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STUDIES OF SOME BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF AVIAN INFLUENZA A/H7 (Hav1) SUBTYPES

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Summary. — Current isolates of the subtypes H7N3 and H7N7 from 1979 to 1981 were examined and compared with the reference strains with regard to their antigenic variability and to their pathogenicity for birds and mammals in order to establish the potentiality of influenza A/H7 virus (Hav1) transmission from birds to mammals. The analysis of the electrophoretic mobility of virus-induced polypeptides and of the double-stranded RNA segments after hybridization revealed equal, similar and deviating patterns. A substantial drift was determined in the surface antigens, especially in the neuraminidase. The avian strains replicated also in mammalian cells and were pathogenic for mammals. All strains examined were reisolated from the infected mammals; they caused more pronounced inflammatory changes in the trachea and lungs of infected mammals than in those of birds.

Key words: influenza A/H7 subtype; genetic variability; replication; pathogenicity; histology

Introduction

Influenza A virus infects humans, pigs, horses, poultry and particularly many wild living birds. The natural history of influenza A virus in animals and the significance for mammals including man of viruses occurring in birds is unknown. Investigations of genetic relations and antigenic similarities (Webster and Laver, 1975; Scholtissek, 1978) indicate that influenza viruses in wild birds may possibly be the evolutionary source of influenza in domestic animals and humans.

We report on studies of the variability of antigens and of pathogenicity of fowl-plague-like A/H7N7 and A/H7N3 isolate from 1979 to 1981 from birds and mammals which were compared with reference strains with regard to the characterization of their genetic variability and their potential for infecting mammals.

Table 1. Comparison of reference and influenza A/H7 virus (Hav1) strains isolated in the G.D.R. in 1979—1981

Virus	Antigenic formula
A/chicken/Brescia/1902	H7 N7 (Hav1 Neq1)
A/FPV/Rostock/45/1936	H7 N1 (Hav1 N1)
A/turkey/England/1963	H7 N3 (Hav1 Nav3)
A/duck/Alberta/48/1976	H7 N3 (Hav1 Nav2)
A/duck/Tennessee/1976	H7 N3 (Hav1 Nav2)
A/tern/Potsdam/342/6/1979	H7 N7 (Hav1 Neq1)
A/tern/Potsdam/343/6/1979	H7 N7 (Hav1 Neq1)
A/gull/Potsdam/1979	H7 N7 (Hav1 Neq1)
A/duck/Potsdam/13/6/1980	H7 N7 (Hav1 Neq1)
A/duck/Potsdam/15/6/1980	H7 N7 (Hav1 Neq1)
A/duck/Potsdam/16/6/1980	H7 N7 (Hav1 Neq1)
A/swan/Potsdam/62/4/1981	H7 N3 (Hav1 Nav2)
A/swan/Potsdam/64/4/1981	H7 N3 (Hav1 Nav2)

Materials and Methods

Viruses. Influenza A virus strains isolated during our surveillance programme in G.D.R. in 1979—1981, which were classified as H7 subtypes, were compared with reference strains and isolates from North America (Table 1). They were identified by haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests carried out as described by Palmer *et al.* (1975) and modified for the NI test according to Aminoff (1961).

Antisera to haemagglutinin (HA) and neuraminidase (NA) antigens of the reference strains were prepared in rabbits and ferrets.

Cell cultures. The replication of the A/H7 strains was followed in avian (chick embryo fibroblasts, CEF) and mammal (MDCK and BHK 21 cells) cultures. The strains were examined by plaque tests both on CEF cells according to Appleyard and Maber (1974) and on permanent cell lines with and without crystalline trypsin. The determination of the HA titre in mammal cell cultures was carried out after the virus had been harvested and again after a passage in chick embryos or cells. The virus was assumed to be capable of replication, when the last determination was positive.

Polypeptide analysis and RNA/RNA hybridization. The analysis of the virus-induced ^{14}C-labelled polypeptides was carried out with polyacrylamide gel electrophoresis (PAGE) modified according to Skehel (1972). The characterization of the ^{3}H-labelled double-stranded polyadenylized RNA and the RNA/RNA hybridization were performed as described by Hay *et al.* (1977). The activity of the RNA-dependent RNA polymerase in vitro was determined using the technique proposed by Hay and Skehel (1979), however, in the presence of 1 mM 1.4-dithioerythrit (DTE) instead of 2-mercaptoethanol and macaloid. Investigations of the thermal stability of the RNA polymerase were carried out as proposed by Kawakami *et al.* (1982).

Animals. The in vivo studies were performed on one-day-old and 3-week-old chickens, young adult ICR mice bred from isolator mice, young adult Syrian hamsters and 10—12-week-old minipigs of the Mini-Lewe breed. The animals had no serological evidence of prior exposure to influenza viruses, and no viruses were isolated from samples taken before inoculation.

The virus titres ranged from 10^5 to 10^7 EID_{50}/0.1 ml of allantoic fluid; 3-week-old chickens were each given 0.2 ml of infectious allantoic fluid orally and intratracheally. In the case of oral and intranasal inoculation, one-day-old chickens were each given 0.1 ml, mice 0.25 ml and 0.1 ml, respectively, and hamsters received 0.25 ml of infectious allantoic fluid in both cases. The intranasal inoculation of mice and hamsters was carried out under ether narcosis. Minipigs were infected intranasally with 1.0 ml infectious allantoic fluid. For 8 days the animals were under daily observation for clinical signs and samples were taken for isolation and histological studies.

Collection of specimens. Cloacal and tracheal swabs were taken daily from hens and later on organ samples were aseptically collected. Every day one mouse and one hamster were sacrificed and trachea, lungs and kidneys were aseptically removed for virus isolation. Nasal swabs were collected from minipigs daily, whilest organ samples (trachea, lungs and kidneys) only on the 3rd and 4th days post infection (p.i.). Swabs of chickens and nasal swabs of minipigs were placed into 1.0 ml of buffered glycerol saline, and titrated in embryonated chicken eggs to determine virus concentrations (Hinshaw *et al.*, 1978). Tissues were weighed, ground in a mortar with sterile sea sand, diluted, and titrated for infectious virus in embryonated chicken eggs (Webster *et al.*, 1981).

Results

Comparison of the RNA segments of influenza virus strains

The influenza virus A/H7 strains isolated in the G.D.R. from 1979 to 1981 belonged predominantly to the subtype H7N7 (Hav1 Neq1), but the subtype H7N3 (Hav1 Nav2) has still occurred.

Within the group of H7-isolates obtained by surveillance programme a close relationship was revealed as evident from polypeptide analysis and RNA hybridization. The isolates from terns A/tern/Potsdam/343/79 and A/tern/Potsdam/342/79 showed the same electrophoretic mobility of double-stranded RNA both in 4% and 7.5% gels (Fig. 1-I).

Fig. 1-II presents the analysis of electrophoretic mobility of double-stranded RNA segments after hybridization of the isolates A/duck/Potsdam/13/80, A/duck/Potsdam/15/80 and A/duck/Potsdam/16/80 (^{3}H-marked cRNS) with the vRNA of A/duck/Potsdam/16/80. The A/duck/Potsdam/16/80 is shown to be identical in all gene segments with the strains isolated from the same animal population. This identity was confirmed by comparison of the virus-induced polypeptides (Fig. 2).

The electrophoretic mobilities of all proteins (P_1, P_2, P_3, HA_0, NP, M, NS) were the both in 8% (Fig. 2-I) and 16% gels. Corresponding results were obtained when comparing A/duck/Potsdam/64/81 and A/duck/Potsdam/62/81 isolates (Fig. 1-III, 2-II). Even between these strains no differences following from genome or/and polypeptide comparisons were found.

The comparison of the electrophoretic mobility of a tern isolate from the Baltic Sea (1979) (A/tern/Potsdam/343/79) with a duck isolate from the country interior (1980) (A/duck/Potsdam/16/80) did not reveal any difference between the double-stranded RNA segments after RNA/RNA hybridization in the 4 and 7.5% polyacrylamide gel (Fig. 1-IV). The swan isolate (A/swan/Potsdam/62/81) deviated already in the antigenic formula of the NA from the tern and the duck isolates. Comparison in 7.5% polyacrylamide gel of the genome of this isolate with the A/duck/Potsdam/16/80, apart from differential electrophoretic mobility of NA gene, showed a difference in the nucleoprotein gene and in the matrix protein gene. Differences in the polymerase gene were evident particularly in the 4% polyacrylamide gel and they were also confirmed by analysis of the virus induced polypeptides.

Fig. 2-III summarizes the electrophoretic mobility of the virus-induced polypeptides of all H7 strains under study. It illustrated the close relationship

Table 2. Antigenic drift of H7 (Hav1) of the influenza virus A/H7 (Hav1)

Influenza virus A	Chick/ Bresc/ 02	FPV/ Rost/ 45/36	Turk/ Engl/ 63	Duck/ Alb/ 48/76	Gull/ Potsd/ 79	Tern/ Potsd/ 342/79	Tern/ Potsd/ 343/79	Duck/ Potsd/ 13/80	Duck/ Potsd/ 15/80	Duck/ Potsd/ 16/80	Swan/ Potsd/ 62/81	Swan/ Potsd/ 64/81
Chick/Brescia/ 02 H7 N7	100											
FPV/Rost/45/36 H7 N1	70*	100										
Turkey/Engl/63 H7 N3	30	100	100									
Duck/Alb/48/76 H7 N3	30	30	30	100								
Gull/Potsd/79 H7 N7	40	40	30	20	100							
Tern/Potsd/342/79	50	50	20	30	50	100						
Tern/Potsd/343/79 H7 N7	30	40	10	20	70	70	100					
Duck/Potsd/13/80 H7 N7	50	50	50	50	100	100	110	100				
Duck/Potsd/15/80 H7 N7	20	20	20	30	30	40	70	100	100			
Duck/Potsd/16/80 H7 N7	30	40	20	20	70	80	110	140	100	100		
Swan/Potsd/62/81 H7 N3	30	50	30	30	60	90	70	80	60	100	100	
Swan/Potsd/64/81 H7 N3	30	50	30	30	50	70	70	100	40	70	100	100

* expressed in per cent relatedness

of the isolates but also the deviations of the compared strains, particularly of their HA. This conclusion was confirmed by RNA/RNA hybridization (results not shown).

RNA polymerase activity of the compared strains

Compared with the strains influenza A/duck/Alberta/48/76, which showed an average 1 hr incorporation of 3 nM ^{3}H-labelled UMP per mg virus protein, strain A/swan/Potsdam/64/81 was found to have incorporation rates of up to 6 nM per mg virus protein without any stimulation immediately after preparation. This indicates a higher RNA polymerase activity of influenza A/swan/Potsdam/64/81. The two strains did not differ in their optimum RNA synthesis temperature which was 32 °C for both. There was a marked reduction in RNA synthesis both at 29 °C and 35 °C.

Examinations of the activity of RNA polymerase in vitro and its dependence on the incubation time and temperature (thermostability) suggest a higher thermostability of RNA polymerase of A/swan/Potsdam/64/81 than that of A/duck/Alberta/48/76 at 32 °C, because a further small increase in the incorporation rates of ^{3}H-labelled UMP in A/swan/Potsdam/64/81 was observed when prolonging the preincubation time over 15 min. At temperatures above 37 °C, also the RNA polymerase of the isolate A/swan/Potsdam/ /64/81 proved to be thermally unstable.

Drift studies of H7 (Hav1)

In representing the cross-reactivity R coefficient as proposed by Archetti and Horsfall (1950), drift studies using chessboard titration on H7 (Hav1) performed with isolates from the G.D.R. and reference strains at first revealed that the HA of the isolates from 1979—1981 were rather closely related or even identical (Table 2). However, some isolates showed a certain drift in relation to each other. This applied to A/duck/Potsdam/15/80 when

Table 3. Drift of N7 (Neq1) of the influenza virus A/H7 (Hav1)

Immune serum	Antigen						
	A/equine/ Prague/ 56	A/chicken/ Germany/ N/49	A/gull/ Potsd/ 79	A/tern/ Potsd/ 342/79	A/duck/ Potsd/ 13/80	A/duck/ Potsd/ 15/80	A/duck/ Potsd/ 16/80
A/equine/Prague/56	100						
A/chicken/Germany/N/49	5*	100					
A/gull/Potsdam/79	4	20	100				
A/tern/Potsdam/342/79	3	14	70	100			
A/duck/Potsdam/13/80	4	16	90	110	100		
A/duck/Potsdam/15/80	4	20	120	70	90	100	
A/duck/Potsdam/16/80	4	13	70	80	100	110	100

* expressed in per cent

Table 4. Replication of influenza virus A/H7 isolates in avian and mammalian cell cultures

Influenza viruses		CEF		MDCK		BHK 21
		Trypsin	Nil	Trypsin	Nil	Trypsin
A/chicken/Brescia/1902	H7 N7	+	+	+	+	+
A/duck/Alberta/48/76	H7 N3	+	−	+	−	(+)
A/duck/Potsdam/13/80	H7 N7	+	−	+	−	+
A/duck/Potsdam/15/80	H7 N7	+	−	+	−	+
A/gull/Potsdam/79	H7 N7	+	+	−	−	+
A/term/Potsdam/342/6/79	H7 N7	+	−	+	−	+
A/swan/Potsdam/64/4/81	H7 N3	+	−	+	+	+

(+) presence or (−) absence of virus replication, respectively

compared with A/gull/Potsdam/79 and A/tern/Potsdam/342/79, and to A/swan/Potsdam/64/81 when compared with A/duck/Potsdam/15/80. All of the H7 isolates showed a considerable drift in comparison with the reference strains. The degree of relationship between H7 (Hav1) of the isolates from the G.D.R., HA of influenza virus strains A/chicken/Brescia/1902, A/FPV/Rostock/ 1936, and the A/duck/Alberta/48/76 was about 20 to 50%. The drift was not related to the time sequence of the isolations. Influenza A/tern/Potsdam/343/79 (degree of relationship 10 to 40%) and A/duck/Potsdam/15/80 (degree of relationship 20 to 30%) showed a rather strong drift.

Drift studies of N7 (Neq 1)

The drift study of the N7 Neq1) showed that the NA of the current virus strains were closely related or even identical. A comparison with the reference strains likewise exhibited a marked drift. Based on the cross reactivity coefficients (R) between N7 (Neq1) the degree of relationships of the G.D.R. isolates with influenza virus A/chicken/Germany/N/49 and influenza virus A/equine/Prague/56 was 13—20% and 3—4%, respectively (Table 3).

Replication in cell cultures

All investigated strains replicated in bird and mammal cell cultures producing plaques in the presence of trypsin (Table 4). Only the influenza virus A/duck/Alberta/48/76 was not always capable of replication in BHK 21 cell cultures. On the other hand, influenza virus strains A/chicken/Brescia/1902 and A/gull/Potsdam/79 replicated in CEF cells also without trypsin. Attempts to infect MDCK cells with A/gull/Potsdam/79 isolate were negative, but it replicated in BHK 21 cells even in the absence of trypsin. However, in MDCK cells replication of influenza virus strains A/chicken/Brescia/1902 and A/swan/Potsdam/64/81 occurred in the absence of trypsin. Thus, influenza virus A/swan/Potsdam/64/81 replicated better in mammalian cell cultures than in CEF cells.

Table 5. Isolation of virus from swabs and organs of birds and mammals experimentally infected with different influenza A/H7 strains

Species	A/duck/Potsdam /13/6/80[a]				A/swan/Potsdam /64/81[a]				A/chicken/ Brescia/1902[a]				A/gull/ Potsdam/79[a]			
	CS	T	L	K	CS	T	L	K	CS	T	L	K	CS	T	L	K
one-day-old chickens	6.5	3.3	2.7	3.3	4.5	2.9	2.9	3.0	5.1	4.5	5.9	5.5	3.5	7.5	7.1	6.9
three-week-old chickens	3.9	6.1	5.5	4.7	5.3	+	+	+	3.5	5.3	5.9	4.9	5.0	6.5	6.9	7.3
mice	NT	6.5	6.5	4.1	NT	6.3	7.1	4.1	NT	5.5	6.9	3.3	NT	7.1	6.1	NT
hamsters	NT	6.1	5.9	3.5	NT	NT	NT	NT	NT	6.1	7.9	4.7	NT	7.9	6.9	3.3

T = Trachea, L = Lung, K = Kidney, CS = Cloacal swab
+ = virus isolated, – = no virus isolated, NT = not tested
[a] Peak virus titre in $\log_{10}$ EID_{50}/ml or $\log_{10}$ EID_{50}/g, respectively

Pathogenicity for chicken

Strains A/chicken/Brescia/1902 and A/gull/Potsdam/79 showed a pronounced pathogenicity for 1-day-old chickens after oral and in 3-week-old chickens after intratracheal inoculations. Within 24 to 48 hr p.i., the infection in all cases resulted in a fulminant disease with fatal outcome. The evidence of viruses in cloacal and tracheal swabs was positive at 18 hr p.i., with only a few exceptions, in all infected animals.

Histological examinations of trachea, lungs, duodenum and kidneys revealed hyperaemic and haemorrhagic changes in one-day-old chickens and tracheitis, interstitial nephritis and enteritis in three-week-old chickens infected with A/chicken/Brescia 1902 and A/gull/Potsdam/79 strains. Other strains did not cause any clinical symptoms in chickens. Cloacal and tracheal swabs provided only sporadic virological evidence, positive results being obtained more frequently following intranasal than oral virus inoculation. Histological investigations also revealed sporadic perivascular and diffuse lympho-histiocytic infiltrations and occasional destructive cell changes in trachea, lungs and kidneys. Upon oral and intranasal applications to mice and hamsters, the strain A/chicken/Brescia/1902 caused severe, in hamsters even lethal clinical symptoms, morphologically manifested by marked tracheitis and pneumonia.

The remaining strains also caused morphologically traceable tracheitis and pneumonia, but without clinical manifestations. No histopathological changes were found in any case in the intestines and kidneys. Results of reisolation of influenza virus strains A/duck/Potsdam/13/80, A/swan/Potsdam/64/81, A/chicken/Brescia/1902 and A/gull/Potsdam/79 from experimentally infected chickens, hamsters and mice are given in Table 5.

Table 6. Recovery of avian influenza A/H7 viruses from swabs and tissues of experimentally inoculated minipigs

Inoculum (log_{10} EID_{50}/0.1 ml)	No. of positive animals/No. of pigs inoculated	Peak virus titre[a]			Virus recovered from nasal passages (days p.i.)	HI antibody response[b]
		Nasal swab	Trachea	Lungs		
A/duck/Potsdam/13/80 (6.1)	3/3	3.5	3.8	<2	1–5	20
A/swan/Potsdam/64/81 (5.1)	3/3	4.1	<2	5.9	1–4	40
A/gull/Potsdam/79 (6.7)	2/3	2.9	–	–	1–4	<10
A/chicken/Brescia/1902 (6.7)	2/3	<1	–	–	1	<10

– = No virus was detected

[a] log_{10} EID_{50}/ml and log_{10} EID_{50}/g, respectively

[b] Swine were bled at 14 days p.i. The HI titre is the reciprocal of the highest dilution of sera inhibiting four haemagglutinating doses of the virus.

All four strains could be isolated in all species from the trachea, lungs and kidneys, although differences were observed with regard to the infectivity and titre of the reisolated virus. The apathogenic strains A/duck/Potsdam/13/80 and A/swan/Potsdam/64/81 were less infectious for one-day-old than for three-week-old chickens. Virological and histological examinations of organs showed that apathogenic strains caused histological changes in the lungs of chickens, mice and particularly hamsters. Remarkable differences in the isolation rate and the virus infectivity were found in pigs infected intranasally with 4 influenza virus A strains (Table 6). Whereas the strains A/duck/Potsdam/13/80 and A/swan/Potsdam/64/81 caused an infection in all inoculated pigs and they could be isolated from trachea and lungs and nasal swabs for several days p.i., the strains A/chicken/Brescia/1902 and A/gull/Potsdam/79, though highly virulent for hens, were found only in nasal swabs of a few pigs. Although it is known that avian influenza viruses grow in the intestines of birds, we were not able to isolate any virus strain tested from the pig intestines. In pigs no clinical symptoms of the illness were observed.

Discussion

The polypeptide analysis and the RNA/RNA hybridization revealed a close genetic relatedness between the strains within the groups of isolates and similarities or differences between the groups of isolates. The isolation of closely related viruses from successive years, from different places and animal species suggests that the pathogens propagate in the bird population. In this connection, various authors point to the special signi-

ficance of birds in the spread of new influenza strains (Webster *et al.*, 1980) and to the resulting possibilities of their recombination (Hinshaw *et al.*, 1980). The latter hypothesis is confirmed by our isolation of H7 isolates with the NA subtype N7 and N3. Marked differences were also detectable in RNA/ /RNA hybridization. Differences in the genetic structure of H7 strains were already described by Markushin *et al.* (1981). The incorporation rate of ^{3}H-labelled nucleoside monophosphate in nM per mg virus protein per 60 min in the in vitro RNA synthesis is a marker of the activity of the RNA-dependent RNA polymerase of the influenza virus. Further investigations of the thermal stability of RNA polymerase under different storage conditions (i.e. temperature, time) of influenza virus strains are necessary to characterize the typical feature of the RNA polymerase.

Further studies on the antigen variations have shown that the influenza virus A/H7 strains, just as the human strains, exhibit some considerable drift of the surface antigens. This was also found by Markushin *et al.* (1981) for the surface antigens of Hav1 viruses. In contrast to previous findings, the drift of the NA N7 (Neq1) was greater than that of the HA H7 (Hav1). We attribute this phenomenon to the lower HA antibody levels and to the higher NA antibody levels in bird populations which we found in our serological studies.

Our investigations on the replication and pathogenicity of the influenza virus A/H7 strains isolated from different bird species revealed that these avian strains propagated so in mammal cell cultures as in mammals, in one case even better than in avian tissues. Also the virus strains which are clinically apathogenic for birds caused occassionally the development of morphologically detectable and in a few cases even destructive changes in trachea, lungs and kidneys of chickens. The morphological process of infection of mammals with viruses pathogenic for birds corresponded completely to that by human strains. In the case of better multiplication of some strains in mammalian cells, animal experiments showed that these strains could be more regularly reisolated from and that they caused more pronounced inflammatory changes in trachea and lungs of mammals than of birds. This was confirmed by experimental infection of minipigs. From results of our experiments follows that virus strains isolated from wild birds, which are apathogenic for hens, can infect pigs and can be isolated for a number of days p.i. from nasal swabs, trachea and lungs. The infection is not accompanied by any clinical symptoms.

Hinshaw *et al.* (1981) examined H1N1 (Hsw1 N1) viruses, which were isolated from mallards, and H7N7 (Hav1 Neq1) viruses which were isolated from seals for their ability to replicate in mammals. The authors found a wide range of hosts including birds and mammals — a fact which is not surprising since in one case a Hsw1 virus and in the other a virus which was isolated from a mammal was involved. For comparison, in our study all investigated H7 (Hav1) virus strains were isolated from birds. Quite obviously, the range of hosts of the avian H7 (Hav1) influenza viruses may occasionally be wider than has been assumed till now and may occasionally or always include mammals.

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Explanation of Figures (Plates VII–VIII):

Fig. 1. Electrophoretic mobility analysis in 4% (I, II) and 7.5% (III, IV) polyacrylamide gel of double stranded RNA after hybridization of with

I – A/tern/Potsdam/343/79, A/tern/Potsdam/342/79 (^{3}H–labelled cRNA) with vRNA of A/tern/Potsdam/342/79,

II – A/duck/Potsdam/15/80, A/duck/Potsdam/16/80, A/duck/Potsdam/13/80 (^{3}H–labelled cRNA) with vRNA of A/duck/Potsdam/16/80,

III – A/swan/Potsdam/62/81, A/swan/Potsdam/64/81 with vRNA of A/swan/Potsdam/64/81,

IV – A/tern/Potsdam/343/79, –, A/duck/Potsdam/16/80, A/swan/Potsdam/62/81 (^{3}H–labelled cRNA) with vRNA of A/duck/Potsdam/16/80

and after treatment with S_1-nuclease.

Fig. 2. Electrophoretic mobility analysis in 8% polyacrylamide gel of virus specific polypeptides of

(I) – A/duck/Potsdam/15/80, A/duck/Potsdam/13/80, A/duck/Potsdam/16/80,

(II) – A/swan/Potsdam/61/81, –, A/swan/Potsdam/64/81,

(III) – A/tern/Potsdam/343/79, A/duck/Alberta/48/76, A/chicken/Brescia/1902, A/gull/ Potsdam/79, A/turkey/England/63, A/FPV/Rostock/45/36, A/duck/Tennessee/1/76, A/duck/Potsdam/15/80, A/duck/Potsdam/13/80, A/duck/Potsdam/16/80, A/swan/Potsdam/62/81, A/swan/Potsdam/64/81, cell control = noninfected CEF.

Monolayer CEF cultures were labelled with ^{14}C-leucin for 15 min at 4 hr p.i. The complete cell monolayer removed with solubilizing solution (8 mol/l urea, 2% SDS, 4% mercaptoethanol) was subjected to electrophoresis.