H ANTIGEN OF *SALMONELLA TYPHOSA*

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Received for publication December 5, 1960

In addition to Vi and O antigens, most strains of *Salmonella typhosa* possess a single phase flagellar H antigen (factor d). Decreases in amount or loss of H antigen have been observed to occur in strains of typhoid bacilli following cultural adaption or growth on minimal medium (Gladstone, 1937; Bacon, Burrows, and Yates 1950). The well known O-901 strain, a variant of the original H-901 culture isolated by Weil and Felix (1920), typifies the permanent loss of H antigen and motility in the typhoid organism. Variation from smooth to rough colonies, usually associated with the loss of the O antigen, can occur without altering the status of the H antigen, since rough, as well as smooth, motile cultures of *S. typhosa* have been recovered. Furthermore, H antigen appears to have no direct relationship to the presence or absence of Vi antigen on the typhoid bacillus, inasmuch as both motile (e.g. Ty2, Panama Carrier) and nonmotile (ViI (Bhatnagar)) Vi-containing strains of *S. typhosa* are known.

The flagellar antigens of the salmonellae have their greatest importance in taxonomy and are generally thought to have little influence on the virulence of this group of organisms or on the protective capacity of vaccines prepared from them (Arkwright, 1927; Raffel, 1953). Recent laboratory studies on two types of typhoid vaccines employed in the Yugoslav field trials have led some investigators to suggest that the H antigen content of vaccines may be of some importance in protecting man against typhoid fever (Edsall et al., 1959). Moreover, Carey and Baron (1958) have recently presented evidence that purified flagellar antigen (factor d) protected mice against intraperitoneal challenge with the fully virulent Ty2 strain of *S. typhosa*. These investigators (*personal communication*), however, doubted the specificity of this protection because heated flagellar antigen also was protective and, in addition, passive mouse protection tests with both mouse and rabbit antisera to the flagellar antigen failed to protect mice against the Ty2 challenge.

The recovery in our laboratory of H antigen-deficient variants of *S. typhosa* from intracerebrally inoculated mice provided an opportunity to re-examine the relationship of H antigen to the virulence of the typhoid bacillus for mice, and to assess the importance of this antigen in the protective properties of typhoid vaccines.

MATERIALS AND METHODS

Cultures. The following strains of *S. typhosa* were employed:

Ty2V, the well known, motile, Vi-containing strain of Felix (Felix and Pitt, 1951). This culture had been passed many times through mice and twice through chimpanzees in this laboratory prior to use in the present study.

Ty2W, a motile, non-Vi variant of the Ty2V strain recovered from a meat extract stab culture in this department.

Mrs. S. (Rough), the rough, motile strain, lacking both Vi and O antigens, isolated from a patient by Felix and Pitt (1935).

H-901, the motile, smooth strain isolated by Weil and Felix (1920).

O-901, the nonmotile variant of the H-901 strain isolated by Felix (1930).

Ty2W-NM, a nonmotile variant of the Ty2W strain recovered from mouse brain following intracerebral inoculation.

H-901-NM, a nonmotile variant of the H-901 strain recovered from mouse brain following intracerebral inoculation.

All strains were maintained on semisolid meat extract agar and passed twice on the solid form of this medium prior to virulence and serological tests. Each of the variant strains could be readily distinguished from its parent by characteristic appearance on meat extract agar when examined by the oblique lighting technique (Landy, 1950),

and single typical colonies were selected for final replating on veal infusion agar, except where otherwise indicated. Five- to six-hour cultures incubated at 37 C were used for all virulence determinations, challenge inocula, immunizing preparations, and as antigens for serological procedures.

Preparation of antisera. Rabbits were inoculated intravenously every other day for 1 week and daily during the second week with increasing volumes of 1×10^9 viable cells/ml until approximately a total of 7 ml was administered. All animals were bled 5 days following final inoculation and sera examined for antibody titers.

Virulence determinations. Intracerebral virulence tests were performed as described by Landy, Gaines, and Sprinz (1957). Briefly, 10-fold dilutions of the infecting culture were made in 1% pancreatic digest of casein in saline and 0.03 ml of each challenge dilution inoculated into the foramen magnum of BALB/c or C_3H/He mice (12 to 16 g) obtained from Microbiological Associates, Bethesda, Maryland. Numbers of viable organisms in challenge dilutions were determined by the usual plate count procedure, using trypticase soy agar (BBL), and each culture was examined for the presence of Vi and O antigens at the time of challenge, employing rabbit antisera to *Paracolobactrum ballerup* and O-901, respectively. Each antigen was tested in the viable as well as heat-killed state (100 C, 1 hr); incubation was at 37 C for 18 hr. All challenge suspensions also were inoculated into a semisolid motility test medium containing 0.005% triphenyl tetrazolium chloride (Kelly and Fulton, 1953) and reactions noted after 18 hr at 37 C. Mortality in inoculated mice was recorded for 28 days and the LD_{50} calculated by the method of Reed and Muench (1938).

Protection tests. Active mouse protection tests were carried out with acetone-killed and dried vaccines prepared from selected strains of *S. typhosa* in a manner similar to that described by Landy (1953). All vaccines consisted of 12 mg dried cells per 50 ml sterile buffered saline (pH 6.8) and represented a concentration of 1×10^9 bacilli/ml. Merthiolate (Lilly) was added (5 mg/50 ml vaccine) as a preservative and all vaccines were stored at 4 C. Mice were inoculated intraperitoneally with 0.5-ml dilutions of each vaccine and challenged by the same route 6 days later with a constant number of viable bacilli representing approximately 10 LD_{50} doses of the challenge culture.

Passive mouse protection tests were performed by inoculating mice intraperitoneally with 0.5-ml dilutions of serum followed by a constant intraperitoneal challenge (10 LD_{50} doses) 1 hr later. All ED_{50} values were determined by the method of Reed and Muench (1938).

Serological tests. Antigens employed for the determination of O and H agglutinin titers of rabbit antisera to the *S. typhosa* variants were prepared from 5- to 6-hr veal infusion agar cultures of O-901 and H-901 strains. H agglutinin titers were also determined with antigens prepared from *Salmonella virginia* (VI, VIII, d) and the Mrs. S. strain of *S. typhosa*. Smooth saline suspensions of the latter culture were obtained only when this organism was cultivated on meat extract agar. Viable and heat-killed cultures were used in all cases. The H-901 agglutination tests were incubated at 52 C and read at 2 hr, whereas the monospecific H tests (Mrs. S. and *S. virginia* strains) and O antibody determinations were read after 18 hr at 37 C. Vi antibody tests on rabbit antisera prepared against the various strains were performed by the hemagglutination technique of Landy and Lamb (1953).

Serological procedures also were employed in direct antigenic analyses of the isolates. Viable and boiled preparations of the Ty2W-NM and H-901-NM cultures were tested against dilutions of an H antiserum to the Mrs. S. strain and against an O-901-adsorbed antiserum to H-901, as well as a Vi antiserum to *P. ballerup*. Readings were taken after 18 hr at 37 C.

Agglutinin adsorption of selected antisera was accomplished by incubating concentrated viable cell suspensions with antiserum at 37 C for 1 hr followed by 4 C overnight. Serum was recovered after centrifugation at 5,000 rev/min for 30 min.

RESULTS

Isolation of variants. During the course of studies in this laboratory on the enhancement of the virulence of a non-Vi variant (Ty2W) of *S. typhosa* by Vi antigen (Gaines, Tully, and Tigertt, 1961), a colonial variant was occasionally isolated from the brains of intracerebrally inoculated mice which, when examined by the oblique lighting technique, resembled the colonial appearance

of the V form of *S. typhosa* strain Ty2. The variant colony, in addition to possessing more color, was smoother and smaller than colonies of the original Ty2W culture and, on subculture to meat extract agar, gave stable colonial forms easily distinguishable from Ty2W. Biochemical reactions in Kligler's iron agar and several carbohydrate media were typical for *S. typhosa*, and the culture was agglutinated by Salmonella O antiserum (IX, XII) in slide tests. On the other hand, motility tests using semisolid agar and hanging drop preparations failed to demonstrate motility even after repeated short-term incubation in broth. The isolate was therefore considered to be a nonmotile variant of Ty2W and designated as Ty2W-NM.

To determine whether nonmotile variants could be recovered from other motile, W form *S. typhosa* cultures, BALB/c mice were inoculated intracerebrally with the H-901 and Mrs. S. strains. No colonial variants were obtained from the brains of mice receiving the Mrs. S. culture but several small colonial types were recovered from mice inoculated with the H-901 strain. These organisms (H-901-NM) were similar to the Ty2W-NM variant in color, size, and lack of demonstrable motility and were confirmed as *S. typhosa* biochemically and by slide agglutination with Salmonella O antiserum.

Serological studies of the nonmotile variants. The results of direct serological examination of the parent and variant cultures (Table 1) show that the nonmotile variants were not agglutinated by H antisera, in contrast to the parent Ty2W and H-901 cells. The failure of these variants to react in an H-901 antiserum adsorbed with O-901 cells, or in a similarly adsorbed H antiserum prepared against the Mrs. S. strain, strongly indicated that these bacilli did not contain H antigen. Additional evidence for the absence of H antigen in the nonmotile variant of H-901 is provided by the failure of an antiserum prepared against this variant to agglutinate the Mrs. S. culture (Table 2). The low titered agglutination of H-901 bacilli by this serum undoubtedly represented the reaction between O antigen and antibody inasmuch as these organisms were not agglutinated by the same serum after it had been adsorbed with O-901 cells. The 1:10 titer of this H-901-NM serum in the test against viable *S. virginia* bacilli was not a specific H antigen-antibody reaction since adsorption of the serum with O-901 cells, whose O antigens differ from those of *S. virginia*, eliminated the observed agglutination.

Despite the inability to demonstrate H antigen in Ty2W-NM by agglutination tests employing H antisera (Table 1), this strain did produce a small amount of H antibody in a rabbit immunized with these cells shortly after they were recovered from mouse brain (October, 1958). As shown in Table 3, an antiserum prepared against this variant agglutinated H-901, Mrs. S., and *S. virginia* cultures in relatively low titer, even after adsorption of the antiserum with O-901 cells, in contrast to the high H agglutinin titer observed with antiserum to the parent Ty2W bacilli. However, after

TABLE 1

Antigenic composition of Salmonella typhosa strains Ty2W, H-901, and their nonmotile variants shown by serological reactions in specific antisera

Antiserum to	Antiserum Adsorbed with	Antibody in Adsorbed Serum	Antigen Preparation									
			Ty2W		Ty2W-NM		H-901		H-901-NM		O-901	
			Viable	HK*	Viable	HK	Viable	HK	Viable	HK	Viable	HK
H-901	O-901	H (d)	5120†	Neg	Neg	Neg	5120	Neg	Neg	Neg	Neg	Neg
Mrs. S.	O-901	H (d)	2560	20	Neg	Neg	2560	10	Neg	Neg	Neg	Neg
O-901	Not adsorbed	O (IX, XII)	1280	1280	2560	1280	5120	1280	5120	1280	2560	1280
Paracolobactrum ballerup	Not adsorbed	Vi	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

* Heat-killed cells (100 C, 1 hr).

† Reciprocal of titer.

Neg = <1:10.

TABLE 2

O and H agglutinin titers of rabbit antisera to Salmonella typhosa strain H-901 and its nonmotile variant

Antiserum		Antigen Preparation								
		S. typhosa						*Salmonella virginia*		Vi antigen sensitized RBC
		H-901		O-901		Mrs. S.				
Prepared against	Adsorbed with	Viable	HK*	Viable	HK	Viable	HK	Viable	HK	
		O, H† (IX, XII, d)	O (IX, XII)	O (IX, XII)	O (IX, XII)	H (d)		H (d)		Vi
H-901	Preimmunization	Neg	Neg	40‡	Neg	—	—	Neg	Neg	Neg
	Not adsorbed	5120	10	2560	1280	—	—	5120	Neg	Neg
	O-901	1280	Neg	Neg	Neg	—	—	2560	Neg	—
	O-901 and Mrs. S.	Neg	Neg	—	—	—	—	Neg	Neg	—
H-901-NM	Preimmunization	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Not adsorbed	40	20	2560	640	Neg	Neg	10	Neg	Neg
	O-901	Neg	Neg	Neg	Neg	—	—	Neg	Neg	—

* Heat-killed cells (100 C, 1 hr).
† Specific antigenic content
‡ Reciprocal of titer.
Neg = <1:10; — = not done; RBC = red blood cells.

adsorption of the variant antiserum with H antigen-containing H-901 cells, agglutination of the organisms was completely eliminated. Following periodic passage on artificial media (meat extract agar) for approximately 10 months, the variant (August, 1959, culture) was re-examined for H antigen content. As observed previously with the original isolate, an H antiserum again failed to agglutinate this culture but, in contrast to the original results, an antiserum prepared against this variant appeared to be entirely devoid of H antibody, as shown by its inability to agglutinate H-901, Mrs. S., or *S. virginia* cells after adsorption with O-901 bacilli (Table 3).

In summary, the results presented in Tables 1, 2, and 3 show that although H antigen was readily demonstrable in the parent cultures (Ty2W and H-901), the variant bacilli either lacked or were deficient in this component. On the other hand, the variants did not differ significantly from their parents in O antigen content, and Vi antigen could not be detected in any of the organisms. Thus, with regard to antigenic composition, the variants appeared to differ from the parent bacilli only in content of H antigen.

Virulence test results. In a series of experiments to determine the relationship of H antigen to virulence of the typhoid bacillus, groups of mice were inoculated intracerebrally with *S. typhosa* strains Ty2W, H-901, or their nonmotile variants. The results of these experiments, compiled in Table 4, show that the virulence of the nonmotile variant of H-901 did not differ significantly from that of the motile parent culture. The nonmotile Ty2W-NM culture, however, was found to be approximately three times more virulent than that of its motile parent and, although this difference between the LD_{50} values of the motile and nonmotile Ty2W was not great, it, nevertheless, was consistent in each of five separate experiments. Furthermore, approximately the same relative differences in intracerebral virulence of the three Ty2 strains was noted when C_3H mice were used for virulence tests.

The importance of Vi antigen for mouse virulence is clearly demonstrated by the low LD_{50} of the Ty2V bacilli shown in Table 4. In view of the greater intracerebral mouse virulence of the nonmotile Ty2W-NM variant, in comparison to that of its motile parent, this variant was submitted to a critical examination for the presence of Vi

TABLE 3

O and H agglutinin titers of rabbit antisera to Salmonella typhosa strain Ty2W and its nonmotile variant

Antiserum		Antigen Preparation								
		S. typhosa						*Salmonella virginia*		Vi antigen sensitized RBC
		H-901		O-901		Mrs. S.				
Prepared against	Adsorbed with	Viable	HK*	Viable	HK	Viable	HK	Viable	HK	
		O, H† (IX, XII, d)	O (IX, XII)	O (IX, XII)	O (IX, XII)	H (d)		H (d)		Vi
Ty2W	Preimmunization	Neg	Neg	10‡	Neg	Neg	Neg	Neg	Neg	Neg
	Not adsorbed	5120	20	5120	2560	1280	10	2560	10	Neg
	O-901	5120	Neg	Neg	Neg	1280	10	5120	20	—
	O-901 and H-901	80	Neg	—	—	Neg	Neg	Neg	Neg	—
Ty2W-NM; Oct 1958	Preimmunization	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Not adsorbed	160	160	5120	2560	160	Neg	160	10	Neg
	O-901	40	Neg	Neg	Neg	40	Neg	80	Neg	—
	O-901 and H-901	Neg	Neg	—	—	Neg	Neg	Neg	Neg	—
Ty-2W-NM; Aug 1959	Preimmunization	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Not adsorbed	160	Neg	2560	1280	20	Neg	Neg	Neg	Neg
	O-901	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	—

* Heat-killed cells (100 C, 1 hr).
† Specific antigenic content.
‡ Reciprocal of titer.
Neg = <1:10; — = not done; RBC = red blood cells.

antigen. The Ty2W-NM culture had been recovered originally from the brains of mice inoculated intracerebrally with the Ty2W strain alone or a Ty2W inoculum containing purified Vi antigen. Thus, it was thought possible that the increased virulence of the variant bacilli might have been the result of W-V reversion. The nonmotile variant, however, failed to agglutinate in a Vi antiserum (Table 1), adsorb Vi antibody, protect mice against a V form challenge (Table 5), stimulate serologically demonstrable or protective Vi antibody in rabbits (Tables 3 and 6), and was not susceptible to Vi bacteriophages. As a consequence, the Ty2W-NM variant was considered devoid of Vi antigen. Moreover, the results of preliminary studies have shown that the virulence of the nonmotile Ty2W-NM variant, like that of its motile parent (Gaines et al., 1961), was enhanced by the simultaneous intracerebral administration of 30 μg of purified Vi antigen. Since the degree of virulence-enhancement was similar for the two cultures, the virulence increase induced by the presence of Vi antigen appeared to be unaffected by the H antigen content of the organisms.

TABLE 4

Intracerebral mouse virulence of V form and motile and nonmotile W form Salmonella typhosa

Mouse Strain	Average LD_{50}				
	Ty2V	Ty2W	Ty2W-NM	H-901	H-901-NM
BALB/c*	371	11,850	3,500	5.6×10^6	5.3×10^6
C_3H†	775	14,800	5,450	—	—

* Average of 5 or 6 virulence tests.
† Average of 2 virulence tests.
— = Not done.

Protection tests. Acetone-killed and dried vaccines prepared from the parent Ty2W culture, its nonmotile Ty2W-NM variant, and from the Ty2V, O-901, and Mrs S. strains of *S. typhosa* were compared for their efficacy in protecting against intraperitoneal challenge with the fully virulent V form culture or its W variant. The re-

TABLE 5

Active mouse protection tests with acetone-killed and dried Salmonella typhosa vaccines

Acetone-Killed and Dried Cell Vaccines	Antigens Present in Vaccine	ED_{50} of Vaccine against Intraperitoneal Challenge with			
		4.5×10^7 *S. typhosa* Ty2V*		7.9×10^8 *S. typhosa* Ty2W†	
		Ml	No. of bacilli	Ml	No. of bacilli
Ty2V	O, H, Vi (IX, XII, d)	0.00016	1.6×10^5	0.104	1.04×10^8
Ty2W	O, H (IX, XII, d)	0.019	1.9×10^7	0.096	9.6×10^7
Ty2W-NM	O (IX, XII)	0.087	8.7×10^7	0.088	8.8×10^7
O-901	O, (IX, XII)	0.079	7.9×10^7	0.13	1.3×10^8
Mrs. S.	H (d)	0.105	1.05×10^8	>0.5	$>5 \times 10^8$

* Of 30 saline controls, there was 1 survivor.

† Of 30 saline controls, there were no survivors.

sults given in Table 5 show that with a Ty2V challenge, representing approximately 15 LD_{50} doses, the ED_{50} values of the non-Vi vaccines ranged from 10^7 to 10^8 cells irrespective of their O and H antigen content. The ED_{50} value for the Vi-containing Ty2V vaccine was 1.6×10^5 bacilli. In contrast, neither the Ty2W vaccine nor any other vaccine tested was effective in protecting against a Ty2W challenge of about 8 LD_{50} doses, and the ED_{50} values for these vaccines against W challenge did not differ significantly. The vaccines prepared from cultures devoid or deficient in H antigen (O-901 and Ty2W-NM) were no less effective than the H antigen-containing Ty2W vaccine and appeared to be somewhat better than the vaccine prepared from the Mrs. S. culture. Furthermore, preliminary experiments employing the intracerebral route of infection have indicated that neither acetone-killed and dried O-901 nor Mrs. S. vaccines was effective in active protection tests against challenge with fully virulent Ty2V cells. More than 2×10^9 Mrs. S. cells and approximately 1.8×10^9 O-901 bacilli were necessary to protect 50% of the mice against an intracerebral challenge with 10 LD_{50} doses of Ty2V cells, whereas only 5×10^5 acetone-killed and dried Ty2V bacilli gave 50% protection against the same challenge. It is quite likely that protection afforded by the other vaccines listed in Table 5 would be of the same order as that of the O-901 and Mrs. S. vaccines when tested against intracerebral challenge.

The results of passive protection tests with rabbit antisera prepared against the vaccine strains, compiled in Table 6, were in agreement with the findings obtained in the active protection experiments. Except for the Ty2V antiserum, none of the antisera offered protection against approximately 17 LD_{50} doses of V form challenge. When a Ty2W challenge of 10 LD_{50} doses was employed, all of the antisera failed to afford effective protection irrespective of O, H, or Vi antibody content. It should be pointed out that normal rabbit serum, in the volume employed (0.1 ml), showed no protective capability against

TABLE 6

Passive protection afforded mice by rabbit antisera to Salmonella typhosa strains of different antigenic composition

Antiserum to Strain	Antibody Titers of Serum			ED_{50} (ml) of Antiserum against Intraperitoneal Challenge with	
	Vi*	O† (IX, XII)	H† (d)	5×10^7 *S. typhosa* Ty2V‡	9.5×10^8 *S. typhosa* Ty2W‡
Ty2V	3840	1280	1280	0.0003	0.1
Ty2W	Neg	2560	5120	>0.1	>0.1
Ty2W-NM	Neg	1280	Neg	>0.1	>0.1
O-901	Neg	1280	Neg	>0.1	>0.1
Mrs. S.	Neg	Neg	2560	>0.1	>0.1

* Hemagglutinin titer reciprocals.

† Bacterial agglutinin titer reciprocals.

‡ There were no survivors in 20 mice receiving saline or 20 mice inoculated with 0.1 ml normal rabbit serum.

Neg = < 1:10.

either challenge but, with 0.2 ml, some nonspecific protection against both V and W form challenges occurred. For this reason, quantities of specific antisera greater than 0.1 ml were not employed.

DISCUSSION

The lack of any significant role for the H antigen in the virulence or protective properties of the salmonellae has been unquestioned since the early work of Braun and Cahn-Bronner (1921), Arkwright (1927), and others. Later, studies on specific strains of *S. typhosa* revealed that a change in colonial characteristics from smooth to rough, with a consequent loss of O antigen, were usually associated with a loss in mouse virulence (Grinnell, 1932; Norton and Dingle, 1935).

The recent field trials with typhoid vaccines carried out in Yugoslavia indicated that the heat-phenol vaccine employed may have been more protective than an alcoholized typhoid vaccine (Cvjetanović, 1957). Laboratory investigations revealed that the only definite difference between these vaccines was the greater stimulation of H antibody production in rabbits by heat-phenol vaccine (Edsall et al., 1959; Standfast, 1960*a*), although, when tested in humans, this difference was not marked (Yugoslav Typhoid Commission, 1957). The observation that heat-phenol vaccines yielded more H antibody than alcoholized vaccines is to be expected, since it is known that treatment of motile bacilli with alcohol not only removes their serological reactivity in H antisera but also decreases their ability to stimulate H antibody (Felix, Rainsford, and Stokes, 1941). In view of this, the suggestion that the H antigen content of the heat-phenol vaccine in the Yugoslav field trial may have accounted for its greater effectiveness in protecting man must be approached with caution. The relationship of superior H antibody production to greater protective properties in man may be one resulting from a fortuitous simultaneous occurrence rather than a true correlation.

The results of the present study on the virulence of antigenically different typhoid bacilli have shown that the loss of H antigen was not associated with the loss of virulence but, on the contrary, the virulence of organisms deficient in this antigen was unaffected or actually slightly increased. The intracerebral virulence of the nonmotile H-901-NM variant was essentially the same as that of its H-containing parent, a finding in agreement with the earlier investigations on nonmotile typhoid bacilli. On the other hand, in tests conducted with the Ty2W-NM variant in two strains of mice, a slight increase in intracerebral virulence over that of the motile parent culture appeared to be associated with the loss of H antigen.

Active immunization tests revealed that a vaccine prepared from a motile W form *S. typhosa* was no more effective in protecting mice against a V or W form challenge than a vaccine prepared from nonmotile W cells devoid of H antigen. Moreover, a vaccine prepared from a strain (Mrs. S.) possessing the flagellar factor d antigen, but lacking Vi and the group D somatic antigens, was likewise ineffective in protecting against V or W challenge. Passive mouse protection tests gave results in close agreement with active tests, in that antisera to W form bacilli afforded little or no protection against V or W challenge, regardless of the presence or absence of H antibody.

It is important to note that, although only a Vi-containing vaccine protected against a V form challenge, this Vi vaccine could not be distinguished from the non-Vi vaccines with respect to protection of mice against the W challenge. Also, only antiserum to Vi-containing cells was effective against a V form challenge and neither Vi nor W antisera was significantly protective against a W form *S. typhosa* challenge. It is evident from the findings presented here, as well as the work of Standfast (1960*b*), that large numbers of non-Vi-containing typhoid bacilli are required to protect mice against a V or W *S. typhosa* challenge. However, the results emphasize again that immunity in mice is dependent upon the presence of Vi antigen, and only vaccines containing this antigen will protect against a fully virulent Vi-containing *S. typhosa* culture.

The results of this study are in accord with recent investigations of D'Allessandro and Comes (1959). These workers, using the technique described by Lipp (1957), explored the ability of typhoid vaccines and purified antigens to prevent bacteremia in mice given a nonlethal intraperitoneal inoculation of typhoid bacilli. Only Vi antigen or Vi-containing cell vaccines

exhibited a definite immunizing effect against *S. typhosa* challenge. Active immunization with H antigen did not protect mice against bacteremia and passive tests were somewhat equivocal.

Thus, the results presented here confirm the earlier reports that the H antigen of *S. typhosa* does not have a significant role in determining the virulence of the typhoid organism for mice. Also, H antigen and antibody appear not to offer any significant degree of protection to mice against Vi-containing or non-Vi strains of *S. typhosa*. What role, if any, this antigen may play in protecting man against typhoid fever still remains to be determined.

ACKNOWLEDGMENT

The technical assistance of Julius A. Currie is gratefully acknowledged.

SUMMARY

Serological studies on nonmotile variants of W form *Salmonella typhosa* recovered from the brains of mice previously inoculated with motile forms indicated that the variants were devoid of, or deficient in, H antigen and appeared to differ from the parent cultures only with respect to this component.

Results of intracerebral virulence tests with these organisms have shown that the loss of H antigen was not associated with loss of virulence but, on the contrary, the virulence of organisms deficient in this component was unaffected or actually slightly increased.

Active and passive mouse protection tests employing various acetone-killed and dried cell vaccines and antisera to these vaccines demonstrated that neither H antigen nor H antibody were effective in protecting against V or W form challenges. Only a Vi-containing vaccine or a Vi antiserum afforded protection against the V challenge, but when a W form challenge was employed, it was not possible to distinguish between V and W form immunizing agents either actively or passively.

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