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Inhibition of colonization of the chicken alimentary tract with *Salmonella typhimurium* gram-negative facultatively anaerobic bacteria

By P. A. BARROW, J. F. TUCKER and J. M. SIMPSON
Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA, UK

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SUMMARY

Oral administration of strains of food poisoning salmonellas to day-old chickens produced a profound inhibition in the subsequent colonization of the caeca by a strain of *Salmonella typhimurium* given one day later. Closely related genera were unable to produce a similar inhibition. The inhibition was not the result of bacteriophages produced by the first strain. Neither was it the result of an immunological response by the host induced by the first strain. In additional experiments in day-old chickens, inhibition of an *Escherichia coli* Nal^r strain and of a *Citrobacter* sp. Nal^r strain was produced by the antibiotic-sensitive forms of the homologous strains while strains from other genera did not produce any inhibition. When an avirulent mutant of *S. typhimurium* was used for pre-treatment a statistically significant reduction in the excretion of the super-infecting *S. typhimurium* Nal^r strain over several weeks was produced. A genus specific inhibition was reproduced *in vitro* by mixed culture experiments. Live cultures were necessary for *in vitro* inhibition. Killed cells or a culture supernatant produced no inhibition.

INTRODUCTION

Oral administration of a suspension of caecal contents or faeces obtained from adult chickens to newly hatched chicks increases their resistance to oral infection by food-poisoning salmonellas to a level associated with adult chickens. This phenomenon has become known as competitive-exclusion and has been demonstrated many times (Nurmi & Rantala, 1973; Lloyd, Cumming & Kent, 1977; Snoeyenbos, Weinack & Smyser, 1978; Barnes, Impey & Cooper, 1980; Dorn & Krabisch, 1981).

One of the problems inherent in the use of such preparations on a commercial scale is the risk of inadvertent simultaneous inoculation with other avian pathogens present in the alimentary tract of the chickens from which the intestinal contents were obtained. This can be overcome by using mixtures of pure cultures of microorganisms obtained from the chicken alimentary tract (Barnes, Impey & Cooper, 1980; Impey, Mead & George, 1982).

The difficulties in preparing and using such mixtures, containing both facultative

and obligate anaerobes suggest that smaller pools or individual protective organisms would be an advantage if they could be found. Soerjadi, Lloyd & Cumming (1978) reported that a strain of *Streptococcus faecalis* was protective although the effect could not be reproduced by other workers (Goren, 1982; Pivnick & Blanchfield, 1982). Rigby, Pettit & Robertson (1977) reported that an unidentified strain of *Clostridium* was also effective. Barrow & Tucker (1986) isolated three strains of *Escherichia coli* which, when administered simultaneously, were partially protective over a period of several weeks. These authors were not able to find a single strain of Gram-negative facultative anaerobe which was protective when dosed on its own. It was considered that for a single strain to be effective in this way it (*a*) must possess the colonization characteristics of salmonella and (*b*) not possess the virulence characteristics of salmonella.

The present study arose from experiments on intestinal colonization of chickens by food poisoning salmonellas in which a very low excretor rate was observed in a group of 4-day-old chickens inoculated orally with a strain of *Salmonella typhimurium*. This appeared to be related to the presence in the chickens of a natural intestinal infection with *S. montevideo*. The chickens had become infected by the ingestion of *S. montevideo* contaminated food.

Similar results were obtained in controlled experiments in which chickens were infected with a strain of *S. typhimurium* resistant to spectinomycin when 1 day old and reinfected with a nalidixic acid resistant mutant of the same strain when 4 days old. Twenty-four hours following the second infection these chickens had ceased to excrete the super-infecting strain, whereas the chickens in a control group inoculated with only the nalidixic acid resistant mutant continued to excrete large numbers of salmonellas for 3–4 weeks. The ability of one salmonella strain to inhibit the intestinal colonization by another was therefore examined in more detail and its practical significance in reducing salmonella infection in poultry was assessed.

MATERIALS AND METHODS

Chickens

All were Light Sussex chickens bred from a specified pathogen-free flock maintained at this station. They were unsexed. Their rearing conditions and diet have been described elsewhere (Smith & Tucker, 1975).

Bacteria

All bacterial strains were maintained on Dorset egg slopes at 4 °C.

Broth cultures were made in 10 ml nutrient broth (Oxoid CM67), incubated at 37 °C for 24 h in a shaking water bath and usually produced viable counts of approximately 10^9 c.f.u./ml. In all experiments the challenge organism was a spontaneous mutant resistant to nalidixic acid (Nal^r) of either *S. typhimurium*, strain F98 of phage type 14(F98), *E. coli* H99 (non-typable) or *Citrobacter* sp. C3.

In vivo *inhibition of* Salmonella typhimurium *F98 Nal*^{r} *by bacterial strains and preparations*

The method used was that described by Barrow & Tucker (1986) in which 0·1 ml of the preparation or the undiluted broth culture to be tested was inoculated orally

into the crop of a group of 10 chickens within 24 h of hatching and immediately before they were given access to food. Twenty four hours later they were inoculated orally with 0·1 ml of a broth culture of the challenge strain diluted to contain 10^5 organisms. Three days later the chickens were killed and viable counts were performed on their caecal contents.

Viable counts were carried out by the method of Miles & Misra (1938) using, as a solid medium, Brilliant Green agar (Oxoid CM263) containing nalidixic acid (20 μg/ml) and novobiocin (1 μg/ml).

Preparation of killed bacterial suspensions and of broth culture supernatants

Three 500 ml Roux flasks of nutrient agar were inoculated with a spectinomycin resistant mutant (Spc^r) of *S. typhimurium* F98 and incubated at 37 °C. The growth from each was harvested and suspended in 10 ml volumes of nutrient broth. One of these was heated at 60 °C for 30 min, 0·1 ml of formalin was added to the second and the third was centrifuged and the pellet resuspended in alcohol. The last two preparations were left at room temperature for 4 h and at 4 °C overnight. They were centrifuged and resuspended in nutrient broth.

A broth culture of F98 Spc^r was centrifuged at 2500 ***g*** for 30 min and the supernatant fluid was passed through a millipore filter, pore size 0·45 μm. A second broth culture was sonicated for 5 min, centrifuged and the supernatant filtered.

Isolation of bacteriophage from feed and preparation of phage resistant mutant of S. typhimurium *F98 Nal^r*

Samples of feed (50 g) were mixed with 30 g nutrient broth powder in 2 l distilled water. This was mixed well to dissolve the nutrient broth and 0·5 ml of a broth culture of F98 was added. After static incubation overnight at 37 °C, 10 ml was withdrawn and centrifuged at 1500 ***g*** for 30 min. The supernatant was decanted and heated at 58 °C for 30 min. This was spotted onto a lawn of 1–10 diluted broth culture of F98 on Tryptose agar (Difco B64). Any phage plaques were purified on a lawn of F98 by picking and mixing a single plaque with a small volume of a broth culture of F98 on a plate of Tryptose agar. The plate was incubated until discrete plaques were visible. A single plaque was picked and replated with F98 and the process repeated twice. A single plaque and some of the surrounding bacterial growth was picked and incubated in nutrient broth until lysis was detected. The preparation was then heated at 58 °C for 30 min and stored at 4 °C until use.

A phage resistant mutant of F98 Nal^r was prepared by flooding nutrient agar plates with mixtures of F98 Nal^r and sufficient phage to produce confluent lysis after incubation. Colonies that grew on plates after further incubation were purified and their phage resistance confirmed.

The inhibitory effect of a rough avirulent strain of S. typhimurium *on faecal excretion of* S. typhimurium *F98 Nal^r by chickens*

Groups of 30 chickens were inoculated before 24 h of age with 0·1 ml of an undiluted broth culture of a somatic antigen minus (rough) mutant of *S. typhimurium* F98. They were then inoculated 24 h later with 10^5 organisms of *S. typhimurium* F98 Nal^r. At weekly intervals cloacal swabs were taken and cultured for salmonella organisms as described by Barrow & Tucker (1986).

Mixed culture experiments

The bacterial strain to be tested for inhibitory activity was incubated for 24 h in nutrient broth. To this culture was added 0·1 ml of a 10^{-4} dilution of a culture of *S. typhimurium* F98 Nal^r. This was incubated further at 37 °C in a shaking water bath and viable counts of the antibiotic resistant mutant were made at intervals thereafter on MacConkey agar containing nalidixic acid.

Virulence of Salmonella typhimurium *mutant in chickens*

Undiluted broth cultures of the rough mutant of *S. typhimurium* and of the parent strain were inoculated in 0·1 ml amounts orally into the crop of groups of 20 chickens within 24 h of hatching. The level of mortality was observed.

RESULTS

In vivo inhibition of S. typhimurium *by bacteria from closely related genera*

Different salmonella serotypes and other bacteria from closely related taxa (Table 1) were assessed for their protective effect after oral inoculation against colonization of the caeca by *S. typhimurium* F98 Nal^r. Compared with the control groups of chickens not pre-inoculated with one of the bacteria tested the only effective inhibition was obtained with salmonella strains. Even amongst these some were more effective than others, the most inhibitory serotypes being *S. typhimurium*, *S. hadar* and *S. anatum*. Inhibition by other serotypes did not appear to be as good since the highest count in the range was sometimes greater than 10^6 per gram of caecal contents. Very good inhibition was also obtained by the parent strain and a rough mutant of *S. typhimurium* F98. The two *S. arizonae* strains and one strain of *S. cholerae-suis* were much less inhibitory for F98 Nal^r.

Amongst the bacteria from other closely related genera none produced an inhibitory effect at all comparable with that produced by most of the salmonella strains. The median counts of F98 Nal^r in the chickens pre-inoculated with all the strains from the taxa examined, including *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, *Providencia* and *Erwinia* were all greater than 10^6 per gram. The citrobacter strains produced some inhibition since the lowest counts of F98 Nal^r in the range were 10^2 per gram or less.

In vivo *inhibition of* Escherichia coli *and* Citrobacter *by bacteria from closely related genera*

A smaller selection of bacteria from the Enterobacteriaceae and also a strain of *Staphylococcus aureus* were assessed for their protective effect in chickens against colonization of the caeca by *E. coli* H99 Nal^r and *Citrobacter* sp. C3 Nal^r (Table 2).

In control groups which were not pre-inoculated with other bacteria the *E. coli* H99 Nal^r strain was present in high numbers (median counts, $\log_{10}$ 8·2, 7·6) while numbers of the *Citrobacter* sp. C3 Nal^r strain were high in one group ($\log_{10}$ 8·0) and somewhat lower in the other ($\log_{10}$ 6·0), one of the chickens in the latter group having no detectable *Citrobacter* organisms in the caeca. In the chickens challenged with *E. coli* H99 Nal^r the only strain to produce a large reduction in the caecal count

Table 1. *The numbers of organisms of* Salmonella typhimurium *F98 in the caecal contents of chickens given this strain one day after inoculation with other closely related bacteria*

Organism tested	Log_{10} no. of viable organisms of F98 Nal^r per g caecal contents of chickens	Organism tested	Log_{10} no. of viable organisms of F98 Nal^r per g caecal contents of 10 chickens
S. typhimurium (1,4,5,12:i:1,2) phage type		*E. coli*	
49	< 2·0 (< 2·0–2·0)*	P20	7·7 (4·7–8·4)*
44	< 2·0 (< 2·0–2·0)	P40	7·6 (2·7–8·4)
40	< 2·0 (< 2·0–2·0)	P80	8·2 (6·6–8·5)
20 (strain 1)	< 2·0 (< 2·0–2·0)	P134	7·5 (4·4–8·4)
20 (strain 2)	< 2·0 (< 2·0–2·0)	M13	7·6 (5·1–8·1)
1	< 2·0 (< 2·0–2·0)	M18	7·8 (< 2·0–8·1)
36	< 2·0 (< 2·0–2·0)	B62	7·6 (2·0–8·5)
104	< 2·0 (< 2·0–2·0)	S6	6·4 (4·3–8·2)
14 (F98)	< 2·0 (< 2·0–2·0)	H99	7·0 (5·6–8·3)
14 (F98) (rough form)	< 2·0 (< 2·0–2·0)	*Citrobacter* sp.	
S. hadar (6,7:z_{10}:e,n,x)	< 2·0 (< 2·0–2·0)	C1	8·0 (< 2·0–9·0)
S. anatum (3,10:e,h:1,6)	< 2·0 (< 2·0–2·0)	C2	7·6 (2·0–8·2)
S. enteritidis (1,9,12:g,m:−)	< 2·0 (< 2·0–2·9)	C3	6·2 (< 2·0–8·6)
S. montevideo (6,7:g.m,s:−)	< 2·0 (< 2·0–3·1)	C5	7·4 (< 2·0–8·5)
S. newport (6,8:e,h:1,2)	< 2·0 (< 2·0–3·6)	C6	7·0 (< 2·0–9·3)
S. agona (1,4,12:f,g,s:−)	< 2·0 (< 2·0–6·1)	*Enterobacter* sp.	8·1 (7·3–8·8)
S. heidelberg (1,4,5,12:r:1,2)	< 2·0 (< 2·0–6·5)	*Klebsiella pneumoniae*	7·4 (5·0–8·0)
S. oranienburg (6,7:m,t:−)	< 2·0 (< 2·0–6·6)	*Proteus mirabilis*	6·7 (< 2·0–7·8)
S. dublin (1,9,12:g,p:−)	< 2·0 (< 2·0–7·2)	*P. rettgeri*	6·9 (2·7–8·7)
S. arizonae		*P. morganii*	8·1 (6·7–8·5)
Strain 1 (unknown)	2·3 (< 2·0–7·2)	*Providencia alcaliquefaciens*	7·7 (2·5–8·4)
Strain 2 (unknown)	3·3 (< 2·0–7·1)	*Erwina* sp.	7·3 (4·2–8·1)
S. cholerae-suis (6,7:c:1,5)	4·9 (< 2·0–8·1)	Nothing	6·9 (4·7–8·0)
Nothing	7·9 (< 2·0–8·8)	Nothing	7·6 (2·3–8·3)
Nothing	7·9 (< 2·0–8·2)	Nothing	8·2 (6·5–8·7)

* The median count is given followed by the range in parentheses.

Table 2. *The numbers of organisms of* Escherichia coli *H99 Nal^r or* Citrobacter *sp. Nal^r in the caecal contents of chickens given either of these strains after inoculation with other bacteria*

Organisms tested	Log_{10} number of viable organisms of the following organisms per gram of caecal contents of chickens	
	E. coli H99 Nal^r	*Citrobacter* sp. C3 Nal^r
Nothing	8·2 (6·6–8·7)*	8·0 (6·0–8·5)*
Nothing	7·6 (7·0–8·4)	6·0 (< 2·0–7·2)
E. coli H99	3·7 (2·7–5·3)	8·4 (6·7–8·9)
Citrobacter sp.	7·9 (5·9–8·7)	< 2·0 (< 2·0–3·6)
S. typhimurium phage type 32	7·7 (7·0–8·3)	7·3 (< 2·0–8·3)
Shigella flexneri	8·6 (6·4–8·9)	6·7 (4·5–8·7)
Staphylococcus aureus	7·7 (7·0–8·8)	8·3 (< 2·0–8·9)

* The median count is given followed by the range in parentheses.

of the challenge strain was the sensitive form of *E. coli* H99. The median count of this group was (log_{10}) 3·7. In the chickens challenged with *Citrobacter* sp. C3 Nal^r the only strain that produced a great reduction in the caecal count of this strain was the sensitive form of the citrobacter. In this case the counts of the challenge strain were very low (median count log_{10} < 2). None of the other organisms produced any inhibition.

In this experiment two additional challenge organisms were used. These were *Shigella flexneri* 59 Nal^r and a tetracycline resistant mutant of a strain of *Staphylococcus aureus*. In the latter case bacterial counts were made on MacConkey containing 25 μg/ml tetracycline. No growth of either of these organisms was demonstrable in the caecal contents of any of the chickens inoculated.

In vivo *inhibition of faecal excretion of* S. typhimurium *F98 Nal^r by an antibiotic sensitive, avirulent rough mutant of this strain*

The effect of pretreatment with an avirulent rough mutant of *S. typhimurium* F98 on the faecal excretion of *S. typhimurium* F98 Nal^r by chickens following challenge with this organism is shown in Table 3. Compared with the untreated group a profound inhibition of excretion of F98 Nal^r was observed in the group treated with the rough strain. The differences between the excretion rate in the two groups was significantly different, $\chi^2 = 24{\cdot}73$. $P = < 0{\cdot}01$.

S. typhimurium F98 Nal^r and the rough mutant were tested for virulence in day-old-chickens by oral inoculation. Whereas *S. typhimurium* F98 Nal^r produced 85% mortality by 3 weeks following inoculation, the rough mutant produced none. No deaths were produced by either strain after 3 weeks post-inoculation.

The effect of pre-treatment with an antibiotic sensitive strain of Salmonella typhimurium *F98 on colonization of the caeca by bacteriophage sensitive and resistant forms of* S. typhimurium *F98 Nal^r*

During studies aimed at understanding the mechanism of the protective effect conferred on the chickens by the presence in the gut of large numbers of

Table 3. *The effect of oral inoculation with an antibiotic sensitive, rough mutant of* Salmonella typhimurium *F98 on the faecal excretion by chickens of* S. typhimurium *F98 Nal*^{r}

Number of weeks after inoculation with F98 Nal^r	The faecal excretion of F98 Nal^r expressed as a percentage by groups of 50 newly hatched chickens after oral inoculation with*					
	An antibiotic-sensitive, rought mutant of *S. typhimurium* followed 24 h later by F98 Nal^r			*S. typhimurium* F98 Nal^r only, after 24 h		
	> 50†	D	T	> 50	D	T
1	0	2	4	28	38	62
2	0	2	2	44	58	72
3	0	4	12	24	56	88
4	0	0	4	6	24	54
5	0	0	0	0	8	32
6	0	2	4	0	6	22
7	0	0	4	2	2	10
7 (caeca)‡	0	0	4	0	0	10

* Differences for the two groups were significant; $\chi^2 = 24{\cdot}727$, $P = 0{\cdot}01$.

† > 50, greater than 50 colonies per plate; D, one or more colonies per plate; T, salmonellas isolated by direct plating or by selenite enrichment.

‡ Figures for caecal contents when birds were slaughtered at the end of the experiment.

salmonellas, phage particles, lytic for *S. typhimurium* F98, were found in the gut. Much smaller numbers were found in uninoculated chickens and in the feed.

To test whether lytic phage activity was involved in the protective effect the phage was isolated and a phage-resistant mutant of F98 Nal^r was prepared. The protective effect of an antibiotic sensitive form of F98 was then assessed against both the phage-sensitive and phage-resistant form of F98 Nal^r. In both cases inhibition occurred there being no growth of F98 Nal^r from caecal swabs suggesting that phage was not involved. Large numbers of phage were re-isolated from the chickens, which were lytic for the phage sensitive F98 Nal^r but showed no lysis on the phage resistant form.

The effect on colonization of Salmonella typhimurium *F98 Nal^r of oral and parental pre-treatment with various preparations of F98 Spc^r*

The effect of oral pre-treatment with various preparations of *S. typhimurium* F98 Spc^r on caecal colonization by F98 Nal^r was assessed. In addition the heat killed growth from the Roux flasks was inoculated in 0·1 ml volumes into two groups of chickens by the intra-muscular and subcutaneous routes respectively and these were challenged to test for any protective effect in the same way. Caecal swabs from all groups were plated on Brilliant Green agar containing spectinomycin and separately nalidixic acid/novobiocin.

From none of the chickens in any of the groups was the Spc^r form of F98 isolated from the caeca. No protective effect was conferred on any of the chickens given

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Table 4. *The* in vitro *inhibition of* S. typhimurium *F98 Nal^r by broth cultures of closely related organisms*

Organism tested	Viable numbers (log_{10}) of F98 Nal^r at h after inoculation into overnight broth cultures of test organisms: 0 h	24 h	(log_{10}) change in viable numbers
S. typhimurium phage type			
14 (F98)	2·0	2·0	0
20	2·9	3·0	+0·1
40	2·9	3·1	+0·2
S. anatum	2·9	2·9	0
S. enteritidis	2·9	3·1	+0·2
S. newport	3·0	2·9	−0·1
S. agona	3·0	2·5	−0·5
S. heidelberg	3·0	3·0	0
S. oranienburg	3·1	2·9	−0·2
S. dublin	3·0	3·0	0
S. arizonae Strain			
1	2·7	5·3	+2·6
2	3·0	5·7	+2·7
S. cholerae-suis strain			
1	2·9	3·0	+0·1
2	< 2·0	2·5	> +0·5
3	2·7	3·5	+0·8
4	2·5	3·6	+1·1
S. gallinarum strain			
1	2·5	4·6	+2·1
2	2·3	4·5	+2·2
3	2·6	5·1	+2·5
4	2·5	6·3	3·8
E. coli strain			
1	3·0	5·9	+2·9
2	2·9	5·6	+2·7
3	2·8	5·9	+3·1
4	2·9	6·8	+3·9
5	2·8	6·4	+3·6
Citrobacter strain			
1	2·8	6·9	+4·1
2	2·8	4·3	+1·5
Enterobacter strain			
1	2·5	6·8	+4·3
2	2·8	7·8	+5·0
Klebsiella pneumoniae	2·8	5·0	+2·2
Proteus vulgaris	2·9	6·5	+3·6
Providencia sp.	2·9	6·5	+3·6
Serratia marcescens	2·7	7·0	+4·3
Shigella flexneri	2·9	8·4	+5·5
Shigella sonnei	2·8	7·4	+4·6
Staphylococcus aureus	2·8	7·0	+4·2
Nothing	2·0	8·4	6·4

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the different inoculations, confluent growth of F98 Nal^r being found on the Brilliant Green/nalidixic acid/novobiocin plates for all groups.

In vitro *inhibition of growth of* S. typhimurium *F98 Nal^r by broth cultures of salmonella strains and organisms from other genera*

Many of the bacteria tested for inhibitory activity against *S. typhimurium* F98 Nal^r in the chicken alimentary tract were also screened as broth cultures for *in vitro* inhibition using the mixed culture tests described in the methods section. The results are shown in Table 4.

In sterile broth F98 Nal^r grew well, the increase in viable numbers after 24 h incubation being ($\log_{10}$) 2·0–8·4. The bacteria under test all grew equally well in broth. Very few of the broth cultures of the bacteria under test, including several *Salmonella* serotypes, *E. coli*, *Citrobacter*, *Klebsiella*, *Proteus*, *Providencia*, *Serratia*, *Shigella* and *Staphylococcus aureus*, allowed the viable numbers of F98 Nal^r to increase to values approaching that obtained in sterile broth. Of the 16 bacterial strains from genera other than *Salmonella*, 15 allowed increases in the viable count of F98 Nal^r of over ($\log_{10}$) 2 and 12 permitted increases of over ($\log_{10}$) 3. One *Citrobacter* strain allowed an increase in the F98 viable count of ($\log_{10}$) 1·5

By contrast many of the salmonella strains produced complete inhhibition of growth of F98 Nal^r. This was particularly true of *S. typhimurium*, 6 other food-poisoning serotypes, *S. dublin* and 1 strain of *S. cholerae-suis*. Four strains of *S. gallinarum* and 2 *S. arizonae* strains allowed growth of F98 comparable to that permitted by strains of the other genera tested.

The inhibitory effects for *S. typhimurium* F98 Nal^r of cultures of the sensitive form of this strain which had been treated in various ways were tested. These included a filtered supernatant of a culture and cultures which had been killed with formalin, ethanol or by heating at 60 °C as described previously. No inhibitory effect was observed at all at 24 h.

DISCUSSION

These results have shown that the colonization of the alimentary tract of the chicken with one salmonella inhibits almost completely subsequent colonization by another salmonella strain.

From previous studies on the phenomenon of competitive exclusion inhibition of caecal colonization with salmonellas required the presence in the alimentary tract of both facultative and obligate anaerobes (Rantala & Nurmi, 1973; Rantala, 1974). Inhibition has been claimed by using single strains of bacteria, namely a *Streptococcus faecalis* strain (Soerjadi, Lloyd & Cumming, 1978) and a strain of *Clostridium* (Rigby, Pettit & Robertson, 1977). Barrow & Tucker (1986) isolated three strains of *E. coli* which were inhibitory for *S. typhimurium* F98 and some other strains in chickens under different conditions. However, no strain of organism from the Enterobacteriaceae has been found which when inoculated on its own was inhibitory for salmonella.

A previous study has observed inhibition between strains of *E. coli* in chickens (Linton *et al.* 1978). They demonstrated partial displacement of antibiotic resistant strains of *E. coli* from the chicken alimentary tract by oral inoculation of large numbers of antibiotic sensitive *E. coli* strains.

The present study has shown that amongst genera from the Enterobacteriaceae only salmonella is naturally inhibitory for organisms from this genus. In addition a strain of citrobacter almost completely inhibited colonization of a Nal^r mutant of the homologous strain, and partial inhibition of a Nal^r mutant of an *E. coli* was produced by the antibiotic sensitive form of the homologous strain. Since *E. coli* is one of the first organisms to establish itself, sometimes in very large numbers, in the chicken alimentary tract after hatching (Smith, 1965; Coloe, Bagust & Ireland, 1984) it is possible that already established indigenous strains might interfere in inhibition studies using *E. coli*. This may also account for the only partial inhibition obtained in older chickens by Linton *et al.* (1978). In contrast, that *Citrobacter* might be expected to be isolated infrequently and salmonella never from specific pathogen-free chickens might explain the much higher level of inhibition obtained with these organisms than that obtained with *E. coli*. A strain of *Shigella flexneri* and *Staphylococcus aureus* did not colonize the alimentary tract extensively enough from any inhibition to be detected.

For the purpose of producing an inhibition for use under commercial conditions the possibility of using live-bacteria free preparations derived from inhibitory organisms was examined. Preparations derived from *S. typhimurium* F98 in which the bacterial cells were killed in a variety of ways were completely ineffective.

Live bacteria were clearly essential for producing the inhibition and the inhibitory effect was therefore unlikely to have been the result of a rapid form of immune response produced by the chicken and stimulated by the inoculated killed organisms, particularly since an inhibition could be produced *in vitro*. This view was also supported by the finding that salmonella from a variety of serotypes possessing different surface antigens were all able to produce inhibition. Neither was the inhibition mediated by the production of large numbers of bacteriophages in the intestine by the first inoculated strain. The inhibitory activity therefore required the presence in the alimentary tract of large numbers of metabolically active salmonella organisms. That this is so is supported by the fact that amongst the salmonella strains tested the extent of the inhibition was to some extent related to the ability of the inoculated strain to colonize the alimentary tract. One of the serotypes known to be a poor colonizer, *S. cholerae-suis* (Smith & Tucker, 1980) was also a poor inhibitor of *S. typhimurium* F98.

Whether the inhibition is caused by a metabolic reaction, similar to that occurring in broth cultures at stationary phase, or is a result of blocking of adhesion sites in the alimentary tract by the first organisms in unclear at this stage. Poor inhibition by poor colonizers could fit either hypothesis equally well. Barrow & Tucker (1986) suggested that competition for adhesion sites was unlikely to be the mechanism of inhibition produced by their three *E. coli* strains but the mechanisms in the two systems could differ.

That a metabolic effect is involved is suggested by the ability to produce similar inhibition *in vitro*. The only strains producing complete inhibition of growth of the *S. typhimurium* strain were those which were most active *in vivo*. A strain of *S. choleraesuis* and two *S. arizonae* strains were only partially inhibitory both *in vivo* and *in vitro* while strains from other genera were generally also much less inhibitory, although *in vitro* at least, they all grew as well as the most inhibitory strains. Thus it is likely that the extent to which strains colonize the gut is not

the only criterion for inhibition amongst salmonella strains, and that a strong inhibitory mechanism specific to closely related biotypes within a genus does occur.

Two levels of inhibition of *S. typhimurium* were observed *in vitro*. Strains and genera which were completely non-inhibitory *in vivo* did produce some inhibition *in vitro* which may have reflected differences in the growth conditions. This lower level of inhibition *in vitro* is likely to be the result of mechanisms such as oxygen depletion as suggested by Wilson, Miles & Parker (1983). The mechanism of the complete inhibition of growth produced by some salmonella strains however, is not understood. The inhibition of organisms by a broth culture of the homologous strain has been observed for a strain of *E. coli*, and in similar experiments with *S. aureus* and *Pseudomonas aeruginosa* (Graham Smith, 1920) but it is surprising that such variation in the extent of inhibition occurs between the closely related genera of the Enterobacteriaceae. The *in vitro* inhibition required the presence of live cells since neither killed cells nor a culture supernatant produced any inhibition. The latter observation suggests that the inhibition is at least not the result of nutrient depletion.

The profound inhibition induced by the avirulent rough mutant of strain F98 lasted for 7 weeks. The rough mutant was avirulent for chickens but at the present time, it would be impossible to assess its ability to produce human food poisoning without volunteer studies. It is unlikely that the British poultry industry would accept the use of an avirulent salmonella strain as a mediator of competitive exclusion without the knowledge that such a strain was as avirulent as the commensal *E. coli* strains also harboured by the chicken and ingested by man. Such avirulence would also have to be stable. This is much more likely if the strain used has been made avirulent by several mechanisms simultaneously. The risk of reversion or conversion to virulence by genetic transfer should then be exceedingly small.

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