grafts. Here, all of the control thymectomized DBA mice had been given intraperitoneal implants of empty diffusion chambers. This type of response would appear to rule out an adjuvant effect of the Millipore material (*10*).

The viability of the thymic tissue in the chamber appears finite (*3*). In many of the chamber-bearing mice that produced antibody, necrotic tissue was found at necropsy, indicating an early beneficial effect similar to that reported in bursectomized chickens bearing bursal implants. In this case there was an enhanced formation of agglutinating antibody to *S. typhimurium* although bursal homografts were uniformly rejected by 8 days (*15*).

The diminished immunologic capacity after neonatal thymectomy, shown by depression of antibody production to most antigens, by depression of delayed hypersensitivity-like reactions, and by inability to reject homografts, has been noted also in the rat and hamster (*16*).

Subcutaneous thymic grafting in the mouse with syngeneic thymic tissue during the first week of life (*8*) or as late as 3 to 4 weeks (*17*) effectively prevented the deficiencies of thymectomy. Cytologic studies with chromosome markers (*18*) showed that the majority of cells multiplying within the thymic graft and in lymphoid organs were of host type and not of thymic graft origin. These results strongly suggested the existence of a factor, noncellular in nature, that contributes to the establishment of immunologic competence, either within the environment of thymic tissue itself or through its elaboration and effect upon seeded cells within lymphoid organs. The results presented here suggest the elaboration of a diffusible thymic factor that acts upon seeded cells in lymphoid organs, but they do not rule out the alternative concept.

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27 December 1963

Neuraminidases and Influenza Virus Infection in Embryonated Eggs

Abstract. *Highly purified neuraminidase from influenza virus prevents embryonated eggs from infection by Lee-B influenza virus. The degree of prevention afforded is equal to that of neuraminidases from* Vibrio cholerae *and from* Clostridium perfringens *when assayed at the same enzyme activity. The preventive effect is much lower against PR-8 virus. The results support the concept that the intact "receptor" containing sialic acid is required for the binding and infection of cells by influenza viruses.*

Stone (*1*) first showed that neuraminidase preparations from *Vibrio cholerae* (RDE) could prevent infection of embryonated eggs by influenza virus. She also showed that intranasal instillations of this enzyme preparation protected mice against lung infection by viruses of the influenza group (*2*).

Fazekas de St. Groth also demonstrated the enzymatic destruction and regeneration of influenza virus receptor groups (*3*). These observations have been interpreted to be the result of the destruction of specific cellular receptor sites by the enzyme, thus preventing adsorption of the virus.

This report concerns the similar behavior of a highly purified neuraminidase isolated from influenza virus and demonstrates that, per unit of enzyme activity, bacterial and viral neuraminidases have the same protective effect.

Neuraminidase with specific activities of about 2.5 units (*4*) per milligram of protein was isolated from Asian influenza virus by the methods of Mayron *et al.* (*5*) as modified by Wilson and Rafelson (*6*). Crystalline neuraminidase from Asian virus has an activity of 5.1 units per milligram of protein. Our preparation was isolated from *Vibrio cholerae* essentially by the method of Ada *et al.* (*7*). A preparation with an activity of 0.015 units per milliliter was obtained (*8*). All preparations lacked protease activity when tested by the casein-splitting procedure (*9*).

Neuraminidase with a specific activity of 20 units per milligram of protein was prepared from a culture filtrate of *Clostridium perfringens* by dialysis. The fraction precipitated between 60 and 75 percent saturation with ammonium sulfate was used.

The activity of viral neuraminidase was determined by incubating 0.2 mg of neuraminlactose, prepared by the procedure of Schneir *et al.* (*10*), with 0.4 ml of 0.1*M* phosphate buffer at *p*H 7.0. One-tenth milliliter of the enzyme containing less than 0.005 units was added, and the mixture was incubated at 37°C. Bacterial neuraminidase was assayed in exactly the same way except that 0.1*M* acetate buffer containing 0.01*M* calcium acetate was used. After 30 minutes or 1 hour at 37°C, the reaction was stopped by adding 0.5 ml of ice-cold 5 percent phos-

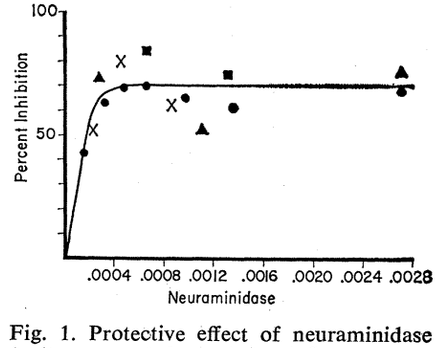

Fig. 1. Protective effect of neuraminidase (units per egg) from Asian influenza virus against Lee-B virus. Results from four separate experiments are shown by the different symbols.

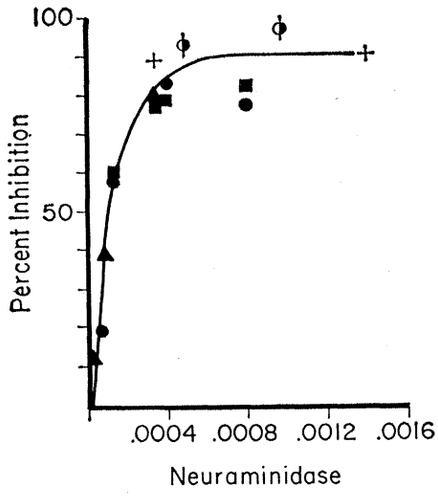

Fig. 2. Protective effect of bacterial neuraminidases (units per egg) against Lee-B virus. *Clostridium perfringens*, solid triangle, cross; *Vibrio cholerae*, half-solid circle, solid square, solid circle.

photungstic acid in 0.1*M* HCl, and the sialic acid liberated was determined on a portion as described by Warren (*11*). The results are expressed as units of enzymatic activity; 1 unit is the amount of enzyme that liberates 1 μmole of sialic acid in 1 minute under the conditions stated.

Eleven-day-old embryonated eggs were used. One-tenth milliliter of the appropriate neuraminidase was injected into the allantoic cavity, and the eggs were returned to the incubator for 2 hours before they were injected with 0.1 ml of diluted infective allantoic fluid containing Lee-B or PR-8 virus. The fluid infected with Lee-B virus had a hemagglutination titer of 640 units per milliliter—$10^{7.2}$ EID_{50}/ml (egg infectious doses, 50 percent effective)—and was diluted 1 to 1000 before administration. The PR-8 infective fluid had a hemagglutination titer of 1280 units per milliliter ($10^{8.6}$ EID_{50}/ml) and was diluted 1 to 100,000 before administration. After 48 hours the infected chorioallantoic fluid was harvested from each egg, and its hemagglutination activity determined by the method of Salk (*12*). In each test group, 10 to 20 eggs were used and the hemagglutination titers were averaged. The percentage protection is expressed as the difference in averages of control and test groups divided by the control times 100.

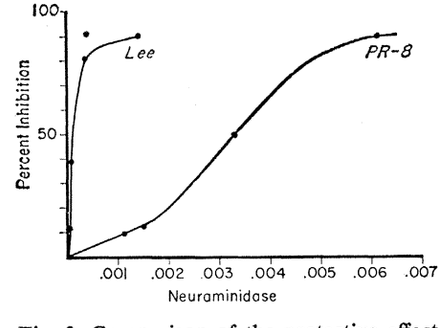

Fig. 3. Comparison of the protective effect of neuraminidase (units per egg) from *Clostridium perfringens* against PR-8 and Lee-B viruses.

The "protective" effect of several concentrations of neuraminidase isolated from Asian influenza virus on infection by Lee-B virus in four typical experiments is shown in Fig. 1. The effect becomes maximal at about 0.0004 units of neuraminidase per egg. Similar results with bacterial neuraminidases are shown in Fig. 2. Again, maximal protection occurs at about 0.0004 units per egg.

It was shown by Stone (*1*) that the protective effect of neuraminidase from *V. cholerae* varied with different viruses. Figure 3 shows that the same is true with the neuraminidase from *C. perfringens* which is more effective against Lee-B than against PR-8 virus.

These results extend previous observations with the neuraminidase of *V. cholerae*, and show that neuraminidase from Asian influenza virus and from *C. perfringens* can also protect cells of the chick embryo against infection by certain strains of influenza virus. The results are in accord with the proposition that specific receptor sites containing sialic acid on infectible cells are required for attachment and attack by influenza viruses, and that different viruses vary in the extent to which they depend on the "intactness" of these receptor sites.

Our experiments clearly show that the protective action is due to neuraminidase since very similar results are obtained with highly purified neuraminidase from several sources. The effect is a temporary one, since after 72 hours the titers of control and protected groups are comparable. This indicates that the receptor sites tend to regenerate over a period of time as previously reported by Stone (*1*).

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6 January 1964

Visual Problem-Solving in a Bottlenose Dolphin

Abstract. *A captive 8-year-old dolphin, well adapted to contact with human beings, was tested by the discrimination method for underwater perception of visual forms or patterns. The animal successfully discriminated 21 of the 25 pairs of stimuli presented. After having learned a particular combination, the dolphin was immediately able to respond to different but related pairs which had been modified in various ways. The memory of the animal for discriminations previously made was excellent.*

Although a good deal of speculation exists on the problem-solving ability of the bottlenose dolphin, *Tursiops truncatus* (Montagu), there is little scientific information on the subject. Many observers, impressed by the striking performances and playful antics of captive specimens, have been quite willing to assign a high order of achievement to this marine mammal. More than 15 years ago McBride and Hebb (*1*) observed that *Tursiops* is a very superior animal indeed, and they ranked it somewhere between the dog and the chimpanzee with respect at least to emotional and motivational behavior. Others have pointed to the size and complexity of the cerebral cortex and higher brain centers (*2*). Since degree of cerebral complexity is often thought to imply behavioral capability, we have