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Desoxycholate-split Influenza Vaccines

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Promising results have been obtained with vaccines prepared from influenza virus disrupted by treatment with desoxycholate. Trials conducted in Australia among children showed that desoxycholate-split vaccine caused no untoward reactions, even when administered in relatively large doses; similar results were obtained in adults. Methods for the large-scale production of such vaccine have therefore been developed in Australia.

During 1969 over 6 million doses of desoxycholate-split A2/Hong Kong vaccine have been administered in that country, which was reached by the Hong Kong virus variant in August 1968. However, planned field trials with this vaccine have been of little value because of the limited nature of the influenza outbreak in Australia as a whole. Nevertheless, the vaccine does appear to have afforded protection in a widespread outbreak among the aboriginal population of central Australia caused by a second wave of Hong Kong influenza.

Webster & Laver (1966b) have found that influenza virus suspensions disrupted by the action of the mild detergent sodium desoxycholate retain not only neuraminidase activity but also the ability to stimulate the production of antihaemagglutinin and neutralizing antibodies in rabbits. As had already been shown with diethyl ether disruption, there was reason to believe that desoxycholate disruption would also remove the specifically human toxicity of parenterally injected inactivated influenza virus vaccines. Furthermore, the use of desoxycholate would avoid the hazards of having to use ether in the large-scale production of these vaccines. For the above reasons preliminary experiments were conducted in Australia in humans, using a desoxycholate-split 1957 A2 Asian influenza vaccine (Webster & Laver, 1966a). This vaccine was found to be non-toxic not only in adults but also in infants given doses (2200 CCA) greatly in excess of those which were demonstrably toxic after injections of untreated vaccine. Unfortunately, simultaneous antibody studies in the infants gave results of limited value because a surprisingly large number had apparently been infected with antigenically related viruses during an epidemic which had occurred in the previous year. The desoxycholate-split vaccine did, nevertheless, stimulate the formation of antibodies in both adults and infants. Following these encouraging results, methods for the large-scale production of desoxycholate-disrupted influenza virus vaccine wers developed. The existing method of production wae retained but with the addition of only a minimum number of essential steps.

METHOD

The method may be briefly summarized as follows. Influenza viruses, grown allantoically in eggs, are deposited by centrifugation, resuspended, sonicated and inactivated with 0.05% formol. Sodium desoxycholate is added to give a final concentration of 1%, and the mixture held at 37°C for 30 minutes with constant stirring. After low-speed centrifugation to remove any aggregated material, the disrupted vaccine is dialysed for 3 days to lower the residual desoxycholate content to less than 100 parts per million.

RESULTS

In order to study the antihaemagglutinin antibody response to desoxycholate-disrupted vaccine in infants, the A/Swine strain (Shope, 1935) was selected to eliminate the possibility of prior exposure in the subjects. The antigenicity of the disrupted vaccine was first demonstrated following intraperitoneal injections in mice, and it was then shown that in adult humans the disrupted vaccine produced no unwanted reactions in any of the subjects after either initial or subsequent injections. The antihaemagglutinin responses in those without pre-

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existing antibody were satisfactory after 2 injections had been given. The results obtained when this vaccine was given to infants are shown in Table 1.

TABLE 1
RESPONSES OF INFANTS TO A/SWINE DESOXYCHOLATE-TREATED VACCINE

Dose equivalent	No. of infants	Reactions	Geometric mean antihaemagglutinin titres	
			Primary	Secondary
0.5 adult	12	Nil	32	110 [a]
0.75 adult	6	Nil	32	790
1.00 adult	6	Nil	30	350 [b]

[a] Only 11 tested.
[b] Only 5 tested.

In this trial in infants no further attempt was made to establish the minimum level for toxic reactions with untreated vaccine, but from our previous experience it is usually of the order of 0.25 of an adult dose in the age-group tested (26 months and younger). However, as a precaution, doses of disrupted vaccine equivalent to 0.1 of an adult dose (50–60 CCA units) were first given to groups of infants and, as there were no toxic reactions, the dose was gradually increased. No febrile or other signs of constitutional upset were observed in any of the infants, whose temperatures were recorded at 4-hourly intervals for 48 hours after injection. All responded with detectable levels of antibody following the first dose of vaccine, and all showed an increase in titre following a booster injection of the same dose size. All antibody titres following the second injections of either 0.75 of a dose or a full adult dose were of a high order. These results show that excellent antibody levels can be obtained in infants when relatively large doses of vaccine are used, and that these can be safely administered after the influenza virus suspensions have been disrupted with sodium desoxycholate.

In Australia, the use of unsplit influenza virus vaccines was abandoned in 1968, and in that year over half a million doses of a disrupted vaccine containing a recent type B strain and the then current A2 strain were used. Considerable data were accumulated relating to antibody responses and the lack of toxic reactions, and these will be published elsewhere. In summary, it can be stated that the disrupted divalent vaccine produced good antibody responses to both strains in adult humans, but it must be remembered that these were booster effects since it was practically impossible to find any individuals in 1968 who did not possess at least low levels of antibody in their prevaccination sera. For obvious reasons it is far more difficult to obtain quotable evidence of the absence of reactions than of their occurrence, but some large institutions which have been conducting immunization campaigns with influenza virus vaccine for many years reported a complete absence of reactions for the first time. It was easier to study the problem of reactions objectively in institutionalized infants, although again there was a surprisingly high percentage of individuals with evidence of antibody from prior infection with A2 viruses, presumably from the mild 1966 outbreak. Response to the type B component of the vaccine was, however, typical of a novel experience; antibody could be detected following a first injection, but much higher titres were obtained after a booster dose.

With the advent of the Hong Kong variant of A2 influenza viruses, it was expected that the antigenic shift would be so great that the disrupted vaccine could be studied as a novel experience in humans. This, however, did not prove to be the case, as evidenced by the failure of second injections of the Hong Kong vaccine to give more than an occasional significant rise in titre. Table 2 shows the

TABLE 2
ANTIHAEMAGGLUTININ RESPONSES OF INFANTS TO A2/NT/68

	Pre-bleed	Primary	Secondary
Geometric means	<10	85	160
Range	All <10	30–110	80–640

responses of infants to monovalent vaccines prepared from an Australian (Northern Territory) Hong Kong-type strain. Only minimal increases in antibody titres were found following second injections.

Since 1967 a total of 160 infants have received 0.5, 0.75, or 1.0 of an adult dose of desoxycholate-disrupted influenza virus vaccines containing A2 virus components. Of these, 96 received vaccine consisting of A2/Hong Kong, either as monovalent vaccine or with 40% type B. No significant toxic

reactions have been found with these vaccines, but, of 64 infants who received bivalent vaccine containing the older A2 virus, 2 showed high temperatures and vomited 12 hours after inoculation. However, it is unlikely that these reactions were due to the vaccines since both children subsequently tolerated a further adult dose 1 month later without any untoward reactions.

During 1969, over 6 million doses of desoxycholate-disrupted vaccine, composed of A2/Hong Kong and recent B type viruses, have been administered in Australia, with very few reported reactions. In the expectation of a widespread epidemic of clinical influenza associated with the new strains, a number of field trials were organized in the southern States, but these have been of little value in assessing protection because of the limited nature of the 1969 outbreak in most of Australia. In the Northern Territory, however, which was the first area to be afflicted by A2/Hong Kong, in 1968, there has recently been a second wave of influenza associated with that virus. Reports reaching us to date indicate that immunization with the disrupted vaccine has provided considerable protection in a widespread epidemic in areas around Alice Springs in central Australia. Full details are not available yet, but Table 3 shows data which are typical of the information obtained to date from the many aboriginal settlements where vaccination was, of course, made available to these most susceptible people (Langsford and White, personal communication, 1969).

TABLE 3
CLINICAL INFLUENZA IN AUSTRALIAN ABORIGINAL SETTLEMENTS (NORTHERN TERRITORY, 1969)

Station	Vaccinated		Non-vaccinated	
	Total No.	Cases of influenza	Total No.	Cases of influenza
Hermannsburg	100	Nil	454	100
Areyonga	80	14	245	66

CONCLUSION

In summary, it has been shown that disruption with desoxycholate considerably improves the safety of influenza virus vaccines. These vaccines exhibit acceptable antigenicity in man, and preliminary protection studies are promising. Further studies will be required to ascertain whether the effectiveness of these vaccines can be improved by increasing the dose beyond the current upper level, which has heretofore been limited because of the toxic reactions which have been observed following the use of untreated influenza virus vaccines.

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