nin for other materials. In the treatment of patients with septic injuries (*5*), fibronectin might perform other functions of clinical relevance, such as augmenting the clearance of tissue debris or denatured proteins; these other roles need further analysis in both experimental and clinical studies.

L. VAN DE WATER
Department of Pathology, Beth Israel Hospital, Boston, Massachusetts 02215
A. T. DESTREE
Department of Cardiology, Children's Hospital Medical Center, Boston, Massachusetts 02115
R. O. HYNES
Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. T. M. Saba and E. Jaffe, *Am. J. Med.* **68**, 577 (1980).
2. D. F. Mosher, *Prog. Hemostasis Thromb.* **5**, 111 (1980); E. Ruoslahti, E. Engvall, E. G. Hayman, *Collagen Res.* **1**, 95 (1981); H. K. Kleinman, R. J. Klebe, G. R. Martin, *J. Cell Biol.* **88**, 473 (1981); R. O. Hynes, *Cell Surf. Rev.* **7**, 97 (1981); *Cell Biology of the Extracellular Matrix* (Plenum, New York, 1982), pp. 295–333; ——— and K. M. Yamada, *J. Cell Biol.* **95**, 369 (1982).
3. J. E. Doran, A. R. Mansberger, A. C. Pease, *J. Reticuloendothel. Soc.* **27**, 471 (1980); P. W. Gudewicz, J. Molnar, M. Z. Lai, D. W. Beezhold, G. E. Siefring, R. B. Credo, L. J. Lorand, *J. Cell Biol.* **87**, 427 (1980); D. Marquette, J. Molnar, K. M. Yamada, D. Schlesinger, S. Darby, P. Van Alten, *Mol. Cell. Biochem.* **36**, 147 (1981).
4. L. Van De Water, S. Schroeder, E. B. Crenshaw, R. O. Hynes, *J. Cell Biol.* **90**, 32 (1981).
5. T. M. Saba, F. A. Blumenstock, W. A. Scovill, M. Bernard, *Science* **201**, 622 (1978); W. A. Scovill, T. M. Saba, F. A. Blumenstock, M. Bernard, S. R. Powers, *Ann. Surg.* **183**, 521 (1978).
6. P. Kuusela, *Nature (London)* **276**, 718 (1978).
7. D. F. Mosher and R. A. Proctor, *Science* **209**, 927 (1980).
8. D. R. Senger and R. O. Hynes, *Cell* **15**, 375 (1978).
9. K. Sekiguchi and S. I. Hakomori, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2661 (1980); B. A. Atherton and R. O. Hynes, unpublished data.
10. K. Alitalo, T. Movi, A. Vaheri, *J. Exp. Med.* **151**, 602 (1980); S. Johansson, K. Rubin, M. Hook, T. Ahlgren, R. Seljelin, *FEBS Lett.* **105**, 313 (1979); B. Villiger, D. G. Kelley, W. Engleman, C. Kuhn, J. A. McDonald, *J. Cell Biol.* **90**, 711 (1981); S. I. Rennard, G. W. Hunninghake, P. B. Bitteman, R. G. Crystal, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7147 (1981).
11. M. E. Lanser and T. M. Saba, *J. Reticuloendothel. Soc.* **30**, 415 (1981).
12. R. A. Proctor, E. Prendergast, D. F. Mosher, *Blood* **59**, 681 (1982); H. A. Verbrugh, P. K. Peterson, D. E. Smith, B.-Y. T. Nguyen, J. R. Hoidal, B. J. Wilkinson, J. Verhoef, L. T. Furcht, *Infect. Immun.* **33**, 811 (1981).
13. H. Isliker, D. H. Bing, R. O. Hynes, *The Immune System*, C. M. Steinberg and S. Lekovits, Eds. (Karger, Basel, 1981), vol. 2, pp. 231–328; E. J. Menzel, J. S. Smolen, L. Liotta, K. B. M. Reid, *FEBS Lett.* **129**, 188 (1981); E. Pearlstein, J. Sorvillo, I. Gigli, *J. Immunol.* **128**, 2036 (1982); D. H. Bing, S. Almeda, H. Isliker, J. Lahav, R. O. Hynes, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4198 (1982).
14. S. W. Kessler, *J. Immunol.* **115**, 1617 (1975).
15. E. Engvall and E. Ruoslahti, *Int. J. Cancer* **20**, 1 (1977).
16. This research was supported by grant R01CA17007 from the National Cancer Institute, by a Muscular Dystrophy postdoctoral fellowship (to L.V.D.W.), and by a National Cancer Institute research career development award (to R.O.H.). Purified collagenase was a gift from C. Little.

30 August 1982; revised 19 October 1982

Bacteria-Free Sea Urchin Larvae: Selective Uptake of Neutral Amino Acids from Seawater

Abstract. *Bacteria-free suspensions of larvae of* Strongylocentrotus purpuratus *(Stimpson) were prepared without the use of antibiotics. Net rates of removal of 18 amino acids, each supplied at 125 nanomoles per liter, and the appearance of ammonia were measured by high-performance liquid chromatography. Taurine and acidic and basic amino acids were not taken up. Removal of neutral amino acids from the medium occurred at rates adequate to contribute to the carbon and nitrogen balance of the larvae at ecologically relevant substrate concentrations.*

Strong antibiotic solutions have been used in the past to produce bacteria-free soft-bodied marine invertebrates, but the process is lengthy and success is not assured (*1*). Antibiotics may kill only certain groups of bacteria and may actually increase total bacterial numbers by removing interspecies competition (*2*). Even if successful, the intensive use of antibiotics may inhibit animal growth, and the toxic effects can persist for several weeks after the initial exposure (*3*). A reliable technique has been introduced whereby axenic marine invertebrate larvae can be obtained by taking advantage of the fact that gametes in the gonad are free of bacteria (*4*).

We have studied axenic larvae of the sea urchin, *Strongylocentrotus purpuratus*, obtained without the use of antibiotics. Adult animals were collected from the Newport Bay area in southern California. When brought to the laboratory, they were injected with several milliliters of 0.5*M* KCl to induce spawning. After this treatment, animals continued to release gametes for at least 30 minutes. The surface spines and tissues on the aboral surface of spawning animals were removed, and the cleaned area was sprayed with 95 percent ethanol. All reagents and equipment were sterilized by autoclaving and aseptic procedures were followed. The animals were handheld over a 10-ml beaker of MBL-seawater (Marine Biological Laboratory) (*5*) in a transfer hood with the gonopores just touching the surface of the water. The first gametes shed were discarded. Subsequent eggs and sperm obtained in this way were sterile (*6*).

The possibility that marine invertebrates may obtain an important nutritional supplement by absorbing dissolved organic material (DOM) directly from seawater has long been of interest to marine biologists (*7*). Previous studies were done in the presence of heterotrophic microorganisms, and therefore bacteria may have contributed to the

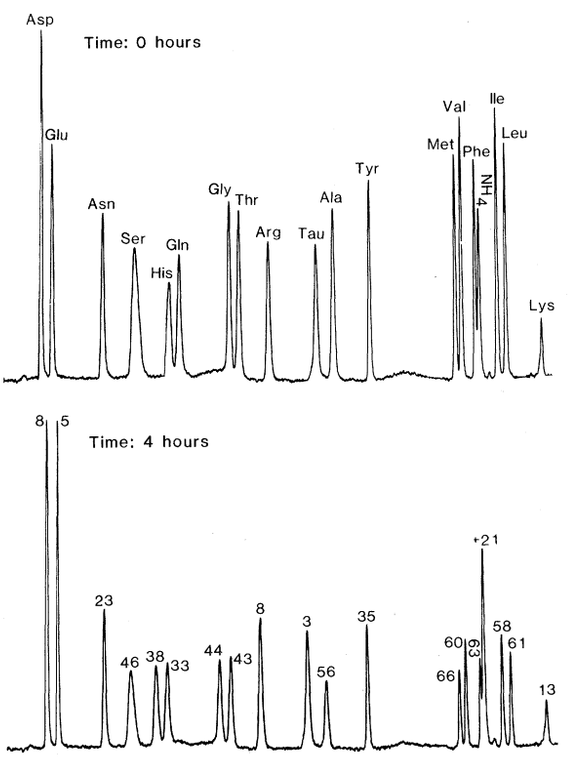

Fig. 1. Chromatograms (HPLC) of the uptake of 18 amino acids and the release of ammonia by axenic sea urchin larvae (*S. purpuratus*). The top tracing (at start) shows the amount of amino acid present in a 400-μl sample. Each amino acid is present at 125 n*M*. The bottom tracing shows the amino acid concentrations after a 4-hour exposure to larvae (102 per milliliter). The numbers above the peaks give the percent of each amino acid removed and the amount of ammonia released. Although the glutamic acid peak has increased in height, the total area has decreased by 5 percent. Concentration is related to area, not peak height (*9*). Amino acid abbreviations are: Asp, aspartic acid; Glu, glutamic acid; Asn, asparagine; Ser, serine; His, histidine; Gln, glutamine; Gly, glycine; Thr, threonine; Arg, arginine; Tau, taurine; Ala, alanine; Tyr, tyrosine; Met, methionine; Val, valine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; Lys, lysine.

apparent uptake of DOM (*8*). Until now, it has not been possible to resolve this issue because axenic soft-bodied marine invertebrates have been unobtainable.

All larvae were grown at 16°C for 5 to 7 days after fertilization. Immediately before an experiment, the larvae were siphoned onto a 48-μm mesh screen and washed several times with MBL-seawater. Samples of larvae (30 to 100 larvae per milliliter) were placed in flasks containing 300 ml of MBL-seawater at 16°C that had been sterilized by passing it through a 0.2-μm membrane filter (Nuclepore). The experimental medium contained 18 amino acids in solution, each present at 125 nmole/liter (Fig. 1). The uptake of these 18 amino acids by axenic larvae was measured by determining the rate of removal of amino acids from solution by high-performance liquid chromatography (HPLC) (*9*).

The percentage of each amino acid removed is given above the individual peak (Fig. 1). Observations at intermediate times verified that depletion of amino acids was exponential. In control experiments without larvae present in the medium, there was no removal of amino acids. Clearly, there are dramatic differences in the rates of uptake of individual amino acids by sea urchin larvae. The most rapid uptake occurs with neutral amino acids. Methionine, phenylalanine, leucine, and valine have the fastest removal rates—66, 63, 61, and 60 percent, respectively. There is no statistically significant uptake of taurine, or of the acidic and basic amino acids. The apparent rates of removal of aspartic acid, glutamic acid, arginine, lysine, and taurine (Fig. 1) are within experimental error (*9*). In another experiment, selective uptake of nine "essential" amino acids by urchin larvae was observed (Fig. 2). Again, the basic amino acids arginine and lysine were not transported; histidine and threonine were taken up, but only at about half the rate of isoleucine, leucine, valine, phenylalanine, and methionine. This pattern of different rates of uptake of neutral, basic, and acidic amino acids was observed in five experiments with axenic larvae from four separate spawnings of sea urchins. In all experiments there was no measurable net loss of amino acids from larvae.

The rate of uptake (data of Fig. 1) by larvae of each amino acid was calculated as a first-order depletion constant (k):

$$k = (\ln [S]_0 - \ln [S]_t)/t$$

where $[S]_0$ is the substrate concentration at the start of the experiment and $[S]_t$ is the concentration after 4 hours.

The uptake rate for each individual neutral amino acid was calculated by multiplying the initial amount supplied by the depletion constant, k. The total uptake from an individual concentration of 125 nM for each of the 13 neutral amino acids was (per larva) 2.75 pmole/hour. During a 4-hour experiment (Fig. 1), the ammonia in the medium increased from 0.429 μM (at the start) to 0.518 μM (at 4 hours).

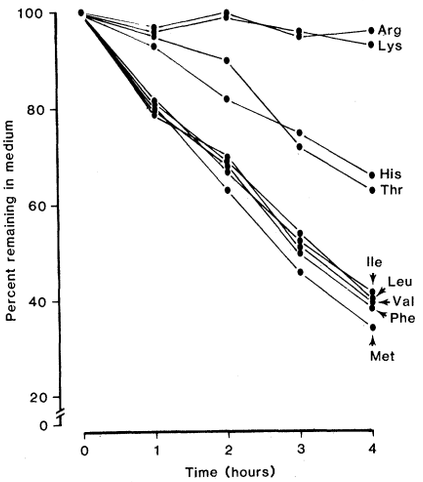

Fig. 2. The simultaneous uptake of nine "essential" amino acids by axenic sea urchin larvae of *S. purpuratus*. Each amino acid was initially present at 125 nM in a medium containing 98 larvae per milliliter. The amino acid concentrations in a 400-μl sample were measured by HPLC. Each data point represents a single value. Standard amino acid abbreviations are used (see legend to Fig. 1).

Amino acid transport in nonaxenic sea urchin larvae has been studied with isotopically labeled substrates (*10*). However, the influx of isotope may not be equivalent to a net entry of that substrate (*11*). We investigated the relationship between influx and net flux by comparing the rate of removal of ^{14}C-labeled substrate to the rate of net removal of amino acid as determined by direct chemical analysis with HPLC. Samples of the medium containing either ^{14}C-labeled serine or leucine were taken every 30 minutes for 4 hours. The samples were acidified, and the radioactivity was determined after several days, with a scintillation counter. In an experiment with 43 larvae per milliliter, the rate of influx of [^{14}C]serine from a 125 nM solution was (per larva) 0.45 pmole/hour; the net flux of serine measured by HPLC was almost identical at 0.48 pmole/hour. In another experiment with a different batch of larvae at 102 per milliliter, the rate of [^{14}C]leucine influx from 125 nM was (per larva) 0.26 pmole/hour; net flux of leucine was 0.28 pmole/hour. In both cases, the influx of ^{14}C label represents net influx of that substrate. This implies net entry of amino acids against very large concentration gradients; however, the thermodynamic work required for their transport is small compared to the energy obtained from their subsequent oxidation (*12*).

The small size and resulting high ratio of surface to volume for marine larvae suggest a priori that they may be well adapted for utilizing DOM (*13*). The potential role of amino acid uptake in the nutrition of *S. purpuratus* larvae can be estimated by comparing the rate of uptake of neutral amino acids to the metabolic requirements of the larva, as measured by oxygen consumption (oxidation of a 1-mg mixture of amino acids requires approximately 1 ml of O_2). Net flux of amino acids may also contribute to growth by providing a supplement to protein reserves in eggs and larvae (*14*). In the experiment presented in Fig. 1, uptake of the 13 neutral amino acids from a total concentration of 1.63 μM was (per larva) 2.75 pmole/hour, or approximately 275 pg/hour. The oxygen consumption for the early larvae of *S. purpuratus* at 16°C is (per larva) 350 pl/hour (*15*). Thus, the uptake of amino acids at 1.3 μM in seawater could account for 79 percent of the energy requirements of the larvae, as estimated by oxygen consumption. The contribution that the uptake of amino nitrogen makes to the nitrogen balance of larvae can be estimated by comparing the rate of uptake of amino nitrogen to the rate of ammonia loss. The amount of ammonia lost to the 300 ml of medium from the larvae (102 per milliliter) during the 4-hour exposure was 267 nmole. This is equivalent to an ammonia loss (per larva) of 2.18 pmole/hour. At an amino acid uptake rate given above of 2.75 pmole/hour, a larva would be accumulating amino nitrogen at a rate of 2.75 pmole/hour. Thus, the uptake of amino acids from a concentration of 1.63 μM would keep the larva in nitrogen balance.

An amino acid concentration of 1.63 μM is high, particularly since it includes only neutral amino acids, but it is within the range reported for surface seawater (*16*). However, the microhabitat of *S. purpuratus* larvae is unknown. Echinoid larvae are sparsely represented in plankton and may be near the sediment when approaching metamorphosis, where an amino acid concentration of 1.63 μM is quite possible, and may be a conservative estimate. In larvae of marine invertebrates, a large percentage of the food intake is used for growth (*17*) and this energy cost is not considered in an estimate of metabolic requirements based on O_2 consumption. Despite these uncer-

tainties, the present work indicates that net influx of amino acids from dilute solution in seawater has the potential to contribute substantially to the carbon and nitrogen requirements of the larvae of *S. purpuratus*.

Since there were no bacteria present in the experimental material used in our study, the larvae must be the biological agent responsible for the observed removal of substrate. Influx of ^{14}C-labeled leucine and serine occurs at rates identical with those observed for net removal of these substrates. Thus, our data provides support for previous observations on uptake and assimilation of DOM by nonaxenic marine invertebrates and for observations based on influx of labeled substrate (*7*).

DONAL T. MANAHAN
JAMES P. DAVIS
GROVER C. STEPHENS
Department of Developmental and Cell Biology, University of California, Irvine 92717

References and Notes

1. R. H. Millar and J. M. Scott [*Nature (London)* **216**, 1139 (1967)] obtained axenic larvae of the European oyster, *Ostrea edulis*, by allowing them to swim for 48 hours in an antibiotic mixture of penicillin, streptomycin, and chloramphenicol made up in sterile seawater. According to their criteria for sterility, 13 out of 17 cultures treated in this way were axenic.
2. H. Hidu and H. S. Tubiash, *Proc. Natl. Shellfish. Assoc.* **54**, 25 (1963).
3. E. Chernin, *Ann. N.Y. Acad. Sci.* **77**, 237 (1959); ——— and A. R. Schork, *Am. J. Hyg.* **69**, 146 (1959); A. D'Agostino, in *Culture of Marine Invertebrate Animals*, W. L. Smith and M. H. Chanley, Eds. (Plenum, New York, 1975), pp. 109–133.
4. C. J. Langdon, in *Proceedings of the Second International Conference on Aquaculture Nutrition* (Univ. of Delaware Press, Lewes, in press) reported that the eggs and sperm of the oyster *Crassostrea gigas* are sterile in the gonad. He obtained axenic suspensions of larvae by removing the gametes directly from the gonad of ripe adults by aseptic techniques.
5. G. M. Cavanaugh, Ed., *Formulae and Methods, IV, of the Marine Biological Laboratory Chemical Room* (Marine Biological Laboratory, Woods Hole, Mass., 1956).
6. Eggs, sperm, and culture water were tested for sterility (i) by incubating portions in 5 ml of Zobell's liquid medium [1 liter of artificial MBL-seawater, 5 g of Bacto-Peptone, and 0.1 g of ferric phosphate] and (ii) by epifluorescence microscopy after acridine orange staining [J. E. Hobbie, R. J. Daley, S. Jasper, *Appl. Environ. Microbiol.* **33**, 1225 (1977)].
7. A. Krogh, *Biol. Rev.* **6**, 412 (1931); C. B. Jørgensen, *ibid.* **51**, 291 (1976); M. G. Stewart, *Annu. Rev. Oceanogr. Mar. Biol.* **17**, 163 (1979); G. C. Stephens, in *Analysis of Marine Ecosystems*, A. L. Longhurst, Ed. (Academic Press, New York, 1981), pp. 271–291.
8. J. M. Sieburth, *Microbial Seascapes* (University Park Press, Baltimore, 1975); A. B. J. Sepers, *Hydrobiology* **52**, 39 (1977); F. L. Castille and A. L. Lawrence, *Comp. Biochem. Physiol. A* **69**, 41 (1979); D. Siebers, *Mar. Biol. Progr. Ser.* **1**, 169 (1979).
9. The amino acids in the medium sample were derivatized by mixing 400 μl of MBL-seawater with 100 μl of an *o*-phthaldialdehyde (OPA) solution [P. Lindroth and K. Mopper, *Anal. Chem.* **51**, 1667 (1979)]. The OPA-derivatives were separated on an Ultrasphere ODS column (150 by 4.6 mm; particle size, 5 μm). Eluent gradients were formed with Beckman model 110A pumps with two buffers of sodium acetate and methanol (flow rate of 1.5 ml/min). The buffers were made up as described by B. N. Jones, S. Paabo, and S. Stein [*J. Liq. Chromatogr.* **4**, 565 (1981)], but with a *p*H of 6.8 to improve the separation of phenylalanine and ammonia. Analysis time for a single sample was 30 minutes. Peaks were monitored with a fluorometer and identified by elution time. The amino acids were quantified with a digital integrator calibrated with known peak standards. The maximum error in measuring the amounts (20 to 50 pmole) of each of the 18 amino acids (except lysine) was ±4 percent. Lysine has a low specific fluorescence making quantification difficult (error, ±8 percent).
10. J. M. Mitchison and J. E. Cummins, *J. Cell Sci.* **1**, 35 (1966); A. Tyler, J. Piatigorsky, H. Ozaki, *Biol. Bull. (Woods Hole, Mass.)* **131**, 204 (1966); B. J. Fry and P. R. Gross, *Dev. Biol.* **21**, 105 (1970); D. Epel, *Exp. Cell Res.* **72**, 74 (1972).
11. R. E. Johannes, S. L. Coward, K. L. Webb, *Comp. Biochem. Physiol.* **29**, 283 (1969); D. T. Manahan, S. H. Wright, G. C. Stephens, M. A. Rice, *Science* **215**, 1253 (1982).
12. G. C. Stephens, *Am. Zool.* **8**, 95 (1968); D. T. Manahan, S. H. Wright, G. C. Stephens, *Am. J. Physiol.*, in press.
13. D. T. Manahan and D. J. Crisp, *Am. Zool.* **22**, 635 (1982).
14. D. Epel and J. D. Johnson, in *Biogenesis and Turnover of Membrane Macromolecules*, J. S. Cook, Ed. (Raven, New York, 1976), pp. 105–120; D. T. Manahan, *Biol. Bull. (Woods Hole, Mass.)*, in press.
15. A. H. Whiteley and F. Baltzer [*Pubbl. Stn. Zool. Napoli* **30**, 402 (1958)] found rates of oxygen consumption of 150 and 320 pl/hour for early echinoplutei of *Paracentrotus lividus*; P. F. Scholander, C. L. Claff, S. L. Sveinsson, and S. I. Scholander [*Biol. Bull. (Woods Hole, Mass.)* **102**, 185 (1952)] reported that the oxygen consumption of early embryos of *S. purpuratus* is 100 to 350 pl/hour at 16°C. The figure we used for our calculation (per larva) of 350 pl/hour is a maximum estimate of oxygen consumption.
16. B. B. North, *Limnol. Oceanogr.* **20**, 20 (1975).
17. D. L. Holland, in *Biochemical and Biophysical Perspectives in Marine Biology*, D. C. Malins and J. R. Sargent, Eds. (Academic Press, London, 1978), vol. 4, pp. 85–119.
18. We thank Dr. C. W. Sullivan for assistance with the sterility testing of larvae and Dr. K. E. Arnold for suggestions on improving the manuscript. Supported in part by NSF grant PCM 78-09576 and Department of Commerce grant NOAA 04-8-M01-89.

23 August 1982; revised 22 November 1982

Swine Influenza–Like Viruses in Turkeys: Potential Source of Virus for Humans?

Abstract. *Influenza A viruses (subtype H1N1), recently isolated from turkeys in different areas of the United States, were determined to be closely related to strains typically associated with pigs. This conclusion was based on comparisons of H1N1 isolates from pigs, humans, ducks, and turkeys with polyclonal and monoclonal antibodies, RNA-RNA competitive hybridization, and replication studies. One of the H1N1 isolates from turkeys caused influenza in a laboratory technician, who displayed fever, respiratory illness, virus shedding, and seroconversion. These results suggest that turkeys as well as pigs are involved in the maintenance of these viruses and their transmission to humans.*

Swine influenza–like viruses (subtype H1N1) are periodically isolated from humans (*1*). There is no doubt that in some cases the viruses in humans originate from pigs. For example, viruses isolated from sick pigs and humans on farms in Wisconsin were antigenically and genetically indistinguishable (*1*). However, there is not always a clear connection between human infections and contact with pigs. The outbreak of swine influenza in soldiers at Fort Dix, New Jersey, in 1976 was not preceded by such contact (*1*), nor was the influenza in a leukemia patient who died of viral pneumonia in Nevada (*2*). This raises the question of whether other sources of swine influenza viruses exist. In this report we describe influenza A isolates (subtype H1N1) from turkeys that are virtually indistinguishable from viruses typically associated with pigs. The potential importance of these turkey viruses in the epidemiology of human influenza was established by the fact that one of the isolates caused an influenza infection in a laboratory technician.

Two viruses were isolated from six adult female turkeys that showed a sudden drop in egg production in Missouri in 1981. In a separate laboratory other H1N1 viruses were recovered from turkeys that had similar problems with egg production in Missouri, Colorado, and Kansas in 1980 to 1981 (*3*). The viruses were grown in 10- to 11-day-old chick embryos and characterized serologically as H1N1 with hyperimmune goat and rabbit antiserum (*4*).

To determine the antigenic relatedness of these turkey viruses to those in other species (*1–3*), we compared a panel of H1N1 viruses from pigs, turkeys, ducks, and humans in hemagglutination inhibition (HI) tests with antisera from ferrets recovering from infection and with monoclonal antibodies (*5*, *6*). Representative strains of the human, swine, and turkey viruses all reacted at high titers with ferret antisera to the human virus (A/NJ/8/76) and the turkey virus (A/Ty/Mo/1/81) (Table 1). The duck viruses did not react with those antisera. The three monoclonal antibodies that recognize different antigenic determinants on H1 strains show that these determinants are shared by the recent turkey, human, and swine viruses but not by the duck viruses.

To examine the other surface antigen of the turkey isolates (the neuraminidase), we compared the H1N1 viruses in neuraminidase inhibition assays (*7*) with the same ferret antisera. The turkey iso-