Replication of Animal Viruses in Differentiating Muscle Cells: Influenza Virus A

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SUMMARY

Cells were cultured from the breast muscle of 11- to 12-day-old chick embryos and were grown under conditions optimal for the development of the cells into terminally differentiated, fused myotubes. Myotubes were infected with influenza virus A/Ann Arbor/6/60(H2N2) at high multiplicity, and synthesis of virus-specific proteins and RNAs was detected by haemadsorption, fluorescence microscopy and/or isotope labelling and electrophoresis techniques. Provided that myotubes were maintained at temperatures below 39 °C after infection, production of virus components and yield of infectious virus in these cells was similar to those observed in infected chick kidney cells. However, if cells were maintained at temperatures of 39° to 40 °C after infection, virus nucleoprotein was prominent in the nuclei, and synthesis of virus-specific polypeptides and of plus-strand RNA was reduced about fourfold to 20-fold compared to that detected at lower temperatures. Moreover, infectious virus was not produced when temperatures of 39 to 40 °C were used during virus replication. The results demonstrate that under suitable conditions avian myotubes formed in culture resemble epithelioid cells in their ability to support the productive replication of influenza virus.

INTRODUCTION

One approach to understanding the role of intracellular host cell functions in virus replication is to study differentiating cell systems, since many cellular activities change during embryogenesis. Previously O'Neill & Kendal (1975) observed that the susceptibility of avian embryonic muscle cells to influenza virus infection appeared to increase during the course of cell development. Thus it was found that myoblasts, the rapidly dividing precursors of muscle cells, were considerably more refractory to infection than were terminally differentiated, fused myotubes. In contrast to myoblasts, myotubes supported the synthesis of virus haemagglutinin and underwent a pronounced cytopathic change when infected with influenza virus. Since the lack of DNA synthesis in myotubes is known to be a major difference between myotubes and myoblasts (Lash, Holtzer & Swift, 1957; Stockdale & Holtzer, 1961; Okazaki & Holtzer, 1966; Stockdale & O'Neill, 1972a), it was speculated that changes in the DNA synthetic activities of the muscle cells after cell fusion

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may have permitted the nuclear event known to be essential for influenza replication (Barry, 1964). Before such a hypothesis could be further examined, we felt it necessary to establish whether influenza infection of myotubes was itself fully productive, or whether it might result only in the synthesis of some virus antigens (such as haemagglutinin), without proper assembly and release of virus. The studies described here have demonstrated that, under conditions of high multiplicity of infection, influenza viruses undergo a fully productive replicative cycle in chicken myotubes, provided that the temperature of cell cultures is maintained below 39 °C during virus growth. Influenza virus thus differs from the Ross River togavirus, which can produce a lytic infection in mouse myoblasts but a persistent infection in myotubes (Eaton & Hapel, 1976). Replication of influenza virus in muscle cells probably also differs from that of Coxsackievirus A13, which replicates best in mouse muscle cell cultures infected during the period of rapid cell fusion (Goldberg & Crowell, 1971). DNA synthesis in myotubes normally occurs at a very low level and has been shown to be repair DNA synthesis (Stockdale & O'Neill, 1972b); however, Rous sarcoma virus, which requires DNA synthesis for replication to occur, can not only infect myoblasts (Kaighn, Ebert & Stott, 1968) but can also induce ³H-thymidine incorporation in myotubes (Lee, Kaighn & Ebert, 1968). In a subsequent paper, we will demonstrate that the DNA viruses vaccinia and herpes simplex type 1 are also capable of infecting avian myoblasts and myotubes and undergoing a productive replicative cycle. These results emphasize the potential value of muscle cell cultures for studying virion and host-specific factors involved in replication of animal viruses.

**METHODS**

**Viruses.** Influenza A/Ann Arbor/6/60(H2N2) was received originally from Dr H. F. Maassab, University of Michigan. The history of this virus includes one passage in chick kidney (CK) cells (Maassab, 1959) and 24 to 25 egg passages. Virus A/NWS/33(HoN1) was originally received from the American Type Culture Collection, and had an extensive passage history in ferrets, mice and eggs. Seed of infected allantoic fluid had infectivity titres of about 10⁶ infectious doses/ml for each virus.

**Media.** Complete media used for culturing muscle cells consisted of 85 parts of minimum essential medium (MEM) in Earle’s balanced salt solution (EBSS) supplemented with 10 parts of horse serum (demonstrated by pre-testing to contain factors supporting muscle cell fusion), four parts of extract prepared from 11- or 12-day-old chick embryos, and one part of fresh 100 × glutamine solution. The medium also contained 100 units of penicillin and 5 μg of streptomycin per ml. After infection by influenza, muscle cells were maintained in medium containing only 2 % horse serum. Medium for growth of chick kidney (CK) and Madin & Darby canine kidney (MDCK) cells consisted of Eagle’s MEM prepared in Hanks’ balanced salt solution (HBSS) containing 10 % foetal calf serum and twice the standard concentration of amino acids and vitamins. Serum-free medium was used during virus growth studies. Overlay medium used for virus plaque titrations consisted of medium 199 in HBSS containing 1 % Tonagar, 0.1 % DEAE-dextran, and 10 μg/ml of L-(tosylamido 2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin. Gentamycin was included at 50 μg/ml. For isotope labelling of virus polypeptides we used Eagle’s MEM containing 10 % of the normal concentration of leucine and 5 μCi of ³H-leucine/ml. Virus RNA was labelled with Eagle’s MEM containing 5 μCi of ³H-uridine/ml.

**Cell cultures.** The pectoral muscles were dissected from 11- or 12-day-old chick embryos, the connective tissue was removed and the tissue was minced to pieces having a size of 1 to 2 mm². Tissue fragments from 4 to 8 embryos were incubated in 5 ml of trypsin solution
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[0.25% in pH 7.2 phosphate buffered saline (PBS)] for 15 min at 37 °C. An equal volume of complete medium was added, and the cells were suspended by repeatedly passing the tissue fragments through the orifice of a Pasteur pipette. Cells were pelleted by centrifugation for 5 min at 500 g, resuspended in fresh complete medium, and then pelleted and suspended once more. The cell suspension was filtered through a double layer of lens paper to remove debris. Fibroblast cells were removed by pre-plating as previously described (O'Neill & Stockdale, 1972) on collagen-coated plastic tissue culture dishes at 39.5 °C for 10 to 15 min. Cells remaining in the supernatant fluid were diluted in complete medium to a concentration of approx. 0.5 x 10^6 cells/ml and plated on collagen-coated plastic tissue culture dishes at a density of about 1.5 x 10^6 cells/cm^2. For fluorescent antibody staining, cells were seeded on 8 x 22 mm glass coverslips that had been soaked in 10% sodium hydroxide, thoroughly washed with distilled water, soaked in 10% NaHCO_3 and 10% EDTA, thoroughly washed with distilled water and then in absolute alcohol, air dried, and sterilized with dry heat. Coverslips were coated with collagen immediately before use. Cells were grown on coverslips kept in tissue culture dishes. All cells were grown and maintained in an atmosphere containing 5% carbon dioxide so that the pH of the medium did not fall below about 7.2. Cells were incubated in a water-jacketed incubator maintained at 39.5 ± 0.5 °C to ensure maximum cell fusion. The temperature was verified with laboratory thermometers that had been calibrated with a certified thermometer. Separate water-jacketed incubators were used to maintain virus-infected cells at various lower temperatures, between 34 and 38 °C.

CK cell cultures were prepared and infected as previously described (Maassab, 1959; Kendal, Galphin & Palmer, 1977a). MDCK cells were initially obtained from Flow Laboratories, Md, and were propagated by the tissue culture unit at the Center for Disease Control.

Immunofluorescence. Coverslip cultures of cells were washed twice in HBSS, fixed for 1 min in an ice-cold acetone–ethanol mixture (50:50, v/v) and allowed to air-dry. One hundred μl of normal or immune rabbit serum diluted appropriately in PBS (see below) was added to each coverslip and allowed to react for 30 min at room temperature in a covered Petri dish. Coverslips were then washed by immersion in PBS and gentle rocking for 30 min at 35 °C. One hundred μl of anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC) diluted in PBS was added to each coverslip and allowed to react for 30 min at room temperature. Coverslips were then washed as described above, mounted in 90% (v/v) glycerol in PBS, pH 9.1, and examined by transmission light microscopy under a Zeiss microscope equipped with a phase/dark field condenser, halogen lamp and FITC excitation filter and matched barrier filter. We used a 16× or 25× objective magnification and an exposure time of 30 s to 2 min with Kodak Tri-X film, processed for a 1000 ASA film speed.

Antisera. Antiserum used for specific staining of influenza A nucleoprotein antigen was prepared by immunizing a rabbit with 250 μg of purified A/PR/8/34(HoN1) virus that had been disrupted by three cycles of freeze-thawing and incubation with 1% Triton X-100 in 0.05 M-potassium chloride and 0.005 M-phosphate buffer, pH 7.2. The treated virus was mixed with an equal volume of Freund's complete adjuvant and inoculated into the rabbit's rear footpads. Blood was collected at 1 month, and serum from this specimen was shown by double immunodiffusion not to react with influenza B and to produce only a single precipitin line with sodium sarkosyl sulphate-disrupted virus A/Ann Arbor/6/60(H2N2). This line was a line of identity with one precipitin band formed when the serum was tested with the homologous A/PR/8/34 virus, but was not a line of identity with the line formed with influenza A matrix protein (Dowdle et al. 1974). Therefore, it was concluded that the
predominant antibody in the serum reactive with A/Ann Arbor/6/60 virus was directed against influenza type A-specific nucleoprotein. FITC-conjugated antibody to rabbit IgG was purchased from Microbiological Associates, Md. Various dilutions of rabbit antibody and the conjugate were used in fluorescent antibody tests with virus-infected or control cell cultures and it was found that maximum specific fluorescence and minimum background non-specific staining were obtained with a 1:10 dilution of rabbit serum and a 1:20 dilution of conjugate.

**Infectivity titrations.** Plaque titrations were carried out in MDCK cells by a procedure similar to that described by Appleyard & Maber (1974). Confluent cell monolayers in 25 cm² flasks were infected with 1-0 ml of virus dilutions in HBSS containing 0-5 % foetal calf serum. Virus was absorbed for 1 h at room temperature with continuous gentle rocking. Excess virus was aspirated and 5 ml of overlay medium was added. After incubation at 35 °C for 48 h, plaques were stained by adding a 2 % Ionagar overlay containing 0-01 % neutral red and were counted on the next day when they had a size of about 3 mm diam. Egg infectivity titrations were carried out as previously described (Kendal *et al.* 1977a).

**Synthesis of virus proteins and RNA.** Cultures of chick muscle or kidney cells were infected with approx. 100 p.f.u. of A/Ann Arbor/6/60 per nucleus for 30 min at room temperature. The excess virus was removed, the cells were washed twice with HBSS and appropriate maintenance medium was added. To label proteins, at 6 h post infection (p.i.) we replaced the maintenance medium with medium containing 10 % of the normal amount of leucine. After depletion of leucine in the cultures for 30 min, ²H-leucine (sp. act. 57·4 Ci/mmol) at a final concentration of 5 μCi/ml was added for 1 h. Cell cultures were washed three times with ice-cold 0·1 m-tris-HCl pH 7·5, and cells were scraped off and frozen. Electrophoresis of polypeptides according to the method of Laemmli (1970) and processing of gels have been previously described (Kendal *et al.* 1977b). In some experiments, labelling of host protein (Nuss, Oppermann & Koch, 1975) was minimized by treating cells with pre-warmed hypertonic medium containing 120 mm excess sodium chloride for 20 min. Virus polypeptides were then labelled by adding medium consisting of EBSS supplemented with 5 % dialysed foetal calf serum and ¹⁴C-amino acid mixture to a concentration of 10 μCi/ml. After 30 min, cell cultures were washed and harvested as above. To label virus plus-strand RNA, at 3 h p.i. we added actinomycin D at a final concentration of 0·2 μg/ml to chick muscle cells or 2 μg/ml to kidney cells. One h later ³H-uridine (sp. act. 43 Ci/mmol) was added to a final concentration of 5 μCi/ml for a labelling period of 3 h. Cells were then washed three times with ice-cold NTE buffer (100 mm-NaCl, 10 mm-tris-HCl, 1 mm-EDTA, pH 7·4) and scraped into B2 buffer (10 mm-tris-HCl, pH 7·4, 10 mm-NaCl and 1·5 mm-MgCl₂). The cells were homogenized with 20 strokes of a pestle, and nuclei were removed by centrifugation for 10 min at 1000 rev/min. SDS was added to the cytoplasmic supernatant fluid to a final concentration of 1 %, and, after one extraction with phenol, the RNA in the aqueous phase was precipitated overnight with two and a half volumes of cold ethanol. The RNA was analysed on 5 to 30 % sucrose gradients or on 3 % polyacrylamide cylinder gels containing 0·5 % agarose as previously described (Cox & Kendal, 1976).

**Materials.** All isotopes were obtained from New England Nuclear, Ma, and reagents for acrylamide gel electrophoresis from Bio-Rad, Ca. Collagen and TPCK-trypsin were obtained from Worthington Biochemicals, NJ, and actinomycin D from Calbiochem, Ca.
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RESULTS

Synthesis of virus proteins

Initial studies to determine whether any virus proteins other than haemagglutinin were synthesized in influenza-infected muscle cells were done by fluorescent antibody staining for nucleoprotein. When myotubes were maintained at 39 to 40 °C after infection (the same
temperature used to grow the cells for maximum fusion), most of the nuclei in the cells fluoresced brightly when cells were fixed at 7 h p.i., and relatively little cytoplasmic fluorescence was seen (Fig. 1). Similar results were obtained if cells were stained from 3 to 14 h p.i. (not shown). Cells infected for longer periods could not be stained because the pronounced cytopathic effect caused contraction of myotubes and release from the surface of the culture dish as described previously (O’Neill & Kendal, 1975). If the myotubes were
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Fig. 3. Polyacrylamide gel electrophoresis of polypeptides synthesized at 35 °C in influenza-infected cells. Polypeptides were labelled with 3H-leucine from 6.5 to 7.5 h p.i. and subjected to electrophoresis on discontinuous 11% polyacrylamide gels. (a) Polypeptides synthesized in myotubes. (b) Polypeptides synthesized in chick kidney cells. Polypeptides (P, HA, NP and M) were identified by comparison with virus polypeptides electrophoresed in parallel gels. (Equivalent numbers of cells were not used.)

maintained at 37 to 38 °C after infection, however, many nuclei exhibited less fluorescence than their surrounding cytoplasm (Fig. 2). These observations indicated that in infected myotubes maintained at 39 to 40 °C, detectable levels of influenza nucleoprotein (NP) were synthesized and that migration of NP out of the nucleus was much reduced compared with that observed at 37 to 38 °C. Fluorescent antibody staining results consistently showed cytoplasmic, rather than nuclear, accumulation of NP when cells were maintained at any temperature between 34 and 38 °C after infection (not shown).

Synthesis of virus proteins in influenza-infected myotubes was further examined by labelling the cells with 3H-leucine and then analysing the polypeptides by high resolution acrylamide gel electrophoresis with a discontinuous buffer system. When infected at 34 to 35 °C, virus polypeptides P, uncleaved HA, NP, M and NS were identified in myotubes (Fig. 3a) in approximately similar proportions to each other as found in CK cells infected in parallel (Fig. 3b). Lack of cleaved HA was similar to our previous results and to results often found by others who have studied the replication of human strains of influenza virus. At the higher temperature of 39 to 40 °C, incorporation of label into polypeptides was reduced about 20-fold in myotubes. To detect virus polypeptides more readily under these conditions, we treated cells with hypertonic salt to suppress host polypeptide synthesis. This
treatment reduced the proportion of the higher mol. wt. virus polypeptides HA and NP in relation to the lower mol. wt. polypeptides M and NS at 34 °C and at 39 to 40 °C (Fig. 4). Although, as shown here, virus-specific polypeptide synthesis and migration of nucleoprotein proceeded less efficiently at the elevated temperatures, the qualitative haemadsorption assay demonstrated that at 39 to 40 °C haemagglutinin became incorporated into myotube plasma membrane in amounts sufficient to readily bind red blood cells (Fig. 5), as has been previously observed when temperature of incubation was not closely monitored (O’Neill & Kendal, 1975).

Synthesis of virus RNA

Demonstration of the synthesis of virus proteins provided circumstantial evidence that virus messenger (complementary) RNA was also being made in infected myotubes. To determine whether synthesis of virion RNA also occurred, we treated infected cells with actinomycin D at 3 h p.i. and labelled them with ^3H-uridine from 4 to 7 h p.i. This protocol is similar to those adopted by other workers to preferentially suppress cellular RNA synthesis and detect virion RNA synthesis, which is the predominant type of RNA made after about 3 to 4 h p.i. in influenza virus infected cells (Scholtissek & Rott, 1970; Pons, 1973). Since muscle cells may be unusually sensitive to the inhibitory activity of actinomycin D (Molinaro et al. 1974), preliminary studies were undertaken which showed that when the drug was used at a concentration of 0.2 μg/ml, RNA synthesis of myotubes was almost completely suppressed (Fig. 6) without causing detectable cytopathic changes to the cells for several hours.

Virus RNA extracted from myotubes that had been infected at 38 °C and had been treated
**Fig. 5.** Haemadsorption of influenza-infected myotubes incubated at 40 °C and assayed for virus haemagglutinin with chicken erythrocytes at 15 h p.i.

**Fig. 6.** Sedimentation analysis of RNA extracted from influenza-infected or mock-infected myotubes treated with actinomycin D and incubated at 38 or 40 °C. Infection, labelling, phenol extraction of RNA and its sedimentation on 15 to 30 % sucrose gradients are described in the text. Results from parallel gradients are shown in one figure for the sake of clarity. Positions of chick ribosomal RNA are indicated by the arrows. ▲—▲, Mock-infected cells at 38 °C; □—□, influenza-infected cells at 40 °C; ●—●, influenza-infected cells at 38 °C. RNA synthesis in mock-infected cells at 40 °C was similar to that shown for cells at 38 °C.
at 3 h p.i. with actinomycin D had a sedimentation profile with a peak at about 18S, and lesser amounts of more slowly sedimenting RNA (Fig. 6). However, when cells were maintained at 39 to 40 °C after infection, the amount of virus RNA was reduced by about fourfold. This observation was reproducible in four similar experiments.

To confirm that influenza RNA synthesis in infected myotubes at temperatures of 34 to 38 °C was equivalent to that in a system known to be productive for influenza virus, we extracted RNAs from influenza-infected myotubes and chicken kidney cells and analysed them by polyacrylamide gel electrophoresis. Although the large RNA species were resolved incompletely in the electrophoresis system used, the close similarity of RNAs in influenza-infected myotubes and chick kidney cells was apparent (Fig. 7). Polyacrylamide gel analysis of the virus-specific RNA synthesized at 40 °C revealed a heterogeneous population of RNA without a typical influenza virus RNA profile.

Fig. 7. Polyacrylamide gel electrophoresis of RNA extracted from influenza-infected myotubes (a) and chick kidney cells (b). RNA synthesized in the presence of actinomycin D at 35 °C as described was analysed on 3 % polyacrylamide gels containing 0·5 % agarose. Electrophoresis was carried out at room temperature for 6 h at 6 mA/gel. Equivalent amounts of RNA were not used.
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Table 1. Production of infectious influenza viruses in cultured avian myotubes

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Virus</th>
<th>Temperature (°C)</th>
<th>Time p.i. (h)</th>
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<td>1</td>
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<tr>
<td>1*</td>
<td>A/AA/6/60</td>
<td>35</td>
<td>5.1</td>
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<td>1†</td>
<td>A/AA/6/60</td>
<td>35</td>
<td>5.7</td>
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<tr>
<td>1†</td>
<td>A/NWS/33</td>
<td>35</td>
<td>5.3</td>
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<tr>
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<td>40</td>
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<tr>
<td>2†</td>
<td>A/NWS/33</td>
<td>40</td>
<td>5.3</td>
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* Infectivity is expressed in log_{10} p.f.u./ml.
† Infectivity is expressed in log_{10} EID_{50}/ml.

Production of infectious virus

Growth curves were carried out to determine whether infectious influenza virus was produced in myotubes. When cells were infected at high multiplicity (10 p.f.u./nucleus) and were maintained at 34 to 35 °C, the virus titre increased about 1.5 to 2 log units by 24 h p.i. (Table 1). However, when cells were maintained at 39 to 40 °C, no increase in virus titre occurred at any time. These observations were consistent both for the influenza strain A/Ann Arbor/6/60(H1N2) and for the strain A/NWS/33 (H3N1). The efficiency of production of infectious viruses in myotubes at 35 °C was determined by calculating yields on the basis of the number of nuclei per culture. It was determined that about 5 to 10 infectious virus particles were produced per nucleus, which was similar to the yield of infectious virus from cultures of chick kidney cells infected and titrated in parallel.

DISCUSSION

The major objective of this study was to determine whether terminally differentiated muscle cells (myotubes) previously shown to be susceptible to infection by influenza virus (O'Neill & Kendal, 1975) were capable of supporting productive virus replication. Results described demonstrate that infected chicken myotubes do indeed support the production of virus proteins, nucleic acids and infectious virus with an efficiency comparable to that of chick kidney epithelial cells (Maassab, 1959), which, similarly to other primary kidney cell cultures such as calf kidney (Lehmann-Grube, 1965) and monkey kidney (Mogabgab, Holmes & Pelon, 1961; Choppin, 1962), have customarily been used as a permissive host system for the growth of influenza viruses.

Although the results are not shown here, we have demonstrated that the swine influenza-like A/New Jersey/8/76(Hsw1N1) virus, which is poorly adapted to avian tissues, infects cultured chicken myotubes, and in collaborative studies with Dr S. Hauschka we have shown that the egg-adapted A/Ann Arbor/6/60 virus readily infects clones of myotubes prepared from human embryonic muscle tissue (unpublished observations). Throughout all studies it has consistently been observed that the influenza virus strains used infect chick or human embryonic fibroblasts with very low efficiency, as judged by haemadsorption and fluorescent antibody staining procedures. The ready infection of myotubes appears therefore to reflect a genuine sensitivity of these cells to influenza viruses instead of being an artifact resulting from host adaptation of the virus strains used.

The finding that at elevated temperatures influenza nucleoprotein accumulates in the host cells' nuclei represents a previously undescribed effect of temperature on influenza
replication. Lack of production of infectious virus when nuclear retention of nucleoprotein occurs is consistent with observations of abortive infections in HeLa cells (Hills, Moffat & Holtermann, 1960) and L cells (Franklin & Breitenfeld, 1969). The elevated temperature of 39 °C is also known, however, to reduce the incorporation of matrix protein into virions of influenza strain A/Ann Arbor/6/60 grown in CK cells (Kendal et al. 1977a). The abortive nature of influenza replication in chick myotubes at temperatures of 39 to 40 °C might therefore result from a combination of effects, including nuclear retention of virus NP, reduced incorporation of M protein into virions, and perhaps other phenomena not yet identified. Similarly, the reduction in virus-specific protein and RNA synthesis observed at 39 and 40 °C may result from the lack of nucleoprotein migration into the cytoplasm, or to other unidentified factors.

Although influenza virus is not normally believed to be disseminated to tissue outside the respiratory tract during infection of man, this has occasionally been reported (Oseasohn, Adelson & Kaji, 1959; Hildebrandt, Maassab & Willis, 1962), including most recently the isolation of influenza virus from the skeletal muscle of a patient suffering from Reye's syndrome (Partin et al. 1976). Additional studies are needed to confirm this observation and, if it is confirmed, to determine whether there are any clinically significant consequences of natural influenza infection of muscle.

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REFERENCES


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