THE STERILIZATION OF ANTI-TYPHOID VACCINE

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Our knowledge of the antigens of the typhoid bacillus has recently been enlarged by the investigations of Felix & Pitt (1934).

It has been recognized that the Vi antigen, newly established by these workers, is not only a very important factor in immunity, but also apparently a more labile substance than the O antigen hitherto regarded as the portion of the typhoid soma vitally concerned with the production of prophylactic immunity.

Sterilization by heating to 58° C. so lowers the efficacy of Vi antigen that the antibody response to it in a rabbit is almost suppressed (Felix *et al.* 1934), while sterilization by formalin so alters its antigenic nature that the resulting antibody, while of high agglutination titre, acts feebly in passive protection and phagocytosis experiments (Felix & Bhatnagar, 1935).

The presence of Vi antigen in anti-typhoid vaccine is known to be essential for the protection of mice against a lethal dose of Vi-containing typhoid bacilli, and one may assume that it will also be of importance for the protection of man against a naturally occurring infection. It becomes, therefore, of practical interest to establish which method of preparation will yield a vaccine containing Vi antigen in its least damaged and most effective form.

A preliminary step in the preparation of an efficient anti-typhoid vaccine is the selection of the most suitable strain. Felix has shown that while the great majority of typhoid strains possesses Vi antigen, only a very small number possesses this antigen to an exceptional degree. It is reasonable to suppose that vaccine prepared from such full Vi strains would protect better than vaccine made with the so-called intermediate strains. And this in the experiment to be described was seen to be very strikingly the case.

Prophylactic value of full and intermediate Vi strains

In a series of eighty-eight mice inoculated with a vaccine prepared with Ty 1, an intermediate Vi strain, thirty-seven animals survived a test dose of 3 M.L.D. of living virulent typhoid culture Ty 2, while of another eighty-eight mice inoculated with similar doses of vaccine prepared with Ty 2, a full Vi strain, double that number, namely, seventy-six, survived the same test dose. Of a control batch of mice immunized with a vaccine made from a strain, H 901, entirely lacking Vi antigen, no animal survived the test dose. The superior protective value of vaccine made with a full Vi strain is, thus, very decidedly proved.

Felix has demonstrated that the excess of Vi antigen possessed by such a strain is expressed in its raised virulence for mice and in its lowered agglutinability by typhoid Vi antibody. It is also possible to demonstrate, and probably quantitatively, the Vi content of a typhoid strain by means of the precipitin test.

Estimation of the Vi content of a typhoid strain by the precipitin test

Extracts of agar grown cultures are prepared by incubation of saline suspensions for 2 hours at 37° C. with subsequent centrifugation; two capillary drops of the supernatant in varying dilution are mixed with five capillary drops of typhoid serum from which all but the Vi antibodies have been absorbed; the readings are taken after standing 2 hours at 37° C. and overnight in the cold room.

A comparison of the precipitation titres of extracts made from the non-Vi strain, H 901, the intermediate Vi strain, Ty 1, and the full Vi strain, Ty 2, is afforded by Table I. To indicate the quantitative possibilities of the test, a series of Ty 2 suspensions was extracted and the precipitation titre found to vary with the density of those suspensions.

Table I. *Vi precipitation*

Strain tested	Vi content as established by virulence and agglutination tests	Density of suspension from which extract was made	Extract dilutions 1/1	1/2	1/4	1/8	1/16	1/32
H 901	Negative	16×10^9 per c.c.	−	−	−	−	−	−
Ty 1	Intermediate	,, ,,	+	+	+	−	−	−
Ty 2	Maximum	,, ,,	+	+	+	+	+	−
Ty 2	Maximum	8×10^9 ,,	+	+	+	+	−	−
Ty 2	Maximum	4×10^9 ,,	+	+	+	−	−	−
Ty 2	Maximum	2×10^9 ,,	+	+	−	−	−	−
Ty 2	Maximum	1×10^9 ,,	+	−	−	−	−	−

The extract prepared from the intermediate strain, Ty 1, has a Vi precipitation titre one-quarter of that yielded by the full Vi strain, Ty 2.

Effect of medium and its *p*H on Vi content of typhoid culture

A further preliminary step is then to assay, by the precipitin test, the Vi content of cultures grown on a variety of media in the hope of finding one which would enhance the production of Vi antigen. A series of the simpler laboratory media was tested. These consisted of ordinary nutrient agar as commonly used at the Lister Institute, the same medium plus 25 per cent broth to decrease the solidity of the medium, or plus 10 per cent horse serum or plus 4 per cent glycerine, agar according to the Veillon formula which gives such an enhanced growth with certain types of bacteria, and Dorset egg, which has a reputation for the maintenance of cultures in a smooth condition. The only media to vary the Vi content of the typhoid culture Ty 2, as shown by the precipitin test, were the Veillon and the Dorset egg; in both cases the extracts

gave lower titres. There was, therefore, no superiority in any of the media modifications tested. Similar results were obtained on testing nutrient agar of *p*H varying from 7·2 to 8·2. Variations of *p*H within this range did not alter the Vi precipitation titre.

The vaccines to be tested were, therefore, prepared with the full Vi strain Ty 2 grown on ordinary nutrient medium of *p*H 7·6, the methods of sterilizing being as diverse as could be arranged.

COMPARISON OF STERILIZATION METHODS

In the preparation of the variously sterilized vaccines, an addition of preservative was dispensed with, so that the effect of sterilization alone upon the efficiency of the vaccine might be observed. In each experiment, one batch of vaccine sterilized in the manner ordinarily employed at the Lister Institute, viz. heating to 53° C. for 70 min. with the subsequent addition of 0·5 per cent phenol, was simultaneously prepared to serve as a standard of control for the other vaccines.

The methods of sterilization comprised heat alone, phenol or alcohol at room temperature, formalin or merthiolate at 37° C., CO_2 under pressure at room temperature, the osmotic action of a concentrated solution of saccharose.

Mice were immunized with equal sized doses of these various vaccines and, after 2–3 weeks, tested with 3–4 M.L.D. of virulent typhoid culture, Ty 2. The vaccine and test doses were chosen of such a size as would give for mice inoculated with the standard 53° C. killed vaccine a survival rate of under rather than over 50 per cent, so that should a more efficient vaccine be among the number tested it might be the more readily detected.

In general, immunization consisted of two doses (50×10^6 and 100×10^6) of vaccine given with an interval of 7 days, but on one occasion in the hope that a better differentiation of the vaccines might be achieved, a single dose, 250×10^6 was administered; the result, however, was the same.

These results were unexpected and disappointing in that no vaccine occasioned a degree of immunity greater than that resulting from the use of the standard 53° C. killed vaccine, all of them approximating to this very closely, as is seen in Table II. Two experiments with smaller numbers of mice, twenty-seven and twenty-two respectively, were performed but have not been included in the table. Sterilization was effected by suspension in 25 per cent galactose (6 days at 37° C.), where the killing was doubtless achieved by the acid produced during fermentation of the sugar and by suspension in 20 per cent sodium chloride (2 days at 37° C.). There was no indication of any superiority in these two methods.

The conclusion to which these experiments leads is that among the various sterilization methods tested here, none has been found to give a better prophylactically immunizing antigen than does the method commonly in use.

This conclusion, however, is only of value when it has been shown what delicacy the mouse test, as employed in these experiments, possesses.

Table II. *Mouse immunization with variously sterilized vaccines*

Exp.	Vaccines	Survival rate	Percentage survivors
1	70 min. at 53° C.—½ % phenol	35 out of 95	37
	½ % phenol at room temp.	29 ,, 93	31
	1/4000 merthiolate at 37° C. (4 days)	34 ,, 91	37
2	70 min. at 53° C.—½ % phenol	46 ,, 127	36
	75 % saccharose at 37° C. (3 days)	46 ,, 127	36
3	70 min. at 53° C.—½ % phenol	42 ,, 107	39
	50 atmos. CO_2 at room temp. (7 hours)	45 ,, 109	41
4	70 min. at 53° C.—½ % phenol	23 ,, 45	51
	1½ hours at 58° C.	16 ,, 40	40
	1/500 formalin at 37° C. (1 day)	22 ,, 42	52
5*	70 min. at 53° C.—½ % phenol	7 ,, 47	15
	70 % alcohol at room temp. (1 day)	4 ,, 47	9

* The percentage of survivors in Exp. 5 is low because the test dose consisted of 5–6 M.L.D. and not the usual 3–4 M.L.D.

Experiments were, therefore, carried out in which mice were inoculated with increasing amounts of a single standard vaccine, and these have shown that only when there is an increase in vaccine dose of at least 50 per cent, is an increase in immunity in the mice detectable.

With an increase in vaccine dose of 100 per cent a more notable increase in immunity results.

Table III. *Increase of immunity following increase in vaccine dose*

Exp.	Vaccine dose	Survival rate	Percentage survivors
1	200×10^6	46 out of 90	51
	300×10^6	57 ,, 90	63
2	200×10^6	26 ,, 57	46
	300×10^6	33 ,, 60	55
3	200×10^6	12 ,, 27	44
	400×10^6	19 ,, 30	63

Table III demonstrates that the effect of raising the vaccine dose by 50 per cent produces, in both Exp. 1 and 2, an increase of roughly 20 per cent in the consequent immunity, while in Exp. 3 one sees that to double the vaccine dose is to improve the immunity response by about 40 per cent.

One must, then, modify the conclusion and say that among the various sterilization methods tested, none has produced an antigen which is 50 per cent better prophylactically than that resulting from the standard method commonly employed.

This conclusion that these ten different sterilizing methods are, within 50 per cent, of equal value in anti-Vi prophylaxis, is remarkable when one considers to what extent the Vi antibody producing powers of a vaccine are damaged by similar treatment. It has already been recalled that heating Vi antigen to 58° C. practically inhibits agglutinin production in the rabbit and that vaccine sterilized by formalin yields a Vi antibody which acts feebly in the

passive protection of mice or in promoting phagocytosis. Yet neither of these sterilizing methods is markedly inferior when used in the preparation of prophylactic vaccines. It would seem, therefore, that the mechanism of active immunity is largely independent of circulating antibody as estimated by agglutination, phagocytosis or passive protection. Perhaps it is important to remember that the typhoid bacillus is not a natural pathogen of the mouse. Previous experiments (Schütze, 1930) with *S. typhi murium* (*B. aertrycke*) seemed to indicate that with this organism, notably infective for the mouse, the somatic antibody titre might to some extent serve as index to immunity, although even here one surmised the existence of a basal immunity independent of circulating O antibody.

CONCLUSIONS

1. The importance of employing a full Vi strain in the preparation of antityphoid vaccine is demonstrated by the fact that with it immunity in mice is 100 per cent greater than when an intermediate Vi strain is used.

2. The precipitin test furnishes a method of estimating the Vi content of a typhoid culture.

3. Attempts to improve the Vi content of a full Vi strain by growth on certain special media or on ordinary agar by varying the *p*H have failed.

4. Vi-containing antityphoid vaccines sterilized in some ten different ways, all immunized mice against 3–4 lethal doses of a full Vi strain to very similar degree. As it has been demonstrated that it needs an increase in vaccine dose of about 50 per cent before an increase in immunity is observable, we may conclude that the various methods of sterilization under examination are, within that limit of accuracy, of equal value in the preparation of typhoid Vi antigen for vaccine purposes.

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