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Relative Efficacy of Embryonated Eggs and Cell Culture for Isolation of Contemporary Influenza Viruses

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The efficiency of embryonated eggs and primary monkey kidney cell culture for isolation of contemporary influenza viruses was compared over three seasons by parallel inoculation of clinical materials. For the type A (H1N1) strains of 1977-1978 and for type B strains eggs were very inefficient, and nearly all viruses were isolated in cell culture alone. For the type A (H1N1) strains of 1978-1979 and for type A (H3N2) strains, isolations were more frequently made in eggs, but even here eggs were not as efficient for primary isolation as were cell cultures. It is concluded that primary monkey kidney cell cultures can be used as the single system for primary isolation of current type A and B influenza viruses.

The finding by Burnet in 1940 that influenza virus could be isolated in embryonated hens' eggs (1) represented a major advance in the ability to work with this agent. Previously inoculation of laboratory animals, especially ferrets, had been required for virus recovery (1, 10). Even after cell cultures became widely used, eggs continued to be the standard host for isolation of influenza viruses, in part because of the lack of availability of primary cell cultures in certain geographic areas (4). However, an additional reason for the continued reliance on eggs was the concern that cell cultures were not as sensitive for isolating the virus. This was clearly the case in the mid-1960s when the H2N2 strains of type A influenza were prevalent (3). Since that time, less attention has been given to defining the relative contribution of eggs for isolation of contemporary influenza strains. In general, it has been considered that eggs were required for optimal isolation of type A strains but were not necessarily needed for type B (5). The present report describes the changing pattern of virus isolation after parallel inoculation of regularly collected clinical specimens into monkey kidney cells and eggs during three respiratory virus disease seasons. It also presents the results of substituting primary cynomolgus for rhesus monkey kidney cell cultures.

Clinical specimens were those collected in the course of the Tecumseh Study of Respiratory Illness. Participants were contacted on a weekly basis to identify the onset of acute respiratory illness episodes (7, 8), and when such illnesses were reported, throat swab specimens were collected within 2 days of onset. The cotton swabs were placed in veal infusion broth enriched with 0.5% bovine serum albumin which contained 500 U of penicillin and 2 μg of amphotericin per ml. The specimens were transported to the laboratory within hours of collection and inoculated into two tubes each of three types of cell culture and into embryonated eggs. The cell culture in which influenza and parainfluenza viruses were usually isolated was primary monkey kidney. During the 1976-1977 and 1977-1978 seasons, the primary monkey kidney was exclusively rhesus in origin. However, in 1978-1979 cynomolgus cells were used for specimens in alternate weeks, and rhesus cells continued to be used in the other weeks. Both types of monkey kidney cells were purchased as tube cultures from Microbiological Associates and were handled as previously described (7). The tubes were hemadsorbed twice weekly with guinea pig erythrocytes. Observation of negative tubes was continued for 3 weeks, after which they were discarded.

Specimens were also inoculated amniotically in 0.2-ml volumes into five 9- to 11-day-old embryonated eggs. This was done regularly with all specimens received, except in occasional weeks in which the number of specimens exceeded the supply of eggs. In such weeks, the specimens were inoculated sequentially until no more eggs were available, and additional specimens were inoculated into cell cultures only. The eggs were incubated at 33°C for 3 days, and at that time, amniotic and allantoic fluids of each egg were harvested separately and tested for presence of hemagglutinating agents, using a 0.5% suspension of chicken erythrocytes. Isolates from cell cultures or eggs were typed by hemagglutination inhibition. Antisera used were produced either in this laboratory in ferrets or at the Center for Disease Control in chickens.

Specimens were collected through the period

from September 1976 to August 1979 and were inoculated in parallel into primary monkey kidney cell cultures and embryonated eggs. During the 3 years, one outbreak each of type B and type A (H3N2) influenza occurred in Tecumseh, and two outbreaks of type A (H1N1) influenza were observed. Frequency of isolation of influenza virus from clinical specimens during that period is shown in Table 1. The table includes all specimens from which an influenza virus was isolated and indicates whether the isolation was made in cell cultures, in eggs, or in both systems. During the type B influenza outbreak of 1976–1977, no isolations were made in eggs without the virus also appearing in cell culture. Indeed, eggs were an inefficient system for isolating these viruses, with nearly 80% of the viruses being recovered in cell culture alone.

The next respiratory viral disease season provided a striking contrast in that two different strains of type A virus were isolated in that year, with a 3-week period in which they cocirculated. The H3N2 (A/Texas-like) strains were much more likely to be isolated in eggs than the type B viruses had been. Nearly 73% of these viruses were isolated in both eggs and cell culture, but even here only two isolates were recovered in eggs alone. The H1N1 viruses (A/USSR-like) isolated in the same period were much more like type B strains in their tropism, with cell cultures clearly the more efficient host for recovery. During this period, to identify the presence of virus in the egg harvests, guinea pig erythrocytes were used along with chicken erythrocytes, since it was found that the former were more likely to detect the H1N1 viruses. The marked difference in isolation pattern of the H3N2 and H1N1 viruses provided an internal technical control, since the clinical specimens containing the two subtypes were being inoculated at the same time into material handled in the same way. In fact, 7 of the 10 H3N2 viruses isolated in the period of cocirculation were isolated in eggs (70%), whereas only one of the nine H1N1 viruses recovered in this period were isolated in this host (11.1%). In 1978–1979, the H1N1 strains reappeared. These A/Brazil-like viruses had undergone a marked change in tropism and now resembled the H3N2 viruses seen the year before. Most of the isolations were made in both eggs and cell cultures; cell cultures alone were responsible for more isolations (25.0%) than were eggs alone (1.8%). In addition, the hemagglutinating characteristics of the new isolates had also changed, with chicken erythrocytes being quite suitable for detecting the presence of virus in egg fluids.

The increasing scarcity of primary rhesus monkey kidney cells during the 1978–1979 respiratory virus disease season prompted a search for alternative hosts for isolation of influenza and parainfluenza viruses (2, 6). The sensitivity of primary cynomolgus monkey kidney cells was first compared with that of the rhesus cells by inoculating two tubes of both cell types with dilutions of original throat swab material containing known viruses. Three specimens containing each of the viruses in question were used, and infectivity endpoints were calculated by the method of Reed and Muench (9). For type A (H3N2) and type B influenza viruses as well as parainfluenza virus types 1 and 3, titers were nearly identical. For type A (H1N1) and parainfluenza type 2 viruses, the titer differences were relatively small (under 1 log) and, if anything, suggested that the cynomolgus cells were more sensitive than the rhesus cells. Thereafter, during the course of the winter of 1978–1979, rhesus and cynomolgus monkey kidney tubes were used in alternate weeks. In that 8-week period, an outbreak of A/Brazil-like (H1N1) influenza occurred; no other virus was isolated with sufficient frequency to permit proper comparison. In the 249 specimens processed during that time, the overall rate of isolation in rhesus monkey kidney cells was 36.1%, and that in the cynomolgus monkey kidney was 29.1%. These values were not significantly different when subjected to the χ^2 test. Therefore, it appears that the conclusion about the usefulness of primary monkey kidney cells as a host system applies to the current, more available cynomolgus cells.

TABLE 1. *Comparative isolation frequency of influenza virus in monkey kidney cells and embryonated eggs, 1976–1979*

System in which viruses were isolated[a]	1976–1977: type B		1977–1978				1978–1979: type A (H1N1)	
			Type A (H3N2)		Type A (H1N1)			
	No.	%	No.	%	No.	%	No.	%
Cell culture alone	22	78.6	14	23.7	36	85.7	14	25.0
Eggs alone	0	0	2	3.4	1	2.4	1	1.8
Both cells and eggs	6	21.4	43	72.9	5	11.9	41	73.2

[a] All clinical specimens were inoculated in parallel into the two host systems.

Since the cynomolgus and rhesus kidney cell systems were similar in ability to detect influenza viruses in clinical material, both could be used to compare isolation rates with those in eggs. Two subtypes of type A influenza as well as type B influenza virus were readily isolated in primary monkey kidney cells. In fact, with the A/USSR-like (H1N1) viruses of 1977–1978 and the type B viruses, eggs were very inefficient for virus isolation, and their use added little to the total number of isolates recovered. Although the proportion of viruses isolated in eggs was higher, even with the other type A viruses, few would have been missed if eggs had not been used. The situation in 1977–1978 was of special interest, since during that winter the difference in the ability to isolate the A/Texas-like (H3N2) and A/USSR-like (H1N1) viruses in eggs was clear, even though the outbreaks overlapped.

Thus, eggs added little in the isolation of the currently prevalent influenza viruses when cell culture systems were in regular use. Although certain continuous cell lines have been proposed as substrates for virus isolation, cynomolgus monkey kidney cells offer the advantage of being a single suitable system not only for influenza but for the parainfluenza viruses as well (2). These observations on contemporary influenza virus strains cannot be generalized to all subtypes. The HswlN1 viruses isolated from humans in 1976 were more efficiently isolated in eggs than cell culture, as were the H2N2 viruses (3). Laboratories with a special interest in influenza will have to continue to use eggs should a new shift in type A strains occur. In addition, eggs are useful for preparation of large pools of influenza viruses; the viruses isolated in cell cultures can immediately be passed in eggs for this purpose.

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