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EXPERIMENTAL STUDY OF INTERFERENCE BETWEEN PERTUSSIS ANTIGENS AND SALK POLIOMYELITIS VACCINE

by

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Combined vaccines for immunization against sesveral diseases of man or animals are widely used. Ramon & Zoeller (1) first used Diphtheria and Tetanus toxoids combined with TAB vaccine and found that there was no interference. The wide acceptance of Diphtheria, Tetanus and Pertussis vaccine (DTP) is the ressult of intensive studies both in experimental animals and field trials. All the evidence indicates that the serological response to each of these antigens is satisfactory when the antigens are used in combination.

The addition of Salk Poliomyelitis vaccine to the combined DTP vaccine has also been the subject of many investigations during the last decade. Kendrick and Brown (2,3) found that the diphtheria and tetanus antitoxin levels in guinea pigs were lower following the combined DTP+Polio vaccine than following triple DTP. They also found the pertussis serologic response in both guinea pigs and monkeys was consistently good regardless of the combination in which it was given. Pontecorvo (4) studied the serological response of a group of young children to 3 monthly intramuscular inoculations of DTP mixed with Polio vaccine. He found a good response to diphtheria, tetanus and polio antigens but a poor response to pertussis vaccine. Wilson & al (5) have used DTP+polio vaccine in children with good response to diphtheria, tetanus and polio but the response to pertussis vaccine was not studied. In order to learn more about the serological response to polio vaccine, when it is used in combination, more experiments with small laboratory

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animals are needed.

The present paper describes the serological response of the rabbit to a combined DTP+Salk polio vaccine.

MATERIALS AND METHODS

DTP - The triple vaccine used in this study was supplied by the Department of Immunology of the Razi Institute. One ml. containing 30 Lf of diphtheria toxoid, 15 Lf of tetanus toxoid and 12 billion of pertussis organisms in phase I was combined with I mg. of aluminium hydroxide. The same composition was used without adjuvent as fluid DTP.

Pertussis vaccine

Twelve billion pertussis organisms in phase I were used with or without adjuvant.

Pertussis soluble antigen (S.A)

S.A. was prepared by shaking a fluid culture of B. pertussis phase 1 in an incubator at 36°C for 48 hours. To the final product containing 25 to 30 billion organisms per ml. 0.01% merthiolate was added and then centrifuged 45 minutes at 6000 r.p.m. The supernate was kept for 3 days at 4°C before use.

This soluble antigen was free from toxicity. 2 subcutaneous inoculations of 0.5 ml. protected 100% mice challenged 12 days later by 2000 ID50 of pertussis organisms (6). In certain experiments 0.5 ml of SA replaced the standard pertussis vaccine in combination with diphtheria and tetanus toxoids (D.T.S.A.).

Poliomyelitis vaccine

Trivalent polio vaccine, which was commercially available has been used in this study. The vaccine is an aqueous preparation of killed polio viruses containing type 1 Mahoney, type 2 MEF-1 and type 3 Saukett. The viruses were inactivated with formaldehyde solution of 1 :4000. Sodium bisulfite, 0.434 gm. per liter, was added to neutralize the formaldehyde. The vaccine was preserved by merthiolate 1 :20,000 and stabilized with sodium ethylenediamine tetraacetate, 7 :20,000. In this work polio vaccine was used alone or combined with DTP, DTSA, pertussis or S.A. with or without adjuvant. In all cases 0.5 ml of the polio vaccine was combined with the other components in syringe just before inoculation.

Test for antibodies

Diphtheria antitoxin was determined by using Lr/100 of a stabilized toxin. Dilution of sera were mixed with the test dose of toxin and 0.1 ml. of the mixture was injected intradermally into guinea pigs. As a control 0.01 A.U of standard antitoxin received from Serum Institute - Copenhagen was mixed with 1 Lr/100 of the same toxin and injected intradermally into the same guinea pig. The reactions were compared and recorded after 3-4 days.

Tetanus antitoxin was tested with L+/1000 of a dried tetanus toxin combined with different dilutions of serum. The Danish reference serum was also used as a control. 0.2 ml of the mixture of toxin-antitoxin was injected subcutaneously in mice. The reactions of mice were recorded daily for 5 days.

The level of pertussis agglutinins was determined by mixing 0.25 ml. of different dilutions of sera with 0.25 ml. of pertussis antigen containing 8 billion organisms per ml. The mixture was shaken and kept in 37°C water-bath for 2 hours. The first reading was made and the tubes were shaken and kept overnight at +4°C. Next day the final reading, based on the highest dilution giving clear agglutination, was made.

Poliomyelitis neutralizing antibodies were determined as we have described elsewhere (7).

Pooled rabbit sera were inactivated at 56°C for 30 minutes. Fourfold serum dilutions were made from 1 :4 to 1 :1024. of each serum dilution were combined with 0.2 ml of the test dose of virus and the mixture incubated for 3 hours at room temperature. The virus test dose was one previously calculated to contain 100 TCID 50 of virus. Controls of normal rabbit serum and homologous hyperimmune polio rabbit serum, as well as a virus titration were included in each test. After incubation, 0.1 ml of each serum-virus mixture was inoculated into each of 2 tissue culture tubes and incubated at 37° C. The tubes were read each day for cytopathic effect. The final reading was taken on the day that the control virus titration showed 100 TCID 50 of virus to be present. If the control titration did not progress to 100 TCID 50, the readings obtained on the 7th day after inoculation were used. We have used Hela cells for titration of Polio antibodies.

Rabbits from the Razi Institute colony were approximately the same age and were kept under the same conditions. All rabbits were inoculated 3 times with 0.5 ml (pertussis, S.A or polio vaccine), 1 ml. (DTP or DTSA) and 1.5 ml (DTP and DTSA + polio vaccine) of the given antigen, 3 weeks apart and were bled 2 weeks after first and 3rd injection.

EXPERIMENTAL RESULTS

A) PERTUSSIS AGGLUTININS

Pertussis antigen: 6 groups of 6 rabbits were injected as indicated in table 1 and figures 1 and 3.

Table 1 - Serological response of Rabbits after injection of Pertussis Vaccine and Pertussis soluble antigen (S.A.)

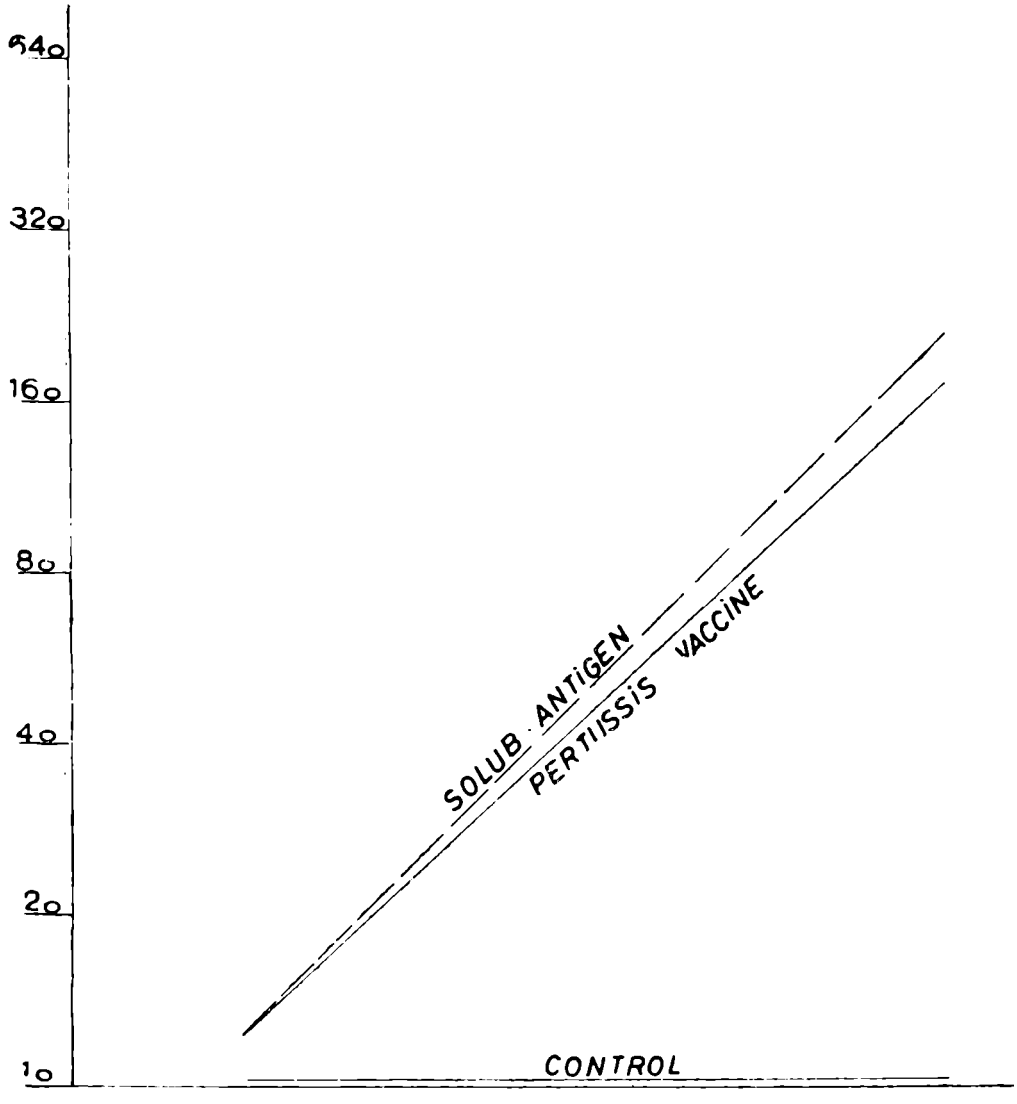
Fluid Antigen	Rabbit N°	Agglutinin titers 3 weeks after		Adsorbed antigen	Rabbit N°	Agglutinin Titers 3 weeks after	
		1st injection	3rd injection			1st injection	3rd injection
D.T.P.	37	80[X]	320	D.T.P.	43	20	80
	38	80	160		44	40	320
	39	20	40		45	20	160
	40	20	40		46	40	160
	41	40	80		47	20	1280
	42	40	40		48	< 10	320
	GM	47 (±10)[x]	113 (±47)		GM	23 (± 5)	387 (± 190)
D.T.P.+ Polio Vaccine	49	160	160	D.T.P. + Polio Vaccine	55	40	80
	50	80	40		56	20	160
	51	80	40		57	40	160
	52	80	20		58	40	160
	53	80	20		59	< 10	160
	54	20	80		60	< 10	160
	GM	83 (±19)	113 (±18)		GM	23	147 (± 12)
D.T.S.A.	61	40	160	D.T.S.A.	67	< 10	160
	62	40	80		68	< 10	80
	63	20	40		69	40	80
	64	20	40		70	20	80
	65	320	320		71	40	40
	66	320	320		72	20	160
	GM	127 (±61)	160 (±54)		GM	20	100 (± 40)
D.T.S.A. + Polio Vaccine	73	20	< 10	D.T.S.A. Polio Vaccine	79	20	20
	74	20	< 10		80	20	20
	75	40	< 10		81	< 10	20
	76	20	< 10		82	< 10	20
	77	20	< 10		83	20	20
	78	40	< 10		84	40	160
	GM	27	< 10		GM	17	43 (± 75)

X = Reciprocal of the highest dilution giving agglutination.

G M = Geometric Mean.

x = The figure in parenthesis indicate the standard errors of the geometric mean.

Fig. 1 - Geometric Mean Titers of Pertussis agglutinins in Rabbits after injection of standard Pertussis vaccine, Pertussis soluble antigen or Polio vaccine combined on Aluminium Hydroxide.



It can be seen that the primary response to pertussis vaccine or S.A. combined with aluminium hydroxide is lower than that of fluid antigen. This may be due to the slow release of antigen. The response to the third injection of antigen mixed with gel was higher however than that of the fluid antigen. The geometric means of the responses show this increase. That no agglutinins were detected in the control group was to be expected.

D.T.P. and D.T.S.A. alone or combined with polio vaccine

Eight groups of 6 rabbits were injected as shown in table 2 and figures

Table 2 - Pertussis Response of rabbits after injection of D.T.P. and D.T.S.A. alone or combined with Polio Vaccine.

Fluid Antigen	Rabbit N°	Agglutinin Titers 3 weeks after 1st injection	Agglutinin Titers 3 weeks after 3rd injection	Adsorbed antigen	Rabbit N°	Agglutinin Titers 3 weeks after 1st injection	Agglutinin Titers 3 weeks after 3rd injection
Pertussis vaccine	1	80x	160	Pertussis vaccine	7	20	80
	2	20	40		8	< 10	160
	3	160	80		9	< 10	160
	4	160	160		10	40	320
	5	40	160		11	< 10	160
	6	40	80		12	20	< 10
	GM	83(±26)+	113 (±22)		GM	13	176 (±39)
S.A	13	40	80	S.A.	19	< 10	320
	14	40	160		20	< 10	160
	15	40	80		21	40	320
	16	40	80		22	< 10	80
	17	80	320		23	40	160
	18	80	160		24	< 10	< 10
	GM	53 (±8)	147 (±122)		GM	13	208 (± 43)
Controls (Polio vaccine)	25	< 10	< 10	Controls (Polio vaccine)	31	< 10	< 10
	26	< 10	< 10		32	< 10	< 10
	27	< 10	< 10		33	< 10	< 10
	28	< 10	< 10		34	< 10	< 10
	29	< 10	< 10		35	< 10	< 10
	30	< 10	< 10		36	< 10	< 10
	GM	< 10	< 10		GM	< 10	< 10

X = Reciprocal of the highest dilution giving agglutination.

x = The figures in parenthesis indicate the standard errors of the geometric mean.

G M = Geometric mean.

Fig. 2 - Geometric Mean Titers of Pertussis agglutinins in Rabbits bled 3 weeks after first and third injections of combined fluid Diphtheria - Tetanus - Pertussis (DTP), Diphtheria - Tetanus - Pertussis soluble antigen (DTSA) alone or combined with fluid Polio vaccine.

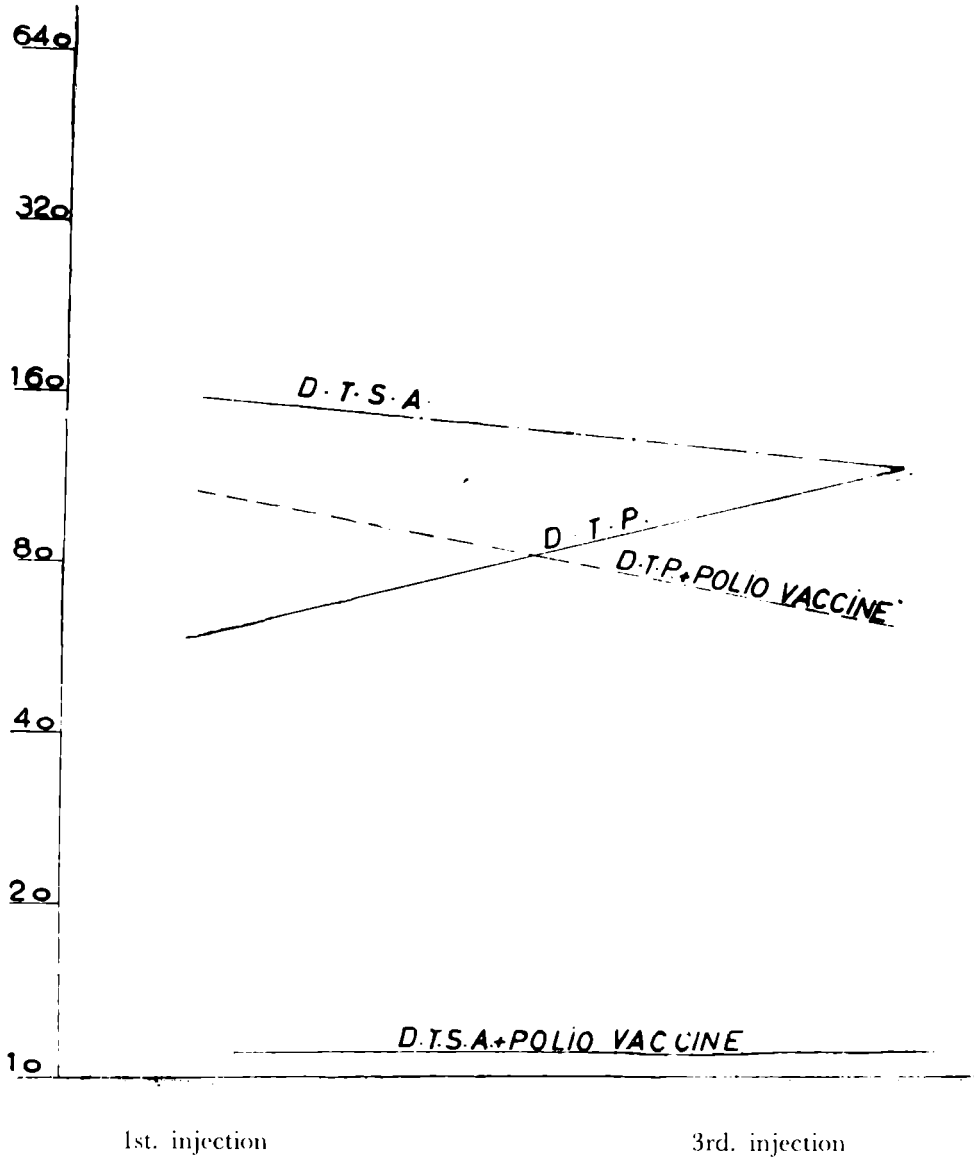
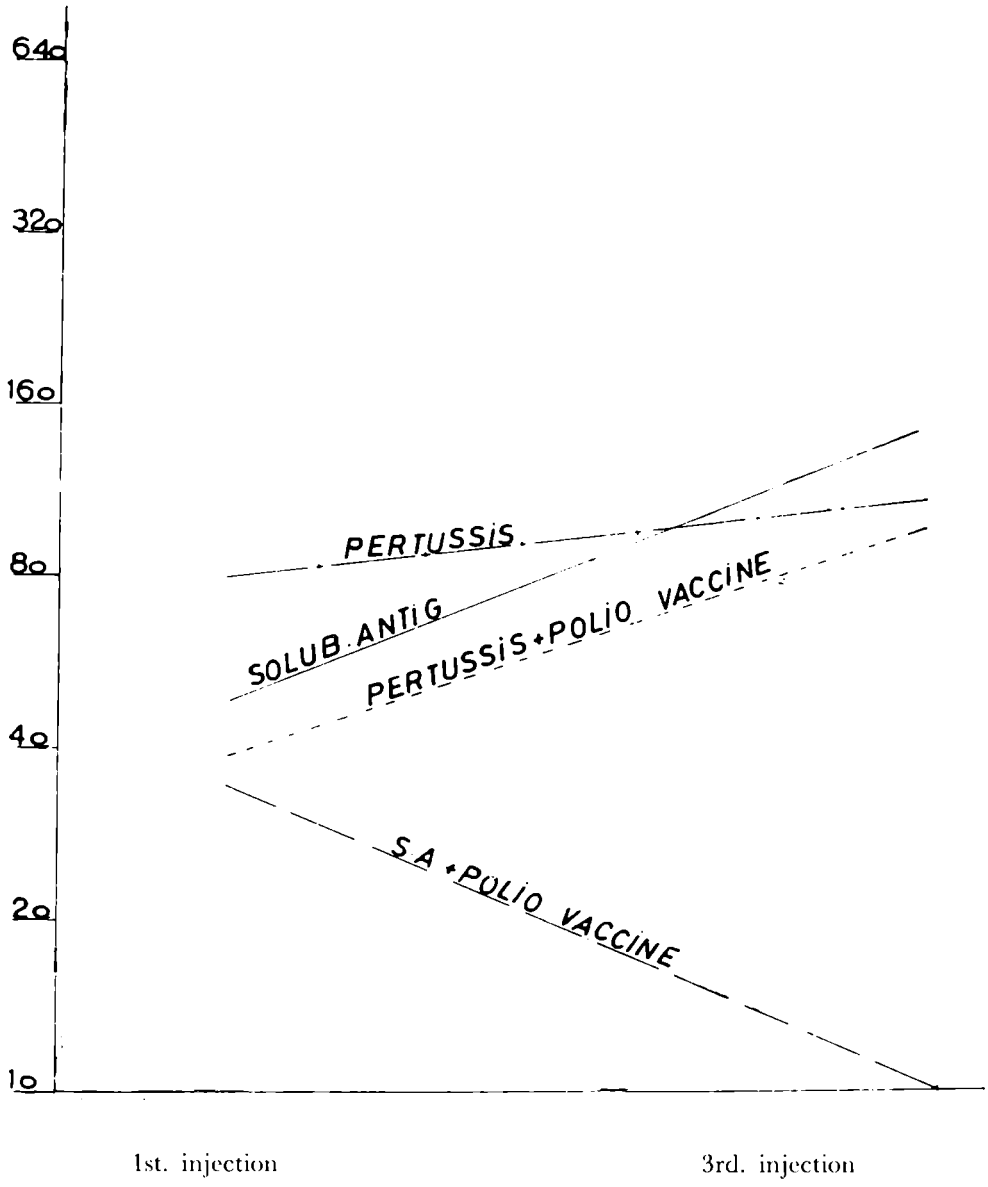


Fig. 3 - Geometric Mean Titers of Pertussis agglutinins in Rabbits after injection of standard fluid Pertussis vaccine and fluid Pertussis soluble antigen alone or combined with fluid Polio vaccine.



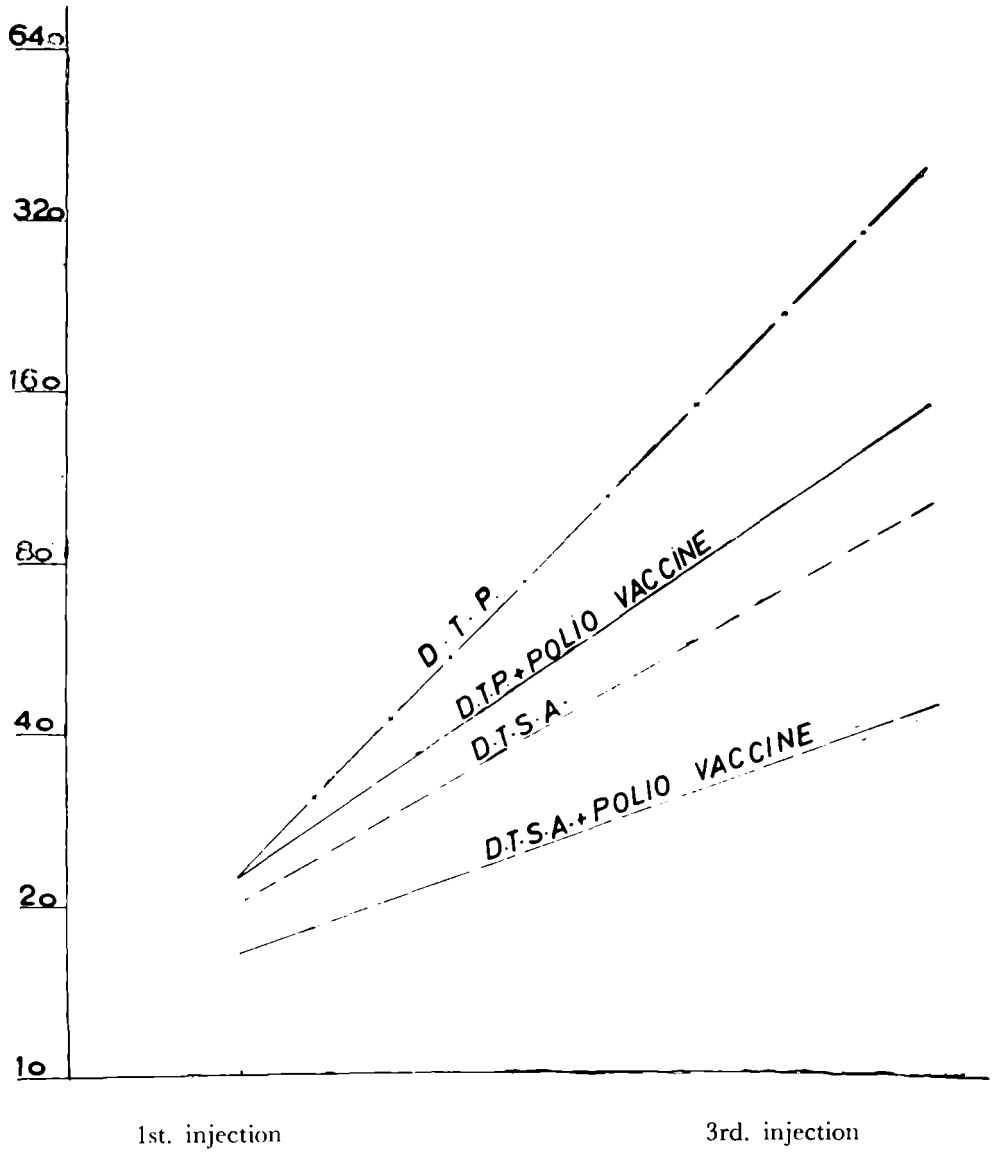
2 & 4. Here again the primary response to fluid D.T.P. or D.T.S.A. is higher than that of antigens adsorbed with aluminium gel. The response to the 3rd injection is also higher for antigens with gel than for the fluid antigens. There is an increase of agglutinins after the 3rd injection in all groups of animals. The response to the first dose of fluid D.T.P.+Polio is on other hand higher than that of the same antigen combined with aluminium hydroxide. Responses to the primary doses of DTSA+Polio either in fluid or gel are poor. The response to the third dose of fluid DTP + Polio is lower than that of primary dose of the same antigen, but it is higher when aluminium hydroxide is added.

In case of DTSA + Polio there was a poor response after 3rd injection when aluminium gel was used. No response was recorded when 3 doses of fluid DTSA+Polio were given. These results show a definite interference when 4 fluild antigens: Diphtheria, Tetanus, Pertussis S.A. and Polio antigens were injected simulataneously. As there is no interference between diphtheria, tetanus and pertussis soluble antigen it is evident that polio vaccine in-

Table 3 - Pertussis response of Rabbits injected with combined Polio vaccine and Pertussis Antigens

Antigen	Rabbit N°	Agglutinin titers 3 weeks after	
		1st. injection	3rd. injection
Pertussis vaccine + Polio	85	20	320
	86	40	40
	87	40	80
	88	20	40
	89	20	80
	90	40	40
	GM	30 (±9)	100 (±45)
S.A. + Polio	91	< 10	< 10
	92	40	< 10
	93	20	< 10
	94	20	< 10
	95	40	< 10
	96	20	< 10
	GM	25 (±12)	< 10

Fig. 4 - Geometric Mean Titers of Pertussis agglutinins in Rabbits bled 3 weeks after first and third injection of combined (DTP) or (DTSA) alone or with Polio vaccine and mixed with Aluminium Hydroxide.



terferes with pertussis antigen. This interference is further demonstrated in the following experiment.

Pertussis soluble antigen combined with polio vaccine

Two groups of rabbits were injected as indicated in table 3 and figure 3. There is some interference between polio and pertussis vaccine. There is, on the other hand, more interference between pertussis soluble antigen and polio vaccine.

B) DIPTHERIA AND TETANUS ANTITOXINS

The response of different groups of rabbits to the first dose of diphtheria antigen was about 0.1 A.U/ml. The response, after 3rd injection of DTP or DTP+Polio, was more than 2 A.U/ml. but between 1 and 2 A.U/ml. when DTSA alone or combined with polio vaccine were injected.

The response to tetanus toxoid of all groups seems to be identical, being approximately 1/50-1/100 A.U/ml. following the first injection and 0.1-0.2 A.U/ml. after the 3rd inoculation.

Poliomyelitis neutralizing antibodies

The serological response of rabbits to the poliomyelitis vaccine is indicated in table 4.

Table 4 - Poliomyelitis neutralizing Antibodies in Rabbit

Antigen Group	Adjuvant	Titers after first and third injection					
		Type I		Type II		Type III	
		1st.	3rd.	1st.	3rd.	1st.	3rd.
D.T.P. + Polio	1mg/Dose	4	256	4	16	64	1024
D.T.S.A. + Polio	Aluminium	4	64	< 4	4	16	64
Polio	Hydroxide	4	256	16	64	16	64
D.T.P. + Polio	Without Adjuvant	16	256	16	64	16	1024
D.T.S.A. + Polio		16	256	64	1024	64	64
Polio		16	256	64	1024	64	1024

All rabbits which received poliomyelitis vaccine alone or in combination with the other antigens show a poor response after the first injection. There is an increase of antibodies for all the types after the third inoculation of polio vaccine regardless of the nature of the combined antigens.

It is worth mentioning that the first response, in all but one of the groups injected with combined antigen and adjuvant, is poorer than the first response in groups inoculated with fluid antigen.

DISCUSSION

From the data presented in tables 1 to 3 and figures 1 to 4 there is a clear interference between whooping-cough antigens and polio vaccine. There could apparently not have been any interaction between different antigens, *in vitro,* as the mixture was prepared in the syringe and was injected immediately after mixing.

The interference was most apparent when pertussis soluble antigen was used. Whereas the addition of DT and aluminium hydroxide improves, to some extent, the serological response to pertussis antigens, the further addition of poliomyelitis vaccine is detrimental to the elaboration of pertussis agglutinins. There is the possibility that some enzymes existing in poliomyelitis vaccine could inactivate the pertussis soluble antigen to a greater extent than the pertussis vaccine.

A marked interference has recently been observed following the immunization of people with a combined diphtheria, tetanus, typhoparatyphoid and Salk polio vaccine (8). In this case the protective value of Salmonella antigens was suppressed by the presence of inactivated polyvalent polio vaccine.

Although the mechanism of this interference is not yet clear, from the practical point of view it is preferable to avoid mixing pertussis vaccine with other prophylactic antigens when poliomyelitis vaccine is to be used.

Summary

An interference is observed between whooping-cough antigens and Salk polio vaccine even if the two components are mixed immediately before use. The phenomenon is more evident when fluid antigens are injected. Pertussis soluble antigen, which gives a good serological response in rabbits, when used alone or combined with DT, is inactivated in the presence of Salk polio vaccine.

Résumé

On observe une interférence entre les antigènes coquelucheux et le vaccin antipoliomyélitique type Salk, même si les deux composants sont mélangés immédiatement avant usage. Le phénomène est plus évident avec les antigènes liquides. L'antigène coquelucheux soluble, qui donne une bonne réponse sérologique chez le lapin, lorsqu'il est utilisé seul ou en combinaison avec le DT, est inactivé en présence du vaccin antipoliomyélitique.

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