Typhoid Vaccine Studies VII: Typhoid-Paratyphoid Vaccine*

LIEUTENANT COLONEL DON LONGFELLOW, M.C., AND MAJOR GEORGE F. LUIPPOLD, SN.C.

Typhoid Research Unit, Army Medical School, Washington, D. C.

FOUR previous communications[1,2,3,4] emanating from the Typhoid Research Unit of the Army Medical School have been concerned with studies of monovalent typhoid vaccine. This paper reports our first investigations since World War I of typhoid-paratyphoid vaccine—often referred to as "triple typhoid" and as "T.A.B." vaccine.

The bacterial content of the typhoid and typhoid-paratyphoid vaccines prepared at the Army Medical School has been changed from time to time since 1916, to meet existing conditions. In 1934 it was simplified to a monovalent typhoid product. These changes have been reviewed in a previous publication.[7]

In September, 1940, the preparation of triple typhoid vaccine was resumed; and since then, the combined T.A.B. product prepared in the Division of Biologic Products of the Army Medical School has consisted of 1,000 million typhoid bacilli and 250 million each of the paratyphoid A and B components per ml. of material. Aside from its modification in bacterial content from any previously used T.A.B. vaccine, the 1940 product is different in respect to the strains of organisms employed in its preparation. The old "Rawlings" strain of *Eberthella typhosa* has been replaced by the more immunogenically active Panama "carrier" strain 58 (Army Medical School culture collection No. 42-A-58); and the strains of *Salmonella paratyphi* and *Salmonella schottmuelleri* are also different from those formerly used.

SELECTION OF STRAINS OF PARATYPHOID ORGANISMS

Selections of strains of *S. paratyphi* and *S. schottmuelleri* to be used in the 1940 type of T.A.B. vaccine were based, in general, on the same criteria as was the selection of the strain of *E. typhosa* in 1935.[5] A number of colonially smooth strains of each species were chosen from the Army Medical School culture collection and from outside sources, and titrated for mouse virulency. The most virulent of each species was selected for observations of cultural characteristics and biochemical behavior. Productivity of agglutinins and of protective substances was next determined; and lastly, the capacity of these organisms (prepared as vaccines) actively to immunize mice against heterologous strains of homologous species was ascertained.

No difficulty was experienced in the selection of a suitable Para B organism. Strain 41-H-6 of the Army Medical School culture collection was highly virulent for mice (m.l.d. 1 to 10 organisms in 5 per cent mucin); it produced a high agglutinin titer in rabbits, and the rabbit antiserum conferred a

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high degree of immunity on mice to an heterologous strain of *S. schottmuelleri.* Culturally and biochemically, strain 41-H-6 was a typical Para B organism, growing luxuriantly on infusion agar and emulsifying readily in physiological saline solution. Prepared as a vaccine, this organism protected mice against several hundred m.l.d.'s of an equally virulent strain of *S. schottmuelleri.*

In the case of the Para A culture, however, the most virulent of the strains titrated (41-N-8, A.M.S.) was found to be relatively avirulent, its m.l.d. in 5 per cent mucin ranging between 1 million and 10 million organisms. Otherwise, except for its comparatively sparse growth on infusion agar, this strain of Para A was fairly satisfactory. It was culturally smooth and biochemically typical of *S. paratyphi,* and it produced a satisfactory agglutinin titer in rabbits; but its productivity of immune substances, as determined by the mouse protection test, was difficult of appraisal because of the enormous numbers of test organisms required to represent multiples of the m.l.d. Prepared as a vaccine, it exhibited a demonstrable protection to mice against other strains of Para A, but here again the higher multiples of the m.l.d. amounted to overwhelming doses of foreign protein against which vaccination could not be expected to protect.

The avirulency of *S. paratyphi* 41-N-8 caused us no little concern; and when we learned from an antigenic analysis made in April, 1941, by Edwards [6] that this strain, as we were carrying it, was deficient in O-antigen I, we instituted a search for an antigenically complete strain which was more virulent than 41-N-8. This search did not end until one year later.

Antigenically complete strains of Para A, as determined by typing with diagnostic sera, were not difficult to find; but invariably they proved to be no more virulent than, or not as virulent as, our strain of 41-N-8. We adhered to the belief that virulency was somehow correlated with immunogenicity—but only because vaccines prepared with these avirulent but antigenically complete strains of *S. paratyphi* did not protect mice as effectively as a vaccine prepared with our antigenically deficient, but somewhat more virulent strain 41-N-8.

As a typical example of the results we obtained when making such comparisons of the immunogenicity of strains of *S. paratyphi,* a representative protocol is reproduced (Table 1) from the first announcement of this finding reported by one of us earlier this year.[7]

Referring to Table 1, strain CA is antigenically complete, being strongly agglutinated by I, II, XII, and *a* diagnostic sera; yet, when prepared as a vaccine, it is relatively inferior to the antigenically deficient strain 41-N-8.

The point of this discussion is that antigenic completeness of an organism, as determined by typing with diagnostic sera, is not synonymous with immunogenic effectiveness, as determined by active immunization of mice with vaccines or by passive immunization of mice with immune sera produced by vaccines. It is desirable, of course, to use a vaccine strain of an organism which is antigenically complete; but the decisive criterion for its acceptance should be the quality of the organism's performance, as a vaccine, in the animal body.

Our search for a replacement for strain 41-N-8 ended in the receipt of strain HA-6 from Dr. A. Felix, of London. This strain (hereafter referred to as 41-N-22) proved to be more virulent than any culture of Para A we had thus far worked with, its m.l.d. for mice ranging between 10,000 and 1 million organisms in 5 per cent mucin, averaging 100,000 organisms. It is culturally smooth and biochemically typical of *S. paratyphi;* upon being typed with diag-

TABLE 1

A Comparison of the Degree of Immunity Produced in Mice by Vaccines Prepared with an Antigenically Complete Strain of S. paratyphi (CA), and by an Antigenically Deficient, but Somewhat More Virulent, Strain of S. paratyphi (41–N–8)

Test No.	Dosage of Test Organisms (Expressed in Powers of 10; Exponent in Parentheses)	Results Expressed as a Fraction, the Denominator of Which Indicates the Number of Mice Subjected to Respective Dosages, While the Numerator Indicates the Number of These that Died — Results in Mice Vaccinated with: Para A (CA) Vaccine (I, II, XII; a)	Para A (41-N-8) Vaccine ((I), II, XII; a)
1.	10(6)	1/4	0/4
	10(7)	4/4	0/4
	10(8)	4/4	2/4
2.	10(6)	0/4	0/4
	10(7)	3/4	0/4
	10(8)	4/4	0/4
3.	10(6)	1/4	0/4
	10(7)	3/4	0/4
	10(8)	4/4	1/4
	10(9)	4/4	4/4
Totals—All mice		28/40	7/40

nostic sera, this culture is antigenically complete; and as a final qualification leading to its adoption, a vaccine prepared with strain 41-N-22 proved to be more immunogenically potent than strain 41-N-8 vaccine. Strain 41-N-8 was thereupon discarded, and strain 41-N-22 has been the parathyphoid A component of our T.A.B. vaccine since March 27, 1942 (T.A.B. Pool No. 119). We are not at all certain that this replacement represents the ultimate in Para A organisms, and we still desire to obtain cultures of this organism from recent isolations for comparative studies.

CIRCULATING PROTECTIVE SUBSTANCES PRODUCED BY T.A.B. VACCINE

As a means of determining the immunologic response of the animal body to vaccination, we know of no method which is more practical and more informative than titration of blood serum for protective substances demonstrable by mouse protection tests.[2]

A number of these tests were performed with sera of young adult males, initially inoculated with T.A.B. vaccine. These individuals had no history of previous typhoid vaccination, nor of any recognized enteric infection. Their sera were titrated for protective substances active against (1) alien strains of the typhoid-paratyphoid organisms comprising T.A.B. vaccine, (2) heterologous types of *Salmonella,* and (3) coliform organisms containing *Salmonella* O-antigens.

Individual Typhoid, Paratyphoid A, and Paratyphoid B Protective Titers—These were determined by routinely performed serum protection tests, and are represented here only in tabulated form. It will be noted that the results of titrations of two groups of individuals have been entered in Tables 2, 3, and 4. Titrations of sera from persons vaccinated with the original 1940 type of T.A.B. vaccine, containing the relatively avirulent strain of *S. paratyphi* 41-N-8, were made soon after this vaccine had been adopted by the Army. Replacement of strain 41-N-8 with the more virulent strain 41-N-22 necessitated a revaluation of the T.A.B. product in respect to the production of protective substances active against alien strains of all three organisms comprising T.A.B. vaccine.

TABLE 2

Typhoid Protective Titers of Sera from 85 Previously Unvaccinated Individuals, Before Inoculation and 14 Days After Inoculation with the Original 1940 Type T.A.B. Vaccine, and of a Comparable Group of 73 Individuals Before and 14 Days After Inoculation with the Present T.A.B. Vaccine Containing a Para A Component Prepared with a Relatively Virulent Strain of S. paratyphi

Minimum Lethal Doses of the Test Organism	Number and Per cent of Persons Whose Sera, Before and After Vaccination, Protected Mice Against the Dosages of Test Organism in Column on Left. Individuals Vaccinated with: T.A.B. Vaccine (With S. paratyphi 41-N-8)				T.A.B. Vaccine (With S. paratyphi 41-N-22)			
	Before Vaccination		After Vaccination		Before Vaccination		After Vaccination	
	No. of Persons	Per cent	No. of Persons	Per cent	No. of Persons	Per cent	No. of Persons	Per cent
100,000	..	.....	2	2.34	..	.....	3	4.10
10,000	..	.....	12	14.11	..	.....	25	34.24
1,000	..	.....	31	36.47	..	.....	19	26.02
100	..	.....	28	32.94	1	1.36	19	26.02
10	1	1.17	12	14.11	3	4.10	7	9.58
1	10	11.76	..	.....	9	12.32	..	.....
Less than 1	74	87.05	..	.....	60	82.19	..	.....
Totals	85	99.98	85	99.97	73	99.97	73	99.96

COMMENT: It will be noted (Table 2) that some improvement in the production of typhoid protective titers is evident in the group vaccinated with the present T.A.B. product, over the titers produced by the T.A.B. vaccine containing strain 41-N-8 of *S. paratyphi*. However, to those who have followed this series of studies, it will also be noted that even the titers resulting from inoculation with the present vaccine are not, in general, as high as those following inoculation with monovalent typhoid vaccine. No explanation of this obvious lowering of protective antibody content is offered, but it is thought to be connected in some way with the multiplicity of reaction-provoking substances in T.A.B. vaccine simultaneously administered, as contrasted to the relatively fewer of such substances present in monovalent typhoid vaccine.[8]

TABLE 3

Paratyphoid A Protective Titers of Sera from 80 Previously Unvaccinated Individuals, Before Inoculation and 14 Days After Inoculation with the Original 1940 Type T.A.B. Vaccine, and of a Comparable Group of 69 Individuals Before and 14 Days After Inoculation with the Present T.A.B. Vaccine Containing a Para A Component Prepared with a Relatively Virulent Strain of S. paratyphi

Minimum Lethal Doses of the Test Organism	Number and Per cent of Persons Whose Sera, Before and After Vaccination, Protected Mice Against the Dosages of Test Organism in Column on Left. Individuals Vaccinated with: T.A.B. Vaccine (With S. paratyphi 41-N-8)				T.A.B. Vaccine (With S. paratyphi 41-N-22)			
	Before Vaccination		After Vaccination		Before Vaccination		After Vaccination	
	No. of Persons	Per cent	No. of Persons	Per cent	No. of Persons	Per cent	No. of Persons	Per cent
1,000	..	.....	..	.....	..	.....	1	1.44
100	..	.....	1	1.2	..	.....	17	24.63
10	..	.....	17	21.2	..	.....	32	46.52
1	5	6.25	48	60.0	3	4.34	19	27.39
Less than 1	75	93.7	14	17.5	66	95.65	..	.....
Totals	80	99.95	80	99.9	69	99.99	69	99.98

COMMENT: These results (Table 3) are characterized by comparatively low protective titers, when expressed as m.l.d.'s of the test organism. They are difficult of interpretation because of the relative avirulency of the test organism, necessitating the administration of enormous numbers of organisms in the challenging doses. And, if the causal organism, in an actual epidemiological situation, were not any more virulent than the test organism used in these tests, the degree of immunity would require restatement in some term other than " m.l.d."

However, replacement of *S. paratyphi* 41-N-8 by the more virulent strain 41-N-22 has, apparently, resulted in the production of a higher average *S. paratyphi* protective titer.

higher than that of the 1940 group, and it is possible that this factor may have influenced the production of comparatively higher titers by the present T.A.B. vaccine.

Cross-immunization against Heterologous Types of Salmonella—Sera of individuals initially vaccinated with T.A.B. vaccine were similarly titrated against various types of *Salmonella.* It was found that significant amounts of protective substances could be demonstrated in such sera, active against *Salmonella typhimurium, Salmonella enteritidis,* and against the Java strain of paratyphoid B, but not against *Salmonella choleraesuis* nor *Salmonella oranienburg;* nor were there any demonstrable protective substances active against *Proteus morganii*—an organism

TABLE 4

Paratyphoid B Protective Titers of Sera from 84 Previously Unvaccinated Individuals, Before Inoculation and 14 Days After Inoculation with the Original 1940 Type T.A.B. Vaccine, and of a Comparable Group of 56 Individuals Before and 14 Days After Inoculation with the Present T.A.B. Vaccine Containing a Para A Component Prepared with a Relatively Virulent Strain of S. paratyphi

	Number and Per cent of Persons Whose Sera, Before and After Vaccination, Protected Mice Against the Dosages of Test Organism in Column on Left. Individuals Vaccinated with:							
	T.A.B. Vaccine (With S. paratyphi 41-N-8)				*T.A.B. Vaccine (With S. paratyphi 41-N-22)*			
Minimum Lethal Doses of the Test Organism	*Before Vaccination*		*After Vaccination*		*Before Vaccination*		*After Vaccination*	
	No. of Persons	*Per cent*	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*
1,000,000	..	.....	..	.....	..	.....	19	33.91
100,000	..	.....	22	26.19	..	.....	25	44.64
10,000	..	.....	37	44.04	..	.....	11	19.64
1,000	..	.....	20	23.8	2	3.57	1	1.78
100	5	5.95	4	4.76	20	35.71	..	.....
10	16	19.04	1	1.19	11	19.64	..	.....
1	31	36.9	..	.....	14	25.00	..	.....
Less than 1	32	38.09	..	.....	9	16.07	..	.....
Totals	84	99.98	84	99.98	56	99.99	56	99.97

COMMENT: The potency of T.A.B. vaccine containing *S. paratyphi* 41-N-22 (Table 4) seems to extend its improved quality to protection against *S. schottmuelleri.* However, it will be noted that the intitial or " natural " immunity of this group of individuals is, in general,

of questionable etiological significance in diarrheal diseases, classified at one time with the *Salmonella* but later withdrawn because of its antigenic alienage.

From these results, it appears that T.A.B. vaccine may be considered an effective immunizing agent against cer-

tain types of *Salmonella* which are antigenically related to the vaccine organisms, but not against the "Suipestifer" or paratyphoid C (O-antigens VI–VII) group of *Salmonella* nor against the antigenically unrelated *Pr. morganii.*

Cross-immunization against Strains of Coliform Organisms containing Salmonella Antigens—Two strains of coliform organisms, each containing a different group of *Salmonella* antigens, were obtained from Dr. Philip R. Edwards of the National Salmonella Center in Lexington, Ky. One of these was designated "Coli Fla. 573," and contained O-antigens I and II (as in paratyphoid A); the other was labeled "Coli 3," and was endowed with O-antigens IV and V (as in paratyphoid B). Although neither of these organisms had been indisputably incriminated as the causal organism of an enteric infection, "Coli 3" was thought to have caused such a condition, based on the absence of any other etiological agent in the stools of the patient and on a significant paratyphoid B agglutinin titer of the patient's serum. Strain "Coli Fla. 573" was as virulent for mice as the more active strains of *S. paratyphi,* its m.l.d. being 1 million organisms in 5 per cent mucin. The "Coli 3" culture was highly virulent for mice, its m.l.d. in mucin being between 100 and 1,000 organisms.

It had previously been learned[7] that these organisms were loosely connected immunogenically with their paratyphoid simulants; that is, "Coli Fla. 573" was capable of evoking a low degree of immunity to paratyphoid A, and "Coli 3," prepared as a vaccine, produced a somewhat higher degree of immunity to paratyphoid B. It was then desired to learn, in the event these coliform organisms were actually pathogens, whether

TABLE 5

Coliform "Coli Fla. 573" Protective Titers of Sera from 57 Previously Unvaccinated Individuals, Before Inoculation and 14 Days After Inoculation with T.A.B. Vaccine

Minimum Lethal Doses of the Test Organism	Number and Per cent of Persons Whose Sera, Before and After Inoculation with T.A.B. Vaccine, Protected Mice Against the Dosages of "Coli Fla. 573" Entered in Column on Left			
	Before Vaccination		After Vaccination	
	No. of Persons	Per cent	No. of Persons	Per cent
100	..	.....	14	24.56
10	..	.....	19	33.33
1	19	33.33	20	35.08
Less than 1	38	66.66	4	7.01
Totals	57	99.99	57	99.98

TABLE 6

Coliform "Coli 3" Protective Titers of Sera from 41 Previously Unvaccinated Individuals, Before Inoculation and 14 Days After Inoculation with T.A.B. Vaccine

Minimum Lethal Doses of the Test Organism	Number and Per cent of Persons Whose Sera, Before and After Inoculation with T.A.B. Vaccine, Protected Mice Against the Dosages of "Coli 3" Entered in Column on Left			
	Before Vaccination		After Vaccination	
	No. of Persons	Per cent	No. of Persons	Per cent
100,000	..	.....	22	53.65
10,000	..	.....	17	41.46
1,000	8	19.51	2	4.87
100	25	60.97	..	.....
10	8	19.51	..	.....
Totals	41	99.99	41	99.98

any demonstrable protective substances active against them were produced in persons inoculated with T.A.B. vaccine. Serum protection tests were therefore performed with the sera of persons initially vaccinated with T.A.B. vaccine, using " Coli Fla. 573 " and " Coli 3 " respectively as test organisms. The results of these determinations have been recorded in Tables 5 and 6.

COMMENT: Vaccination of humans with T.A.B. vaccine (Table 5) produced demonstrable protective substances active against this coliform organism containing paratyphoid A O-antigens I and II; but, as in the case of serum protection tests against *S. paratyphi,* interpretation of results is difficult because of the relative avirulency of the test organism and the necessity for administering enormous numbers in the challenging doses.

COMMENT: These results (Table 6) indicate that vaccination of persons with T.A.B. vaccine produced significant quantities of protective substances in the blood serum of these individuals, active against the coliform organism containing paratyphoid B O-antigens IV and V. It is interesting to note that, as in the case of titration of these sera for paratyphoid B protective substances, the titers of the sera collected before vaccination are relatively high.

Interest in these coliform organisms is centered around a statistical analysis of the incidence of enteric infections, made in 1937 by Callender and Inmon.[9] These observers noted that a significant drop in the morbidity of all diarrheal diseases in the United States Army followed, by some months, the introduction of *S. schottmuelleri* in typhoid vaccine, and that an equally significant increase in these diseases followed the withdrawal of the Para B fraction from T.A.B. vaccine. They realized that " the antigenic factors in typhoid [and paratyphoid] immunization by the U. S. Army may have an influence on the morbidity of diarrheal diseases . . ."; and, referring to the apparent correlation between changes in morbidity and changes in the components of enteric vaccine, they added: " Whether this series of events is due to immunization or not, the coincidence is striking."

Members of genera of the family *Enterobacteriaceae* other than the *Salmonella* are, of course, responsible for much of this heterogeneous group of " diarrheal diseases." Coliform organisms have often been suspected of having some etiological significance, but have never been definitely incriminated as causal organisms of human enteric infection; and even more often, probably, these organisms have escaped attempts to incriminate them solely because of their coliform cultural characteristics.

One instance of the reverse of this was brought to our attention about a year ago—that is, an instance wherein the cultural characteristics of an organism were overlooked (or not determined), the culture being hastily typed with diagnostic *Salmonella* sera and reported as *S. paratyphi* because of its high agglutinability by Para A serum. Later, this culture was found to ferment lactose slowly, and it was subsequently identified as a coliform organism containing Para A antigens.

The question arises—How often, if this latter procedure were followed, would coliform organisms, suspected of playing an etiological rôle in diarrheas, be found to contain *Salmonella* antigens? And this suggests another question: Are these particular coliform organisms the only—or the most common—representatives of the *Escherichia* capable of producing enteric symptoms in man? If so, the connection between morbidity of diarrheal diseases and the antigenic content of enteric vaccines becomes evident. A potentially productive field for investigation of this type of enteric infection is open to the epidemiologist.

SUMMARY

Studies have been presented on the special problems involved in the selection of desirable vaccine strains of organisms comprising T.A.B. vaccine, and on the production of circulating protective substances by vaccination with T.A.B. vaccine.

The apparent relationship of typhoid-paratyphoid immunization to the morbidity of all diarrheal diseases in the United States Army has been briefly discussed.

Vaccine strains of organisms were chosen on the basis of their virulence for mice on the one hand, and for their immunogenic potency as demonstrated by active immunization of mice, on the other.

Initial vaccination of young adult males with T.A.B. vaccine produced significant amounts of protective substances in the blood serum active against *E. typhosa, S. paratyphi, S. schottmuelleri,* the Java strain of paratyphoid B, *S. enteritidis, S. typhimurium,* and against two coliform organisms containing *Salmonella* O-antigens I and II, and IV and V, respectively. Such vaccination failed to produce, by the method used for detection, demonstrable protective substances active against *S. choleraesuis, S. oranienburg,* and *Pr. morganii.*

Replacement of the originally selected strain of *S. paratyphi* as the Para A component of T.A.B. vaccine with a relatively more virulent strain of this organism was followed by a general improvement in the potency of the combined product, as evidenced by significantly higher protective titers for *E. typhosa, S. paratyphi,* and *S. schottmuelleri.*

Coliform organisms containing *Salmonella* antigens offer an interesting group of possible etiological agents for study, particularly in view of the antigenic and immunogenic relationship between these and the T.A.B. vaccine organisms. It is probable that, if coliform organisms suspected of causing or inciting enteric infections were typed with diagnostic *Salmonella* sera, many more of such antigenic relationships would be revealed.

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