Notes

Mouse Challenge with *Salmonella typhosa* T 5501 in Testing the Potency of Typhoid Vaccines

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It was observed in the course of the recent field trials of typhoid vaccines in Yugoslavia [b] that the group of persons immunized with alcohol-killed vaccine showed higher Vi antibody titres than the group immunized with heat-killed, phenol-preserved vaccine, but that the latter afforded significantly better protection than the former. This indicates that the Vi antibodies have no significant value in the protection of man against typhoid fever, although they are of primary importance in mouse protection. [c, d]

This observation indicates the necessity of overcoming the influence of Vi antigen and antibodies in mice in order to obtain a correlation between the protection in man and that in mice. In the passive mouse-protection test, this may be done in either of two ways: (*a*) removing Vi antibodies from the serum of immunized rabbits, or (*b*) using a W strain for challenge. The latter is the more practical procedure.

A. F. B. Standfast has reported to the authors in a personal communication (1956) on the use of strain T 5501—a pure W strain—as a challenge strain for mice. The present note is a report on our experiments with this challenge strain, kindly provided by him.

LD_{50} of *Salmonella typhosa* T 5501 suspended in saline. The test animals used were Swiss mice of either sex, weighing 18-20 g. An 18-hour culture of the challenge strain was grown on nutrient agar and suspended in saline; 0.5 ml of the suspension was injected intraperitoneally. The mice were observed for a period of three days.

The pooled results of five experiments are as follows:

Dose (opacity units/ml)	*No. of animals dying/Total tested*	*Percentage of deaths*	*LD_{50} (opacity units/ml)*
20	54/59	91	
4	31/60	52	4
1.6	0/60	0	

LD_{50} of *S. typhosa* suspended in semi-fluid thioglycollate-agar medium. Since we considered that it would be of advantage to obtain a lower LD_{50} than the above, we conducted certain experiments to that end. These experiments demonstrated that *S. typhosa* grown and suspended in semi-

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[b] Yugoslav Typhoid Commission (1957) *Bull. Wld Hlth Org.*, **16**, 897
[c] Felix, A. & Pitt, R. M. (1935) *J. Hyg. (Lond.)*, **35**, 428
[d] Landy, M. (1953) *Amer. J. Hyg.*, **58**, 148

fluid thioglycollate-agar is more virulent for mice than when suspended in saline. The pooled results of five experiments with this medium were:

Dose (opacity units/ml)	*No. of animals dying/Total tested*	*Percentage of deaths*	*LD_{50} (opacity units/ml)*
1.00	50/50	100	
0.25	31/50	62	0.16
0.06	9/50	18	

As will be seen from the above figures, the use of this medium for both growth and suspension decreases the LD_{50} some 25 times. If the organisms are grown on the surface of nutrient agar and merely suspended in the semi-fluid thioglycollate-agar medium, the LD_{50} is 0.25 opacity unit per ml. Omission of the agar component from the semi-fluid medium has almost no effect, the LD_{50} then being 0.18 opacity unit per ml.

Passive mouse protection with *S. typhosa* T 5501. Mice were injected subcutaneously with 0.5 ml of a series of fivefold dilutions of serum and challenged 48 hours later with a 1/10 dilution of a culture of *S. typhosa* T 5501 grown for 18 hours in semi-fluid thioglycollate-agar medium and diluted in fresh growth medium. The challenge dose, equivalent to 4-10 LD_{50}, was given intraperitoneally in 0.5 ml volumes. The mice were observed for three days. The results below illustrate the differences in protective power of various antisera:

Antiserum	*Serum dose (ml)* *					*Control mice * (no serum)*	*ED_{50} (ml)*
	0.25	*0.05*	*0.01*	*0.002*	*0.0004*		
Normal	10/10	10/10				10/10	>0.25
Shigella flexneri	10/10	9/10	8/10	10/10	10/10	10/10	>0.25
Salmonella gaminara	12/40	32/40	39/40			38/40	0.12
Salmonella typhosa (Ty 2)		4/50	18/50	44/50	44/50	48/50	0.007
Escherichia coli (5396/38)	25/30					28/30	>0.25
Salmonella loma-linda	5/30	17/40	28/40	30/40	9/10	38/40	0.03

* The fractions represent the number of mice dying over the number tested.

The agglutination titres of the antisera tested were as follows:

	Agglutination titres			*ED_{50} (ml)*
	H 901	*O 901*	*Vi I*	
Normal	—	—	—	>0.25
Shigella flexneri	—	—	—	>0.25
Salmonella gaminara	4000	—	—	0.12
Salmonella typhosa (Ty 2)	8000	8000	160	0.0007
Escherichia coli (5396/38)	—	—	320	>0.25
Salmonella loma-linda	6400	3200	32	0.03

These experiments demonstrate that normal serum, anti-*flexneri* serum and anti-*coli* serum, which contain Vi antibodies, have no protective value against the challenge strain used. Anti-*gaminara* serum gives a low degree of protection. The protection afforded by the anti-*loma-linda* serum is of the same order as that afforded by the anti-*typhosa* (Ty 2) serum. The results suggest that the mechanisms of immunity in mice against experimental typhoid infection are quite different with the Ty 2 strain and with the T 5501 strain. Although Vi antibody is of primary importance in the protection of mice against infection with the Ty 2 strain, O antibody (or possibly some other antibody against an undetermined somatic antigen) plays an important role in their protection against T 5501.

Protective value of alcohol and phenol vaccines. A culture of the Ty 2 strain was harvested after growth on nutrient agar and suspended in saline. This culture was divided into two lots, one of which was used to prepare heat-killed and phenol-preserved vaccine and the other to prepare alcohol-killed and alcohol-preserved vaccine.

Ten rabbits (five for each vaccine) were immunized by Felix's method.[e] The sera of the immunized rabbits were pooled and passive mouse-protection tests were performed as described above. The results were as follows:

		Alcohol vaccine	*Phenol vaccine*
O-agglutination titre		2560	1280
Serum dose (ml)*	0.03	1/20	2/20
	0.0075	16/20	8/20
	0.0019	16/20	17/20
Control*		16/20	
ED_{50} (ml)		0.011	0.007

* Fractions represent the number of mice dying over the number tested.

These figures show that 0.0075 ml of immune serum obtained with alcohol vaccine gave no observable protection, whereas the same amount of immune serum obtained with phenol vaccine afforded significant protection ($\chi^2=5.1$).

Conclusions. Our results confirm the suitability of strain T 5501 for use in challenging mice. When the organisms were suspended in saline, the LD_{50} obtained in our experiments was greater than that which we understand to have been obtained by Standfast, but it can be much decreased by suspending the organisms in semi-fluid thioglycollate-agar medium.

The interference of Vi antibodies in mouse-protection tests is circumvented by the use of strain T 5501 for challenge, and our preliminary experiments indicate the possibility of a potency test which may yield results correlating with the protection afforded to man by different typhoid vaccines. Further experiments towards the development of such a test are in progress.

[e] Described by Grabar, J. & Le Minor, S. (1955) *Ann. Inst. Pasteur*, **88**, 601