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Diphtheria–tetanus–pertussis vaccine combined with *Bordetella parapertussis* vaccine

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INTRODUCTION

As a general rule whooping cough due to *Bordetella parapertussis* is, clinically, a milder type of illness than that resulting from infection with *B. pertussis*, but Zuelzer & Wheeler (1947) have described severe parapertussis infections, some of which were fatal. According to Lautrop (1958) and Vysoká-Burianová (1960) parapertussis infections are widespread and occur among children who have been immunized against pertussis as well as those who have not been vaccinated against whooping cough. Until it was appreciated that *B. pertussis* and *B. parapertussis* were antigenically not related the failure of pertussis vaccines to protect children against parapertussis infections tended to discredit pertussis vaccines and it was the recognition of the absence of any cross-protection between the two infections that induced Rambar, Howell & Deneholz (1947) to prepare a combined pertussis–parapertussis vaccine with which a group of children were inoculated and subsequently examined for the development of specific antibodies by opsonocytophagy.

The importance of *B. parapertussis* infection in the epidemiology and aetiology of whooping cough is now well recognized and in order to study further the prophylaxis of the two infections it was decided to prepare, after suitable preliminary trials, a combined pertussis–parapertussis vaccine which could be used eventually for the immunization of children. The protective value of the vaccines would be assessed by the production of agglutinins for the pertussis and parapertussis components of the vaccines. Tests would first be undertaken on batches of mice and then on groups of children.

EXPERIMENTAL METHODS

Eight experimental batches of vaccine were prepared from freshly isolated strains of *B. pertussis* and *B. parapertussis*. These vaccines were inactivated by heating at 56° C. for 30 min., merthiolate 1:10,000 was then added. The number of bacterial cells in the vaccine was determined according to the U.S.A. National Opacity Standard. Female mice from the same strain and weighing 14 g. were inoculated by the method described by Evans & Perkins (1953). Agglutinin titres were determined by my own modification (Köhler-Kubelka, 1957) of Detlor's (1951) micro-agglutination test. Later, a vaccine was prepared from *B. parapertussis*, strains 16/58 and 50/59. One half of this vaccine was mixed with Ser. 13 routine diphtheria–tetanus–pertussis (DTP) vaccine while the other half was used as a parapertussis monovalent vaccine. Different groups of mice were inoculated with

the monovalent parapertussis, DTP and the mixture of the DTP and the parapertussis (DTPParap) vaccines and the agglutinin titres were subsequently determined for the pertussis and the parapertussis components.

The actual compositions of the vaccines were as follows: monovalent parapertussis contained 1×10^9 organisms per dose of 0·5 ml.; DTPParap. contained in 0·5 ml. 1×10^9 parapertussis; 12×10^9 pertussis organisms; 20 Lf diphtheria toxoid and 10 BU tetanus toxoid with 2 mg. $AlPO_4$ and merthiolate to a final concentration of 1:10,000 and the DTP had the same composition as the DTPParap. except that it did not contain the parapertussis element.

After the safety and potency tests, as laid down by the National Institutes of Health, Bethesda, had been carried out on mice, seventeen unvaccinated children were inoculated with DTPParap. and twelve unvaccinated children were inoculated with DTP vaccine. Both groups of children received three doses of their respective vaccine in 0·5 ml. amounts at monthly intervals. Local and general reactions were recorded after each inoculation and one month after the last injection the children were bled and their sera examined to determine the agglutinin titres for *B. pertussis* and *B. parapertussis*.

RESULTS

The average agglutinin titres in mice inoculated twice with a dose of 10^9 organisms of eight different strains each of *B. pertussis* and *B. parapertussis* are shown in Table 1. It will be noted that the mean figures for pertussis and parapertussis respectively were 1:422 and 1:9976, that is to say, the titres for the mice inoculated with *B. parapertussis* were some 23 times higher than those found in mice inoculated with *B. pertussis*. There were no cross-reactions.

Table 1. *The ability of sixteen different pertussis and parapertussis vaccines to produce agglutinin in mice*

No. of mice	Vaccine	Average agglutinin titre with		Number of oganisms × 10^9	
		Pertussis	Parapertussis	Pertussis	Para-pertussis
12	Parapertussis 29/56	0	2,420	—	2
13	27/56	0	7,040	—	2
13	37/56	0	12,658	—	2
15	16/58	0	21,851	—	2
13	36/58	0	2,528	—	2
15	11/58	0	9,232	—	2
11	32/59	0	12,096	—	2
11	50/59	0	11,991	—	2
14	Pertussis 25/55	736	0	2	—
15	24/55	480	0	2	—
11	60/55	263	0	2	—
13	19/56	332	0	2	—
14	27/60	381	0	2	—
13	88/60	464	0	2	—
13	19/61	250	0	2	—
15	46/61	471	0	2	—
	Average titre	**422**	**9,976**	—	—

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The results of inoculating mice with the three vaccines: monovalent parapertussis, DTPParap and DTP vaccines, in so far as agglutination reactions with the antigens of *B. parapertussis* and *B. pertussis* are concerned, are shown in Table 2 and it is evident, although the *B. parapertussis* element of these vaccines contain only one-twelfth the number of organisms that are present in the *B. pertussis* element, that the agglutinin response for the former was approximately double that for the latter. Even in the case of the combined vaccines DTP and DTPParap. in which the agglutinin response was lower for both organisms the parapertussis titres were the higher, as the mean figure shows. Again there were no cross-reactions.

Table 2. *The ability of different vaccines to produce agglutinin in mice*

No. of mice	Vaccine	Average agglutinin titre with		Number of organisms $\times 10^9$	
		Pertussis	Parapertussis	Pertussis	Parapertussis
15	Parapertussis	0	920	0	0·2
14	Di-Te-Per	451	0	2·4	0
14	Di-Te-Per-Paraper	269	474	2·4	0·2

Table 3. *The ability of combined vaccines Di-Te-Per and Di-Te-Per-Paraper to produce agglutinin in children*

No. of children	Vaccine	Average agglutinin titre with		Number of organisms $\times 10^9$	
		Pertussis	Parapertussis	Pertussis	Parapertussis
12	Di-Te-Per	1400	0	36	0
17	Di-Te-Per-Paraper	1068	1401	36	3

The results of the tests in children are perhaps not so striking (see Table 3); the agglutinin levels are rather better for both organisms and although the parapertussis titres are still higher than those for pertussis, the difference is not so marked. It must not be forgotten, however, that there were twelve times more organisms in the pertussis component than in the parapertussis component.

In so far as the clinical reactions to the vaccine injections are concerned there was no observable difference between the different types of vaccines: a small proportion of children in each group had raised temperatures up to 38·4° C. with slight redness and infiltration at the site of inoculation but nothing more.

DISCUSSION

Owing to the general mildness of the majority of parapertussis infections it is very difficult to assess the protective value of vaccines prepared from *Bordetella parapertussis* cultures by clinical observations. Normally comparatively few of these infections are severe enough to be brought to the notice of the attending medical practitioner. Thus while large-scale clinical field trials are feasible for the

assessment of the protective value of *B. pertussis* vaccines against *B. pertussis* infections, no such simple procedure is available for the study of prophylaxis against *B. parapertussis* infections. The experiments described in this paper indicate that *B. parapertussis* is far more agglutinogenic than *B. pertussis* and accepting the fact that pertussis vaccines prepared in accordance with the recommendations of the National Institutes of Health, U.S.A., are thought to offer a reasonable degree of protection when they induce an agglutinin response in the serum of inoculated individuals of titres in the region of 1:300, it was felt that a combined vaccine which induced agglutin titres of 1:1000 or more for both organisms indicated a degree of protection against parapertussis infections at least equal to that offered by pertussis vaccine. The mouse protection test offers a method for assessing the potency of pertussis vaccine, but owing to the relatively low mouse-virulence of *B. parapertussis* such a method is not suitable for the assessment of vaccines prepared from this organism. It is therefore suggested that in the absence of any more reliable method, the estimation of agglutinin levels induced by *B. parapertussis* vaccines might be employed for the assessment of the prophylactic value of such vaccines.

In view of the increasing frequency of parapertussis infections and the possible complicating epidemiological problems arising out of this, it is recommended that immunization against whooping cough, since there is no antigenic relationship between the two organisms responsible, should be carried out with a vaccine containing both *B. pertussis* and *B. parapertussis* in suitable proportions.

SUMMARY

Investigations carried out to ascertain the ability of various strains of *Bordetella pertussis* and *B. parapertussis* to produce agglutinins have shown that the agglutinin response is considerably greater with *B. parapertussis*.

Children inoculated with a combined vaccine in which the parapertussis element contained *B. parapertussis* in only one-twelfth of the concentration of *B. pertussis* in the pertussis element showed agglutinins in their sera in titres well above 1:300 for both organisms. There were no cross-reactions and the serological responses were specific throughout. The vaccine used was the standard diphtheria–tetanus–pertussis (DTP) prophylactic to which had been added a vaccine prepared from recently isolated strains of *B. parapertussis*.

Agglutinin titres of both whooping cough components with the combined vaccine were somewhat lower in mice than was the case when monovalent vaccines were used, but they were considered to be satisfactory.

It is suggested that the agglutination production test in mice could be used for the assessment of protective power of *B. parapertussis* vaccines against infection.

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