merkamine solutions (0·15 M) for 18 h at +4 °C (Bichurina, 1972). The nose and throat swabs were inoculated into the amniotic cavity of 10- to 11-day-old chicken embryos for influenza virus isolation.

The following four influenza A virus strains isolated in Leningrad in the spring of 1980 were studied in greater detail: A/Leningrad/527/80, A/Leningrad/549/80, A/Leningrad/553/80 and A/Leningrad/586/80. The reference and recombinant influenza A virus strains used in this study included A/Swine/1976/31 (H1N1), A/WSN/33 (H1N1), A/PR/8/34 (H1N1), A/FM/47 (H1N1), A/Netherlands/36/56 (H1N1), A/Khabarovsk/74/77 (H1N1), A/Singapore/1/57 (H2N2), A/Japan/305/57 (H2N2), A/Netherlands/65/63 (H2N2), A/England/12/64 (H2N2), A/Taiwan/1/64 (H2N2), A/Leningrad/29/65 (H2N2), A/Hong Kong/1/68 (H3N2), A/Victoria/35/72 (H3N2), A/Texas/1/77 (H3N2), R-7 ($Heq_{A/eq/Prague/1/56}N1_{A/Khabarovsk/74/77}$) (produced by Dr Gorev, Influenza Institute, Leningrad, U.S.S.R.), X-7 ($HO_{A/WSN/33}N2_{A/Singapore/1/57}$) (produced by Dr Kilbourne, U.S.A.), R-9 ($Heq_{A/Prague/1/56}N2_{A/Texas/1/77}$) (produced by Dr Gorev). Haemagglutinin antigens were isolated from the viruses using the method described by Laver (1978).

Antigenic characterization of the HA was carried out by conventional HI cross-reactions using 4 HA units of the antigen, 1% suspension of chicken erythrocytes and strain-specific antisera from laboratory animals heated at 56 °C for 1 h and prepared according to the method of Webster & Laver (1967).

Hierarchical relationships between the HA antigens of different viruses were analysed on the basis of the 'relatedness' and 'symmetry' criteria with modifications (Paramonova *et al.* 1978).

We used the conventional neuraminidase inhibition (NAI) test recommended by W.H.O. and modified by Paramonova & Golubev (1975).

The viral proteins were studied in 10% slab polyacrylamide gel by the method of Laemmli (1970).

RNA studies

The electrophoretic mobility of both single-stranded virion-type RNA and hybridization mixtures of viral and complementary RNA (vRNA–cRNA) was studied. The unlabelled RNAs were extracted using SDS-phenol, and were subsequently precipitated with 2·5 vols. of ethanol. The extracted material of single-stranded virion-type RNA was analysed by electrophoresis in a 2·7% slab polyacrylamide gel in the presence of 6 M urea. Electrophoresis was carried out at

Table 1. *The results of haemagglutination inhibition tests on paired sera collected from six Leningrad children's-home children with ARD syndrome in the spring of* 1980

Patient	Patient	Days after onset of illness	Sample of blood	Antibody titres to influenza-A viruses			
				H0N1	H1N1	H2N2	H3N2
2	S.L.	24	I	0	0	0	0
			II	0	0	20	0
124	K.G.	19	I	0	0	10	0
			II	0	0	40	0
129	Ch.Yu.	32	I	0	0	0	0
			II	0	0	20	0
175	W.S.	13	I	0	0	20	0
			II	0	0	40	0
188	B.Zh.	35	I	0	0	20	0
			II	0	0	80	0
260	N.I.	29	I	0	0	80	0
			II	0	0	320	0

80 V for 19 h at 27 °C. After electrophoresis, the gels were stained with ethidium bromide (2 μg/ml) and photographed in ultraviolet light (Zhilinskaya, 1980).

The electrophoretic mobility of ^{3}H-labelled single-stranded RNA was also studied by PAGE. RNA–RNA hybridization was performed according to technique of Hay, Skehel & Webster (1979) using ^{3}H-labelled complementary RNA extracted from infected cells.

RESULTS

Virological studies

From September 1979 to May 1980 three influenza outbreaks were registered in a children's home in Leningrad: they occurred in September–October 1979, January–March 1980 and in April–May 1980.

During the first outbreak no influenza virus was isolated from children. According to serological data this outbreak was caused by several viruses. Thus, seroconversions were shown to the HA of the following viruses: A/Khabarovsk/74/77 (H1N1), A/Texas/1/77 (H3N2) and B/Hong Kong/72. A total of four influenza virus strains similar to A/Texas/1/77 (H3N2) were derived from four affected children during the second outbreak.

The third outbreak occurred between 15 April and 27 May 1980. Of the 68 children under observation, 50 (73·5%) were ill. Of five groups studied only one avoided ARD cases.

There were no cases of ARD in the children's home personnel prior to the third outbreak. By the end of the latter, ARD symptoms were shown in two nurses, but they were able to work. Five influenza A virus strains were isolated from four children.

At the same time, pupils aged 15–17 years at one of the boarding schools under observation were examined. An ARD outbreak had begun in this community on

Table 2. *Haemagglutination inhibition reactions of viruses isolated in Leningrad in May–June* 1980 *and of A/Singapore/1/57 reference strain employing hyperimmune rat sera*

Sera to ...	A/Swine/1976/31 (H1N1)	A/WSN/33 (H1N1)	A/PR/8/34 (H1N1)	A/FM/47 (H1N1)	A/Netherlands/36/56 (H1N1)
A/Leningrad/527/80	20	20	20	20	20
A/Leningrad/549/80	20	20	20	20	20
A/Leningrad/553/80	20	20	20	20	20
A/Leningrad/566/80	20	20	20	20	20
A/Singapore/1/57	20	20	20	20	20

Sera to ...	A/Singapore/1/57 (H2N2)	A/Hong Kong/1/68 (H3N2)	A/Victoria/35/72 (H3N2)	A/Texas/1/77 (H3N2)	A/Leningrad/549/80
A/Leningrad/527/80	320	20	20	20	640
A/Leningrad/549/80	320	20	20	20	640
A/Leningrad/553/80	320	20	20	20	320
A/Leningrad/566/80	320	20	20	20	320
A/Singapore/1/57	640	20	20	20	640

2 May 1980 in two groups, each of 25 pupils. Between 2 and 27 May 1980, 13 of the 50 pupils became ill, of whom 7 were subjected to virological examination. An influenza B virus strain identical to B/Singapore/222/79 virus was derived from one of the groups; from the other was obtained an influenza A virus strain. The latter was isolated on 21 May 1980.

At the same time we studied the patient E (aged 24 years) with ARD from a household. A/Leningrad/617/80 was isolated from her on the third day of illness. There were no other cases with ARD in the household.

We failed to ascertain any possible connexion between these three foci of type-A influenza or possible invasion of infection from outside of the foci of infection.

Thus, a total of seven influenza A virus strains were obtained from patients in three foci of influenza located in different parts of the city from 15 April to 27 May 1980. Viruses with high haemagglutinating titres (512–1024) were isolated after primary intra-amniotic inoculation of 10-day-old chick embryos. All three viruses were identified as H2N2 influenza A viruses in the HI test.

Details of virus isolations

We would like to describe the cases of virus isolation in greater detail. Five influenza A(H2N2)/80 virus strains were isolated from four children's-home children aged 2–3 years:

(1) A boy, W.S., became ill with ARD on 1 May 1980 without fever. An influenza strain, A/Leningrad/444/80, was derived from him on 9 April 1980, i.e. 3 weeks before the onset of illness. Antibodies to A(H2N2) virus were detected 13 days after the onset of disease (titre, 40). He did not show significant seroconversions to the HA antigens of other serosubtype viruses.

(2) A boy, Sheh.A., was healthy. An influenza strain A/Leningrad/528/80 was obtained from him on 12 May 1980. The antibody titre to the HA of A(H2N2) virus

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Table 3. *Antigenic characterization of the neuraminidase of influenza A/Leningrad/80 viruses by neuraminidase inhibition tests using hyperimmune rabbit sera*

Viruses	Sera to viruses				
	A/FM/1/47 (H1N1)	R-7 (HeqN1)	X-7 (H0N2)	A/Texas/1/77 (H3N2)	R-9 (HeqN2)
A/FM/1/47 (H1N1)	**3500**	1000	—*	—	—
A/Khabarovsk/74/77 (H1N1)	400	**800**	—	—	—
A/Singapore/1/57 (H2N2)	—	—	**1280**	—	—
A/Texas/1/77 (H3N2)	—	—	—	**2560**	**2560**
A/Leningrad/553/80	—	—	1200	—	—
A/Leningrad/527/80	—	—	1280	—	—
A/Leningrad/549/80	—	—	1280	—	—
A/Leningrad/566/80	—	—	1100	—	—

* Dashes indicate a titre of < 40.

was 10 on 8 April 1980, 20 on 13 May 1980 (i.e. on the day after virus isolation) and < 10 on 7 June and on 13 July 1980.

(3) A boy, T.S., became ill with ARD on 11 May 1980 with a temperature of 38·4 °C. An influenza strain, A/Leningrad/530/80, was isolated from him on 12 May 1980. The antibody titre to the HA of A(H2N2) virus was 20 on 7 December 1979, on 12 February 1980, on 8 April 1980 and on 13 May 1980; it was < 20 on 3 July 1980.

(4) A girl, N.Ju., is of particular interest. She was ill with ARD from 7 to 21 April 1980 but without fever. An influenza strain, A/Texas/1/77 (H3N2), was isolated from the girl on 14 April 1980. She became ill with ARD a second time on 13 May 1980 with a temperature of 38·5 °C. Two influenza virus strains, A/Leningrad/549/80 (H2N2) and A/Leningrad/566/80 (H2N2), were isolated from her in the course of infection on 19 and 26 May 1980 respectively. Antibodies to A(H2N2) virus were not detectable on 13 May 1980. The antibody titre to the HA of A(H2N2) virus was 40 on 1 June 1980 (i.e. 18 days after the onset of reinfection).

A boarding-school pupil, B., of 16 years of age from whom an H2N2 influenza A virus strain (A/Leningrad/553/80) was isolated on 21 May 1980 (6 days after the onset of illness) had no HI antibody rises to H2 haemagglutinin.

At the same time we studied patient E., with ARD, aged 24 years, from a private household. A/Leningrad/617/80 (H2N2) was isolated from her on 5 June 1980 (on the third day of illness). The antibody titre to the HA of A(H2N2) was 10 on that day and 25 days after onset of illness (in the course of convalescence) was 40.

Serological studies

As mentioned above, all children's-home children were under continuous serological examination every 2–2·5 months. As is seen in Table 1, six children with ARD had a fourfold antibody titre rise only to the HA of H2N2 influenza A virus in the course of illness, an H2N2 strain being isolated from one of them (no. 175).

In addition, of great interest to us are the following children.

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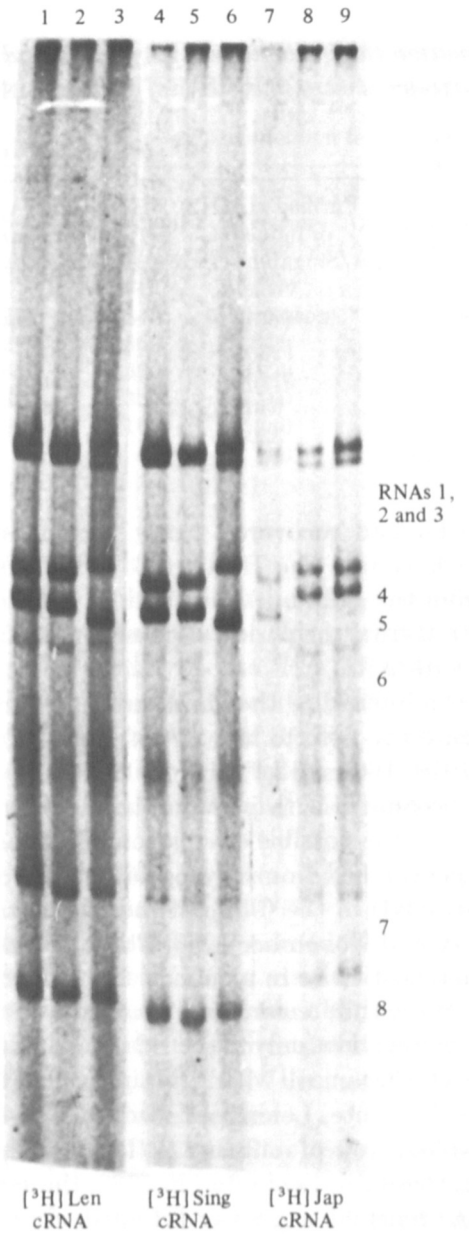

Fig. 1. Polyacrylamide gel electrophoresis of hybridization mixture of viral and ^{3}H-labelled complementary RNA of A/Leningrad/549/80, A/Singapore/1/57 and A/Japan/305/57 ^{3}H-labelled RNA complementary in sequence to the appropriate virus genome RNA was prepared from infected chick embryo fibroblasts. The double-stranded RNA hybrids were formed by hybridizing with the appropriate purified virus RNAs.

The nine hybridization mixtures from which the double-stranded RNAs were separated by polyacrylamide gel electrophoresis were: 1, [^{3}H]Leningrad cRNA × vRNA; 2, [^{3}H]Leningrad cRNA × Singapore vRNA; 3, [^{3}H]Leningrad cRNA × Japan vRNA; 4, [^{3}H]Singapore cRNA × Singapore vRNA; 5, [^{3}H]Singapore cRNA × Leningrad vRNA; 6, [^{3}H]Singapore cRNA × Japan vRNA; 7, [^{3}H]Japan cRNA × Japan vRNA; 8, [^{3}H]Japan cRNA × Leningrad vRNA; 9, [^{3}H]Japan cRNA × Singapore vRNA.

The virus genome RNAs used were: 1, Leningrad/549/80; 2, Singapore/1/57; 3, Japan/305/57; 4, Singapore/1/57; 5, Leningrad/549/80; 6, Japan/305/57; 7, Japan/305/57; 8, Leningrad/549/80; 9, Singapore/1/57.

Table 4. *Haemagglutination inhibition titres of A/Leningrad/549/80 virus and some reference strains employing antisera to the HA of influenza A(H2N2) viruses isolated in* 1957–1964

	Antisera to		
Influenza A(H2N2) viruses	Purified HA(H2) of influenza A/Singapore/1/57 virus (goat origin)	Purified HA(H2) of influenza A/Japan/305/57 virus (rabbit origin)	Purified HA(H2) of influenza A/Taiwan/1/64 virus (ferret origin)
A/Singapore/1/57	1280	640	20
A/Japan/305/57	5120	2560	20
A/Taiwan/1/64	640	1280	320
A/Leningrad/549/80	320	640	20

(1) A girl, P.N., who had recovered from bronchitis was admitted to the children's home from hospital. The HI titre to H2 haemagglutinin in a serum specimen obtained from the girl on 9 April and on 13 May 1980 was 320; on 16 September 1980, < 20. During the period of observation (from April to September 1980) she was not ill with ARD.

(2) A girl, S.A., was admitted to the children's home from a day nursery on 5 October 1979. Her antibody titre to the HA of A(H2N2) was 320 on 1 October and on 7 December 1979, 160 on 12 February 1980, 320 on 9 April 1980 and 10 on 13 May 1980. She became ill with pneumonia on 10 May 1980.

Further information on the possible circulation of influenza A(H2N2) viruses in Leningrad in 1980 was obtained from serological studies of 599 children and 543 adults hospitalized for ARD in the Clinic of the Influenza Institute, Leningrad, U.S.S.R., between May and November 1980. These clinics are located in various parts of the city. A diagnostic rise in antibody titres only to H2 haemagglutinin was observed in 3·5 % of the children and in 4·5 % of the adults. During the autumn of 1980 significant seroconversions only to the H2 haemagglutinin were documented also in three neonates who became ill with ARD in the first 5 days after birth (Prof. Fridman, The Pasteur Institute, Leningrad, personal communication).

To ascertain an aetiologic role of influenza A/Leningrad/80 (H2N2) virus in the genesis of influenza morbidity in Leningrad in 1980, the seven random-paired sera (from patients who had fourfold or greater HI antibody rises to both H2 and H3 haemagglutinins) were examined. The sera were treated with merkamine as described above (Bichurina, 1972). The second serum specimen was obtained from the patients about a week after the onset of the illness.

Treatment of paired sera from a child of 3 years (no. 172) and from an adult aged 48 years (no. 189) with merkamine removed antibodies to the HA of influenza A(H2N2) virus but not to that of A(H3N2) virus. Sensitivity of antibodies to the H2 haemagglutinin with merkamine suggests that they were IgM antibodies. This suggests that the patients were freshly infected with A(H2N2) virus. This is supported also by non-sensitivity to merkamine treatment of antibodies to the H3 haemagglutinin.

Table 5. *Antigenic cross-reactions of A/Leningrad/549/80 virus and some reference strains as indicated by haemagglutination inhibition titres*

	Post-infection ferret sera to					
Viruses	A/Singapore/1/57 (H2N2)	A/Japan/305/57 (H2N2)	A/Netherlands/65/63 (H2N2)	A/England/12/64 (H2N2)	A/Leningrad/29/65 (H2N2)	A/Leningrad/549/80
A/Singapore/1/57	**1280**	640	1280	80	320	1280
A/Japan/305/57	1280	**2560**	2560	1280	1280	1280
A/Netherlands/65/63	640	320	**5120**	640	640	640
A/England/12/64	320	320	2560	**2560**	2560	320
A/Leningrad/29/65	320	160	640	640	**2560**	160
A/Leningrad/549/80	80	40	40	40	40	**320**

Characterization of the virus isolates

In Table 2 we present the results of HI reactions of the four strains isolated in Leningrad employing several rat antisera to the influenza A viruses H1N1, H2N2 and H3N2. The antigenic profile of the HA of these four viruses and of A/Singapore/1/57 (H2N2) was found to be identical.

Data on the antigenic characterization of the NA of influenza A viruses isolated in Leningrad in 1980 is given in Table 3. As is seen in the table, the NA of these viruses belongs to serosubtype N2 and is highly related to that of A/Singapore/1/57.

Comparative analyses of the virus-induced polypeptides of viruses A/Leningrad/527/80, A/Leningrad/549/80, A/Leningrad/553/80 and A/Singapore/1/57 reference strain were carried out and no differences in the polypeptide composition of these three viruses as compared with that of A/Singapore/1/57 virus was detected (data not presented).

Similarly, polyacrylamide gel electrophoresis of single-stranded virion RNA of A/Leningrad/553/80 and A/Singapore/1/57 viruses detected no differences in the migration of RNA segments of these viruses.

Fig. 1 shows the results of analysis of hybridization mixtures of viral and ^{3}H-labelled complementary RNA of the viruses A/Leningrad/549/80, A/Singapore/1/57 and A/Japan/305/57 (Hay *et al.* 1979). As is seen in the figure, the genome composition of A/Leningrad/549/80 virus is more like that of A/Singapore/1/57 than of A/Japan/305/57. The same holds true for the other virus strains isolated in Leningrad in 1980 (Zhilinskaya & Stamkulova, 1981).

Fig. 1 also shows that the A/Leningrad/549/80 virus is not identical in gene 7 to the A/Singapore/1/57 reference strain. This was demonstrated by polyacrylamide gel electrophoresis of the hybridization mixtures of ^{3}H-labelled complementary RNA of A/Leningrad/549/80 virus and viral RNA of both the same virus and A/Singapore/1/57. In addition, analysis of hybridization mixtures showed that gene 8 of the A/Leningrad/549/80 virus was not identical to that of the A/Singapore/1/57 virus.

Table 4 shows the results of HI tests with A/Leningrad/549/80 virus employing sera from goats, rabbits and ferrets inoculated with the purified HA of H2N2 influenza A viruses, isolated in 1957–64. As is seen in the table, the HA of the above virus is related to influenza A(H2N2) viruses isolated in 1957 but not in 1964.

We demonstrated that the above virus reacted poorly with A/Singapore/1/57 and A/Japan/305/57 antisera, and failed to react with the sera from those ferrets which have had influenza caused by A(H2N2) viruses isolated in 1963–5 (Table 5). On the other hand, the A/Leningrad/549/80 serum neutralized the A/Singapore/1/57 and A/Japan/305/57 viruses and (to a lesser extent) the viruses from 1963–5.

DISCUSSION

The main question regarding origin of new 'unusual' virus isolates concerns their authenticity. As indicated above, we have carried out the necessary work on the collection of blood specimens, and on the isolation and identification of viruses as suggested previously by the W.H.O.

The W.H.O. requirements suggest re-isolation of new 'unusual' viruses from both the original specimens and a series of specimens that can be obtained from the same or other representatives of a given population. The data of the present paper meet these requirements. Besides the re-isolation of viruses from the same specimen carried out by an independent laboratory, it is worth mentioning the following:

(1) H2N2 influenza A virus was isolated on two occasions, at a 7-day interval, from the girl N. Ju. aged 1·2 years.

(2) A total of five influenza A(H2N2)/80 virus strains were derived from four children's-home children for some days. Similar viruses were isolated from one boarding-school pupil and from a woman aged 24 years (sporadic case within a household). All these foci are located in various parts of Leningrad. The viruses were isolated during several weeks in May of 1980.

W.H.O. requirements include evidence of significant rises in antibody titre to the virus isolate taking into account anamnestic responses. The data presented indicate that fourfold or greater rising titres only to H2 haemagglutinin was observed in two patients from whom the virus was isolated, in four other ill children and in 3·5% of the children and in 4·5% of the adults admitted to the Clinic of the Influenza Institute, Leningrad, for ARD in 1980.

The biochemical analysis allowed the following conclusions: the viruses studied (A/Leningrad/80 isolates) belong to H2N2 serosubtype influenza A virus strains; the A/Leningrad/80 (H2N2) viruses are similar but not identical to A/Singapore/1/57 (H2N2) virus. They differ in a characteristic of the HA antigenic profile in details of the polypeptide composition and in their gene composition.

The data presented by Moore *et al.* (1981) concerning type A influenza H3N2 virus anachronisms is based on similar data. The viruses isolated were of high haemagglutinating activity. In addition, similar occurrences of new influenza viruses took place in the past. Some isolates obtained at the beginning of influenza A(H2N2) pandemic period (1957–68) had similar properties (Smorodintseff &

Korovin, 1961). the same holds true for a set of viruses designated A(H1N1)/77 (Sominina, Lisok & Korchanova, 1979).

In 1979 Hope-Simpson put forward a hypothesis that the antigenic drift of influenza A virus occurs during persistence of viruses in human carrier-hosts. From this hypothesis, influenza A virus, having caused influenzal illness, rapidly becomes latent in the tissues of the human host. The antigenic anachronism of type A influenza H2N2 virus recorded in Leningrad in 1980 appear to support Hope-Simpson's hypothesis, although the mechanisms of the survival of viruses in man in the inter-pandemic period, as well as the cause and form of their activation, must differ from those responsible for antigenic drift.

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