THE VIRULENCE AND IMMUNOGENICITY OF STRAINS DERIVED FROM SALMONELLA TYPHOSA 58 (PANAMA CARRIER)

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Since the early days of immunology, it has been considered that high virulence and recency of isolation from pathological material were essential attributes of strains of bacteria to be used in production of superior immunizing agents. In spite of a surprising paucity of supporting factual information this concept has pesisted.

Perhaps in no field of investigative immunology has the theory of an essential relationship of virulence to immunogenicity been accepted more widely than it has been in relation to the development of immunizing agents against typhoid. As early as 1910, Leishman (1) pointed out that employment of highly virulent cultures of *Salmonella typhosa* appeared to have certain theoretical advantages in the preparation of typhoid vaccines. Perry, Findlay, and Bensted (2), who employed active-immunity protection tests on mice, concluded that the more highly virulent the strains of *S. typhosa* employed in preparing vaccines, the greater was the immunizing activity of the resultant products. Similar conclusions were reached by Arkwright (3), Grinnell (4), and Brown (5). In many of these earlier investigations, however, virulence and avirulence was not clearly distinguished from smooth-rough variation of strains; in fact, virulence and smoothness (also avirulence and roughness) generally were considered essentially synonymous.

One of the most comprehensive studies of the relationship of virulence to antigenicity of *S. typhosa* cultures was conducted by Siler and his associates at the Army Medical School (6). This investigation included a study of seven strains of varied virulence and it was found that, as measured by active-immunity mouse-protection tests and by "O" agglutinin and mouse-protective antibody responses in human volunteers, vaccines prepared from virulent strains were more highly immunogenic than were those prepared from avirulent strains.

Felix (7) likewise reported that highly virulent strains of *S. typhosa* exhibited the greatest immunizing activity. He placed major emphasis on the rôle played by Vi antigen both in virulence and in immunogenicity.

There is, however, no unanimity of opinion in regard to any essential relationship between virulence and immunogenicity. This is evidenced more by the development of certain immunizing agents and methods than by any specific investigations of the phenomenon itself. It is not difficult to understand why there is no unanimity of opinion in regard to the supposed mutual interdependent relationship of virulence and immunogenicity since there has been a marked lack of uniformity in the experimental techniques employed in the various investigations and most of the published

conclusions have been based on experiments inadequate both in scope and in design. In a recent review of the available evidence, Dubos (8) concluded that, in view of the meager information available, the popularly accepted concept was an unwarranted generalization. Furthermore, he concluded that immunogenicity of organisms was dependent upon the presence of specific cellular structures or components capable of eliciting an immune response in the host and that the presence of such components was not necessarily interdependent with virulence. In many instances the presence and activity of such components has been well established, particularly in the pneumococci (capsular polysaccharides), the streptococci (M antigen), and even in the group of Gram-negative enteric organisms (O antigens, Vi antigen). Thus, Dubos' contention can be accepted without serious reservation.

Recent isolation of an avirulent culture of *S. typhosa* from Boxill (9), the carrier of strain 58 (Panama Carrier), and the *in vitro* production by nutritional means, of an avirulent culture of the parent strain, have permitted a direct comparison of the immunogenicity of virulent and avirulent strains of *S. typhosa* of common origin. It was the objective of the investigation reported here to compare, by laboratory means, the immunogenicity of these closely related virulent and avirulent strains of *S. typhosa*.

EXPERIMENTAL

Strains Used.—Four strains of *S. typhosa* were employed in this study. They were related in so far as they were isolated, or were derived from isolates, from the same carrier, Boxill, the source of the strain employed as routine for production of typhoid vaccine by the Army Medical Department. The designation, history of isolation, and maintenance of these strains is as follows:—

Strain 58.—The regular vaccine strain, isolated from Boxill in 1934 and maintained in the lyophilized state since 1936.

Strain 58V.—A culture isolated from Boxill in August, 1948.

Strain 58B.—The original strain 58 maintained in a chemically defined amino acids–inorganic salts–glucose medium[1] for 47 daily transfers.

Strain 58A.—The original strain 58 maintained in the chemically defined medium without glucose for 46 daily transfers.

Laboratory studies revealed that strains 58 and 58B were mixtures of V and W forms (10) although the former were predominant. Strain 58V contained only V forms and strain 58A contained only W form organisms. Aside from the occurrence of W forms, strains 58, 58V, and 58B qualitatively were identical in their content of somatic antigens (IX, XII, and Vi), and in regard to their other morphological, cultural, and biochemical characteristics. Strain 58A, containing only W form organisms, was devoid of Vi antigen.

Virulence Tests.—The virulence for mice of each of the strains of *S. typhosa* was determined by means of the intraperitoneal injection of graded doses of 16 hour cultures of the organisms suspended in 5 per cent hog gastric mucin (Wilson 1701-W). Cultures for preparation of

[1] The amino acids–inorganic salts medium had the following composition: *dl*-alanine, *dl*-valine, *l*-leucine, amino-acetic acid, *l*-proline, *l*-hydroxyproline, *dl*-aspartic acid, and *l*-glutamic acid, M/1500; *dl*-methionine, *dl*-phenylalanine, *l*-tyrosine, *l*-arginine, *l*-histidine, *dl*-lysine, and *l*-cystine, M/4000; *l*-tryptophane, M/20,000; KH_2PO_4, M/30; $MgSO_4 \cdot 7H_2O$, M/6000; pH 7.6. Glucose, as sterile supplement, M/80.

challenge suspensions of strains 58 and 58V were grown on veal infusion media while cultures of strains 58B and 58A were grown in the chemically defined amino acids–inorganic salts medium, with and without glucose, respectively.

The mice employed in these virulence tests were 14 to 16 gm. white Swiss mice (Bagg strain) equally divided as to sex. In all mouse tests included in these studies, selection and assignment of mice to jars in groups of five, location of the groups (jars) in the test-animal room, and the

TABLE I

Virulence for Mice of S. typhosa, Strains 58 and 58V

Challenge dose (in 0.5 ml. of 5 per cent mucin)	Strain 58	Strain 58V
5	13/20*	
50	18/20	
500	17/20	
5,000	19/20	
30,000		1/20
300,000		3/20
3,000,000		8/20
30,000,000		20/20

* Numerators denote number of deaths in 72 hours; denominators, total mice injected.

TABLE II

Virulence for Mice of S. typhosa, Strains 58B and 58A

Challenge dose (in 0.5 ml. of 5 per cent mucin)	Strain 58B	Strain 58A
3	1/20*	
30	9/20	
300	10/20	
3,000	17/20	
80,000		1/20
800,000		6/20
8,000,000		16/20

* Numerators denote number of deaths in 72 hours; denominators, total mice injected.

order of injection by groups all were determined by randomization procedures. The temperature of the test-animal room was maintained at 80° ± 1°F.

Results of the virulence tests, in terms of the number of mice dying in 72 hours out of the total injected, are presented in Tables I and II.

As can be seen from the results presented in Tables I and II, strains 58 and 58B were typically virulent strains (LD_{50}'s = <5 and 180 organisms, respectively), while strains 58V and 58A were relatively avirulent (LD_{50}'s = 3,000,000 and 1,750,000 organisms, respectively).

Preparation of Vaccines.—The method employed for the production of experimental vaccines was essentially the same as that employed as routine by the Army Medical Department

in preparation of typhoid vaccine for the Armed Forces (11). Thus, the organisms were grown on solid media for 24 hours at 37°C., suspended in saline, killed by heating to 56°C. for 1 hour, and preserved with 0.5 per cent phenol. Routine sterility, safety, and toxicity tests were run on each vaccine and identification tests were performed by immunization of rabbits with three injections of 0.5 ml. subcutaneously, 1.0 ml. and 1.0 ml. intravenously at 7 day intervals. One week following immunization all rabbits were bled and the sera were titrated for typhoid H and O agglutinins. On the basis of these agglutination tests, no differences in the agglutinogenic activity of the experimental vaccines were observed.

TABLE III

*Survival of Mice Immunized with Vaccines Prepared from S. typhosa, Strains 58 and 58V, and Challenged with Strain 63**

Vaccine made from	Vaccine dose, *ml.*			
	0.003	0.012	0.048	None (controls)
Strain 58 (virulent)	3/15‡	9/15	13/15	1/19
Strain 58V (avirulent)	8/14	12/15	15/15	

* Challenge dose, 5,000 organisms suspended in 0.5 ml. of 5 per cent mucin.

‡ Numerators denote number of survivors at 72 hours; denominators, total mice tested.

TABLE IV

*Survival of Mice Immunized with Vaccines Prepared from S. typhosa, Strains 58B and 58A, and Challenged with Strain 63**

Vaccine made from	Vaccine dose, *ml.*				
	0.006	.012	0.024	0.048	None (controls)
Strain 58B (virulent)	9/30‡	14/30	17/29	26/30	2/13
Strain 58A (avirulent)	7/30	8/29	15/30	18/30	

* Challenge dose, 10,000 organisms suspended in 0.5 ml. of 5 per cent mucin.

‡ Numerators denote number of survivors at 72 hours; denominators, total mice tested.

Mouse-Protection Potency Tests

Mouse-protection potency tests on the experimental vaccines were run in pairs, *i.e.* vaccines prepared from strains 58 and 58V were compared directly in one assay, while vaccines from strains 58B and 58A were compared in another. The assay procedure employed was based on the use of graded doses of vaccine and a constant challenge dose of viable *S. typhosa* suspended in 5 per cent mucin since a previous study (12) indicated that use of this method permits comparisons of greater precision than are obtained by procedures more commonly employed (13, 14). The mice employed in these assays were 15 to 17 gm., male, white Swiss mice (CFW strain). All immunizing injections were made intraperitoneally, employing appropriate dilutions of vaccine made up to 0.5 ml. in each case, and 6 days later all mice were challenged by the same route with mucin suspensions of the test organism *S. typhosa*, strain

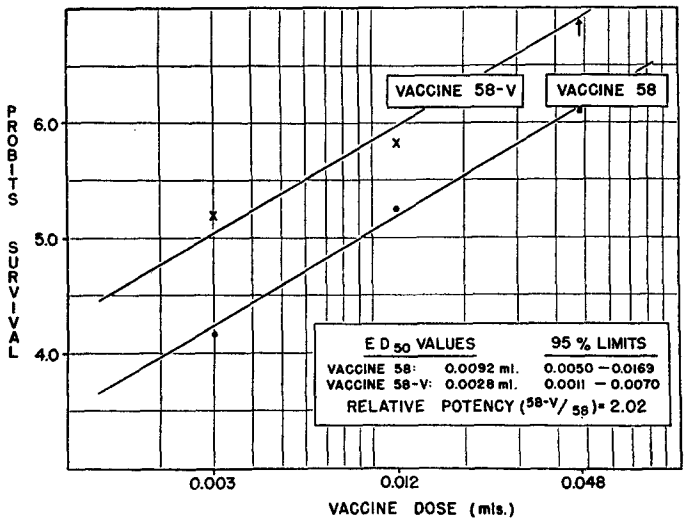

FIG. 1. Mouse-protection potency tests on vaccines prepared from naturally occurring virulent (58) and avirulent (58V) strains of *S. typhosa*.

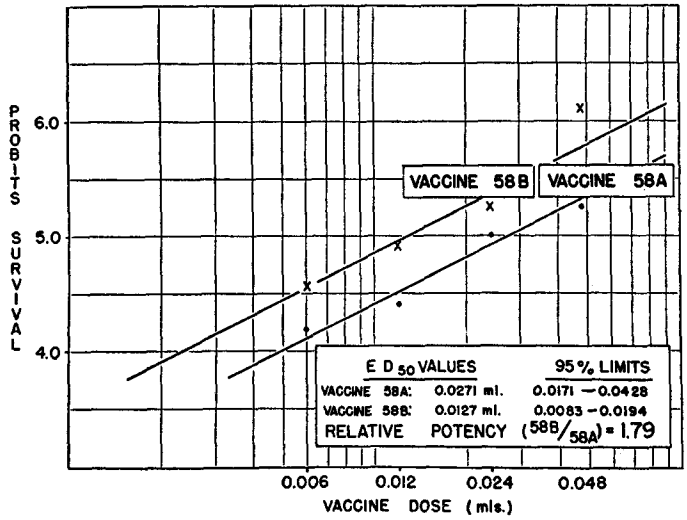

FIG. 2. Mouse-protection potency tests on vaccines prepared from *S. typhosa* strains 58A and 58B.

63 (6). Deaths among the challenged mice within 72 hours were recorded and the absolute potency of each vaccine, in terms of ED_{50}'s, and the relative potency of each pair of vaccines were estimated by the methods proposed by Bliss (15, 16). The results of these tests are presented in Tables III and IV, and in Figs. 1 and 2.

The estimates of absolute and relative protective potency of the two vaccines prepared from virulent strains revealed no conclusive evidence of superior immunogenicity of these products over that of those prepared from the avirulent strains. Repeated tests have yielded relative potencies of these pairs of vaccines made from virulent and avirulent strains varying from 0.97 to 2.02. In each case the 95 per cent confidence limits of the experimentally determined ED_{50} values overlapped to such an extent that there was no statistically significant evidence of any true differences in potency.

TABLE V

Typhoid O Agglutinin Titers of Sera from Individuals Immunized with Vaccines Prepared from S. typhosa, Strains 58B and 58A

Vaccine made from	No. of persons	O agglutinin titers					Mean titers (geometric)
		1:80	1:160	1:320	1:640	1:1,280	
Strain 58B (virulent)	11	1	2	4	3	1	341
Strain 58A (avirulent)	13	2	3	5	2	1	273

TABLE VI

Typhoid H Agglutinin Titers of Sera from Individuals Immunized with Vaccines Prepared from S. typhosa, Strains 58B and 58A

Vaccine made from	No. of persons	H agglutinin titers					Mean titers (geometric)
		1:80	1:160	1:320	1:640	1:1,280	
Strain 58B (virulent)	11	2	0	5	2	2	362
Strain 58A (avirulent)	13	0	4	4	4	1	355

Immunological Response of Human Beings

A series of 26 individuals[2] was immunized with vaccines prepared from virulent (58B) and avirulent (58A) strains of *S. typhosa*; 13 individuals with each product. None of the individuals included in the study had a history of typhoid or typhoid-like infections and none previously had been immunized with typhoid vaccine.

Individuals were selected at random for immunization with each vaccine and three injections of 0.5 ml. each were given at weekly intervals. A complete record of systemic and local reactions following each injection was obtained. The observed reactions, as a whole, were mild and were essentially identical both in occurrence and severity in the two groups.

[2] The authors wish to express their appreciation to Dr. Harry E. Morton, Department of Bacteriology, School of Medicine, University of Pennsylvania, for his cooperation in this investigation, and to students of the Department who served as volunteer subjects in the study of the antibody response to immunization with the experimental vaccines.

Blood specimens were obtained from each individual immediately prior, and 21 days subsequent to, immunization. All serum specimens were titrated for typhoid H and O agglutinins and for mouse-protective antibodies. No evidence was obtained of the existence of specific immune antibodies in any of the serum specimens collected prior to immunization.

Sera from two individuals immunized with vaccine prepared from strain 58B failed to yield agglutination and mouse-protection antibody determinations both of which were satisfactory. The distribution of O and H agglutinin titers and geometric mean titers of the remaining 24 individuals in the two immunization groups is presented in Tables V and VI. While the mean titers were slightly higher in the group given vaccine prepared from strain 58B, the difference was not significant[3].

The method of determining the mouse-protective potency of the sera was based on observations previously reported (17) that assays based on graded doses of serum and a constant challenge dose of viable organisms yield more reliable results than do tests in which graded challenge doses and a constant serum dose are employed. A two dose serum protection test was used, the doses differing by a fourfold increment and so selected as to bracket the expected ED_{50} value determined by preliminary screening tests. Male, white Swiss mice (CFW strain), weighing 15 to 17 gm., were used in all serum assays. Serum dilutions were injected intraperitoneally and all mice were challenged 1 hour later by the same route with 1,000 *S. typhosa*, strain 63, suspended in 5 per cent mucin. Twenty mice (four groups of five each) were tested at each serum dose. Tests were considered satisfactory only when 1 to 9 mice given the smaller serum dose, and 11 to 19 given the higher serum dose, survived for 72 hours. Tests were repeated until two satisfactory assays were obtained on each serum specimen. In each test the relative potency of each serum (16), in terms of mouse-protection units, was calculated in reference to a standard antiserum arbitrarily designated as possessing 100 mouse-protective units per ml. The individual mouse-protective potencies, means, and standard deviations of such potencies of the sera from each study group are presented in Table VII.

The observed close agreement between the mean mouse-protective potencies obviously was a chance circumstance when the standard deviations of the two series are note. The entire data, in terms of mouse-protective units, were analyzed statistically by analysis of variance (18). Results of the analysis are presented in Table VIII.

The analysis presented in Table VIII reveals the futility of attempting to draw conclusions from mouse-protective potency data such as were obtained in this study. It can be seen that although differences between the sera of individuals were significantly greater than were those between repeat tests on the same sera, the former contributed overwhelmingly more to the total variation than did differences in responses to the two types of vaccine. In fact, it

[3] Additional typhoid O and H agglutinin titrations were run on serum specimens obtained from the same study individuals at approximately 90 and 180 days following immunization. At each postimmunization period the geometric mean agglutinin titers were significantly lower than at the preceding period, but at no time did the titers of the two immunization groups differ significantly.

TABLE VII

Mouse-Protective Potency of Sera from Individuals Immunized with Vaccines Prepared from S. typhosa, Strains 58B and 58A

Vaccines made from strain 58B (virulent)				Vaccines made from strain 58A (avirulent)			
Serum No.	Protective potency*			Serum No.	Protective potency*		
	1st test	2d test	Mean		1st test	2d test	Mean
6	226	245	236	1	401	455	428
8	76	100	88	3	207	237	222
10	42	139	91	5	189	153	171
12	316	153	235	7	125	178	152
14	172	259	216	9	595	873	734
16	90	142	116	11	358	634	496
18	1,148	800	974	13	136	437	287
20	258	597	428	15	212	500	356
22	240	225	233	17	167	185	176
24	153	217	185	19	321	497	409
26	316	216	266	21	250	373	312
—	—	—	—	23	189	282	236
—	—	—	—	25	232	351	292
Group mean potency.....	279			328			
Standard deviation‡.....	250			161			

* Determined in relation to a reference serum arbitrarily designated as having a mouse-protective potency of 100.

‡ Calculated from mean potencies of individuals.

TABLE VIII

Analysis of Variance of Mouse-Protective Potency Data Presented in Table VII

Source of variation	Degrees of freedom	Mean square	Variance ratio
Between vaccine groups.....	1	29,447	0.34‡
Between individuals within groups.....	22	86,353	5.91***
Between repeat tests on individual sera.....	24	14,618	

‡ Not significant at the 5 per cent level.

*** Exceeds the 0.1 per cent level of significance.

can be stated that it is extremely improbable that there should be such close agreement between immunization groups when the sera of different individuals and the repeat tests themselves were subject to such great variation. If there is a true difference in response to vaccines of these two types, it is obvious that new and far more reproducible testing procedures must be developed be-

fore the significance of such differences can be established. Certainly, no evidence was obtained in this study indicating that there were any true differences in the responses to the two products employed.

DISCUSSION

The experimental results reported in this investigation fail to indicate the existence of any essential relationship between the virulence, as measured in mice, and the immunogenicity of the strains of *S. typhosa* employed. Furthermore, no quantitative differences in the immunogenicity of strains of different virulence were established. The latter may have resulted from the absence of any appreciable true differences or the inadequacy of the methods employed to establish the existence of such differences beyond the limits of experimental uncertainty.

It is becoming more generally recognized that virulence, rather than being a specific, intrinsic attribute of an organism, is a manifestation of a combination of factors pertaining to the infectious agent, the affected host, and other factors both known and unknown. In other studies recently completed in these laboratories (19), it was found that the degree of difference in virulence for mice of strains 58 and 58V (also 58B and 58A) depended upon the method of testing for virulence. Thus, the distinction between the virulence of these strains was marked when tested by the intraperitoneal-mucin technique but was of questionable certainty when tested by the intracerebral injection of saline suspensions of the organisms.

Under both experimental and natural conditions, there undoubtedly are many factors complementing the infective process which either are unrecognized or for which no quantitative methods of estimation are available. If in any one particular situation one factor (or a combination of factors) uniquely essential to those conditions is absent or is inadequate to complement the process, failure to obtain evidence of virulence would result. Thus, experimental demonstration of avirulence may be the result of fortuitous circumstances rather than of any true inadequacy of the potential capability of the test organisms to parasitize the host.

The possibility of apparent marked differences in virulence of strains of organisms resulting from chance circumstances can largely be eliminated by proper planning and execution of experiments. For example, the concomitant testing of virulent and avirulent strains such as 58 and 58V (or 58B and 58A) in a single experiment with the environmental conditions and such reagents as mucin being common to both, and unknown differences in resistance of individual mice or progressive changes in challenge suspensions being distributed throughout the experiment by proper randomization, tend to minimize the possible introduction of bias resulting from chance association of unknown inadequate biological factors with an experimental factor of prime interest.

In this study, strains 58 and 58V were equal in regard to their content of known somatic antigens IX, XII, and Vi. The prime difference between these strains was the inability of the latter, when suspended in 5 per cent mucin, to exhibit comparable virulence for mice by intraperitoneal injection. This observation clearly indicates that Vi antigen, regardless of its rôle in immunogenicity, is not an independent virulence entity.

Strains 58B and 58A differed in that the former was predominantly a V form strain (a few W form organisms were present) while 58A was a pure W form strain. Thus, these two strains were characterized by the presence and absence, respectively, of Vi antigen. The difference in virulence of these two strains ordinarily would have been assumed to be conditioned by this difference in Vi antigen content except for the aforementioned observation of virulence and avirulence of strains 58 and 58V, both of which contained Vi antigen.

Failure to demonstrate virulence of strains 58V and 58A indicates that some factor or component essential to or complementing the experimental infective process was missing; a factor possessed by strains 58 and 58B. All that can be surmised regarding its nature is that it is independent of the somatic antigens IX, XII, and Vi; it is not a definitive characteristic of V form strains; and it is not of demonstrated immunogenic importance. If the component responsible for the differentiation between virulent and avirulent strains were immunogenically active, it is possible that it was destroyed or rendered inactive by the heat or phenol employed in preparing the experimental vaccines. Felix and Bhatnagar (20) have reported such effects of various killing and preserving agents on Vi antigen and, while the explanation of the findings reported here cannot very well be based on Vi antigen, other immunologically active components could exhibit comparable lability. It has been established that merthiolate is a mild killing agent and does not destroy Vi antigen (21). Vaccines prepared in these laboratories from strains 58, 58V, 58B, and 58A using merthiolate as a killing and preserving agent have shown immunizing activity entirely comparable to those produced by the heat-phenol method. These results cast doubt on, but do not negate, the foregoing supposition.

The comparable protective potency of vaccines produced from strains 58B and 58A, as measured by active-immunity mouse-protection tests, may appear to cast doubt on the rôle of Vi antigen in determining the immunogenicity of typhoid vaccine. Failure to obtain conclusive evidence of difference in the potency of these two products could have resulted from (*a*) non-existence of any difference, (*b*) a quantitative difference of such minor magnitude that the experimental design selected was of insufficient scope and sensitivity for its detection with certainty, or (*c*) inappropriateness of the selected experimental conditions for detecting the existence of a difference, either qualitative or quantitative.

Although no conclusive evidence was obtained of a difference in potency of

vaccines made from strains 58B and 58A, the findings do not necessarily preclude the existence of a true difference. Strain 58B was predominantly a V form strain and did contain Vi antigen while strain 58A did not. Other active-immunity mouse-protection tests conducted in these laboratories, employing somewhat dissimilar experimental conditions, repeatedly have indicated that vaccines prepared from V form strains are more highly immunogenic than are those prepared from W form strains. The experimentally determined ratio of the protective potencies of these two products, 1.79 (Fig. 2), was well within the 95 per cent confidence limits of the ratio and, therefore, no statistical justification existed for stating that a difference in potency existed. Likewise, there was no justification for stating that a difference did not exist. The observed difference could well have been a chance occurrence but the best estimate of relative potency was 1.79. Establishment of statistical significance of a true difference of this magnitude would have required a more elaborate or extensive experiment.

It is conceivable that the experimental conditions selected, such as strain of mice, strain of challenge organisms, etc., inadvertently were such as to preclude or at least to minimize the demonstration of differences in potency of the experimental vaccines. In another series of investigations currently being pursued in these laboratories it has been found, for example, that *S. typhosa*, strain 63, is markedly less virulent than is strain 58 but that demonstration of this difference can be accomplished only by the deliberate use of relatively resistant mice or other experimental factors unfavorable to high mouse mortality. In all active-immunity mouse-protection tests presented in this study, strain 63 was used as the challenge organism. Differences in the protective potency of the vaccines being studied might well have been more clearly evident if other testing conditions, such as a more highly virulent challenge strain, had been employed. Studies in this regard are now in progress and will be made the basis of a separate report.

Regardless of minor differences in potency of vaccines prepared from strains 58B and 58A, which could be dependent upon the presence or absence of Vi antigen, it appears that whatever the nature of the factor essential to the virulence of strains of *S. typhosa* it is not of demonstrable immunogenic importance. This is evidenced particularly by the relative potency (Fig. 1) of vaccines prepared from the naturally occurring virulent and avirulent strains 58 and 58V. Such a conclusion is strictly valid only on the assumption that survival of vaccinated mice, when subjected to challenge, is dependent upon the existence of a true state of immunity resulting from injection of the vaccines. While the true immunological significance of the mouse-protection test has not been established incontrovertibly, results of cross-protection tests (22) have confirmed the specificity of the method.

The results of the O and H agglutinin titrations run on sera from human

beings (Tables V and VI) immunized with vaccines prepared from virulent and avirulent strains likewise failed to furnish any evidence of a difference in the immunogenic potency of the two products. Although agglutinin response is considered to be less biologically significant than is mouse-protective potency (23), the close comparability of the responses to the two vaccines affords important supportive evidence.

Results of the mouse-protection tests on the human sera likewise afforded no evidence of a qualitative or quantitative difference in the response to vaccines made from relatively virulent and avirulent stains. Of equal or greater interest, however, was the observation of the extreme variations in the results of such tests performed on sera from different individuals given the same vaccine and even in the results of repeat tests on the same sera. These findings emphasize the lack of reliability of quantitative data based on mouse-protection tests and reveal the need for further developmental study of the technique and its utilization in immunological investigations.

It is appreciated that the results presented here do not necessarily refute the concept that recently isolated, fully virulent, smooth strains of *S. typhosa* are to be preferred for the production of typhoid vaccines. Rather, these results may merely emphasize the inadequacies of the laboratory techniques available for evaluation of such a concept. The strains considered to be virulent and avirulent may well be of equal virulence, either high or low, for man. The true state of protective immunity engendered in man by injection of vaccines made from these strains actually may be of entirely different orders of efficacy. If, however, these true states are so contradictory to the reported experimental findings, further research of this nature not only may be fruitless,—it may be misleading.

SUMMARY

An investigation of the immunogenicity of vaccines prepared from closely related virulent and avirulent strains of *S. typhosa* is reported.

No evidence was obtained of any essential relationship between the virulence of the strains employed and their immunogenicity, as determined by active-immunity mouse-protection tests and by the agglutinin and mouse-protective antibody response in human beings.

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