Protective Effect of Intravenous Mumps Vaccine in Early Neonatal Mice.* (27390)

WALDON B. WACKER, MURRAY M. LIPTON, AND ALEX J. STEIGMAN
Kentucky Child Health Foundation Laboratory, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Ky.

Resistance of adult mice to certain virus diseases to which neonates are susceptible is being investigated by technics used in studies in immunologic unresponsiveness. Reported here are observations with mumps virus. Overman and Kilham's suckling mouse adapted mumps virus was chosen because of a direct correlation between resistance to infection with age, and antibody production(1, 2,3). During these investigations, we noted that the usual susceptibility of the neonatal mouse to fatal mumps encephalitis was markedly diminished by intravenous injection of mumps vaccine 24 hours before intracerebral challenge with the living virus(4). The following experiments were therefore done to confirm and extend this observation.

Materials and methods. Neonatal Swiss Webster mice of known age were used. Material for intravenous injection consisted of 20-fold concentrated mumps vaccine in 0.1 M phosphate buffer with 0.1% formalin.† Twenty-fold concentrated influenza PR8 vaccine was also used and was prepared as follows: a pool of virus was obtained by intraallantoic injection of 11-day-old embryonated hens' eggs with 0.1 ml of 1% seed inoculum, and harvest of infected allantoic fluids after 2 days of further incubation at 37°C. The pool was clarified by centrifugation at 1,500 rpm for 15 minutes and virus was absorbed onto thrice washed and packed chicken erythrocytes in the cold, eluted in phosphate buffered saline pH 7.2 at 37°C for 3 hours, cells were removed by centrifugation at 3,000 rpm for 10 minutes and virus was then concentrated by ultracentrifugation in the Spinco Model L at 25,000 rpm for 1 hour. The pellets of packed virus were resuspended in 1/20 the original volume by addition of phosphate buffered saline containing 0.1% formalin, and thoroughly mixed by aspiration through syringe and needle. Mumps and influenza vaccines were stored at 4°C and had hemagglutinin titers (HA) of 1:2048 and 1:16,384, respectively. Both vaccines were 3-12 months old at time of use. Neonatal mice were given 0.05 ml of this material intravenously. The Kilham-Overman strain of mumps virus‡ adapted to the suckling mouse was given by the intracerebral route to determine susceptibility. The dose was 0.01 ml of a 10% suspension of infected mouse brain, which titered between 2.5 and 3.7 log LD_{50}. In all of our experiments, there were approximately 50 mice per group made up of 6 to 8 litters.

Individual and pooled mouse sera were examined for mumps antibody by the standard hemagglutination inhibition test (HI). Prior to the test, sera were treated to diminish in titer the normal serum inhibitor for mumps virus and the normal hemagglutinin for chicken erythrocytes. Receptor destroying enzyme (RDE) was prepared by filtration of a 24-hour-old broth culture of Vibrio comma strain 4Z through a Selas 02 filter, and was stored at −20°C. To 1 volume of serum was added 4 volumes of RDE containing 1,000 units of penicillin and 1,000 μg of streptomycin. After incubation overnight at 37°C, RDE was neutralized by addition of 5 volumes of 1% sodium citrate. Sera were then absorbed with thrice washed and packed chicken erythrocytes, in the ratio of 2 drops of cells to 1 ml of treated sera.

Results. In Fig. 1 is shown the sequence of events which occur when normal neonatal mice less than 24 hours old were injected intracerebrally with mumps virus. Following an incubation period of 8 to 10 days, mice develop symptoms of encephalitis. A few of the mice die soon after onset of symptoms,

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but most remain in a weakened or prostrate condition for several days before death occurs. Of interest here are the rather long incubation period before onset of symptoms, the prolonged period over which deaths occur, and the near 100% mortality.

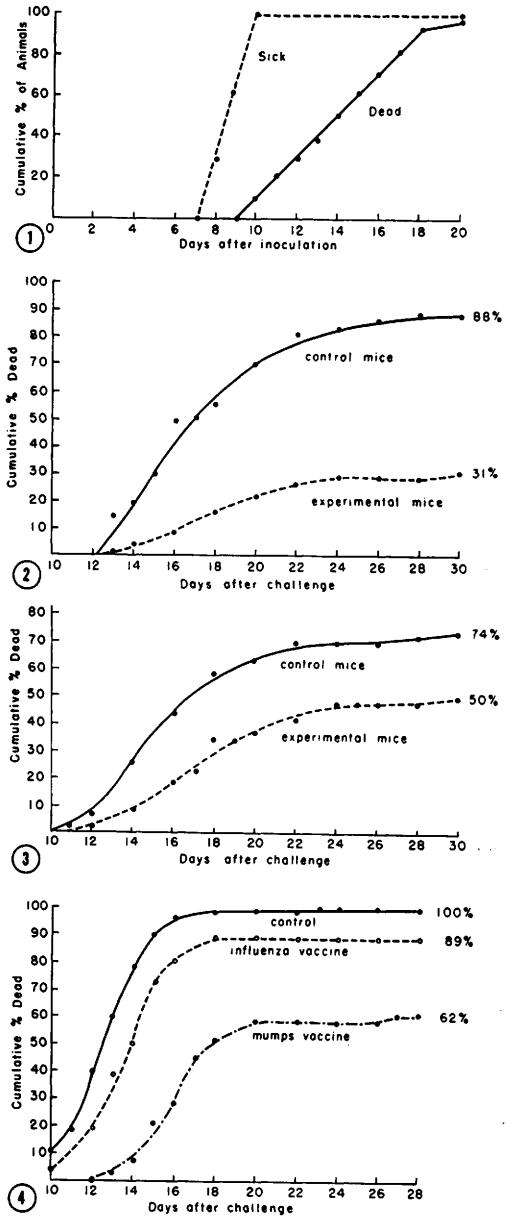

FIG. 1. Symptoms and death following intracerebral inoculation of mice <24 hr old with suckling mouse adapted mumps virus.

FIG. 2. Effect of mumps vaccine given intravenously to mice <4 hr old on development of fatal mumps encephalitis.

FIG. 3. Effect of mumps vaccine given intravenously to mice 4-24 hr old on development of fatal mumps encephalitis.

FIG. 4. Protective effect of mumps vaccine *vs* influenza vaccine (given intravenously to mice <24 hr old) in fatal mumps encephalitis.

This is the typical pattern seen in mice inoculated intracerebrally at less than 24 hours of age. The use of slightly older mice (24 to 48 hours old) results in a delay of 1 to 3 days in onset of symptoms, and a greater number of mice recover from the infection. Mice 3 to 7 days old at time of challenge have mortality rates of about 50% and 0%, respectively. Thus, the mouse during the first 7 days of life undergoes a change from complete susceptibility to complete resistance. These results confirm the findings of Overman(2).

Resistance is enhanced if the neonatal mice are injected intravenously with mumps vaccine approximately 24 hours prior to intracerebral challenge with the living mumps virus. In Fig. 2 are shown the results of an experiment in which newborn mice less than 4 hours of age were injected intravenously with mumps vaccine. Untreated mice of the same age were set aside as controls. Twenty-four hours later both groups of mice were inoculated intracerebrally. Symptoms of mumps encephalitis began about the 10th day, and by the 12th to 13th day all of the mice in both groups were sick. The vaccine thus did not prevent infection. It did, however, lessen the severity of symptoms from which many mice recovered. In the experimental group symptoms progressed to frank prostration and death in only 31% of the animals, whereas in the control group 88% of the mice became prostrate and died. The remaining animals in both groups recovered completely from the infection and appeared normal at 6 weeks of age. In other experimental controls, intravenous injection of neonatal mice with 0.1% formalin in phosphate buffered saline alone had no significant effect on susceptibility to fatal mumps encephalitis.

Similar, but less pronounced differences, were obtained with slightly older mice (Fig. 3). Here the mice were 4 to 24 hours old at time of vaccination and 28 to 48 hours old at time of challenge. Mice in both groups developed symptoms of mumps encephalitis,

which terminated fatally in 50% of experimental mice and 74% of control mice. This experiment was done simultaneously with the previous one, and with the same reagents. Comparison of Fig. 2 and 3 shows that among the controls there were fewer deaths in the older group (74%) than in the younger group (88%). This result is a reflection of the increasing resistance with age to mumps virus. It will also be noted that a greater percent of the older experimental animals died than did the younger, indicating that the protective effect of intravenous vaccination diminishes rapidly with age of the animal. In a similar experiment, mice were vaccinated at 2 days of age and challenged 24 hours later. About the same number (50%) of animals died in the vaccinated as in the unvaccinated control group. The protective effect of vaccination therefore diminishes with age at injection, and is largely dissipated by the time animals are 3 days old.

This protective effect might be due to viral interference or to antibody production, or perhaps a combination of both. To study the problem further the effect of a heterologous vaccine on this susceptibility of neonatal mice was therefore investigated. Influenza virus was chosen because of its relationship to mumps virus in the red cell receptor gradient series. In the experiment shown in Fig. 4 two groups of mice less than 24 hours old were injected intravenously, one with concentrated mumps vaccine and the second with concentrated influenza vaccine. A third group was set aside as untreated controls. Twenty-four hours later animals in the 3 groups were inoculated intracerebrally to determine their susceptibility to mumps virus.

All of the mice became ill with mumps encephalitis. Symptoms at onset and for several days thereafter were somewhat less pronounced in the 2 vaccinated groups than in the controls, and deaths occurred much earlier in the control group. By the 12th day, 40% of the controls had died as against 18% and 0% of the mice given influenza and mumps vaccine, respectively. Nearly all observed deaths occurred by the 18th day, and survivors to that time usually recovered. At termination of the experiment 100, 89 and 62% of the controls, influenza treated and mumps treated mice, respectively, had died. Similar results were obtained in a second experiment in which the challenge inoculum was a 2% suspension of infected mouse brain. Thus, the sparing effect, although less than that obtained with mumps vaccine, was also produced by influenza vaccine.

To study the possible role of antibody in this phenomenon, we examined sera from some of the mice in experimental and control groups at intervals after injection and after recovery from encephalitis. In agreement with the results of Overman(2), we have been unable to detect mumps antibody by the HI test in mice 7 days of age or less when previously vaccinated at birth. However, at the 10th day of age, there was some suggestion of antibody, manifest in the experimental group as serum HI titers of 1:10 or 1:20 and in the control group at 1:10. At the 20th day of age, serum HI titers of 1:20 or 1:40 were found in both groups. Mice 4 to 6 weeks old recovered from mumps encephalitis, had antibody titers of 1:40 or 1:80. Differences in HI titer between mice of the 2 groups did not exceed the 2-fold technical-error range in any given age group.

In another experiment, groups of suckling mice of selected age and adult mice were injected intravenously or intraperitoneally with 0.05 ml of 20× mumps vaccine to determine the effect of age on antibody response. Two weeks after injection mice were bled and HI titers were obtained on sera pooled from 4 to 5 mice in each age group. For controls, pooled sera from normal unvaccinated mice were similarly examined. There were increased antibody titers with age of the animal (Table I). Sera of mice injected soon after birth had HI titers of 1:10 two weeks later, in contrast to titers of 1:80 found 2 weeks after injection of 21-day-old mice. Of particular interest were the increased antibody titers occuring with maturation of the animals, which may have been a factor in the recovery of mice from mumps encephalitis.

Discussion. Suckling mice are susceptible to many viral agents to which adult mice are resistant. Interpretation of this phenomenon

TABLE I. Relation of Antibody Response to Age of Animal Following Inoculation with Mumps Vaccine.

Age at immunization*	Serum HI titer when bled 14 days later†
4 hr	1:10
24 "	1:10
4 days	1:20
8 "	1:20
10 "	1:40
21 "	1:80
Over 6 wk	1:80

* Through age 4 days, inj. intrav. Over 4 days, inj. intraper.
† Serum titers of control mice of all ages <1:10.

varies with the investigator and system used. Newborn mice were given concentrated mumps vaccine intravenously to study the role of antibody in age dependent resistance. These experiments were designed to break through the resistance of older mice to mumps virus by the mechanisms operative in immunologic tolerance. Rather than increasing susceptibility, however, this treatment enhanced resistance of suckling mice to fatal mumps encephalitis. Workers who first noted changes with age in susceptibility to mumps virus ascribed this in hamsters and mice to development of the immune mechanism(1,2,3). Our studies in part confirm these observations. In addition, passive immunization of suckling mice during the incubation period was reported to prevent fatal infection(3).

It seems unlikely, however, that differences in recovery rates between vaccinated and unvaccinated mice can be attributed solely to antibody, because: 1. In neonates injected intracerebrally or intravenously, antibody was not detected until after the 7th day of life, an age when passive immunization against this virus has been reported to be ineffective (3). Although failure to detect antibody in young suckling mice may be due to inefficient technical methods, it seems significant that similar problems were not encountered in older vaccinated mice. 2. Following intracerebral injection with living virus, there was no significant difference in antibody titers between vaccinated and unvaccinated mice, from the earliest time it could be detected through the 20th day. 3. The protective effect of vaccine was greatest in newborn mice, less in 1-day-old mice, and least in 2-day-old mice, a diminishing effect with age that is the opposite of what might be expected from antibody production. 4. The protection lacked immunologic specificity, being also partially induced by influenza vaccine.

The last mentioned finding suggests that viral interference may be involved here. Reciprocal interference between mumps and influenza viruses occurs in the embryonated egg(5). Timing and dosage relationships used in our experiments are of the order used to demonstrate interference. Thus, a very large dose of concentrated inactive virus, approximately equivalent to ½ the estimated blood volume of the newborn mouse, was given 24 hours before injection of a relatively small amount of living virus. Unlike technics used in demonstration of viral interference, however, the 2 injections were given by different routes. Histologic lesions caused in the brain by mumps virus occur primarily in and around blood vessels which have been suggested as the sites of virus growth(3,6). In addition, increased intravascular pressure from intravenous injection may favor extrusion of virus from capillary walls into central nervous system tissue. It is therefore possible that cells in which intracerebral mumps virus multiply may also be exposed to inactive virus given intravenously.

The diminution of sparing effect by vaccination with advancing age above the 4-hour group may be related to changes in capillary permeability occurring with age. Thus, fewer inactive viral particles may pass through capillary walls of the central nervous system in older than younger neonates. Viral multiplication may then be suppressed by interference more readily in younger neonates. (In this connection, trypan blue given intravenously was found to color the brain substances of 1-day-old mice more densely than of 3-day-old mice.)

Although this explanation favors the classical type of viral interference, interferon induced by vaccination may be a factor in these results. Production of interferon in tissues of infected animals has been reported(7,8), and recently has been suggested as the cause

of interference between virus inocula given at distant sites(9). However, the lack of effectiveness of mumps vaccine in older mice may make the role of interferon unlikely.

From these considerations it seems likely that viral multiplication was partially suppressed by interference, and was further inhibited as the immune mechanism developed. This interpretation takes into account the observation that vaccinated animals developed encephalitis, though less severely than unvaccinated controls, and further that there was evidence of recovery between the 16-18 day, when according to Overman(3,4) mice have fully developed antibody-forming mechanisms. The possible sparing effects of other myxoviruses, as well as unrelated viruses will be studied.

Summary. A striking degree of protection from fatal mumps encephalitis is afforded mice if they are injected intravenously with mumps vaccine when less than 4 hours old. A less marked but very significant degree of protection is also afforded by influenza vaccine used in this same way, but none at all using the formalin diluent alone. With advancing age above the four-hour group this sparing effect diminishes. These results may be due to combined effects of viral interference and antibody production.

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Alteration of Bile Salts by Bacteria.* (27391)

O. W. PORTMAN,† S. SHAH, A. ANTONIS, AND B. JORGENSEN
(Introduced by F. J. Stare)
Department of Nutrition, Harvard University School of Public Health, Boston, Mass.

A variety of studies of the effects of microorganisms on sterols and steroids has been made. Talalay(1) has reviewed many of these, including certain studies of bile acids. The use of aerobic conditions in which hydroxyl groupings were converted to ketones has been the most commonly reported experimental technic. The influence of bile salts on growth of microorganisms has also been studied extensively, particularly in working out methods for isolation of bile salt resistant organisms(2).

In the whole animal the bile salts present in the intestinal tract undoubtedly account, in part at least, for the paucity of microorganisms in the small intestine and the character of the microflora in the large intestine. The microorganisms of the large intestine modify the chemical nature of the bile acids and cholesterol carried from the upper gastrointestinal tract. The most prominent bile acid in the feces of animals(3) and man(4) is often deoxycholic acid. It has been demonstrated that cholic acid in the intact mammalian organism is reduced to deoxycholic (3α, 12α-dihydroxycholanic) acid(5,6,7,8), although keto-bile acid derivatives of cholic acid have also been found in feces(6,9). Bergstrom(10) considers that deoxycholate

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