THE USE OF LIVE VACCINES IN EXPERIMENTAL *SALMONELLA GALLINARUM* INFECTION IN CHICKENS WITH OBSERVATIONS ON THEIR INTERFERENCE EFFECT

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Although some reports, mainly of field observations, are favourable, the bulk of the available evidence indicates that dead vaccines prepared from cultures of *Salmonella gallinarum* are of no value in the control of fowl typhoid (for example, McNutt, 1926; Simms, 1946; Wilson, 1946; Hall, MacDonald & Legenhausen, 1949). Since it was found in recent studies that naturally recovered chickens were completely immune to re-infection (Smith, 1955*a*), it was decided to re-investigate the production of immunity to *Salm. gallinarum* infection by the use of vaccines.

Apart from confirming the comparative uselessness of dead vaccines, the present report is concerned mainly with two attenuated live vaccines, one smooth and one rough, both of which were very effective in preventing the development of experimental infection. Investigations on an interference phenomenon, noted when these vaccines were employed at the same time as, or shortly after, oral infection with *Salm. gallinarum*, are also described.

MATERIALS AND METHODS

Unless otherwise stated in the text, all the materials and methods employed were exactly as described below.

Chickens. Sex-linked Brown Leghorn × Barred Rock cockerels from a salmonella-free flock were used.

Diet. Up to 5 weeks of age, chickens were fed on a conventional type of chick mash. After this time, they were maintained on a mash of the following composition, provided *ad lib.*: fish meal, 10%; decorticated ground nut meal, 5%; dried yeast, 2½%; maize meal, 10%; wheat meal, 15%; barley meal, 17%; oat meal, 15%; middlings, 19%; dried grass meal, 5%; minerals, 1½%.

Salm. gallinarum *cultures.* Vaccines, agglutination suspensions and challenge inocula were prepared from a smooth strain of *Salm. gallinarum*, strain 9, which was maintained throughout on Dorset egg medium. Freeze-dried specimens of strain 9 prepared at the commencement of the work were compared with the Dorset egg cultures from time to time; no variation in the virulence of the latter was noted. Two other smooth strains of *Salm. gallinarum*, nos. L and 584, were employed in some experiments. The smooth (S) or rough (R) nature of cultures was assessed by the acriflavine slide test (Braun & Bonestell, 1947.)

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(A) *Dead vaccines*

(1) *Killed cultures.* These were prepared from 48 hr. cultures grown on nutrient agar. They were washed off with 70% ethanol, centrifuged and resuspended in sterile normal saline. These suspensions were then adjusted by means of a nephelometer to contain 10^9 bacteria per ml.

(2) *Phage-lysed cultures.* A broth culture of strain 9 was lysed by means of bacteriophage and filtered through a Seitz E.K. filter.

(3) *Formolized tissue vaccine.* The livers of chickens, dead as a result of infection with strain 9, were macerated and a 20% suspension in sterile normal saline prepared, the resulting suspension containing approximately 40×10^6 viable *Salm. gallinarum* per ml. This was then incubated at 37° C. for 24 hr. after adding 0·3% formalin. Both phage-lysed and tissue vaccines were tested for sterility before they were used.

(B) *Live vaccines*

The exact method of preparation of live vaccines is described later. They were all employed as 24 hr. broth cultures containing approximately 50×10^7 viable bacteria per ml. and were administered subcutaneously in 1 ml. amounts.

Agglutination tests

Tube method. Twofold serial dilutions of sera in normal saline, commencing at 1 in 12·5 and in volumes of 0·5 ml., were set up in agglutination tubes and an equal volume of a suspension of *Salm. gallinarum*, strain 9, added. The latter was prepared in exactly the same manner as the dead culture vaccine. The tubes were incubated at 50° C. for 20 hr. before being read.

Plate method. This was performed in the manner described by Gordon & Brander (1942), using whole blood and a stained antigen prepared from strain 9.

The examination of faeces for Salm. gallinarum. Cloacal swabs, taken so as to include a liberal portion of faeces, were incubated at 37° C. for 24 hr. in selenite F medium (Hobbs & Allison, 1945). Subcultures were then made on to plates of deoxycholate-citrate agar containing 1% each of sucrose and salicin (Smith & Buxton, 1951). The plates were incubated at 37° C. for 24 hr. and examined for the presence of *Salm. gallinarum*.

The examination of egg contents for Salm. gallinarum. Any faecal material was first removed from each egg by means of a piece of cotton-wool soaked in 70% ethanol. The egg was then dipped in 70% ethanol and flamed, the process being repeated once. It was carefully cracked with a sterile knife and the contents tipped into a flask containing 150 ml. of selenite F medium, which was examined for *Salm. gallinarum* in the manner previously described.

Method of producing challenge infection. Chickens were fasted for 18 hr. and then given 0·1 ml. of an 18 hr. broth culture of strain 9, standardized on every occasion to the same nephelometer reading, in 1 ml. of distilled water containing 0·3 g. of alkali, the purpose of which was to neutralize the bactericidal action of the gastric juice in the gizzard. This amount of culture contained approximately 50 million viable *Salm. gallinarum* bacteria, and it was deposited in the oesophagus by means

of a Pasteur pipette, care being taken to avoid contaminating the epiglottis. The alkali employed was a powder of the following composition: powdered chalk, 40 %; colloidal kaolin, 43 %; magnesium trisilicate, 17 %.

Technique of experiments. Normally, chickens were vaccinated at 5–6 weeks of age. When live vaccines were employed the faeces of the vaccinated chickens were examined for the presence of *Salm. gallinarum* at 1 and 2 weeks later. Unless otherwise stated, the immunity of the chickens was challenged 3 weeks after vaccination, the sera of the chickens being examined at the same time for agglutinins to *Salm. gallinarum*. A group of non-vaccinated control chickens was included in each experiment. Chickens that died were examined to determine whether they had succumbed from either the acute or the chronic form of the disease, the lesions of which have been described previously (Smith, 1955*a*). Deaths from the acute form usually occur within 14 days of infection, later deaths being of the chronic type. Experiments were terminated 3 weeks after challenge, since deaths are relatively uncommon after this time. The surviving chickens were killed, their organs examined for lesions of the chronic form of the disease and their faeces for *Salm. gallinarum*. In some experiments, the blood of the survivors was also examined for agglutinins to *Salm. gallinarum*.

RESULTS

(A) *Dead vaccines*

The immunizing potency of one or two injections of dead cultures of *Salm. gallinarum* administered by different routes to groups of 35 chickens is illustrated in Table 1. The first and second injections, in 1 ml. amounts, were given 21 and 10 days respectively before challenge with strain 9. Apart from a slight increase in survival times, two doses of vaccine given by either the intramuscular or

Table 1. *The effect of dead cultures in producing immunity to* Salm. gallinarum *infection in groups of* 35 *chickens*

Route of vaccination	No. of times vaccinated	Average agglutination titre to *Salm. gallinarum*	No. of 35 chickens dead by the following days after challenge: 7–10 (acute)	10–14 (total acute)	15–21 (total acute and chronic)	No. of survivors: Total	With severe lesions	With mild lesions	Faecal positive*
Intravenous	Twice	1/1250 (2)	0	5	12	23	8	7	8
Intravenous	Once	1/125 (3)	2	14	18	17	4	9	9
Intramuscular	Twice	1/150 (5)	6	16	26	9	2	5	2
Subcutaneous	Twice	1/30 (23)	9	16	23	12	3	6	3
Controls	—	0	18	20	25	10	1	6	3

The 1st and 2nd vaccinations were carried out 21 and 10 days respectively before challenge. The figures in parentheses refer to the number of vaccinated chickens with no demonstrable agglutinins to *Salm. gallinarum*.

* Positive for *Salm. gallinarum*.

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subcutaneous route had little effect on the course of the disease. More satisfactory results were noted in the group that received two intravenous injections of vaccine, the total death-rate being only 12 compared with 25 unvaccinated controls. Of these 12, only 5 died from the acute form of the disease compared with 20 controls. However, lesions of the chronic form of the disease were found in 15 of the 23 survivors of the intravenous group, showing that in only a small number of chickens was there any evidence of complete immunity. Less satisfactory results were obtained in the group that received only one intravenous injection of vaccine. The average agglutination titre of the sera of chickens in the group that received two injections of vaccine intravenously was very much higher than in the other groups. However, when chickens in all the vaccinated groups were compared individually, no obvious correlation was observed between agglutination titre and survival time.

The results of vaccinating groups of 30 chickens subcutaneously with two 3 ml. injections of either filtered phage-lysed cultures of *Salm. gallinarum* or formolized suspensions of livers of chickens that had died from fowl typhoid, and then infecting them with strain 9 by mouth 21 days later, indicated that these procedures had, at the most, only a slightly beneficial effect on the course of the disease. As in the previous experiment no positive correlation was noted in individual chickens between agglutination titre and survival time.

(B) *Live vaccines*

The immunity produced by live cultures of Salm. gallinarum

Nine live vaccines, five smooth and four rough, prepared from strain 9 by various methods, were examined. All except one of the smooth vaccines, 9S, were rejected because they were too virulent. Similarly, all except one of the rough vaccines, 9R, were rejected because either the chickens vaccinated with them were found to excrete the vaccinal strain in their faeces and to develop agglutinins to smooth *Salm. gallinarum*, or the immunity they produced, although considerable, was not equal to that produced by 9R.

Table 2. *The effect of live vaccines in producing immunity to* Salm. gallinarum *infection in groups of* 15 *chickens*

Vaccine (1 ml. subcutaneously 3 weeks before challenge)	No. of chickens that died from vaccination	No. of chickens excreting vaccine strain in faeces at 7 and/or 14 days	Average agglutination titre produced by vaccine*	No. of chickens that died when challenged	No. of survivors		
					With severe lesions	With mild lesions	Faecal positive
9S smooth	0	0	1/1800	0	0	0	0
9R rough	0	0	0†	0	1	4	0
Controls	—	—	0	11	3	2	3

* To smooth *Salm. gallinarum*, 9.

† The serum of one chicken gave a slight agglutination reaction at 1 in 25; a negative reaction was obtained with the plate test.

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The results obtained with vaccines 9S and 9R are illustrated in Table 2. The lesions found at the end of the experiment in 5 of the chickens vaccinated with 9R were probably due to the challenge strain, since in neither experiment were chickens killed several weeks after vaccination with this strain ever found to possess lesions in their organs. After challenge, the sera of several of the chickens vaccinated with 9R developed titres of 1/25 to 1/400 to the challenge strain indicating that slight infection had occurred.

Both 9S and 9R had been prepared by continuous passage of strain 9 in a semi-synthetic medium at 20° C. A phage preparation active on this strain was added at one stage in the experiment that led to the production of 9R. After the final passages, the cultures of 9R and 9S were streaked on nutrient agar and single colonies selected after incubation. The semi-synthetic medium employed had the following composition: KH_2PO_4, 1·0 g.; Na_2HPO_4, $12H_2O$, 6·25 g.; sodium citrate, 1·5 g.; ammonium chloride, 1·0 g.; $Mg.SO_4$, $7H_2O$, 0·01 g.; $CaCl_2$, 0·0005 g.; $ZnSO_4$, 0·0001 g.; $CuSO_4$, 0·0001 g.; glucose, 1·0 g.; iron citrate, 0·0006 g.; distilled water to 1000 ml. A sterile solution of fraction V albumen was added in amounts sufficient to permit only a very slight growth of the vaccinal culture.

The immunizing ability of vaccine 9S against heterologous strains of Salm. gallinarum

The effect of using two strains of *Salm. gallinarum*, 584 and L, instead of strain 9, to challenge the immunity of groups of 20 chickens vaccinated 3 weeks previously with vaccine 9S is illustrated in Table 3. Apart from the fact that two of them

Table 3. *The immunizing ability in groups of 20 chickens of vaccine 9S against heterologous strains of* Salm. gallinarum

Vaccine	Infecting strain no.	No. of 20 chickens that died after challenge	No. of survivors: With severe lesions	No. of survivors: With mild lesions	No. of survivors: Faecal positive
9S	584	0	0	0	2
None	584	12	3	2	5
9S	L	0	0	1	2
None	L	10	4	4	6

were excreting *Salm. gallinarum* in their faeces 3 weeks after challenge, the vaccinated chickens exhibited a satisfactory immunity against infection with either of the two strains used for challenge.

The virulence of vaccines 9S and 9R for 1-day-old chicks: the effect of passage

The results of injecting groups of 20 1-day-old chicks intramuscularly with 0·1 ml. of a 24 hr. broth culture of either vaccine 9S, or vaccine 9R, or with the strain of *Salm. gallinarum* no. 9 from which they were derived, are shown in Table 4. None of the chicks injected with strain 9R died, neither did any of them appear unwell. Vaccine 9S was definitely virulent but less so than strain 9 as judged both by death-rate and survival times. Passage of 9S through chicks ten times did

not result in any increase in virulence. In later experiments, 9R was occasionally isolated from the eggs of laying hens a week or two after vaccination; in no case was change to the smooth form ever noted.

Table 4. *The virulence of vaccines 9R and 9S for 1-day-old chicks: the effect of passage*

Infecting culture	No. of 20 chicks that had died by the following days after infection								
	3	4	5	6	7	8	9	10	10+
9R	0	0	0	0	0	0	0	0	0
9S	1	4	12	12	13	13	13	13	14
9S 1st passage	2	8	17	17	17	17	17	17	17
9S 2nd passage	1	6	8	10	12	13	13	13	13
9S 3rd passage	1	4	9	11	12	13	13	13	13
9S 5th passage	2	8	12	14	14	14	14	14	14
9S 10th passage	1	4	5	8	11	11	11	11	11
9*	16	20	20	20	20	20	20	20	20

The infecting dose was 0·1 ml. of a 24 hr. broth culture given *intramuscularly*.
* Strain from which vaccines 9R and 9S were prepared.

The vaccination of seven-week-old chickens with 9S: the effect on weight gain, faecal excretion and future egg production

The total weekly weight of 70 7-week-old chickens vaccinated with 9S and of a similar number of unvaccinated chickens is illustrated in Table 5. The vaccinated chickens gained slightly less weight than the unvaccinated at 1, 2 and 3 weeks after vaccination. Weights were the same, however, at the fourth and subsequent weeks. No sign of ill-health was noted in the chickens at any time. *Salm. gallinarum* was

Table 5. *The effect of vaccination with 9S on the weight gain of 70 7-week-old chickens*

Chickens	Total weight in lb. of 70 chickens at the following weeks after vaccination				
	0	1	2	3	4
Vaccinated	84	102·5	118	146	193·5
Non-vaccinated	84	105	124	153	193

isolated from the faeces of 2 of the 70 chickens at 7 days after vaccination and from another 2 at 14 days, but not at 21 days. The females in both groups commenced laying eggs at approximately the same time. In the sixth week after commencement of lay 28 vaccinated hens laid 103 eggs and 34 unvaccinated hens laid 119. The contents of the first 100 eggs laid by the vaccinated hens were examined for *Salm. gallinarum*; the results were completely negative.

The persistence of agglutinins in the sera of chickens vaccinated with 9S

The average agglutination titres of the sera of a group of chickens at different times after vaccination when 7 weeks old with 9S is shown in Table 6. High titres noted 3 weeks after vaccination declined fairly rapidly by 12 weeks, after which the

decline was much slower, an average agglutination titre of 1/25 still being found at 34 weeks. No difference was noted between the persistence of agglutinins in males and in females. The fact that half of the group had been vaccinated with 0·2 ml. of a broth culture of strain 9S instead of the customary 1·0 ml. also made no difference.

Table 6. *The persistence of agglutinins to* Salm. gallinarum *in the sera of chicks vaccinated with 9S at 7 weeks of age*

Time after vaccination (weeks)	No. of chickens examined	Average agglutination titre	No. of chickens with no demonstrable agglutinins
3	70	1/900	4 (5·7 %)
7½	63	1/150	14 (22 %)
12	52	1/50	24 (46 %)
20	26	1/25	15 (58 %)
34	17	1/25	9 (53 %)

The duration of the immunity produced by 9S in chickens vaccinated when 7 weeks old

The result of challenging, at different times, the immunity of the chickens vaccinated with 9S described in the previous experiment is shown in Table 7. At 4 and 12 weeks after vaccination, the immunity produced by 9S was complete, but at 20 weeks two of the twelve vaccinated chickens, compared with ten unvaccinated controls, died when challenged. The chickens infected at 20 weeks were

Table 7. *The duration of the immunity produced by 9S in chickens vaccinated when seven weeks old*

Time after vaccination when challenged (weeks)	Chickens	No. per group	No. that died when challenged	No. of survivors			
				Total	With severe lesions	With mild lesions	Faecal positive
4	Vaccinated	12	0	12	0	0	0
	Control	12	8	4	1	2	1
12	Vaccinated	12	0	12	0	0	2
	Control	12	9	3	2	1	3
20	Vaccinated	12	2	10	—	—	0
	Control	12	10	2	—	—	0
34	Vaccinated	17	0	17	0	1	0
	Control	7	7	0	0	0	0

all actively laying hens in the early stages of the first laying period. Egg production ceased completely in the controls. It was reduced in the vaccinated hens for a period of approximately 14 days, commencing 10 days after challenge. The first 90 eggs laid by the vaccinated chickens after challenge were examined for *Salm. gallinarum*; this organism was isolated from one of them. Seventeen vaccinated chickens, comprising 11 hens at the very end of their first lay and 6 cockerels,

challenged 34 weeks after vaccination were completely immune; all of seven unvaccinated cockerels died. Half of the vaccinated chickens in these experiments had been vaccinated with 1·0 ml. of a broth culture of 9S and the other half with 0·2 ml.; no difference was noted in the immunity of these two groups.

The duration of the immunity produced by 9R in chickens vaccinated when 7 weeks old

The result of challenging the immunity of chickens at 4, 8 and 12 weeks after vaccination when 7 weeks old with 1·0 ml. of a broth culture of 9R is illustrated in Table 8. The total number of chickens vaccinated was 65; none of these was found to be excreting 9R in the faeces at 7 or 14 days after vaccination. At 21 days after vaccination, all were negative to the plate-agglutination test, but the serum of one gave a slight degree of agglutination with *Salm. gallinarum* at 1/25 in the tube test.

Table 8. *The duration of the immunity produced by 9R in chickens vaccinated when 7 weeks old*

Time after vaccination when challenged (weeks)	Chickens	No. per group	No. that died when challenged	No. of survivors			
				Total	With severe lesions	With mild lesions	Faecal positive
4	Vaccinated	20	0	20	2	5	1
	Controls	20	15	5	2	3	3
12	Vaccinated	20	0	20	0	6	1
	Controls	20	13	7	2	5	4
12	Vaccinated	20	3	17	0	3	1
	Controls	20	12	8	2	4	3

None of each of the two groups of 20 chickens challenged at 4 or 8 weeks after vaccination died, although a small proportion of them when killed had lesions of the chronic form of the disease. Eighteen of the 20 survivors challenged at 4 weeks and 12 of those challenged at 8 weeks developed agglutinins to *Salm. gallinarum* following challenge, the average titres being 1/250 and 1/150 respectively; the average titres of the surviving control chickens for each group were 1/1500 and 1/600 respectively.

Immunity 12 weeks after vaccination appeared less complete, 3 of 20 chickens dying compared with 12 of 20 unvaccinated chickens. Lesions, all mild, were found, however, in only 3 of the 17 survivors of the vaccinated group.

The immunizing effect of vaccines 9S and 9R in laying hens: their influence on egg production

The results of challenging the immunity of groups of 10 laying hens 3 weeks after vaccination with 9S or 9R, together with the egg-production records of each group before and after vaccination and challenge, are shown in Table 9. Both vaccines produced a complete immunity to the challenge infection. Vaccination with 9S had a marked depressing effect on egg production, which was not further affected by

challenge, egg production increasing during the 3 weeks after challenge. During the fourth week after challenge, blood samples were taken from the hens vaccinated with 9S, a procedure that was followed by a sharp decline in egg production; in view of this no further egg records were kept of the hens in this group. The hens vaccinated with 9R produced fewer eggs in the second week after vaccination than did the controls, but production increased considerably during the third week. A decline was also noted in the third week after challenge. However, in the fourth and fifth weeks, the hens in this group were laying considerably more eggs than were the unchallenged control hens. Egg production ceased almost completely in the group of control hens that were challenged, but commenced again during the fourth week after challenge.

Table 9. *The immunizing effect of 9S and 9R when used in groups of 10 laying hens: their influence on egg production*

Vaccine	Average agglutination titre produced	No. of 10 hens that died when challenged *	No. of survivors faecal positive	Total no. of eggs laid in following weeks: Before vaccination 1	After vaccination 1	2	3	After challenge 1	2	3	4	5
9R	0†	0	0	44	40	23	37	37	33	17	48	60
9S	1/1600	0	0	35	29	14	8	14	18	25	—	—
None (challenged)	0	8	0	48	56	52	48	24	1	1	3	9
None (not challenged)	—	—	—	41	57	53	43	40	47	38	33	25

* Three weeks after vaccination.

† The sera of 2 chickens gave a slight reaction at 1 in 25; both were negative by the plate test.

Cultural examination of 190 eggs laid by the 9R group of hens during the first 6 weeks after vaccination yielded 9R from 9 (4·7%), all laid during the first 3 weeks. None of the 190 laid after challenge was found to contain the challenge strain 9. Of 108 eggs laid by the 9S group in the first 6 weeks after vaccination, 5 (5%), all laid in the first 10 days, yielded 9S. *Salm. gallinarum* was not recovered from any of the 108 eggs laid after challenge. *Salm. gallinarum* was found in one of the 25 eggs laid by the control chickens during the first 20 days after challenge.

The effect of vaccinating chickens with 9S at different times before and after challenge

The immunity possessed by groups of 30 chickens vaccinated with 9S at different times in relation to the time of challenge is illustrated in Table 10. None of 30 chickens vaccinated at either 9, 6, 3 or 0 days before challenge died, compared with 21 of 30 unvaccinated controls. However, as the interval between vaccination and challenge decreased, chronic lesions were found more frequently in the vaccinated chickens; 14 of the 30 chickens vaccinated and challenged at the same time were so affected. The beneficial effects of vaccination were still obvious, but

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to a lesser degree, in chickens vaccinated 1 or 2 days *after* challenge. When the interval was 3, 4 or 5 days the course of the disease in the vaccinated chickens closely resembled that in the unvaccinated controls. The influence of vaccination 6 or 7 days after challenge was probably adverse, but most of the chickens vaccinated at this time were very ill and several died on the day of vaccination.

Table 10. *The effect of vaccinating groups of* 30 *chickens with* 9*S at different times before and after challenge*

Time of vaccination in relation to challenge	Total no. of 30 chickens that were dead by the following days after infection												No. of survivors			
	6	7	8	9	10	11	12	13	14	15	16	16+	Total	With severe lesions	With mild lesions	Faecal positive
9 days before	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	1
6 days before	0	0	0	0	0	0	0	0	0	0	0	0	30	1	4	3
3 days before	0	0	0	0	0	0	0	0	0	0	0	0	30	3	8	2
Same time	0	0	0	0	0	0	0	0	0	0	0	0	30	3	11	6
1 day after	0	0	0	0	1	1	2	2	2	2	2	3	27	7	5	13
2 days after	0	1	2	3	7	7	7	9	9	9	9	9	21	5	5	9
3 days after	1	5	6	8	12	13	15	18	19	21	22	22	8	4	3	—
4 days after	0	5	9	12	15	17	17	20	21	22	22	22	8	4	3	—
5 days after	0	2	4	5	9	13	13	14	14	17	17	20	10	6	3	—
6 days after	3	10	16	19	23	23	24	25	26	27	27	27	3	2	1	—
7 days after	0	6	9	15	18	20	22	23	23	25	25	26	4	2	2	—
Not vaccinated	1	4	8	13	17	18	19	21	21	21	21	21	9	3	3	2

The effect of injecting chickens with 9*S*, 9*R and other materials at the same time as they were infected with strain* 9

The course of *Salm. gallinarum* infection in chickens injected with 9S, 9R or other materials at the same time as they were infected by mouth with strain 9 is illustrated in Table 11. A similar type of interference phenomenon was noted with 9R as with 9S, although examination of the survivors indicated that it was probably slightly less effective in the case of 9R. The injection of heat-killed cultures of strain 9 either intravenously or subcutaneously had, by comparison, only a slight influence on the course of the disease. Somewhat similar effects were obtained by injecting broth cultures of two strains of *Bacterium coli* as were obtained with the dead cultures of strain 9, better results being obtained with *B. coli* A, a pathogenic strain, than with *B. coli* B, a non-pathogenic strain. *B. coli* A when injected into chickens produced either death within 4 days or survival; it was necessary to employ 40 chickens in this experiment to obtain 30 that survived the *B. coli* A infection. Of the two 'non-specific' materials examined, the intravenous injection of 2·0 ml. of autoclaved milk possibly had a slightly beneficial effect on the course of the disease. The results of injecting 0·8 ml. of indian ink intravenously were quite different; 20 chickens so injected died from *Salm. gallinarum* infection within 7 days, all 30 being dead by 11 days.

The interference phenomenon was also noted when 9S was injected at the same time as infection by mouth with a different culture of *Salm. gallinarum*, strain L; no effect was observed when heat-killed 9S was used instead of live 9S.

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Table 11. *The effect of injecting groups of* 30 *chickens with* 9*S*, 9*R and other materials at the same time as they were infected with strain* 9

Material injected	Total no. of 30 chickens dead by the following days after infection												No. of survivors			
	6	7	8	9	10	11	12	13	14	15	16	16+	Total	With severe lesions	With mild lesions	Faecal positive
9S	0	0	0	0	0	0	0	0	0	0	0	0	30	3	11	6
9R	0	0	0	0	0	0	0	0	1	1	1	1	29	11	9	14
1·0 ml. heat-killed culture of strain 9 intravenously	0	0	2	5	9	13	13	13	13	13	13	14	16	3	9	8
1·0 ml. heat-killed culture of strain 9 subcutaneously	0	1	3	5	5	9	11	12	13	13	14	18	12	6	3	5
1·0 ml. broth culture of *B. coli* A subcutaneously	0	0	0	2	3	4	5	6	8	8	8	9	21	12	4	9
1·0 ml. broth culture of *B. coli* B subcutaneously	0	0	1	1	3	7	7	9	9	12	12	16	14	8	3	8
2 ml. autoclaved cow's milk intravenously	0	0	2	3	6	10	10	12	12	14	15	16	14	7	3	5
0·8 ml. indian ink intravenously	8	20	24	27	30	30	30	30	30	30	30	30	0	0	0	0
None	0	1	4	8	13	17	18	19	21	21	21	21	9	3	3	2

DISCUSSION

It was found in previous work (Smith, 1955*b*) that chickens in which furazolidone treatment was commenced as late as 6 days after oral infection with *Salm. gallinarum* were usually susceptible to re-infection; a solid immunity, however, was exhibited by chickens in which treatment was commenced after this time. It was apparent from this observation that a relatively powerful stimulus was necessary to provide a satisfactory degree of immunity, much more powerful than that which would be expected to be produced by dead cultures of *Salm. gallinarum*. The present results have shown that this, in fact, is the case. The smooth vaccine, 9S, produced a more complete immunity than did the rough vaccine 9R. Whether this was due to the fact that it was more virulent and hence produced a greater immunogenic stimulus and/or because it might contain some immunogenic component not present in 9R, is not known. At the commencement of the studies it was naturally assumed that the smooth O antibodies were associated with immunity. This view was supported by the observation that the chickens vaccinated intravenously (twice) with dead cultures possessed higher serum agglutinins and also a slight but better immunity than those vaccinated by other routes. However, when the individual chickens in each group were compared, no correlation was found between the level of serum agglutinins and the length of survival time. Furthermore, when it was found that the live rough vaccine, 9R, produced a good immunity in

chickens yet did not produce antibodies that agglutinated smooth strains of *Salm. gallinarum*, it became apparent that this view might need revising. Later work (Smith, 1956*a*) too, showed that a live, incompletely rough, strain of *Salm. dublin* produced in chickens a high level of O agglutinins against a smooth *Salm. gallinarum* antigen yet, in contrast to a smooth *Salm. dublin* strain, it produced no immunity against *Salm. gallinarum* infection. It is clearly extremely difficult to determine the precise part played by any single factor in immunity to an infection such as that caused by *Salm. gallinarum* in chickens since many inter-related factors obviously operate. The dangers of over-simplification must be avoided.

It is difficult to account for the fact that, of the chickens vaccinated at 7 weeks of age with 9S, those challenged 20 weeks later, during the active laying period, were not completely immune to challenge, but those challenged at 34 weeks, at the end of the laying period, were. Previous work (Smith, 1955*a*) with unvaccinated chickens had shown little difference to exist between the susceptibility of chickens at these two periods, a fact that was confirmed by comparing the results for the unvaccinated control chickens in the present experiments. It can only be assumed that the immune mechanisms, as distinct from the normal defence mechanisms, functioned more satisfactorily in the chickens at the end of the laying period than in those in the actively laying period.

The interference effect was first observed during the study of immunity to virus infection and was thought to be a feature of such systems only. A somewhat similar phenomenon has now been found to apply fairly generally in bacterial immunity. This does not infer, of course, that the mechanism by which it is brought about is the same in both systems. Apart from its discovery here in *Salm. gallinarum* infection, the phenomenon has also been noted in mice infected with *Haemophilus pertussis* (Evans and Perkins, 1954, 1955) and *Bact. coli* (Rowley, 1955) and in rats infected with *Pasteurella pestis* (Parry, 1955). In the present studies, interference was still noted to occur in chickens injected with strain 9S two, but not three, days after oral infection with *Salm. gallinarum*. It is significant in this respect that bacteraemia does not usually occur in chickens until 3 days after oral infection (Smith, 1955*a*). None of the other substances, including heat-killed *Salm. gallinarum*, evoked an interference effect in any way so marked as did the live smooth and rough vaccines, 9S and 9R, indicating that the effect of these live vaccines appeared to be specific in this respect. Indian ink was employed in the interference experiments because Parry found it produced increased resistance in *Pasteurella pestis* infection. Although the amount of ink was less than that employed by Parry, the chickens so injected exhibited a very decreased resistance to *Salm. gallinarum* infection compared with control chickens, probably because the indian ink had blocked the reticulo-endothelial system to a degree that had impaired its satisfactory functioning. The fact that fewer chickens injected with the pathogenic strain of *Bact. coli*, strain A, at the same time as they were given *Salm. gallinarum* by mouth died from the latter infection, compared with chickens given *Salm. gallinarum* only, was unexpected. It could be argued that the *Bact. coli* itself had eliminated from the experiment the chickens most susceptible to *Salm. gallinarum* infection, but this explanation could not wholly account for

the low death-rate observed. It would appear, from this experiment, that the simultaneous exposure of chickens to two quite different infections had resulted in the overall disease picture being less severe than that which would have been expected to occur from one of them (*Salm. gallinarum*).

From the practical point of view, the live vaccines 9S and 9R might well be of considerable value in the control of *Salm. gallinarum* infection in the field. Vaccine 9S would appear to be the most useful since the immunity it produces is more complete and more prolonged. Vaccine 9R, however, has two advantages. First, it does not produce agglutinins detectable by the plate test and, consequently, it is conceivable that it could be employed in flocks in which *Salm. pullorum* infection is controlled by blood testing. Secondly, it produced only a slight drop in egg production when used in laying hens. Although another drop occurred when the hens vaccinated with this culture were infected, it is noteworthy that at 4–5 weeks after infection they were laying considerably more eggs than were the corresponding group of uninfected control hens, indicating that vaccination and subsequent infection only inhibited egg production temporarily and appeared to have no effect on the total number of eggs laid during a laying period. Since the interference phenomenon occurred with both live vaccines, they could probably be employed with advantage during an outbreak of *Salm. gallinarum* infection in a flock of chickens since many of the chickens would be uninfected or in the early stages of the disease. There would be no advantage in vaccinating chickens in the later stages of the disease. In fact, the results indicate that the course of the disease may be adversely influenced if chickens critically ill are vaccinated with 9S. The fact that passage of 9S through chicks did not increase its virulence and the fact that 9R was never found to revert to the smooth form either *in vivo* or *in vitro* increase confidence that these changes would not readily occur in the field. It would be wise, however, in the case of 9S to vaccinate only a few chickens in any flock as a preliminary since it is known that different breeds of chickens vary in their susceptibility to *Salm. gallinarum* infection (Smith, 1956*b*), some breeds being more susceptible than the one employed in the present study. Eggs laid by hens during the first few weeks after vaccination with 9S should not be used for hatching as some of them may contain this vaccinal strain. This would be of less importance in the case of 9R as this strain appeared to be harmless for day-old chicks. It should be borne in mind also that the recommendations made here are based on observations on experimental *Salm. gallinarum* infection; quite different conditions might apply in natural outbreaks of this disease.

SUMMARY

1. A good immunity was produced in chickens against oral infection with *Salm. gallinarum* by the use of either of two live attenuated vaccines, one smooth, 9S and one rough, 9R. Dead vaccines had little or no effect.

2. The immunity possessed by chickens vaccinated with 9R at 7 weeks of age commenced to wane 12 weeks later. Chickens similarly vaccinated with 9S were completely immune 4, 12 and 34 weeks later. When tested during the active laying

period (at 20 weeks after vaccination) their immunity, although substantial, was not complete.

3. Vaccine 9R did not produce demonstrable agglutinins against smooth *Salm. gallinarum* in chickens. It was non-lethal to 1-day-old chicks and was never found to revert to the smooth virulent form *in vitro* or *in vivo*. By contrast, vaccine 9S produced agglutinins and was lethal for 1-day-old chicks, its virulence not being increased by passage. No deaths were ever observed when this vaccine was used in either chickens of 5–12 weeks old or laying hens.

4. Although both vaccines produced a good immunity when employed in laying hens, vaccination with 9S was accompanied by a marked reduction in egg production; this was not the case with 9R.

5. An immunity of the interference type was also evoked by both live vaccines. This immunity was still produced to some extent in chickens injected with 9S, 2 days *after* they were infected with *Salm. gallinarum* by mouth.

6. The interference effect could not be produced by the use of either dead *Salm. gallinarum* vaccines or certain non-specific substances. The intravenous injection of indian ink had a marked adverse effect on the course of the disease. Injecting chickens with a virulent strain of *Bacterium coli* at the same time as they were infected with *Salm. gallinarum* resulted in the latter infection running a milder course than would normally have been expected.

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