Use of Alum-Treated Pertussis Vaccine, and of Alum-Precipitated Combined Pertussis Vaccine and Diphtheria Toxoid, for Active Immunization*

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THE study of active immunization against pertussis, in Grand Rapids, Mich., has been the subject of several reports.[1, 2, 3] Field Series I, comprising 4,212 children, was the subject of a paper read at the meeting of the American Public Health Association in Kansas City, 1938. The total quantity of vaccine was 70 billion organisms given in four weekly subcutaneous doses of 1, 1.5, 1.5, and 3 ml. respectively, the last amount given in two bilateral injections. The pertussis incidence in the vaccinated group was 2.3 per 100 person-years, in comparison with 15.1 among the unvaccinated controls. The secondary attack rates [3] were calculated for all the susceptible children in the families in which pertussis occurred and found to be 36.4 in the vaccinated group in comparison with 92.0 in the unvaccinated group, in the age band 1 to 6, inclusive. An indication of what has happened in the series since it was closed for compilation of records in November, 1937, is found in an analysis of pertussis cases reported to the Grand Rapids Health Department. A tabulation of these reported cases was made from the beginning of the study in 1934 through December, 1940, and compared with those cases discovered in the field study. The results are shown in Table 1.

Of 2,074 cases of pertussis reported to the City Health Department during the period of study, 181 were found among the study children of Series I; 8 in the vaccine group, and 173 among the unvaccinated controls. From the close of the study through December 31, 1940, there were 1,353 cases reported to the Health Department of which 117 were found among the study children; 10 in the vaccinated group, and 107 in the control group. The relatively larger difference in the ratio of cases in the test and control groups, when based on those reported than when determined by the field study analysis, is easily explained by the case histories available in the study records. In general, only the clinically typical

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‡ In coöperation with the City Health Department of Grand Rapids, Mich., Dr. C. C. Slemons, Health Officer.

TABLE 1

Field Series I. Pertussis Cases Among the Children of Study Series I, Which Were Reported to the City Health Department

Found and Reported Cases of Pertussis	Study Period: Mar. 1, 1934, Through Oct. 31, 1937				After Study: Nov. 1, 1937, Through Dec. 31, 1940			
	Control Group	Vaccine Group	Total	C/V Ratio	Control Group	Vaccine Group	Total	C/V Ratio
Found by regular visiting in study Series I	348	52	400	6.7	Study series terminated			
Reported to City Health Dept. among children of Series I	173	8	181	21.6	107	10	117	10.7
Total reported cases for whole city during same period	...	..	2,074	....	...	..	1,353	....

cases were reported, the milder ones were not.

After completion of Field Series I, which gave good evidence of an immunizing antigen in the vaccine used, it seemed logical to investigate certain problems related to the type of vaccine and dosage schedule from the standpoint both of protection and practicability. In Field Series II, the object was twofold: first, to test the efficacy of a smaller quantity of vaccine than that used in Series I but given over a longer period of time; and second, to test an alum-precipitated vaccine preliminary to its combined use with diphtheria toxoid in a study of simultaneous immunization against pertussis and diphtheria. In Field Series III, the use of alum-precipitated, combined pertussis vaccine and diphtheria toxoid is being investigated.

FIELD SERIES II. ALUM-PRECIPITATED COMPARED WITH "STANDARD" PERTUSSIS VACCINE AS AN ACTIVE IMMUNIZING AGENT

GENERAL PLAN OF INVESTIGATION

The study area, the method of entry and withdrawal of test and control children, the plan for obtaining follow-up information, and the system of records, were all essentially as described for Field Series I.[2]

Selection of test and control groups—The age group under study was defined as including from 6 months up to but not including the 3rd birthday. The children were presumably susceptible, that is, with a record of neither previous vaccination against pertussis nor an attack of the disease.

As in the first field series, the vaccinated group was made up of children who were brought to the city immunization clinic for pertussis vaccination. The receiving nurse designated the entry history blanks alternately for alum and standard vaccine, and the injected children were entered as of the date of last injection. The control children, as in Series I, were designated at random from the city department of health immunization file of preschool children. After the information blanks were received from the clinic for a group of children who had completed their immunization, an approximately equal number of children, within the age group of the study and in the same districts as the injected children, were called on by the nurses for verification of their histories and suitability for inclusion in the study. These children if found to be presumably susceptible were entered as controls as of the date designated. There were only 40 col-

TABLE 2

Field Series II. Number of Children and Average Experience in Months, According to Reason for Withdrawal from Study

Reasons for Withdrawal	Number of Children				Average Experience in Months			
	All Groups	Vaccine Groups		Control Group	All Groups	Vaccine Groups		Control Group
		Alum	Standard			Alum	Standard	
All reasons	2,751	655	993	1,103	16.0	18.6	16.1	14.4
End of study	1,533	441	668	424	20.9	22.4	19.4	21.6
Pertussis	158	16	23	119	17.4	17.7	14.1	18.0
Moved	658	188	281	189	9.6	10.0	9.3	9.8
Vaccination of controls	355	...	...	355	7.4	....	....	7.4
Other *	47	10	21	16	6.9	9.5	5.5	7.3

* Includes 4 deaths from other causes in the control group, and 2 deaths in each of the other groups.

ored children in the whole series and they are tabulated along with the others.

Period of observation—The study period for the series extended from January 1, 1938, through December, 1940. The period of observation for any particular child was the number of months from entry to withdrawal. A record was terminated for any of the following reasons: attack of pertussis; moved out of study area; vaccination of control; death; a few miscellaneous reasons, such as lack of coöperation; and, finally, close of study December 31, 1940, for compilation of records and analysis. The numbers of children withdrawn for various reasons in the vaccinated and control groups, and the average experience per child in each group, are shown in Table 2.

Comparison of vaccinated and control groups—It is well recognized as pointed out by Bell [5] that it is impossible to select identical study groups, and that, even if it were possible to make all attributes identical at the moment of selection, they would not remain so. An effort was made, however, as previously described, to obtain reasonably comparable groups by designating in the control group presumably susceptible children of the same age and geographical distribution as the vaccinated children. In Table 3 a comparison is given of several factors which seem to have some obvious relation to comparability between groups.

The standard vaccine and control groups are seen to be approximately the same size. The alum vaccine group, according to method of choosing children for vaccination, should be the same as the standard. The reason for its smaller size is to be found largely in the shortage of alum vaccine supply at one period in the study, at which time the injections were continued with

TABLE 3

Field Series II. Comparison of Vaccine and Control Groups with Respect to Factors Related to Selection

		All Groups	Vaccine Groups		Control Group
			Alum	Standard	
(a)	Number of children	2,751	655	993	1,103
(b)	Mean length of experience in months	16.0	18.6	16.1	14.4
(c)	Mean age at entry in months	14.0	13.7	13.8	14.4
(d)	Males per 100 females	111	107	114	111
(e)	Average interval between nursing visits	2.7	2.7	2.8	2.6
(f)	Per cent attacked by measles, scarlet fever, and chicken pox	8.1	6.4	8.1	9.1
(g)	Upper respiratory infections other than pertussis, per 100 person-years	42.8	45.9	42.0	41.4

standard vaccine. The records of children who received both types of vaccine were eliminated from the analysis.

The somewhat shorter average experience of the control children in comparison with vaccinated children is explained by the withdrawal of many because of pertussis vaccination. There is no real difference in the mean age of the groups, in the sex ratio, or in the average interval between nursing visits. Finally, the incidence of scarlet fever, measles, and chicken pox, as well as the relative frequency of upper respiratory infections other than pertussis, indicates that the opportunity for exposure to some of the common childhood diseases was approximately the same in the vaccinated and control groups. The differences are within the limits of sampling variation.

A comparison of the number of three-person families, that is, of families in which it may be assumed there is only one child, suggests some difference between the vaccinated and control groups. Of the families with vaccinated children, 47 per cent were three-person families in comparison with 20 per cent of the families of the control children.

VACCINE USED *

"Standard" pertussis vaccine—The term "standard" vaccine is applied to the type of product used in Series I,[2] since the results obtained with it form the basis for comparison with the results of subsequent study series. This vaccine was a 10 billion per ml. suspension in saline of *Hemophilus pertussis* organisms with smooth characteristics, as indicated by tests just previous to their use. The killing agent was merthiolate.

Alum-precipitated vaccine—A suspension of *Hemophilus pertussis* was prepared just as for the standard vaccine used in Series I.[2] It was then precipitated with alum, essentially as described by Harrison, Franklin, and Bell,[4] and by Bell,[5] by the addition of 1 per cent potassium alum in the presence of 0.27 per cent sodium bicarbonate.

A point worthy of comment is the ease and speed with which the precipitated vaccine shakes into a homogeneous suspension.

Dosage schedule—A total of 3 ml. of vaccine was given in 3 subcutaneous injections of 1 ml. each, alternately in the two arms, with an interval of 1 week between the first and second, and an interval of 4 weeks between the second and third injections.

Immediate reactions to the injections—The immediate reactions after injection of the standard vaccine in this series were of no consequence. In general, there was a slight local reaction, with the formation of subcutaneous nodules which often persisted for several weeks. Following the injection of alum vaccine, the subcutaneous nodules were a little more marked. In one instance, a sterile abscess occurred. Bell[5] observed that such a reaction may be associated with a too superficial injection.

DEFINITIONS WITH RESPECT TO DIAGNOSIS, SEVERITY OF ATTACK, AND EXPOSURE

The same definitions were used for Series II as for Series I. Primarily, the diagnosis of pertussis in study children and in source cases was based upon a detailed case history taken by the nurse. All available supplementary information was used in making the final decision, such as the judgment of the attending physician or members of the health department, cough plate findings, and records of exposure. An attack was considered *moderate* if the child had characteristic whooping and

* The vaccine was prepared in the Biologic Products Division of the Michigan Department of Health Laboratories, Dr. J. T. Tripp, Associate Director.

vomiting, an uncomplicated disease duration of approximately 4 to 6 weeks, and no evidence of marked interference with nutrition. In a *severe* attack the paroxysms of coughing and whooping were unusually severe and frequent, there was marked loss in weight or complications such as bronchopneumonia or prolonged bronchitis. The *mild* attack had only occasional whooping or vomiting, no obvious interference with nutrition, and usually lasted no more than 4 weeks. Under *very mild* attacks were included coughs of not more than 3 weeks' duration in which characteristic symptoms were absent and the diagnosis depended upon either a record of definite exposure or positive cough plate findings. The average interval between onset of attack and the first visit for obtaining the history was 1.8 months. Over 82 per cent of the histories were secured within 2½ months after onset of first symptoms.

Exposures in this report are tabulated as occurring in own household or outside of own household; that is, familial and community exposures, respectively. Each recorded exposure is based upon exposure to a source case in which the diagnosis was established on the basis of a written case history and under the same criteria used for the attacks among the study children. Exposure must have occurred within 3 weeks of the onset date of the source case, and a particular attack was related to a source case only if it occurred within an arbitrarily designated period of 30 days after onset of the source case; that is, the onset date of the attack must be within 51 days of the onset of the source case.

RESULTS OF FIELD SERIES II

Pertussis incidence in vaccinated and control groups—In Table 4 is shown the pertussis incidence in terms of attacks per 100 person-years for alum and standard vaccine groups, and control groups.

Of the total 158 pertussis attacks which occurred in the whole series, 1.6, 1.8, and 9.0 attacks per 100 person-years occurred in alum, standard, and control groups, respectively.

In comparing each of the vaccinated groups with the control group, the difference obviously is significant. Comparing the two vaccinated groups, the difference is not significant.

Comparison of incidence in Field Series II with incidence in Series I—While it is not possible to compare directly the incidence in Series II with the previously reported Series I, it seems possible to make a reasonable comparison through the medium of the control groups. The attacks per 100 person-years in the control groups of Series I and II were 15.1 and 9.0, respectively. If it can be assumed that these rates give a fair indication of normal expectancy within the study groups, the expected incidence in the vaccinated groups of Series II, if the results were similar to those of Series I, can be derived by a simple proportion, since the incidence in the vaccine group in Series I is known to be 2.3. By this

TABLE 4

Field Series II. Incidence of Pertussis in Vaccinated and Control Groups, Based on Period at Risk

	All Groups	*Vaccine Groups*		*Control Group*
		Alum	*Standard*	
Number of children	2,751	655	993	1,103
Person-months experience	43,659	12,142	15,596	15,921
Number of attacks	158	16	23	119
Attacks per 100 person-years	4.3	1.6	1.8	9.0

TABLE 5

Field Series II. Severity of Pertussis Attacks in Vaccinated and Control Groups

Severity Rating	All Groups		Vaccine Groups: Alum		Vaccine Groups: Standard		Control Group	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
All attacks	158	100	16	100	23	100	119	100
Very mild	24	15	7	44	7	31	10	8
Mild	47	30	9	56	9	39	29	24
Moderate	71	45	..	..	6	26	65	55
Severe	16	10	..	..	1	4	15	13

means, the expected number of attacks per 100 person-years in the vaccinated group is calculated to be 1.4. The actual incidence in the alum group was 1.6 and in the standard group, 1.8. In relation to the amount of experience, the difference between the expected and actual incidence is not significant with respect to either alum or standard group. This suggests that both the alum and standard vaccines in a total dosage of 30 billion organisms over a period of 5 weeks may be as effective as standard vaccine, 70 billion organisms, given over a period of 3 weeks.

Severity of attack—As pointed out repeatedly, the rating of the severity of pertussis is rough at best. Every effort was made to avoid bias and to use the same criteria, already outlined, for rating the attacks among vaccinated and control children. It was not possible, however, in many instances to avoid information on the part of the visiting nurse as to whether the patient had had vaccine injections. During the relatively frequent visits, such information was apt to be proffered by the mother.

As found also in Series I, the pertussis attacks in Series II were relatively milder in the vaccinated than in the control children. This is shown in Table 5.

The tabulation indicates further that there was a tendency toward even milder attacks in the alum group in comparison with the standard. It is seen that 100 per cent of the attacks in the alum group were mild or very mild, in comparison with 70 per cent in the standard group and 32 per cent among the controls. A tabulation of severity and mean age gave no explanation of the difference in severity in the vaccinated and control groups. It is pointed out that the numbers of attacks in the vaccinated groups are small and the comparison therefore is considered only as suggestive.

Attacks of pertussis related to exposures—Exposure information was obtained in connection with the regularly scheduled nursing visits so that some of the exposures were recorded before the diagnosis of the attack which may have followed. Other exposures were recorded during the visits for obtaining the case histories on suspected attacks of pertussis.

There were no known exposures, that is, exposures sufficiently well defined to be accepted as such according to the definitions, among 2,751 of the children. Of the remaining 175 children with known recorded exposures, 50 were in the alum group, 52 in the standard, and 73 in the control group. Per hundred person-years experience there were 4.4 known recorded exposures among all vaccinated children and 5.5 among controls. A correlation of exposures with subsequent attacks is shown in Table 6.

Of the children without acceptable records of exposure, 1 per cent contracted pertussis in the alum group, 1

TABLE 6

Field Series II. Proportion of Children Attacked in Relation to Their Exposure Records

Record with Respect to Exposure	Alum Vaccine Group			Standard Vaccine Group			Control Group		
	Number of Children	Children Attacked		Number of Children	Children Attacked		Number of Children	Children Attacked	
		Number	Per cent		Number	Per cent		Number	Per cent
All records	655	16	2.4	993	23	2.3	1,103	119	10.8
No known exposures	605	5	0.8	941	8	0.9	1,028	52	5.1
All known exposures	50	11	22.0	52	15	28.8	73	65	89.0
Exposed in own household	17	7	41.2*	18	9	50.0*	54	53	98.1*
Exposed outside of own household	33	4	12.1	34	6	17.6	19	12	63.2

* Secondary attack rates.

per cent in the standard, and 5 per cent in the control group. Of the children with accepted records of exposure, 22 per cent in the alum group contracted pertussis, 29 per cent in the standard, and 89 per cent in the control group. Following exposure in own household, 41 per cent were attacked in the alum group, 50 per cent in the standard, and 98 per cent in the control group. The number of attacks is relatively small, and the larger percentage following exposure in the standard compared with the alum group is not significant.

Secondary attack rates—Since by definition all the susceptible children of a particular age band in the study families were entered in the study, the secondary attack rates for the vaccinated and control groups are identical with the proportions of children attacked following exposure in their own households, just referred to in connection with Table 6.

SUMMARY OF RESULTS OF SERIES II

The data of Series II indicate protection of a large proportion of children injected with alum-precipitated pertussis vaccine in a total dosage of 30 billion organisms, given over a period of 5 weeks.

The results in no way contraindicate the use of alum vaccine, and form a basis for its trial in combination with diphtheria toxoid.

FIELD SERIES III. ALUM-PRECIPITATED COMBINED PERTUSSIS VACCINE AND DIPHTHERIA TOXOID FOR ACTIVE IMMUNIZATION

The use of several immunizing antigens simultaneously is not new. Even in the injection of a single strain vaccine we know we are introducing several separate antigens, and each will stimulate its own antibody. Experimentally in laboratory animals, many known antigens have been mixed for injection and each found to produce an individual antigenic stimulus. The most familiar example of mixed antigens for human immunization is triple typhoid, paratyphoid A and B vaccine. Smallpox vaccine frequently is given at the time of last injection of diphtheria toxoid although not mixed with it. Recently, combined diphtheria and tetanus toxoid has been used, apparently with success.

With the multiplicity of accepted immunization procedures for children it is logical that appropriate combinations of antigens for active immunization be studied in order to reduce the number

of required injections, thereby lessening discomfort for the child and family, and simplifying administrative procedure. A number of workers have recognized that simultaneous immunization against diphtheria and pertussis would be a particularly desirable and reasonable procedure if its effectiveness could be demonstrated. Bordet,[6] in 1936 announced that he and Ramon had plans for studying the use of such a product.

Since the findings of Series II in no way contraindicated the use of alum pertussis vaccine, it was considered safe and reasonable to test an alum-precipitated, combined pertussis vaccine and diphtheria toxoid. A study group was started January 1, 1940, in comparison with a standard vaccine group and a control group. These three groups comprise Field Series III, the subject of a previous preliminary report by the author,[13] and of this interim report. Except for the substitution of alum-precipitated combined diphtheria toxoid and pertussis vaccine for the alum-precipitated pertussis vaccine, the methods of procedure were the same in all respects as for Series II.

THE COMBINED ANTIGENS *

Preparation of vaccine—H. pertussis cultures were grown and harvested as for standard vaccine, and the merthiolate-killed packed organisms suspended in crude diphtheria toxoid to make a final count of 10 billion organisms per ml. This mixture was precipitated by the addition of sterile potassium alum solution in amounts which ranged between 1.5 and 2.0 per cent. In each case, the exact amount of alum required for maximum precipitation was determined by preliminary titrations of aliquots with varying amounts of alum. The calculated amount of alum was added to the bulk and the precipitate washed twice with saline, and brought back to the original volume with saline and the final product preserved with merthiolate 1:10,000.

The usual sterility, safety, and potency tests for diphtheria toxoid and for pertussis vaccine, respectively, were done before release of the product. Each ml. of the final antigen contained 10 billion *H. pertussis* organisms and the amount of diphtheria toxoid which on antigenic test would produce more than two units of antitoxin in guinea pigs.

Dosage schedule—Just as in Series II, three injections were given, with an interval of 1 week between the first and second injections and 4 weeks between the second and third. The first dose was 1 ml. of " standard " pertussis vaccine, and the second and third were each 1 ml. of the combined antigen. This provided the same dosage of pertussis vaccine as used for Series II, and the dosage of alum-precipitated diphtheria toxoid which is recommended by the Michigan Department of Health.

Immediate reactions—The immediate reactions, in general, resemble those which follow the injection of alum-precipitated pertussis vaccine or alum-precipitated diphtheria toxoid. Records on local and general reactions were obtained on more than 900 children by nurses' visits the day after injection, and further visits if indicated. Reactions were recorded as none, slight, moderate, or marked, according to a predesignated code. Marked local reactions were based upon an intensely sore area of 2 or more inches in diameter. A marked general reaction was defined to include such symptoms as vomiting, or high fever. As recorded, the majority of reactions were none or slight; only a few were marked. In three known instances, a sterile abscess occurred. Under Series II in this paper, reference has been made to the observation of Bell [5] in this connection.

* The combined antigen was prepared by the Biologic Products Division, Michigan Department of Health. A separate communication will be made on the details of technic.

TABLE 7

Field Series III. Diphtheria Antitoxin Titrations in Children Before and After Immunization with Alum-Precipitated Combined Diphtheria Toxoid and Pertussis Vaccine: Number and Per cent at Various Antitoxin Levels

Number of Diphtheria Antitoxin Units	Time of Test in Relation to Time of Injection										
	Group A (6)	Group B (19) Before and Once After			Group C (9) Before and Twice After			Group D (111) Once After, Only		All Children (145) All Tests	
	Before, Only	Before	5–8 Mo.	11–20 Mo.	Before	5–8 Mo.	11–12 Mo.	5–8 Mo.	11–14 Mo.	Before	After
	Number										
Under 0.001	6	19	0	0	9	0	0	0	0	34	0
0.001–0.01 *	..	..	1	0	..	0	0	1	0	..	2
0.01 –0.1	..	..	3	3	..	0	0	7	11	..	24
0.1 –0.2	..	..	1	4	..	2	1	8	13	..	29
0.2 –1.0	..	..	6	1	..	7	7	19	31	..	71
1.0 and over	..	..	0	0	..	0	1	5	16	..	22
All tests	6	19	11	8	9	9	9	40	71	34	148
	Per cent										
Under 0.001	100	100	0.0	0.0	100	0.0	0.0	0.0	0.0	100	0.0
0.001–0.01	...	...	9.1	0.0	...	0.0	0.0	2.5	0.0	...	1.4
0.01 –0.1	...	...	27.3	37.5	...	0.0	0.0	17.5	15.5	...	16.2
0.1 –0.2	...	...	9.1	50.0	...	22.2	11.1	20.0	18.3	...	19.6
0.2 –1.0	...	...	54.5	12.5	...	77.8	77.8	47.5	43.7	...	47.9
1.0 and over	...	...	0.0	0.0	...	0.0	11.1	12.5	22.5	...	14.9

* Note: 0.001–0.01 means up to but not including 0.01.

TABLE 7A

Cumulative Results of Diphtheria Antitoxin Titrations Before and After Combined Pertussis-Diphtheria Immunization: Derived from Table 7

Number of Antitoxin Units	Before Immunization		5 to 20 Months After Immunization	
	No.	Per cent	No.	Per cent
Under 0.001	34	100.0	...	....
0.001 or more	..	....	148	100.0
0.01 or more	..	....	146	98.6
0.1 or more	..	....	122	82.4
0.2 or more	..	....	93	62.8
1.0 or more	..	....	22	14.9

IMMUNE RESPONSE OF THE INJECTED CHILDREN

Diphtheria antitoxin—As a criterion of antitoxic level, titrations * were done by the method of Fraser [7] in which mixtures of varying amounts of serum and constant amounts of toxin were tested for neutralization by skin injections in the rabbit. Tests were compared with controls on the same rabbit, in which known amounts of antitoxin and toxin were used. Schick tests of the children prior to immunization have been avoided since they would complicate the interpretation of antigenic response to the combined antigen, due to the antigen in the Schick test material. Titrations have been made with the sera from 34 children before, and from 148 after injection of the combined antigen. The children were selected on the basis of ease of bleeding. The results are shown in Table 7, and cumulated in 7A.

* Diphtheria antitoxin titrations were made by the Biologic Products Division, Michigan Department of Health.

None of the children tested before immunization had as much as 0.001 unit of antitoxin and every one of those tested 5 to 20 months after immunization had at least that amount. As the tabulation indicates, 98.6 per cent of them had a level of 0.01 unit or more, and 82.4 per cent, a level of 0.1 or more. It is of interest to compare these levels with those obtained by Volk and Bunney [8] in the Saginaw County, Mich., study. The determinations in both series were done in the same laboratory. In the age group from 2 to 5 years, 4 months after injection of two doses of alum-precipitated diphtheria toxoid, 97 per cent of the children had at least 0.01 unit, and 53 per cent at least 0.1 unit; at 12 months, the respective proportions at these two levels were 93 and 51 per cent. It should be pointed out that these authors were dealing with rural children, while the children in this study were largely urban. The results indicate that after injection with the combined antigen, the antitoxin reaches fully as high levels as after injection with an equivalent amount of alum-precipitated toxoid given alone.

Pertussis antibodies—As a criterion of response to the pertussis antigen the opsonocytophagic test as reported by Kendrick, Gibbs, and Sprick [9] was used. In addition, some complement-fixation tests and agglutination tests were done. A total of 545 opsonic tests before and 311 tests after immunization are summarized in Table 8.

Of the opsonic reactions before immunization 99.3 per cent were negative or weak; 4½ to 14 months following immunization 79.7 per cent had reactions at least moderately strong, a reaction considered to indicate a significant antigenic response. Of these tests, 180 were on the same children before and after immunization, and the results were in line with those for the whole group, as recorded in Table 8.

Of 27 children tested by the complement-fixation method before injection, the reactions were negative in 26, and doubtful in 1. Six months to 1 year after immunization, 135 were tested; 105 were positive, 5 doubtful, and 15 negative.

Agglutination tests were done on 62 children from 6 to 15 months after completion of immunization and the titers were as follows: 5 were negative in 1:10 dilution; 9 had a titer of 1:10; 35 had titers 1:20 through 1:100; 13 had titers 1:250 or higher.

The results of the complement-fixation and agglutination tests indicate good response to the pertussis antigen.

INTERIM TABULATION OF RESULTS RELATED TO PROTECTION

During the period from the beginning of Series III on January 1, 1940, through September 30, 1941, the study series has come to include 2,194 children of whom 847 were injected with combined, and 380 with standard vaccine, and 967 were unvaccinated controls. The experience and incidence in

TABLE 8

Field Series III. Opsonocytophagic Tests with Pertussis Antigen Before and After Immunization with Combined Diphtheria Toxoid and Pertussis Vaccine

Time of Tests with Respect to Completion of Immunization	*Number of Children*	*Per cent of Children with Opsonic Reaction*	
		Negative to Weak	*Moderately Strong or Strong*
Before immunization	545	99.3	0.7
After immunization	311	20.3	79.7
4¼ to 5½ months	38	13.2	86.8
6 to 8 months	179	20.7	79.3
11 to 14 months	94	22.3	77.7

NOTE: Of these tests, 180 were on the same children before and after immunziation.

TABLE 9

Field Series III. Alum-Precipitated Combined Diphtheria Toxoid and Pertussis Vaccine: Interim Tabulation of Pertussis Incidence in Test and Control Groups

Time at Risk and Attacks	All Groups	Vaccine Groups		Control Group
		Combined	Standard	
Number of children	2,194	847	380	967
Person-years	1,536	610	386	540
Attacks	69	4	6	59
Attacks per 100 person-years	4.5	0.7	1.6	10.9

terms of attacks per 100 person-years are indicated in Table 9.

The children in this Series III are still under observation, additions are being made from week to week, and a final conclusion regarding protection is not being drawn. In this interim report, however, the incidence of 10.9 in the control group and of 1.6 in the standard vaccine group closely parallel the findings of 9.0 and 1.8, respectively, for Series II. Reason for particular encouragement is found in the low incidence of 0.7 for the combined vaccine group.

SUMMARY OF THE RESULTS OF SERIES III

Titrations of diphtheria antitoxin levels before and after injection indicate as good response to the toxoid in the alum-precipitated, combined pertussis vaccine and diphtheria toxoid, as to diphtheria toxoid alone. Also, opsonocytophagic as well as complement-fixation and agglutination tests, indicate that pertussis antibodies are stimulated by the pertussis vaccine.

An interim tabulation of incidence indicates 0.7 attack per 100 person-years in the combined vaccine group in comparison with 1.6 in the standard vaccine group, and 10.9 in the control group.

COMMENT

The question of dosage in relation to pertussis vaccination has received considerable discussion in various articles during the past several years. There is no experimental basis at present for saying that one or another schedule is optimum. It can only be said that with a particular procedure, the results were as found. There has been a tendency on the part of some workers to increase the total dosage as an answer to incomplete protection in any study series, and heavier suspensions such as "double strength" vaccine are in use. The total amount undoubtedly is important, but investigations should cover more thoroughly the other phases of the problem: the type of vaccine, the number of injections and the period over which they are given, and the several questions associated with secondary stimulus. In view of inconclusive data as to the duration of immunity it would seem reasonable to study the advisability of re-immunization just before the child goes to school. Limited experience suggests that a single, relatively small injection may provide an adequate secondary stimulus.

In the study of *total dosage,* the desirability of giving no more than is required for a good degree of protection should be recognized. Investigations of the *type of vaccine* must take into consideration the possible value of toxic products of the pertussis organism as immunizing agents, in the light of the work of Evans [10] and others. Also, the possibility should be explored further of *combining* the pertussis antigen with other antigens, such as diphtheria toxoid. On the subject of *interval* between injections, Faber,[11] Maclean,[12] and others, have called at-

tention to its possible importance. Bell,[5] in his recent study of alum-precipitated pertussis vaccine used a 4 week interval between two injections. His results as well as those included in this paper suggest that the subject can be investigated further with profit. Field Series III in Grand Rapids, for which an interim report is made, is expected to throw more light on several phases of the problem as more experience is accumulated with a dosage schedule of 30 billion organisms given over a period of 5 weeks.

GENERAL SUMMARY AND CONCLUSIONS

The results indicate a good degree of protection against pertussis, following a dosage schedule of 30 billion organisms distributed in 3 injections over a period of 5 weeks. Also, the results appear similar to those in a previous series in which 70 billion organisms were injected over a period of 3 weeks. These observations suggest that the period over which the vaccine is administered should receive as much consideration as the total dosage.

Following the use of alum-precipitated, combined pertussis vaccine and diphtheria toxoid, good antigenic response to both the pertussis antigen and diphtheria toxoid was demonstrated by immunological tests. An interim tabulation on incidence indicates protection against pertussis following injection of the combined antigen.

A more definite conclusion on protection as well as on the question of an adequate dosage schedule awaits further observation.

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