STUDIES ON INFECTION AND IMMUNITY IN EXPERIMENTAL TYPHOID FEVER

III. Effect of Prophylactic Immunization

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Immunization of human beings against typhoid fever has been practiced for more than 60 years, probably having been first performed by Wright in 1897 (1). Large scale active immunization was first employed in the British Armies in South Africa during the Boer War, with controversial results (2). Later, more systematic trials were carried out, mainly in India (3).

As a consequence of the fairly successful experiences of the British in immunizing their troops, antityphoid vaccination was initiated on a voluntary basis in the United States Army in 1908 by Major F. F. Russell of the Army Medical Corps (4). Compulsory vaccination for all Army recruits was made effective in 1911, and by the end of that year, 85 per cent of all Army personnel had been immunized (5). Within the next few years, antityphoid vaccination was made obligatory for all military services. Meanwhile, the extensive British experience in India, which provided an opportunity for comparing typhoid rates in unvaccinated *versus* vaccinated volunteers, was very carefully examined by Greenwood and Yule (6), who concluded that the efficacy of typhoid immunization had been clearly established. Subsequent reports of various investigators generally have indicated that antityphoid vaccination has been an effective, if not the dominant, factor in lowering the incidence and case fatality rates of typhoid fever in military organizations.

On the other hand, these studies have been criticized on the ground that few, if any of them, were adequately controlled. In most instances, controls were simply those individuals who happened not to be vaccinated, without regard to any differences in degree of exposure between the two groups. Thus, on the basis of available data, some epidemiologists (*e.g.*, Cockburn, 2), while not denying the apparent protection afforded by typhoid vaccine, have questioned the validity of the conclusions cited above. This

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doubt was increased further by a number of reports during the past two decades on the occurrence of unexpected numbers of cases of typhoid fever in vaccinated groups. Limited outbreaks of this disease in American soldiers in the Mediterranean (7) and Pacific (8) areas during World War II have been described, and extensive outbreaks have been reported among vaccinated British soldiers in the Middle East (9–11) and elsewhere (12). Indeed, the findings of Marmion *et al.* (11) led to some uncertainty regarding the degree of immunity induced by the disease itself.

Consequently, a critical re-evaluation of antityphoid immunization was undertaken several years ago at this institution. Since it was impractical at that time to carry out definitive studies on this problem in man, a suitable experimental animal was sought.

Laboratory testing of typhoid vaccines has been performed customarily in mice, and, although it is possible to distinguish between different types and batches of vaccines by the mouse protection test, a direct correlation between the mouse - protective potency of a vaccine and its ability to protect man against infection with the typhoid bacillus has not yet been established. Hence, it was felt that more pertinent information on typhoid prophylaxis might be obtained if a species closely related to man were employed. Accordingly, the chimpanzee was selected as the animal of choice for re-evaluating the effectiveness of antityphoid immunizing agents. It was first necessary to define the conditions under which this disease could be established consistently in the chimpanzee. An account of these studies is given in the first communication in this series (13). The earlier results of Grünbaum (14) and Metchnikoff and Besredka (15, 16) were confirmed and extended; it was demonstrated that a disease with clinical, laboratory, and histopathological findings closely resembling those occurring in human typhoid infections could be produced in chimpanzees. In a subsequent report (17), it was shown that recovery from this disease conferred immunity to a second challenge.

The next phase of these studies, described in the present report, deals with the capability of immunizing agents to protect chimpanzees against this experimental infection.

Materials and Methods

Experimental Animals.—During the course of this investigation, which extended over a period of 3 years, a total of sixty-seven young chimpanzees of both sexes ranging in weight from 14 to 35 pounds were used. These animals were obtained from the Congo and West Africa, and were housed and maintained at this institution for at least 2 months prior to this study. Several stool cultures from each animal were examined to ascertain that they were free of Group D *Salmonellae.*

Challenge Organisms.—*Salmonella typhosa* strain Ty2, phage-type E_1, and strain 2593, phage-type T, were utilized as challenge cultures. The characteristics of these strains and their infectivity for chimpanzees are given in a previous publication (13).

Challenge Procedures.—The preparation of the challenge suspensions and the method of challenge were the same as those employed previously (13). A 5 hour veal infusion agar culture of either Ty2 or 2593 was harvested in saline and the chimpanzees challenged orally by feeding each a banana inoculated with the desired number of organisms. The number of viable bacilli contained in the dose administered was determined tubidimetrically and checked by subsequent viable count. The mouse virulence of each challenge culture was ascertained by intra-

peritoneal inoculation, employing saline suspensions of the organisms. LD_{60} values of approximately 3×10^6 bacilli, as calculated by the method of Reed and Muench (18) were obtained. The presence of Vi and O antigens in the challenge inocula and the O-inagglutinability of these challenges were confirmed by serological tests with rabbit antisera produced as previously described (17).

Bacteriological Procedures.—Stool specimens were cultured regularly on duplicate SS agar plates and occasionally in selenite-F enrichment broth, on MacConkey agar and Wilson-Blair bismuth sulfite agar. Lactose-negative colonies were transferred to Kligler's iron agar slants to ascertain biochemical changes characteristic of *S. typhosa*. From time to time, confirmatory checks of these cultures were made by more extensive biochemical procedures and by serological methods. Blood was cultured by inoculating 2 to 4 ml into 40 ml of trypticase soy broth containing 0.1 per cent agar and 0.1 per cent sodium citrate. When bacterial growth was evident, or after 7 days of incubation in the absence of discernible growth, subcultures were made to SS agar. Colonies suspected of being *S. typhosa* were handled in the same manner as stool culture isolates. Blood cultures were considered negative if growth of *S. typhosa* failed to occur within 7 days.

Clinical and Laboratory Observation of Challenged Animals.—For the duration of the experiment after challenge (generally 28 days), the course of infection was followed by daily bacteriological examination of stools, blood cultures, either daily or every other day, close observation for alterations in appetite, appearance or behavior, and periodic examination of serum for the presence and titer of O, H, and Vi antibodies. Rectal temperatures were taken daily, and an animal was considered to be febrile when its temperature was greater than 1.5°F above its mean prechallenge normal temperature.

Serological Examinations.—Serum titers of antibody to the flagellar and somatic components of *S. typhosa* were determined by the usual bacterial agglutination procedures employed by the Department of the Army (19), while Vi antibody was measured by the hemagglutination technique of Landy and Lamb (20).

Immunizing Products.—A monovalent acetone-killed and dried typhoid vaccine (AK) was prepared from the Ty2 strain of *S. typhosa*. This vaccine was made according to the method described by Landy (21) except for the omission of the paratyphoid components. On reconstitution to 50 ml with distilled water, each 0.5 ml dose contained approximately 500×10^6 organisms. The heat-killed, phenol-preserved vaccine (HP) employed was the standard fluid typhoid and paratyphoid vaccine used as routine by the Armed Services. The product utilized in these studies was Lot 5802-6211272, prepared by Eli Lilly Company, Indianapolis. Each 0.5 ml dose contained 500×10^6 *S. typhosa* 58, 125×10^6 *S. paratyphi*, and 125×10^6 *S. schottmuelleri*. Both vaccines met or exceeded the potency requirements of the National Institutes of Health (22), which are based on mucin suspension of the challenge cultures. In mouse protection tests employing saline challenges, it was observed by one of us (SG) that AK vaccines prepared from *S. typhosa* Ty2 were approximately 30 to 70 times more potent than typical HP typhoid-paratyphoid vaccines. Comparable results in experiments utilizing both types of challenge have been reported for similar vaccines by Edsall *et al.* (23).

In addition to the aforementioned vaccines, purified Vi and O antigens were used as immunizing agents. The Vi antigen utilized was the highly polymerized, acidic polysaccharide consisting of repeating units of *N*-acetyl-D-galactosaminuronic acid (24–26) and had been isolated from a Vi strain of *Escherichia coli* (27). The purified O antigen, derived from *S. typhosa* 0-901 (28), was a phosphorylated lipopolysaccharide.

RESULTS

The issues explored in the present study were: (*a*) whether typhoid vaccine would confer protection against the experimental disease, (*b*) comparison of the

effectiveness in chimpanzees of typhoid vaccines of different immunogenic potency in mice, and (*c*) the capacity of the purified Vi and O antigens, either singly or in combination, to induce immunity to infection.

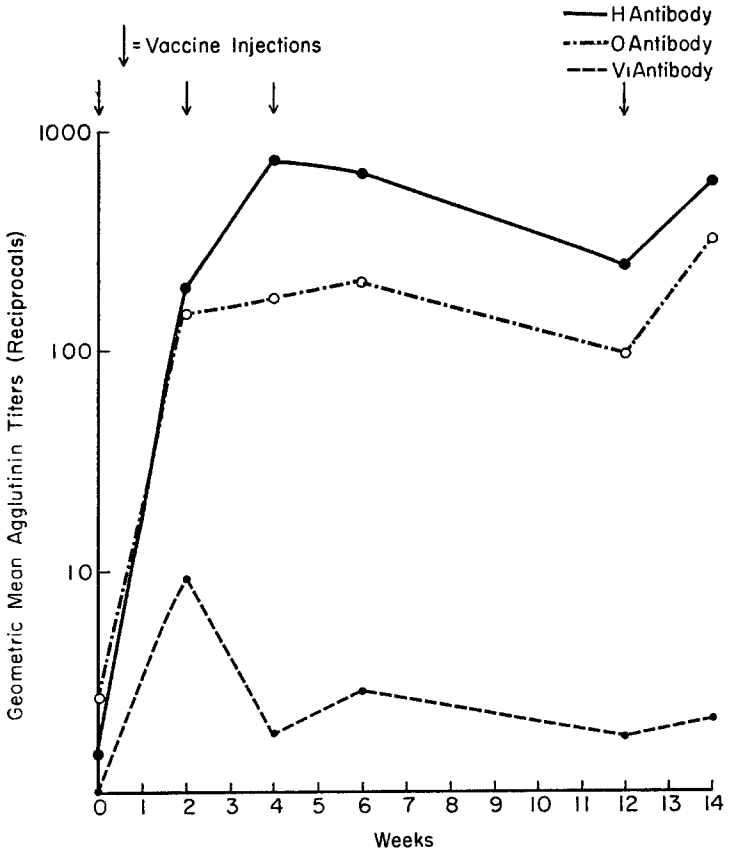

FIG. 1. Antibody responses of chimpanzees to AK vaccine

Antibody Responses Following Immunization.—

A total of 23 chimpanzees were immunized with AK vaccine by subcutaneous inoculation with three 0.5 ml doses at 14 day intervals, followed by a 0.5 ml booster injection *via* the same route 2 months later. The geometric mean antibody responses of this group of animals are given in Fig. 1. Ten additional chimpanzees, inoculated by the same schedule, were immunized with HP vaccine, and produced the mean antibody levels shown in Fig. 2.

As seen in these figures, both vaccines stimulated the production of good O and H antibody levels. The O responses to the 2 products were essentially the same, but greater H antibody titers were attained following the administration of the AK vaccine. In contrast to the O and H responses, however, Vi antibody

titers were low in both instances. These findings in the chimpanzee are essentially comparable to those observed in man under similar immunizing regimens.

With respect to the isolated antigens, 4 chimpanzees were injected with purified Vi antigen, 4 with purified O antigen, and 4 with both Vi and O. All animals received 4 subcutaneous injections, each consisting of 40 micrograms of antigen, at intervals indicated in Fig. 3. The levels of antibody produced by

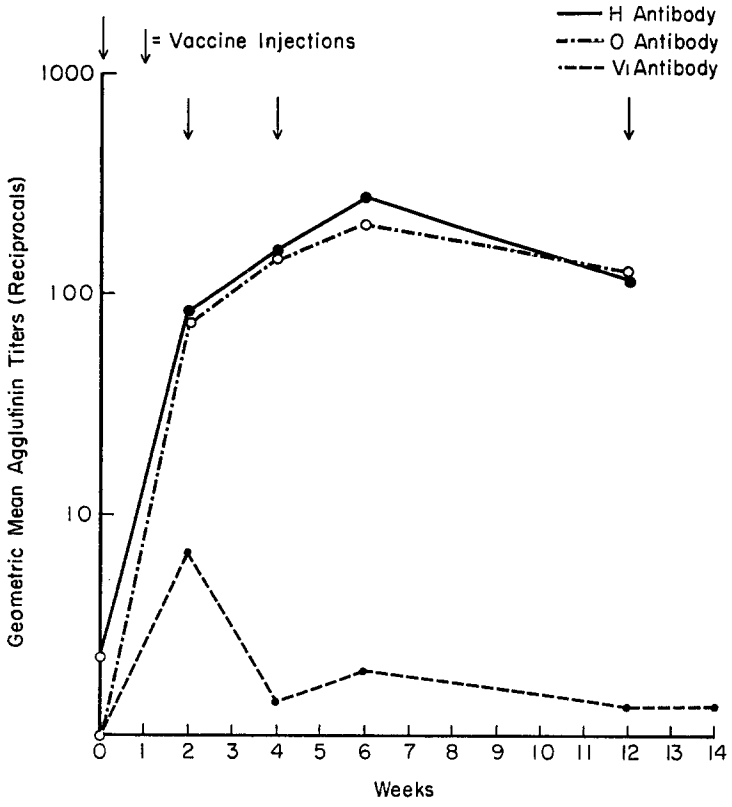

FIG. 2. Antibody responses of chimpanzees to HP vaccine

these animals resembled those seen in chimpanzees following immunization with whole organism vaccines; *i.e.*, O antibody levels were good, but Vi responses were low. In man, on the other hand, Landy *et al.* (29) showed that immunization with isolated antigens resulted in satisfactory levels of Vi as well as O antibody.

Protection Provided by AK Vaccine.—

Ten chimpanzees were inoculated with AK vaccine, and 2 weeks after the booster inoculations these animals together with 7 unvaccinated control chimpanzees were challenged orally with 5 billion viable Ty2 bacilli. The responses of these animals are given in Table I.

Bacteriemia could be demonstrated in 5 of the 7 unvaccinated control chimpanzees, the first positive cultures appearing 4 to 8 days after challenge. The number of positive blood cultures per animal varied from one to six and the periods of bacteriemia ranged from 1 to 10 days. In contrast, none of the 10 immunized chimpanzees showed any evidence of bacteriemia at any time.

Positive stool cultures were obtained from all but one of the animals within

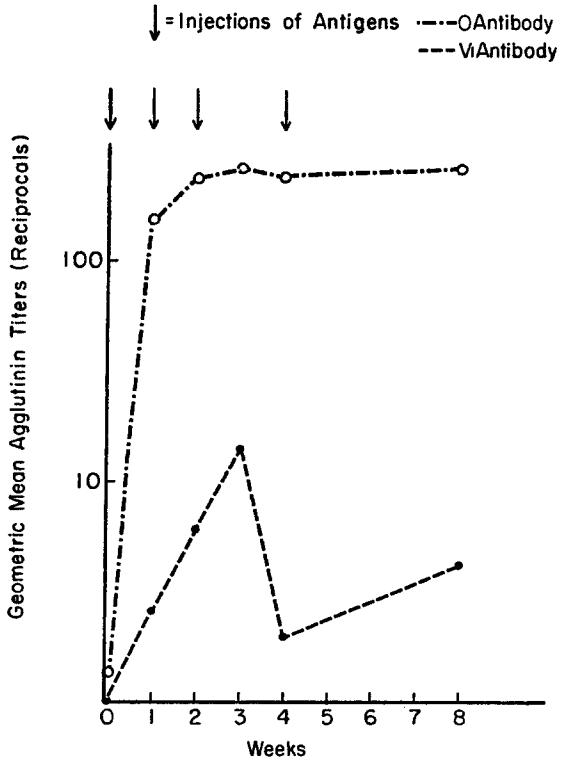

FIG. 3. Antibody responses of chimpanzees to purified Vi and O antigens

24 hours following challenge, a finding indicating that the animals had been exposed to viable typhoid bacilli throughout the gastrointestinal tract. After the 2nd day and until the 5th day, *S. typhosa* could not be isolated from the stools of any of the chimpanzees. Differentiation between the two groups appeared from the 5th postchallenge day, on; 5 of the 7 controls exhibited 1 or more positive stools during the remainder of the experiment. (Of the 5 animals showing bacteriemia, 4 had positive stool cultures; the other stool-positive animal was one of the 2 in which bacteriemia was not demonstrated.) In contrast, only 2 of the 10 vaccinated controls were positive. It is of interest that 1 of the 2 stool-positive immunized animals became a temporary carrier and

continued to shed typhoid bacilli in his stool intermittently for 90 days after challenge (Chimpanzee Jet referred to in Paper I (13)).

Pyrexia of varying degree and duration was noted in the same 5 unvaccinated chimpanzees in which bacteriemia was evident. On the other hand, of the 10 vaccinated animals, only one showed a short febrile period (3 days), with a peak of 1.8°F above its average prechallenge temperature. Lethargy was clearly evident in 4 of the control chimpanzees, and 3 of these 4 also were anorectic. In contrast, all of the immunized group remained lively and continued to eat well. Weight losses of approximately 9 and 11 per cent were observed in 2 of the control animals which had been markedly anorectic for 10 and 14 days, respectively. The weights of all other chimpanzees in either group remained essentially the same during the 4 weeks following challenge.

Examination of sera obtained at approximately weekly intervals after chal-

TABLE I

Response of AK-Vaccinated and Unvaccinated Chimpanzees to Oral Challenge with 5 × 10^9 S. typhosa Ty2

Group	No. of animals	Animals showing						
		Bacteriemia		Fever		Antibody rises		
		No. of animals	No. of days	No. of animals	No. of days	Vi	O	H
Immunized	10	0	0	1	3	0	0	0
Unimmunized	7	5	16	5	32	5	7	7

lenge revealed that all 7 control chimpanzees, in which prechallenge typhoid antibodies were absent, showed development of O and H agglutinins, while the 5 with bacteriemia produced Vi antibody as well. The positive serological findings on the 2 apparently healthy controls provided evidence for subclinical infection in these animals, since it had been shown (13) that antibody response to ingested typhoid bacilli occurred only if living organisms were fed. In the vaccinated chimpanzees, neither the moderately high O and H antibody levels nor the low Vi titers resulting from immunization increased following challenge, but on the contrary, tapered off.

Comparison of the Immune Status at 2 and 14 Weeks Following Vaccination.— The results presented in Table I show that monovalent AK vaccine was highly protective when the animals were challenged 2 weeks following a booster inoculation, a time at which the immune state was presumed to be maximal.

To ascertain the effectiveness of this vaccine for a longer interval between immunization and challenge, the responses of chimpanzees challenged 3½ months (14 weeks) following booster inoculations were compared with those observed in animals challenged at 2 weeks. At

the same time a group of chimpanzees immunized with the standard HP vaccine were challenged 2 weeks after a booster inoculation with this product. The responses of these animals as well as an unimmunized group to challenge with 3 billion viable Ty2 bacilli are illustrated in Table II.

Bacteriemia was demonstrated in 4 out of 5 of the unvaccinated controls (group IV), the first positive cultures appearing from the 4th to the 6th day after challenge. The number of positive blood cultures per animal varied from 2 to 7, and the total period of bacteriemia for this group was 21 days (an average of 4.2 days per animal). On the other hand, bacteriemia of short duration (1 and 2 days) was seen in 2 of the 4 chimpanzees in group I (AK, 14 week), and 1 day of bacteriemia occurred in 1 animal in group III (HP, 2 week).

Fever of varying degree and duration was observed in 4 of the 5 control

TABLE II

Response of AK- and HP-Vaccinated Chimpanzees to Ingestion of 3 × 10^9 S. typhosa Ty2

Group	Immunized with	No. of animals	Wks. booster to challenge	Animals showing						
				Bacteriemia		Fever		Antibody rises		
				No. of animals	No. of days	No. of animals	No. of days	Vi	O	H
I	AK vaccine	4	14	2	3	2	6	0	3	2
II	AK vaccine	4	2	0	0	3	4	0	0	0
III	HP vaccine	5	2	1	1	2	3	0	0	1
IV	Unimmunized	5	—	4	21	4	22	1	5	5

chimpanzees. In the immunized animals, 2 of the 4 in group I, 3 of the 4 in group II, and 2 of the 5 in group III exhibited febrile responses. However, there were 22 days of fever in the control group (4.4 days per animal) in contrast to only 6, 4, and 3 days of fever (averages of 1.5, 1, and 0.6 per animal), respectively, in groups I, II, and III.

Little difference was apparent between the control animals and any of the vaccinated groups as regards the number of positive stool cultures. The usual early positive isolates were obtained from 14 of the 18 animals in this experiment during the first few days following challenge, but after the 3rd day, all stools were negative for *S. typhosa* except for a single positive in one animal of group II and 3 positives in one control chimpanzee.

Tests on serum specimens obtained 10, 19, and 28 days following challenge showed development of O and H agglutinins in all 5 of the control animals, but only 1 of them produced detectable Vi antibody. In the group of 4 animals challenged 14 weeks after booster inoculation with AK vaccine (group I), slight to moderate increases in O and H agglutinin titers above those seen at

the time of challenge occurred in 3 and 2 animals, respectively. Vi antibody was not apparent immediately prior to challenge and could not be demonstrated during the postchallenge period. All of the animals challenged 2 weeks following booster inoculations (groups II and III) exhibited moderate to high O and H agglutinin titers at the time of challenge, irrespective of the type of vaccine employed. Prechallenge Vi antibody was evident in 2 of the 4 group II chimpanzees and in 1 of the 5 group III animals. Following challenge, antibody titers in these 2 groups either remained the same or declined, except for a slightly increased H agglutinin titer in one group III animal.

Protection Afforded by Vaccines against Challenge with a "Wild" Strain of S. TYPHOSA.—It is evident that under the test conditions employed in this study, AK or HP vaccines effectively immunized chimpanzees against challenge with the stable, mouse virulent Ty2 strain of *S. typhosa*. Since this strain

TABLE III

Response of AK- and HP-Vaccinated Chimpanzees to Oral Challenge with 100 × 10⁹ S. typhosa 2593 ("Wild" Strain)

Immunized with	No. of animals	Wks. booster to challenge	Animals showing						
			Bacteriemia		Fever		Antibody rises		
			No. of animals	No. of days	No. of animals	No. of days	Vi	O	H
AK vaccine.....	5	2	2	6	1	2	0	1	4
HP vaccine.....	5	2	1	1	0	0	0	0	3
Unimmunized.....	5	—	5	20	1	8	2	5	5

had been carried in the laboratory for several decades, it was important to determine whether the typhoid vaccines under study would protect against challenge with a more recently isolated typhoid culture.

Accordingly, groups of chimpanzees were immunized with these vaccines in the usual manner, and challenged orally 2 weeks later with 100 billion viable *S. typhosa* 2593, a strain which had been isolated recently from a carrier responsible for a case of typhoid fever. It should be pointed out that although strain 2593 was a freshly isolated culture virulent for mice, the disease it produced in unimmunized chimpanzees was somewhat milder than that resulting from a Ty2 challenge; *i.e.*, there occurred fewer instances of fever, lethargy, and anorexia.

The results of this experiment, summarized in Table III, show that both vaccines afforded protection to chimpanzees fed the "wild" strain of *S. typhosa*. The AK vaccine in this experiment appeared to be somewhat less effective than the HP product, but this difference may not be significant. In any case, the number of positive blood cultures and the duration of bacteriemia was markedly reduced in the vaccinated chimpanzees, and moreover, bacteriemia

appeared later than in the controls. Febrile response was observed in only 2 of the 15 animals in this experiment, but positive stool cultures were obtained on 13 occasions in 4 of the 5 control chimpanzees, in contrast to only 4 positives in 3 of the 10 vaccinated animals. Typical O and H agglutinin development occurred in all of the controls, and 2 of these animals exhibited low Vi antibody titers in response to challenge. In the immunized chimpanzees, the high prechallenge O and H antibody titers remained unchanged, declined, or increased slightly (one 2-fold dilution). Vi antibody could not be detected in any of the vaccinated animals immediately prior to or following challenge.

Immunization with Isolated Typhoid Antigens.—An area of major concern in this laboratory has been the characterization of the Vi and O antigens of *S. typhosa* with a view toward their ultimate use as a replacement for the bacterial vaccine, provided their ability to protect against typhoid fever could be demonstrated. Studies on the immunogenic properties of these antigens in mice had been reported by several investigators (30–33). Landy and his coworkers (32, 34, 35) showed that Vi was highly effective in protecting these animals against intraperitoneal or intracerebral challenge with fully virulent typhoid bacilli, whereas the O antigen was relatively ineffective. In view of these results in mice and the observations that isolated Vi and O antigens stimulated excellent antibody production in man (29), these components were examined for their effectiveness in protecting chimpanzees challenged orally with *S. typhosa.*

Groups of animals were given 3 weekly injections of 40 micrograms each of either Vi (Group V), O (Group O), or both (Group V-O), followed by a booster inoculation 2 weeks thereafter. All injections were made subcutaneously, employing 0.5 ml volumes. A fourth group of uninoculated chimpanzees served as controls. One month subsequent to the booster injections the animals were challenged with 3 billion viable Ty2 bacilli; their responses are presented in Table IV.

Typhoid bacilli were cultured from the blood of all except one control and 2 Vi-immunized chimpanzees. However, the duration of bacteriemia in the O-immunized and control chimpanzees was approximately 2 to 3 times longer than that seen in the animals previously inoculated with Vi antigen alone or with Vi plus O. The proportion of chimpanzees exhibiting a febrile response was high in all groups except the Vi-immunized animals, only one of which became febrile. Furthermore, the duration of fever in this latter group was only one-fourth that of the controls, while the length of febrile periods seen in the animals of groups O and V-O was intermediate. Positive stool isolates were obtained irregularly from all groups from the 2nd day on; however, the 8 animals receiving Vi antigen yielded a total of 8 positive isolates, whereas 32 positive specimens were cultured from the other 9 animals in the experiment.

All 5 control chimpanzees, including the asymptomatic animal, free of typhoid antibodies at the time of challenge, produced good O agglutinin levels.

Two developed moderately high Vi antibody titers. Six of the 8 animals given O antigen developed O agglutinin titers greater than the levels present at the time of challenge, while all of the chimpanzees in group V (O-negative pre-challenge) formed O antibody. With regard to Vi antibody, 3 group O animals, all 4 of which were negative prior to challenge, produced measurable Vi hemagglutinin levels. Only 1 of group V and 2 of group V-O chimpanzees exhibited Vi antibody immediately prior to challenge, and their Vi levels did not increase as a result of infection. The remaining animals of these two groups, which were negative for Vi antibody at the time of challenge, remained negative subsequent to challenge. H antibody levels were not determined in this experiment.

TABLE IV

Protection Afforded Chimpanzees by Purified Vi and O Antigens against Oral Challenge with 3×10^9 S. typhosa Ty2

Group	Immunized with	No. of animals	Wks. booster to challenge	Animals showing					
				Bacteriemia		Fever		Antibody rises	
				No. of animals	No. of days	No. of animals	No. of days	Vi	O
V	Vi antigen	4	4	2	6	1	8	0	4
O	O antigen	4	4	4	17	3	19	3	4
V-O	Vi and O antigens	4	4	4	9	3	24	0	2
Unimmunized	—	5	—	4	17	4	32	2	5

DISCUSSION

It is evident from the results of this study that typhoid vaccines afforded chimpanzees significant protection against challenge capable of inducing disease in non-immunized animals. Two types of whole bacterial vaccines provided excellent protection against infection with homologous or heterologous strains. The monovalent acetone-killed and dried vaccine prepared from strain Ty2 produced in 10 chimpanzees a solid immunity against challenge with this strain. In contrast, 5 of 7 control animals manifested laboratory and clinical evidence of disease, while the remaining 2 showed inapparent infection. In another experiment, average periods of approximately 5 days of bacteriemia and fever were observed in 4 of the 5 unimmunized chimpanzees infected with this strain; in contrast, none of the 4 vaccinated animals exhibited bacteriemia, and the average duration of fever was only slightly over 1 day. When a "wild" heterologous strain was employed as challenge, 3 of 5 immunized chimpanzees were completely asymptomatic, and the infection in the remaining 2 was

definitely mitigated. All 5 controls exhibited frank infection, although febrile response to this strain was minimal.

Heat-killed, phenol-preserved vaccine also was highly effective against challenge with either Ty2 or the "wild" strain. Only 3 days of fever occurred in the 10 animals given this type of vaccine, and only 2 positive blood cultures were obtained.

In the animals challenged after a 14-week interval following immunization, 2 of the 4 vaccinated animals exhibited 6 days of fever and 3 days of bacteriemia. Although the protection afforded this group was less than that seen in the animals challenged 2 weeks following the same immunizing regimen, it was nevertheless considerable when compared with the findings in the 5 control chimpanzees. Thus, the results of this limited experiment suggest that the immunity conferred by typhoid vaccine extends for at least several months, and hence cannot be regarded simply as a transient non-specific enhancement of resistance or as an interference effect.

The suggestion has been made that strains of bacilli freshly isolated from active cases be utilized in the preparation of typhoid vaccines. It is of interest in this regard that, in the present study, vaccines prepared from laboratory strains of the typhoid bacillus stimulated resistance not only against homologous challenge but against heterologous challenge as well. The Ty2 vaccine was effective against a homologous and a heterologous strain, while the HP vaccine, prepared from *S. typhosa* 58, immunized against 2 heterologous strains. The 3 strains involved (Ty2, 58, and 2593) differ in origin, date of isolation, history of handling, and in phage type (13, 36–38). However, despite quantitative differences, they all contain the 3 major antigens of the typhoid bacillus. The protection afforded by these vaccines thus may be regarded as supporting the concept that, whether carried in the laboratory or of clinical origin, strains which possess the necessary antigenic composition may be utilized in the preparation of vaccines, provided they are sufficiently stable. It does not appear, therefore, that the source and freshness of isolation of the culture are critical factors in the selection of vaccine strains.

Since 1934, when Felix and Pitt (39) first associated Vi antigen with the virulence of *S. typhosa* for mice, and the subsequent demonstration (40) that this antigen or Vi antibody would protect these animals against a virulent challenge, it has been generally accepted that the presence of this component in typhoid vaccines was desirable. This opinion has received further support from the reports of Findlay (41) and Felix and Anderson (42), which indicated that the mouse virulence and Vi antigen content of organisms isolated from human cases of typhoid fever were directly related to the severity of the disease. Consequently, considerable emphasis has been given to selecting Vi-containing typhoid bacilli for vaccine strains, and to the employment of methods in

vaccine preparation which would result in maximal retention of this antigen (21, 43, 44).

The present study has shown that the resistance of chimpanzees to infection with typhoid bacilli was significantly increased by the administration of Vi-containing whole organism vaccines, regardless of their manner of preparation. The protection provided by the heat-killed, phenol-preserved vaccine was essentially the same as that afforded by the acetone-killed product, although the latter type of vaccine presumably contains a greater quantity of Vi antigen (21). Whether similar vaccines prepared from non-Vi variants of these strains would protect under the same conditions remains for future work to determine. It is noteworthy, however, that in mice, such non-Vi vaccines are relatively ineffective in protecting against virulent challenge.

The finding that vaccines of markedly different immunogenic potency for mice produced apparently equal immunity in chimpanzees is of particular interest. It is pointed out that the chimpanzee experiments were not designed to establish quantitative relationships such as those developed in mice. For the latter purpose, vaccines were administered in single, graded quantities to determine the minimal immunogenic dose. In contrast, chimpanzees were given a series of 3 injections of vaccine and a booster, a quantity which might conceivably have obscured any difference in the protection afforded by the two products. On the other hand, it may be that, despite the limited numbers of animals employed in these experiments, the results obtained represent the actual relative potencies of the AK and HP vaccines for chimpanzees. If so, mouse tests for vaccine potency do not accurately reflect the efficacy of vaccine for primates. The relationship between vaccine potency for mice and primates, however, remains to be established.

With respect to individual antigenic components, the protection provided chimpanzees by the isolated antigens was less than that induced by the whole vaccines. When Vi antigen alone was employed, fever and bacteriemia occurred, but in fewer animals and with shorter duration than in the unimmunized group. On the other hand, the findings in the animals immunized with O antigen alone or with O antigen in combination with Vi were similar to those seen in the controls, except that the group immunized with the Vi-O preparation manifested a shorter period of bacteriemia and a smaller number of post-challenge antibody increases. It is noteworthy that Vi antigen alone did mitigate the reaction of primates to oral challenge with *S. typhosa*. However, in assessing the magnitude of the protection induced by this purified antigen, it should be borne in mind that the vaccines and isolated antigens were not compared in the same experiment. Since the responses of the chimpanzees varied appreciably from one experiment to another, and the challenges undoubtedly fluctuated from one test to another despite uniform handling and

standardization, it would be premature to draw final conclusions regarding the comparative performance of whole vaccines *versus* purified antigens. Clearly, the findings with isolated Vi antigen justify further study so as to evaluate more accurately the effectiveness of this antigen in immunization of man against typhoid fever.

SUMMARY

A study was made of the efficacy of various antityphoid immunizing agents in immunizing chimpanzees against typhoid fever produced by feeding viable *S. typhosa.*

It was found that both acetone-killed and heat-killed, phenol-preserved typhoid vaccines were effective in protecting against infection induced with either homologous or heterologous strains of typhoid bacilli.

Purified O antigen induced no discernible protection, but some immunity was afforded by the administration of purified Vi antigen.

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