A STUDY IN ACTIVE IMMUNIZATION AGAINST PERTUSSIS[1]

By

PEARL KENDRICK AND GRACE ELDERING

WITH STATISTICAL ANALYSES OF THE DATA BY ANTHONY J. BOROWSKI

(Received for publication January 25th, 1939)

The present study of pertussis immunization in Grand Rapids had its beginning in 1932 when a study of the practicability of laboratory diagnostic methods in pertussis was undertaken. The relative uniformity with which *H. pertussis* could be isolated during the first weeks of disease under properly controlled conditions emphasized the etiological association of the organism with the disease and raised the question as to why attempts to produce active immunization with pertussis vaccine had given such irregular and inconclusive results. In February, 1933, a study series was started to observe the protective effect of pertussis vaccine given to a child after exposure to pertussis in his own household but before the development of symptoms. The administrative problem was so complicated and the interpretation of results so difficult that toward the latter part of the year this plan was abandoned in favor of a study of the protective value of vaccine completely administered prior to exposure. Also this seemed a more fundamental approach to the public health problem of pertussis control. A progress report on the preventive study was made by the authors (1) in January, 1936, after which the series under observation was extended. On November 1, 1937, the records were closed for purposes of compilation and analysis.

Since the publication of the authors' progress report, several papers have been added to the literature on the use of "Phase I" or "smooth" *H. pertussis* suspensions as vaccine for pertussis immunization. Among these, Doull, Shibley and McClelland (2) reported a study in Cleveland in which the distribution of pertussis attacks was not significantly different in the vaccine-injected and control groups. Silverthorne and Fraser (3) recently reported two attacks of whooping cough among 747 vaccinated children compared with 23 attacks among 161 controls. Singer-Brooks (4) and Miller (5) present data which show a higher degree of protection in a vaccinated group than in a control group. Sauer (6) has reaffirmed his finding of protection following the use of pertussis vaccine. Madsen (7), basing his opinion on the Denmark experience, believes that pertussis vaccine used at times of epidemics, lightens attacks and reduces mortality. He considers the immunizing effect to be of short duration.

The investigation which is here the subject of final report was undertaken to ascertain the degree of protection

[1] From the Michigan Department of Health, Bureau of Laboratories, Western Michigan Division, Grand Rapids, Mich.; with the co-operation of the City Health Department of Grand Rapids, John L. Lavan, M.D., Health Officer; Fred Miller, M.D., School Physician in charge of immunization clinics.

Financial assistance in this investigation has been given through several Works Progress Administration projects, several emergency funds from the City of Grand Rapids and private sources; and finally, in connection with the statistical analysis of the data, by the Subcommittee on Evaluation of Administrative Practices of the American Public Health Association.

against clinical attacks of pertussis afforded by a method of vaccination subsequently to be described, during a limited time period to a group of children between the ages of 8 months and 6 years in comparison with an unvaccinated group as nearly similar as possible in regard to susceptibility and exposure.

The persons responsible for obtaining and recording the data of any field study are of such importance to the character of the results that a few comments on personnel seem required. Because the study grew naturally by expansion out of the diagnostic bacteriological work, the investigation was planned and directed from the laboratory. Advantage was taken of the opportunities for consultation with various members of the Michigan Department of Health, particularly of the Bureau of Epidemiology, and for close co-operation with the City Health Department of Grand Rapids. The cultural studies and technical procedures connected with vaccine production were carried out by laboratory staff bacteriologists. Facilities were available for obtaining considerable familiarity with pertussis cultures through continuous experience with an unusually large volume of cough-plate diagnostic work. The injections were given in city immunization clinics by regular health department staff members. House visits for obtaining follow-up information were made by staff members of the Bureau of Public Health Nursing; nurses with special training in public health procedures. This bureau and the clinics were assisted by trained nurses furnished through W.P.A. The nurses' field work was unified throughout the study by the same supervising nurse from the Bureau of Public Health Nursing. Most of the clerical work in connection with records was done by a staff of clerks furnished by federal projects, these clerks being under the intimate supervision of the authors. The chief clerk was the same person throughout the study, thus lending uniformity and consistency to the clerical procedures.

General plan of investigation

Field study area. The study was confined to the city of Grand Rapids and environs in Kent County, Michigan. The estimated population of Grand Rapids, metropolitan area, census of 1930, was 168,650. In general, the population of Grand Rapids is considered unusually stable, approximately 60 per cent of the families being home owners. American-born persons of American parents make up 49 per cent of the population. Of the 51 per cent who are foreign or native-born of foreign-born parents, about one third are Dutch, one sixth Polish and the remainder various nationalities.

The City Department of Health has divided the city into eighteen geographical districts, particularly for the convenience of its Bureau of Public Health Nursing in which a plan of generalized nursing is followed. The populations of these districts are not known but the school populations have, in general, a similar distribution, the range of the middle 50 per cent of the districts having school populations from 1,500 to 2,300. Roughly, the social status may be considered similar for the majority of the population in any one district. These eighteen city districts plus East Grand Rapids and the other environs in Kent County constitute the twenty geographical units used for classifying the material in the study. The eighteen city districts furnished approximately 90 per cent of the children.

Key list and permanent record card files. The completed key list contained

a chronological entry of all persons admitted to the study series from the beginning, comprising 5,815 entries. For each child admitted, a permanent record card bearing all relevant information was filed. If, upon a subsequent follow-up visit, the history showed that a child did not fulfill the conditions of the study, the particular permanent record card was transferred from the "main active file" to an "invalid file." Into this file also were transferred cards of all children for whom no information had been obtained subsequent to entry, such as for children who could not be located upon the first follow-up visit. All records received through private physicians were kept in a separate file. In reviewing the records prior to analysis, the earliest designated controls were likewise separated out into a special file. These control children were chosen by district nurses under conditions not as clearly defined as later, when the lists of presumably susceptible children became available. The source of these lists will be described under "Test and control groups." All these early controls therefore were filed separately and were excluded from the main analyses.

All the entries in the chronological list are accounted for as follows:

File I. Main, active file: Complete vaccine series: 1,815 injected and 2,397 control children	4,212
File II. Private physicians' file	376
File III. Invalid file: 248 vaccine and 308 controls	556
File IV. Early controls	364
Incomplete vaccine series	177
Colored and unknown race	120
Original records lost, 4 injected and 6 controls	10
Total entries in Key List	5,815

The main analyses and the tabulations were confined solely to the group of 4,212 white children in file I. Unless otherwise specified, all discussions and statements refer to this particular group.

Duration of study. The period of observation extended from March 1st, 1934 to November 1st, 1937, covering 44 months.

Admittance to study. At the beginning, the age range was from 8 months up to, but not including, the sixth birthday. The upper limit was reduced early in the study to 4 years; that is, up to, but not including, the fifth birthday. Only presumably susceptible children were accepted in either test or control group; i.e., children with a record of no known attack of pertussis and no injections of pertussis vaccine.

Test and control groups. All children who received pertussis vaccine according to the plan of investigation constituted the vaccine-injected or test group. This group was made up of all children of acceptable age and history who presented themselves at the city immunization clinics for pertussis vaccination. The fact that the injected children presented themselves at the clinic for vaccination introduces an element of selection. Comparative analyses of test and control groups must be depended upon to discover whether this factor was important.

As a basis for the designation of controls, lists were prepared of children of acceptable age and history in the various districts. These control lists were made from the card file records of a house-to-house preschool immunization survey under F.E.R.A. sponsored by the City Health Department, the records containing the history of infection and immunization with regard to diphtheria, smallpox and whooping cough. The cards and lists were kept up to date by the addition of births and new records of infection or immunization. As soon as information blanks on vaccine injec-

tions were received from the immunization clinics, an approximately equal sample of children of the same age, and in the same districts as the injected children, was selected at random from the control lists. Calls were then made by the nurses to verify the record and to assure co-operation in obtaining follow-up information as to exposure to pertussis or attacks of the disease.

In general, the date of entry designated the time a child began his period at risk; and the disposition date designated the time the child was removed from observation for a reason to be defined under "period of observation." Each vaccine-injected child was entered on the date of the last dose of vaccine. Each control child was dated for entry when chosen from the presumably-susceptible list. In either vaccine or control families, a baby of less than required age at time of family entry, automatically was entered as a control on the date he became 8 months of age.

Follow-up information. The recorded follow-up information depended first of all on house visits by nurses at stated intervals. At first, visits were scheduled every 3 to 4 months but in November, 1935, the interval was reduced to 2 months. In addition to the scheduled visits, City Health Department lists of reported cases of whooping cough as well as daily records of cough plates received in the laboratory were checked with the permanent card file of the study; if any of the names were found, nurses were sent to make immediate house visits. Further, nurses from the Bureau of Public Health Nursing were on the alert for information regarding pertussis exposures or extended coughs, on their visits connected with their regular district responsibilities.

Records. Special forms were used for recording information on vaccine inoculation, nurses' visits, exposure to pertussis, case histories and cough-plate findings. A permanent record card was kept for each child. A nurses' visit card file was an automatic record of calls due. A transcription sheet was prepared for each child, based upon a code and a manual of definitions. Data were transferred to punch cards from the transcription sheets for analysis.

Period of observation. All active records were closed on the date of the last effective visit prior to November 1st, 1937, a closing date designated for compilation of the records. During the course of the study, records were terminated (1) at onset of an attack of pertussis; (2) on the last effective visit prior to loss by death, removal or inability on the part of the nurse to locate a family or to obtain necessary information; and (3) on the date of the first injection in the instance of controls who were given pertussis vaccine.

The vaccine used

In line with recent studies on bacterial dissociation in relation to antigenicity; in line also with the procedures of those workers who have made favorable reports on the efficacy of pertussis vaccines, particular attention was given to the pertussis cultures used in the preparation of the vaccine employed in the study. *H. pertussis* cultures were isolated from routine diagnostic cough-plates and maintained on a modified Bordet-Gengou medium containing at least 15 per cent blood and were examined just before use as to their agglutinability with specific antiserum, their morphology and growth characteristics including hemolytic zone on Bordet-Gengou medium, and their ability to produce a necrotic skin reaction in the rabbit. Only recently isolated cultures with smooth strain characteristics were

selected. The number of cultures included in each lot of vaccine varied but the average number was five. The average age of the cultures from the time of collection of the cough plate to the vaccine filling date was 2.2 months.

The method of vaccine production has been described in a progress report (1) and in greater detail by Kendrick, Lawson and Miller (8) in the report of the Committee on Standard Methods of the American Public Health Association. In brief, the 72-hour growth of *H. pertussis* on Bordet-Gengou medium was washed off in a small amount of saline. The suspension was filtered through a thin layer of cotton to free it from particles of medium, immediately centrifuged, and the supernatant fluid discarded. The organisms were resuspended in saline containing a killing agent, either merthiolate 1:10,000 or 0.5 per cent phenol, and stored in the cold room until sterile, usually about 1 week. Phenol, or phenol plus merthiolate, was used in about one fourth of the lots of vaccine while merthiolate alone was the killing agent in the remainder. The killed suspension was standardized to 10 billion organisms per cc, and was then ready for filling. In addition to the usual sterility and safety tests the vaccine was tested for agglutinability with specific antiserum and ability to produce agglutinins in the rabbit. The finished vaccine was stored at about 8 to 10° C until used.

Size and number of vaccine lots. Because of physical limitations vaccine production was on a small scale and was almost continuously in progress throughout the study. Thirty-nine lots were prepared with an average of 820 cc per lot.

Vaccine storage interval. The age of the vaccine from date of filling to date of injection is a matter of interest. Fifty per cent of the vaccine was less than 2.4 months old; 70 per cent was less than 6 months; of the remainder, about half had been stored for 10 months or longer.

Vaccine dosage and method of administration. The standard dose was 7 cc of vaccine, divided into four weekly doses of 1 cc, 1.5 cc, 1.5 cc, and 3 cc, respectively, with the last amount injected bilaterally, 1.5 cc in each arm. All injections were made subcutaneously in the arm, care being taken to select a different area for each dose.

Immediate reactions to vaccine injections. Following arbitrary definitions, local and systemic reactions were classified roughly as no reaction, slight, moderate and severe, and were recorded on the vaccine inoculation blanks. With the exception of a group of 87 nursery school children who were observed 24 hours after injections, the records were made from the report of the mother when she returned to the clinic with the child for the next dose of vaccine. According to these reports most of the children showed slight to moderate local reactions, consisting of soreness and induration, and slight or no systemic reactions. As a rule temperatures were not taken. There was one systemic reaction in which convulsions occurred, and two others which were classed as severe based upon vomiting and relatively high temperature. The 1,815 children represent 9,075 separate injections and in no instance was there abscess formation or breaking down of the indurated area. A rather consistent correlation was observed between size of dose and degree of resultant soreness.

Composition of vaccine and control groups

Size of sample and period of observation. In table 1 is shown the size of the

TABLE 1

Number of children in test and control groups and length of stay in study

Groups	Number of children and period of observation			
	Persons	Person-years	Months in study	
			Number	Average per person
Both groups	4,212	4,575	54,899	13.0
Injected	1,815	2,268	27,212	15.0
Control	2,397	2,307	27,687	11.6

samples observed in the vaccine-injected and control groups both from the standpoint of numbers of persons and of person-months or person-years, in order to take account of the period at risk.

The total period of observation extended over 44 consecutive months, thus taking into account seasonal factors. During this period the size of the groups changed with the development of the study. Children were entered at irregular intervals and others withdrawn for any of the reasons defined. This fluctuation in the size of the two study groups is best shown in table 2 which was prepared by the experience table method.

This tabulation shows the size of the two groups at the beginning of each

TABLE 2

Experience table for all children in the study

Month of study	Number of children								Person-months at risk	
	At first of month		Entered during month		Reasons for withdrawals					
					Pertussis attacks		All other reasons			
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Total			1,815	2,397	52	348	1,763	2,049	27,212	27,687
1	1	0	24	15					13	8
2	25	15	61	20					56	25
3	86	35	6	5					89	38
4	92	40	2	2				1	93	40
5	94	41	1	4			1		94	43
6	94	45	72	16					130	53
7	166	61	8	4		1			170	62
8	174	64	1	0					175	64
9	175	64	3	2		1	1	1	176	64
10	177	65	4	4		1			179	66
11	181	68	0	2		3		1	181	67
12	181	66	3	3		1	1		182	67
13	183	68	1	4		3	1		183	68
14	183	69	4	2	1		1	4	184	68
15	185	67	34	15		1		3	202	73
16	219	78	1	14		2		1	220	83
17	220	89	14	19	1	1			226	98
18	233	107	78	309	2	2	1	4	270	259
19	308	410	29	19		4		4	323	416
20	337	421	14	17	1	5	4	2	341	426

TABLE 2—*Continued*

Month of study	Number of children								Person-months at risk	
	At first of month		Entered during month		Reasons for withdrawals					
					Pertussis attacks		All other reasons			
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
21	346	431	8	17		2	1	8	349	435
22	353	438	6	26		2		3	356	448
23	359	459	82	127		2	1	14	399	515
24	440	570	132	161		2	1	18	505	641
25	571	711	76	77		3	2	28	608	734
26	645	757	121	176	2	4	2	24	704	831
27	762	905	93	182	1	7	7	27	805	979
28	847	1,053	60	94		9	11	26	871	1,082
29	896	1,112	103	123		11	12	28	942	1,154
30	987	1,196	79	135	2	5	10	42	1,021	1,240
31	1,054	1,284	207	182		3	21	60	1,147	1,343
32	1,240	1,403	150	250	1	7	35	100	1,297	1,474
33	1,354	1,546	83	173	4	23	30	91	1,379	1,576
34	1,403	1,605	79	66	4	19	30	38	1,426	1,609
35	1,448	1,614	45	26	2	21	11	41	1,464	1,596
36	1,480	1,578	60	19	3	24	8	21	1,504	1,565
37	1,529	1,552	5	18	2	33	19	37	1,521	1,526
38	1,513	1,500	10	14	7	35	25	40	1,502	1,469
39	1,491	1,439	18	17	8	33	29	57	1,481	1,403
40	1,472	1,366	13	12	3	20	73	83	1,441	1,320
41	1,409	1,275	12	17	5	40	74	73	1,375	1,227
42	1,342	1,179	9	6	2	14	407	404	1,112	973
43	882	767	3	3	1	4	819	691	474	421
44	65	75					57	74	36	38
	8	1					6	1	5	
	2						2		1	

month of the study. It shows also, for each month, the number of children entered, the number withdrawn because they contracted pertussis, or for other reasons, and the person-months at risk.

The 4,212 children in the study were at risk for 54,899 months, or an average of 13.0 months per child. In the vaccine-injected group, 1,815 children had 27,212 months of experience or an average of 15.0 months per child; the 2,397 control children were at risk for 27,687 months or an average of 11.6 months per child. A correlation of the length of stay in months with the reasons for terminating the records is shown in table 3.

The range of the length of stay in months for the middle 50 per cent of all 4,212 children was 6.5 to 17.7; of the injected group, 8.2 to 17.8; of the control group, 5.2 to 14.9. While the average period of observation, therefore, for the whole group was 13.0 months per child, 25 per cent of the children were under observation less than 6.5 months, and 75 per cent less than 17.7 months. The

TABLE 3

Length of stay for all children in test and control groups according to reasons for withdrawal from study

Reasons for withdrawal	Number of children and mean length of stay (*months*)					
	Both groups		Injected group		Control group	
	Children	Months	Children	Months	Children	Months
Total	4212	13.0	1815	15.0	2397	11.6
End of study	2938	15.0	1531	15.9	1407	14.1
Pertussis	400	9.0	52	10.7	348	8.8
Moved	537	8.8	232	9.9	305	8.0
Vaccine injection of controls	337	7.0			337	7.0

longest period for any individual was 41.6 months.

As shown in table 1, the person-months experience in both vaccine and control groups is similar. The average period at risk per child, however, was 3.4 months longer in the injected than in the control group. A reason may be found in the fact that 337 control children were removed from the study by subsequent immunization, a factor which did not enter in the vaccine group. In addition, a larger number of records was terminated for pertussis attacks in the control than in the vaccine group. For reasons other than pertussis, vaccination or normal termination at close of the study, the per cent of the records terminated was the same in both vaccine and control groups.

Distribution of sex and age at time of entry. Males and females were evenly distributed in test and control groups, males making up 52.5 per cent of the controls and 50.0 per cent of the injected children. In table 4 is shown the age distribution by sex in the two groups.

Comparing the test and control groups, the mean age in the test group for both males and females was 29.7 months; for males 29.4 and, for females, 29.7. For the control group, the mean age was 28.6; for males 28.5 and, for females, 28.7 months. The difference between the mean ages of the injected and control children is only twice the standard error and therefore cannot be considered significant. An analysis by quartile distribution showed that the middle 50 per cent of the children in the two groups had very similar age distribution since 75 per cent of the children in both groups in the study were 42 months or less of age. There was a slight discrepancy in the first quartile; those in the control group having an average age of 11 months compared with 15 for the injected group. Reference

TABLE 4

Distribution of ages in test and control groups according to sex

Groups in study	Age (*months*)	
	Mean age	Age range of middle 50 per cent
Total	29.1	13–42
Males	29.0	13–42
Females	29.2	13–42
Injected	29.7	15–42
Males	29.4	15–41
Females	29.7	15–43
Control	28.6	11–42
Males	28.5	10–42
Females	28.7	11–42

has been made to this point in connection with the automatic entry, as controls, of all babies in study families at 8 months of age. In general, it may be stated that there was a similar age distribution in both groups.

Distribution of samples in city. For any number of vaccine-injected children in a particular district of the city a relatively equal number of controls was chosen in the same geographical area. The number of children and their per cent of distribution in the twenty districts is shown for both groups in table 5.

It will be seen that there is no marked discrepancy between the groups in any one district although some districts contributed a larger number of children to the study than others.

Family size. Any marked difference in the number of persons in a family as between controls and injected children might be an indicator of lack of comparability in other respects, such as social status and more particularly in the opportunity for exposure to pertussis. Counting all members of the immediate family of each child, the average size of the families from which control children were taken was 4.6 compared with 4.4 in the vaccine group. The difference in mean depends largely on the difference in the number of three-person families. Among the vaccine children, 24 per cent were from three-person families compared with 16 per cent of the controls. In other words, about one fourth of the injected children were the only children in their families at time of entry, compared with one sixth of the controls.

An analysis of the families according to the distribution of study children without reference to other members in the family is shown in table 6.

It should be borne in mind that the tabulation is based on family descriptions, at entry, of the children in the study and does not allow for changes in

TABLE 5

Distribution of children in test and control groups according to districts

Number of districts	Groups in study			
	Injected		Control	
	Number	Per cent	Number	Per cent
Total	1815	100.0	2397	100.0
1	54	3.0	73	3.1
2	36	2.0	49	2.0
3	190	10.5	219	9.1
4	109	6.0	157	6.6
5	54	3.0	80	3.3
6	46	2.5	70	2.9
7	63	3.5	68	2.8
8	111	6.1	149	6.2
9	64	3.5	86	3.6
10	59	3.3	94	3.9
11	135	7.4	146	6.1
12	94	5.2	135	5.6
13	139	7.7	176	7.3
14	55	3.0	104	4.3
15	125	6.9	164	6.8
16	124	6.8	151	6.3
17	50	2.8	77	3.2
18	107	5.9	141	5.9
19	9	.5	3	.1
20	191	10.5	255	10.6

TABLE 6

Distribution of test and control children in families

Family description as to test and control children in family	Distribution of children in families		
	Children	Families	
		Number	Per cent of total families
Total families	4212	2907	100.0
Test children only	1517	1223	42.0
Control children only	2027	1453	50.0
Both test and control	668	231	8.0

status as the study progressed, such as entry, as a control, of a baby in the family on reaching 8 months of age, or withdrawal of a control child for vaccination and his re-entry with a revised family description. There is, then, some overlapping of families. For example, a child who started as a control and later received vaccine under the limitations of the study was withdrawn as a control and re-entered as if in a new family. The analysis recorded in table 6 indicates that 42 per cent of the 2,907 study families contained only vaccine-injected children, 50 per cent only control, and 8 per cent both test and control children.

Incidence of communicable diseases other than pertussis.[2] As an index of comparability between test and control groups, as regards exposure to infection with common communicable diseases, a comparison was made of the incidence of measles and scarlet fever in the two groups. The City Health Department records of reported cases were accumulated for measles and scarlet fever over the entire 44 months of study and the cases which occurred in the study series were tabulated. The numbers and per cents of attack are shown in table 7.

There is a striking similarity of experience with reference to scarlet fever and measles in test and control groups, suggesting that there is no difference between the groups with respect to exposure to these communicable diseases. It is inferred that whatever the factors which determined the selection of the vaccinated group, they did not involve lessened exposure to acute infectious diseases of the respiratory group which are widely distributed in such populations.

Follow-up nursing visits in test and control groups. As shown in table 8 there was a total of 23,961 visits recorded for both groups, with an average of 5.7 per child and an average interval between visits of 2.3 months.

The reason for the larger average number of 6.5 visits per child in the vaccine group as compared with 5.1 for the control group is evident when it is recalled that the average length of stay per child was 3.6 months longer for the test than for the control child. The average interval between visits, however, was alike for both groups, suggesting that the two groups had been followed in a similar manner. The number of visits per child in the whole study ranged from one to twenty but, regardless of the total number of visits per child, the interval between visits was approximately the same.

[2] This device was used at the suggestion of the late Dr. Wade H. Frost.

TABLE 7

Comparison of test and control groups with respect to attacks of scarlet fever and measles reported to the health department during period of study

Study series		Number of attacks and per cent of children attacked					
		Scarlet fever		Measles		Both diseases	
Groups	Number	Attacks	Per cent	Attacks	Per cent	Attacks	Per cent
Both.....	4212	98	2.3	365	8.7	463	11.0
Injected...	1815	42	2.3	161	8.8	203	11.2
Control...	2397	56	2.3	204	8.5	260	10.8

TABLE 8

Nursing visits for test and control groups

Study series			Nursing visits		Average interval between visits in months
Groups	Number	Person-months	Number	Average per child	
Both.....	4,212	54,899	23,961	5.7	2.3
Injected...	1,815	27,212	11,759	6.5	2.3
Control...	2,397	27,687	12,202	5.1	2.3

Conclusion on comparability of test and control groups. By the analyses just discussed, the injected and control groups were found to be of relatively similar size with similar periods of observation; they had approximately equal distributions as to age, sex and geographical districts, and were relatively similar in family size; there was a striking similarity in the incidence of childhood communicable diseases other than pertussis; finally, there was similarity in the procedures and character of the observations of the children throughout the period of study. For the purposes of comparison with respect to this investigation, it seems reasonable to assume that there was an unbiased allocation to the test and control groups and that the groups were similar in respect to conditions affecting susceptibility and exposure to pertussis infection.

Incidence of pertussis in test and control groups

Definitions with respect to diagnosis and severity rating of pertussis attacks. The difficulties associated with diagnosing an attack of pertussis with certainty, particularly one in which the usually accepted clinical criteria are lacking or at least are not prominent, do not need emphasis. Also, any attempt to rate satisfactorily the severity of an attack can give nothing more than a relative classification which at best is rough.

In this study information relevant to the diagnosis was obtained from several sources. Physicians from the City Health Department helped with their clinical opinions. Advantage was taken of the co-operation of private physicians when they were in attendance. Of the children who contracted pertussis, 62.6 per cent in the control group and 40.6 in the vaccine group were attended by their family physicians.

Some information was obtained from cough-plate findings but positive results were necessarily limited to instances in which attacks were located in their earlier stages. Of the children for whom a pertussis diagnosis was accepted, 69 per cent of those in the injected group had cough-plates taken and, in the control group, 67 per cent. Positive findings were obtained in 6 per cent of the plates in the test group and in 24 per cent in the control group.

Primarily, the diagnosis of pertussis in this study was based upon detailed case histories obtained and recorded by visiting nurses. These histories were all examined independently by one of the authors and by a member of the Bureau of Epidemiology of the Michigan Department of Health. They were rated as to severity according to the more or less arbitrary set of definitions given in the progress report (1). An attack was considered *moderate* if the child had characteristic whooping and 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 broncho-pneumonia or prolonged bronchitis. The *light* attack had only occasional whooping or vomiting, no obvious interference with nutrition and usually lasted no more than 4 weeks. Under *very light* attacks were included coughs usually of less than 4 weeks but at least 1 week's duration in which there was no history of whooping or vomiting, and diagnosis was dependent upon either a record of definite exposure or positive cough-plate findings.

In connection with basing the diagnosis on case histories obtained by nurses, it is a matter of importance to

know how soon after the onset of attack the information was obtained. Analysing the records for the interval which elapsed between onset of attack and the date the case history was started, the mean interval in connection with all case histories was 46 days. The quartile distribution shows that 50 per cent of all the case histories were started within 32 days after onset and 75 per cent of them within 55 days after onset of case.

Pertussis incidence in vaccine-injected and control groups. The experience of the test and control groups has been analysed on the basis of persons and also, in order to take account of variations in the period at risk, on the basis of person-years. The basic information from which the period at risk was calculated has been discussed with reference to table 2. In table 9 is shown the pertussis incidence in both groups.

On the basis of 348 attacks for the 2,307 person-years experience in the control group and 52 attacks for the 2,268 person-years in the test group, there were 15.1 annual pertussis attacks per 100 in the control group compared with 2.3 in the injected group. The difference of 12.8 is equal to 15.4 times the standard error. Assuming that attacks were distributed in a purely random fashion in two groups of this size, a difference as great as that observed would be expected to occur by chance alone only once in many millions of trials.

TABLE 9

Incidence of pertussis in test and control groups based on period at risk

Time at risk and subsequent attack	Groups in study		
	Both groups	Injected	Control
Number of children	4212	1815	2397
Person-years	4575	2268	2307
Number of attacks	400	52	348
Annual pertussis attack rate per 100	8.7	2.3	15.1

Quartile distribution of pertussis incidence over period of study. As a consistency check, the total incidence of pertussis in the study has been analysed to show quartile distribution. The results are shown in table 10.

In every period there was a higher rate of pertussis in the control than in the injected group and the difference is statistically significant.

Pertussis incidence in the study compared with reported incidence in Grand Rapids. As would be expected, the pertussis incidence in the control group rose and fell with the incidence in the community. Due to more complete finding of cases, however, the incidence was

TABLE 10

Incidence of pertussis in test and control groups during entire period of observation and by quartiles of time period

Quartiles 11 months each	Both groups			Injected group			Control group		
	Person-years	Number of attacks	Annual attack rate per 100	Person-years	Number of attacks	Annual attack rate per 100	Person-years	Number of attacks	Annual attack rate per 100
Total	4575	400	8.7	2268	52	2.3	2307	348	15.1
First	157	6	3.8	113	0	0.0	44	6	13.6
Second	440	28	6.4	236	5	2.1	204	23	11.3
Third	1771	86	4.9	807	10	1.2	964	76	7.9
Fourth	2207	280	12.7	1112	37	3.3	1095	243	22.2

higher in the control group of the study than in the general population. In the study, several cases in a particular family would be recorded as individual cases while the reports in the Health Department frequently included only one of several cases in any particular family. Also, the systematic visiting revealed many otherwise missed cases particularly the very mild ones.

Distribution of pertussis incidence with respect to districts in the city. In the twenty geographical areas recognized for the study there was a statistically significant difference between the two groups in all but one, in which district the incidence for both test and control groups was 8.0 per cent based upon four attacks during a risk of 50 person-years, and six attacks during 75 person-years, respectively.

Severity of pertussis attacks

Consistent with the difference in incidence of attacks in the injected and control group was the relatively mild character of the attacks that did occur in the injected group. An analysis of the 400 attacks of pertussis as to the per cent classified in the various severity ratings is given in table 11.

The tabulation shows that the usual moderate attack constituted 23.1 per cent of the attacks in the injected group in comparison with 60.1 per cent among the controls. The two cases rated severe in the injected group did not have broncho-pneumonia or other serious complications but were rated on the basis of relatively frequent paroxysms and a long course of disease. These two severe attacks formed 3.8 per cent of the attacks in the injected group compared with 45 severe attacks (13.1 per cent) of the attacks in the control group. A large discrepancy between the two groups is seen in the per cent of light plus very light attacks in the two groups, 73 per cent in the injected group compared with 27 per cent among controls.

Since, by definition, the "very light" attack had no history of whooping or vomiting and may be considered clinically a questionable, abortive attack, it is interesting to note the effect of eliminating all such attacks and basing the incidence only on recognizable clinical attacks. By such an analysis the annual attacks per 100 in test and control groups, respectively, would be 1.4 and 14.5, instead of the recorded 2.3 and 15.1 in the two groups as shown in table 9. It is evident that by elimination of the

TABLE 11

Severity of pertussis attacks in test and control groups

Severity rating	Number and per cent of attacks					
	Both groups		Injected group		Control group	
	Number	Per cent	Number	Per cent	Number	Per cent
Total attacks.....	400		52		348	
All attacks rated..	395	100.0	52	100.0	343	100.0
Very light....	35	8.9	21	40.4	14	4.1
Light.....	95	24.0	17	32.7	78	22.7
Moderate.....	218	55.2	12	23.1	206	60.1
Severe.....	47	11.9	2	3.8	45	13.1
Rating unknown..	5		0		5	

very light attacks there is an even greater difference between the lowered rates of pertussis incidence in test and control groups than when such attacks were included.

Exposure to pertussis correlated with subsequent attacks

Definitions with regard to exposures. From the beginning, one important objective in the study was to obtain as exact information as possible with regard to exposures to pertussis and subsequent related attacks. To do this it was necessary to adhere to certain well understood definitions.

First of all, to be recorded as an exposure there must have been definitely recorded contact with a source case for which a written case history was obtained and a diagnosis made on the same basis as were attacks in the test and control groups. In defining exposures, the period of infectivity of the source case must be taken into account. It has been found by various workers, for example Kendrick and Eldering (9), that a relatively high per cent of positive cough-plate results may be obtained during the first 3 weeks after onset; there is a sharp decline up to the end of the fifth week of disease, after which only an occasional patient with pertussis is found to harbor the organism. Any exposure, therefore, to be considered at all, should have occurred within 5 weeks of onset of the source case, and to be recorded a definite exposure, it should have occurred within 21 days. In evaluating exposures, the incubation period of pertussis also must be taken into consideration. Lawson (10), in connection with his study of attacks, found the incubation period to be 13.05 ± 7.6 days. It may be mentioned that, in general agreement with Lawson's finding, subsequent analysis showed the mean incubation period of the attacks in this study to be 15.4 ± 1.3 days. In defining incubation period in relation to acceptance of exposure histories, allowance was made for the longest possible incubation period and for the memory factor, and the maximum period was set arbitrarily at 30 days. The longest period, therefore, that could elapse between onset of the source case and subsequent attacks was 21 plus 30 days, or 51 days, in connection with a definite exposure; 35 plus 30, or 65 days, with an indefinite exposure. Any record of exposure outside these limits was not counted. Furthermore, a definite exposure must have been intimate and indoors, it may have been continuous in the child's own household from the date of onset of the first source case, or it may have been definite in another household and may have lasted for 30 minutes, an afternoon, a day or several days. An *indefinite* exposure occurred under conditions less intimate than for the definite. Indefinite exposures may have included contacts with a source case out-of-doors and also they may have been later than the twenty-first day after onset of the source case but never later than the thirty-fifth day.

It will be understood from the limitations of the definitions that many exposures mentioned by the families were excluded for lack of specific information or failure to meet the defined requirements. This explains in part the relatively large number of pertussis attacks in which the exposures were *unknown*. This is discussed later in the text.

Exposures to pertussis correlated with number of subsequent attacks of pertussis. From the systematic questioning during the regular nursing visits to the 4,212 children in the study, it was discovered that 3,467 of them gave no history of attacks or of exposure sufficiently well defined to be accepted as such ac-

cording to the definitions. There were 570 with acceptable records of exposures. Some of these children had records of several exposures. In such instances, only the most intimate exposure was included in the tabulation, or, if pertussis infection occurred, the one related to the attack. In addition to the 570 exposures known by histories of contact with source cases, 175 other unrecognized exposures were revealed by pertussis attacks in the absence of acceptable exposure histories. In some of these instances there was no record at all of exposure; in others, exposures were reported by the family but the information proved insufficient to satisfy the definitions. In table 12 is shown the correlation of exposures with subsequent attacks.

Of the 570 children with exposures known by history of contact with source cases, 225 (39.5 per cent) contracted pertussis.

Of 297 exposed children in the test group, 12.8 per cent contracted the disease and of 273 in the control group, 68.5 per cent.

In the test group 34.9 per cent of all children who were exposed in their own households contracted pertussis compared with 89.4 per cent in the control group.

When exposed definitely in other households, 5.0 per cent contracted pertussis in the test group compared with 55.7 per cent in the control group.

Of those children who had exposures classed as indefinite, 3.5 per cent were attacked with pertussis in the test group compared with 19.2 per cent in the control group.

It has been pointed out that there were 175 attacks of pertussis without acceptable records of contact with source cases. It is significant that there were only 14 such instances in the vaccine group compared with 161 in the control group. Assuming comparability of the two groups, this strongly suggests a considerably larger number of unrecognized exposures in the vaccine group which

TABLE 12

Persons in the study series exposed to pertussis according to "type" of exposure and proportions of those exposed who were attacked

	Classification according to history of exposure				No history of exposure
	Definite in own household	Definite in other household	Indefinite	Total	
Both groups					
No. of exposures...	243	161	166	570	3642
Attacks.....	172	39	14	225	175
Per cent.....	70.8	24.2	8.4	39.5	4.8
Vaccine group					
No. of exposures...	83	100	114	297	1518
Attacks.....	29	5	4	38	14
Per cent.....	34.9	5.0	3.5	12.8	0.9
Control group					
No. of exposures...	160	61	52	273	2124
Attacks.....	143	34	10	187	161
Per cent.....	89.4	55.7	19.2	68.5	7.6

were not followed by pertussis. This implication is consistent with the wide difference in the test and control groups as to the attacks which resulted from the known exposures.

Comparing the different types of known exposures, the data show that in both test and control groups, the greatest risk of infection is provided by definite exposure in a child's own household. There is a progressively lower attack rate as the conditions of exposure become relatively less intimate. As between test and control groups, there is a consistently lower per cent of attack in the test group for all types of exposures.

Coughs not diagnosed pertussis but immediately preceded by exposure. In order to take precaution against the exclusion of abortive or atypical attacks of pertussis, case histories were obtained for coughs not diagnosed pertussis if they were within the time limit after exposure as previously defined, with respect to period of infectivity of the source case and incubation period. As noted before, "very light" attacks of pertussis included any cough of more than 1 week which occurred within 30 days following a definite exposure. In addition to these coughs there were other upper respiratory infections including coughs of less than a week's duration and those diagnosed bronchitis or influenza by the family physicians. There were 32 such infections recorded in the injected and thirteen in the control group.

The occurrence in the vaccine group of more coughs not diagnosed pertussis but immediately preceded by exposure than in the control group raises the question whether they may not include a number of mild abortive attacks of pertussis. If, for discussion, we should add all exposure-connected coughs not diagnosed as pertussis to the number of pertussis attacks in both groups, the per cents of attacks following all known exposures would be 23.6 and 72.3 in the test and control groups respectively, instead of the 12.8 and 68.5 per cent recorded in table 12.

Some factors which might influence the protective results of pertussis vaccination

If it may be assumed that the difference observed in this study between test and control groups is explained on the basis of protection conferred by the injected vaccine, then the question may be asked as to what factors might determine the degree of such protection. Several questions were considered during the analysis of the data from the standpoint of their possible correlation with the 52 attacks of pertussis which occurred in the test group. It must be remembered that such a small number is not sufficient to form the basis for final conclusions.

Factors related to the vaccine. If the suitability of the vaccine as to antigenicity of the cultures used and method of preparation can be accepted, questions still arise concerning the care and administration of the product.

The vaccine storage interval is a point about which there is too little information with respect to any bacterial vaccine. Comparing the extremes of the storage period range represented in the test group as to incidence of pertussis, the following results were observed. Among 1,074 children, representing 1,056 person-years of observation, in which the vaccine used had had a storage interval of 1 to 3 months inclusive, there were 26 attacks of pertussis, an incidence of 2.5 per hundred person-years. Among 236 children, representing 435 person-years, injected with vaccine stored 9 months to 1 year there were six-

teen attacks, an incidence of 3.7 per cent. The difference in incidence of 1.2 is only 1.28 times the standard error, suggesting that up to approximately 1 year, the storage period did not have a significant influence upon incidence. There are no data upon a longer storage interval and further work is needed to clarify this point.

It was not possible to make a significant comparison of different *lots of vaccine* used in the study as to pertussis incidence following their use because of the relatively few attacks in the injected children and also the relatively large number of small lots.

Factors related to dosage. Size of dose, interval and method of injection are complicated factors difficult of analysis. For the study series analysed the conditions have been defined. An interesting point in addition is to be found in the incompletely-injected series. Among these was a group of 126 children who received 4 cc of vaccine distributed over the same period as the complete series, with an incidence of pertussis of 2.8 per 100 person-years in comparison with 2.3 for the "completely" injected group analysed in this study. This group with the total dosage of 4 cc was relatively small and the incidence, therefore, is not really comparable with that reported for the study. It is of interest, however, to note that there was an indication of a considerably lower incidence than that expected from the findings in the control group, although it was higher than in the main injected group of 1,815. It is of further interest to know what happened to the remaining 51 children in the incomplete series, for whom the total dosage varied between 1.5 and 3.75 cc. Although this number was too small for comparison with the other groups, the incidence of pertussis in these children was 5.0 per 100 person-years. This should not be construed, however, as indicating a direct relation between quantity of vaccine injected and incidence. The problem is more complicated than that and it may be found, aside from any possible modification of the vaccine, that the period over which the vaccine injections are distributed is a matter of importance. This problem is under continued investigation.

The time required for immunity to develop is an important question in connection with any type of active immunization. An analysis of exposures and attacks was made with respect to the interval between vaccination and exposure, by 3-month intervals. The incidence for the first of the 3-month intervals was no different from that for the prolonged interval of 24 months. Here again, the relatively few attacks make it impossible to draw conclusions.

Factors related to the children injected. An analysis of the pertussis attacks which occurred in the test group, per 1,000 person-months experience, according to the children's age *at the time of vaccine injection,* showed that, within the age range of the study, there was no concentration of attacks with respect to the age at vaccination. Another factor considered was *sex.* Compared with the incidence of 15.1 for all the control group, the incidence among male controls was 14.6 and among females 15.6. In the injected group, compared with the incidence of 2.3 for all, the incidence among males was 2.3 and among females 2.2. The difference between males and females in both groups was statistically not significant and there was no indication that sex had any measurable influence on whether or not an injected child contracted pertussis.

A factor which stands out in relation to the incidence of pertussis in vaccinated children is the *intimacy of ex-*

posure. As emphasized with reference to table 12, it may be expected that the incidence will be higher following intimate exposures than after more casual ones.

An interpretation of the observed results in terms of expectancy in a given population

Even if the results of such an investigation as this are accepted as valid, there are various obstacles to translating them directly into terms of what might be expected to happen in the general population. As shown in table 2, there was a constant fluctuation in the number of children at risk in the study and the children were under observation for varying periods; also the risk at different times varied with the rise and fall of the curve of incidence of whooping cough for the community in which they lived. In order to facilitate the interpretation of the data a life experience table has been constructed in which a definite number of persons is assumed to stay throughout the study unless eliminated for the particular factor under consideration. In table 13 the material has been treated in such a way.

The object of this tabulation was to apply the results of the study, with respect to pertussis, to two groups of 1,000 children each, assuming that all had en-

TABLE 13

Results of the study applied to 1,000 vaccine-injected and 1,000 non-injected controls

Month of study	Per cent attacked with pertussis during period		Per cent not attacked during period		Number of pertussis cases		Number out of original 1,000 not attacked at end of each month from beginning of study	
	Test	Control	Test	Control	Test	Control	Test	Control
1. March—1934..	0.0	0.0	100.0	100.0	0	0	1000	1000
2. April.....	0.0	0.0	100.0	100.0	0	0	1000	1000
3. May.....	0.0	0.0	100.0	100.0	0	0	1000	1000
4. June.....	0.0	0.0	100.0	100.0	0	0	1000	1000
5. July.....	0.0	0.0	100.0	100.0	0	0	1000	1000
6. August.....	0.0	0.0	100.0	100.0	0	0	1000	1000
7. September.....	0.0	1.6	100.0	98.4	0	16	1000	984
8. October.....	0.0	0.0	100.0	100.0	0	0	1000	984
9. November.....	0.0	1.6	100.0	98.4	0	16	1000	968
10. December.....	0.0	1.5	100.0	98.5	0	15	1000	953
11. January—1935	0.0	4.5	100.0	95.5	0	43	1000	910
12. February.....	0.0	1.5	100.0	98.5	0	14	1000	896
13. March.....	0.0	4.4	100.0	95.6	0	39	1000	857
14. April.....	0.5	0.0	99.5	100.0	5	0	995	857
15. May.....	0.0	1.4	100.0	98.6	0	12	995	845
16. June.....	0.0	2.4	100.0	97.6	0	20	995	825
17. July.....	0.4	1.0	99.6	99.0	4	8	991	817
18. August.....	0.7	0.8	99.3	99.2	7	7	984	810
19. September.....	0.0	1.0	100.0	99.0	0	8	984	802
20. October.....	0.3	1.2	99.7	98.8	3	10	981	792
21. November.....	0.0	0.5	100.0	99.5	0	4	981	788
22. December.....	0.0	0.4	100.0	99.6	0	3	981	785

TABLE 13—*Continued*

Month of study	Per cent attacked with pertussis during period		Per cent not attacked during period		Number of pertussis cases		Number out of original 1,000 not attacked at end of each month from beginning of study	
	Test	Control	Test	Control	Test	Control	Test	Control
23. January—1936	0.0	0.4	100.0	99.6	0	3	981	782
24. February	0.0	0.3	100.0	99.7	0	2	981	780
25. March	0.0	0.4	100.0	99.6	0	3	981	777
26. April	0.3	0.5	99.7	99.5	3	4	978	773
27. May	0.1	0.7	99.9	99.3	1	5	977	768
28. June	0.0	0.8	100.0	99.2	0	6	977	762
29. July	0.0	1.0	100.0	99.0	0	8	977	754
30. August	0.2	0.4	99.8	99.6	2	3	975	751
31. September	0.0	0.2	100.0	99.8	0	2	975	749
32. October	0.1	0.5	99.9	99.5	1	4	974	745
33. November	0.3	1.5	99.7	98.5	3	11	971	734
34. December	0.3	1.2	99.7	98.8	3	9	968	725
35. January—1937	0.1	1.3	99.9	98.7	1	9	967	716
36. February	0.2	1.5	99.8	98.5	2	11	965	705
37. March	0.1	2.2	99.9	97.8	1	16	964	689
38. April	0.5	2.4	99.5	97.6	5	17	959	672
39. May	0.5	2.4	99.5	97.6	5	16	954	656
40. June	0.2	1.5	99.8	98.5	2	10	952	646
41. July	0.4	3.3	99.6	96.7	4	21	948	625
42. August	0.2	1.4	99.8	98.6	2	9	946	616
43. September	0.2	1.0	99.8	99.0	2	6	944	610

tered the study at the beginning of the period of observation and all had stayed throughout except for those who contracted pertussis. It is assumed that the two groups were entirely comparable under the definition of the study except for one point; the vaccine injection of one group. The incidence of pertussis for each month was calculated on the basis of the number of children actually at risk for that particular month, according to the experience recorded in table 2. On this basis it is seen that at the end of the observation period of 44 months, 94.4 per cent of the injected children would have remained without having contracted pertussis in contrast to 61.0 per cent of the non-injected group. The difference of 33.4 in per cent is equal to 17.9 times the standard error, in general agreement with the difference found for the actual incidence in the study for the test and control groups.

SUMMARY

A series of 4,212 children of which 1,815 were injected with pertussis vaccine and 2,397 were non-injected controls, was observed over a period of 44 months. The total observation may be expressed as 4,575 person-years, 2,268 for the injected group and 2,307 for the controls.

There were 400 attacks of pertussis, 52 in the vaccine-injected and 348 in the control group. Based on person-years experience, there were 2.3 annual attacks per 100 in the injected group and

15.1 in the control group. Expressed statistically, the difference is equal to fifteen times the standard error, indicating that the probability of such a difference occurring by chance alone is one in several million trials.

A correlation of all known exposures with subsequent attacks shows that 12.8 per hundred exposures in the injected group were followed by pertussis, compared with 68.5 in the control group.

The pertussis attacks which occurred in the vaccine group were less severe than those which occurred in the control group.

Conclusion

Under the conditions of the study there was a statistically significant difference in incidence of pertussis between the vaccine-injected and the control groups, indicating that substantial protection had been afforded to the injected group.

Acknowledgments

It is not possible to give due credit to all agencies and individuals co-operating in the study. We acknowledge with gratitude the interest of local physicians who lent their support in many ways including aid in immunization clinics. We cannot praise too highly the Bureau of Public Health Nursing of the City Health Department of Grand Rapids for the important part its nurses played in the study, first under the direction of Miss Bosse Randall, and later of Mrs. Mary Otterbein. Direct supervision of the pertussis field work done by the nurses was the responsibility of Miss Mildred Lineberger. Various staff members of the Michigan Department of Health have given invaluable assistance. Especial thanks are due Dr. Filip C. Forsbeck who assisted in the formulation of plans and methods of collecting information, gave helpful guidance, and reviewed all case histories. To Miss Dorothy Harmelink we are indebted for her devoted attention to the records. We are obligated to Dr. Haven Emerson's Sub-Committee on Evaluation of Administrative Practices of the American Public Health Association for the opportunity of carrying out a statistical analysis of the data and to various of its members for helpful criticism. To Dr. Kenneth F. Maxcy we are grateful for constructive suggestions in connection with final revision of the manuscript. Finally, the authors cannot express adequately their gratitude for the privilege of receiving helpful criticism and encouragement from the late Dr. Wade H. Frost.

References

1. Kendrick, P., and Eldering, G.
1936 Progress report on pertussis immunization. Amer. Jour. Pub. Health, *26:* 8–12.
2. Doull, J. A., Shibley, G. S., and McClelland, J. E.
1936 Active immunization against whooping cough. Interim report of the Cleveland experience. Amer. Jour. Pub. Health, *26:* 1097–1105.
3. Silverthorne, N., and Fraser, D.
1938 Whooping cough. Canad. Med. Assoc. Jour., *38:* 556–559.
4. Singer-Brooks, C.
1938 A controlled study of pertussis prophylaxis. Jour. Pediat., *13:* 292–296.
5. Miller, J.
1938 A controlled study in pertussis immunization. Jour. Pediat., *13:* 290–291.
6. Sauer, L.
1937 Municipal control of whooping cough. Jour. Amer. Med. Assn., *109:* 487–488.

7. Madsen, T.
1937 Abraham Flexner Lectures, Vanderbilt University, W. & W. Co. P. 172.
8. Kendrick, P., Lawson, G. M., and Miller, J. J.
1936 Tentative methods for the bacteriological diagnosis and control of whooping cough. Amer. Pub. Health Assn. Year Book, 1935–1936, 200–206.
9. Kendrick, P., and Eldering, G.
1935 Significance of bacteriological methods in the diagnosis and control of whooping cough. Amer. Jour. Pub. Health, *25:* 147–155.
10. Lawson, G. M.
1932 Studies on *H. pertussis.* Thesis, Harvard School of Public Health.