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Human Febrile Response to Influenza Virus or Its Ether Isolated Hemagglutinins. (32180)

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An earlier study(1) has shown that monovalent influenza virus vaccines prepared from two strains of influenza A virus processed to yield either intact virus vaccines or vaccines composed of the ether isolated hemagglutinins induced equivalent hemagglutinating inhibiting (HI) and neutralizing antibodies in children and adults. Additionally, the isolated hemagglutinin vaccines failed to induce febrile responses in children, while the same or lower doses of intact virus were clearly pyrogenic. The earlier study did not include type B influenza virus, and the processing had included a lanthanum precipitation step designed to reduce or eliminate the soluble (S) influenza antigens. It was important therefore, to determine if ether treatment alone, without lanthanum precipitation, would render the antigens nonpyrogenic for children; and if treatment of type B influenza virus would reduce its reactivity in infants.

Only minimal antibody response could be anticipated from the single vaccine dose in the very young subjects who participated in this study. However, it was of interest to compare the immunogenicity of the standard and extracted antigen vaccines under these adverse conditions.

Methods and materials. Virus. The strains of influenza virus used for vaccine production were Asian/170, Type A-2 and Maryland/59, Type B obtained from the Division of Biologics Standards, National Institutes of Health.

Vaccines. Intact Virus Vaccine. The virus was concentrated approximately 20-fold and partially purified by differential centrifugation in the Sharples and International Centrifuges. A portion of the concentrate was inactivated by addition of formalin to a final concentration of 1 part in 2,000 and incubation at 37°C for 48 hours. The chick cell agglutinating (CCA) activity(2) of the inactive concentrate was determined and the maximum test strength vaccine prepared by dilution of an appropriate volume of the inactive concentrate with isotonic saline containing 1 part in 10,000 each of thimerosal and formalin and buffered to pH 7.2 with 0.01 molar phosphate.

Isolated hemagglutinin vaccine. To the remaining aliquot of the active monovalent concentrate from the Sharples and International Centrifuges was added 1 mg/ml of Tween 80, and the mixture was treated with 2 volumes of fresh anesthetic ether at 4°C for 5 hours. Constant agitation was maintained during the period of extraction. After separation, the ether phase was decanted and to remove residual ether the aqueous phase was aerated with nitrogen and held under vacuum at room temperature. Formalin and thimerosal were each added to a final concentration of 1 part in 10,000.

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TABLE I. Number of Subjects Assigned to the Designated Vaccine Group.

CCA units per ml	Virus type A-2 Vaccine		Virus type B Vaccine	
	Standard	Extracted	Standard	Extracted
200	10	10	10	10
400	11		10	
800	10	10	10	11
1600		10		10
Total	31	30	30	31

Final preparation and standardization of vaccines. Four vaccine strengths of each standard or ether treated monovalent concentrate were prepared for study. These contained 1600, 800, 400 and 200 CCA units/ml respectively. The vaccines of lesser antigen concentration were prepared from the maximum strength vaccine by diluting appropriate quantities of that vaccine in isotonic buffered saline containing formalin and the preservative.

Since there is some question about the utility of the CCA test as a method of standardizing ether treated influenza hemagglutinin vaccines(3,4,5) the maximum test strength ether treated vaccine was prepared by diluting this material to the same extent as the standard virus preparation without regard to its post-ether CCA titer. Each member of this series was then assigned the titer of its intact counterpart. Thus, the maximum test strength vaccine for both series contained the same amount of non-ether soluble material.

Subjects for study. Vaccine reactivity was measured in 122 infants and young children attending a well baby clinic. These were allocated to the various test groups as shown in Table I. All subjects were free of clinical evidence of infection, and none had a history of previous respiratory vaccine treatment. No preinjection rectal temperature exceeded 100.9°F. The age range was from 4 months to 5 years, 9 months with a median age of one year, 4 months. Vaccines were given, 0.5 ml per dose, by intramuscular injection in the left deltoid region. Rectal temperatures were taken prior to and 24 hours following injection. The nature and dose of the vaccine given was unknown to the observers who recorded reactions.

Serological response. Homologous HI antibody titers were measured in sera taken before inoculation and 2 to 4 weeks after. All sera were heated at 56°C for 30 minutes and sera to be tested for type A-2 antibodies were treated with M/90 KIO_4. Details of the test procedures are given elsewhere(5).

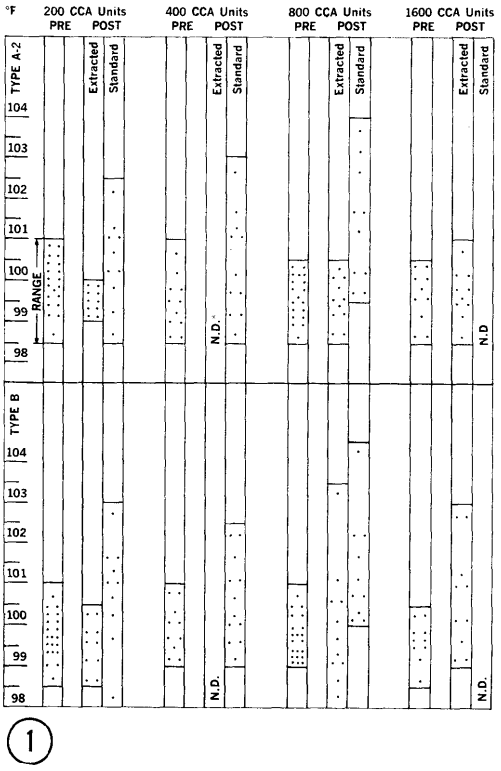
Results. Groups of 10 or 11 children were given 0.5 ml of the least concentrated material (nominally 100 CCA units). If significant temperature elevations did not occur, an additional 10 or 11 children were given 0.5 ml of a higher strength as shown in Table I. This regime was continued until a significant febrile response was observed or until the maximum test strength vaccine had been administered.

Fig. 1 shows the distribution of pre- and 24-hour postvaccination rectal temperatures of children receiving various quantities of either standard or isolated hemagglutinin vaccine prepared from types A-2 or B influenza virus.

The difference in febrile response by the two groups given the type A-2 vaccine is striking. Twenty-four hours after receiving standard vaccine, 15 of 31 children had a rectal temperature higher than the highest preinjection temperature. In contrast, not a single child had a rectal temperature above the preinjection temperature range 24 hours after receiving the isolated hemagglutinin vaccine.

The differential in febrile response of children given the two kinds of type B vaccine was not as pronounced. Again, about 50%, or 16 of 30 children receiving standard vaccine, had a rectal temperature above the preinjection temperature range 24 hours after inoculation. But 7 of 31 children receiving the hemagglutinin vaccine also had elevated temperatures at this time. While these latter responses occurred only in children receiving the two more concentrated vaccines (400 and 800 CCA units/0.5 ml dose, respectively, antigen levels considerably in excess of those useful in routine immunization), they nevertheless indicate that not all fever-inducing material was removed by ether from the type B virus.

The data in Fig. 2 compare febrile response



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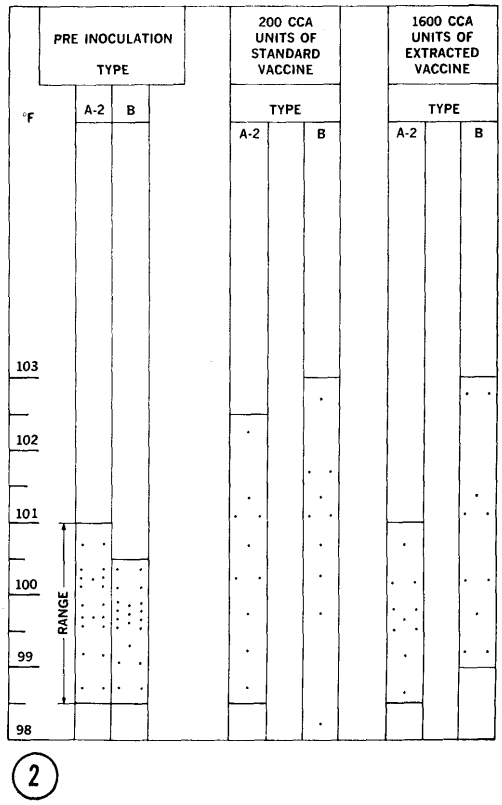

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FIG. 1. Pre and post rectal temperatures of infants given 0.5 ml of the indicated influenza virus or ether-extracted hemagglutinin vaccine.
* Not done.
FIG. 2. Comparative pyrogenicity of 0.5 ml doses of the 200 and 1600 CCA unit standard and ether-extracted vaccines.

to the least concentrated standard virus vaccine with response to the most concentrated virus antigen vaccine for types A-2 and B virus respectively. For either virus serotype, the hemagglutinin vaccine retains no more pyrogenic material than the corresponding standard vaccine diluted to an eighth the antigenic mass of the treated vaccine.

Within each set of vaccines (Fig. 1) there is a tendency for febrile response to increase with increasing vaccine concentration. However, a valid quantitation of the dose-response relationship could not be made.

Paired sera from 109 children were available for serologic evaluation. Of these, 35 responded to vaccination with a 2-fold or greater rise in antibody titer. The generally low response rate following the single dose in these children limited the size of the responding group and precluded statistical evaluation of a dose-response relationship. However, while only 11 (23%) of 53 children given the 200 or 400 CCA/ml vaccines had an antibody increase, 24 (43%) of 56 recipients of the 800 or 1600 CCA/ml vaccines responded to vaccination. Thus, higher antigen levels did elicit a better conversion rate. Table II gives the pre- and post antibody titers of the 35 children who responded to vaccination.

It is known(6,7) that immunologically inexperienced children respond very poorly to a single dose of vaccine and that previous vaccination or infection with influenza enhances a child's responsiveness. These observations were reaffirmed in the present study. Of the 109 children studied serologically, 13 had antibody to the stimulating strain prior to vaccination. Ninety-two per cent (12 of 13) of these preinoculation-posi-

TABLE II. Distribution of Pre- and Post Inoculation HI Antibody Titers of Children Responding* to the Indicated Vaccine.

Virus type	Vaccine type	Serum	Homologous serum titer†								
			<5	5	10	20	40	80	160	320	Total
A-2	Standard	Pre	4‡	2	2						
		Post		1	1	1	4	1			8
	Ether treated	Pre	6	2	2						
		Post		1	1	1	2	2	1	2	10
B	Standard	Pre	7								
		Post		3	1				2	1	7
	Ether treated	Pre	6	2	2						
		Post		3		3	2	1	1		10
Total		Pre	23	6	6						
		Post		8	3	5	8	4	4	3	35

* 35 of the 109 children responded to vaccination.
† Reciprocal of serum dilution.
‡ Number with indicated titer.

tive children responded. In contrast, only 24% (23 of 96) preinoculation-negative children had an antibody rise.

Discussion. There seems little doubt that the bothersome side reactions usually associated with influenza immunization can now be reduced, if not eliminated, from type B strain influenza vaccine as well as from type A. Further, such reduction is not dependent on the removal of non-ether soluble material from the virus suspension, since, in the present study, nothing was eliminated from the suspension beyond that which dissolved in the ether phase.

Detailed information on the influenza virus or antigen dose *vs* human infant febrile response relationship was not obtained. In fact, it now appears this information may not be needed. Antigen levels usually restricted to adult immunization can be given quite innocuously in children using ether treated vaccine.

Predictably, most of the children seem not to have encountered influenza either through illness or vaccination prior to this study. With their low resistance to infection, such children may well be the prime reservoir for human infection(6). If elimination of influenza as a public health hazard is to be achieved, the findings of this study reaffirm the need for and feasibility of a broad spectrum influenza vaccine for general use in the pediatric cohort.

Summary. Febrile response of infants to increasing concentrations of standard, intact influenza virus or its ether isolated antigens is compared. Types A-2 and B virus were used. At maximum antigen doses ether treatment eliminated fever induction by the A-2 strain and reduced this property for the B strain. For either serotype, the standard virus vaccine contains at least as much pyrogen as did the corresponding isolated antigen vaccine at 8 times the antigenic mass.

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