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Degree of Immunity Induced by Killed Vaccines to Experimental Salmonellosis in Mice

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Killed vaccines, deoxycholate-extracted or heated, were shown to induce an effective degree of immunity which protected against death (100%), prevented extensive multiplication, and left the mice with low residual salmonella populations in spleen and liver after intravenous (iv) or intraperitoneal (ip) challenge with virulent *Salmonella typhimurium*. Protection was most effective against the ip challenge route and less effective against the iv route. A study of the kinetics of the population of bacteria in the spleens and livers of immunized animals showed that after ip challenge there was an initial reduction of 99% at 6 hr after challenge, maintenance of levels of less than 10^3 bacteria per organ, and a final population of 10^2 to 10^3 per organ at 21 days. With iv challenge, after an initial reduction of 90% at 6 hr, growth ensued to levels above 10^6 bacteria per organ until 8 days, followed by a steady decline yielding residual populations of 10^3 to 10^4 in some cases. Organ hypertrophy correlated with bacterial population. Morbidity was prevented (as measured by gain in body weight) by immunization against ip challenge but not against iv challenge. Killed vaccines protected by their ability to induce an immune state which reduced the initial challenge population, prevented extensive multiplication, yet allowed "cellular immunity" to develop due to response to the living challenge infection itself. The consequence was a low-level carrier state similar to that induced by recovery from sublethal virulent infection.

The weight of evidence clearly shows that living vaccines in salmonella infections are more effective than killed vaccines (4). The type of immunity produced is predominantly cellular rather than humoral, although the precise role of the humoral factors in interaction with cellular factors has yet to be elucidated. Yet, with living vaccines, persistence of the vaccine strain appears to be required for the persistence of cellular immunity (4, 6), hence the immunized animal is actually a carrier. Although such an animal rapidly eliminates the challenge infection, it does not eliminate the vaccine strain at the same rate, even at the height of immunity. In fact, survivors of a virulent salmonella infection are for the most part persistent carriers, i.e., they do not sterilize their tissues (13). Such an immunity has been designated in the older literature as "infection immunity" (21) which persists as long as the infection and then is presumably lost.

The question arises: Can one expect a killed vaccine to give a higher degree of immunity than that which can be achieved by survival from a virulent, sublethal infection? Perhaps the goal of a tissue-sterilizing immunity is unattainable. In this report we present evidence for a lesser degree of immunity achievable by killed vaccines which, firstly, prevents the death of the animal, secondly, prevents extensive net multiplication of the bacteria, and thirdly, results in a residual population (carrier state) equivalent to or less than that found after sublethal, virulent infection of normal animals. Such a state is an enhanced resistance which enables the host to cope with the initial assault of virulent infection and which then allows the host to develop a state of further enhancement by the development of the "nonspecific" (15) cellular immunity resulting from the challenge infection itself.

This paper reports the levels of bacterial population in challenged mice which were previously immunized with various killed vaccines and describes an immune state which leaves the animals as residual carriers of very low populations, but essentially healthy in terms of body weight and organ hyperplasia, in some cases. The degree of protection induced by a vaccine preparation termed WCR, the whole-cell residue after extraction of living cells with 2% deoxycholate, is compared with heat-killed whole-cell vaccines. Protection was assessed by survival of the animals, their ability to clear or reduce the challenge

infection from their tissues, the degree of hypertrophy of reticuloendothelial organs (spleen and liver), and ability to gain weight during the observation period.

MATERIALS AND METHODS

Bacterial cultures. *Salmonella typhimurium* strain Suc LL has been described (12); its LD_{50} is approximately 1.0×10^4 by the ip route and 1.0×10^6 by the iv route. A second, more virulent strain, SR11 (12), was also employed. Its LD_{50} was approximately 5.0×10^2 ip and 2.0×10^5 iv.

Vaccines. The WCR vaccine previously described (3) consists of the residue after extraction of living cells with 2% deoxycholate in 0.2 M tris(hydroxymethyl)aminomethane (Tris) buffer, *p*H 8.0, at 37 C. Two preparations of heat-killed cells were also employed, one killed at 56 C for 2 hr, termed K56, and the other killed at 100 C for 1 hr, termed K100. Another preparation of WCR was made at a higher optical density of cells (1.0), with the hope of increasing the yield of the product, and at the higher temperature of 56 C; this was termed WCR56. The above vaccines were prepared from Suc LL. One other vaccine was prepared in the same manner as WCR56 but from the more virulent strain SR11, with the hope of increasing potency; it was termed WCR56S. Table 1 gives a summary of the vaccines employed. All vaccines were tested for sterility by plating on Trypticase-soy agar (Difco) and by subculturing heavy inocula in Trypticase soy broth (Difco). Vaccines were lyophilized, and weighed amounts were resuspended at the time of use in sterile saline.

Immunization and challenge. Female white Swiss mice (27 to 31 g) from the University of Hawaii colony were immunized by the ip route with 0.25 mg of vaccine per inoculation. Multiple inoculations were spaced 7 days apart. All immunized animals were challenged by the ip or iv route with 100 LD_{50} of either Suc LL or SR11 along with the appropriate control of nonimmunized mice.

Assessment of infection. The animals were observed for death for 21 days postchallenge. On day 21, surviving animals were killed and weighed. Liver and spleen were removed aseptically, weighed, and homogenized with sterile sand with a mortar and pestle. Appropriate dilutions were plated on freshly prepared SS agar (Difco), and colony counts were performed to determine the number of viable bacteria per organ. The plating efficiency of SS agar has been shown to be only slightly less than that of nonselective medium (~85%) with organ homogenates in this laboratory (*unpublished data*). Time course of infection was studied by sacrificing at intervals 9 to 10 randomly selected animals from larger groups which had been immunized and challenged identically.

TABLE 1. *Vaccines employed*

Vaccine	Conditions of preparation			Endotoxicity[c]
	OD[a]	DOC[b]	Temp (C)	
WCR	0.3	2%	37	−
WCR56	1.0	2%	56	−
WCR56S[d]	1.0	2%	56	−
K100	1.0	0	100	+
K56	1.0	0	56	+

[a] Optical density of cells during treatment procedure, measured at 600 nm on a Spectronic 20 spectrophotometer.

[b] Deoxycholate (2%) in 0.2 M Tris buffer, *p*H 8.0.

[c] +, LD_{50} of 1 to 2 mg; −, LD_{50} > 6.0 mg.

[d] Made from strain SR11; all others made from strain Suc LL.

RESULTS

Protection against death. WCR and K56 readily enabled animals to survive an ip challenge of 100 LD_{50} of either *Salmonella* strain, a dosage which killed all controls (Table 2). Only one immunizing inoculation was necessary to achieve such protection. The K56 vaccine was consistently more effective than WCR against iv challenge and protected fully against Suc LL challenge. The K100 vaccine was as effective in protection as the WRC and K56 vaccines which had received less drastic treatment. On the other hand, the WCR56 vaccine, whose treatment combined mild heat (56 C) and deoxycholate extraction, was much less effective. In an attempt to determine whether this was an effect of the virulence of the strain employed, SR11, which is more virulent than Suc LL, was employed as starting material. It was as ineffective as the WCR56 vaccine. It appears that the combination of heat with deoxycholate either extracted some of the immunizing antigen(s) or partially inactivated it. Evidence favoring the last statement lies in the response-to-dose effect with these two vaccines; thus, an insufficient dose may account for their relatively poor showing.

Full protection against iv challenge was only successful with the K56 vaccine against Suc LL, but a level of 78% was achieved by WCR (group B3). The K56 also protected well, 60 to 80% against the more virulent SR11 challenge (D groups). In general, the K56 vaccine was most effective, followed closely by WCR. K100 was not tested against iv challenges in this experiment, but it was tested in a later experiment (see Fig. 1).

Protection measured by visceral infection. The data in Table 3 are presented as both means and medians, since the variance in such data is very large due to the occasional aberrant animal. The least degree of infection, measured 21 days after challenge, was achieved against ip challenge with Suc LL by both WCR and K56 as shown by small residual bacterial populations, less than 10^3 viable salmonella left in both organs. Slightly higher populations were found after challenge with the more virulent strain SR11 (C groups). The heat-killed vaccine K100, while protecting

well against death, was not as effective as K56 or WCR in protecting against infection (Table 3, A groups).

The residual bacterial populations were usually higher after iv challenge (B groups) than after ip challenge, except that the K56 immunization was considerably more effective than WCR against Suc LL challenge. On the other hand,

TABLE 2. *Comparison of killed vaccines*

Group	No. of inoculations[a]	Challenge strain and route[b]	Survival postchallenge (%)[c] when vaccinated with					
			WCR	K56	K100	WCR56	WCR56S	None
A	1	Suc LL, ip	100	90	88	20	10	0
	2		100	100	100	50	50	0
	3		90	100	100	90	80	0
B	1	Suc LL, iv	50	100	—[d]	40	30	0
	2		60	100	—	40	40	0
	3		78	100	—	90	50	0
C	1	SR11, ip	100	100	—	20	30	0
	2		100	100	—	40	50	0
	3		100	89	—	10	70	0
D	1	SR11, iv	40	60	—	0	0	0
	2		40	70	—	0	20	0
	3		67	80	—	0	50	0

[a] Inoculations of 0.25 mg (dry weight) of vaccine, spaced 7 days apart when more than one was given.
[b] Challenges were 100 LD_{50}.
[c] Nine to 10 mice per group; observation period was 21 days.
[d] —, No data.

TABLE 3. *Comparison of killed vaccines: bacteria in organs of survivors 21 days postchallenge*

Group[a]	Log of mean no. of bacteria per organ (and log median) when vaccination was with				
	WCR	K56	K100	WCR56	WCR56S
Spleen					
A 1	2.6 (2.6)	2.4 (2.2)	2.9 (3.2)	3.6[b]	2.9[b]
2	2.1 (1.8)	2.2 (2.1)	3.5 (2.9)	3.2 (3.2)	2.9 (2.5)
3	1.9 (1.0)	2.4 (2.2)	3.4 (2.5)	2.7 (2.7)	2.9 (2.7)
B 1	4.8 (3.2)	2.7 (3.4)		>6.0[b]	3.3[b]
2	4.3 (3.3)	2.8 (3.3)		>6.0[b]	3.2[b]
3	5.2 (2.9)	2.5 (3.1)		>6.0 (3.8)	4.7 (3.4)
C 1	3.2 (3.2)	3.1 (2.9)		2.8[b]	>6.0[b]
2	3.1 (2.7)	3.0 (2.7)		3.7[b]	3.1 (3.1)
3	3.0 (2.4)	2.7 (2.7)		5.0[b]	3.2 (3.0)
D 1	>6.0[b]	>6.0 (4.2)		—[b]	—[b]
2	>6.0[b]	>6.0 (3.6)		—[b]	>5.0[b]
3	1.9 (2.9)	>6.0 (4.1)		—[b]	3.1[b]
Liver					
A 1	2.4 (1.9)	1.7 (0)	2.2 (1.6)	3.2 (1.6)[b]	2.4[b]
2	1.2 (0)	2.2 (2.0)	2.5 (1.1)	2.8 (2.9)	2.9 (2.1)
3	1.2 (0)	2.2 (1.9)	4.8 (3.7)	2.3 (2.1)	2.8 (2.4)
B 1	5.3 (3.1)	2.6 (2.5)		>6.0[b]	2.9[b]
2	5.0 (3.1)	2.8 (2.5)		3.5[b]	3.1[b]
3	2.5 (1.6)	2.7 (1.9)		>6.0 (3.8)	2.9 (2.9)
C 1	2.9 (2.8)	3.9 (2.9)		1.9[b]	>4.0[b]
2	5.4 (2.4)	3.0 (2.6)		3.6[b]	2.9 (2.7)
3	2.9 (1.9)	3.1 (2.8)		>6.0[b]	3.3 (2.9)
D 1	>6.0[b]	>6.0 (>6.0)		—[b]	
2	>6.0[b]	>6.0 (3.4)		—[b]	>5.0[b]
3	1.9 (1.6)	>6.0 (3.3)		—[b]	2.5[b]

[a] For immunization, challenge, and vaccine see Table 2.
[b] Survival less than 50%.

challenge with SR11 by the iv route (D groups) was not resisted except when three inoculations were given with WCR. The WCR56 and WCR56S vaccines were unsuccessful in regard to infection of survivors except in group A.

In general, where protection was achieved by any vaccine, a trend toward dose-response was evident; i.e., multiple inoculations left smaller residual populations than single inoculations, and SR11 left slightly higher populations, by ~ 1 log, than Suc LL.

Hypertrophy of organs. Normal spleens in this mouse strain averaged $0.53 \pm 0.06\%$ and normal livers averaged $5.9 \pm 0.2\%$ of body weight. In no case had the organs returned to normal size by 21 days after challenge (Table 4). However, in general, the organs with low bacterial populations were smaller, suggesting an approach to normal, e.g., A3 group with WCR and K56.

Course of infection over a 21-day period in immunized animals. The question arose as to the kinetics of the in vivo bacterial population during the period of observation. Were the animals resistant at the time of challenge or did they develop the resistance in a combination with the secondary stimulus of the challenge infection? The following experiment was designed to study the time course of the infection in immunized animals.

Animals were immunized by three inoculations with one of three vaccines (WCR, K56, or K100) and challenged ip or iv with Suc LL, and groups of seven or eight animals were sacrificed at intervals, up to 21 days, and assessed for infection. The in vivo bacterial populations are shown in Fig. 1. In all three immunization procedures, the bacterial populations in both spleen and liver were held at the initial 6-hr level after ip challenge throughout the 21-day period, with a trend toward elimination after 18 days. At no time did the median population in either organ rise above 10^3.

After iv challenge there was an initial rise in numbers during the first 4 days to a high level of 10^6 and greater per organ but with no deaths, followed by a progressive decrease starting at day 8 until levels approaching the ip residual population were reached. Again, a trend toward elimination was evident. This curve is similar to that of response of mice to an LD_{50} infection (12).

The results are further corroborated by data on the size of liver and spleen (Fig. 2). These curves parallel the bacterial populations, with slight maxima at day 8 followed by a trend to return to normal in ip-challenged animals. On the other hand, the organs of iv-challenged mice showed a peak of hypertrophy ($\sim 5 \times$ normal for spleens and $\sim 2 \times$ normal for liver) 8 days after challenge, with a subsequent rapid trend toward normality which was not quite attained by 21 days.

Illness as evidenced by body weight. Immunized animals challenged ip tended to maintain body weight, as compared to normal uninoculated animals, although only the group immunized with WCR actually gained weight as the normal animals did, commencing 12 days postchallenge. These animals were essentially not ill and ate and drank as did normal animals. After iv challenge, the animals lost weight extensively as compared to the normal uninoculated controls during the first 12 days but started showing return to normal by 21 days. Thus, they were ill during the first 2 weeks and progressively recovered in the third

TABLE 4. *Comparison of killed vaccines: mean organ weights (per cent total body weight) of survivors, 21 days after challenge*

Group[a]	Spleen wt (% total body wt)			Liver wt (% total body wt)		
	WCR	K56	K100	WCR	K56	K100
A 1	1.17 ± 0.11	1.23 ± 0.14	2.59 ± 0.22	7.3 ± 0.21	6.8 ± 0.44	6.27 ± 0.29
2	0.77 ± 0.13	1.13 ± 0.07	2.40 ± 0.09	6.2 ± 0.18	6.6 ± 0.30	6.8 ± 0.19
3	0.65 ± 0.06	0.84 ± 0.06	2.39 ± 0.15	5.7 ± 0.30	6.4 ± 0.19	6.5 ± 0.26
B 1	2.33 ± 0.38	1.63 ± 0.12	—[b]	8.4 ± 0.03	8.0 ± 0.32	—
2	2.31 ± 0.32	1.79 ± 0.10	—	7.9 ± 0.57	7.0 ± 0.25	—
3	1.59 ± 0.22	1.14 ± 0.12	—	7.0 ± 0.61	7.4 ± 0.25	—
C 1	1.32 ± 0.13	1.42 ± 0.12	—	7.7 ± 0.28	7.0 ± 0.23	—
2	0.92 ± 0.10	0.90 ± 0.12	—	6.7 ± 0.36	6.9 ± 0.22	—
3	1.11 ± 0.15	0.76 ± 0.07	—	7.4 ± 0.32	6.7 ± 0.18	—
D 1		1.78 ± 0.14	—	—	9.4 ± 0.77	—
2		1.99 ± 0.28	—	—	8.1 ± 0.58	—
3	1.17 ± 0.09	2.10 ± 0.26	—	6.8 ± 0.24	9.0 ± 0.74	—

[a] For immunization, challenge, and vaccine see Table 2.
[b] —, No data or survival less than 50%

week as shown by return to more normal feeding habits (Fig. 3).

State of immunity at the time of challenge. Regardless of the vaccine, animals showed a high degree of immunity in terms of reduction of the challenge 6 hr after ip challenge (Table 5). Either very few organisms reached the reticulo-endothelial cells of spleen and liver (i.e., were sequestered in the peritoneal cavity) or very few organisms survived the 6-hr period. After iv challenge, organisms remained in the blood, but the uptake by phagocytic cells of the organ was considerable. Yet a sequestering is evident in comparison with the control animals, whose tissue

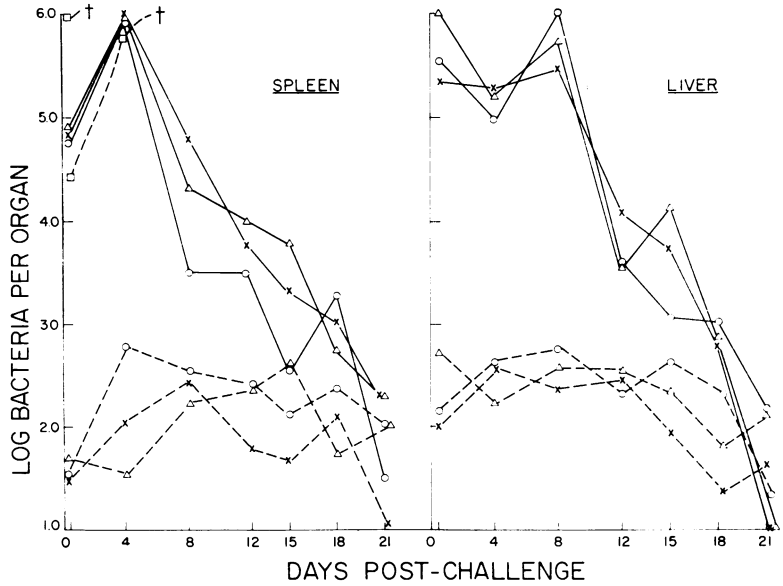

FIG. 1. *Bacterial populations in organs of mice immunized with WCR* (×), *K56* (○), *or K100* (△) *after intravenous (solid line) or intraperitoneal (broken line) challenge with S. typhimurium. Nonimmunized mice* (□); *death of all animals in group* (†). *Each point represents the mean of determinations for seven or eight mice.*

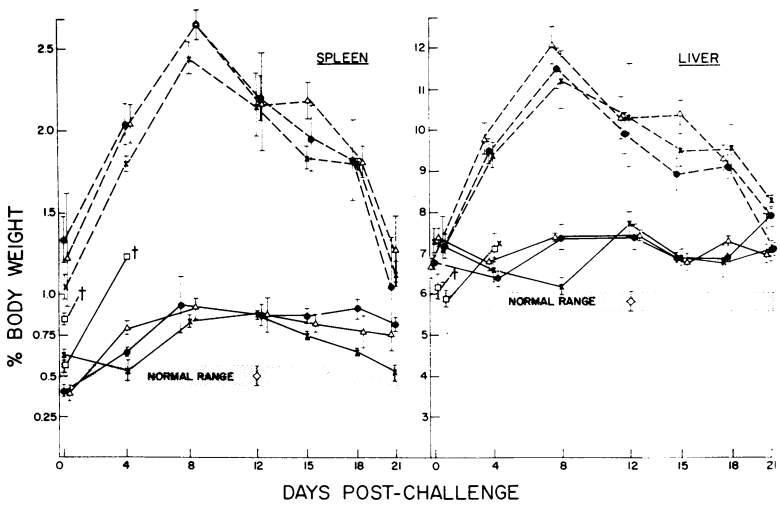

FIG. 2. *Size of spleen and liver, expressed as per cent body weight, of mice immunized with three vaccines after intravenous or intraperitoneal challenge with S. typhimurium. (See Fig. 1 for symbols.) Normal range is that of normal, nonimmunized and nonchallenged animals, maintained under similar conditions, originally randomly selected from the same batch as the test animals.*

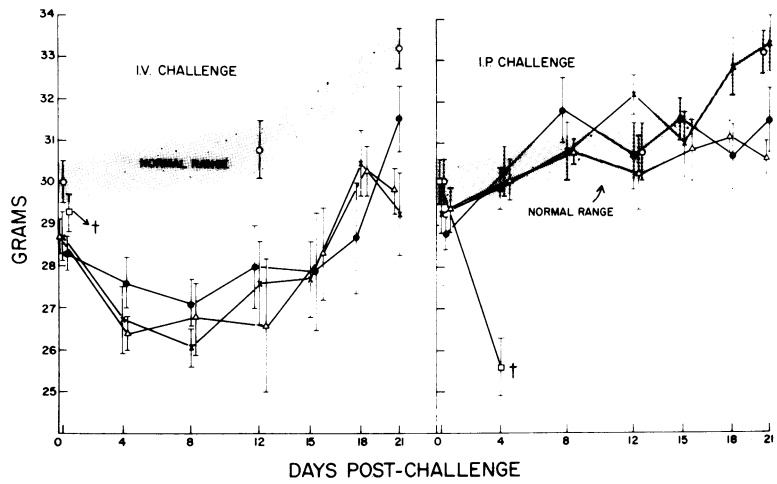

FIG. 3. *Body weight of immunized mice after challenge with S. typhimurium. (See Fig. 1 and 2 for symbols.)*

TABLE 5. *Initial populations of bacteria in organs of immunized animals 6 hr after challenge*

Oragan	Immunizing agent	Bacteria per organ		Per cent of control[a]	Bacteria in blood	
		Mean	Median		Positive culture/total	Average score
Spleen (ip challenge)	WCR	46	34	0.13	0/8	0
	K56	196	33	0.13	0/8	0
	K100	571	50	0.19	0/8	0
	None	29,089	26,000	100	8/8	3.9+
Liver (ip challenge)	WCR	111	100	0.38		
	W	196	143	0.55		
	H	559	503	2.04		
	None	27,730	26,000	100		
Spleen (iv challenge)	WCR	49,025	68,500	6.9	6/8	0.75+
	K56	70,267	48,000	5.8	8/8	1.0+
	K100	87,165	80,000	8.0	8/8	1.0+
	None	$>10^6$	$>10^6$	100	8/8	2.4+
Liver (iv challenge)	WCR	224,846	215,000	22		
	W	318,599	358,000	36		
	H	$>10^6$	$>10^6$	100		
	None	$>10^6$	$>10^6$	100		

[a] Based on median.
[b] Scored as 4+ to 0 on viable count of one drop of heart blood.

populations were 12- to 20-fold higher. The effects are probably due to a combination of opsonization, phagocytosis, and considerable kill of the bacteria in the reticuloendothelial system of immune animals.

DISCUSSION

These experiments demonstrate that it is relatively easy to protect animals against death due to experimentally induced parenteral challenge by immunization with either WCR or heat-killed vaccines. These findings confirm our previous reports (3, 14) and those of others (18, 20) on nonliving vaccines. Protection against ip challenge is more effective than protection against iv challenge, and the WCR vaccine, which lacks endotoxic activity (3), measures up well compared to the heat-killed preparations which con-

tain endotoxin, but there was essentially no great difference among the three preparations.

Collins, MacKaness, and Blanden (6) point out that, in addition to survival, the criterion of clearance of the bacteria from the tissue is of utmost importance in assessing immunity. In agreement with them, we have assessed this parameter in these experiments, finding a reduction in levels of infection within the observation period. The preliminary report (3) that three inoculations of WCR protected completely against infection was not borne out. In no case were such animals able to sterilize their tissues completely, but the level of bacteria at 21 days of infection was extremely low.

Attempts to increase the yield of preparation of the WCR vaccine by increasing the numbers of bacteria 3.3-fold (from optical density 0.3 to optical density 1.0) and enhancing kill by extracting at 56 C rather than 37 C yielded poor immunogens (WCR56, WCR56S) on a weight basis when compared with WCR in terms of either protection or residual in vivo population. These data are included for comparison with the more effective vaccines to show that the immunogen is critical and that one is not dealing with a host population which is readily immunized.

The organ weights correlated with the residual bacterial populations and although none had returned to normal by day 21 they had almost attained that level when the animals had been immunized with three inoculations of WCR after ip challenge. In general, the organ weights of WCR-immunized mice were lower than those of mice immunized with heat-killed vaccines.

The behavior of the in vivo populations during the 21-day observation period brings out some interesting points. After ip challenge, the initial visceral populations (6 hr postchallenge) were greatly reduced, yielding populations of a fraction of 1% of those found in the controls. These populations were lower than those reported by Collins (5), and they confirm his finding that there is a drastic reduction of the original population in immune animals. The reduction was not as dramatic after iv challenge, but the effect was still demonstrable. After ip challenge, the population remained at low levels, fluctuating below log 3.0 in both liver and spleen with a tendency downward after day 12. These figures are below the range of residual population which Hobson (13) found in survivors of a virulent infection. After iv challenge, an initial rise occurred in the organs from day 4 to day 8, followed by a steady decline to relatively low levels by day 21. Apparently, the immunized animals had a sufficient initial immunity to suppress the multiplication of the original inocula below the lethal level, as discussed above, and at about the eighth day onward exhibited the developing immunity due to the challenge infection itself. This was especially dramatic after iv challenge, probably because of the greater stimulation of the immune system by this systemic route. The organ weights paralleled the bacterial populations and reflected the tendency toward recovery.

As an index of illness, the body weight data are most instructive. After ip challenge the WCR-immunized animals gained weight at essentially the same rate as normal uninfected animals. The animals immunized with heat-killed cells differed from the normal animals only toward the end of the observation period. As judged by using body weight as an index of illness, probably a reflection of feeding activity, these animals were not ill; hence, there was protection against morbidity as well as mortality. The effects of iv challenge were quite different. The immunized animals exhibited a profound weight loss during the first 12 days after challenge, showing that there was no protection against morbidity after this severe route of challenge.

Immunization with killed vaccines does afford a practical level of immunity in that it provides the animal with sufficient resistance to reduce the original challenge inoculum and subsequently to suppress net multiplication, maintaining the population below the lethal level, or 10^9 to 10^{10} per mouse (19). This can be done to a degree adequate to prevent the clinical illness, as shown by the normal weight gain, after ip but not iv challenge. Intraperitoneal challenge probably is more analogous to natural infection, which must pass lymphoid and epithelial barriers, than is iv challenge, which is an unnatural route and a severe test of immunity. It can be hypothesized that after initial suppression, the presence of the living challenge organisms further allows the development of cellular immunity, which further suppresses the population (as was especially evident in the iv population curve), leaving the animal with a residual population similar to the recovered carrier state. This again points up the role of antibody, functioning as an opsonin, in the immunized animal at the initial stage of infection and subsequently at higher levels during the secondary response induced by the challenge (14). The importance of opsonin in survival has been recently re-emphasized by Ornellas et al. (17).

The results reported in this work differ from those of Collins (5) who show progressive proliferation of bacteria in animals immunized with killed vaccines in the first 8 to 10 days of infection. The discrepancy can be explained by the model system which has been chosen. Admittedly,

the degree of virulence of the salmonella, the degree of natural resistance, and the degree of immunizability of the mouse strain can be varied within wide limits. As has been repeatedly demonstrated by the work of Gowen (8, 9) and others (10), one can, by manipulation of these parameters, set up an almost infinite set of variables to yield great differences in resistance or immunizability. Investigators should justify the model system chosen for the experimental work in some terms relating to the real world. In nature, salmonellosis encounters relatively resistant hosts in animals and man. For the most part, salmonella septicemias or enteric fevers are relatively rare in spite of a high rate of gastroenteritis (1). Typhoid fever, caused by a host-specific pathogen in man, was highly infectious when conditions were more conducive to its transmission, nevertheless its case fatality rate was relatively low, even in its heyday before the advent of antibiotics. Kliegler and Bachi reported mortality rates varying from 3 to 8.9% in the preantibiotic era (quoted in reference 16), and Wilson and Miles (21) present data showing case fatality rates varying from 6 to 23% between the ages of 10 and 60 years. In such terms, man is a fairly resistant animal to typhoid, especially since such figures are based on morbidity during outbreaks and fail to account for the true base which would include subclinical infections. If one chooses the "artificial" white mouse or inbred strains, it is possible to find hosts which are susceptible to very low numbers of bacteria administered by the parenteral route, e.g., LD_{50} values of 10 to 100. Although the bacterial strains employed in this laboratory exhibit LD_{50} values of 10^3 to 10^4 with this strain of mouse and so would not be classified as highly virulent, a case can be advanced that this model system is perhaps more realistic in terms of assessing a protective vaccine. In man, killed vaccines have been shown to be efficacious in controlled mass trials in reducing morbidity (7, 11, 22). Undoubtedly, better vaccines can be developed, especially vaccines similar to WCR employed in this study, which would have a reduced endotoxicity yet would be immunogenic, so that dose would not be limited by the essential toxicity of the vaccine.

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