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IMMUNIZATION OF MICE AND GUINEA-PIGS AGAINST *SALMONELLA DUBLIN* INFECTION WITH LIVE AND INACTIVATED VACCINE

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ABSTRACT

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The immunogenicity of a number of avirulent rough *Salmonella dublin* mutants was compared in mice and guinea-pigs.

Live vaccine prepared from Strain HB 1/17 at doses of 5×10^7 per mouse usually gave an immunity of between 70 and 80% but in certain experiments the results were more variable and always poorer. This strain gave a cross protection of 28,5% to *S. typhimurium* in mice. In guinea-pigs it evoked an average protection of approximately 46% to homologous challenge and approximately 26% to challenge with *S. typhimurium*.

Strain 5765 protected up to 80% of mice against *S. dublin* infection and was generally superior to Strain HB 1/17 in this respect. It was, however, less effective in protecting mice against *S. typhimurium* (20%). In guinea-pigs it was also less effective than Strain HB 1/17, giving 34% protection against homologous and 20% against heterologous challenge.

Other strains also produced immunity in mice but they were not studied in detail.

Formalin-inactivated alum-precipitated vaccine prepared from avirulent smooth strain and containing 0,5% packed cells proved to be extremely effective in protecting mice against *S. dublin* infection. It produced an average immunity of 75% and was often 100% effective. It also protected 60% of mice against challenge with *S. typhimurium*. In guinea-pigs it was, however, totally ineffective against challenge with both *S. dublin* and *S. typhimurium*.

INTRODUCTION

The question of whether a better immunity to *Salmonella* infections is evoked by live or inactivated vaccines is a controversial one. This theme has been extensively studied, using *S. typhimurium* infection in mice as the experimental model, by Cameron & Fuls (1974), who showed that a very good immunity can be induced by means of a formalin-inactivated alum-precipitated vaccine. Similarly, a good immunity was obtained by immunizing mice with a number of avirulent rough mutants of *S. typhimurium*, though, unfortunately only mutants that retained some degree of residual virulence were effective. This finding naturally precludes their use in a live vaccine and, since the paratyphoid vaccine currently produced at this institute is prepared from a rough avirulent mutant of *S. dublin*, a combined live vaccine could not be formulated.

With the exception of the report by Henning (1953), little definitive work has been done on the efficacy of inactivated *S. dublin* vaccines. He was able to immunize mice effectively with a formalin-inactivated vaccine and also found such a vaccine to be effective in protecting calves against *S. dublin* infection. Botes (1964), however, contended that such a vaccine deteriorated rapidly on storage and he obtained far better results with a live vaccine. Smith (1965) also reported very good results with a live vaccine.

Since no finality regarding the relative value of live or inactivated vaccines has been established, experiments were undertaken to elucidate the question of live versus inactivated *S. dublin* vaccines.

MATERIALS AND METHODS

Experimental animals

Mice: Conventionally reared male albino mice obtained from the colony maintained at this institute were housed in plastic cages on wood shavings and fed a balanced ration. All immunization experiments were done in 6-week-old male mice whereas 8-week-old animals were used for the virulence assays.

Guinea-pigs: Young male albino guinea-pigs which were reared at this institute were used in all the experiments. They were fed a balance pelleted ration supplemented with fresh green lucerne.

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Bacterial strains

Rough strains: S. dublin strains HWS 51 and HWS 17A were obtained from Dr H. Williams-Smith*. Strain HB 1/17 is currently used for vaccine production at Onderstepoort and has been described by Botes (1964). It agglutinates in acriflavine and has a low pathogenicity for mice. Strains 5765, 5565K1, 6792, 6785 and HB 2267 57X were available local strains. All the strains were maintained in the lyophilized state.

Virulent smooth strains: S. dublin strain 2652V was originally isolated from a case of calf paratyphoid (Botes, unpublished data, 1964). *S. typhimurium* strain 2656V has been described previously (Cameron & Fuls, 1974).

Characterization of rough strains of S. dublin

Virulence for mice: The strains to be tested were grown at 37° C on nutrient agar overnight, the growth washed off, suspended in tryptone water and nephelometrically adjusted to give a density of approximately 1×10^9 bacteria/ml. Five-fold dilutions of this suspension were prepared and 0,2 ml of each dilution injected intraperitoneally and subcutaneously into 6–8-week-old male mice. Deaths were recorded for 14 days.

Agglutination in acriflavine: In order to determine their degree of roughness, the ability of strains to agglutinate in acriflavine solutions was determined as described previously (Cameron, Fuls & Van Reenen, 1972).

Preparation and titration of pathogenicity of challenge strains

Since it was desirable to use challenge doses of bacteria which would cause a protracted disease with deaths occurring over a period of 10-12 days, graded doses of bacteria were injected intraperitoneally into mice and guinea-pigs in order to find satisfactory challenge doses.

The challenge strains *S. dublin* 2652V and *S. typhimurium* 2656V were grown in D15 broth (Schlect & Westphal, 1966) shake cultures at 37° C for 24h. The bacteria were collected by centrifugation, dense suspensions were prepared in BLP (Cameron & Fuls, 1974) and 1,0 ml aliquots lyophilized *in vacuo*.

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The number of viable bacteria/vial was determined by means of plate counts. Dilutions were made according to these counts to give doses of approximately 5×10^7, 1×10^7, 2×10^6 and 4×10^5 for mice and $2,5\times10^8$, 1×10^8, 5×10^7, 2×10^7 and 1×10^7 for guinea pigs. Groups of 10 mice and 6 guinea-pigs were injected intraperitoneally with each of the above-mentioned dosages and deaths recorded for 14 days.

Based on the outcome of the titrations, appropriate doses were selected for challenging immunized animals and controls (see below).

Preparation of vaccines

Live fresh vaccines: All the rough *S. dublin* strains used for vaccine production were grown on nutrient agar at 37 °C for 24 h and the growth suspended in tryptone water. The density was nephelometrically adjusted to contain approximately 1×10^9 or $2,5–3,0\times10^8$ bacteria/ml. From these suspensions 5-fold dilutions were made in tryptone water in order to obtain the desired dosage of bacteria for each particular experiment. In one experiment two combined vaccines were prepared from strains HB 1/17 and 5765. Equal numbers of each strain were mixed to give total doses of 1×10^8 and 5×10^7 live bacteria/mouse.

Formalin inactivated alum precipitated vaccine: The procedure followed was exactly as described by Cameron & Fuls (1974) except that *S. dublin* 2656V was used instead of *S. typhimurium*. The final vaccines contained either 0,1 % or 0,5 % packed cells.

Immunization of experimental animals

Mice: Groups of 24 mice were immunized with varying doses of live bacteria of a number of different strains or with inactivated vaccine. Doses of 0,2 ml of either dilutions of live vaccines or inactivated vaccine were administered subcutaneously. Depending on the requirements of a particular experiment a single injection or 2 injections at an interval of 14 days were given. When 2 injections were given, the 1st was given at 4 weeks of age and the 2nd at 6 weeks of age. For the live vaccines doses of approximately 1×10^8, 5×10^7, 1×10^7, 2×10^6, 4×10^5 and 8×10^4 live bacteria/mouse were employed.

Guinea-pigs: Groups of 8 or 12 guinea-pigs were used throughout and 1,0 ml of either live or inactivated vaccine was injected subcutaneously. For the live vaccines doses of either approximately 1×10^9 or 3×10^8 bacteria/guinea-pig were used. When 2 injections were used, they were given 14 days apart.

Challenge of experimental animals

Mice: Immunized mice were challenged 14 days after the last injection of vaccine. The number of animals in each group was reduced to 20 and 20 non-immunized control animals were challenged simultaneously. Ten mice in each group were challenged by injecting intraperitoneally approximately 5×10^7 *S. dublin* 2652V or approximately 3×10^6 *S. typhimurium* and 10 mice were challenged with approximately 1×10^7 *S. dublin* 2652V or approximately 6×10^5 *S. typhimurium* 2656V. Deaths were recorded for 14 days and the percentage protection calculated as outlined previously (Cameron & Fuls, 1974).

Guinea-pigs: Four or 6 animals were challenged by injecting intraperitoneally approximately $2,5\times10^8$ and 4 or 6 with approximately 5×10^7 live bacteria of either *S. dublin* or *S. typhimurium*. Deaths were recorded for 14 days.

RESULTS

Characterization of rough strains

Agglutination in acriflavine: The agglutinability of the strains in different concentrations of acriflavine is shown in Table 1. On the basis of these results strains HWS 51, HWS 17A, HB 1/17 and HB 2267 57X can be classified as rough, while strains 5765, 5565 K1, 6792 and 6785 cannot be distinguished from virulent ones.

Virulence for mice: The pathogenicity of various dosages of *S. dublin* strains injected intraperitoneally and subcutaneously into mice is shown in Table 2. None of the strains was completely avirulent and strains HWS 17A and 5565 K1 in particular killed numerous mice. A noteworthy observation is that strain 6792 was the least virulent despite the fact that it did not agglutinate in acriflavine.

Infectivity of challenge strain

Examples of titrations of *S. dublin* 2652V in mice and guinea-pigs are given in Tables 3a and 3b respectively.

In mice doses of approximately 5×10^7 and 1×10^7 bacteria of *S. dublin* 2652V given intraperitoneally resulted in an infection that killed them in 3–10 days. These 2 levels of exposure were used in all the immunity experiments in mice.

TABLE 1 Agglutination of *S. dublin* strains in acriflavine

Strain	Acriflavine concentration				
	1: 500	1: 1000	1: 2000	1: 5000	1: 10000
HWS 51	++++	++++	++	—	—
HWS 17A	++++	+++	+++	+	—
HB 1/17	++++	++++	+++	+	—
5765	—	—	—	—	—
5565 (K1)	—	—	—	—	—
6792	—	—	—	—	—
6785	—	—	—	—	—
HB 2267 57X	++++	++++	++++	++	—

++++ = Rapid coarse agglutination
+++ = Distinct agglutination
++ = Fine agglutination
+ = Slight agglutination
— = No agglutination

TABLE 2 Virulence of *S. dublin* strains for mice

Strain	Bacteria/ mouse	Intraperitoneally		Subcutaneously	
		Deaths/6 mice	Total deaths/ 18 mice	Deaths/6 mice	Total deaths/ 18 mice
HWS 51	2×10^8	6	11	4	6
	5×10^7	4		2	
	1×10^7	1		0	
HWS 17A	2×10^8	6	17	5	11
	5×10^7	5		5	
	1×10^7	6		1	
HB 1/17	2×10^8	6	6	2	7
	5×10^7	0		3	
	1×10^7	0		1	
5765	2×10^8	6	13	2	3
	5×10^7	4		1	
	1×10^7	3		0	
5565 K1	2×10^8	6	14	6	18
	5×10^7	6		6	
	1×10^7	2		6	
6792	2×10^8	0	3	1	3
	5×10^7	3		1	
	1×10^7	0		1	
6785	2×10^8	0	4	5	12
	5×10^7	4		5	
	1×10^7	0		2	
HB 2267 57X	2×10^8	5	5	0	0
	5×10^7	0		0	
	1×10^7	0		0	

TABLE 3a Titration of *S. dublin* 2652V in mice by the intraperitoneal route

Approximate dosage /mouse	Cumulative deaths/10 mice													
	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5×10^7	0	0	3	4	7	8	9	10						
1×10^7	0	0	0	2	3	4	6	7	9	10				
2×10^6	0	0	0	1	1	2	2	3	4	5	6	7	7	7
4×10^5	0	0	0	0	1	1	5	5	6	7	8	8	8	8

TABLE 3b Titration of infectivity *S. dublin* 2652V in guinea-pigs by the intraperitoneal route

Approximate dosage /guinea-pig	Cumulative deaths/6 guinea-pigs													
	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
$2{,}5 \times 10^8$	0	2	3	3	3	5	5	6						
1×10^8	0	0	0	0	0	0	2	4	4	4	4	4	4	4
5×10^7	0	0	0	0	0	2	4	6						
2×10^7	0	0	0	0	0	1	4	5	5	6				
1×10^7	0	0	0	0	0	0	0	0	1	3	3	5	5	5

The infectivity of different doses of *S. dublin* 2652V in guinea-pigs was more erratic. Nevertheless doses of approximately $2,5 \times 10^8$ and 5×10^7 bacteria consistently killed all experimental animals within 8–10 days. These challenge levels were consequently used in all the immunity experiments in guinea-pigs.

Immunogenicity of S. dublin strains in a live vaccine

Mice: The immunity induced in mice by various strains is shown in Table 4a.

The efficacy of the strains varied with the challenge and immunizing doses but the average percentage protection of all the strains at immunizing doses of approximately 1×10^7 and 2×10^6 was at least 57,5% (strain HWS 17A). The only exception was strain HB 2267 57X which only afforded 25% protection.

All the strains could not, however, be used in all the ensuing experiments: many had to be omitted for practical reasons. Strains HWS 17A and 5565 K1 were considered to be too virulent (Table 2) and strain 5565 K1 grows poorly, so they were discarded. The immunogenicity of the 4 remaining local strains, HB 1/17, 5765, 6792 and 6785, was very similar and an arbitrary decision was taken to employ Strains HB 1/17 and 5765 in further experiments in mice.

Guinea-pigs: As shown in Table 4b there was a marked variation in the results obtained in different experiments and it is difficult to draw definite conclusions. However, as in the case of mice, Strain HB 2267 57X is obviously a poor immunogen while Strains HB 1/17 and 5765 are good.

Attempted immunization against S. typhimurium infection with S. dublin *strains*

Mice: Despite repeated experiments, it is clear from the data shown in Table 5a that none of the *S. dublin* strains afforded any appreciable immunity to challenge with *S. typhimurium* 2656V. The only interesting finding is that while Strain 5765 produces a better immunity to *S. dublin* than Strain HB 1/17 (Table 4a), the reverse is true when the mice are challenged with *S. typhimurium*.

Guinea-pigs: The results in guinea-pigs (Table 5b) were equally poor. Under our experimental conditions Strains HWS 51, HB 1/17 and 5765 were almost equally ineffective and produced only 19–25% immunity to *S. typhimurium*.

Immunization with combined live S. dublin *vaccines*

Mice: Immunization with a combination of Strains HB 1/17 and 5765 was superior to either of the strains alone when the mice were challenged with *S. dublin* (Table 6a). Whereas Strains HB 1/17 and 5765 gave an immunity of 45% and 77,5% respectively, the combined vaccine protected 82,5% of mice. As found earlier the superiority of Strain 5765 over Strain HB 1/17 for mice is noteworthy (Table 4a).

TABLE 4a Comparison of the immunogenicity of *S. dublin* rough mutants for mice against infection with *S. dublin* 2652V

Strain	Approximate vaccine dose	%Protection to challenge with $\pm 5 \times 10^7$ bacteria	% Protection to challenge with $\pm 1 \times 10^7$ bacteria	Average % protection
HWS 51	1×10^7	80	80	70
	2×10^6	40	80	
	4×10^5	nt	nt	nt
	8×10^4	nt	nt	
HWS. 17A	1×10^7	20	20	57,5
	2×10^6	100	90	
	4×10^5	nt	nt	nt
	8×10^4	nt	nt	
HB 1/17	1×10^7	40	80	60
	2×10^6	60	60	
	4×10^5	40	60	25
	8×10^4	0	0	
5765	1×10^7	100	80	75
	2×10^6	60	60	
	4×10^5	40	60	45
	8×10^4	20	60	
5565 (K1)	1×10^7	80	80	85
	2×10^6	100	80	
	4×10^5	60	80	45
	8×10^4	20	20	
6792	1×10^7	60	60	60
	2×10^6	60	60	
	4×10^5	nt	nt	nt
	8×10^4	nt	nt	
6785	1×10^7	100	80	75
	2×10^6	40	80	
	4×10^5	nt	nt	nt
	8×10^4	nt	nt	
HB 2267 57 X	1×10^7	0	40	25
	2×10^6	0	60	
	4×10^5	0	80	30
	8×10^4	20	20	

nt = not tested

TABLE 4b Comparison of the immunogenicity of *S. dublin* rough strains for guinea-pigs

Vaccine strain	Experiment No.	Survivors/4 guinea-pigs to challenge with ± 2,5 × 10^8 bacteria	Survivors/4 guinea-pigs to challenge with ± 5 × 10^7 bacteria	Total survivors
HWS 51	1	0	2	$^4/_{16}$
	2	0	2	
HB 1/17	1	4	3	$^{18}/_{32}$
	2	1	4	
	3	2	2	
	4	0	2	
5765	1	4	4	$^{11}/_{32}$
	2	0	1	
	3	0	0	
	4	0	2	
5565	1	0	3	$^5/_{16}$
	2	0	2	
6792	1	1	4	$^5/_8$
6785	1	2	2	$^5/_8$
HB 2267 57X	1	0	0	$^0/_8$
Controls	1	0	0	$^0/_{32}$
	2	0	0	
	3	0	0	
	4	0	0	

All guinea-pigs were immunized with approximately 3×10^8 bacteria

TABLE 5a Protection of mice immunized with *S. dublin* strains and challenged with *S. typhimurium* 2656V

Vaccine strain	Experiment No.	Approximate dosage/mouse	% protection to challenge with ±3× 10^6 bacteria	% protection to challenge with ± 6 × 10^5 bacteria	Average % Protection
HWS 51	1	5 × 10^7	0	20	20
	2	5 × 10^7	40	30	
	3	5 × 10^7	40	10	
	4	5 × 10^7	20	0	
HB 1/17	1	5 × 10^7	80	70	28,75
	2	5 × 10^7	20	50	
	3	5 × 10^7	0	0	
	4	5 × 10^7	0	10	
	5	1 × 10^7	50	40	
5765	1	5 × 10^7	20	60	22,5
	2	5 × 10^7	20	40	
	3	5 × 10^7	10	20	
	4	5 × 10^7	0	10	
	5	1 × 10^7	20	10	

TABLE 5b Attempted immunization of guinea pigs with *S. dublin* rough mutants against challenge with *S. typhimurium* 2656V

Vaccine strain	Experiment No.	Survivors/4 guinea-pigs challenged with ±2,5 × 10^8 bacteria	Survivors/4 guinea-pigs challenged with ±5 × 10^7 bacteria	Total survivors
HWS 51	1	1	0	$^4/_{16}$
	2	2	1	
HB 1/17	1	0	4	$^8/_{32}$
	2	0	2	
	3	0	0	
	4	0	2	
5765	1	0	1	$^6/_{32}$
	2	0	1	
	3	1	2	
	4	0	1	
Controls	1	0	0	$^0/_{32}$
	2	0	0	
	3	0	0	
	4	0	0	

All guinea-pigs were immunized with ± 3 × 10^8 live bacteria subcutaneoulsy

TABLE 6a Immunization of mice with combined *S. dublin* live vaccines against challenge with *S. dublin* and *S. typhimurium*

Vaccine strains	Total vaccine dose/mouse	% protection against challenge with $\pm 5 \times 10^7$ *S. dublin* bacteria	% protection against challenge with $\pm 1 \times 10^7$ *S. dublin* bacteria	Average % protection against challenge with *S. dublin*	% protection against challenge with $\pm 3 \times 10^6$ *S. tm* bacteria	% protection against challenge with $\pm 6 \times 10^5$ *S. tm* bacteria	Average % protection against challenge with *S. tm*
HB 1/17	1×10^8	10	70	45	30	40	37,5
HB 1/17	5×10^7	30	70				
5765	1×10^8	70	90	77,5	60	10	37,5
5765	5×10^7	80	70		50	30	
Combined	1×10^8	50	100	82,5	40	30	37,5
Combined	5×10^7	80	100		50	30	

S. *tm* = *S. typhimurium*

TABLE 6b Immunization of guinea-pigs with combined *S. dublin* live vaccines against challenge with *S. dublin* and *S. typhimurium*

Vaccine strain	Survivors/6 guinea-pigs challenged with $\pm 2,5 \times 10^8$ *S. dublin* bacteria	Survivors/6 guinea-pigs challenged with $\pm 5 \times 10^7$ *S. dublin* bacteria	Total Survivors/12 guinea-pigs challenged with *S. dublin*	Survivors/6 guinea-pigs challenged with $\pm 2,5 \times 10^8$ *S. tm* bacteria	Survivors/6 guinea-pigs challenged with $\pm 5 \times 10^7$ *S. tm* bacteria	Total Survivors/12 guinea-pigs challenged with *S. tm*
HB 1/17	2	5	7	2	5	7
5765	0	1	1	1	4	5
Combined	2	5	7	0	3	3
Controls	0	0	0	0	0	0

All guinea-pigs were immunized with $\pm 1 \times 10^9$ live bacteria subcutaneously
S. tm = *S. typhimurium*

TABLE 7a Immunization of mice with formalin-inactivated *S. dublin* vaccine against challenge with *S. dublin* and *S. typhimurium*

Exp. No.	Vaccine concentration (% pcv)	No. of injections	% protection to challenge with $\pm 5 \times 10^7$ *S. dublin* bacteria	% Protection to challenge with $\pm 1 \times 10^7$ *S. dublin* bacteria	Average % protection to challenge with *S. dublin*	% protection to challenge with $\pm 3 \times 10^6$ *S. tm* bacteria	% protection to challenge with $\pm 6 \times 10^5$ *S. tm* bacteria	Average % protection to challenge with *S. tm*
1	0,1	2	20	0	10	nt	nt	—
2	0,5	1	50	60	75	50	50	60
3	0,5	1	100	80		50	80	
4	0,5	2	100	60		80	50	

S. tm = *S. typhimurium*

TABLE 7b Immunization of guinea-pigs with formalin-inactivated *S. dublin* vaccine against infection with *S. dublin* and *S. typhimurium*

Experiment No.	Vaccine concentration (% pcv)	No. of injections	Survivors/4 guinea-pigs challenged with $\pm 2,5 \times 10^8$ *S. dublin* bacteria	Survivors/4 guinea-pigs challenged with $\pm 5 \times 10^7$ *S. dublin* bacteria	Total survivors challenged with *S. dublin*	Survivors/4 guinea-pigs challenged with $\pm 2,5 \times 10^8$ *S. tm* bacteria	Survivors/4 guinea-pigs challenged with $\pm 5 \times 10^7$ *S. tm* bacteria	Total survivors challenged with *S. tm*
1	0,1	1	0	2	2/24	nt	nt	
2	0,5	1	0	0		nt	nt	
3	0,5	1	0	0		nt	nt	
4	0,1	2	0	0	2/16	0	0	0/16
5	0,5	1	0	2		0	0	
Controls	0	0	0	0	0	0	0	0

nt — not tested

Combination of the 2 above-mentioned strains had no beneficial effect when the mice were challenged with *S. typhimurium*. In fact all the groups showed an average protection of 37,5% (Table 6a).

Guinea-pigs: The results in guinea-pigs were essentially similar (Table 6b). As shown previously (Table 4b), Strain HB 1/17 was superior to Strain 5765 with respect to homologous challenge while they were essentially identical with respect to heterologous challenge (Table 5b). In neither instance was any advantage gained by combining the 2 strains.

Immunization with formalin-inactivated alum-precipitated vaccines

Mice: A vaccine containing 0,1% packed cells was ineffective, but a single injection of vaccine containing 0,5% packed cells consistently gave a good protection (Table 7a). The administration of 2 injections did not improve the degree of protection. Moreover, the inactivated vaccine also protected an average of 60% of the mice against challenge with *S. typhimurium*, which is appreciably superior to the 20–28,5% obtained with live vaccines (Table 5a).

Guinea-pigs: The results obtained with formalin inactivated vaccines in guinea-pigs were very poor (Table 7b). When immunized animals were challenged with *S. dublin*, a protection of less than 8% was recorded and no cross protection whatsoever could be demonstrated against *S. typhimurium*.

Discussion

Despite the availability and reported efficacy of *S. dublin* live vaccine (Botes, 1964), calf paratyphoid has remained a major problem (Cameron, unpublished data, 1974), especially in calf-rearing establishments where injudicious administration of antibiotics probably jeopardizes the immunogenicity of a live vaccine. Moreover, cases of paratyphoid are periodically encountered in calves under 2 weeks of age before they can be effectively immunized by means of a live vaccine. Previously it has been found that a safe live *S. typhimurium* vaccine could not be formulated (Cameron & Fuls, 1974) and this precluded the production of a combined live *S. dublin*/*S. typhimurium* vaccine. Attention was therefore directed towards either finding an *S. dublin* strain that also protects against *S. typhimurium* or formulating an effective inactivated vaccine.

A number of important observations were made relating to *Salmonella* immunity in general and *S. dublin* immunity in particular. It was found that certain strains of *S. dublin*, such as 5765, 6792 and 6785, do not agglutinate in acriflavine although they have a low virulence for mice. They are not, therefore, typical rough strains, such as Strains HB 1/17 and HWS 51, and cannot readily be distinguished from virulent strains. Consequently it would be difficult to employ them for routine vaccine production. On the other hand not all rough strains are immunogenic, e.g. Strain HB 2267 57X. Similar poorly immunogenic strains of *S. typhimurium* have been described previously (Cameron & Fuls, 1974).

Another interesting finding was the discrepancy in immunogenicity of Strains HB 1/17 and 5765 for mice and guinea-pigs. Whereas Strain 5765 was superior to Strain HB 1/17 in mice, the reverse was true in guinea-pigs. Conversely, although of a low order, Strain HB 1/17 produced a better cross immunity to *S. typhimurium* in mice than Strain 5765.

Furthermore, we were unable to obtain the same degree of cross immunity that was reported by Smith (1965) in mice. He demonstrated that his strain of *S. dublin* (HWS 51) produced a good cross protection to both *S. typhimurium* and *S. cholerae-suis*. We could not confirm these findings either with *S. dublin* strain HWS 51 or with our strains. Similarly Smith & Halls (1966) reported that Strain HWS 51 would protect guinea-pigs against *S. typhimurium* and *S. cholerae-suis*. Although our results were similar to theirs with respect to homologous challenge with *S. dublin* we were unable to demonstrate cross protection to challenge with *S. typhimurium*.

Our results with inactivated vaccines in mice agree with those of Henning (1953), who was also able to demonstrate a good immunity to homologous challenge. As in the case of live vaccines there was, however, a marked species variation. Whereas an inactivated *S. dublin* vaccine afforded a high level of immunity to both *S. dublin* and *S. typhimurium* in mice, it was ineffective in guinea-pigs. The results obtained in 1 species cannot, therefore, be extrapolated to another.

In the light of the finding that individual species vary in their response to *Salmonella* immunization a reassessment of the efficacy of both live and inactivated vaccines must be undertaken in calves.

References

BOTES, H. J. W., 1964. Calf paratyphoid immunity: Evaluation of formol-killed and live attenuated vaccines. *Bull. Off. int. Epiz.*, 62, 581–605.

CAMERON, C. M. & FULS, W. J. P., 1974. Comparative study on the immunogenicity of live and inactivated *Salmonella typhimurium* vaccines in mice. *Onderstepoort J. vet. Res.* 41,81–92.

CAMERON, C. M., FULS, W. J. P. & VAN REENEN, LUCILLE, 1972. Characterization of eight rough mutants of *Salmonella gallinarum*. *Onderstepoort J. vet. Res.*, 34, 139–146.

HENNING, M. W. 1953. Calf paratyphoid II. Artificial immunization. *Onderstepoort J. vet. Res.*, 26, 25–44.

SCHLECT, S. & WESTPHAL, O., 1966. Wachestum und lipopolysaccharid (O-Antigen)-gehalt von Salmonellen bei züchtung auf Agarnährböden. *Abl. Bakt. I. Orig.*, 200, 241–259.

SMITH, H. W., 1965. The immunization of mice, calves and pigs against *Salmonella dublin* and *S. cholerae-suis* infections. *J. Hyg. Camb.*, 63, 117–135.

SMITH, H. W. & HALLS, SHEILA, 1966. The immunity produced by a rough *Salmonella dublin* variant against *Salmonella typhimurium* and *Salmonella cholerae-suis* infection in guinea-pigs. *J. Hyg. Camb.*, 64, 357–359.

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