cortex and the 17-ketosteroid determinations[10] which measure its androgenic activity. The test appears to correlate best with the excretion of 11-hydroxysteriods as estimated by chemical means.[11]

SUMMARY

Two striking and easily determined changes (a decrease in circulating eosinophils and a rise in excretion of uric acid) consistently follow the administration of a single dose of 25 mg. of purified pituitary adrenocorticotropic hormone to normal subjects and patients with diseases not involving the adrenal cortex. It is suggested that a decrease of 50 per cent or more in the urinary uric acid—creatinine ratio indicates adequate adrenal cortical reserve. The test as outlined appears to be a measure of the adrenal reserve of hormones regulating carbohydrate, fat and protein metabolism. The use of this test in the diagnosis of adrenal insufficiency is discussed.

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DIPHTHERIA IMMUNIZATION

Use of an Alum-Precipitated Mixture of Pertussis Vaccine and Diphtheria Toxoid

JOSEPH A. BELL, M. D.
SENIOR SURGEON
UNITED STATES PUBLIC HEALTH SERVICE
Bethesda, Md.

There has been a downward trend in United States mortality from diphtheria and pertussis, but current reports show that several thousand young children continue to die every year from these diseases. Many deaths from these diseases will be prevented when effective prophylactic agents are extensively used. Such agents will be used more extensively when demonstrated improvements are made which increase their protective efficiency, decrease the number of reactions and simplify their method of administration.

To these ends considerable progress has been made in recent years. Diphtheria prophylactic agents have progressively improved from the old toxin-antitoxin mixtures to fluid toxoid and, now, alum-precipitated diphtheria toxoid. In 1938 Harrison and his associates[1] introduced alum-precipitated pertussis vaccine and it became evident that the method for immunizing children might be simplified through mixing precipitated pertussis and diphtheria antigens for simultaneous administration. In 1940 Schutze concluded from animal experiments that the admixture of alum-precipitated toxoid and pertussis vaccine does not impair the antigenic potency of either product.[2] In 1938 to 1940 Volk and Bunney[3] found that two doses of alum-precipitated diphtheria toxoid, when given to children 8 months of age and older with a three-week interval between doses, were the simplest and most effective procedure for immunization against diphtheria. Simultaneously in 1938 to 1941 Bell[4] found that two doses of alum-precipitated pertussis vaccine, when given to children 2 months to 2 years of age with a four-week interval between doses, conferred substantial protection against pertussis. Successful immunization at this young age was later confirmed by Sako and his associates.[5] From 1941 to date many reports[6] have appeared which are favorable to the use of an alum-precipitated mixture of these antigens; however, reports covering well controlled trials in the general population are scanty. This is unfortunate because clinical trial and experience constitute the ultimate test of the value of any prophylactic agent.

A clinical trial of a prophylactic agent presents many difficulties and may easily be fraught with errors unless properly planned and carried out. There are many known and unknown attributes which influence immunologic phenomena, and unless a clinical trial takes all these influences into account, one can never be certain that a particular phenomenon was the result of a particular treatment given. This study of an alum-precipitated mixture of pertussis vaccine and diphtheria toxoid utilizes a method for evaluating the immunity conferred by this product which takes account of all known and unknown attributes which might influence immunity to these diseases. The data are too extensive for immediate analysis and inclusion in a single report; hence this report is limited to the aspects of the study which relate to diphtheria immunization. This report outlines the objectives and fundamental criteria which were predetermined as essential to account for all attributes which might influence diphtheria immunity. It describes the preparation of the antigens used and the procedures used to fulfill the fundamental criteria, and it makes an analytic check (tables 1, 2 and 3) on the effectiveness with which the criteria were fulfilled. The report identifies all known attributes which influenced diphtheria immunity in this study and takes account of these and unknown influences in evaluating the effects of the study product given.

OBJECTIVES

In 1941 a fully controlled epidemiologic trial of an alum-precipitated mixture of

Due to lack of space, the many persons who contributed to this study cannot all be mentioned in this initial report. Their particular contributions will be described in later more detailed reports. Dr. J. P. Leake conducted the study during part of the war years and Public Health Nurses Anne Hodges, Ruth Sargent and Edna McCaleb made the routine visits and recorded the basic observations of the study.

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From the Division of Infectious Diseases, National Institute of Health, Bethesda, Md.

Medical Director J. P. Leake conducted this study in the author's absence during part of the years 1943 to 1945.

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diphtheria toxoid and pertussis vaccine was undertaken in Norfolk, Va. The trial was carried out in a manner to show what could be expected through routine use of this product in young children of the community. The main objectives were to answer the four questions: (*a*) Will two doses of an alum-precipitated mixture of diphtheria toxoid and pertussis vaccine protect young children against diphtheria as well as ordinary alum-precipitated diphtheria toxoid? (*b*) Will the mixture give substantial protection against clinical pertussis? (*c*) Will the mixture protect against both diphtheria and pertussis when given to children 2 to 5 months of age? (*d*) Will the mixture cause more local or general reactions than ordinary alum-precipitated diphtheria toxoid?

Table 1.—*A Comparison of the Number of Births and Families in the Study Area with the Number of Study Children and Families by Sex, Color and Family Size.*

		White		Negro		Total	
		Number	Percentage	Number	Percentage	Number	Percentage
Children	Sex						
Number of reported births in study area Jan. 1941 through April 1943	M	4,228		1,645		5,873	
	F	3,614		1,487		5,101	
	Total	7,842		3,132		10,974	
Number and percentage entering study	M	299	7.1	348	21.2	647	11.0
	F	292	8.1	299	20.1	591	11.6
	Total	591	7.5	647	20.7	1,238	11.3
Families	Size						
Estimated no. families in area with children < 10 years old	1 child	4,956		1,804		6,762	
	> 1 child	2,643		2,017		4,660	
	Total	7,599		3,821		11,420	
Number and percentage entering study	1 child	287	5.8	240	13.3	527	7.8
	> 1 child	279	10.6	358	17.7	637	13.7
	Total	566	7.4	598	15.7	1,164	10.2

FUNDAMENTAL CRITERIA FOR STUDY

To answer the foregoing questions on diphtheria immunity, it was predetermined that the following fundamental criteria would be essential for an adequate and fully controlled clinical trial of the mixed product. The criteria are listed as outlined in 1941 and all have been meticulously adhered to throughout the study.

(*a*) An alum-precipitated mixed or test product and an alum-precipitated unmixed or control product must be prepared so that each dose of each product contains the same quantity of alum-precipitated diphtheria toxoid from the same lot of crude toxoid. The test and control products must be prepared so that each will be identical in all respects except that each dose of the alum-precipitated diphtheria toxoid in the mixed product will contain 10,000,000,000 killed Hemophilus pertussis organisms.

(*b*) A study group of children must be preselected so as to be an adequate and representative sample of young children in a sizable United States community who are routinely available for voluntary immunizations. This is essential so that the results of the study may safely be projected to reflect the results expected from general use of the study products.

(*c*) A predetermined strictly random sampling procedure, based on a simple attribute entirely unrelated to immunologic phenomena, must be used to divide the study children into a test and a control group. This is essential to insure that all known and unknown attributes which might influence the occurrence of diphtheria or pertussis immunity (aside from the study products given) would be distributed between the two groups as equally as possible within the range of chance sampling variation.

(*d*) No person other than the visiting nurse observers and the physicians conducting the study should know that a study of pertussis vaccine is involved, and neither the nurses nor the physicians should know which child belonged to the test or control group, or which child received which study product, or whether any one child received the same product as any other child. This is essential to neutralize any possible human bias on the part of observers or informants in the recognition of immunologic phenomena. This is also essential to maintenance of the test and control groups as strictly random samples of the combined groups throughout the period of observation. When no one knows that pertussis vaccine has been given to any of the study children, no disproportionate treatment can be given to either of the random groups and all known and unknown attributes will have a nearly equal influence on each group; e.g., a nearly equal proportion of each group will receive pertussis vaccine by private physicians, and a nearly equal proportion of each group will depend on immunization rather than shielding their child from exposures to pertussis and other diseases.

(*e*) An intensive effort must be made to see that two doses of the test or control product are given to each study child as selected by the predetermined sampling process, that the products be given with a four-week interval between doses to children in the age band 2 to 23 months and that the products be given without inquiry or regard to a prior history of pertussis or pertussis vaccination. All children who receive at least one dose of the study product must be placed under uniform and routine monthly observation as long as they remain in the study area by one of the two specially trained public health nurses.[7]

7. Public Health Nurses Anne Hodges and Ruth Sargent carried out the previous study [1] and were the mainstays of the present study through the period covered by this report. Most of the Schick tests were carried out by Public Health Nurse Edna McCaleb under my supervision and that of Dr. J. P. Leake, who read and interpreted the Schick reactions in all children having any reaction whatsoever.

(f) A controlled Schick test is to be used as an index of diphtheria immunity, and a single lot of Schick toxin is to be used throughout the study, providing tests on animals indicate its potency is maintained. An intensive effort must be made to give Schick tests to all children remaining under observation one year after receiving the study products and simultaneously to give Schick tests to the mothers of such children. All disease diagnoses must be made, and all Schick reactions read and classified by me or by Dr. J. P. Leake.

Table 2.—*A Comparison of Children Born in the Odd with Those Born in the Even Months According to Specified Attributes*

Attributes	Month of Birth of Child Odd	Even	Total	Percentage Odd
Total no. of children entering study	646	592	1,238	52.2
Male	337	310	647	52.1
Female	309	282	591	52.3
	146	128	274	53.3
Health section at entry 4-6, 9-11	166	151	317	52.4
Health section at entry 1, 12, 14 Total white	312	279	591	52.8
Health section at entry 2,3,13	105	112	217	48.4
Health section at entry 7,8	229	201	430	53.3
Total Negro	334	313	647	51.6
1st dose injected Jan-June 1942	337	324	661	51.0
1st dose injected July 1942-June 1943	309	268	577	53.6
1st dose age 2-4 months	281	256	537	52.3
1st dose age 5-23 months	365	336	701	52.1
Others age <10 in household at entry	366	345	711	51.5
No others age < 10 in household at entry	280	247	527	53.1
Mothers of children given Schick test	367	340	707	51.9
Lost from observation prior to Schick test	103	100	203	50.7
Observed and given Schick test	540	485	1,025	52.7
Rec'd 2 doses mixed product	461	11	472	97.7
Rec'd 2 doses unmixed product	13	410	423	3.1
Other (1 dose aa, 1 dose, etc.)	66	64	130	50

PREPARATION OF STUDY PRODUCTS

The study products used were prepared by Parke, Davis and Company according to my specifications. They were purchased in 1941 in a quantity sufficient for the entire study and stored until used at 4 to 7 C. A single lot of fluid diphtheria toxoid was used in the preparation of both the mixed and unmixed product. For the mixed product 2 parts of the toxoid were mixed with 1 part of a suspension containing 30,000,000,000 phase I H. pertussis organisms per cubic centimeter which were grown on Sauer's blood agar and killed with a 1:10,000 solution of "merthiolate." For the unmixed product 2 parts of the toxoid were mixed with 1 part isotonic solution of sodium chloride instead of the pertussis suspension. Separately the mixed and unmixed products were then precipitated by adding 4 per cent alum solution and the precipitates washed twice. The products were passed through a colloid machine in order to obtain a fine division of the alum precipitate and finally were subjected to the usual sterility and safety tests. Titrations of both the mixed and unmixed products indicated that there was slightly less than 0.1 per cent alum equivalent in each. The crude toxoid had a least fatal dose value of 9, and each dose of the mixed and unmixed products by calculation had a least fatal dose value of 6 and an L+ value of 0.30 unit per dose.[8] Each dose of the mixed and unmixed product contained the same quanity of diphtheria toxoid and differed only in that each dose of the mixed product contained 10,000,000,000 killed H. pertussis organisms.

STUDY PROCEDURE

The study is being conducted in cooperation with the Norfolk City Union of the King's Daughters Visiting Nurse Association and with the assistance of the state and local health departments and physicians. The study area is in Virginia and comprises the cities of Norfolk and South Norfolk and the Tanners Creek District of Norfolk County. The population of the entire area is estimated at 250,000, of which 69 per cent are white and 31 per cent Negro. The study area was divided into fourteen geographic subdivisions in an effort to group people according to their usual associations at schools, churches, theaters and shopping centers. Each geographic section contained one King's Daughters Well Baby Health Station, and diphtheria immunization was urged for all infants 2 to 23 months old who attended these stations from January 1942 to July 1943. Using the current birth certificates in Norfolk, the Health

8. Dr. D. R. Mathieson, under direction of Dr. Noble Ferry and the late Dr. L. T. Clark, all of Parke, Davis and Company laboratories, carried out a few laboratory tests with the mixed product. The results were: "A. 100 per cent of sixteen 270 Gm. guinea pigs survived six weeks when challenged in groups of 4 with 10, 20, 40 and 80 minimum lethal doses of Corynebacterium diphtheriae toxin, respectively. Three such guinea pigs out of 4 similarly survived 160 minimum lethal doses. All had previously received a 1 cc. subcutaneous dose of the mixed antigen. Five such guinea pigs used as controls received no previous antigen and all died within six weeks after receiving only 1 minimum lethal dose of the same diphtheria toxin. B. Eighteen guinea pigs inoculated with the mixed antigen and then sacrificed fourteen days after receiving 10 to 160 minimum lethal doses of diphtheria toxin showed not the slightest vestige of reaction to the toxin, whereas the 1 control guinea pig given only 1 minimum lethal dose died in four days showing pleural effusion and massive hemorrhages of the adrenals, subcutaneous tissue, and abdominal muscles at the site of inoculation."

Department sent letters to the mothers of all 2 to 12 month old children urging immunization against diphtheria and smallpox. Local health department personnel administered the study products, and neither they nor the recipients knew that pertussis vaccine was involved in the study. The mixed and unmixed products were in 20 dose identical vials, each of which was numbered and labeled only "A-P Diphtheria Toxoid." If a code combination of the digits of a vial number was odd, it was to be given to children born in an odd month; i.e., January, March, May, etc. If the code combination was even, it was to be given to children born in an even month, February, April, June, etc. Thus birth in an odd or even month was the basis for dividing the children into a random test group and a control group each of which would have a nearly equal distribution of all known and unknown attributes which might influence diphtheria or pertussis immunity. At the health station an identifying record card was prepared for each child, and the card turned over to the Public Health Service visiting nurses after the child had received two doses, or in any event after two months had elapsed since the first dose. The visiting nurses prepared household rosters and made routine monthly visits, and more frequent visits during the occurrence of communicable disease, to all study children and have continued such visits to date. Under my close supervision and that of Dr. Leake the nurses made a record of all known and suspected attributes which might influence diphtheria or pertussis immunity. No child has been dropped from the study unless he permanently moved away from the area or otherwise became non locatable. Neither the visiting nurses nor I knew which children received which product, or which child belonged to which group, or whether any child received the same product as any other child.

Table 3.— *A Time Comparison of Schick Test Results and Laboratory Tests for Potency of the Schick Toxin Used-Lot AS3*

		Clinic No.	No. Tested	No. +	% +	Test No.	No. of G. Pigs	Cc. Diluted Toxin Per Pig.	Avg. No. of Days Survived
	July					I	5	5	3.9
1942							5	5	4.4
	Sept.					II	5	5	3.4
	Jan.	I	220	29	13	III	5	5	3.4
	Feb– March								
	May	II	618	81	13	IV	5	5	4.2
	June								
1943									
	July					V	2	5	3.7
							2	7	3.8
	Aug-Sept.	III	279	39	14				
	Nov.	IV	201	32	16				
	Feb.	V	190	34	18				
1944									
	May-July	VI	207	26	13				
1945	Feb-May	VII	17	2	12				
1946									
1947	Jan.					VI	4	5	4.1
							1	5	9.5
Total		I-VII	1,732	243	14	I-VI	2	5	4.2
							2	7	3.8

From February 1943 to July 1944, six regular Schick testing clinics were held and an effort made to give the Schick test to all children who received the first dose of the study products twelve or more months previously. In February and again in May 1945, an effort was made to give the Schick test to the few remaining children who were under observation and who had not been tested previously at the regular clinics. All mothers who accompanied children at the time of the Schick test were invited to have a Schick test. A single lot of Schick

toxin, stabilized with human serum,[9] was used throughout the study. It was tested for potency in guinea pigs at various intervals. All Schick reactions were read on the fourth or seventh day by me or by Dr. J. P. Leake. All were classified as either positive or negative, as a decision had to be made with respect to further immunization.[10]

in the area who are routinely available for voluntary immunization. The method of selection must be relied on to establish representativeness, but a few crude checks are available. If the study group is representative it would be expected to have a disproportionately large number of children from large families, to have a disproportionately large number of Negro

TABLE 4.—*Results of Schick Tests in Children 1 Year After Receiving the Mixed and Unmixed Product According to Specified Attributes.*

Attributes	Product Received: Mixed (Odd) No.	No.	+%+	"P"*	Unmixed (Even) No.	No.	+%+	"P"	Total No.	No.	+%+	"P"
1. Received one dose	42	8	19	.001	55	23	42	.000	97	31	32	.000
Received two doses	472	24	5		423	63	15		895	87	10	
2. Section 4-6, 9-10	106	3	3	.938	86	11	13	.250	192	14	7	.352
Section 1, 2, 14	97	2	2		83	16	19		180	18	10	
3. Section 2, 3, 13	90	7	8	.943	87	15	17	.312	177	22	12	.308
Section 7, 8	179	12	7		167	21	13		346	33	10	
4. Male	243	13	5	.787	229	36	16	.604	472	49	10	.481
Female	229	11	5		194	27	14		423	38	9	
5. Measles, chickenpox, mumps	117	4	3	.482	91	13	14	.854	208	17	8	.390
No measles, chickenpox, mumps	355	20	6		332	50	15		687	70	10	
6. W.C. vaccine outside study	86	2	2	.309	74	14	19	.284	160	16	10	.895
No W. C. vaccine outside study	386	22	6		349	49	14		735	71	10	
7. Mother Schick positive	57	1	2	.366	50	6	12	.485	107	7	7	.223
Mother Schick negative	282	16	6		244	42	17		526	58	11	
8. Given injection before July 1942	239	12	5	.952	224	32	14	.710	463	44	10	.820
Given injection after July 1942	233	12	5		199	31	16		432	43	10	
9. < 6 weeks between doses	334	14	4	.169	308	48	16	.514	642	62	10	.919
> 5 weeks between doses	138	10	7		115	15	13		253	25	10	
10. 2d dose to Schick< 11 mo.	191	12	6	.329	178	23	13	.331	369	35	10	.842
2d dose to Schick > 10 mo.	281	12	4		245	40	16		526	52	10	
11. Schick at< 18 mo. old	232	14	6	.356	220	37	17	.247	452	51	11	.111
Schick at >17 mo. old	240	10	4		203	26	13		443	36	8	
12. 2d dose age 2-5 months	201	15	8	.070	187	40	21	.000	388	55	14	.000
2d dose age> 5 months	271	9	3		236	23	10		507	32	6	
13. Breast fed at 1st dose	220	16	7	.111	205	36	18	.202	425	52	12	.031
Not breast fed at 1st dose	231	8	4		193	25	13		424	33	8	
14. White	203	5	3	.041	169	27	16	.610	372	32	9	.341
Negro	269	19	7		254	36	14		523	55	11	
15. Others age< 10 in household	291	22	8	.004	250	41	16	.296	541	63	12	.016
No others age< 10 in household	181	2	1		173	22	13		354	24	7	

* "P" equals the probability of occurrence of this distribution by chance.

EFFECTIVENESS WITH WHICH CRITERIA WERE CARRIED OUT

Table 1 was prepared to check the effectiveness with which the method pursued in obtaining the study children provided a study group which was an adequate and representative sample of all young children

children, and to have no disproportion by sex.[11] Table 1 compares the study group with estimates prepared from vital statistics reports on color, sex and family size. It shows that the expectations were fulfilled. This crude but confirmatory check permits the belief that the study group is adequate in size and representative to the extent that the results from the use of the study products may be projected to portray that which would result from their routine use in the study area.

Table 2 was prepared to check the effectiveness with which the sampling procedure was carried out to divide the study children into a comparable test and control group The predetermined and meticulously executed random sampling procedure based on an odd or even month of birth (an attribute entirely unrelated to diphtheria or pertussis immunity) should divide the study children into a test and a control group so that each group will have a nearly equal distribution of all known and unknown attributes which might influence the occurrence of diphtheria or pertussis immunity aside from the influence of the study products given. This can be checked by observing whether all known and suspected

9. The Schick toxin and the heated control material used were prepared by Dr Geoffrey Edsall at the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Health (Edsall, G., and Wyman, L.: Am. J. Pub. Health 34:365, 1944). In October 1942 he sent to the National Institute of Health a quantity of the diluted material sufficient for the entire study. It was promptly placed in the refrigerator, where the temperature was kept between 4 and 7 C. A quantity sufficient only for each clinic was transported to Norfolk in ice and kept on ice until the day of use.

10. Many of the Schick reactions were observed on the second, fourth and seventh day. Observation consisted of measuring and recording the transverse and longitudinal diameters of each zone of reaction at the test and control site and noting the presence of redness, edema, wrinkling and desquamation. Reactions were classified according to the area of clear definite redness on the fourth to seventh day following injection. The area was computed by assuming it equivalent to a circle with a diameter equal to the mean of the vertical and transverse measurements. If the area of redness at the test site exceeded the area of redness (if any) at the control site by the area of a circle 10 mm. in diameter, the reaction was classified positive; otherwise the reaction was classified negative The reaction was classified negative in the few instances in which definite evidence existed that the maximum local reaction at the test site occurred within forty-eight hours after injection and the nature of the reaction was similar to that at the control site. The reaction was classified as positive in a few instances (particularly in the Negro where discolorations are less evident than in the white skin) in which, because of the intensity and duration of the reaction, the original observer (J.A.B. or J.P.L.) judged it to be positive even though the measured area of redness was slightly less than has been set forth. All reactions were observed and classified without knowledge as to what immunizations the child may have received.

11. These are the characteristics expected in groups of children who respond to routine public pleas for immunization and constitute the mass of children generally immunized.

attributes are equally distributed within reasonable limits of chance sampling variation. It must be pointed out, however, that only a check can be made and that the meticulous execution of an adequate and predetermined sampling procedure is the all important factor. In the absence of such a procedure there can be no assurance that a disproportionate distribution of an unknown attribute may not have occurred and significantly influenced the results in an unknown manner and to an unknown extent.

Table 2 shows that 52.2 per cent of the total 1,238 children who entered the study were born in an odd month. It lists a few known and suspected attributes which might influence diphtheria or pertussis immunity in this study. (Others may be observed in table 4.) It will be noted that in all attributes except those relating to the study products given, close to 52 per cent of the children were born in an odd month. It is also pertinent to note that some 97 per cent of the children receiving two doses of the same product received the product intended. Twenty-four children, 13 odd and 11 even, received two doses of the wrong product because they gave the wrong birth date at both visits to the clinic for injection. They are hereafter tabulated according to the product received. Thirty-three children, 24 odd and 9 even, received one dose of each product. A few of these gave the wrong birth date at one clinic, but the disproportion was due entirely to the fact that in the early part of the study the persons giving the study products thought that the number zero on a vial was an odd number. This was promptly corrected, and although these 33 children have been carried under observation, they are not counted in tabulations comparing the study products. In table 4 it is pertinent to note that of the small percentage of children who received pertussis vaccine prior to Schick test from sources outside the study 53.8 per cent were born in an odd month. This confirms the statement that none of the parents knew that pertussis vaccine was involved in the study. It is obvious that the procedures used in this study gave two groups of children, one of which received two doses of the mixed and one of which received two doses of the unmixed product, and each of which had a nearly equal distribution of all known and suspected attributes which might influence diphtheria or pertussis immunity in these children. Aside from the study products given, no one of these attributes deviated beyond what might be expected through chance sampling variation, thus confirming that the sampling procedure was effectively carried out and insuring that all unknown attributes are similarly distributed between the two groups.

Table 3 was prepared to check whether the single lot of Schick toxin used throughout the Schick testing period remained of uniform potency. In each of the seven groups of mothers and children given Schick tests at various time intervals there was no significant variation in the percentage of positive reactions. In the laboratory tests all animals used died and had pathologic changes demonstrating death from diphtheria toxin. There was no significant variation in the average survival time at the different dates on which the toxin was tested. Thus it is concluded that the Schick toxin used remained remarkably uniform in potency throughout its long period of use.

RESULTS

Table 4 was prepared to identify attributes which may have influenced the Schick reaction. It lists all known and suspected attributes which might influence diphtheria immunity and shows for each the results of Schick tests performed one year after immunization. It shows the number of children tested according to the product received and the number and percentage who were Schick positive; i.e., who failed to be immunized. The "P" is the calculated probability of a difference in the number of Schick reactors as great or greater than observed occurring through chance sampling variation of independent attributes. Yates modification of Chi square was used in computing the "P" on all fourfold tables having less than 10 persons in any cell. In attribute 1, 32 per cent of the children who received only one dose of a known product were Schick positive as compared with 10 per cent who received two doses. This difference is consistent and significant both in the children receiving the mixed and in those receiving the unmixed product. On account of this finding the remainder of table 4 includes only children who received two doses of the same known product. It will be noted that children receiving the unmixed product had about three times as many Schick positive reactions as those receiving the mixed product, and this difference is consistent and fairly uniform throughout all attributes tested. It will be noted that attributes 2 to 11 inclusive were not significantly associated with the Schick reaction in this experience.[12] In attribute 12, age at time of injection, it is significant that children who received both their doses before they were 6 months of age had a higher proportion of Schick positive reactions than those who received at least one of their doses when they were 6 months of age or over. It will be noted that there is an association of questionable significance between the Schick reaction and the remaining attributes 13, 14 and 15. In summary of table 4 it appears that the Schick reaction is definitely correlated with the type of study product received, the number of doses received and the age at time of injection. There appears to be a correlation of questionable significance between the Schick reaction and the attributes concerning breast feeding at time of first dose, race and the presence or absence of other children in the household less than 10 years of age. There appears to be no correlation between the Schick reaction and any of the other attributes observed.

The significant correlations observed in table 4 do not suffice to answer the questions propounded on diphtheria immunity. For example, it is obvious that the group of children given injections at the earlier age must include a large proportion of children who were still feeding at the breast, and it must be determined whether age at time of injection or breast feeding or both were responsible for the correlations. Other similar but perhaps not so obvious relationships may exist between the various attributes listed in table 4. Thus it is necessary to examine further all the correlations observed in order to answer the questions propounded on diphtheria immunity. This involves a multitude of tabulations, far too many for publication, and hence only table 5 is presented to exemplify the procedure used.

Table 5 was prepared to determine whether the questionable correlation between the Schick reaction and breast feeding at time of first dose was due to breast

12. Some of these attributes are known to be correlated with the Schick reaction in the general population; e.g., no. 11, age at time of Schick test. This study was designed to minimize the influence of attributes not pertinent to the questions propounded. For the vast majority of children the age spread at time of the Schick test was less than fourteen months, and thus no significant age correlation was to be expected.

feeding or to the result of interrelationship with other attributes. It shows the results of the Schick test according to breast feeding at first dose among the 849 children who received two doses of the study product and on whom a history of breast feeding was obtained. The total group of 849 children are subdivided according to the age at time of injection, the presence or absence of other children less than 10 years of age in their household, and the study product received. In each subdivision the number of children tested and the observed number of Schick positive reactions is recorded and the "T," the theoretically expected number of reaction after adjusting for each of the other three attributes. The adjustment did not alter the significance of any of these attributes. Each was then checked against all other attributes listed in table 4, and the observed differences were remarkably consistent so far as the small numbers would permit comparison. Thus it is evident that only four of the attributes observed are significantly correlated with the Schick reaction in this study and that each of these four is independently significant after taking account of all other attributes: 1. Children receiving two doses of either product had disproportionately fewer Schick positive reactions than children receiving only one dose of the same product. 2. Children receiving their second dose of either product

Table 5.-- *Results of Schick Test by Duration of Breast Feeding After Adjusting for Influence of Other Attributes*

Product Rec'd	No. other children in h'hold	Age at Last dose, months	Breast Feeding at First Dose: Breast Fed No.	Breast Fed No.+	Breast Fed "T" +	Not Breast Fed No.	Not Breast Fed No.+	Not Breast Fed "T" +	Total No.	Total No.+	Total %+
Mixed	None	2-5	43	0	1.2	27	2	0.8	70	2	2.9
		>5	33	0	-	70	0	-	103	0	-
	1 or more	2-5	89	11	9.0	39	2	4.0	128	13	10.2
		>5	55	5	3.3	95	4	5.7	150	9	6.0
Unmixed	None	2-5	56	6	8.4	24	6	3.6	80	12	15.0
		>5	20	0	2.0	61	8	6.0	81	8	9.9
	1 or more	2-5	76	23	20.6	20	3	5.4	96	26	27.1
		>5	53	7	5.6	88	8	9.4	141	15	10.6
	Total		425	52	50.1	424	33	34.9	849	85	

Computation of Chi Square, Adjusted Experience

	Breast Fed Observed	Breast Fed Theoretical	Not B. Fed Observed	Not B. Fed Theoretical	Total
Schick positive	52	50.1	33	34.9	85
Schick negative	373	374.9	391	389.1	764
Total	425		424		849

Adjusted
Chi Square = 0.1944
"P" = .659
Unadjusted
Chi Square = 4.6700
"P" = .031

positive reactions, is computed. The computation is based on the assumption that breast feeding exerted no influence on the Schick reaction, and the "T" is derived by applying the percentage positive in the total of each subdivision to the number of children tested. By summation it is found that among the 425 children who were still feeding at the breast at first dose 52 Schick positive reactions were observed and 50 Schick positive reactions were expected after adjusting for the influence of other attributes. Thus it is concluded that breast feeding did not significantly influence the Schick reaction in this experience. A table was prepared and treated precisely in the same manner as table 5 except that race was substituted for breast feeding. The computed "P" was .688, and it is concluded that race did not significantly influence the Schick reaction in this experience.

Similar tables were prepared to determine whether the age at last dose, the presence of other children less than 10 years old in the household, the product received the number of doses received influenced the Schick at 6 to 23 months of age had disproportionately fewer Schick positive reactions than children receiving their second dose of the same product at 2 to 5 months of age. 3. Children receiving the mixed product had disproportionately fewer Schick positive reactions than children receiving the unmixed product. After adjusting for the combined influence of significant attributes the ratio of the percentage of Schick positive reactions among children receiving the mixed as compared with those receiving the unmixed product was 1:3. 4. Children living in single child households had disproportionately fewer Schick positive reactions than children living in households with other children less than 10 years of age.[13]

To the foregoing observations an over-all consideration should be added. In the absence of artificial immunization few children 6 to 30 months of age are immune to diphtheria, whereas adults for the most part are immune. In this study a total of 1,025 children of median age 18 months were given Schick tests one

13. This observation would appear to be incompatible with existing theories on the natural acquisition of diphtheria immunity. It is receiving further study.

year after they were given only one or two doses of the mixed or unmixed study products, and 90.6 per cent were found immune, whereas of the 707 mothers given Schick tests 82.3 per cent were found immune, as measured by the Schick test. Thus the over-all effort to immunize at a young age was highly successful. With respect to the age of immunization and the products given it should be noted that although children 2 to 5 months of age were not immunized as well as children 6 to 23 months of age, as indicated by the Schick test, the mixed product was so superior to the unmixed product that it appeared to be a better immunizing agent at the young age than the unmixed product at any age. Actually 8 per cent (15 out of 201) of the children who received both doses of the mixed product at 2 to 5 months of age had Schick positive reactions, as compared with 11 per cent (19 out of 175) of the children who received both doses of the unmixed product at 6 to 23 months of age.

SUMMARY AND CONCLUSIONS

A method has been described for designing and carrying out a fully controlled clinical trial of a prophylactic agent for immunizing young children in the general population against diphtheria.

The routine use of an alum-precipitated mixture of diphtheria toxoid and pertussis vaccine as prepared for this study and given in two doses with a four-week interval between doses to young children in Norfolk, Va., and a similar community, not only should result in a better protection against diphtheria, as measured by the Schick test, than that which would result from the similar use of ordinary unmixed alum-precipitated diphtheria toxoid but also should be effective for diphtheria immunization when given to infants 2 to 5 months of age.

The use of a Schick test performed one year after immunization as an index of diphtheria immunity revealed:

1. Children who received two doses of the unmixed alum-precipitated diphtheria toxoid had three times as many failures to immunize against diphtheria as children who received two doses of the alum-precipitated mixture of diphtheria toxoid and pertussis vaccine. Children who received only one dose of these products exhibited a similar difference.

2. Children who received only one dose had three times as many failures to immunize against diphtheria as children who received two doses. This was true no matter which product was given.

3. Children who received both doses at 2 to 5 months of age had twice as many failures to immunize against diphtheria as children who received at least one of their doses at 6 to 23 months of age. This was true no matter which product was given.

4. Children who received two doses of the mixed product before they were 6 months of age had fewer failures to immunize against diphtheria than children receiving the ordinary unmixed product at any age from 2 to 23 months.

ABSTRACT OF DISCUSSION

DR. HAROLD B. WOOD, Harrisburg, Pa.: These laborious investigations are worth while, and the investigators deserve thanks from everybody. In the immunization of children there is a question of to what extent Schick testing should be done. Frequently city or state organizations have certain amounts appropriated for such work. Is it worth while to give Schick tests to those children who have received their immunizing doses? I do not think so. Where the appropriation is limited, it is much better, to consider that a certain percentage are immunized already from their first series of doses, and use the rest of the money to immunize more children, rather than to go back and pick the Schick test for those children who have already received their immunizing doses. That is the problem we are up against frequently, and it is better, I believe, to carry on that plan. The new toxoid toxin-antitoxin which Dr. Ross explained seems to be an advantage because, if there is one thing we need in the Schick testing, it is a better way to determine definitely what is positive and what is negative. It is much easier to get children and their parents out for the immunizing doses than it is for the Schick retesting to determine the definite positive and what is negative. It is much easier to get children and their parents out for the immuch easier to get children and their parents out for the immunizing doses than it is for the Schick retesting to determine the definite positive immunity granted; therefore, why bother with that in general work? Of course, these remarks have no reference whatsoever to children in institutions which are under definite, lengthy control. Reprints of papers like these should always go into the library of the Hygienic Laboratory and also to the principal state authorities, if the authors are willing to send them.

DR. MAXWELL STILLERMAN, Great Neck, Long Island, N. Y.: What was the incidence of sterile abscess reactions in your series?

DR. JAMES P. LEAKE, Washington, D. C.: May I remark that my associates and I think the preferable term is "cyst" and not "sterile abscess." They are not sterile abscesses but little cysts, which tend to absorb themselves.

DR. JOSEPH A. BELL, Bethesda, Md.: The mixed product as prepared and used in this study at 2 to 5 months of age was a better immunizing agent, that is, produced disproportionately fewer Schick positive reactions, than the unmixed product given at any age from 2 to 23 months. However, neither the mixed nor the unmixed product immunized as well when given at 2 to 5 months of age as when given at 6 to 23 months of age. Table 4, attribute 12, shows the number of Schick positive reactions by age at time of injection. There was no significant association between the mothers' and children's Schick reaction in this study. The mothers were given Schick tests not only to give evidence with respect to the potency of the Schick toxin but also because it was considered possible that some association between the mothers' and children's Schick reaction would occur. If the children had been given Schick tests when less than 6 months of age and if they had received no diphtheria toxoid prior to the Schick test, some association between the mothers' and children's Schick reaction might have been evident. In this study all the children received diphtheria toxoid one year prior to the Schick test. With respect to Dr. Stillerman's question, the local and general reactions resulting from the use of the mixed and unmixed products in this study were so few and insignificant that to date no tabulations of their occurrence have been made. The Health Department personnel who administered the study products were instructed to use a different needle for each injection and to inject the products deeply into the loose subcutaneous tissue. I do not know whether the lack of reactions was the result of the method of injection or the small number of H. pertussis organisms injected with the mixed product, or whether it was due to other factors.

Whales.—Since the period of gestation is stated to be just over ten months for blue whales and eleven and a half months for fin whales, and the addition of the suckling period of six or seven months gives a total of about eighteen month from conception before the calf can fend for itself, it is obvious that the legal size limit has been fixed too low to be effective in preserving the stock Hamilton, J. E., *Nature*, June 12, 1948.