J. Hyg., Camb. (1985), 95, 363–374
Printed in Great Britain

Acquisition of genes from an O18:K1:H7 ColV$^+$ strain of *Escherichia coli* renders intracranially-inoculated *E. coli* K12 highly virulent for chickens, ducks and guinea-pigs but not mice

By H. WILLIAMS SMITH and M. B. HUGGINS

Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire, PE17 2DA

(*Received* 13 *March*, 1985; *accepted* 15 *May*, 1985)

SUMMARY

The virulence of intracranially-inoculated mutant forms of an O18ac:K1:H7 ColV$^+$ strain of *Escherichia coli* (designated MW) that lacked different combinations of its O and K antigens and ColV, and of an *E. coli* K12 strain to which these characters had been transmitted was studied in mice, chickens, ducks and guinea-pigs.

The O18$^+$K1$^+$ColV$^+$ form of MW was highly virulent for chickens and mice but the corresponding form of K12 was only highly virulent for chickens; the O18$^-$K1$^-$ColV$^-$ forms of both strains were of low virulence for chickens and mice. K1 was more important than O18 or ColV in determining virulence for both animal species. Ducks and guinea-pigs resembled chickens, not mice, in their response to infection with the O18$^+$K1$^+$ColV$^+$ form of K12.

Pathogenesis studies revealed that the virulence of the forms of MW and K12 was associated with their ability to proliferate in the central nervous system; only low numbers of organisms were found in the blood and spleen of inoculated animals.

The O18$^+$K1$^+$ColV$^+$ form of K12 multiplied in mouse brain and in mouse blood *in vitro*; its multiplication in chicken blood was partially inhibited. Agglutinins to this and other forms of K12 were found in chicken serum but not in mouse serum. Large doses of mouse serum given to chickens and large doses of chicken serum given to mice did not alter the manner in which these animals responded to K12 O18$^+$K1$^+$ColV$^+$ infection. Vaccination protected chickens and mice against lethal intracranial infection with the O18$^+$K1$^+$ColV$^+$ forms of K12 or MW; it produced a much stronger immunity in mice against intraperitoneal challenge than against intracranial challenge.

INTRODUCTION

In a previous study (Smith & Huggins, 1980) the virulence of mutants of an O18ac:K1:H7 ColV$^+$ *Escherichia coli* strain (designated MW) was assessed by inoculating them intramuscularly or intraperitoneally into chickens and mice. Those that lacked the O18 or K1 antigens or the ColV plasmid were less virulent

to *E. coli* K12. The resulting mutants were slightly more virulent than the K12 parent strain but the virulence of all of them, including one possessing the three characters, did not approach that of MW. Pathogenesis studies indicated that the MW and K12 mutants were of low virulence because they were less competent than MW in overcoming the defence mechanisms of the host. It was decided, therefore, to study the behaviour of the K12 mutants in a host site where the defences were weak. This was achieved by inoculating them intracranially because then they would be in a confined region away from the defence mechanisms of the reticulo-endothelial system and, presumably, exposed only to those of the fluid and cellular components of the blood. Chickens and mice were again used but, because different results were obtained with them, ducks and guinea-pigs were included in some of the experiments. The results are reported in this paper.

METHODS

Bacterial strains, their cultivation and enumeration

The properties of the O18ac:K1:H7 ColV$^+$ strain MW and the methods employed to isolate the mutants lacking different combinations of its O18 and K1 antigens and the ColV plasmid and to transfer these three characters by conjugation to an *E. coli* K12 strain resistant to nalidixic acid have been described previously; as have the methods of cultivating the mutants and estimating their numbers in broth cultures and in tissues of inoculated animals (Smith & Huggins, 1980). In experiments requiring estimation of the numbers of MW and K12 organisms in mixtures, MW was used as a spontaneous mutant resistant to spectinomycin and the counts were performed on two sets of plates of MacConkey's medium, one containing sodium nalidixate and one containing spectinomycin, both at concentrations of 20 μg ml^{-1}.

Experimental animals

Unless stated, the chickens were from this institute's specified-pathogen-free (SPF) Light Sussex flock and the mice were from its Tuck no.1 colony. Some experiments were also performed in the institute's guinea-pigs and SPF White Leghorn chickens and in mice of the ICI no.1 strain and ducklings obtained from commercial breeders. Unless stated, the chickens were aged 25 days, the mice aged 42 days and the ducks and guinea-pigs aged 7 days.

Virulence tests

Tenfold dilutions of broth cultures in normal saline were inoculated intracranially in 0·02 ml amounts into groups of 10 chickens, mice or ducks or 20 guinea-pigs; all were lightly anaesthetized with halothane. The inoculation site was in the mid-line immediately in front of the junction of the parietal and frontal bones, the needle being inserted sufficiently deep to enter the outer region of the cerebrum. After inoculation the animals were observed at least twice daily until it was apparent that no more would die. When the inoculation doses gave responses such that median lethal doses (LD_{50}) were estimable, the method of Spearmann–Karber (Finney, 1971) was adopted. When the data did not allow an estimate of the LD_{50}, a range of values for the $\log_{10}$ (of the number of organisms) that embraced the

observed 50 % mortality point was quoted. For experiments in which the observed mortalities did not embrace 50 %, the LD_{50} value was quoted as being either greater than or less than a specified number ($\geqslant$ when no animals died in the group given the highest dose and $>$ when less than 50 % of the animals in this group died or $\leqslant$ when all the animals died in the group given the lowest dose and $<$ when more than 50 % of the animals in this group died). All decisions on choice of statistical method and their performance was kindly dealt with by Mr D. E. Walters of the Agricultural Research Council's Statistics Group, Department of Applied Biology, Cambridge.

Survival of bacterial mutants in brain and blood in vitro

A total of 15 g of brain collected aseptically from 65 mice was ground in a pestle and mortar and 7 ml of normal saline added. The resulting suspension in 0·5 g amounts was placed in 5 ml screw-capped bottles, inoculated with 10^3 viable organisms of a broth culture of the mutant under test and incubated in a shaking water bath at 37 °C. At intervals, bottles were removed from the bath and the number of organisms in their contents was estimated.

Twelve ml of blood from 15 mice or 6 chickens was collected in 0·5 ml of heparin solution and distributed equally into four 5 ml screw-capped bottles. Each bottle was inoculated with 10^4 viable organisms of a broth culture of the strain under test and incubated in a stationary water bath at 37 °C for mouse blood and 41 °C for chicken blood, the body temperatures of the animals from which the specimens were obtained. At intervals, 0·1 ml was removed from each bottle and its bacterial content determined.

Vaccination procedure

Broth cultures were killed by the addition of 0·25 % formaldehyde and emulsified in an equal volume of Freund's incomplete adjuvant. Animals were given three intramuscular inoculations of this material at weekly intervals; the dose for chickens was 1·0 ml and for mice was 0·2 ml. Control animals were treated similarly except that the broth cultures in the inocula were replaced by plain broth. Chickens were 7 days old when they received their first inoculation and mice were 35 days old; their immunity was challenged at 15 days after the final inoculation.

RESULTS

Virulence of different forms of MW and K12 administered intracranially

(*a*) *For chickens*

The results of intracranial inoculation experiments designed to assess the virulence for chickens of MW and K12 and of mutant forms of MW deficient in different combinations of the O18 and K1 antigens and its ColV plasmid and of forms of K12 that had acquired these characters during conjugation are summarized in Table 1. All the forms of MW and K12 that possessed K1 were lethal in low dosage; the LD_{50} for the form of K12 that possessed ColV or ColV and O18 in addition to K1 was similar to that of MW itself, i.e. its $O18^+K1^+ColV^+$ form. The forms of MW and K12 that lacked K1, especially those that in addition lacked O18, ColV or both characters, were less virulent. The survival times of groups of

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

Table 1. *Lethality by the intracranial route of different forms of MW and K12 for chickens*

	$\log_{10}$ (LD_{50} of viable organisms)			
	MW form		K12 form	
$O18^+K1^+ColV^+$	$\leqslant 0{\cdot}6$	(3·6)	$< 0{\cdot}6$	($> 8{\cdot}5$)
$O18^+K1^+ColV^-$	$< 0{\cdot}6$	(6·4)	1·9	($\geqslant 8{\cdot}5$)
$O18^+K1^-ColV^+$	1·9	($\geqslant 8{\cdot}5$)	2·9	($\geqslant 8{\cdot}5$)
$O18^-K1^+ColV^+$	0·6–1·6	(7·9)	$< 0{\cdot}6$	($> 8{\cdot}5$)
$O18^+K1^-ColV^-$	3·7	($\geqslant 8{\cdot}5$)	4·6–5·6	($\geqslant 8{\cdot}5$)
$O18^-K1^+ColV^-$	0·6–1·6	(8·0–8·5)	1·8	($> 8{\cdot}5$)
$O18^-K1^-ColV^+$	5·6	($\geqslant 8{\cdot}5$)	6·6	($\geqslant 8{\cdot}5$)
$O18^-K1^-ColV^-$	6·1	($\geqslant 8{\cdot}5$)	6·6–7·6	($\geqslant 8{\cdot}5$)

The figures in parenthesis are the results obtained by Smith & Huggins (1980) for intramuscular inoculation.

$\geqslant$, No animals died in the group given the highest dose; $>$, the number of animals that died in this group was too low to determine the LD_{50}. $\leqslant$, all the animals died in the group given the lowest dose; $<$, the number of animals that died in this group was too high to determine the LD_{50}.

Table 2. *The survival times of groups of chickens inoculated intracranially with $O18^+K1^+ColV^+$ and $O18^-K1^-ColV^-$ forms of MW and K12*

Dose of viable organisms	Form	No. of 15 chickens that had died by the following days after inoculation						
		½	1	1½	2	3	4	5
150	MW $O18^+K1^+ColV^+$	13	13	13	13	13	13	13
	K12 $O18^+K1^+ColV^+$	6	11	14	14	14	15	15
15	MW $O18^+K1^+ColV^+$	9	13	14	14	14	14	14
	K12 $O18^+K1^+ColV^+$	0	1	9	12	12	13	13
1·5	MW $O18^+K1^+ColV^+$	5	6	6	6	6	6	6
	K12 $O18^+K1^+ColV^+$	0	1	5	8	8	10	10
10^7	MW $O18^-K1^-ColV^-$	4	4	7	8	8	9	9
	K12 $O18^-K1^-ColV^-$	6	9	10	10	10	11	11
10^6	K12 $O18^-K1^-ColV^-$	0	1	1	1	1	2	2
	K12 $O18^-K1^-ColV^-$	1	2	4	4	4	4	4

15 chickens inoculated with different doses of the $O18^+K1^+ColV^+$ and $O18^-K1^-ColV^-$ forms of K12 and MW are given in Table 2. Although the chickens inoculated with the $O18^+K1^+ColV^+$ form of K12 tended to survive longer than those inoculated with the corresponding form of MW there was little difference in the total mortality caused by the two forms; it is probable that in some cases both forms were lethal in doses of only one viable organism. There was no difference in survival time or mortality between the groups of chickens inoculated with the $O18^-K1^-ColV^-$ form of K12 or MW.

The experiments with the different forms of MW and K12 were repeated in chickens aged one day and in chickens of the White Leghorn breed. Also, those with the $O18^+K1^+ColV^+$ forms of K12 and MW were repeated in chickens aged

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

Table 3. *Lethality by the intracranial route of different forms of MW and K12 for mice*

Form	$\log_{10}$ (LD_{50} of viable organisms) MW form	K12 form
$O18^+K1^+ColV^+$	1·2 (4·4)	5·7 (> 8·0)
$O18^+K1^+ColV^-$	2·1 (5·7)	5·8 (> 8·0)
$O18^+K1^-ColV^+$	5·3 (> 8·0)	6·6–7·6 (⩾ 8·0)
$O18^-K1^+ColV^+$	1·2 (> 7·3)	6·0 (> 8·0)
$O18^+K1^-ColV^-$	5·8 (—)	6·6–7·6 (—)
$O18^-K1^+ColV^-$	2·7 (—)	5·6–6·6 (—)
$O18^-K1^-ColV^+$	6·7 (⩾ 8·0)	6·6–7·6 (⩾ 8·0)
$O18^-K1^-ColV^-$	6·6–7·6 (⩾ 8·0)	7·6–8·6 (⩾ 8·0)

The figures in parenthesis are the results obtained by Smith & Huggins (1980) for intraperitoneal inoculation.

—, No observation; for other abbreviations see Table 1.

Table 4. *Numbers of organisms in the tissues of chickens killed at different times after they had been inoculated intracranially with* 10 *or* 10^3 *viable organisms of the* $O18^+K1^+ColV^+$ *form of MW and K12*

No. of organisms inoculated	Time after inoculation (h)	$\log_{10}$ [no. of viable organisms (g tissue)$^{-1}$] MW form in Brain	Blood	Spleen	K12 form in Brain	Blood	Spleen
10	8	3·2	< 2·0	3·0	3·3	< 2·0	2·0
	16	8·0	4·0	5·3	5·5	2·3	4·5
	24	9·5	5·7	6·0	9·3	< 2·0	4·5
	32	—	—	—	9·3	3·0	3·8
	40	—	—	—	—	—	—
10^3	5min	2·0	< 2·0	< 2·0	2·0	< 2·0	< 2·0
	8	7·5	2·7	4·5	6·0	< 2·0	3·3
	16	9·5	4·7	6·0	9·3	4·7	5·5
	24	—	—	—	9·5	< 2·0	3·3
	32	—	—	—	—	—	—

Three chickens given each form were killed at each of the stated times and the numbers of organisms in their tissues were estimated; the medians of each set of three counts for each particular tissue are shown.

—, Insufficient numbers of the chickens given this form were surviving at this time to permit observations to be made.

42 and 66 days. The results of all these experiments were essentially the same as those illustrated in Table 1.

(*b*) *For mice*

The results of giving the different forms of MW and K12 to mice are summarized in Table 3. Their responses to infection with the different forms of MW, in general, resembled those of the chickens except that slightly higher doses were required to kill them and they survived slightly longer; the survival times of the mice

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

inoculated with the MW O18$^+$K1$^+$ColV$^+$ form resembled those quoted in Table 9 for control mice. Unlike the chickens, the mice were highly resistant to lethal infection with all the forms of K12, even the O18$^+$K1$^+$ColV$^+$ form.

The experiments with the O18$^+$K1$^+$ColV$^+$ forms of MW and K12 were repeated in mice aged 25 days and in mice of the ICI no.1 strain. The results were essentially the same as those quoted in Table 3. The LD_{50}s of these forms of MW and K12 for the mice aged 25 days were, $\log_{10}$, 2·1 and 5·6–6·6 organisms respectively; the corresponding figures for the ICI no.1 strain were 1·6–2·6 and 5·6–6·6 respectively.

(*c*) *For ducks*

The results of inoculating ducks with the O18$^+$K1$^+$ColV$^+$ and O18$^-$K1$^-$ColV$^-$ forms of MW and K12 resembled those obtained in chickens. The LD_{50}s of the O18$^+$K1$^+$ColV$^+$ forms of MW and K12 for these animals were, $\log_{10}$, 0·06 and 0·2 viable organisms; the correspondiong figures for the O18$^-$K1$^-$ColV$^-$ forms of MW and K12 were 5·6–6·6 and $\geqslant$ 6·6 viable organisms respectively.

(*d*) *For guinea pigs*

The experiments with the O18$^+$K1$^+$ColV$^+$ forms of MW and K12 and with the O18$^-$K1$^-$ColV$^-$ form of K12 were also repeated in guinea-pigs. The results resembled those that had been obtained in chickens. The LD_{50}s of the O18$^+$K1$^+$ColV$^+$ forms of MW and K12 were, $\log_{10}$, $< 0{\cdot}6$ and 0·3–1·3 respectively; the LD_{50} for the O18$^-$K1$^-$ColV$^-$ form of K12 was $> 6{\cdot}3$.

Fate of different forms of MW and K12 inoculated intracranially into chickens and mice

Chickens

Estimating the numbers of organisms in the tissues of chickens killed at different times after they had been inoculated intracranially with 10 or 10^3 viable organisms of the O18$^+$K1$^+$ColV$^+$ form of MW or K12, revealed that both forms proliferated in the brain, the MW form being present in larger numbers than the K12 form at 8 h in the chickens inoculated with 10^3 viable organisms and at 16 h in the chickens inoculated with 10 viable organisms (Table 4). In the chickens examined after these times, all of which were severely ill, the numbers of the MW and K12 forms in the brain were about the same. The numbers, especially of the K12 form, were much lower in the spleen and blood than in the brain.

The above experiment at the 10^3 dose level was repeated using the O18$^-$K1$^-$ColV$^-$ forms of MW and K12, 3 chickens given each form being examined at 5 min and 8, 16, 24, 32, 40 and 48 h after inoculation. None of the 42 chickens became unwell. Approximately, $\log_{10}$, 2·0 viable organisms g^{-1} were isolated from the brains of the 6 chickens killed at 5 min after inoculation. The K12 form, $\log_{10}$, 2·5 viable organisms g^{-1}, was found in the brain of one of the chickens examined at 32 h and the MW form, $\log_{10}$, 3·3 viable organisms g^{-1}, was found in the brain of one examined at 40 h. No organisms were isolated from the brains of the remaining 34 chickens; neither were they isolated from the blood or spleen of any of the 42 employed in the experiment.

Table 5. *Numbers of organisms in the tissues of mice killed at different times after they had been inoculated intracranially with* 10^3 *viable organisms of the O18⁺K1⁺ColV⁺ forms of MW and K12*

Time after inoculation (h)	$\log_{10}$ [no. of viable organisms (g tissue)$^{-1}$] of MW form in Brain	MW form in Blood	MW form in Spleen	K12 form in Brain	K12 form in Blood	K12 form in Spleen
5min	2·3	< 2·0	< 2·0	2·5	< 2·0	< 2·0
8	6·6	2·3	< 2·0	2·3	< 2·0	< 2·0
16	7·2	2·3	2·0	3·3	< 2·0	< 2·0
24	8·2	2·0	< 2·0	2·3	< 2·0	< 2·0
32	8·6	2·0	2·8	4·0	< 2·0	< 2·0
40	9·0	2·7	2·3	2·7	< 2·0	< 2·0
48	7·6	< 2·0	< 2·0	2·0	< 2·0	< 2·0
72	8·6	2·9	3·3	4·3	2·6	< 2·0

Three mice were killed at each of the stated times; for other details see Table 4.

Table 6. *Numbers of organisms in the brain of mice killed at different times after they had been inoculated intracranially with a mixture of* 10^3 *viable organisms of the O18⁺K1⁺ColV⁺ forms of MW and K12*

Time after inoculation (h)	$\log_{10}$ [no. of viable organisms (g brain)$^{-1}$] of MW form	K12 form
5min	2·6	2·6
8	5·2	3·3
16	8·3	4·2
24	9·0	5·7
32	8·5	4·7
40	8·8	5·5
48	8·7	4·7
72	8·7	3·3

Three mice given the mixture were killed at each of the stated times and the numbers of the two kinds of organisms in the brain were estimated by performing the counts on MacConkey's agar containing spectinomycin (for MW) and sodium nalidixate (for K12); the median K12 count of each set of three counts is shown together with its corresponding MW count.

Mice

High numbers of organisms were found in the brains of mice at 8 h or more after they had been inoculated with 10^3 viable organisms of the O18⁺K1⁺ColV⁺ form of MW; the number of organisms found in the brains of mice inoculated with the corresponding form of K12 were always low (Table 5). Only very low numbers of organisms were found in the blood and spleen of the mice inoculated with the MW form; the K12 form was only found in one of the 24 mice examined. Some of the mice that had been set aside for this experiment died 48 h after inoculation with MW; none survived longer than 72 h. All the mice inoculated with the K12 form remained well.

The numbers of K12 organisms were usually higher in the brains of mice

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

Table 7. *Numbers of organisms in a 68% suspension of pooled mouse brain at different times after adding the O18⁺K1⁺ColV⁺ form of MW and K12 and incubating at 37 °C*

Time after inoculation (h)	$\log_{10}$ [no. of viable organisms (g brain)$^{-1}$] of	
	MW form	K12 form
0	3·0	3·0
1	3·9	3·8
3	5·3	5·2
5	7·8	7·5
8	9·2	8·6
12	9·3	9·2

Table 8. *Numbers of organisms in pooled heparinized mouse or chicken blood at different times after adding the O18⁺K1⁺ColV⁺ and O18⁻K1⁻ColV⁻ forms of MW and K12*

	$\log_{10}$ [no. of viable organisms (g tissue^{-1}]							
	O18⁺K1⁺ColV⁺ form of				O18⁻K1⁻ColV⁻ form of			
	MW in		K12 in		MW in		K12 in	
Time after inoculation (h)	Chicken blood	Mouse blood	Chicken blood	Mouse blood	Chicken blood	Mouse blood	Chicken blood	Mouse blood
0	3·5	3·5	3·2	3·2	3·4	3·4	3·2	3·2
½	3·5	3·6	3·0	3·2	3·3	3·4	3·2	3·3
1	3·8	3·6	2·4	3·3	2·3	3·4	2·3	3·2
1½	3·2	3·9	2·5	3·4	1·7	3·5	< 1·0	3·3
3	4·7	5·4	3·4	3·9	2·6	4·2	< 1·0	3·7
5	5·3	6·9	5·2	4·3	1·9	4·7	< 1·0	3·6

The mouse blood was incubated at 37 °C and the chicken blood at 41 °C.

inoculated with a mixture of the O18⁺K1⁺ColV⁺ forms of MW and K12 than in the brains of mice inoculated with the K12 form only but they were still much lower than the numbers of the MW form (Table 6).

The effect of incubating forms of MW and K12 in mouse brain and in mouse and chicken blood in vitro

The O18⁺K1⁺ColV⁺ forms of MW and K12 multiplied at the same rapid rate in a freshly-prepared suspension of pooled mouse brain in normal saline (Table 7).

The numbers of organisms of the O18⁺K1⁺ColV⁺ form of K12 and of the O18⁻K1⁻ColV⁻ forms of K12 and MW had decreased 1 h after incubation in fresh pooled heparinized chicken blood, those of the O18⁺K1⁺ColV⁺ form of K12 increasing later; the numbers of organisms of the O18⁺K1⁺ColV⁺ form of MW had not decreased (Table 8). No decrease was observed in the count of all four forms incubated in mouse blood; all, except the O18⁻K1⁻ColV⁻ form of K12, multiplied.

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

Table 9. *Immunity produced in chickens against intracranial challenge with the O18⁺K1⁺ColV⁺ form of the MW strain by vaccination with this form*

Challenge dose (log_{10} viable organisms)	Vaccinal status of chickens	No. of 20 chickens that had died by the following days after challenge			
		1	2	3	7
5·3	+	17	20	20	20
	−	20	20	20	20
4·3	+	12	19	20	20
	−	18	20	20	20
3·3	+	6	12	18	19
	−	12	20	20	20
2·3	+	0	9	14	15
	−	9	20	20	20
1·3	+	0	3	8	8
	−	5	20	20	20
0·3	+	0	0	0	0
	−	3	11	11	11

+, Vaccinated; −, unvaccinated.

Three doses of vaccine consisting of formaldehyde-killed organisms in Freund's incomplete adjuvant were given intramuscularly to groups of 7-day-old chickens at 7 day intervals; control animals were only given the adjuvant. Their immunity was challenged 15 days after the last dose.

Agglutination tests with pooled mouse and chicken serum and different forms of K12

In slide tests, pooled chicken serum strongly agglutinated the O18⁺K1⁺ColV⁺, the O18⁺K1⁻ColV⁺ and the O18⁻K1⁻ColV⁻ forms, but not the O18⁻K1⁺ColV⁺ form, of K12; pooled mouse serum did not agglutinate any of the four forms. These and the subsequent experiments were performed to obtain additional evidence on whether or not serum might be involved in the differing susceptibility of chickens and mice to infection with the MW and K12 forms.

The effect of chicken or mouse serum on the lethality of the O18⁺K1⁺ColV⁺ form of K12 for mice or chickens

Ten mice given 3 ml of pooled chicken serum subcutaneously were inoculated intracranially 24 h later with, log_{10}, 4·6 viable organisms of the O18⁺K1⁺ColV⁺ form of K12, a dose calculated as being just insufficient to kill normal mice; only one died. The experiment was repeated in chickens aged 5 days giving them pooled mouse serum instead of chicken serum and a dose of, log_{10}, 1·0 viable organisms, an amount just sufficient to kill normal chickens; only one survived.

Immunity produced in chickens and mice against intracranial and intraperitoneal challenge with the O18⁺K1⁺ColV⁺ form of MW or K12

A slight but definite immunity against intracranial challenge with the O18⁺K1⁺ColV⁺ form of K12 was detected in groups of chickens that had been given a dead vaccine prepared from this form; immunity was expressed as decreased

Table 10. *Immunity produced in mice against intracranial challenge with the O18⁺K1⁺ColV⁺ form of the K12 strain by vaccination with this form*

Challenge dose (log_{10} viable organisms)	Vaccinal status of mice	No. of 20 mice that had died by the following days after challenge							
		1	2	3	4	5	6	7	14
4·0	+	2	4	6	7	11	15	17	18
	−	1	7	11	11	13	16	18	19
3·5	+	0	1	1	4	4	6	9	11
	−	0	2	5	10	10	12	14	18
3·0	+	1	2	3	3	3	3	3	3
	−	0	0	6	7	10	12	13	17
2·5	+	1	1	3	4	5	5	5	8
	−	0	2	10	10	16	17	17	19
2·0	+	0	0	0	0	2	2	4	4
	−	0	2	8	10	12	12	12	12

For details see Table 9; the mice were 35 days old when vaccination was commenced.

Table 11. *Immunity produced in mice against intraperitoneal challenge with the O18⁺K1⁺ColV⁺ form of the MW strain by vaccination with this form*

Challenge dose (log_{10} viable organisms)	Vaccinal status of mice	No. of 20 mice that had died by the following days after challenge			
		1	2	5	14
6·5	+	0	0	0	0
	−	19	20	20	20
6·0	+	0	0	0	0
	−	8	16	17	18
5·5	+	0	0	0	0
	−	7	15	15	15
5·0	+	0	0	0	0
	−	0	1	1	1

For details see Tables 9 and 10.

mortality in the groups challenged with low doses of the O18⁺K1⁺ColV⁺ form and by increased survival times in the groups challenged with higher doses (Table 9). Similar results were obtained when the experiment was repeated in mice vaccinated and challenged with the O18⁺K1⁺ColV⁺ form of MW (Table 10). A much stronger immunity was observed in vaccinated mice challenged intraperitoneally instead of intracranially (Table 11).

DISCUSSION

As anticipated, most of the forms of MW and K12 were much more virulent for chickens and mice when they were given intracranially than when they had been given intramuscularly or intraperitoneally (Smith & Huggins, 1980). The fact that

https://doi.org/10.1017/S0022172400062781 Published online by Cambridge University Press

many of them differed greatly in virulence when given intracranially, however, indicated that the defences of the central nervous system were not inconsequential. As was the case following intramuscular and intraperitoneal inoculation, the K1 antigen conferred a high degree of virulence on intracranially-inoculated MW forms. On the assumption that the main defences of the central nervous system are the components of the blood, this increase in virulence was expected because Smith & Huggins (1980) had found that the K1 derivatives exhibited an increased resistance to the bactericidal activity of serum. K1 serum resistance has since been noted and studied more fully by other workers (Gemski, Cross & Sadoff, 1980; Agueero & Cabello, 1984; Pluschke *et al.* 1983; Pluschke & Achtman, 1984), the classical complement pathway being shown to be important in the particular susceptibility of newborns to $K1^+$ infections (Pluschke & Achtman, 1984). The K1 antigen, too, has also been found to endow organisms with increased resistance to phagocytic killing (Cross *et al.* 1984). Unlike the position of intramuscularly and intraperitoneally-inoculated MW forms (Smith & Huggins, 1980), the virulence genes of the ColV plasmid, be they those that encode iron transport systems (Williams, 1979; Williams & Warner, 1980; Stuart, Greenwood & Luke, 1980) or complement resistance (Binns, Davis & Hardy, 1979; Binns, Mayden & Levine, 1982; Nilius & Savage, 1984), only slightly increased the virulence of these forms when given intracranially. The O18 genes, though, had a greater effect, an effect that was expected because O18 increased serum resistance (Smith & Huggins, 1980); this increased resistance was also observed by Pluschke *et al.* (1983) and Pluschke & Achtman (1984) in their studies on the impact of different O antigens in $K1^+$strains on virulence. The increased virulence, though, was only slight in the case of the mice. The differences between the responses of chickens and mice to intracranial inoculation was much more marked when the results from the different forms of K12 were compared. All were of low virulence for mice but some, especially those possessing the K1 antigen, were highly virulent for chickens, and also for ducks and guinea pigs, to the extent that probably one organism was sufficient to produce a lethal infection, an observation that emphasized the vulnerability of some species of animals to intracranial invasion of certain kinds of *E. coli*, such as those possessing the K1 antigen.

No explanation could be found for the difference between mice and chickens, ducks and guinea-pigs in the manner in which they responded to intracranial inoculation with the K12 forms. Low doses of the $O18^+K1^+ColV^+$ form of K12 failed to proliferate in the central nervous system of mice when inoculated alone or when inoculated with the corresponding form of MW which could proliferate there. No inhibitors for this K12 form could be demonstrated in mouse brain and, although it was possible to protect mice against lethal infection with the corresponding form of MW and chickens against lethal infection with the K12 form by vaccination, the ability of the K12 form to survive apparently unharmed in normal mouse blood but not in normal chicken blood and the failure to demonstrate agglutinins for it in mouse serum but not in chicken serum, did not suggest that antibody was involved. Furthermore, the fact that the administration of large doses of normal mouse serum to chickens and large doses of normal chicken serum to mice did not alter the manner in which they reacted to infection with the K12 form suggests that the property responsible for mice being highly resistant to

infection with this form and for chickens being highly susceptible to it does not reside in their sera. Whatever the reason for this difference between mice and chickens, ducks and guinea-pigs, it provides one more example of the danger in extrapolating between species.

We are grateful to Mrs Theresa Berrill for her capable technical help. Our thanks are also due to Professor P. M. Biggs, Mrs Shirley Pilcher, Miss Betty Royston and Mr Peter Townsend for assistance in a variety of ways.

REFERENCES

Agueero, M. & Cabello, F. C. (1983). Relative contribution of ColV plasmid and K1 antigen to the pathogenicity of *Escherichia coli*. *Infection and Immunity* **40**, 359–368.

Binns, M. M., Davies, D. L. & Hardy, K. G. (1979). Cloned fragments of the plasmid ColV, I-K94 specifying virulence and serum resistance. *Nature* **297**, 776–781.

Binns, M. M., Mayden, J. & Levine, R. P. (1982). Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV, I-K94. *Infection and Immunity* **35**, 654–659.

Cross, A. S., Gemski, P., Sadoff, J. C., Orskov, F. & Orskov, I. (1984). The importance of the K1 capsule in invasive infections caused by *Escherichia coli*. *Journal of Infectious Diseases* **149**, 184–193.

Finney, D. J. (1971). *Statistical Method in Biological Assay*, 2nd edn, p. 524. London: Charles Griffin.

Gemski, P., Cross, A.S. & Sadoff, J. C. (1980). K1 antigen-associated resistance to the bacteriocidal activity of serum. *FEMS Microbiological letters* **9**, 193–197.

Nilius, A. M. & Savage, D. C. (1984). Serum resistance encoded by Colicin V plasmids in *Escherichia coli* and its relationship to the plasmid transfer system. *Infection and Immunity* **43**, 947–953.

Pluschke, G. & Achtman, M. (1984). Degree of antibody-independant activation of the classical complement pathway by K1 *Escherichia coli* differs with O antigen type and correlates with virulence in newborns. *Infection and Immunity* **43**, 684–692.

Pluschke, G., Mayden, J., Achtman, M. & Levine, R. P. (1983). Role of capsule and the O antigen in resistance of O18:K1 *E. coli* to complement-mediated killing. *Infection and Immunity* **42**, 907–913.

Smith, H., Williams & Huggins, M. B. (1980). The association of the O18, K1 and H7 antigens and the ColV plasmid of a strain of *Escherichia coli* with its virulence and immunogenicity. *Journal of General Microbiology* **121**, 387–400.

Stuart, S. J., Greenwood, K. T. & Luke, R. K. J. (1980). Hydroxamate-mediated transport of iron controlled by ColV plasmids. *Journal of Bacteriology* **143**, 35–42.

Williams, P. H. (1979). Novel iron uptake system specified by ColV plasmids: an important component in the virulence of invasive strains of *Escherichia coli*. *Infection and Immunity* **26**, 925–932.

Williams, P. H. & Warner, P. J. (1980). ColV plasmid-mediated colicin V-independent iron uptake system of invasive strains of *Escherichia coli*. *Infection and Immunity* **29**, 411–416.