Bacterial Allergy Increases Susceptibility to Influenza Virus in Mice.* (22037)

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We found previously that the vaccination of mice with *H. pertussis* vaccine increased their susceptibility to infection with several unrelated species of Gram-negative bacteria; such mice succumbed from a smaller number of live bacteria than normal mice. These results stimulated our interest in determining whether sensitization of mice with *H. pertussis* vaccine could make them more susceptible to virus. Experiments on this subject so far performed with influenza virus are described below.

Materials and methods. We used the CFW strain female mice of different ages: young mature females weighing about 22 g and weanlings weighing 10-12 g. Influenza virus A, strain WS, harvested in chick embryo allantoic fluid, with a hemagglutinin titer of 1:1280 was used for the intranasal inoculation of the mice. Sensitization was performed by the intraperitoneal injection of 15 billion cells of commercial *H. pertussis* vaccine. Experiments with sensitized mice were started 5-7 days after vaccination with *H. pertussis* and mice were observed for 2 weeks after the administration of the virus.

Results. Immunization with *H. pertussis* antigen considerably reduced the resistance to virus in mice of different ages. Normal and sensitized mice died within 5-10 days after an inoculation with the virus; however, the lethal dose of the virus for sensitized mice was much smaller than for normal mice of the same age group (Table I).

The young weanling mice which had been inoculated with the *H. pertussis* vaccine had become hypersensitive and could be shocked with the homologous antigen. The shocking dose of *H. pertussis* nucleoprotein had no effect on the normal, but killed 3 out of 6 of the sensitized weanlings. Adult mice injected with the same number of *H. pertussis* cells became sensitized to a higher degree; the same shocking dose of nucleoprotein produced up to 80-90% of anaphylactic deaths among the sensitized adult mice.

Four control groups of sensitized mice were used to demonstrate the mode of action of the influenza virus (Table II). When the influenza virus was inactivated and inoculated intranasally into sensitized mice it did not kill them, in contrast to the active virus, which in a dilution of 1:10000 given intranasally killed most of the sensitized mice. However, active virus, even undiluted, was well tolerated by sensitized adult mice if it was injected intraperitoneally.

Special studies were performed on the effect of sensitization by *H. pertussis* on the protection of mice by specific anti-viral antibodies. Immune serum was prepared by the injection of the same WS strain of influenza virus into rabbits. The immune serum had an anti-hemagglutinin titer of 1:5000. We studied the effect of the antibodies on the inactivation of the virus *in vitro* and *in vivo*. In

TABLE I. Susceptibility to Influenza Virus of Normal and Sensitized Mice of Different Ages.

Virus given .05 ml intranasally in dil.	Adult mice			Weanling mice		
	Normal	Sensitized	P value	Normal	Sensitized	P value
1: 1000	15/30	18/23	$.10 > P > .05$	31/35		
1: 10000	1/17	13/17	$P < .001$	16/22		
1:100000				6/19	15/18	$P < .01$

No. of dead mice in numerator; total No. of mice in denominator.

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TABLE II. Different Pretreatments of Influenza Virus and Different Routes of Administration to Sensitized Adult Mice.

Material used for inoculum	Route of admin. and dilution	Results
Virus inactivated 30 min. at 56°C	Intranasally, .05 ml, dil. 1:100	0/ 6
Virus inactivated by incubation with 1% formalin at room temp., 24 hr	*Idem*	0/ 6
Allantoic fluid from non-infected eggs, same age as those used for harvest of virus	Intranasally, .05 ml, undil.	0/ 5
Active virus	Intraper., .2 ml, undil.	0/ 6
Idem	Intranasally, .05 ml, dil. 1:10000	13/17

the first series of experiments the virus in a dilution of 1:10 was mixed with an equal volume of antiserum diluted 1:10. After 30 minutes incubation at room temperature, .05 ml of the mixture was administered intranasally to each mouse. As a control, virus diluted in saline 1:20 was given intranasally in .05 ml volume. Normal and sensitized mice were used for this test and, since the results of both groups were the same, the data were combined. Virus inactivated with antiserum *in vitro* was not infectious for either normal or sensitized mice.

Different results were obtained in experiments in which mice received antiviral serum intraperitoneally and virus intranasally. In this case the protection of sensitized mice was very poor in comparison to normal mice. In one experiment some mice received immune serum undiluted; others in dilutions of 1:10 and 1:100; since protection with serum undiluted and diluted 1:10 was about the same in each group of normal and sensitized mice, the data were combined. Half of the mice in our tests were sensitized by *H. pertussis* vaccine as described above. In these experiments mice started to die 5-10 days after the injection of virus and were kept under observation for 2 weeks (Table III). An autopsy of the dead mice revealed lesions in the lungs typical of an influenza virus infection.

Discussion. The striking effect of the *H. pertussis* vaccine on the sensitization of mice and guinea pigs was described by the author and his collaborator in 1947(1). The development of increased sensitivity to histamine after vaccination with *H. pertussis* was observed by us in 1948(2), and since that time various studies of this phenomenon have been performed(3-5,8). In 1953 we observed(6) that vaccination with *H. pertussis,* which produced an immunity to the homologous organism, at the same time lowered the resistance of the mice to non-related infections with different Gram-negative bacteria and increased their susceptibility to *Shigella* endotoxin(7).

(1) Vaccination of mice of different ages with the *H. pertussis* antigen increased their sensitivity to the influenza virus (Tables I and III). The increase in the death rate in vaccinated mice in comparison to the normal animals of the same group could not be attributed to anaphylactic shock, but rather to the lowering of their resistance to an influenza virus infection. In vaccinated mice, as in normal animals, death occurred between 5-10 days after the administration of the virus. However, sensitized mice died from a smaller dose of virus. An autopsy of these mice showed that death was due to influenza infection with typical lesions of the lungs.

(2) Incubation with specific antiserum inactivated virus and rendered it non-infective for normal and for sensitized mice.

(3) Injecting antiserum intraperitoneally into normal mice produced considerable protection to intranasal inoculation of the virus.

TABLE III. Protection of Normal and Sensitized Mice by Antiviral Antibodies against Virus Administered Intranasally in Volume of .05 ml Diluted 1:20.

Serum inj. intraper. .2 ml	Mice vaccinated by *H. pertussis* vaccine	Normal (not vaccinated) mice	P value
Undil. and dil. 1:10	21/22	10/25	P <.001
Dil. 1:100	6/ 6	6/ 6	
No serum (control)	11/11	10/10	

On the contrary similar antiserum treatment gave very poor protection to the virus in sensitized mice. Apparently the state of hypersensitivity impaired the protecting qualities of the antibodies.

The observations presented in this report may have special interest for prophylactic work. Due to the present-day mass immunization of children with various bacterial antigens (including *H. pertussis*) and the possible increase of the number of cases of bacterial allergy, the regular test for the safety of a new vaccine on normal animals may not be adequate. Our studies offer a very simple technic for testing a new vaccine on hypersensitive mice.

Summary. Experiments with mice indicate that hypersensitivity induced by the *H. pertussis* antigen can increase susceptibility to influenza virus.

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New Olive Oil Emulsion for Lipase and New Observations Concerning "Serum Lipase". (22038)

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Emulsions prepared from olive oil, or similar fats, tend to separate on standing after short periods of time. For tests of lipase activity stable emulsions are required. In addition, there is in these tests a correlation between the degree of emulsification and the amount of lipase activity detected(1). In the present paper a simple method for preparing a stable emulsion of olive oil is described. With this emulsion the "lipase activity" of various sera was determined. In addition the following points were investigated: (a) the activation of serum lipase by Lima bean trypsin inhibitor, (b) a method for demonstrating and determining antilipase in serum, (c) other observations on lipase.

Methods. Preparation of a stable olive oil emulsion. The emulsion is a conventional olive oil-acacia mixture to which there is added 0.25% of a new hydrophilic colloid "Carbopol 934" (B. F. Goodrich Chemical Co.). Carbopol 934, having carboxyl groups, disperses in water to yield a low viscosity acid solution which on neutralization turns into a stable gel. Toluene (0.5%) is also incorporated into the emulsion to prevent bacterial growth. Control tests demonstrated that toluene does not inhibit lipase action. One gram Carbopol 934 and 200 ml of distilled water were mixed in Waring blendor until dispersion was complete (5 minutes). A solution of 5 g of acacia (McKesson, U.S.P.) in 100 ml of distilled water was added to the Carbopol solution; the mixture was stirred in a Waring blendor for 5 minutes and then filtered through glass wool. The blendor must be thoroughly washed at this time to remove the small particles of Carbopol which would interfere because Carbopol is a strong acid. The colloid mixture was then returned to the blendor, and 100 ml of olive oil (Fisher, U.S.P.) were added in a slow stream during 5 minutes with stirring. Without discontinuing mixing, N sodium hydroxide was then added dropwise until a pH of 7.00 was obtained. Usually 10.3 ml N sodium hydroxide