Pullorum Disease and Fowl Typhoid with particular reference to their control and prevention

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Thesis for the Degree of
Doctor of Veterinary Medicine and Surgery

by

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Introduction

Although many types of salmonella have been isolated from domestic poultry, only two are responsible for specific diseases, Salmonella pullorum the cause of pullorum disease (previously known as bacillary white diarrhoea or B.W.D.) and Salmonella gallinarum the cause of fowl typhoid. Both organisms are non-motile and antigenically identical and are distinguishable only by slightly differing fermentative reactions; these differences are so insignificant that some authorities, mostly European, regard them as variants of a single organism. This view is not acceptable to most British and American bacteriologists who consider the organisms to represent distinct types giving rise to separate diseases of widely differing epidemiology.

Fowl typhoid, mainly a disease of adult fowls, has shown a striking increase in incidence during the past decade and chicks are now becoming more often affected than formerly. The incidence of pullorum disease on the other hand has waned, but through altered methods of poultry husbandry, the effect of individual outbreaks is very much greater.

Both are important diseases of poultry which may cause high mortality with consequent serious economic loss. The thesis describes the author's investigations into the epidemiology of these diseases and methods of

control and prevention during the past 30 years.

An earlier review and other relevant papers published by the writer during the period are attached for reference.

PULLORUM DISEASE

Historical

Pullorum disease first became prominent towards the end of last century in the U.S.A. where it was known to poultry farmers as "White diarrhoea". The causative organism, later classified by systematic bacteriologists as Salmonella pullorum was first isolated in 1899 by Rettger (1900) who described the disease as a "Fatal septicaemia of young chicks". It later became known as bacillary white diarrhoea (Rettger and Stoneburn 1909) and more recently as pullorum disease.

There is no record of its introduction into Great Britain. The small losses in hen-hatched chicks would not attract much attention and it was not until the great expansion in poultry keeping which occurred after the 1914-1918 war that pullorum disease became prominent. With the growing use of artificial incubation losses increased rapidly and the disease presented a serious problem to the recently established industry. In a review of the incidence of poultry diseases in Scotland during the period 1913 to 1932, Matheson and Wilson (1933) noted that out of 559 outbreaks of disease affecting chicks, pullorum disease was responsible for 278, almost half of which occurred during the last two years of the survey.

Investigations into the nature and mode of transmission of the disease were carried out in U.S.A.

and in 1909, Rettger and Stoneburn reported the isolation of the causative organism from the ovaries of hens whose progeny had succumbed to the disease and from the yolks of a proportion of the eggs laid. Later Rettger, Kirkpatrick and Jones (1914) established that infected chicks may become permanent carriers and transmit the disease to their progeny by way of the infected egg. Infected chick down was shown to be a means of transmitting the disease within the incubator (Hinshaw, Upp and Moore 1926). In 1931 Wilson (1948) found that an acute outbreak with a high mortality could be produced by spraying day-old chicks with a broth culture of Salm. pullorum, which suggested that inhalation and not ingestion was the usual method of infection.

The possibility of detecting carriers of the disease by the agglutination test was reported by Jones (1913) and from 1914 onward the test was used in the U.S.A. for this purpose. In several states official eradication programmes were in force in the early 1920's and have been expanded greatly since then.

Facilities for the routine agglutination testing of flocks in Great Britain were first provided at the Ministry of Agriculture and Fisheries Veterinary Laboratory at Weybridge in 1924 and at the Royal (Dick) Veterinary College, Edinburgh, in 1925. Testing at first was on a very small scale; at Edinburgh less than 14,000 tests were carried out during the first five

years. In the next year almost as many tests were carried out as during the former period and in the following year the number had multiplied five-fold. The impetus to increased testing was provided by the serious and widespread losses from pullorum disease which followed the advent of the mammoth incubator which gradually replaced the multiple small still-air machines which had been in vogue until then. Not only were very much larger numbers of eggs set in a single machine, but the action of the air-circulating fans caused infection to be carried to all parts of the incubator and its continuous use, with one third of the eggs hatching each week, permitted a carry-over of infection to successive hatches.

The establishment of hatcheries drawing supplies of hatching eggs from several sources and distributing chicks over a wide area, with consequent increased risks of acquisition, propagation and dissemination of pullorum disease, focussed further attention on preventive measures and regular agglutination testing of hatchery-supply farms became the accepted procedure.

Testing had been encouraged by the Department of Agriculture for Scotland by giving an additional bonus in 1929 to participants in the Poultry Improvement Scheme, which had been in existence since 1912, who had their stock tested, and in 1931 it became compulsory and remained so in the Accredited Poultry Scheme which

superseded the former scheme in that year. By its organised and continued use a great reduction in the incidence of pullorum disease has been effected.

Pullorum disease is essentially one of chicks, but from time to time outbreaks occur in adult fowls, usually through the feeding of infected incubator waste, bakers' sweepings or similar material containing egg products. During the period under review ten outbreaks in turkey poults and two outbreaks in ducklings were recorded; all were associated with contact with infected chicks.

The organism is seldom pathogenic for man but several cases of food poisoning have been reported in the literature in which infected eggs were implicated. An extensive outbreak affecting over 400 people was described by Mitchell, Garlock & Broh-Kahn (1946) and a recent outbreak involved three children in a hospital who had raw eggs in milk a short time before (Anon 1956).

Transmission of the disease within the incubator

The effect of exposing chicks to varying weights of infection artificially produced within the incubator was investigated by the writer in 1931 (Wilson 1948, 1955a). At that time it was commonly believed that ingestion of food and water contaminated with Salm. pullorum was chiefly responsible for natural outbreaks but difficulty was experienced in reproducing the disease experimentally in chicks which received drinking water to which washings of agar cultures of recently isolated Salm. pullorum were added.

Results were uncertain; even in day-old chicks the mortality was never as high as in natural outbreaks and chicks over three days old were refractory as far as the production of disease with any appreciable mortality was concerned.

The examination of almost 300 chicks which had died from pullorum disease, from over 70 outbreaks, showed 66.4 per cent to have gross lesions in the lungs. This was considered suggestive of inhalation infection, an opinion which had previously been expressed by Bunyea and Hall (1929).

It was found that by spraying a suspension of Salm. pullorum over healthy day-old chicks and returning them to the incubator to dry off, a mortality of up to 100 per cent could be produced. The death rate varied according to the proportion of chicks sprayed, e.g. to

produce an outbreak with an almost negligible mortality and a high proportion of carriers amongst the survivors it was sufficient to spray the down of the heads of two chicks or of the back of one chick and place them in an incubator with a group of healthy chicks. When equal amounts of Salm. pullorum suspension were administered to equivalent groups of chicks by spraying and adding to the drinking water, the mortality resulting from the former method was about four times greater than that from the latter.

As a result of these experiments, a standard procedure has been adopted for infecting day-old chicks with any of the salmonellae. Saline washings of nutrient agar cultures of the test organism are used at the rate of 10 ml. for 150 to 200 day-old chicks. During the process of spraying and for 30 minutes afterwards the chicks are held either in an incubator at 100°F. or in a large cardboard container in a warm room. They are then divided into random groups for experimentation.

Spraying with Salm. pullorum and Salm. gallinarum results in a high mortality. The course of the disease follows a standard pattern. Deaths begin on the fifth day and reach a peak on the sixth day with about the same number of deaths on the seventh day as on the fifth, e.g. in three experiments involving groups of 45 to 48 chicks the daily death rates were, fifth day 9, 9, 10, sixth day 24, 23, 23, and seventh day 9, 8 and 11. With

other members of the Salmonella group, the mortality is variable; strains appear to differ considerably in pathogenicity.

Apart from the ease of administration and regularity of infection, this method has the additional advantage that as it closely simulates the natural mode of dissemination of infection and produces outbreaks of disease similar to those occurring in the field, it is likely to provide a reliable indication of the practical value of any drug or other control measure which proves successful experimentally.

From the results of these early experiments it was evident that the severity of an outbreak of pullorum disease is related to the weight of infection to which chicks are exposed within the incubator. In natural outbreaks this depends on the virulence of the organism and its concentration in the large amount of fluff and dust circulating in the incubator during the process of hatching, to which the chicks are exposed from the time of "pipping" of the eggs to their removal as finished chicks for dispatch or transfer to the brooder.

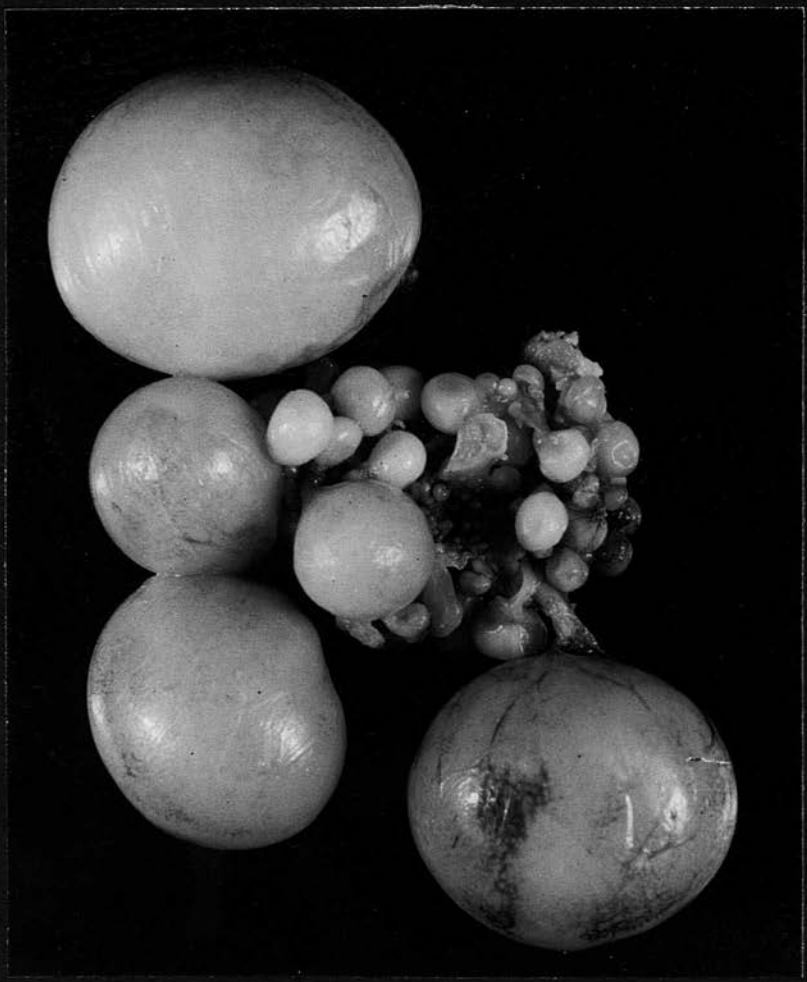
At hatching time viable Salm. pullorum is present in the egg contents only, so the final concentration of infection to which chicks are exposed depends on the number of infected eggs which hatch. Contamination of the outside of the shell which is of major importance in the causation of Salmonellosis plays little if any

part in the spread of pullorum disease. Jones (1910) was unable to isolate the organism from artificially infected shells after 21 days' incubation and Gwatkin (1926) found that it died out on the shell after 12 days at 37°C. A large number of tests have been carried out by the writer with many strains of the organism and it has invariably died on egg shells in the incubator, in from three to four days.

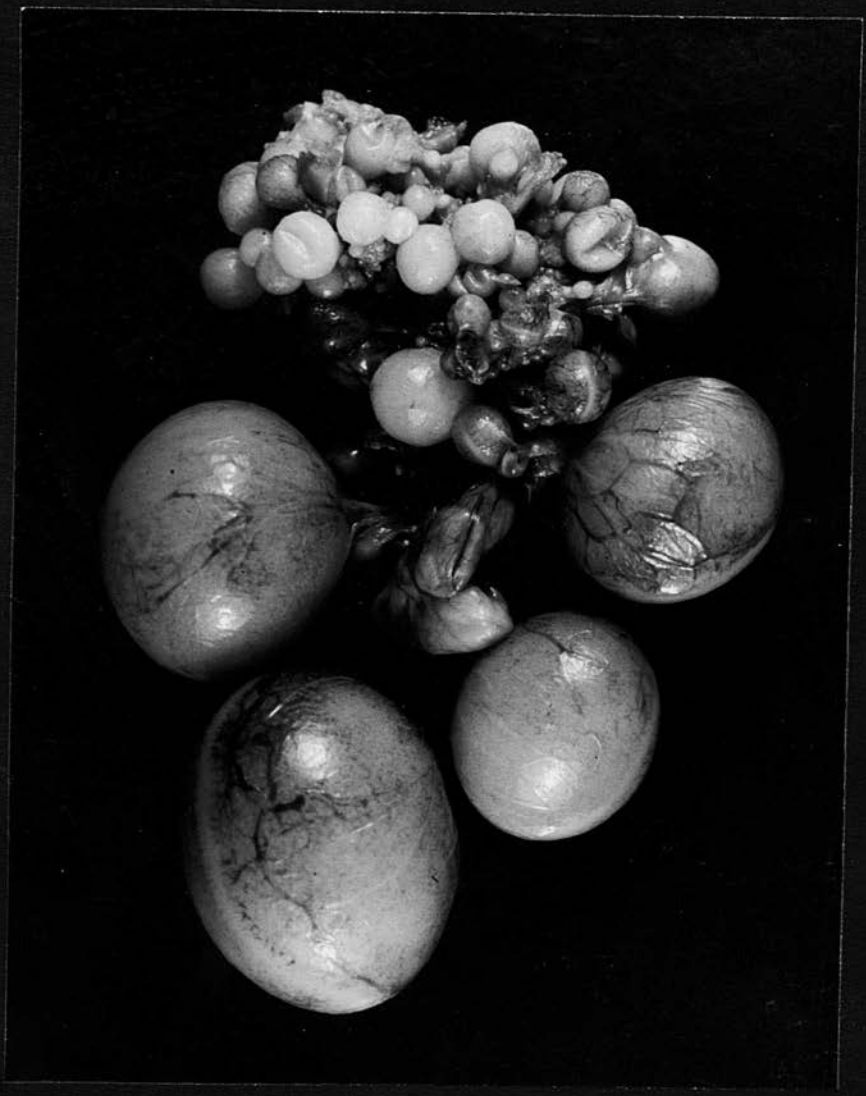
The infected egg

There is great variation in the number of infected eggs laid by carriers. Rettger and Stoneburn (1909) found nine of 44, Gage, Paige and Hyland (1914) 32 of 619, Gwatkin (1925) 20 of 240, Doyle (1925) nine of 341, Kaupp and Dearstyne (1927) 96 of 1,313 and 131 of 2,505 and Wilson (1931) 25 of 310.

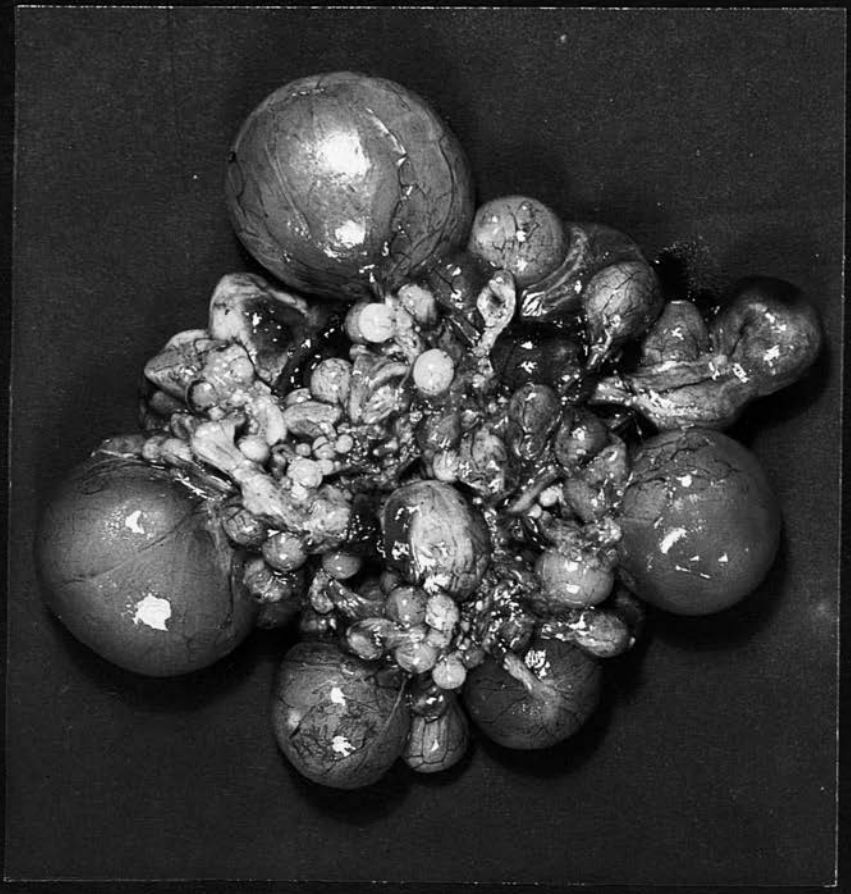
In the period under review, autopsies on more than 1,000 reactors revealed striking differences in the ovaries. Some were fully active and showed no macroscopic evidence of disease and in others pathological changes were slight and often consisted of a single small shrunken or large mis-shapen ovule in an otherwise normal ovary. In some cases normal and diseased ovules were present in about equal proportions and in others degenerate ovules only were present, often attached to the ovary by long stalks. Sometimes pathological ovules had been shed from the ovary and were found free



Normal ovary
Laying fowl



Diseased ovary
several small distorted ovules
Laying fowl



Diseased ovary
numerous small distorted ovules
Laying fowl

Diseased ovary
very large distorted ovules
Laying fowl

Diseased ovary
two very large distorted ovules
Non-laying fowl

Diseased ovary
complete degeneration
many large & small distorted ovules
with long stalks

in the abdomen or adhesed to the peritoneum. In some birds, the ovary was completely quiescent with the ovules undeveloped and apparently normal.

A series of photographs shows typically affected ovaries.

The wide range of ovarian changes suggested a corresponding diversity in the number of infected eggs laid by reactors and the proportion and periodicity of such eggs were studied by trap-nesting eight reactors, selected at random, for a period of eight months.

The procedure used for the bacteriological examination of eggs was that described by Wilson (1945) except that cultures were not made from the outside of the shell and the selective medium used was selenite F medium (Hobbs and Allison 1945) modified by the substitution of the Sorenson salt ($Na_2HPO_4 2H_2O$) by sodium beta-glycero-phosphate ($Na_2C_3H_5(OH)_2PO_4$) 1 per cent. Trials have shown that this modification not only ensures a better inhibitory effect but also results in stronger growths of Salm. pullorum.

The results are summarised in Table I.

Salm. pullorum was isolated from 137 of 578 eggs laid (23.7 per cent) with an individual variation of 0 to 58 (71 per cent).

Some birds laid single infected eggs sporadically, some in groups of two and three and one laid a clutch of three, two clutches of six and a sequence of nine, all

TABLE I

The incidence and periodicity of infected eggs laid by carriers of *Salm. pullorum*

	LS 280			RIR 300			LS 296			LS 299			RIR 1463			RIR 73			RIR 96			RIR 295		
	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern	Eggs Laid	Eggs +	Pattern
4/2/57 to 10/3/57	12	0		25	10	4/2 2/1	8	6	3/2	18	3	3/1	19	1		5	1		13	9	9 in succession	7	4	4 in succession
11/3 to 14/4	21	0		26	4	1/2 2/1	10	3	1/1 1/2	10	2	2/1	20	1		1	0		10	7	6 in succession	12	3	1/2 1/1
15/4 to 22/5	28	0		26	9	2/2 5/1	16	6	1/3 3/1	0	0		5	0		2	0		9	8	6 in succession & 1/2	18	5	1/2 3/1
23/5 to 30/6	9	0		28	9	1/3 1/2 4/1	4	3	1/3	0	0		9	2	2/1	0	0		6	3	3 in succession	10	3	1/2 1/1
1/7 to 7/8	27	0		26	8	1/3 5/1	2	0		0	0		23	1		0	0		0	0		12	2	2/1
8/8 to 15/9	14	0		21	10	1/4 1/3 1/2 1/1	0	0		0	0	killed	23	6	1/4 2/1	0	0		0	0		0	0	
16/9 to 23/10	16	0		12	8	1/4 4/1							15	6	2/2 2/1									
	127	0		164	58	35.4%	40	18	45%	28	5	17.8%	114	17	14.9%	8	1	12.5%	38	27	71%	59	17	28.8%

\+ = *Salm. pullorum* isolated
1/2 = 1 sequence of 2+ eggs
2/1 = 2 single + eggs

MacConkey plate
Typical growth of normal
Salm. pullorum on left
no growth of atypical strain of
Salm. pullorum on right

Blood-agar plate
Both typical and atypical
strains of Salm. pullorum
grow equally well

within a short period, after which it ceased to lay. The low egg production of five of the eight birds is characteristic of many carriers.

The higher incidence of positive isolations compared with those reported by earlier workers is probably attributable to the superior media now available.

Salm. pullorum is recognised to be a delicate and sparse grower on ordinary media. Several strains have been isolated at Lasswade which failed to grow on MacConkey media but grew well on blood agar and for many years it has been a routine procedure to make cultures on both these media from every batch of chicks received for diagnosis.

The agglutination test in the control and prevention of pullorum disease

General testing programme 1926 to 1939

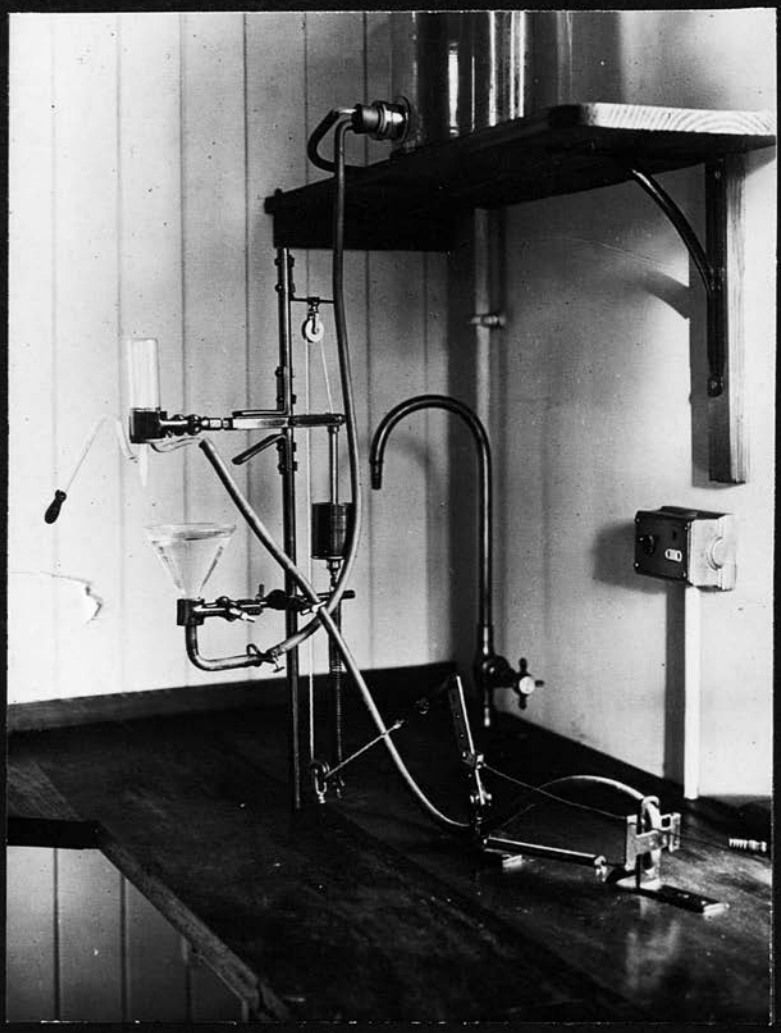
The results of agglutination testing of flocks in Scotland during the period 1926 to 1939, are summarised in Table II. The figures refer to blood samples from flocks in the official schemes of the Department of Agriculture for Scotland and from general flocks.

During the early years few flocks were tested and the carrier rate revealed was high. This was reflected in the prevalence of the disease in chicks concurrently received for post-mortem diagnosis. From 1927 to 1930 of 472 chicks examined by the writer, Salm. pullorum was isolated from 288 (61 per cent).

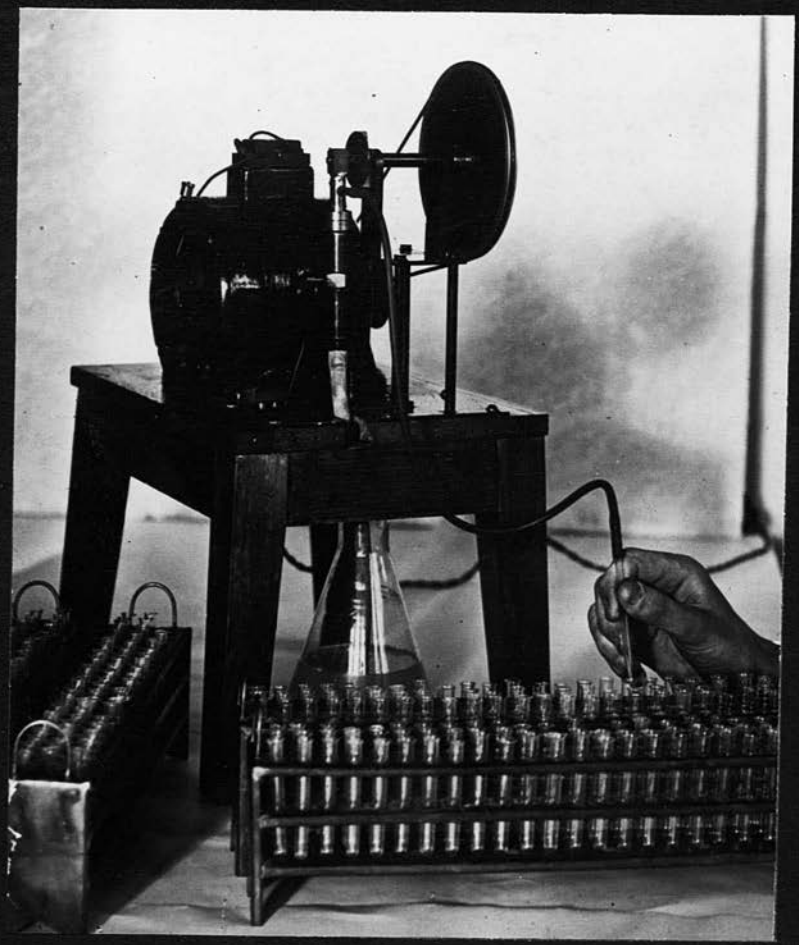
TABLE II

Results of Agglutination Tests
Scotland 1926-1939

Year	Bloods Tested	Bloods Positive	Percent Positive
1926	608	127	20.8
1927	1448	344	23.7
1928	762	104	13.1
1929	3673	360	9.8
1930	7224	742	10.3
1931	13484	1579	11.7
1932	77821	5722	7.4
1933	93222	5120	5.6
1934	116882	4792	4.1
1935	125784	4780	3.8
1936	121622	2797	2.3
1937	120208	2529	2.1
1938	164872	3956	2.4



Pipetting apparatus
For transferring measured amounts of serum from the blood phials to the agglutination tubes



Automatic pipette
Electrically driven
delivers 1 ml. antigen
at rate of 80 per minute

The beneficial effect of testing was shown by the comparative absence of reactors in the progeny of tested flocks, and the steady overall reduction in the percentage of reactors in a greatly increasing number of samples received annually. Systematic retesting was not carried out except in the case of participants in the official schemes, in which it was compulsory. As a result, in 1938 while the overall percentage of reactors was 2.4 it was only 0.5 per cent in official flocks and no outbreak of pullorum disease was recorded in them. The proportion of females and males tested was about 12 to 1 and the proportional reactor rate 8 to 1 e.g. in 1931 approximately 12,500 females were tested ans 1,564 (12.5 per cent) reacted. Of just over 1,000 males tested 15 (1.5 per cent) reacted.

Until 1929 three dilutions were used for each test (1/25, 1/50 and 1/100) but from 1930 the test consisted of a single tube only, at a dilution of 1/30 and later 1/20, and to cope with the increasing number of tests the operation was mechanised. A pipetting apparatus, based on that described by Beaudette (1929) for measuring serum, was built by the writer and by its use an experienced operator could transfer between 500 and 600 samples of serum per hour from the blood phials to the agglutination tubes. Antigen was added by means of the apparatus described by Sheather (1928) but this proved to be slow and was replaced in 1930 by a simple

TABLE III

Results of Agglutination Tests Accredited Flocks in Scotland 1939-1956

Year	Flocks	Flocks with no reactors	Percent with no reactors	Bloods tested	Bloods positive	Percent positive	Outbreaks pullorum disease
1939	108	79	73	36,814	164	0.44	0
1940	102	78	76	34,396	204	0.59	1
1941	112	93	83	31,354	122	0.39	1
1942	135	101	75	39,464	157	0.40	8
1943	157	129	82	49,000	363	0.74	4
1944	226	163	72	113,643	637	0.60	15
1945	478	400	84	235,635	653	0.28	13
1946	730	566	78	354,203	1,147	0.32	15
1947	700	564	81	376,639	1,109	0.29	8
1948	789	719	91	474,576	421	0.09	9
1949	862	766	89	554,425	790	0.14	8
1950	922	831	90	610,739	1,365	0.22	9
1951	953	881	92	610,551	898	0.13	9
1952	943	881	92	614,120	718	0.12	5
1953	892	826	93	611,513	846	0.12	6
1954	816	781	96	546,646	370	0.06	3
1955	777	744	95	542,063	370	0.06	3
1956	732	697	95	556,580	558	0.1	3

electrically driven pump, built on the spring ball-valve principle, which automatically delivered one millilitre of antigen at the rate of 80 per minute. By the use of these machines the mechanical part of the testing programme was undertaken by a single worker.

Testing of Flocks in Official Schemes - 1939 to 1956

The results of agglutination testing of accredited flocks in Scotland, during the above period, given in Table III, indicate steady progress towards the eradication of pullorum disease.

In 1939 the number of flocks participating in official schemes was relatively small and provided about one fifth of all samples received for testing. About three quarters of the accredited stations were completely free from reactors and the overall percentage of reactors was less than 0.5. During the last three years of the survey, with seven times as many stations, 95 per cent are without reactors and the percentage has been reduced to less than 0.1. This exemplifies the effect of repeated retesting at regular intervals, until clear, of all flocks in which reactors are revealed at the initial test.

The increased number of stations in which reactors were present in 1944 is attributable to the influx of new flocks into the Accredited Scheme as a result of the ending of the Preferential Feeding Stuffs Scheme which had been instituted as a temporary war-time measure to

TABLE IV

Results of Agglutination Tests
Preferential Feeding Stuffs Scheme 1941-1943

	Flocks	Flocks with no reactors	Percent with no reactors	Bloods tested	Bloods positive	Percent positive
1941	493	278	56	106,603	3,565	3.3
1942	539	312	58	144,669	2,895	2.0
1943	515	345	67	138,000	1,513	1.1

TABLE V

Results of Agglutination Tests

Probationer Flocks 1939-1956

	Flocks	Flocks with no reactors	Percent with no reactors	Bloods tested	Bloods positive	Percent positive
1939	23	11	48	4,067	284	6.9
1940	33	27	82	7,961	46	0.6
1941	44	28	64	17,492	249	1.4
1942	42	24	57	16,272	310	1.9
1943	131	79	60	32,000	538	1.7
1944	598	407	68	222,875	2,670	1.2
1945	396	267	67	155,119	1,168	0.8
1946	198	125	63	91,427	971	1.1
1947	301	200	66	117,769	1,190	1.0
1948	410	303	74	142,590	1,000	0.7
1949	387	289	75	161,828	1,383	0.9
1950	232	178	77	106,309	419	0.4
1951	124	105	85	49,612	148	0.2
1952	93	86	92	33,241	94	0.3
1953	115	104	91	42,917	195	0.4
1954	107	100	93	35,878	75	0.2
1955	87	77	88	36,189	77	0.2
1956	104	95	91	52,397	201	0.4

facilitate the allocation of poultry feeding stuffs. When flocks participating in this scheme were tested in 1941, some possibly for the first time, reactors were present in almost half with an incidence of three per cent (almost ten times as great as in accredited flocks).

The figures are given in Table IV.

Although retesting was not carried out in flocks where reactors were found, the single annual agglutination test brought about a significant reduction in the reactor rate within the short time the scheme was in force. In 1943 when it ended the percentage of reactors was 1.1 compared with 0.74 for accredited flocks.

Before a flock is accepted for the Accredited Scheme it undergoes a period of probation during which the stock is subjected to agglutination testing; the results of these tests are in Table V and confirm the value of organised agglutination testing in the elimination of pullorum disease. In 1939 reactors were present in more than half the flocks with an incidence of nearly seven per cent. During the last five years about 90 per cent of the stations have been without reactors and the average incidence has been between 0.2 and 0.4 per cent.

For comparative purposes some testing figures from the U.S.A. are given in Table VI. Large scale agglutination testing was begun there in the middle and late 1920's and the percentage of positive birds in different states ranged from 2.4 to over 20. The

TABLE VI

Agglutination Testing in U.S.A.
Some State results in 1953

	Birds Tested	Percentage Positive
Delaware	546,379	0.021
Maine	1,365,314	0.027
Maryland	815,250	0.18
Massachusetts	1,155,359	0.04
New Hampshire	1,512,219	0.00006
New Jersey	1,025,449	0.035
New York	810,619	0.0035
North Carolina	1,668,830	0.056
Vermont	234,282	0.09

TABLE VII

Results of Agglutination Tests
Accredited Turkeys
1946-1956

	Scotland		England	
Year	Tested	Failed	Tested	Failed
1946	818	4	5655	10
	21		450	1
1947	154	1	10118	24
	86		1120	2
1948	2199		14592	43
	289		1768	2
1949	3707	2	19278	3
	443		1936	
1950	3189	1	21779	2
	886		944	
1951	3882		17127	6
	1297		0	
1952	6659		25492	1
	179		399	
1953	7056		30777	6
	262		5851	2
1954	9459		38841	11
	1953		5268	1
1955	14384		55488	8
	715		3089	
1956	12595		71860	
	750		7412	
	70983	8	339244	122

The lower annual figures refer to probationer flocks

figures show the great reduction in the carrier rate that has been achieved during a slightly longer comparable testing period.

Pullorum disease in turkeys is a serious problem in parts of the U.S.A. and in other countries where large scale turkey rearing is practised. The disease is rare in Scotland, where there are few large turkey breeding farms. It will be seen from Table VII that only 8 reactors have been found during the past 11 years. The English figures, given for comparison, also show a low incidence.

In 1948 the rapid whole blood test which had been in operation in England since 1942, became the official method of testing flocks in the Accredited and Poultry Improvement Schemes. The antigen is prepared at Lasswade and issued to the Ministry's Divisional Veterinary Officers whose staff are responsible for carrying out the test.

The Rapid Whole Blood Test for Pullorum Disease

Following the work of Huddleston and Carlston (1926) on a rapid serum test for contagious abortion, Runnells, Coon, Farley and Thorp (1927) showed that a similar test could be used for pullorum disease. Schaffer, MacDonald, Hall and Bunyea (1931) and Schaffer and Bunyea (1933) demonstrated that a rapid test using whole blood and a dense suspension of Salm. pullorum stained with crystal violet was an effective method for the detection of carriers and the test now in general use is based largely on their work. Following a series of comparative laboratory and field trials carried out by the writer in 1935, in which almost complete agreement was obtained between the rapid and tube tests, stained antigen was produced at the Royal (Dick) Veterinany College, and used extensively in commercial flocks, particularly in Lancashire.

The rapid whole blood test was adopted as the official test for fowls in the Accredited Scheme in England in 1942 and in Scotland in 1948 and for turkeys in both countries in 1954. Its chief advantages are that the fowls need only be handled once and reactors disclosed are immediately available for disposal. With the tube test much handling may be necessary in the search for reactors; there is a possibility of faulty identification through clerical errors in

completing the card accompanying the samples and a risk of breakage of tubes and deterioration of the samples during transit. In spite of these obvious drawbacks the tube test remains the standard method of testing both for fowls and turkeys in several states in U.S.A.

Interpretation of Rapid Whole Blood Test

Typically positive and negative bloods present no difficulty and need not be discussed but doubtful reactions characterised by the formation of smaller clumps, lighter in colour and occurring either throughout the mixed blood and antigen or more commonly at the periphery may be confusing. A reaction of this nature may be indicative of a low agglutination titre for Salm. pullorum or Salm. gallinarum or may be associated with agglutinins for other bacteria which have common antigenic factors with Salm. pullorum e.g. Salm. enteriditis, Salm. typhimurium and paracolon and coliform bacteria. A second test two to three weeks later is usually sufficient to determine the significance of the reaction or birds may be submitted for bacteriological examination. A very weak reaction is classified in the field as "pin-point" and is generally considered to be of little pathological significance. Stronger reactions are denoted "doubtful" and qualified -, +, ++, +++ as appropriate. Results of bacteriological examinations over a period of years confirm the general accuracy of the field assessments. Salmonella serotypes

TABLE VIII

Bacteriological Examination of Reactors
to the Rapid Whole Blood Test
Accredited Stations 1956-1957

Type	Number	Number Positive	Per Cent Positive	Salm. pullorum	Salm. gallinarum	Salm. typhimurium
Pin Point	81	10	12.3	7	0	3
Doubtful	61	18	29.5	14	4	0
Multiple	264	197	74.6	145	44	8
Single	158	124	78.5	97	25	2
Total	564	349		263	73	13
Per Cent				75.4	20.9	3.7

+ = Positive salmonella isolation.

were isolated from only 10 of 81 (12 per cent) "pin-point" reactors and from 18 of 61 (30 per cent) doubtful reactors.

Birds are also submitted for bacteriological examination when positive reactors occur in a flock which has been previously clear; of 264 received during 1956 and 1957 Salm. pullorum was isolated from 154, Salm. gallinarum from 44 and Salm. typhimurium from 3. The incidence of Salm. gallinarum is higher than would be expected from accredited flocks generally but many of the birds submitted were from the north of England, where fowl typhoid is very prevalent in some areas.

A single reacting bird is always submitted for bacteriological examination. In a flock with a recent history of reactors it usually represents a bird which was too immature at the time of the previous test, but single reactors sometimes appear in flocks which have been clear for many years. During 1956 and 1957, 158 single reactors were examined; Salm. pullorum was isolated from 97, Salm. gallinarum from 25 and Salm. typhimurium from 2.

The bacteriological examination of over 500 pin-point, doubtful and positive reactors is summarised in Table VIII Approximately 75 per cent were infected with pullorum disease, 20 per cent with fowl typhoid and less than 4 per cent with Salm. typhimurium.

TABLE IX

Positive isolations of Salm. pullorum in chicks submitted for diagnosis

	Accredited Stations		Purchased from Accredited Stations		Non-designated		Total batches of chicks examined
	Scotland	England	Scotland	England	Scotland	England	
1953	6	14	26	7	32	37	1706
1954	3	16	17	10	27	34	1530
1955	3	11	14	53	34	26	1590
1956	3	17	15	13	52	53	1723
1957	0	5	19	10	51	30	2013
TOTAL	15	63	91	93	196	180	8562

Total Batches of Chicks Examined

	Accredited	Non-designated
1953	1,000	706
1954	940	590
1955	994	596
1956	958	765
1957	1,144	869
Total	5,036	3,526

Incidence of pullorum disease in Accredited Flocks

During the period 1939 to 1956 there were 120 outbreaks of pullorum disease on Accredited farms in Scotland. From 1938 until 1941 when the number of stations was small and practically stationary there were only two outbreaks but as has been shown in Table III a greatly increased incidence coincided with the sudden expansion of the Accredited Scheme in 1942 and was maintained for three years during each of which there was an average annual increase of more than 100,000 tests. During the next five years the incidence remained steady at either eight or nine outbreaks since when the gradual decline in the number of stations has been accompanied by a marked fall in the number of outbreaks. In 1957, the figures for which are not included in Table III, pullorum disease was not diagnosed on any Accredited Station in Scotland.

The comparative incidence of pullorum disease in accredited stocks, in stock purchased from accredited stations and in non-designated stock in Scotland, and in the six northern counties of England for which Lasswade also caters, is shown in Table IX and provides evidence of the value of the disease control methods in force in the Accredited Scheme and indicates what might be achieved by their extension to poultry flocks in general.

Therapeutic treatment

Smith (1954) showed that furazolidone was effective against pullorum infection in chicks and concluded that the most satisfactory therapy was 0.04 per cent of the drug in the mash continuously for 10 to 14 days.

Wilson (1955a) compared the effect of furazolidone at this level with sulphaquinoxaline (0.05 per cent) in the drinking water. When furazolidone was administered 72 hours after infection none of the treated chicks died, compared with a mortality of 94 per cent in the control group, and Salm. pullorum was isolated from one chick only of 48 destroyed after treatment had ceased. There was a mortality of 33 per cent in the group which received sulphaquinoxaline and Salm. pullorum was isolated from all the survivors.

Furazolidone is unlikely to be as effective in natural outbreaks for while the weight of infection to which chicks are exposed is likely to be lighter than in the artificially produced disease the disease will be more advanced when treatment is begun. It is probable that the death rate will be higher and a greater proportion of the surviving birds will be carriers. In an experimental outbreak when treatment was delayed until the chicks started to die the final mortality was about one third of that of untreated controls and more than

30 per cent of the survivors excreted the organism.

The treatment of adult carriers of Salm. pullorum with furazolidone was investigated by Wilson (1956a). It failed in most cases to cause a significant or permanent lowering of the agglutination titre and Salm. pullorum persisted, usually in the distorted ovules, but was never isolated from any egg laid after treatment.

FOWL TYPHOID

Historical

The earliest recorded outbreaks of fowl typhoid were in Northern Italy (Peroncitto 1877) and in England (Klein 1889). Beaudette (1925, 1930) reviewed the early history of the disease and considered that it first occurred in U.S.A. in 1894 (Moore 1895). An account of its later distribution in U.S.A. is provided by Moore (1946$_a$).

In Great Britain, fowl typhoid appeared for many years to remain localised, becoming endemic in parts of Wales and the neighbouring counties of England, and in some districts in the north of England, notable in Yorkshire. The disease has existed in Northern Ireland for many years but until 1948 it only occurred sporadically and showed no tendancy to spread. The number of outbreaks never exceeded one to two per year but in 1948 five outbreaks were diagnosed and this number had increased to 61 in 1950. Apart from seven sporadic outbreaks in different areas of Northern Ireland all the other outbreaks during the period 1948 to 1950 occurred in a comparatively restricted area of County Down (Luke, Gordon and Gracey, 1951). In Scotland, the disease was practically confined to the Island of Lewis where it was believed to have been introduced from the mainland of Scotland early this century by the importation of fowls under the auspices of the Congested Districts Board

(Wilson, 1940).

It was generally supposed that the prevalence of fowl typhoid in a particular part of the country was indicative of an unhygienic environment, often the result of the continued practice of backward methods of poultry husbandry which had been given up elsewhere. Thus the disease was common where fixed poultry houses were in use. These were often stone-built and with earthen floors, difficult if not impossible to clean and disinfect satisfactorily even if an attempt were made to do so, and necessitating the continuous use for many years of the adjacent land for poultry keeping.

During the last decade there has been a striking increase in the incidence of fowl typhoid. Accurate figures for the overall incidence of the disease are not available but from 1953 to 1956 the number of cases diagnosed at the laboratories of the Ministry of Agriculture, Fisheries and Food, at Weybridge, Lasswade and the Veterinary Investigation Centres, rose each year by about 100. In 1956 the total number was 572 but this dropped to 483 in 1957.

Not only has the disease increased in districts where it was considered to be endemic but it has appeared in areas where previously it was unknown. An exception to this general statement is the almost complete disappearance of fowl typhoid from the Isle of Lewis, where it had been a serious source of loss for

TABLE X

Fowl typhoid

Location of Scottish outbreaks

		Lasswade	North of Scotland Veterinary Investigation Laboratory	West of Scotland Veterinary Investigation Laboratory
1953	6	2 Caithness 1 Rossshire 1 Ayrshire 1 Midlothian 1 Kirkcudbright	7 6 Aberdeenshire 1 Lewis	0
1954	10	3 Ayrshire 2 Moray 2 Midlothian 1 Aberdeenshire 1 Stirlingshire 1 Wigtownshire	14 13 Aberdeenshire 1 Lewis	0
1955	5	2 Stirlingshire 1 Aberdeenshire 1 Banffshire 1 Midlothian	6 3 Aberdeenshire 1 Banffshire 1 Kincardine 1 Moray	1 Ayrshire
1956	7	2 Aberdeenshire 2 Stirlingshire 1 Dumbartonshire 1 East Lothian 1 Peeblesshire	5 4 Aberdeenshire 1 Lewis	0
1957	7	3 Ayrshire 1 Berwickshire 1 Dumfries-shire 1 Lanarkshire 1 West Lothian	2 2 Aberdeenshire	0
	35		34	1

There were no outbreaks diagnosed at the East of Scotland Veterinary Investigation Centre.

more than 50 years; in some years it was responsible for the death of about half the total poultry population. Only four outbreaks have been diagnosed since 1951, when following widespread outbreaks of Newcastle disease in the Western Isles a high proportion of the poultry population was slaughtered and the premises depopulated and disinfected under the terms of the Fowl Pest Order.

Although fowl typhoid has now a wider distribution in Scotland than formerly, outbreaks have been sporadic and widely separated and there has been no tendency towards local spread. The locations of the outbreaks are given in Table X. Fowls are most commonly affected; outbreaks in turkeys are unusual (only six have been met during the period of the survey) and the disease is rare in other species. Fowls of any age may be affected but laying pullets appear to be particularly susceptible. Somewhat surprisingly outbreaks in young chicks as a result of incubator infection, have been relatively rare in Great Britain but are increasing. In 1956 and 1957 there were 13 and 11 outbreaks respectively and in 1956 three outbreaks occurred in turkey poults.

A striking feature of the increased incidence of fowl typhoid throughout the country is that outbreaks have not been confined as formerly to unsatisfactory and unhygienic premises but have occurred on farms on which the poultry stock and methods of husbandry are of

a high standard. It is difficult to give any ready reason for these changes. It might have been thought, with some justification, that the general large-scale agglutination testing of poultry for pullorum disease, which has proved so successful in eliminating that disease from most flocks and in drastically reducing the carrier rate in the remainder, would have resulted in a lowered incidence of fowl typhoid because the same test detects carriers of both diseases.

In most poultry-keeping areas, where up-to-date methods of husbandry and disease prevention are practised by the majority of poultry farmers, there are odd nondescript premises with inferior stock and faulty methods of management, and where agglutination testing is neglected and these may provide reservoirs of infection from which disease may spread to surrounding farms. This is supported by the results of a survey the writer carried out in 1953 in the North and East Ridings of Yorkshire, to compare the prevalence of fowl typhoid carriers in accredited and non-designated flocks. A proportion of fowls which gave positive reactions at the routine test for Salm. pullorum was obtained for bacteriological examination. Of 79 reactors from 15 accredited flocks Salm. gallinarum was isolated from 20 (3 flocks), Salm. pullorum from 55 (8 flocks), dual infection with Salm. gallinarum and Salm. pullorum from

TABLE XI

Outbreaks of Fowl Typhoid
in Scotland and North of England,
diagnosed at Lasswade.

	Scotland		England	
	Accredited	Non-designated	Accredited	Non-designated
1953	1	5	9	63
1954	0	10	14	55
1955	0	5	13	74
1956	2	5	23	110
1957	0	7	19	85
Total,	3	32	78	387

The total number of outbreaks of infectious disease investigated is not available but the numbers of birds received for examination from accredited and non-designated flocks provide a means of comparison of the prevalence of fowl typhoid in these flocks.

Number of fowls received for examination

	Accredited	Non-designated
1953	2,536	4,613
1954	2,461	4,657
1955	2,488	5,779
1956	3,533	7,523
1957	3,943	6,593
Total	14,961	29,165

a single flock and Salm. typhimurium from another. From 262 reactors from 31 non-designated flocks Salm. gallinarum was present in 201 (18 flocks) Salm. pullorum in 52 (4 flocks) and in 9 flocks there was evidence of dual infection representing an incidence of Salm. gallinarum infection of 87 per cent compared with an incidence of 27 per cent in accredited flocks.

The benefit of regular agglutination testing and the other disease control measures practised on accredited farms is demonstrated by the low incidence of fowl typhoid on them compared with the incidence on non-designated farms. The comparative figures for the period 1953 to 1957 are given in Table XI.

Changed methods of husbandry, and the more general keeping of poultry on farms other than specialised poultry farms, may have played a part in the increased incidence of the disease and changes in the causative organism itself may be partly responsible; strains of Salm. gallinarum from some outbreaks appear to have a greater virulence, and to be more tenacious, than formerly.

Epidemiology

The causative organism is present in the natural secretions of birds affected with the acute disease, and there is rapid spread of the disease by the ingestion of food and water soiled by infected droppings. Infection may also be carried on the boots and clothing of workers and on feeding utensils and other equipment in regular use on the farm.

A proportion of recovered birds become carriers; some of them may excrete Salm. gallinarum in the droppings intermittently for several months. Many carriers however, cease eliminating the organism in the droppings soon after recovery, and the chief focus of remaining infection is the diseased ovary.

The hatching of one or more infected eggs in the incubator may result in an outbreak of the disease in chicks. The use of infertile eggs and unhatched eggs for feeding to poultry, or the careless disposal of incubator waste, may lead to the occurrence of the disease in adult stock. The prevalence of the vice of egg eating may also sometimes be responsible.

The improper disposal of carcases of birds which have died from the disease is one of the commonest means of spreading fowl typhoid. Not infrequently carcases are left unburied, and thrown under hedges or into ponds or ditches where they may be accessible to cats, dogs,

rats or wild birds, with the result that portions of the carcase may be transported to neighbouring farms and provide fresh foci of infection.

Salm. gallinarum has been recorded by various investigators in several of the commoner wild birds, and at least once in the intestines of a rat (Luke, Gordon and Gracey, 1951) so that these species may act as more than merely mechanical carriers of the disease. Jennings (1954) isolated the organism from a blackbird and a curlew and Harbourne (1955) from rooks and from a pigeon and a partridge from areas where the disease was rife.

It is important, however, not to exaggerate the significance of the occasional isolation of Salm. gallinarum from wild birds. It is probable that a few individuals acquire transient infection from a local outbreak of the disease in poultry but from the results of the examination at Lasswade of nearly 2,000 wild birds, representing over 70 species, it appears unlikely that there is any general or widespread infection of wild birds. Salm. gallinarum was isolated from 36 birds; from sparrows, blackbirds and wood pigeons from the sites of outbreaks and from a single grouse, owl and goldfinch.

The organism is readily destroyed by the usual disinfectants and by exposure to direct sunlight. Ground does not appear to remain infected for long after the removal of infected birds, which are the main source of infection.

The factors which precipitate an outbreak are not always recognised; but in many cases over-population, sometimes temporary, is responsible by permitting a build-up of infection; for example, serious outbreaks have followed a spell of wintry weather when birds have been confined to the houses for several days on end, and a disastrous outbreak in turkeys originated in a small and over-crowded pen in which broody turkey hens were isolated.

The infected egg

The importance of the infected egg as a means of spreading fowl typhoid has been noted and investigations have been made into the proportion of such eggs laid by recovered birds.

Records in the literature are scanty; published figures include, 9 per cent (Moore 1946_b) 10 per cent (Simms 1946) and 6 per cent (Hall, Legenhausen and MacDonald 1949_a). Doyle (1926) failed to recover Salm. gallinarum from any of 140 eggs laid by carriers and this was the earlier experience of Wilson (1948). Later, Wilson (1955_b) found 108 positive eggs out of

TABLE XII

Bacteriological examination of eggs from fowl typhoid carriers during a period of 9 months

Bird	Eggs laid	Eggs +	Per cent +	Distribution of + eggs
1	62	9	15	1st egg laid was + followed by 19- then single + eggs at long intervals
2	99	37	37	One sequence of 5+ 3/3+, 10/2+ and 3/1+ spread over whole period
3	128	35	27	1/3+ several 2+ and single + evenly distributed throughout
4	102	9	9	Single + eggs at regular intervals in early stages. Last 36 eggs -
5	72	27	38	1/3+, 4/2+ remainder single + at intervals throughout period
6	31	25	81	Sequences of 6, 9 and 6 + eggs with single healthy eggs intervening.
7	120	54	45	1/9+, 1/5+, 6/3+, 5/2+ and 13/1+ throughout period
8	151	8	5	Single + eggs with 40, 25, 44 and 14 healthy eggs intervening.
	965	204	27	

\+ = Salm. gallinarum isolated
1/4+ = A sequence of 4+ eggs
4/1+ = 4 single + eggs

1,843 eggs laid by 21 carrier fowls (18 per cent). The individual production of infected eggs varied from 5 to almost 40 per cent. From another group of 14 recovered birds, 53 positive eggs were detected from 150 eggs laid (35 per cent) during a period of two to three months, with an individual variation of 0 to 80 per cent. As a result of advanced ovarian degenerative changes one bird did not lay any eggs and the egg production of eight of the others was between one and nine; five of these birds did not lay positive eggs (Wilson 1956a).

A study of the distribution of infected and healthy eggs throughout the laying season was made by keeping a pen of 14 carriers under observation for nine months (November to July). The birds were in individual cages on wire floors and all eggs were examined bacteriologically by the method already described. After three months the eggs of six birds which had not produced a positive egg were discarded.

The results are in Table XII.

Very wide individual differences exist in the production of infected eggs, ranging from five to 80 per cent and the varying pattern of their distribution as described earlier in carriers of Salm. pullorum was evident; some birds tended to lay single infected eggs or groups of two or three and others sequences of five and six and in two cases as many as nine.

These investigations confirm the potential danger

of the eggs laid by recovered birds in the spread of the disease.

The Viability of Salm. gallinarum in different habitats

The association of outbreaks of fowl typhoid with the careless disposal of carcases left unburied or improperly buried or thrown into ponds or ditches led to an investigation of the survival of Salm. gallinarum in carcases under such conditions artificially reproduced in the laboratory.

The carcases of ten fowls which had died of acute fowl typhoid five to seven days after inoculation with a virulent strain of Salm. gallinarum were buried in a plot of land at a depth of about two feet and at the same time five carcases, similarly prepared, were placed in a large water tank situated outside and exposed to normal weather fluctuations throughout the period of the experiment which was from January to October.

Salm. gallinarum was viable in carcases exhumed after 98 days, 140 days and 186 days but not in the remaining carcases which were exhumed after 249 and 260 days respectively.

The organism was isolated from carcases which had been in static water for 80 days and 123 days but not in the remains of carcases which had been immersed for longer periods.

The carcases of 15 birds which had died from fowl

TABLE XIII

Viability of Salm. gallinarum in different environments (in days)

Material	Conditions	0°C.	Room Temp.	Outside Temp.
Carcase	Buried			186
	Immersed in static water			123
	Stored in refrigerator			486
Chaff	Dry Sterile	1138*	900*	
	Moist Sterile	95	74	
	Natural State	103	63	100
Sand	Dry Sterile	370*	228*	
	Moist Sterile	45	29	
	Natural	106	106	
Faeces	Dry Sterile	439*	228*	
	Moist Sterile	50	45	
	Normal	29	24	31
Deep Litter		48	102	70
Water	Static	41	38	41

* The figures are the maximum obtained from several strains tested. The average survival figure is much lower.

typhoid were retained in a cold store at 0°C for varying periods of time. Salm. gallinarum was isolated from ten carcases after storage for up to 486 days. Carcases removed at 545 and 561 days showed evidence of decomposition and this was advanced in the three remaining carcases which were removed after 572 days of storage; Salm. gallinarum was not isolated from these birds.

The lengthy survival of Salm. gallinarum in carcases stresses their potential danger as sources of infection.

Further viability studies showed that Salm. gallinarum is capable of living for a very long time under experimental conditions, particularly in a dry environment. Strains vary in their viability; four strains persisted in dry sterilised chaff for 439, 466, 1,125 and 1,138 days respectively. The organism died more rapidly in normal chaff, usually in 60 to 100 days. In dry sand, strains lived for from 98 to 370 days, in sterilised dry faeces for 70 to 182 days, in sterilised moist faeces for about 50 days and in normal faeces for 26 to 31 days. Odd strains persisted very much longer in some media e.g. two strains persisted in dry faeces at 0°C for 416 and 439 days but this was exceptional. In deep litter, Salm. gallinarum usually died out after 60 to 70 days and in static water strains were never viable longer than 38 to 41 days.

The main findings are summarised in Table XIII.

Spread of fowl typhoid by wild birds

The peculiar disposition of single deaths from fowl typhoid in widely separated pens on premises where fowl typhoid had recently occurred in birds kept wholly intensively led to an investigation of the method of spread.

Agglutination tests showed that there were no carriers in the flock but sporadic losses continued after special precautions were taken to eliminate the hands, clothing and boots of workers, and equipment and utensils as the possible means of spread. The litter and droppings from the infected house had been placed in a central manure pit on which large numbers of wild birds, mostly sparrows and blackbirds fed regularly; they also fed at feeding troughs in the hen runs.

Circumstantial evidence that the disease was spread by these birds was provided by the deaths from acute fowl typhoid of two fowls in coops with open fronts, while 18 birds kept alongside in similar coops with the fronts netted to prevent access of birds remained healthy. A number of blackbirds and sparrows were trapped near the contaminated manure pit and although none showed evidence of gross disease Salm. gallinarum was isolated from both species. It appeared that the infection in the wild bird was transient; specimens examined later were uniformly negative.

Spread of fowl typhoid by the house fly (Musca domestica) and the blue-bottle (Calliphora erythrocephala)

Circumstantial evidence that fowl typhoid might be transmitted by the blue-bottle and house fly was obtained when two of six turkeys housed in a wire floored verandah with solid roof and netted sides died from the acute disease. The verandah was situated at a distance of 24 feet from the infected litter and droppings which attracted large numbers of flies and blue-bottles. It was protected on three sides by walls eight feet high which enclosed the stacked manure and although this minimised the chances of air-borne infection the possibility that it occurred cannot be entirely eliminated.

Control and Prevention

In the past the customary recommendations for dealing with fowl typhoid have been prompt slaughter of ailing birds and the use of the agglutination test to detect carriers, followed by vaccination of the remainder of the flock and attention to disinfection and improved methods of hygiene and husbandry. In general, this procedure was reasonably successful in controlling the disease, but within recent years there has been a growing reluctance to make use of the agglutination test and to carry out adequate disinfection and a tendency to rely more on vaccination and chemotherapy.

There is much to recommend the retention of the agglutination test. It is the best and quickest method of removing the main source of infection which is the carrier fowl. The agglutination test is always carried out when the disease occurs in any flock in the official poultry schemes, and it should be done also in non-designated flocks producing chicks or hatching eggs.

The "carrier", apart from remaining a potent source of infection is often an uneconomic egg-producer on account of the degenerative changes in the ovary associated with the disease and although recovered birds are generally resistant to further exposure to the disease (Smith, 1955) immunity may be only temporary. The disease may flare up and deaths from virulent fowl typhoid may occur (Wilson 1954). This was also noted

by Hall, MacDonald and Legenhausen (1949) who reported that of 55 birds which had reacted strongly for about a year 14 eventually died of acute fowl typhoid.

Therapeutic Treatment

Drugs of the sulphonamide series are effective in reducing mortality from fowl typhoid, but a high proportion of recovered birds become carriers of the disease and for this reason the use of sulphonamides is not to be recommended. Furazolidone is to be preferred (Smith, 1955_b; Wilson 1955_a); a concentration of 0.04 per cent in the mash for a period of ten days is recommended. It is more effective in reducing mortality and, provided sick birds are treated in the early stages of the disease, the carrier rate is low.

Results of flock treatment are sometimes unsatisfactory; there may be a marked improvement during the period of administration of the drug and for a short time afterwards but, unless the birds are removed to fresh quarters after treatment (and sometimes even when this is done) the disease may reappear.

Furazolidone is probably most effective in outbreaks of fowl typhoid in batches of chicks which have been exposed to infection about the same time, for example within the incubator. If treatment is begun immediately the disease is diagnosed, results are good and few of the treated birds become carriers. When treatment is

delayed, it is less effective and a high proportion of survivors continue to excrete Salm. gallinarum (Wilson, 1955_a).

The effect of furazolidone on "carriers" was investigated by Wilson (1956_a). It was found that although there was a temporary fall in titre in some birds, a majority remained positive to the agglutination test and in quite a high proportion Salm. gallinarum could be isolated from the ovary (particularly from the distorted ovules) and occasionally from other organs. Treated birds, however, almost invariably ceased to lay infected eggs. Later work has confirmed these findings.

Vaccination against fowl typhoid

For many years a stock vaccine was available to poultry farmers from the Ministry of Agriculture's Veterinary Laboratory at Weybridge. It is unnecessary here to describe its preparation, but briefly it consisted of broth cultures of Salm. gallinarum killed by the addition of formalin and phenol. It was difficult to assess its real value for, while good results apparently followed its use, in many cases preliminary testing leading to the removal of carriers and improved hygienic procedures must have played an important part in reducing infection, and some of the success ascribed to vaccination may have been largely attributable to these measures.

Vaccination against fowl typhoid with killed cultures of Salm. gallinarum is carried out to a limited extent in the United States of America but there is a dearth of evidence to substantiate its efficacy. Hall, MacDonald, and Legenhausen (1949) reported the results of trials with 15 different dead vaccines, none of which was successful. When the interval between vaccination and exposure was short (two to three weeks) mortality was less than when the interval was over three weeks. After 30 days there was no indication of protection.

In Northern Ireland some success has been claimed for a dead autogenous vaccine which appeared to give protection for six to eight weeks (Luke, Gordon and Gracey, 1951).

Small-scale experiments (Wilson, 1946) suggested that a dead vaccine, either stock or autogenous gave little protection against fowl typhoid and later work confirmed this (Wilson, 1947_a). Good results were obtained with an attenuated live vaccine but there were practical difficulties attendant on its use in the field. Apart from the dangers inherent in the administration of a potentially pathogenic organism vaccinated birds reacted to the agglutination test for pullorum disease and as this would have precluded its use in accredited flocks, work with living vaccines was discontinued.

During 1953 and 1954 further trials with dead vaccines were carried out by Wilson (1956_b) but they

provided no protection against experimentally induced fowl typhoid and a large-scale field trial with one of the vaccines showed it to be ineffective in preventing the natural disease.

The failure of dead vaccines to induce immunity in fowls against a strong challenge with Salm. gallinarum is perhaps not surprising. Although killed vaccines are widely used in protecting man against typhoid, it has been pointed out (Topley and Wilson, 1955) that while T.A.B. vaccination probably affords some degree of protection against slight and occasional exposure to infection it has little or no effect when exposure is severe and frequent; against paratyphoid it appears to be even less efficacious. It is considered that vaccination would form a poor substitute for sanitary control in a population exposed to a severe and continuous risk of infection.

Live vaccines

When it became apparent that dead vaccines were incapable of stimulating an effective immunity against fowl typhoid further trials with a living attenuated vaccine were made (Wilson, 1956_b). The vaccine strain proved to be insufficiently attenuated and all the vaccinated birds developed fowl typhoid of varying severity; one third of the birds died from the acute disease. When challenged 112 days later apart from a

temporary cessation of egg production, all except one of the vaccinated birds remained normal, whereas all the controls showed typical symptoms of fowl typhoid with profuse sulphur-yellow diarrhoea, and 13 out of 15 died from the acute disease.

Although no conclusions of practical value could be drawn from this experiment in which the solid immunity present must be considered to be associated with recovery from active disease, it suggested the prophylactic value of a living vaccine in natural outbreaks of fowl typhoid provided its virulence could be sufficiently attenuated.

Smith (1956) developed two such vaccines, one smooth (9S) and one rough (9R). The former produced the better immunity. Chickens vaccinated with it at seven weeks of age were completely immune 34 weeks later, whereas the immunity induced by 9R began to wane after 12 weeks. The rough vaccine did not upset day-old chicks when injected intra-muscularly but the smooth vaccine was lethal for birds of that age. Both vaccines produced a good immunity in laying hens. 9S caused a marked reduction in egg production, of a temporary nature, and vaccinated birds subsequently reacted to the agglutination test. Vaccination with 9R interfered only slightly with egg production and did not produce agglutinins.

Vaccination trials with 9S and 9R vaccines at Lasswade

In 1956 the writer began vaccination studies with 9S and 9R vaccines prepared at Lasswade by the methods used by Smith (1956) from cultures supplied by him. The main objectives were to determine the degree of risk attached to the use of the vaccines and to investigate the possibility of the application of mass vaccination of chicks by spraying, as has been done successfully for Newcastle disease in North America following the work of Hitchner and Johnson (1948) Johnson and Gross (1951 and 1952) and Crawley (1953).

Vaccination of laying fowls with 9S and 9R

Laying pullets were chosen as birds of that age appear to be particularly susceptible to fowl typhoid and any adverse effect of vaccination can be readily detected and measured by the degree of impairment of egg production.

Three groups of 25, 27 and 24, related six month old pullets were kept under observation for four weeks (2nd May 1956 to 30th May 1956), in individual battery cages, in strictly comparable positions in the same room, to minimise any effect of environment on egg production. During this period they laid 435,422 and 406 eggs respectively. Group 1 was then vaccinated with 9S, Group 2 with 9R and Group 3 with a living vaccine prepared from Salm. gallinarum (Fewson) after 100

TABLE XIV

Effect of vaccination with living vaccines, and later challenge with virulent Salm. gallinarum, on egg-production and egg-transmission of the organism.

Vaccine	9S			9R			F			Unvaccinated Controls		
No.of birds	25			27			24			14		
Week ending	Eggs Laid	Eggs +	Deaths	Eggs Laid	Eggs +	Deaths	Eggs Laid	Eggs +	Deaths	Eggs Laid	Eggs +	Deaths
9.5.56	97			102			91					
16.5.56	121			108			109					
23.5.56	111			108			105					
30.5.56	106			104			101					
TOTALS	435			422			406					
VACCINATION												
6.6.56	87	2		89			74		1			
13.6.56	47	4		66	6		21	1	4			
20.6.56	59	10		59	7		12	2				
27.6.56	83	10	1	66	3		29	2	1			
4.7.56	94	11		71	6		43	4				
11.7.56	91	14		78	6		39		1			
18.7.56	89	8		80	7		43	1				
25.7.56	78	17		85	4		40	1		40		
TOTALS	628	76	1	594	39		301	11	8	40		
CHALLENGE												
1.8.56	74	7		67	3		46	1		36		
8.8.56	73	9		71	4		48	5	✱ 1	19		1
15.8.56	74	15		74	5		52	6		6		3
22.8.56	78	10		72	6	1	41	6		4		1
29.8.56	72	9		61			46	5		12		
5.9.56	77	14		56	1		36	5		9	1	
12.9.56	64	8		49	4	1	27	1		8		
19.9.56	49	2		33			30			12	1	1
26.9.56	51	4		34	1		24			7	1	
3.10.56	50	5		35	1		16	5		12	1	
10.10.56	41	2		30	2	1	8			11		1
17.10.56	35	1		23			10	1		11	3	
24.10.56	17	1		19		1	8			1		
31.10.56	12			10	1		4			1		
TOTALS	767	87		634	28	4	396	35	0	149	7	7

\+ = Salm. gallinarum isolated.

✱ = Death caused by vaccination.

subcultivations on agar at three day intervals.

The effects of vaccination are shown in Table XIV.

There was a drop in egg production in all groups, six to eight days after vaccination. This was most marked in Group 3 in which many of the birds developed symptoms of fowl typhoid and six birds died from the acute disease. Several of the birds vaccinated with 9S were upset; two developed characteristic symptoms of fowl typhoid and one of them died from the chronic disease 26 days after vaccination. Egg production returned to normal in three to four weeks. Apart from a fall in egg production vaccination with 9R had no adverse effect. During the two months following vaccination two thirds of the birds in the 9S group and about half of the birds in the 9R group excreted the vaccinal strains in a proportion of their eggs (10 per cent and 7 per cent respectively of all eggs laid). About 4 per cent of the eggs laid by the birds in Group 3 contained Salm. gallinarum.

When challenged two months after vaccination there were no deaths from fowl typhoid in the group vaccinated with 9S. During the following three months 87 of 767 eggs laid (11 per cent) contained Salm. gallinarum and when the birds were destroyed for bacteriological examination the organism was isolated from over 80 per cent. All had reacted to the agglutination test.

Challenge produced no immediate ill effects in the

9R group but four birds died of chronic fowl typhoid 24, 45, 69 and 89 days later. About half of the birds excreted the vaccinal strain in a proportion of their eggs (5 per cent of all eggs laid by the group) but the challenge strain was not isolated from any egg. When the birds were destroyed the rough strain was isolated from about half of them and the challenge strain from a single bird only.

The birds vaccinated with the Fewson vaccine were completely unaffected by challenge. (The gradual decline in egg production in all three groups is probably a seasonal one). The single bird which died 15 days after challenge was in the advanced stage of chronic fowl typhoid, probably the result of vaccination 69 days earlier.

Challenge produced a serious disease in the unvaccinated controls resulting in almost complete cessation of egg production and a mortality of 50 per cent.

The trials confirmed Smith's findings that 9S produced a superior and more lasting immunity than 9R but the upset to the birds resulting from vaccination, which was greater than he experienced, suggests that it is too virulent to be used with complete safety in the field. The inevitable production of agglutinins is an added defect.

The protection against the acute disease afforded

TABLE XV

Effect of spray vaccination of chicks

Vaccine	Vaccination at day-old			Challenge at 2 months			Challenge at 5 months			Bacteriological examination of survivors		
	No. birds	Deaths F.T.	%	No. birds	Deaths	%	No. birds	Deaths	%	No. birds	+	%+
9S	114	7	6.1	47	0	0	49	0	0	46	19	41.3
9R	118	6	5.1	60	6	10	42	4	9.5	35	15	42.9
F	110	44	40	-	-	-	-	-	-	-	-	-
Unvaccinated controls	100	0	0	60	30	50	48	15	31.2	31	7	22.6

\- = Not challenged.
\+ = *Salm. gallinarum* isolated.

by the more bland 9R and the non-stimulation of agglutinins against smooth strains of Salm. gallinarum indicate its greater suitability for use in the field.

Application of vaccines by spraying

Three groups of day old chicks were sprayed with suspensions of Salm. gallinarum 9S, 9R and Fewson (sub-culture 100) respectively, at the rate of 10 ml. per 50 chicks (1 ml. = 50 x 10^7 viable organisms (Miles & Misra 1938)).

The operation was carried out by placing the chicks in a deep cardboard container and spraying the suspension over them with an atomiser of the "Flit-gun" type. The chicks were kept in the container, in close contact with each other, for 30 minutes and then placed in electrically-heated, wire-floored brooders and reared in the usual manner. Fowl typhoid developed in all three groups with mortalities of 6, 5 and 40 per cent respectively.

A proportion of the chicks in the rough and smooth groups was challenged at two months. There was no mortality in the 9S group but 10 per cent of the chicks in the 9R group and 50 per cent of the unvaccinated controls died from fowl typhoid. The remaining birds in both groups were challenged at 5 months; there were no deaths in the 9S group but approximately 10 per cent of the 9R group and 30 per cent of the unvaccinated

controls died from fowl typhoid.

When survivors were examined bacteriologically five months later Salm. gallinarum was isolated from 41 per cent of the 9S group, 38 per cent of the 9R group and 22 per cent of the controls.

The results of the trials are summarised in Table XV.

Although the smooth vaccine proved to be a highly effective immunising agent its use appeared to be too hazardous and insufficient protection was afforded by the rough vaccine. It is apparent that the spray method of vaccination has no practical application in the control of the disease.

Conclusion

The research which has been described in the thesis confirms that the agglutination test is the most important factor in preventing pullorum disease. By its regular and repeated use the incidence of the disease can be quickly reduced and free flocks established. The disease is perpetuated by the continued existence of low-grade hatcheries; it would seem possible to eradicate it by extending testing to all breeding flocks.

The agglutination test is also of value in preventing and controlling fowl typhoid, which despite its increased incidence remains mainly a disease of the poorer type of farm which constitutes a continuing source of infection.

The use of modified living vaccines appears to provide a promising means of prevention and therapeutic treatment with furazolidone is useful within the limitations which have been described.

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Appendix

Published papers for reference

1. Fowl typhoid in Scotland (1940).

2. Fowl typhoid. Certain aspects of the experimentally produced disease (1946).

3. Avian salmonellosis (1948).

4. The use of furazolidone in the treatment of infections of day-old chicks with S. pullorum, S. gallinarum, S. typhimurium and S. thompson (1955).

5. The treatment of carriers of Salmonella pullorum and Salmonella gallinarum with furazolidone (1956).

6. Fowl typhoid. The effect of vaccination on the natural and experimental disease (1956).

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FOWL TYPHOID IN SCOTLAND

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It is generally believed that in Great Britain fowl typhoid (an acute infectious disease of fowls caused by Bacterium gallinarum) is more or less confined to Wales and the contiguous counties of England, although from time to time sporadic outbreaks occur elsewhere.

My experience tended to confirm that view, for after carrying out a large number of post-mortem examinations of fowls from all parts of Scotland during the period 1927-1936, only two outbreaks were confirmed.

In 1936 it was brought to my notice that a disease had been prevalent for many years on the island of Lewis (Outer Hebrides), causing heavy losses and possessing symptoms suggestive of fowl typhoid. It is believed by the inhabitants that this disease, which is known in Lewis as Galair na Cearc (Gaelic—the disease of the hen) was introduced to the island by poultry imported from the mainland about thirty years ago under the auspices of the Congested Districts Board, and it is said that heavy mortality has been experienced since that date. Through the co-operation of Miss C. Matheson, then Poultry Instructress in Lewis, and later Miss A. MacKinnon, her successor, I was able to obtain an account of this disease and also a supply of dead birds from several outbreaks, and to show that the disease was in fact fowl typhoid.

It is generally considered that fowl typhoid is associated with an unhygienic environment where poultry husbandry is backward and the use of poultry houses with earthen floors is common. The position in certain parts of Lewis supports that view. Poultry keeping is not carried on as a specialised branch of farming, and the methods in common use on many of the crofts are extremely unsatisfactory. The farming land is split up into crofts, a number of crofts comprising a village or township. The poultry population per croft may be as small as twenty head, but some carry much larger stocks. In most cases fowls are kept in stone houses with earthen floors, and roam over ground which has been used by poultry for generations, coming into contact with fowls from adjacent crofts or cottages.

The type of house described is difficult to clean and impossible, even if an attempt is made, to disinfect. Little attention is paid to sanitation or the disposal of dead birds, and faulty methods of feeding prevail; all combining to provide an environment ideal for the spread of infectious disease.

Symptoms

There is usually marked loss of appetite, drowsiness, and progressive weakness, accompanied by the passage of liquid fæces, yellowish green in colour.

In some accounts of the symptoms forwarded with bodies for examination there is a history of " whitening of the comb and wattles " (anæmia), whereas in others there is marked " darkening " (congestion) of comb and wattles. This is in agreement with the symptoms generally described.

The period of illness is usually short, and mortality is high. The disease is always more prevalent in spring and summer, and outbreaks are more frequent after rain, due, it is suggested, to the birds drinking from the puddles formed by the rain on the badly drained ground.

Autopsy and Method of Diagnosis

Post-mortem examination usually revealed some degree of enlargement of the liver, which was often congested and dark in colour. The multiple minute areas of necrosis in the liver described in some textbooks were not encountered. In many cases no gross or suggestive lesions were present at autopsy.

Cultures were made from the liver and bone marrow on MacConkey's bile salt medium, and non-lactose fermenting colonies were inoculated into tubes of glucose, lactose, mannitol, dulcitol, maltose and litmus milk, and agglutination tests carried out with the positive sera.

Bacterium gallinarum, the causative organism, forms acid but no gas in glucose, mannitol, dulcitol and maltose. There is a marked late alkalinity in litmus milk, while the organism is serologically indistinguishable from salmonella pullorum, the causative organism of bacillary white diarrhœa.

Control Measures

In view of the geographical situation of Lewis and the almost certain absence of fowl typhoid in birds likely to be imported from the mainland, it was felt that a good opportunity was presented for testing the efficiency of the measures at present available for controlling and ultimately eradicating the disease.

A scheme was evolved whereby if the disease was suspected birds could be forwarded for examination. If fowl typhoid was diagnosed, all poultry stock on the farm was subjected to the agglutination test. Reactors were

culled and half the stock were vaccinated with an autogenous vaccine (killed culture), the remaining birds acting as controls.

The response to the scheme was very poor, and in view of this and also of the fact that only an odd crofter in a community responded, leaving disease centres all around, no deductions could be made regarding the value of the control measures; nevertheless the number of outbreaks diagnosed was sufficient to show that the disease is very prevalent in certain districts of Lewis.

A new scheme has now been arranged whereby it is proposed to confine attention to a given area, in which all crofters are agreeable to take advantage of the scheme and also to assist by the disinfection of their premises.

The selected area involves thirty-nine crofts and is one in which in recent years fowl typhoid has been particularly severe.

It is hoped that by demonstrating the value of preventative measures, other villages and townships may be encouraged to attempt similar eradication measures.

It is of interest that within recent years in certain parts of the island there has been a marked improvement in the poultry stock, and where proper methods of management are in vogue and the fowls kept semi-intensively, fowl typhoid is practically unknown. This is a further demonstration of the important part played by sanitation and management in eradicating the disease.

Summary

A disease known locally as Galair na Cearc, which has been prevalent in Lewis for many years, has been shown to be fowl typhoid.

The backward methods of poultry keeping and lack of proper hygienic measures are of major importance in causing epidemics, as where more modern methods are employed the disease is almost unknown.

A scheme for attempted eradication by means of agglutination tests, vaccination and sanitary measures has been commenced.

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Fowl Typhoid

Certain Aspects of the Experimentally Produced Disease

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Fowl typhoid was first recorded in Great Britain in 1888 by Klein (1889). The disease appears to have a more or less local distribution being endemic in certain areas, *e.g.*, in Wales and neighbouring counties of England and in some districts of the North of England, although sporadic outbreaks are encountered in other parts of the country. In Scotland, the disease is chiefly confined to parts of the West Highlands and to the islands off the north-west coast (Wilson 1940).

It is caused by *Salmonella gallinarum*, an organism possessing close relationship with *Salmonella pullorum*, from which it is serologically indistinguishable. The only method of differentiation of these organisms is their behaviour in litmus milk and in dulcitol and maltose. *S. gallinarum* gives rise to well marked late alkalinity in litmus milk and produces acid in maltose and dulcitol, whereas typical strains of *S. pullorum* do not attack either maltose or dulcitol. Atypical strains, however, occasionally ferment maltose or very rarely dulcitol. It will be apparent, therefore, that the difference between these organisms is a minute one; indeed, some workers consider that both represent identical species.

It is generally stated that fowl typhoid is most common in farms where somewhat primitive and unhygienic conditions prevail, being particularly prevalent on the type of farm where fixed hen houses, often stone-built with earthen floors, are in use, and where overcrowding, accompanied by infrequent cleansing of the houses, obtains. Outbreaks do occur, however, from time to time on farms run on modern lines where the standard of hygiene is high.

The factors which precipitate an outbreak are not always recognized, but in many cases over-crowding, sometimes of a temporary nature, is of importance, *e.g.*, a serious outbreak followed a spell of wintry weather when, due to snow, birds were confined to the houses for several days on end and in the only outbreak in turkeys I have seen the disease originated in a small pen in which "broody" turkey hens were isolated and which was over-crowded.

In investigating several outbreaks of fowl typhoid certain aspects of the disease appeared to require further consideration and the present paper describes experimental work carried out during 1942-43 in attempts to elucidate these points.

1. The apparent variation in virulence of *S. gallinarum*.
2. The spread of infection by contact.
3. The value of vaccination as a preventive measure.
4. The behaviour of "carriers" of *S. pullorum* when exposed to infection.
5. The agglutination test as a means of diagnosis of "carriers."

VARIATION IN VIRULENCE OF *S. gallinarum*

In natural outbreaks there appeared to be considerable variation in the virulence of the organism and this was shown to occur when attempts were made to set up the disease artificially. In some cases the inoculation of healthy fowls with relatively large doses of recently-isolated strains failed to produce evidence of disease; other strains produced symptoms of illness which were sometimes severe but deaths seldom resulted.

Biester and Devries (1943) state that agar cultures rapidly lose their pathogenic character; this was evident also from our experiments which showed that the virulence dropped considerably even after a single sub-cultivation and that a pathogenic strain may become avirulent after two or three sub-cultures. A culture, isolated from a cockerel which had been infected by contact, with naturally affected hens remained virulent, through repeated sub-cultivation for ten weeks, but this was exceptional.

It was shown that the virulence of a strain could be enhanced rapidly by passage through fowls, but it was usually necessary to kill the first bird of the series to be inoculated, when symptoms were evident. The increase in virulence by passage is well exemplified in natural outbreaks.

SPREAD OF INFECTION BY CONTACT

In this series of experiments identical pens were used, each having a floor space of 12 feet by 6 feet. The floors which were made of cement concrete were cleaned at infrequent intervals to allow for accumulation of infection and so to simulate the environment most favourable for spread of the disease under natural conditions. Infection was produced by survivors of a natural outbreak, all of which reacted to a titre of 1 : 320 and the healthy fowls used were Brown Leghorns except in the case of the *pullorum* "carriers."

The carriers of *S. gallinarum* were survivors of acute outbreaks obtained from farms which had had a clear test history for bacillary white diarrhoea. The *pullorum* carriers were carefully chosen from a flock, whose history was known, and the presence of *pullorum* infection was verified by bacteriological examination. Disease was also confirmed by the isolation of the organism from eggs laid by the carriers when under observation.

Experiment 1

A healthy Brown Leghorn cockerel was placed in a newly disinfected pen with eight "carriers" and died from acute fowl typhoid in seven days. A second cockerel introduced immediately following the death of the first died in eleven days after having shown symptoms of severe illness for several days before death. The culture obtained from the first cockerel was highly virulent, three out of four birds inoculated with it dying in six, seven and eight days respectively; the fourth bird was very sick but recovered.

Experiment 2

A Brown Leghorn cockerel, a Brown Leghorn hen and four *pullorum* "carriers" were placed in a pen with four fowl typhoid "carriers." The Brown Leghorn cockerel died after a period of illness but a bacteriological examination was negative. A second Brown Leghorn cockerel was then introduced and died from fowl typhoid after an exposure of 102 days.

Experiment 3

A Brown Leghorn cockerel and three Brown Leghorn hens were isolated with three "carriers" and remained healthy throughout the experiment which lasted for four months.

The results obtained from these experiments were in accordance with observations of natural outbreaks and suggested that the spread of fowl typhoid by contact required a high concentration of infection.

SYMPTOMS OF THE EXPERIMENTAL DISEASE

These do not differ materially from those observed in natural outbreaks. The first noticeable symptom was the appearance of a sulphur-yellow colouration of the droppings, which was very slight at first. In birds kept on wire floors, it was noted that in the early stages only a few yellow "splashes" were visible amongst the droppings which were otherwise normal and "formed." Later, as the consistency of the droppings became watery the alteration in colour was most marked, the excreta being entirely bright sulphur-yellow or with an additional greenish tinge; there also occurred inappetence, extreme dejection, and coma resulting in death. Thirst was marked in the early stages and the temperature in some cases reached 112°. Congestion of the comb and wattles followed by cyanosis was frequently noted, yet most writers describe an anaemia of the comb and wattles and suggest that this is a means of differentiating fowl typhoid from fowl cholera, in which cyanosis is usually a prominent feature. Birds which became comatose seldom recovered.

CHANGES IN THE BLOOD

Very marked changes were noted in the blood of sick fowls characterized chiefly by a severe leucocytosis. Myelocytes and other immature white cells were present and polymorphonuclear leucocytes in various stages of maturation were seen. There was a marked reduction in the red cell count and erythroblasts and many basophilic and immature erythrocytes were noted in blood films.

Post-mortem APPEARANCES

Lesions in the experimentally-infected fowl corresponded closely

with those seen in natural outbreaks with one notable exception; the "bronzed" liver described in text books and elsewhere was not noted in any case. Birds dying were examined almost immediately after death and the liver at that time presented an appearance similar to that found in many cases of leukaemia. It was enlarged and congested, being pinkish red in colour with paler areas. The colouration slowly changed and if kept for several hours the typical "bronze" appearance developed, suggesting that in some cases at least it is associated with *post-mortem* changes.

A striking feature in almost every case was the marked inflammatory changes in the intestines. In per-acute cases there was a haemorrhagic duodenitis and in the acute disease there was a reddening and swelling of the mucous membrane, sometimes extending throughout the entire length of the small intestine, accompanied by a reddish tinged gelatinous exudate. As in natural cases there was a distortion of the ovary indistinguishable from that occurring in cases of *S. pullorum* infection and in one case an "egg peritonitis" associated with *gallinarum* was noted.

Isolation of *S. gallinarum*

In acute experimental cases the organism was isolated from the blood, liver, spleen, lungs, kidneys, ovary or testes, oviduct, intestines and bone marrow. It grew readily on agar and McConkey media. It was recoverable from the ovary in the more chronic cases a considerable time after inoculation, and in the natural cases which were used as "carriers" of infection.

The yolks of about 100 eggs from natural and experimental cases were examined bacteriologically with negative results. In view of recent work by Wilson (1945), who showed that in the case of outbreaks of acute disease in chicks and ducklings due to infection with *S. thomson*, infection was introduced into the incubator by faecal contamination of the outside of the egg shells, it is unfortunate that the shells were not cultured as it may be that this is a possible source of some of the outbreaks occasionally met with in young chicks.

The Value of Vaccination as a Preventive Measure

Fowls inoculated with stock vaccine and others with autogenous vaccine (both composed of killed broth cultures), birds which had withstood infection in contact experiments, *pullorum* "carriers" and healthy fowls were used in a preliminary experiment. A culture obtained from one of the Brown Leghorn cockerels from the contact experiment was passaged through fowls and the period between injection and death was reduced to three days. After a further single sub-cultivation 1 ml. of saline washings corresponding in concentration to Brown's Tube No. 8 was injected subcutaneously into each bird, all of which were kept in cages with wire floors to obviate any possibility of added infection.

The results are noted in Table I.

Table I

Description of experimental fowl	No. inoculated	No. died of fowl typhoid
Healthy birds which had proved resistant to fowl typhoid in contact experiments	4	—
Fowls vaccinated with stock vaccine	2	—
Fowls vaccinated with autogenous vaccine	4	—
S. pullorum "carriers"	3	—
Healthy controls	4	2 (21 and 23 days)

As only two controls died and as the period between injection and death was fairly long, it was considered that the single sub-cultivation after passage had probably reduced the virulence of the organism and that although the surviving birds might have possessed some degree of protection, no real conclusions could be drawn. In passing, it is of interest to note that washings from an agar culture obtained from one of the dead birds was lethal for a healthy bird in two days; this is a further demonstration of the effect of passage on the virulence of the organism.

The culture obtained from the Brown Leghorn cockerel which had died after an exposure period of 102 days was passaged through a Cambar hen which died in ten days and was then inoculated into a Brown Leghorn cockerel, two Brown Leghorn hens, a Rhode Island Red hen and a Cross hen, which died in eight, eight, nine and 15 days respectively, the Cross hen recovering after a period of severe illness. A culture from the Brown Leghorn hen dying at eight days was sub-cultured and was used in the next experiment, all birds receiving 1 ml. of washings equivalent to Brown's Tube No. 8, subcutaneously. Fowls which had previously received a heat-killed autogenous vaccine, autogenous vaccine plus 1 ml. *S. gallinarum* culture which had become avirulent by repeated sub-cultivation, *S. pullorum* "carriers" and healthy controls were used.

The results are shown in Table II.

Table II

Description of experimental fowl	No. inoculated	No. died of fowl typhoid
Fowls vaccinated with autogenous vaccine	2	2 (4 and 9 days)
Fowls vaccinated with autogenous vaccine and later live culture	2	Remained healthy
S. pullorum "carriers"	2	"
Healthy controls	2	2 (6 and 8 days)

From these results it appeared that a heat-killed autogenous vaccine was not of much value as a protective agent by itself but that by the addition of a live culture it was effective in conferring immunity. *S. pullorum* "carriers" again remained healthy.

This experiment was repeated, and, while fresh birds were being vaccinated with stock vaccine, autogenous vaccine, and autogenous vaccine plus attenuated culture, the culture previously used was kept alive by subcultivation; it was again shown that virulence had been lost during this process, as three fowls inoculated with it, although they became sick, did not die. They were destroyed nine days after injection and a large dose (4 ml. Brown's tube No. 8) of the organism isolated killed a hen in three days. After two further passages, the virulence of the organism was restored, 1 ml. killing in six days. The prepared fowls were then inoculated, each receiving 1 ml.; the results are shown in Table III.

Table III

Description of fowl	No. inoculated	No. died of fowl typhoid and period between injection and death
Vaccinated stock vaccine		
Brown Leghorn male		5 days
Brown Leghorn hen		7 days
Brown Leghorn hen	4	3
White Leghorn hen		14 days
Autogenous vaccine		
Brown Leghorn hen		18 days
Brown Leghorn hen		9 days
Brown Leghorn hen	4	2
Brown Leghorn hen		
Autogenous vaccine plus live culture		
Brown Leghorn hen	4	0
Brown Leghorn hen		
Rhode Island Red hen		
Rhode Island Red hen		
Salmonella pullorum carriers	3	0
Healthy controls		
Brown Leghorn male		5 days
Brown Leghorn hen		
Rhode Island Red hen	4	2
Rhode Island Red hen		8 days

All the inoculated birds, except those which received autogenous vaccine plus live attenuated culture and two of the *pullorum* "carriers," were obviously ill, showing inappetence, sulphur-yellow diarrhoea, dejection, and even periods of coma. It seems highly probable that if they had been exposed to the variations of temperature and to the other conditions to which fowls kept under natural conditions are subjected they would have succumbed. The use of wire floors prevented any accumulation of infection to which birds would have been exposed in the field, but, on the other hand, the initial dose of organisms was probably considerably greater than that which might have been naturally acquired. It is of interest to note that only one of the *pullorum* "carriers" showed symptoms of illness, and in it the disease was milder and the diarrhoea was less profuse.

The experiments tended to suggest that the use of a dead vaccine, either autogenous or mixed, is of doubtful value, at least under experimental and somewhat unnatural conditions. This was not entirely unexpected as its use in field outbreaks evokes mixed opinions. In some cases good results appear to follow, but the preliminary testing, leading to removal of carriers may play an important part in limiting losses, and some of the credit ascribed to vaccination may thus be misplaced. In no case has a "carrier" of *S. pullorum* succumbed to experimental inoculation but the numbers are too small to eliminate the part played by "chance." It may be recalled, however, that Lambert (1933) showed that selection for resistance to pullorum infection resulted in a decided decrease in mortality from that disease and that some protection to fowl typhoid was also afforded.

The present experiments showed that the use of a live attenuated culture produced a solid and lasting immunity, but the general use of this method in the field could not be justified owing to the possibility of recovery and subsequent enhancement of virulence which might result in a serious outbreak. In the event, however, of the occurrence of an outbreak which threatened to decimate a large and valuable flock, an owner, with a full knowledge of the underlying danger, might justifiably be prepared to run this risk.

Agglutination Tests

It was found that after vaccination with dead culture agglutinins were only rarely demonstrable ; thus the use of this method of prevention should not complicate subsequent agglutination testing for carriers of *S. pullorum*. This is of particular importance in the case of flocks under the Accredited Scheme where annual routine testing is carried out.

Following the injection of a similar amount of live culture, agglutinins were demonstrable in six to seven days and gradually increased in amount up to about 14 days when a titre of 1 : 320 to 1 : 640 was commonly noted, even although no symptoms of illness had been shown. In birds which showed symptoms of the acute disease death frequently took place before the formation of agglutinins and it was concluded that in many cases the production of measurable agglutinins coincided with commencing recovery. The titre varies greatly in individual birds. In birds which have recovered from the acute disease after a period of severe illness and in some of the birds vaccinated with dead culture followed by injection with live culture the titre was very high, reaching 1 : 20,480 in several cases, and up to 1 : 40,000 in a single case. It is of interest to note that 1 : 320 is the highest titre encountered in any of our field cases but there is no doubt that much higher titres occur naturally. In some cases the titre tended to fall after a few weeks and in certain birds, both naturally and artificially infected, after a period of months agglutinins ceased to be demonstrable, and bacteriological examinations were negative. A number of birds which showed falling titres were destroyed, and a bacteriological examination of several which showed a low agglutinin level failed to reveal *S. gallinarum*. It would appear, therefore, that agglutinins may persist for some time after the elimination of the causative organism.

It has been suggested that it is important to carry out a preliminary agglutination test before vaccination in order to eliminate the possibility of vaccinating birds which are in the incubative stage of the disease, but it appears from our work that the test would not discern such birds. Agglutination tests should however, always be carried out as a routine procedure in order to eliminate " carriers " as a possible source of further infection.

Rapid Whole-Blood Test

This test if it proved reliable, appeared to possess certain advantages over the tube test, the chief of which perhaps was the speed with which it could be carried out after a positive diagnosis had been obtained in the laboratory. In addition, reactors could be eliminated immediately, thus avoiding the delay of several days which is inevitable in the case of the tube test. The rapid whole-blood test using *S. pullorum* stained antigen was, therefore, carried out in two natural outbreaks and the results were controlled by tube tests in the laboratory. Complete agreement to both tests was obtained. In addition, tests were carried out in experimentally affected fowls and these confirmed that the rapid whole-blood test was an accurate and efficient method of testing for fowl typhoid in the field.

As a result of the many comparative tests carried out, a rather striking and perhaps surprising fact became evident, namely that the speed of the reaction and the type and size of the clumps obtained in the rapid test in many cases bore little relation to the titre. Thus, birds which had been classified as " strongly positive " to the rapid test included samples of relatively low, medium and high titres. The converse was also true and poor reactions characterized ny slowness in development and fine granulation rather than floculation were noted in samples which had titres of 1 : 40, 1 : 2,000, 1 : 5,000 and in one case, 1 : 10,000 respectively.

In one of the natural outbreaks a number of " pin point " granular reactions were evident. These were considered to be insignificant and the tube tests were negative in every case. In a retest at a later outbreak several similar reactions were encountered and the fowls responsible for them were obtained for bacteriological examination, but in no case was *S. pullorum* or *S. gallinarum* recovered. It may be that sometimes such reactions represent early infection, with commencing agglutinin formation, but it does appear that a granular reaction can occur without pathological significance. This phenomenon is well known to all who have carried out rapid testing on a large scale. In some cases it is probably due to non-specific agglutinins, and in others the presence of late lactose-fermenting strains of *B. coli* may be responsible. It appears in other cases to be associated with haemoglobin or some other substance present in whole blood, but not in serum, as it has frequently been noted that serum from a fowl, the blood of which shows this phenomenon, gives a completely negative result with stained antigen and in tube tests.

In testing a flock, therefore, in which fowl typhoid has been diagnosed care is necessary to differentiate this apparently non-significant reaction from that which might occur in a bird in the early stages of the disease, and it may be considered desirable in the interest of safety, to remove such cases from the flock, especially if they are few in number, or to isolate them, pending a further test.

Summary

1. *S. gallinarum* shows a marked variation in virulence and the inoculation of susceptible fowls with a recently isolated strain causing losses in the field may fail to reproduce the disease.

2. Virulence is lost by subcultivation but can be enhanced by passage.

3. The successful spread of fowl typhoid by contact requires a considerable concentration of infection.

4. The experiments tend to suggest that the use of dead vaccine, either mixed or autogenous, is of doubtful value, at least under experimental and somewhat unnatural conditions.

5. Recovered birds possess a solid immunity and birds vaccinated with dead culture, and later injected with live culture are resistant under experimental conditions.

6. In no case has a " carrier " of *S. pullorum* died from fowl typhoid, following injection or exposure, but the number used is too small to obviate the part played by chance.

7. There is a suggestion that some strains are more susceptible than others ; *e.g.*, in the case of Dr. Greenwood's Brown Leghorns a shorter incubation period and a higher death rate were commonly noted.

8. The male birds used in the experiments invariably showed symptoms before the females and the proportional death rate was higher.

9. Although the organism was readily isolated from the ovary of both naturally and artificially infected hens, it was never recovered from eggs laid by " carriers " or recovered birds.

10. The rapid whole-blood test using *S. pullorum* stained antigen is a useful and accurate method of detecting " carriers."

11. The " degree " of reaction to the rapid test is not always a good indication of the titre, and care is necessary in differentiating between " pin point " or granular reactions of no pathological significance and those shown by positive cases of low titre.

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No. 5.—Avian Salmonellosis *

By

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Members, N.V.M.A., met again in the Cambridge Hall, Southport, on the morning of Tuesday, September 14th, 1948, when there was discussed at the first session, at 9.30 a.m., the paper by Mr. J. E. Wilson, of Lasswade, on the subject of "Avian Salmonellosis." The attendance at the outset was disappointing.

The chair was taken by Mr. G. O. Davies, D.V.SC., M.R.C.V.S., D.V.H., of Liverpool.

The chairman, in introducing Mr. Wilson, said that the subject before the meeting was one which had had a great deal of prominence in the past few years. He would ask Mr. Wilson to amplify some of the remarks in his paper.

INTRODUCTION

Mr. J. E. WILSON: I would first like to express my thanks to the provisional committee for being asked to read a paper at this Congress, and to say how much I appreciate the honour.

My first reaction to the invitation was to decline, as I did not feel capable of dealing adequately with the complex subject of avian salmonellosis, particularly before an audience of such diverse interests. However, after consideration, I decided that a general review of our present knowledge, incorporating some of our own findings and dealing principally with prevention and control, might be of interest, and the paper has been prepared on these lines.

Although nowadays in this country salmonellosis is generally accepted as the disease produced by members of the group other than *S. pullorum* or *S. gallinarum*, bacillary white diarrhoea and fowl typhoid have been included in the interest of completeness.

It can be stated that the latter diseases need no longer present a problem as they can be readily diagnosed and adequate control measures are available. The agglutination test is an efficient method of detecting carriers and by its organised use flocks can be completely cleared of both diseases. Occasionally some difficulty may be met with in certain flocks in interpreting doubtful reactions of a "pin-point" type, either as a result of infection with other salmonellas, or with certain strains of *B. coli* or through non-specific factors not well understood, but retesting usually clarifies the position.

If a variant type of *S. pullorum*, similar to that described by Younie in Canada, became common in this country some preliminary difficulty might be caused, but it has been shown that an antigen containing both variant and stock strains is effective in detecting carriers.

Unfortunately, coinciding with the decline in bacillary white diarrhoea and fowl typhoid, there has been a great increase in infections with other members of the group, many new to this country, and it is impossible to escape the conclusion that they were introduced as a result of the incorporation of American spray-dried egg powder in poultry mashes.

The high incidence of *S. thompson* infection is difficult to explain, as it is not a common poultry pathogen in America, nor is it commonly found in rats and mice in this country, but under modern conditions of poultry husbandry, where many thousands of eggs are set in a mammoth incubator and the resultant chicks distributed literally throughout the country, the introduction of any type results in a rapid and widespread dissemination. Fortunately, many species are not highly pathogenic for humans; it is usually only the very young and the old who are strongly susceptible, and even where serious outbreaks have occurred in hatcheries, and the organism has been isolated from dust, fluff and floor sweepings throughout the buildings, we have no records of illness amongst the operators. It seems to me that fairly rapid passage through chicks lowers the virulence for man; we do know from our own work that it may be necessary to passage a human strain through several lots of chicks before the virulence is sufficient to cause appreciable losses.

The agglutination test is not a practical method for the detection of carriers. Cloacal swabbing is uncertain, as the passage of organisms in the faeces is intermittent; in any case, it is impracticable on a large scale.

Attention must, therefore, be concentrated on hygienic measures, chief of which is probably incubator disinfection. To be effective this must be done within a few hours of setting before there is any chance of shell penetration. Formaldehyde is still the best available agent and it may be used in stronger concentrations than those given in the paper, which are the recommendations of the Ministry and appear to be effective in practice. We are at present experimenting with other aerosols, but, unfortunately, although they are quite effective as aerial disinfectants, they have little effect on surface contamination, and in the control of salmonellosis this is absolutely essential, as the contaminated egg shell is the most likely method of incubator infection.

The use of sulphonamides is discussed, and while they may be contra-indicated in outbreaks of bacillary white diarrhoea in prospective breeding stock, it is obvious that they may be useful in selected cases, and there seems to be no reason why they should not be used in fowl typhoid.

I think, however, that this type of treatment will prove most useful in conjunction with the hygienic measures described in salmonellosis due to other types, more particularly in large-scale outbreaks in hatcheries where the wholesale destruction of an entire hatch is unlikely to be considered.

Mr. Wilson's paper, which had been circulated to the members, was as follows:

It has become the custom to interpret Salmonellosis of poultry as the disease produced by any member of this group of organisms except *S. pullorum*, the cause of bacillary white diarrhoea or pullorum disease, and *S. gallinarum*, the causative organism of fowl typhoid. In the interest of completeness, however, these diseases are included in the present paper in which it is intended to review their salient features with particular reference to control and prevention.

Almost fifty years have passed since Rettger (1900) first described an outbreak of bacillary white diarrhoea in chicks in Indiana, shown by Rettger and Harvey (1908) to be due to *S. pullorum* (then *Bacterium pullorum*) and it was as long ago as 1878 that fowl typhoid was first observed by Peroncitto in Northern Italy, and later described in England by Klein in 1889. With the passing of time the great changes in the poultry industry associated with its vast expansion and the introduction of intensive methods of incubation and rearing have placed the former disease in a category of ever increasing importance; the latter has been almost eliminated as a potent source of loss.

Other members of the group, chiefly *S. typhi-murium* (*S. aertrycke*) and *S. enteritidis* Gaertner have long been recognised as pathogens of man and animals but within recent years infections with many types, some new to this country, have become more common and now represent a serious cause of loss in young chicks and an important potential source of food poisoning in man.

GENERAL CHARACTERISTICS OF SALMONELLA

There are more than 180 named types in this group of coliform bacilli which neither ferment lactose nor produce indol. All are motile, possessing H (flagellar) and O (Somatic) antigens, except *S. pullorum* and *S. gallinarum*, which are non-motile and have only somatic antigens. Classification depends on their antigenic structure and in some which are antigenically identical, on their fermentative reactions. These characteristics are shown in Tables I and II.

* Presented to the Sixty-sixth Annual Meeting of the National Veterinary Medical Association at Southport on September 14th, 1948.

TABLE I

ABSTRACTED FROM TOPLEY AND WILSON

	Production of gas	Mannitol	Dulcitol	Sorbitol	Inositol	Maltose	Arabinose	Xylose	Trehalose	Dextrin	Litmus milk	H_2S production
S. paratyphosum A.	+	+	‡‡	+	—	+	+	—	+	+	Acid or neut.	‡‡
S. typhi-murium	+	+	+	+	‡‡	+	+	+	+	+	Alk.	+
S. thompson	+	+	+	+	+	+	+	+	+	+	Alk.	+
S. oranienburg	+	+	+	—	+	+	+	+	+	+	Alk.	+
S. bareilly	+	+	+	+	+	+	+	+	+	+	Alk.	+
S. pullorum	+	+	—	‡‡	—	—	+	‡‡	‡‡	—	Acid or neut.	+
S. gallinarum	—	+	‡‡	‡‡	—	+	‡‡	‡‡	‡‡	+	Alk.	‡‡
S. enteritidis	+	+	+	+	—	+	+	+	+	+	Alk.	+
S. london	+	+	+	+	+	+	+	+	+	+	Alk.	+
S. anatum	+	+	+	+	—	+	+	+	+	+	Alk.	+

+ = Positive.
— = Negative.
‡‡ = Variable or delayed fermentation.

TABLE II

KAUFFMANN-WHITE SCHEMA

Group	Type	O-Antigen	H-Antigen	
			1-phase	2-phase
Group A	*S. paratyphi* A	[1].II.XII . . .	a	— 1-5
Group B	*S. typhi-murium*	[1].IV..[V].XII . . .	i	1, 2, 3 . . .
	S. california	IV.XII . . .	g, m, t . . .	—
Group C	*S. thompson*	$VI_1.VI_2$.VII . . .	k	1, 5 . . .
	S. montevideo	$VI_1.VI_2$.VII . . .	g, m, s . . .	—
	S. oranienburg	$VI_1.VI_2$.VII . . .	m, t . . .	—
	S. bareilly	$VI_1.VI_2$.VII . . .	y	1, 5 . . .
Group D	*S. pullorum*	IX.XII . . .	—	—
	S. gallinarum	IX.XII . . .	—	—
	S. enteritidis	[1].IX.XII . . .	g, m . . .	—
Group E	*S. london*	III.X.XXVI . . .	l, v . . .	1, 6 . . .
	S. give		l, v . . .	1, 7 . . .
	S. anatum		e, h . . .	1, 6 . . .
	S. meleagrids		e, h . . .	l, w . . .
Other groups	*S. worthington*	I.XIII.XXIII.	l, w . . .	z . . .

[] = These antigens may be missing
. . . = Very much abbreviated formula

Some somatic antigens are common to more than one group, *e.g.*, XII is present in Groups B and D so that birds infected with members of either group react either partially or completely with *S. pullorum* antigen.

The close relationship between *S. pullorum* and *S. gallinarum* will be seen. Both are antigenically identical, and while they may generally be distinguished by their biochemical reactions, occasionally atypical strains of *S. pullorum* produce acid in maltose, failure to reduce dulcitol and absence of alkalinity in litmus milk being the only differential features.

The pathogenicity of the group is due to endotoxins elaborated in the body of the organism and it has been shown by Martin (1934) and Boivin and his co-workers (1933, 1934, 1935) that the toxic fraction of *S. typhi-murium* and *S. enteritidis* consists of a polysaccharide which Topley and Wilson suggest is itself the somatic antigen of the bacterial cell. It has been suggested that the action of the toxin is to produce a hyperglycaemia. The young of the species are most susceptible to infection and secondary factors such as brooding faults and improper feeding aggravate losses.

Bacillary White Diarrhoea

It was not until several years after the disease was first described by Rettger in 1900 that it became of importance in America. There is no record of its introduction into this country but it is probable that it has been present for a long time; the small losses in hen-hatched chicks would not attract much attention. The great expansion of the poultry industry after the 1914-18 war brought it into prominence and publications by Doyle (1925), Dalling, Mason and Gordon (1927, 1928, 1929) and Dalling and Warrack (1930) added to the knowledge already available from the work of American investigators.

It was early recognised that a large number of recovered birds are carriers, the germ being localised in the ovary. As a result the yolks of a proportion of the eggs laid contain *S. pullorum* and the hatching of such an egg may infect many chicks within the incubator during "drying off" after hatching. The number of eggs laid by carriers varies considerably. Rettger and Stoneburn (1909) found nine out of 44, Hadley *et al.* (1917) six out of 55, Gwatkin (1925) 20 out of 240, Doyle (1925) nine out of 341, Kaupp (1927) 96 out of 1,313 and 131 of 2,505 and Wilson (1931) 25 out of 310. The severity of an outbreak depends on the virulence of the organism and the number of infected eggs which hatch, determining the concentration of infection on the down and fluff present in the machine after hatching. Contamination of the outside of the shell with *S. pullorum* probably plays little part in the spread of the disease (cf. other Salmonellas). Jones (1910) was unable to isolate the organism from the external surface of shells which had been saturated with it after 21 days' incubation and Gwatkin (1927) showed that no growth occurred after 12 days at 37° C., although control pieces of infected shell stored at room temperature gave growths up to 44 days, when the supply of shell gave out. Some workers record difficulty in setting up the disease artificially. Wilson (1931) found that an acute outbreak with high mortality could be produced by spraying day-old chicks with a broth culture and leaving them in the incubator until they had dried off. To set up a mild outbreak with a low death-rate but producing a large proportion of "carriers" it was sufficient to damp the down over the back of a single chick or the down on the heads of two chicks with a recently isolated culture and place the treated chick or chicks with the required number of contacts in an incubator for a few hours.

Infection spreads in the brooder by ingestion of food and water contaminated by the organism and by the inhalation of germ-laden dust.

There is a marked variation in the resistance of different strains of chicks. Roberts *et al.* (1939) have shown that a high degree of resistance can be built up by selection and they suggest that the difference between resistant and susceptible chicks is due to an inherited differential in the number of lymphocytes present immediately after hatching.

Environmental conditions play an important part in the expression of the disease. Chilling or other brooding faults aggravate losses. There may be a high death-rate in chicks subjected to lengthy delays during transit while in the infected chicks retained on the farm and not subjected to similar rigours no significant mortality may occur. This may be misleading in determining the source of infection.

The disease is essentially one of chicks although outbreaks occur in turkey and pheasant chicks and more rarely in ducklings. Gordon and Buxton (1946) record a similar experience and also the isolation of the organism from a guinea fowl and from a sparrow. Its occurrence in the last named species had already been recorded by Dalling *et al.* (1928).

Hendrickson and Hilbert (1931) and Hinshaw and Hoffman (1937) described outbreaks in ducklings in America; in both cases there was a history of contact with infected chicks. Cass and Wilkins (1947) isolated the organism from a wild pheasant which had died from injury and quote outbreaks in game farms.

In America bacillary white diarrhoea is a serious disease of turkeys and according to Hinshaw (1937) the cycle of infection is the same as in the fowl. The same writer (1943) isolated the organism from five out of 945 eggs laid by 22 reactors and later (1945) from two out of ten and in ten out of 72 eggs picked at random from a pen of turkey reactors. Gauger (1947) found six positive eggs out of seven laid by one bird. In this country, on the other hand, outbreaks are relatively rare, and very few reactors are demonstrated by the routine agglutination test.

From time to time outbreaks of bacillary white diarrhoea occur in adult birds, often as a result of feeding unhatched eggs from the incubator—which practice is sometimes induced by the marked decrease in fertility and hatchability of eggs from an affected flock. Considerable mortality may be caused but the most serious aspect is the large number of "carriers" which result; almost every bird in the flock may show a positive reaction.

Feeding swill or baker's waste is sometimes responsible and egg eating, which may follow the breaking of eggs through inadequate nesting facilities, causes further spread. Failure to remove reactors from a flock may lead to the infection of contacts. We have found over a number of years that, experimentally, spread of infection by contact does not readily occur in adult birds but it would appear that under field conditions it is of greater importance. This is in agreement with the work of Rettger, Kirkpatrick and Card (1919), Edwards and Hull (1929), Kernhamp (1930), Gwatkin (1945). The male bird appears to play little part in the dissemination of the disease. Rabbits are especially susceptible to artificial infection; cats and mice are slightly susceptible and rats are resistant.

Symptoms

Deaths may occur during the first few days after hatching without symptoms having been shown; often there is excessive chirping; the chicks appear uncomfortable, cease feeding and huddle near the source of heat with ruffled down and drooping wings. Some chicks show a staggering gait and convulsions are sometimes seen. As a result of diarrhoea the down around the vent may become pasted causing impaction of the cloaca and giving the chick a distended appearance. In many outbreaks diarrhoea is absent and deaths occur from pneumonia or septicaemia following a primary inhalation pneumonia. Some recovered chicks are stunted in growth.

Mortality is usually high and may reach 80 to 100 per cent.; rarely it may be so low that the presence of disease is not suspected. Deaths occurring during the first few days are indicative of infection in the incubator; a delayed mortality suggests that infection has occurred in the brooder.

Post-mortem Examination

Often there are no significant lesions; the lungs and kidneys are congested and the yolk is partially absorbed. In many chicks, however, there are yellow or greyish yellow nodules in the lungs varying in size from that of a pin head to that of a hemp seed or larger. Several nodules may coalesce and replace most of the normal lung tissue. Nodules may also be present in the myocardium and in the musculature of the gizzard.

Similar lesions occur in infections with *S. typhi-murium* and *S. thompson* and less commonly with *B. coli* and nodules indistinguishable to the naked eye are associated with aspergillosis. In aspergillosis the air sacs frequently contain yellow caseous material but as the air sacs are sometimes affected in salmonellosis and, in addition, the two diseases may co-exist, differential diagnosis in the field is impossible. A bacteriological examination is necessary in every investigation into chick mortality.

In many infected adult fowls necrotic lesions are present in the myocardium and there may be pericarditis with adhesions. The chief alterations, however, are in the ovary, the yolks of which may be markedly distorted. In some cases, no normal ova remain, the ovary being replaced by a number of small spherical greyish brown sacs containing clear thickish brownish fluid with flocculi. Some are attached to the ovary by stalks of varying length, some are shed and may be free in the abdominal cavity or adherent to the peritoneum. Similar ovarian lesions occur in carriers of *S. gallinarum*.

In other birds there may be slight distortion of one or more ovules with the contents normal in consistency or inspissated. The remainder of the ovary is composed of apparently normal ova in varying stages of maturation. This type also occurs in fowl typhoid and in some cases of infection with *S. typhi-murium* and *S. thompson* in the hen and duck (Wilson, 1947). Impaction of the oviduct and egg peritonitis are sometimes seen

Bacteriological Procedure

In submitting chicks for bacteriological examination several bodies should be sent as the examination of a single chick is an unsatisfactory method of obtaining a diagnosis where losses are occurring. They should be adequately packed and accompanied by a description of the outbreak.

Sowings are made from the liver and lungs of individual chicks on McConkey medium and the pooled intestinal contents of several chicks are placed in flasks of tetrathionate broth or biselenite broth (Leifson). In the adult fowl pure cultures can usually be obtained from the distorted ova or from the myocardium by direct plating on McConkey medium. For more critical work it is necessary to remove the entire ovary and large samples of the other organs, including the gall bladder, grind up with sterile sand and inoculate into tetrathionate broth or selenite broth (Leifson). After incubation for 24 hours at 37° plates are examined and subcultivations on McConkey medium are made from the broth cultures. Colonies of *S. pullorum* are small, clear and discrete and even when numerous there is little change in the coloration of the medium. Colonies of *S. gallinarum* are usually larger and slightly more opaque and the medium presents a brownish yellow tinge. With *S. thompson* and *S. typhi-murium* there is a tendency for colonies to be still larger and the colour change of the McConkey medium is intensified. Suspicious colonies are picked off and grown on agar slopes and in broth for agglutination tests, in peptone water for tests for motility and for indol formation and fermentation tests are carried out. Cultures other than *S. pullorum* or *S. gallinarum* are tested by preliminary slide agglutination tests with group sera supplied by the Standards Laboratory, Oxford, and positive results are confirmed by tube tests following the methods described by Bridges and Taylor (1944). It is sometimes necessary to send cultures to a Salmonella Reference Laboratory for identification. McConkey medium is satisfactory for the isolation of salmonella from the internal organs of chicks but a wholly successful medium for the isolation of the organism from the intestinal contents has not been found. Brilliant green as recommended by Kerr (1930) gives uncertain results and the use of gentian violet 1/40,000 as described by Morcos *et al.* (1946), while having a considerable inhibitory effect is confusing. Tetrathionate broth and desoxycholate-citrate-agar (Leifson) are useful for salmonellas other than *S. pullorum* and *S. gallinarum;* for these we have found biselenite broth (Leifson) the most satisfactory medium. If several specimens are examined by the procedure described there is little risk of failure to isolate any salmonella which may be present even when chicks have been dead for some time before receipt. It has been shown that *S. pullorum* has marked powers of resistance against putrefactive changes and can be isolated from badly decomposed carcases a week or more after death.

Control Measures

Where a high mortality has occurred survivors should be destroyed and bodies burned. In small batches this procedure should be adopted even in less serious outbreaks. In a mild outbreak with a large number of survivors it may be considered desirable to rear them in isolation until they are sufficiently mature for table use, *e.g.*, as petit poussin. Survivors should never be reared as prospective breeding stock or for commercial egg production as about 40 to 50 per cent. may be carriers; apart from the risk of infection, such birds usually show a reduced egg production. The use of drugs of the sulphonamide series will be discussed later.

Litter and droppings from the contaminated brooder should be sprayed with disinfectant and buried, or burned, and the brooder and utensils thoroughly disinfected. The incubator and fittings must be thoroughly cleansed and disinfected before re-use and if the disease is suspected in any hatch the hatching compartment should be fumigated or sprayed with disinfectant before removing debris, dust, fluff, etc. An alternative method is to remove dust and fluff by means of a vacuum cleaner. The fumigation of incubators with formaldehyde will be dealt with later.

There may be an increased embryonic mortality in eggs from affected flocks but even where the disease is not suspected care should be taken in the proper disposal of infertile eggs and dead-in-shell. They should never be fed to hens or pigs but should be burned in an incinerator or buried in a place to which fowls do not have access. Pending disposal, they should be stored in bins provided with lids to keep out flies which may play a part in the further spread of the disease, *e.g.*, Gwatkin and Mitchell (1944) showed that chicks died from pullorum disease after access to food contaminated by infected flies and to the flies themselves, some of which were probably eaten by the chicks. The organism was recovered from the feet and wings of artificially infected flies six hours after exposure and from the gastro-intestinal tract up to five days; infection may persist even longer but bacteriological examinations were not made after that time. Runs may remain infective for a long time, *e.g.*, Kerr (1930) recorded survival of the organism

in faecal emulsions for three months and Allan and Jacob (1930) found that it remained virulent for 14 months in contaminated soil.

Infection may also be introduced by purchased stock and second-hand equipment including chick boxes, incubators and brooders. Van Rockel *et al.* (1941) showed that the organism can remain viable on a dry cloth for more than seven years. The risk of infection from wild birds must be slight. During war-time conditions, the use of swill and baker's waste containing egg products may have been responsible for some outbreaks. Custom hatching, *i.e.*, the incubation of eggs from several flocks in one or more incubators on a single premises, is a frequent source of infection and custom sexing, a process necessitating the handling of successive batches of young chicks from numerous sources by one or more operators in a room often badly designed (from a hygienic point of view) for the purpose, is sometimes responsible for the introduction and spread of the disease. Even when reasonable facilities are provided the nature of the operation and the speed at which it is carried out tend to make chick-sexing dangerous. Where a travelling sexer is employed, he should be provided with an overall, it should be sterilised after using and retained on the premises until next required. A high standard of hygiene in the sexing room is important but chief attention should be paid to the prevention of infection in the incubator by systematic testing of breeding flocks.

The Agglutination Test

The possibility of detecting carriers by the agglutination test was first explored by Jones (1913) but the use of this method did not become general until much later. In England, a laboratory undertaking this work was opened in Weybridge in 1924 and in the following year routine testing was begun in Scotland on a small scale at the Royal (Dick) Veterinary College. It is interesting to recall that in 1927 records show that approximately 20 per cent. of all birds tested were carriers.

The introduction of the Mammoth incubator within the next few years provided an impetus to further testing and by its organised and continuous use a great reduction in the incidence of bacillary white diarrhoea has been effected. In Scotland during 1947 the percentage of positive bloods from Accredited flocks was 0·29; in flocks on probation, some of which were being tested for the first time, it was almost four times as great (1·01); 804 stations out of 1,025 were completely free from reactors.

There is a great variation in the titre of infected birds both in individuals and between the sexes. In the female it ranges from 1/25 to 1/1,000 and higher. In the male it is much lower—1/10 to 1/80, rarely 1/200. Young chicks of both sexes are equally susceptible but with the development of sexual maturity the male bird tends to throw off infection. There is a fall in titre and many male reactors kept in isolation become completely negative. In the female, with the development of the ovary and the onset of egg production the titre rises and usually persists at a significant level throughout life. In some birds, however, there is a fluctuating titre. It may fall below that employed in the routine test, giving a negative reaction, while a later test may again be positive. This type of bird has been called an occasional reactor. The "residual" reactor has been described by Dalling and Warrack (1930). Beaudette (1923) demonstrated the presence of specific agglutinins in the albumen of eggs laid by carriers; birds with a high serum titre had a high albumen titre. May (1924) also found agglutinins in albumen and showed that the titre was always lower than the corresponding blood titre but Doyle (1925) did not confirm these findings.

For many years the tube agglutination test was employed but recently the rapid whole blood test has been almost universally adopted. It is not, however, a new test as following the work of Huddleson and Carlson (1926) on a rapid serum test for contagious abortion, Runnells *et al.* (1927) showed that a similar test could be used for bacillary white diarrhoea. Schaffer *et al.* (1931) and Schaffer and Bunyea (1933) demonstrated that a rapid test using whole blood and a dense suspension of *S. pullorum* stained with crystal violet was an effective method for the detection of carriers and the test now in general use is largely based on their work. It has been used in commercial flocks in this country and in America for many years and was adopted as the official test for the Accredited Scheme in England in 1942 and in Scotland in 1948. There is no difference in the accuracy of the tube and rapid test in the fowl but American work suggests that the tube test is more sensitive in the turkey (Bushnell (1945), Corpron *et al.* (1947)). The chief advantages of the newer procedure are that only a single handling of the birds is necessary and any reactors present are readily available. In the tube test considerable handling is sometimes necessary in the search for reactors and there is a risk of faulty identification through clerical errors in completing the record card accompanying the samples.

A positive reaction is characterised by the formation of deep purple clumps with clearing occurring in many cases almost immediately the loopful of blood is mixed with the antigen or when the plate is rocked gently after mixing. Any reaction which develops after two minutes is considered to be of no pathological significance. There is considerable variation in the size of clumps—a sample with a high titre may give only a granular type of reaction, whereas one of lower titre may show much heavier clumping.

Doubtful reactions characterised by the formation of pin point clumps, lighter in colour and occurring either throughout the mixture or at the periphery without clearing, are sometimes seen and may be confusing. Their exact significance is often difficult to determine. Sometimes they may be associated with residual infections with other salmonella but frequently they occur in flocks in which no trouble has been experienced in rearing healthy chicks.

Doubtful reactions may also be indicative of a low, but rising, titre of agglutinins for *S. pullorum*, in which case a re-test two to three weeks later usually gives a definite result. Doubtful reactions due to this cause may be largely avoided if pullets are not tested until a proportion of their number are in lay and the remainder appear to be about to come into production. Previously this occurred when birds were five to six months old but under present conditions the date may be postponed for a month or even longer.

A further factor which may complicate testing is the occurrence of a variant strain of *S. pullorum*. There is nothing to suggest that this problem exists in this country at present but a strain of this type was reported in Canada by Younie (1941). The disease produced was similar to bacillary white diarrhoea in every way except that the mortality rate was low in young chicks. Gwatkin and Bond (1945) showed that in a pen experimentally infected with the variant strain the standard antigen would have failed to detect approximately 89 per cent. of the infected birds. A combined antigen made from variant and stock strains was effective so that a similar method could be adopted here should it become necessary.

An intradermal test (Pullorin test) was first suggested by Ward and Gallagher (1917) and from time to time other workers have investigated this aspect. There is general agreement that it is unreliable (Beach (1919), Sherago & Benson (1919), Morcos *et al.* (1946)). In view of the simpler procedure of the rapid whole blood test, even if it were possible to develop an efficient intradermal test, its use would be contra-indicated.

It seems probable that if the form of registration and suspension of sales envisaged by the Poultry Technical Committee could be extended to all hatcheries and suppliers of day-old chicks, losses from bacillary white diarrhoea could be reduced to insignificance; meantime the uncontrolled hatchery presents a problem.

Fowl Typhoid

It has already been noted that fowl typhoid was first recorded in Great Britain in 1888 by Klein, and outbreaks of varying severity have been described. It now appears to have a more or less local distribution, being endemic in certain areas of Wales and neighbouring counties of England, and in some districts in the North of England. In Scotland the disease is chiefly confined to parts of the West Highlands and to the islands off the north west coast (Wilson 1940). Sporadic outbreaks are encountered in other parts of the country. The identification of *S. gallinarum* and its close relationship to *S. pullorum* have already been discussed. Its virulence varies greatly and frequently difficulty is experienced in setting up the disease experimentally, even when recently isolated cultures are used. Virulence is rapidly lost by sub-cultivation on laboratory media, but can be regained and enhanced by passage through fowls.

Fowls are most commonly affected but turkeys are also susceptible, and there are records of the disease in the guinea fowl. Ducks and geese appear to be resistant, *e.g.*, Moore (1946a) recorded the disease in chicks, turkeys and guinea fowl, but not in ducks or geese which were on free range and associating closely with the affected birds.

It is generally agreed that the disease is most common on farms where somewhat primitive and unhygienic conditions prevail, being particularly prevalent where fixed hen houses, often stone built with earthen floors, are in use and where overcrowding accompanied by infrequent cleansing of the houses obtains. Outbreaks are less likely where routine testing for bacillary white diarrhoea is practised but they do occur, from time to time, on farms run on modern lines and on which the standard of hygiene is high. Some recovered birds are carriers, and the usual method of infection is ingestion of food and water contaminated by the organism. In a serious outbreak in this country infection was said to have been introduced during the process of carrying out the rapid test; it was held that the antigen contained live *S. gallinarum* and heavy damages were awarded to the plaintiff.

The factors which precipitate an outbreak are not always recog-

nised but overcrowding is of importance, *e.g.*, severe outbreaks followed a spell of wintry weather when, due to snow, birds were confined to the houses for several days on end, and an extensive outbreak in turkeys originated in a small pen in which "broody" turkey hens were isolated and which was overcrowded.

Wilson (1946) confirmed experimentally that spread by contact requires a considerable concentration of infection and that there is a varying susceptibility in individuals and in breeds, and between the sexes. Male birds show symptoms earlier than females and the proportional death rate is higher.

The disease is most common in adult stock. Occasionally outbreaks in chicks occur, and some workers consider that the cycle of infection is similar to that of *S. pullorum* (Beaudette (1925), Beach & Davis (1927) and Hinshaw & Taylor (1933)). Although the causative organism is readily isolated from the ovaries of infected hens it has never been recovered from the eggs laid by "carriers" or recovered birds in any of our experiments; this would appear to be the general experience of workers in this country.

Symptoms

The first noticeable symptom is the appearance of "splashes" of sulphur yellow coloration amongst the droppings which are otherwise normal and "formed." Later the droppings become watery in consistency and entirely bright sulphur yellow or tinged with green. Thirst is most marked and there is inappetence and extreme dejection, followed by coma and death. The comb and wattles are frequently cyanosed, but may be anaemic. The mortality may be high. Blood changes are characterised by a severe leucocytosis and myelocytes and other immature forms are present. There is a marked reduction in the erythrocyte count and erythroblasts and immature erythrocytes can be seen in blood films.

Post-mortem Appearances

In birds examined shortly after death the liver is enlarged and congested, pinkish red in colour with paler areas; in some, multiple small areas of necrosis may be seen. The bronzed appearance described in many text books is rarely evident in fresh cases, being frequently associated with *post-mortem* changes. There is often a haemorrhagic duodenitis and in very acute cases reddening and swelling of the mucous membrane may extend throughout the small intestine. A varying degree of distortion of the ovary similar to that occurring in bacillary white diarrhoea is usually present.

Diagnosis

This depends on the isolation and identification of the causative organism, by the methods already described. In acute cases it is present in the blood, liver, spleen, lungs, kidneys, ovary or testes, oviduct, intestines and bone marrow. In carriers, it is generally localised in the ovary and intestines.

Control

All obviously affected birds should be slaughtered and the bodies burned or buried in quicklime. The remaining birds should be tested by the agglutination test, the reactors removed and the remainder vaccinated. Thorough disinfection of the houses, utensils, etc., should be carried out. In fixed houses with earthen floors it is necessary to remove the top spit of soil and the surrounding land should be limed and rested.

The rapid whole blood test is particularly valuable as it avoids the inevitable delay of several days associated with the tube test, permitting the speedy removal of carriers which may be disseminating large numbers of organisms, and it reduces the handling of stock to a minimum, as birds which pass the test can be vaccinated before being released.

In the acute disease death commonly occurs before agglutinin formation commences; in fact, it would appear that the production of agglutinins is indicative of impending recovery. The titre of recovered birds varies greatly. In field cases it is usually about 1/320 but in experimental cases it may be as high as 1/40,000. Some birds show a falling titre followed by complete recovery. Paille (1935) reported that agglutinins are present in the yolks of affected birds, and that the titres are practically the same as in the respective sera.

A dead stock vaccine is generally used and reports of its efficacy in the field are variable. Wilson (1946) showed that, under experimental and artificial conditions, it is of doubtful value, and it may be that some of the success ascribed to vaccination is mainly due to the removal of "carriers" following the preliminary agglutination test. After the use of a dead vaccine agglutinins are rarely demonstrable or are transient so that this method of prevention does not complicate subsequent routine testing for *S. pullorum*. This is of particular importance in flocks in the Accredited Scheme.

It was shown that a live attenuated vaccine produced a solid lasting immunity but the attendant risks would preclude its use except perhaps in very exceptional circumstances. There was a suggestion that natural "carriers" of *S. pullorum* are resistant to artificial infection with *S. gallinarum* and it will be recalled that Lambert (1933) showed that selection for resistance to pullorum infection, besides resulting in a decided decrease in mortality from that disease, also afforded some protection against fowl typhoid.

The use of sulphonamides will be discussed later.

Infection with Salmonella other than *S. pullorum* and *S. gallinarum*

It has already been noted that salmonella infections in poultry have been recognised for many years, much of the early interest of continental and British workers being concentrated on the disease in the duck and on the presence of the organism in ducks' eggs which had been implicated as a cause of food poisoning in man.

At the World's Poultry Congress in Leipzig in 1936, a special session was given to a discussion on this problem, obviously considered of great importance by continental workers.

The organisms implicated—*S. typhi-murium* (previously known as *S. aertrycke*, Breslau bacillus) and *S. enteritidis gaertner* had been described in poultry in this country by Doyle (1927), Warrack and Dalling (1932 and 1933) and Hole (1932) but the incidence of outbreaks appeared to be low compared with those in Germany and elsewhere on the Continent. Garside and Gordon (1940 and 1943) described a serious outbreak in ducks and ducklings associated with the same organisms and Wilson (1944) recorded several outbreaks in chicks associated with *S. thompson* and one due to a mixed infection with *S. thompson* and *S. typhi-murium*. This would appear to be the first published account of *S. thompson* infection in chicks in this country although Gordon and Buxton (1945a) recognised it as a poultry pathogen in 1943. In 1946, these workers described the occurrence of *S. california, S. bareilly, S. montevideo, S. london and S. anatum*, for the first time in chicks in Great Britain and *S. give* and *S. worthington* were later isolated by Wilson (1947). Dual infection sometimes occurs in a single chick, *e.g.*, *S. pullorum* and *S. thompson*, *S. typhi-murium* and *S. thompson*, *S. thompson* and *S. give*.

A probable source of some of these new types may have been the incorporation in poultry food of dried egg powder, unfit for human consumption. It will be recalled that in July, 1942, the first issue of American spray dried egg was made to the public, and following this a substantial increase occurred in the incidence of outbreaks of food poisoning in man. Of the 24 new species of salmonella isolated from human cases the six commonest were those which headed the list of strains isolated from dried egg, *e.g., S. oranienburg, montevideo, maleagridis, anatum, tennessee* and *bareilly*. (M.R.C. Special Report series, No. 260). These types although new to this country are common pathogens of American poultry. According to Soloway (1948) as many as six types may be present in a single sample of dried egg.

The disease is of great importance in poultry and also from the public health aspect as the increase in the number and size of hatcheries results in the widespread dissemination of stock which may be potential reservoirs of infection. The general tendency to use incubators of greater capacity involving the setting of eggs from diverse sources intensifies the risk of infection.

The disease is essentially one of young stock, the adult being merely a "carrier" and the possible producer of contaminated eggs; chicks, ducklings, turkeys, goslings, pigeons, canaries, parrots and other cage birds are susceptible.

Sources of Infection

It has long been recognised that rats and mice are frequently infected with *S. typhi-murium* and *S. enteritidis* and it was generally considered by workers in this country who, apart from Dalling and Warrack (1932), had consistently failed to find the causative organism in eggs, that food contaminated with rat or mouse faeces was the usual source of infection, *e.g.*, in an outbreak described by Wilson (1944) *S. typhi-murium* was isolated from mouse faeces present in dried milk powder and *S. thompson* was isolated from the intestines of mice caught in the brooder house; these were believed to represent the sources of infection. The disease recurred in the following season and it was then shown that infection had been introduced into the incubator by eggs, the shells of which had been contaminated with salmonella acquired during passage through the cloaca in the process of laying. This was confirmed by the demonstration of "carriers" in the flock, by cloacal swabbing. It may therefore have been that the mice acquired the disease through contact with infected chicks and disseminated rather than introduced the disease.

Incubator infection as a result of contamination of the egg shells was recognised as a source of infection in America many years ago (Beach, 1936) but for some reason it appears to have been

overlooked in this country, and none of the earlier published work gives any indication that cultures were ever made from the outside of the shell.

In the duck, it may be that direct transmission of infection sometimes takes place as salmonella have been recorded in the yolk of duck eggs. (Warrack & Dalling (1932, 1933), Wilson (1945a), Buxton & Gordon (1945b) and others.) It seems certain that this rarely, if ever, occurs in chicks as most workers have consistently failed to isolate the organism in the contents of hen eggs.

In an addendum to a paper published by Wilson (1945) it was stated that *S. thompson* had been isolated from the yolk and white of duck and hen eggs laid by survivors of an outbreak. It was later confirmed that the organism is present in freshly laid duck eggs but further experiments tended to suggest that it may have penetrated the shells of the hens' eggs during the period of storage which was sometimes 14 days or longer. No attempt was made to keep the eggs apart from each other during this period so that contamination by contact may also have occurred. In all, 1,023 eggs were examined and *S. thompson* was isolated from 60: on the outside of the shell of 45 and in the contents of 17. Buxton and Gordon (1947) examined 774 eggs laid by pullets and found the shells of 5·4 per cent. of the untrapped eggs infected, whereas in trapped eggs only a single egg shell was contaminated. They failed to isolate *S. thompson* from egg meat.

The rate of penetration depends on conditions of moisture and temperature. According to Schaaf (1936) it took place in ducks' eggs in the incubator in five days, whereas Lerche (1936) found that at room temperature penetration did not occur until 15 days. Buxton and Gordon (1947) record that penetration occurred in incubated eggs within seven days, while only one yolk became affected out of ten stored at room temperature for 14 days and one out of ten stored under the same conditions for 21 days.

Wilson (1945b) showed that the virulence of the organism appeared to play a part in the rate of penetration and found that after passage it could occur in three days; with less virulent strains the period was longer. It was possible to set up experimental outbreaks by hatching eggs, the shells of which had been painted with a culture of *S. thompson*, but when the virulence of the culture had been enhanced by passage, the majority of the embryos died late in incubation. Untreated eggs became contaminated during the process of daily turning which was done by hand.

Under normal conditions, therefore, the organism probably penetrates the shell during the first week of incubation and multiplies in the yolk. At the time of hatching and during drying off infection occurs as a result of inhalation of germ-laden dust and fluff, a large amount of which is present in the circulating air in forced draught machines.

Secondary spread may take place in the brooder through contamination of food and water by infected droppings or through chicks picking dried faeces which frequently adhere to the wire floors and supporting bars. Primary brooder infection may arise from the use of food contaminated by rat or mouse droppings or by flies. Attention has already been drawn to the work of Gwatkin and Mitchell (1944) and McNeil and Hinshaw (1944) also record the isolation of *S. typhi-murium* from flies and from snakes and cats from infected turkey ranches. Adult pigeons are frequently carriers of *S. typhi-murium*. Felsenfeld and Young (1945) showed that salmonella could survive for several weeks on vegetables kept at room temperature and suggested that this may be a further source of infection. It is probably of greater importance in man than in poultry. Scott (1940) in addition to *S. cholera-suis*, isolated *S. typhi-murium*, *S. thompson* and *S. enteritidis* from the mesenteric lymph nodes of pigs which may therefore be possible reservoirs of infection. Later investigators (M.R.C. Special Report Series No. 260) reported that out of 133 strains isolated 75 belonged to species not previously recorded and since all but three of the new species had already been isolated in dried egg it was considered that they had been introduced to pigs by this medium.

The Disease in Chicks

Deaths usually occur within the first 12 days, the peak period being between the 6th and 10th days. Mortality varies from negligible proportions to 20 per cent. in some outbreaks; in others it may reach 80 per cent. or higher. Environmental conditions probably play an important part, factors such as chilling or improper feeding aggravating losses.

The lesions commonly present have already been described and in addition joint infections sometimes occur in turkeys and pigeons (Brunett (1930), Durant & McDougle (1932), Higgins *et al.* (1944) and Wilson (1948)).

In chicks infected with the acute disease the causative organism is present in all the internal organs, but in a few weeks it is generally recoverable from the intestinal contents only; in some the gall bladder also remains infected. Many birds recover completely and cease to eliminate organisms in the droppings.

S. typhi-murium and *S. thompson* have been isolated from cloacal swabs from "carriers" for periods up to nine to 16 months and these organisms have been isolated from distorted ovules in fowls which gave positive and doubtful results to tube tests and rapid whole blood tests for *S. pullorum* (Wilson, 1947 and 1948).

Buxton and Gordon (1947) record the isolation of *S. thompson* from 75 per cent. of random faecal samples from carriers during the ninth month of observation and from 42 per cent. during the tenth month. The rate of infection steadily decreased and only two remained carriers at 18 months. It seems probable, however, that in many outbreaks the majority of infected birds remain carriers for a much shorter period; in many of our experiments with *S. typhi-murium* it has not been possible to produce carriers excreting the organism for more than a few weeks and it seems probable that where the disease persists re-infection may be responsible in some cases and infection of the gall bladder in others.

Control Measures

It is a sound policy to advise destruction of survivors, especially when the mortality rate is high. Where, however, a very large number of chicks remain it may be necessary for economic reasons to rear them to an age suitable for table purposes, *e.g., petit poussin*. The use of sulphonamides in this connection will be discussed later.

Thorough disinfection of the incubator and brooder should be carried out and when a fresh batch of eggs are set they should be fumigated with formaldehyde as soon as practicable, certainly within 24 hours. During rearing, strict hygienic measures should be practised. Food and water should be placed where they cannot be contaminated by droppings. Particular attention should be paid to water troughs as water containing small amounts of poultry mash is an excellent medium for the multiplication of salmonella. According to Gauger and Greaves (1946) scrubbing and rinsing the troughs several times is not sufficient to prevent infection but daily scalding is effective.

Prevention

Most workers are agreed that the agglutination test is unlikely to become a practical method for the detection of carriers. Many birds eliminating the organism in their droppings fail to react and there are technical difficulties in carrying out large scale testing of the type necessary. Cloacal swabbing is also uncertain as the passing of organisms in the faeces is intermittent and in any event it is impractical on a commercial scale.

Attention must, therefore, be concentrated on hygienic measures of which incubator fumigation is one of the most important. This will be discussed later. Ample nest accommodation should be provided in the laying houses and the nest linings should be replaced frequently. Badly soiled eggs should not be retained for hatching. If for any reason this is unavoidable, they should be soaked in five per cent. dettol solution and the faeces removed by swabbing. The solution used should always be at a temperature slightly higher than that of the eggs. Less badly soiled eggs should be swabbed with a cloth moistened with dettol; the indiscriminate wiping with a damp rag as practised by some farmers merely spreads infection to other eggs. Cursory dipping for a few seconds in methylated spirits is useless as it has been shown that *S. pullorum* on egg shells resists the action of 95 per cent. methyl alcohol for five to 55 minutes and of a freshly made solution of chloride of lime (1 lb. to 50 gallons of water) for up to half an hour (Gwatkin, 1926).

During the period of storage, which should always be as short as possible, the eggs should be kept in a cool room and the incubator should be fumigated as soon after setting as practicable. As it is customary for a fresh batch of eggs to be added each week to replace those removed to the hatching compartment, fumigation of the incubating chamber should be repeated each time this is done. In some hatcheries chicks are also fumigated during hatching, first soon after it commences and again 12 to 24 hours later. It is also a good practice to fumigate the hatcher before removing the debris remaining after hatching is completed, to prevent spread of contaminated down and fluff throughout the incubator room. The provision of a separate hatching compartment is valuable and in large premises it may be possible to have the setters and hatchers in separate rooms. The complete isolation of brooding rooms from the incubator room is an important feature.

Infection is cumulative and in large hatcheries the provision of duplicate brooding equipment, preferably in separate rooms, is of great value as it allows adequate time for proper cleansing and disinfection before re-use, otherwise the continual passage of infected chicks through the brooders may result in a massive accumulation of infection of exalted virulence.

Vermin control should be practised continually and food should be stored in rat and mouse proof containers.

These methods have been found to be effective in practice and it

is of interest that since their institution in two large hatcheries in which heavy losses from salmonellosis had been experienced in successive years, in one no further outbreaks occurred and in the other there have been two isolated breakdowns both coincident with failure to fumigate.

Incubator Fumigation

Although fumigation of the incubator and its contents with formaldehyde during the process of incubation has only recently become common in this country, it was first used 40 years ago by Pernot (1908) who obtained good results by placing a small dish of formalin in the incubator when the eggs were "pipping" and leaving it there until the chicks were removed. Gwatkin (1927), unaware of this earlier work, recorded successful fumigation of a still air incubator six to eight times during incubation without any adverse effect on hatchability. Under modern conditions, in large forced-draught incubators, two methods of releasing the gas are usualiy employed. In one, the requisite amount of formaldehyde (20 c.c. per 100 cubic feet of incubator space) is soaked up by a piece of cheese cloth about a yard square which is then hung up as near the fan as practicable. Evaporation occurs in a few minutes. In the second method, 35 c.c. of formaldehyde and 17·5 grammes of potassium permanganate are required for each 100 cubic feet of incubator space, the materials being placed in a wide enamelled basin with sides sufficiently high to prevent splashing. The organisms on the egg shells are usually destroyed within 30 minutes but contaminated down and fluff require a longer exposure so that the doors should be kept shut for an hour or more.

For fumigation of still air incubators it is necessary to use bigger quantities of the agents; 2·5 c.c. of formaldehyde and 1·8 grammes of potassium permanganate per cubic foot of air space (Gwatkin, 1929). Sometimes difficulty is experienced in preventing diffusion in small machines.

The humidity of the air in the incubator is important as it affects the germicidal effectiveness of the formaldehyde. About 68 per cent. is the optimum (wet bulb 90° F. and dry bulb 100° F.), (Bushnell *et al.*, 1929). Fumigation carried out with similar amounts of the reagents in conditions of low humidity was ineffective. Eggs can withstand repeated fumigation with two to three times the above strength without harmful effect to the developing embryo, which Marcellus *et al.* (1930) showed to be most susceptible to injury between the 24th and the 96th hours. Insko *et al.* (1941) confirmed these results and claimed that embryos may be exposed to concentrations of formaldehyde obtained by mixing 105 to 140 c.c. of formaldehyde and 57 to 70 grammes of potassium permanganate per 100 cubic feet of incubator space for one hour without serious loss. This critical period of embryonic susceptibility is, however, avoided in fumigation for the control of salmonellosis as this should always be completed within a few hours of setting. Where significant mortality occurs in the embryos it is usually found to be confined to certain egg trays, which have probably been exposed to an excessive concentration of gas. To prevent this in large incubators it is better to use several smaller containers rather than one large one. It has also been suggested by Wright *et al.* (1944) that mortality in the embryos can be prevented by neutralising the fumigant after the period of exposure by introducing into the machines a volume of 25 per cent. ammonium hydroxide equal to one half the volume of formalin used in fumigation. This may be done by soaking a cheese cloth or in larger machines by sprinkling on the floor. The same authors point out that variations in the purity of potassium permanganate sometimes make it difficult to compute the exact amount to use. They suggest that the residue after fumigation should be tested by the addition of a small amount of formalin. If formaldehyde is driven off, or if the addition of water to the residue produces a violet colour, too much potassium permanganate has been used. If, on the other hand, the residue is moist and the addition of a few crystals of potassium permanganate causes rapid liberation of formaldehyde, not enough has been used. If insufficient potassium permanganate is used all the formaldehyde will not be liberated from the formalin, and a lethal concentration of fumigant will not be produced; an excess has no adverse effect on embryonic development but increases the cost of fumigating. Fuller accounts of the above method are given by Graham and Michael (1932) and Godfrey *et al.* (1946). A similar procedure may be used to disinfect other poultry equipment, and simple fumigation chambers have been erected on some farms for this purpose. In conjunction with incubator fumigation, the floors and lower walls of the incubator rooms should be sprayed with disinfectant to prevent re-infection of the treated machines when the doors are opened.

Fumigation may not always be effective in completely eliminating mortality from salmonellosis, as infection may remain in some inaccessible part of the incubator, and the possibility of occasional direct egg transmission cannot be entirely overlooked. The practice should not necessarily be adopted as a routine procedure, but it would seem to be a sound precaution, particularly when a large number of pullet eggs are incubated, e.g., for the production of first crosses which are in constant demand.

At the present time, formaldehyde is the best fumigant but it may be that a superior one will become available. Following the successful war-time use of aerosols, such as propylene and triethylene glycol, for the reduction of pathogenic bacteria in the air of buildings, Gwatkin (1947) tried them for the disinfection of incubators. The results were disappointing; they had little effect on egg shells contaminated with *S. pullorum* and the organism grew in 1/100 glycol in broth.

The report of the Commission on Air-borne Infections (1946) showed that pneumococci could grow in broth containing five per cent. glycol but were killed in air by a concentration of one gramme in 10,000,000 c.c. of air. Even if these agents have a similar effect on *S. pullorum*, their apparent failure to kill the organism on the surface of the egg shell would limit their use. It is claimed that aerosols having a lethal effect on organisms on non-porous surfaces are now available and it may be that these substances released by an atomiser of the Phantomyser type will provide an effective method of incubator sterilisation.

THE USE OF SULPHONAMIDES IN SALMONELLOSIS

Within recent years drugs of the sulphonamide series have been used in *S. pullorum* infection and there is no doubt that mortality in chicks can be reduced very considerably by their use.

Severens *et al.* (1945) showed that sulphamerazine and sulphadiazine were the most effective; 0·5 per cent. of the former being as beneficial as two per cent. of the latter. They suggested that treated birds were unlikely to become carriers. On the other hand, Bottorff and Kiser (1947) while reporting a similar reduction in mortality, found that 90 per cent. of the survivors remained carriers and Pomeroy *et al.* (1948) also found carriers amongst the survivors in every experimental group. They reported that the sulphonamides had little effect on the disease in the turkey, whereas Mullen (1946) stated that mortality was definitely reduced.

Hammond (1945) considered that it was possible to eradicate fowl typhoid from a flock by adding two grammes of sulphathiazole to each gallon of drinking water from time to time. This treatment had no adverse effect on egg production but shell quality was poor for one or two days afer administration. Moore (1946b) showed that while sulphamerazine was effective in reducing mortality from fowl typhoid, treated birds remained "carriers." Holtman and Fisher (1947) preferred sodium sulphamerazine as affected birds tended to stop eating but continued to drink. There was slight toxicity and treated birds did not put on weight. *S. gallinarum* was not present in the intestinal contents 24 hours after treatment but could persist in the gall bladder which might represent a source of re-infection. Adult carriers became negative after five months. Pomeroy *et al.* (1948) reported that sulphadiazine, sulphamerazine and sulphamezathine reduced mortality from *S. typhimurium* in chicks by 50 per cent. but proved only half as effective when fed to poults.

There will be general agreement that in bacillary white diarrhoea, treatment with sulphonamides is contra-indicated in chicks intended for prospective breeding stock, but when it is considered necessary for economic reasons to rear survivors for table purposes there can be little objection to the use of these drugs. Infected chicks should be placed on wire floors and given 0·2 per cent. sodium sulphamerazine or sodium sulphamezathine as drinking water for eight consecutive days. Treatment should not be prolonged beyond this period or growth is retarded.

Similar treatment might also be commenced immediately an outbreak of fowl typhoid is diagnosed, in conjunction with the other control measures already described. Dosing has no effect on the titre of the adult carrier and does not interfere with subsequent agglutination testing.

It is probable, however, that sulphonamide treatment will prove of most value in salmonellosis other than *S. pullorum* and *S. gallinarum*, particularly in large-scale outbreaks in hatcheries retaining chicks for the three-week-old chick trade and for the supply of growing pullets, where the wholesale destruction of an entire batch will not readily be countenanced by the owner.

Preliminary experiments we have carried out show that while carriers may still remain after the prescribed period of treatment, their number is very materially reduced and it is suggested that destruction of the worst affected groups and medicinal treatment of the remainder, in conjunction with the preventive measures already described, is the most practical method of dealing with large outbreaks.

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Discussion

The chairman then called upon Dr. R. F. Gordon, D.SC., M.R.C.V.S., Director, Poultry Research Station, Animal Health Trust, to open the discussion.

Dr. R. F. Gordon: In opening this discussion I would first of all take the opportunity of congratulating Mr. Wilson on his excellent survey of the present extent of our knowledge of avian salmonella infections. I have seldom had the pleasure of listening to a more concise and lucid review of the literature which has accumulated on any subject. Not only has Mr. Wilson completely covered this subject from all angles, but he has selected his information with considerable care, and the bibliography which he has consulted constitutes, in my opinion, one of the outstanding contributions which have been made on this subject. If I have one criticism to offer it would be that he has covered the subject too fully, and that there is little of a provocative nature on which to base a discussion. I am reduced, therefore, to merely dotting his Is and stroking his Ts.

At the recent World Poultry Congress at Copenhagen, I had the privilege of acting as chairman to the session devoted to salmonellosis, and it was quite clear from the papers given that in all countries the problem in poultry has two main aspects. The first is the economic loss to the industry as a result of salmonellosis, and the second the public health problem. As Hinshaw has pointed out: "There are few genera of micro-organism which can infect so wide a range of hosts. There are now reported more than 150 antigenic types of salmonella, and of these, few, if any, are limited to one host, and new hosts are continually being found. There may well be no truly avian or human types, in fact such a description frequently means only priority in isolation." Some 60 members of the salmonella group have now been reported as the cause of outbreaks of disease in the avian species, and of these at least 56 have also been found in man.

It is difficult to assess the economic loss from any disease, but since the salmonella group contains two of the commonest causes of epidemics in chicks and adult poultry, *e.g.*, *S. pullorum* (B.W.D.), *S. gallinarum* (fowl typhoid), the salmonella group must be regarded as the most important cause of bacterial disease in the avian species. The Poultry Technical committee in their 1936 report stated that in Great Britain the poultry industry suffered an annual loss from disease of approximately £4,000,000. They stressed that a very high proportion of this loss—certainly half—occurred in the chick-rearing stage. Gordon and Buxton (1946) in a survey of chicks' diseases isolated salmonellae from approximately 30 per cent. of 6,600 groups of chicks examined during the twelve years 1933-44. Assuming that only half the estimated economic loss of £4,000,000 occurred in the chick stage, then salmonella infections were responsible for nearly £1,000,000 per annum. In Canada, Beily (1933) stated that the loss from B.W.D. alone amounted to $2,500,000,000. Numerous workers in other countries have come to the same conclusion, and in South Africa Henning (1939) stated that infection of birds with different salmonella types was much more varied and widespread than in mammals and that the losses involved were probably far greater than those from any other cause. In estimating the economic loss from avian salmonellosis, cognizance must be taken of the fact that heavy losses occur through the adverse effects on the survivor fowl. Various workers have reported that the fertility and hatchability of eggs from reactor hens is reduced by 20 per cent. to 30 per cent., and that the viability of infected groups is some 60 per cent. below that of non-infected fowls. Egg production is similarly reduced and numerous workers have produced ample evidence to show the marked difference in the average egg yields of reactor and non-reactor fowls.

The position regarding outbreaks caused by other salmonella organisms is even more serious, and Gordon and Buxton (1946)

in their survey indicated the increase which has occurred since the war years from this cause. The total outbreaks recorded in the first nine years of the survey 1933-44 were 100, or an average of 11·1 per year, and for the last three years, 1941-44, 113 outbreaks, or an average of 37·6 per year—a numerical increase of more than 200 per cent. This increase has been maintained, and during the last year more than 181 outbreaks were confirmed in the eight months from January to August, 1948. In addition to this numerical increase Mr. Wilson has drawn attention to the change in the types isolated, and enumerated the eight organisms isolated for the first time in this country from 1944 onwards. To this list must be added nine further types isolated for the first time in poultry in Great Britain, Buxton (private communication) giving in all a total of 20 salmonella types which have been identified from poultry in this country, in addition to *S. pullorum* and *S. gallinarum*. When one considers that all of these types are potential pathogens to humans, the importance of this group of diseases as a public health problem cannot be over-stressed. Even *S. pullorum* has recently been incriminated in several cases of human food poisoning (Judefind, 1947), and there are, of course, numerous references to outbreaks of human food poisoning from the consumption of improperly cooked duck eggs (Gordon and Buxton, 1945).

There have also been extensive studies on the occurrence of salmonella types in dried egg powder, and as Hinshaw (1948) points out, such contaminated products are a public health hazard when used as uncooked or slightly cooked food such as meringues and egg drinks, and that this is important, since salmonellosis is a disease of the young and such preparations are favoured by children. Goresline (1948) states that the salmonella problem in eggs and egg powders is a serious one and a challenge which must be met in the near future. The fact that billions of eggs have been consumed in the home and used in egg products without attention being drawn to serious outbreaks should not lull us into indifference.

In considering the control of B.W.D., Mr. Wilson refers to the value of the agglutination test, both the serum tube and the rapid whole blood methods, in the detection of carriers of this disease. In discussing the rapid test which was introduced into the accredited scheme in Scotland in 1948, he refers to some of the American work which had been carried out on the comparative value of the two methods of testing. In England the rapid method was adopted in the Ministry's accredited Poultry Breeding Scheme during 1942 as a result of certain war-time conditions, and during the year 1942-43 a large field scale experiment was carried out (Gordon, 1947). In all, 82 flocks involving 15,440 birds were tested simultaneously by the two methods, and an agreement of 98·76 per cent. was established. Only 1·9 per cent. of the birds gave doubtful reactions to the rapid method. The majority of these doubtful reactions are associated with low serum titres to *S. pullorum* or to the presence of somatic antigen XII in other salmonella types and in some coliform and paracolon bacilli.

Experimental work showed that in interpreting doubtful reactions consideration must be given to the pullorum history of the flock. In infected flocks pin-point or doubtful reactions should be regarded as positive and indicative of infection, whereas in non-infected flocks such reactors can often be disregarded. In field work it is usually desirable to isolate these doubtful reactors and to submit them to a subsequent test at a later date. In the experiment referred to, the birds which gave discrepant results at the comparative test, together with a large proportion of the birds which reacted doubtfully to the rapid test, were examined culturally. Three hundred and sixty-seven birds were examined in this way, and when the isolation of *S. pullorum* was used as a criterion of accuracy the rapid test gave a maximum accuracy of 92·1 per cent. and the tube test 90·2 per cent. It has also been shown that fowl which have had access to rat virus poison composed of *S. enteritidis* reacted positively to the rapid test. Recent work at Weybridge has shown that an alcoholised antigen has better keeping qualities, greater specificity and reacts more quickly. The rapid whole-blood test has been in use officially in England for five years and has, with few exceptions, given complete satisfaction. The number of flocks in which controlled testing has been carried out has been increased from 307 to 3,099. The average number of reactors per station has dropped during the same period from 10 to 4·5, 75 flocks have been free for six years, 371 for five years, 600 for four years and 885 for three years. During 1946-47 just over 2,000,000 birds were tested with only 0·74 per cent. reactors. A further interesting outcome of controlled testing is that the percentage incidence of B.W.D. has been consistently less in chicks received from accredited poultry breeding flocks. Expressed as a percentage of the total groups of chicks from which *S. pullorum* has been isolated, only 18 per cent. occurred in chicks from accredited flocks, and 81·2 per cent. in chicks from non-designated flocks.

Mr. Wilson has also referred to the complications which have arisen in U.S.A. as a result of the occurrence of so-called *S. pullorum* variant. Edwards and Bruner (1946) have shown, however, that there are three components in the somatic antigen XII of *S. pullorum*, and that quantitative variations, especially of antigen XII_3, may have accounted for these doubtful reactions. Over 100 strains isolated from outbreaks in England have been examined antigenically at Weybridge and all except one have been found to contain XII_3 in variable amounts, and in all cases this antigen was detected by the rapid test carried out with routine stained antigen prepared at Weybridge and which contains the full complement of XII_3.

The present official policy in the accredited scheme is that all birds on accredited farms must be subjected to the test, and one wonders whether in flocks where there has been a clean history for a number of years it would not be preferable to restrict testing to the breeding flock only, so that the service could be extended to include flocks at present outside the scheme. Mr. Wilson mentions that B.W.D. is a serious cause of disease in turkeys in the U.S.A., but in Great Britain the organism was only isolated on two occasions in turkey poults during the twelve-year survey carried out by Gordon and Buxton, and in the routine testing of some 10,000 turkey blood samples annually it was extremely rarely that a reactor was found.

There is very little that one can add to Mr. Wilson's summary of our present knowledge on the epidemiology of these other salmonella infections, and it has been fairly clearly shown in recent years that the commonest method of propagation is the contamination of the egg shells with infected faeces and subsequent penetration of the baccilli through the shell to the yolk, under certain conditions of incubation.

Mr. Wilson has quite rightly stressed the importance of hygiene in the hatchery control of salmonella infections, and has referred at some length to incubator fumigation. It has recently been shown, however, that up to four times the officially accepted concentrations of formaldehyde may be used with more satisfactory results (Wright *et al.*, 1946).

The work on the epidemiology of salmonella infections has demonstrated the necessity for fumigation immediately the eggs are set. The second fumigation should be carried out when the eggs are transferred to the hatchery, and finally after the hatch has been taken off but before cleansing is carried out. At least one large hatchery in this country has carried out routine fumigation with these amounts without any adverse effects on incubation. Mr. Wilson has also drawn attention to the possible use of aerosols in incubator sterilisation. Pilot experiments at Weybridge, using aeryl I, atomised by a phantomiser for a length of time and in concentrations in excess of those recommended by the manufacturers, showed this method to be ineffective in controlling surface infection of certain materials (wool, fluff and egg shell) infected with *S. pullorum*. The value of aerosols in controlling aerial contamination in hatcheries or brooder houses has yet to be investigated, but would appear to be a fruitful line of research.

The use of sulphonamide drugs in the treatment of avian salmonellosis infections is debatable. A number of workers have shown that mortality can be materially reduced by this method but that a high percentage of the survivors so treated remain as carriers. Bearing in mind the fact that the cycle of infection is via the egg, and the undoubted progress which has been made by controlled blood testing, one must be chary of recommending treatment except in selected cases and under the strictest supervision. Treatment should not be used on breeding flocks, and it would appear that at the moment it should be restricted to chicks intended for table or for battery egg production and maintained in the strictest isolation. I would welcome Mr. Wilson's view on whether the possibility of carriers being produced would prevent the use of sulphonamides in the case of salmonella organisms pathogenic to man, since such carriers might presumably lay infected eggs. I also wonder whether he has any information on the possibility of sulphonamide-resistant strains being produced.

Probably the most fruitful line for the attack of avian salmonellosis is dipping of hatching eggs in germicidal solutions. Mr. Wilson has referred to his experiments in this respect with 5

per cent. dettol. Buxton (private communication) carried out preliminary tests using potassium tellurite. Owing to the necessity for using a relatively high concentration of the substance in water, the resultant expense might preclude its use, although it is not yet known what maximum number of eggs can be dipped in a given volume without the latter losing its bactericidal activity. In a recent American publication reference is made to a method in which eggs in wire baskets were immersed in a detergent solution which had been heated to 140° F. The eggs were then sprayed with water at the same temperature, the faecal material which had been loosened by the detergent being then washed off.

Pritsker (1941) refers to the disinfection of egg shells by cooling the egg to 8° to 10° C. and dipping in 0·5 per cent. formalin warmed to 23° C. Eggs have also been dipped in sodium hydroxide, sodium orthophenylphenate, and quaternary ammonium chloride without reducing hatchability (Olsen and McNally, 1947).

To summarise, although B.W.D. is still a serious economic problem most outbreaks can be satisfactorily controlled by the blood testing of breeding flocks and hygienic methods in the hatchery and brooder, while the controlled testing and veterinary supervision of the flocks included in the accredited scheme has resulted in a nucleus of pullorum-free flocks being established.

The problem with regard to the other salmonella organisms, however, is a much more difficult proposition and, from both the economic and public health aspects, a serious one. Not only is there the loss from chicken mortality, but the knowledge of dangerous impurities in any food product gives rise to an aversion to that product in the consuming public.

As Goresline (1948) points out, only an occasional egg is contaminated with salmonella organisms and only a small number of persons can be infected at one time when shell eggs are consumed. However, in the commercial processing of liquid, dried or frozen eggs, thousands of eggs are mixed in one vat and a single egg may contaminate an entire vat. The number of persons involved in a food poisoning outbreak from a commercial product may be quite large, since each portion of food prepared from a single contaminated container has an equal inoculation. Elimination of all salmonella types from poultry flocks is an impracticable proposition and the problem to a large extent is in the hands of the egg producers and the processors of egg products. The veterinary profession can be of the greatest assistance by advising on flock management, clean flock production and packing and hatchery hygiene.

The 20 types which have been isolated in Great Britain are:—

1. Pullorum
2. Gallinarum
3. Typhi-murium
4. Enteriditis
5. Thompson
6. Montevideo
7. Bareilly
8. California
9. Anatum
10. Worthington
11. Derby
12. London
13. Oranienburg
14. Bovis malrificans
15. Cubana
16. Brancaster
17. Concord
18. Kentucky
19. Dublin
20. Give

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The Use of Furazolidone in the Treatment of Infections of Day-old Chicks with *S. pullorum*, *S. gallinarum*, *S. typhi-murium* and *S. thompson*

BY

J. E. WILSON

Lasswade

Introduction

FURAZOLIDONE (N-(5-nitro-2-furfurylidene)-3-amino-2 oxazolidone), first synthesised by Stillman and Dodd of Easton Laboratories, U.S.A., and shown by Yurchenco, Yurchenco and Piepoli (1953) to have pronounced anti-bacterial action against gram-negative bacilli, including salmonella, has attracted attention in the U.S.A. and in this country chiefly perhaps as a potentially effective agent in the treatment and prevention of fowl typhoid.

Its use in fowl typhoid was first suggested by Harwood (1954) who found it highly effective in controlling a serious outbreak of this disease in turkeys in which there was a mortality of 100 per cent. in an untreated control pen. Successful results have also been described in U.S.A. by Grumbles, Wills & Boney (1954), Harwood & Stunz (1954), and Lucas (1955). In Great Britain, Williams Smith (1954a) reported that when 90 nine-week-old chicks experimentally infected with fowl typhoid were given furazolidone at a concentration of 0.02 per cent. in the mash, two died and only one of the 88 survivors developed agglutinins for *Salmonella gallinarum*. A full account of the chemotherapy of experimental fowl typhoid was later provided by the same worker (Williams Smith, 1955). Furazolidone was greatly superior to sulphonamides, dihydrostreptomycin, chloramphenicol, terramycin and aureomycin in the treatment of experimental fowl typhoid in one-day-old chicks and nine-week-old chickens. Williams Smith (1954b) showed that furazolidone was also effective against *S. pullorum* infection in chicks and concluded that the most satisfactory therapy was 0.04 per cent. of the drug in the mash continuously for 10 to 14 days.

Following a diagnosis of *Salmonella* infection in chicks, the disposal of survivors often presents a difficult problem. In the official schemes slaughter is usually recommended. Where there has been a heavy mortality an owner will usually readily consent to this procedure but in mild outbreaks where deaths are few there is generally unwillingness to comply; a compromise may be reached by retaining the chicks and disposing of them for table purposes at the earliest opportunity.

It is from the less severe outbreaks that proportionately larger numbers of carriers usually stem. It seemed that furazolidone, by controlling mortality and lowering the incidence of carriers, might be even more valuable in the treatment of salmonellosis of young stock than in fowl typhoid.

The present paper describes a series of experiments begun in the summer of 1954 to determine the value of the drug against experimental infections of day-old chicks with *S. pullorum*, *S. gallinarum*, *S. typhi-murium* and *S. thompson*. The results, using a concentration of 0.04 per cent. of the drug, in the mash, are in general agreement with those of Williams Smith (1954b); at the lower concentration (0.02 per cent.) they are less satisfactory than those described in his original paper (Williams Smith, 1954a). As the type of outbreaks produced closely approximates those occurring naturally, it is considered that the results obtained are likely to be valid under field conditions.

Material and Methods

Pure bred Light Sussex, White Wyandotte, Brown Leghorn, Rhode Island Red and Black Leghorn/Rhode Island Red cross day-old chicks were used. The test organisms were grown on agar from broth

cultures of the dried organism. Saline washings were standardised to Tube 6 Brown's scale. In Experiment 1 chicks were infected by introducing 0.5 ml. of the suspension into the crop by means of a pipette. This method of individual dosing was also used by Williams Smith (1954a). It is tedious and time-consuming and has little to recommend it as a means of infecting day-old chicks.

In experiments 2, 3 and 4, chicks were infected by the spray method devised by the writer nearly 25 years ago (Wilson, 1931). Although at that time it was commonly believed that ingestion of food and water contaminated by *S. pullorum* was chiefly responsible for natural outbreaks of bacillary white diarrhoea, difficulty was experienced in reproducing the disease experimentally in chicks which received drinking water to which washings of agar cultures of *S. pullorum* were added.

Results were uncertain; even in day-old chicks the mortality was never high and chicks over three days old were refractory as far as the production of disease with any appreciable mortality was concerned. As the disease is egg-borne, infection within the incubator by inhalation of germs on dust and fluff appeared more likely and it was found that by spraying a suspension of *S. pullorum* over healthy day-old chicks and returning them to the incubator to dry off, disease having a mortality of 100 per cent. could be set up. The death rate varied according to the proportion of chicks sprayed, *e.g.*, to produce an outbreak with an almost negligible mortality and a high proportion of carriers amongst the survivors it was sufficient to spray the down of the heads of two chicks and place them in an incubator with a group of healthy chicks. When equal amounts of *S. pullorum* suspension were administered to equivalent groups of chicks by spraying and adding to the drinking water, the mortality resulting from the former method was about four times greater than that from the latter.

As a result of these experiments, a standard procedure was adopted for infecting day-old chicks with any of the *Salmonella* group of organisms. Saline washings of nutrient agar cultures of the test organism standardised to Tube 6 Brown's scale are used at the rate of 10 ml. for 150 to 200 day-old chicks. During the process of spraying and for 30 minutes afterwards the chicks are held either in an incubator at 100° F. or in a large cardboard container in a warm room. They are then divided into random groups for experimentation.

Following spraying with *S. pullorum* and *S. gallinarum* a mortality rate of 100 per cent. may be expected. The course of the disease follows a standard pattern. Deaths begin on the fifth day and reach a peak on the sixth day with about the same number of deaths on the seventh day as on the fifth, *e.g.*, in three experiments involving groups of 45 to 48 chicks the daily death rates were, fifth day 9, 9, 10, sixth day 24, 23, 23, and seventh day 9, 8, and 11. With other members of the *Salmonella* group, the mortality is variable; strains appear to differ considerably in pathogenicity.

Apart from the ease of administration and regularity of infection, this method has the additional advantage that as it closely simulates the natural mode of dissemination of infection and produces outbreaks of disease similar to those occurring in the field, it is likely to provide a reliable indication of the practical value of any drug or other control measure proving successful experimentally.

Bacteriological Examination of Chicks

Autopsies were performed on all chicks which died or were destroyed during the experiments. Bacteriological procedures varied according to circumstances. In infections with *S. pullorum* and *S. gallinarum*, chicks from the untreated control groups invariably showed gross evidence of disease (enlarged liver and spleen, nodules in lungs and myocardium) and direct cultures from the livers on MacConkey medium were sufficient to isolate the organism. Chicks which died or were destroyed from all other groups were examined more minutely as it was essential to determine if they were carriers. The liver and lungs of each chick were removed aseptically, ground up in a Griffiths tube and added to 100 ml. of Selenite F medium (Leifson, 1936) in which the disodium salt was substituted for the two phosphate salts and the *p*H adjusted by N/1 HCl (Hobbs & Allison, 1945). After incubation at 37° C. for 20 hours, subcultures were made on MacConkey media which were examined for the presence of salmonella after 20 to 24 hours' incubation.

In the early experiments the entire intestinal tract of each chick was minced up and cultured in Selenite F. media and examined for salmonella by the method already described. In later experiments an emulsion of the intestinal contents in normal saline was substituted. Samples of faeces from the various groups were also examined during the course of the experiments but cloacal swabbing was not carried out. Previous experience has shown the limitations of the latter method.

Results

Experiment 1.

One hundred and fifty day-old chicks were infected by introducing a suspension of *S. gallinarum* directly into the crop as previously described, and divided into two random groups.

Two chicks, which inadvertently received some of the suspension into the trachea, died 48 hours later, with massive nodule formation of both lungs. Twenty-four hours after infection one group of chicks was given a commercial chick mash to which furazolidone was added to give a concentration of 0.02 per cent. Treatment was continued for five days. The other group received mash only. The results are shown in Table I (*opposite*).

Treatment was highly effective in controlling the disease. Losses in the treated pen during the first 21 days amounted to four chicks (5.3 per cent.), compared with 68 (90.6 per cent.) in the control pen. It is apparent that the drug was not completely bactericidal. Twenty days after treatment ceased a serious breakdown occurred; nine chicks died during

FIG. 1.—Hearts of chicks infected with *S. pullorum* (Experiment 4).

17 = Treatment with Furazolidone.
3 and 32 = Treated with Sulphaquinoxaline.
29, 43, NN3 = Untreated controls.

FIG. 2.—Lungs of chicks infected with *S. pullorum* (Experiment 4).
Top = Treated with Furazolidone.
Middle = Treated with Sulphaquinoxaline.
Bottom = Untreated controls.

TABLE I

THE EFFECT OF TREATMENT WITH FURAZOLIDONE (0.02 PER CENT.) FOR FIVE DAYS STARTING 24 HOURS AFTER INFECTION OF DAY-OLD CHICKS WITH *S. gallinarum*.

Age of chicks (days)	Group 1 75 chicks 0·02 Furazolidone	Group 2 75 chicks Control
	Deaths	Deaths
1–7	2	47
8–14	1	19
15–21	1	2
22–28	7	0
29–35	6	0
36–42	3	0
Total deaths	20 (26·7%)	68 (90·6%)

the next four days and deaths continued sporadically for a fortnight. Later, all surviving birds were tested by the rapid whole blood test. Six were positive and from four of them *S. gallinarum* was isolated. The organism was also isolated from six birds which had given negative reactions to the test.

Experiment 2.

In some parts of the U.S.A. fowl typhoid presents a serious problem in young chicks. This is not so in this country where the disease in chicks has been rare. Until last year the writer had not come across any outbreak which appeared to be egg-borne, although bacteriological examination of large numbers of eggs laid by carriers, survivors of the naturally and experimentally produced disease had shown that many contained *S. gallinarum*. With the increased incidence of fowl typhoid in some parts of Great Britain it is probable that the disease in chicks arising from incubator infection will become more common. To test the efficiency of furazolidone against an experimentally produced incubator infection, chicks were infected by the spray method already described. An additional group of chicks was included to study the effect of delayed treatment. The results are shown in Table II.

TABLE II

THE EFFECT OF TREATMENT WITH FURAZOLIDONE (0·02 PER CENT.) FOR FIVE DAYS, BEGUN 24 AND 72 HOURS AFTER INFECTION OF TWO GROUPS OF DAY-OLD CHICKS WITH *S. gallinarum*

Age of chicks (days	Group 1 47 chicks Furazolidone 24 hours after infection	Group 2 47 chicks Furazolidone 72 hours after infection	Group 3 46 chicks Control
	Deaths	Deaths	Deaths
1–7	0	0	42
8–14	6	4	2
15–21	9	2	1
22-28	0	0	1
Total deaths	15 (31·9%)	6 (12·8%)	46 (100%)

The marked protective effect of furazolidone 0.02 per cent. is again clearly shown. During the period of treatment mortality was completely prevented, while it was almost 100 per cent. in the control group. As in the previous experiment a breakdown occurred after treatment had ceased, six days afterwards in brooder 1, and four days afterwards in brooder 2; in both cases 12 days after initial infection. It is not possible to draw any valid conclusion on the effect of the delayed administration of furazolidone, but the results confirm that a concentration of 0.02 per cent. is not bactericidal.

Three more deaths from fowl typhoid occurred in brooder 2, 36 days, 48 days and 113 days after initial infection. All surviving birds were negative to agglutination tests (rapid whole blood and tube methods) and *S. gallinarum* was isolated from one bird only.

Experiment 3.

This experiment was designed to test the efficiency of furazolidone against *S. typhi-murium* infection in chicks. In view of the results of the earlier experiments, the drug was fed at two levels, 0.02 per cent. and 0.04 per cent. and treatment which began 72 hours after infection was continued 12 days.

TABLE III

THE EFFECT OF TREATMENT WITH FURAZOLIDONE (0·02 AND 0·04 PER CENT.) FOR 12 DAYS STARTING 72 HOURS AFTER INFECTION OF DAY-OLD CHICKS WITH *S. typhi-murium*

Age of chicks	Group 1 34 chicks Furazolidone 0·02 per cent.	Group 2 32 chicks Furazolidone 0·04 per cent.	Group 3 33 chicks Control
	Deaths	Deaths	Deaths
1–7	2	1	3
8–14	0	0	1
15–21	0	0	0
22–28	0	1	0
Survivors	32	30	29
	S. typh.	*S. typh.*	*S. typh.*
Bacteriological Examination	+ − 11 21	+ − 4 26	+ − 18 11

This experiment was only partially successful as a virulent outbreak of salmonellosis did not develop. This was not entirely unexpected as in the past it has proved difficult to induce a significant mortality in newly hatched chicks by artificial infection with *S. typhi-murium* even when the virulence of the organism has been increased by passage or the resistance of the chicks has been lowered by keeping at a reduced temperature (Wilson, 1945).

The number of chicks dying from salmonellosis is too small to allow significant deductions to be made. A death rate in the control group double that of the treated pen, however, suggests that the drug may be helpful in controlling outbreaks of the disease. The surviving chicks were destroyed, some at six weeks of age, the rest at eight weeks. The effect of treatment on the incidence of carriers is most striking. There were only four out of 30 in the group receiving 0.04 per cent. furazolidone, 11 out of 32 receiving 0.02 per cent. of the drug compared with 18 out of 29 in the control pen.

Experiment 4.

This experiment was designed to compare the effect of furazolidone (0.04 per cent. in the food for seven days), and sulphaquinoxaline (0.05 per cent. in the drinking water for five days), against artificial infection of day-old chicks with *S. pullorum*. Treatment with both drugs was begun 72 hours after infection. Results are shown in Table IV.

TABLE IV

THE EFFECT OF TREATMENT WITH FURAZOLIDONE (0·04 PER CENT.) AND SULPHAQUINOXALINE (0·05 PER CENT.) BEGUN 72 HOURS AFTER THE INFECTION OF 80 DAY-OLD CHICKS WITH *S. pullorum*.

Age of chicks (days)	Group 1 50 chicks Furazolidone 0·04 per cent. for 7 days	Group 2 49 chicks Sulphaquinoxaline 0·05 per cent. for 5 days	Group 3 49 chicks Control
	Deaths*	Deaths	Deaths
1–7	0	10	32
8–14	0	5	14
Intercurrent deaths	2	3	0
Survivors	48	31	3
S. pullorum +	1	31	3

* From pullorum disease.

The protective effect of furazolidone is apparent. None of the chicks in the treated pen ever showed evidence of illness, and the only two deaths which occurred were from accidental causes. *S. pullorum* was isolated from one of these chicks and from one of the 48 chicks destroyed immediately after treatment had ceased.

During the same period 45 out of 48 control chicks died. All had extensive lung lesions, and in many, necrotic nodules in the myocardium were also present. There were similar lesions in the three survivors destroyed at the end of the experiment.

Ten of the chicks receiving sulphaquinoxaline died from pullorum disease during treatment and five, 48 hours after treatment had stopped. There was gross evidence of the disease in every one of the 31 survivors from 30 of which *S. pullorum* was isolated by direct culture from the liver. The organism was isolated from the intestinal contents of 27 of the chicks. The presence of advanced disease in the survivors suggested that if the period of the experiment had been extended the death rate would have been very much greater.

Experiment 5.

The foregoing trials have shown the effectiveness of furazolidone under certain limited and well-defined experimental conditions. Its usefulness in the field would depend largely on its effect when treatment is delayed until the presence of disease is established, except perhaps on the relatively rare occasions when a previous history indicates the possibility of infection. The continuous feeding of a drug on the chance of chicks becoming infected is to be deprecated. This experiment was designed to create conditions simulating as closely as possible the circumstances which might arise in the field.

A group of 49 healthy day-old chicks was placed in a brooder near those containing the infected chicks of Experiment 4 in the hope that they might become infected. To ensure that any spread was completely natural, and not aided or hindered in any way, the attendant was left unaware of the nature of the experiment.

On the sixth day (a day after the first deaths in Experiment 4) there were six deaths from pullorum disease and treatment at 0.04 per cent. concentration was begun and continued for 10 days. Many of the chicks were obviously ill and four died the next day. A marked improvement in the appearance of the chicks took place and there were no further deaths for three days. During the remaining six days of treatment there were five single deaths.

Three more deaths occurred during the next three weeks when the experiment ended. Of the 28 survivors, three were undersized but the remainder appeared to be normal. *S. pullorum* was isolated from 10 (35.7 per cent.). The total death rate from pullorum disease, including those before treatment began was 18 (36.7 per cent.).

Experiment 6.

This experiment was to test the effect of furazolidone against infection of day-old chicks with *S. thompson*. After spraying, the chicks were divided into four random groups, one of which received furazolidone 0.04 per cent. for seven days starting 72 hours after infection and another was left untreated as a control. The remaining groups were kept for several hours at room temperature to ascertain the effect of chilling on the severity of the disease. By delaying treatment of one of those lots until deaths occurred it was hoped to compare the value of the drug in circumstances similar to those of a natural outbreak. This proved impracticable through failure of the disease to develop. There were single deaths only in the three untreated groups. All chicks were destroyed for bacteriological examination, three to four weeks later. Although the internal organs appeared normal profuse growths of *S. thompson* were obtained from the liver, spleen and lungs of every one of the 144 untreated chicks and from the intestinal contents of 134. The organism was isolated from the internal organs of only 12 of the 48 treated chicks but it was present in the intestinal contents of 45.

It is difficult to explain the latter findings; reinfection during the interval between cessation of treatment and time of slaughter may have been responsible. The treated pen was in close proximity to three times the number of untreated chicks, all excreting infection. From this and other experiences it would appear that to obtain a true assessment of the efficacy of furazolidone, experimental chicks should be destroyed and examined bacteriologically as soon as treatment is ended. On the other hand, keeping them under observation for a further period may provide a more realistic indication of the value of the drug under natural conditions of chick rearing.

Discussion

The experimental results confirm the value of furazolidone in artificial infections with *S. pullorum* and *S. gallinarum*. At a concentration of 0.02 per cent. in the mash, deaths were prevented during the period of treatment but breakdowns invariably occurred later. A concentration of 0.04 per cent. gave complete protection to chicks against infection with *S. pullorum* sufficiently severe to cause a mortality of 95 per cent. in untreated chicks, and only one chick was later found to be bacteriologically positive. The appearance of the internal organs of the treated chicks was striking; they were completely normal whereas there was extensive nodule formation in the lungs and heart of everyone of the control chicks and those treated with sulphaquinoxaline. This is well illustrated in Figs. 1 and 2.

The effect of the drug on other salmonellae was less definitely determined, but the great reduction in the carrier rate in chicks infected with *S. typhi-murium* suggests that it may play a useful part in controlling this disease. The position of *S. thompson* is similar. The presence of the organism in only 25 per cent. of the treated chicks compared to 100 per cent. of the untreated is suggestive and it would appear that following a diagnosis of salmonellosis a course of furazolidone at a concentration of 0.04 per cent. for 7 to 10 days represents a sound treatment especially in birds intended for the table, such as broilers, turkeys and ducklings.

The ready isolation of *S. thompson* from apparently healthy chicks, as judged by the absence of symptoms of illness during life, and lesions of disease *post-mortem*, raises doubts regarding the significance of the isolation of salmonella from similar chicks received for diagnosis. In such cases the organism would appear to be of low pathogenicity or perhaps even avirulent, and unlikely to be the primary cause of losses.

It is improbable that furazolidone will prove as effective against *S. pullorum* and *S. gallinarum* in the field, for while the weight of infection to which chicks are exposed will generally be lighter (mortality is usually less than 100 per cent. in natural outbreaks), the disease will be more advanced when treatment is begun. The death rate is likely to be higher, and a greater proportion of the surviving birds will probably be carriers. Often the occurrence of deaths is the first indication of the presence of disease in young chicks and there will be a further delay while a diagnosis is obtained. Experiment 5 provides a possible example of what may happen. When treatment was delayed until chicks started to die, deaths continued for a short time but this was followed by rapid control of the disease. The final mortality was just over a third of that of the untreated group in Experiment 4, and more than 30 per cent. of the survivors were excreting the organism. This cannot be regarded as a controlled experiment as there is no means of assessing the weights of infection to which the two groups of chicks were exposed.

The effect of treatment of "carriers" is still uncertain. Gordon and Tucker (1955) reported that at both 0.02 per cent. and 0.04 per cent. fed continuously for 10 days to 13 birds positive to the rapid whole blood test in the field, 11 were subsequently found to be free from *S. pullorum* infection. When the drug was used at 0.04 per cent., seven out of eight birds became negative to the agglutination test 60 to 84 days after the termination of treatment. In preliminary experiments here, with similar birds we have not observed a significant fall in titre and *S. pullorum* has been isolated from some of the treated birds sacrificed for bacteriological examination.

S. pullorum continues to be isolated from a proportion of eggs laid by birds in the control pen but has not been found in the eggs of the treated birds. This work is continuing.

In dealing with the natural outbreaks a concentration of not less than 0.04 per cent. furazolidone should be used and treatment should be continued for 7 to 10 days. Points of great practical importance are, the commencement of treatment at the earliest possible moment and the prevention of reinfection by thorough cleansing and disinfection of brooding equipment and utensils, or the removal of chicks to fresh quarters, immediately after treatment.

Summary

(1) Treatment with furazolidone, 0.02 per cent. in the diet, prevented deaths in groups of young chicks artificially infected with *S. pullorum* and *S. gallinarum*, but breakdowns occurred later. Better results were obtained with double the concentration (0.04 per cent.).

(2) When treatment was delayed until deaths occurred it was less effective but still resulted in a significant reduction in mortality.

(3) Furazolidone was superior to sulphaquinoxaline not only in preventing mortality but in reducing the number of carriers in surviving birds.

(4) Results in infection with *S. typhi-murium* and *S. thompson* were promising but not conclusive.

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The Treatment of Carriers of *Salmonella pullorum* and *Salmonella gallinarum* with Furazolidone

BY

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IN an earlier paper (Wilson, 1955a) describing the effect of furazolidone in artificially produced infections in young chicks with *S. pullorum*, *S. gallinarum*, and *S. typhi-murium*, a brief reference was made to the treatment of adult "carriers." The results obtained differed from those of Gordon & Tucker (1955) who reported that following treatment with furazolidone 0.02 per cent. for 10 days, three of four birds showed no evidence of *S. pullorum* or other salmonella infection at autopsy, while two of the birds showed a marked reduction in serum titre. When the concentration of the drug was increased to 0.04 per cent., seven of the nine treated birds became negative to the serum tube agglutination test between 60 and 84 days after the cessation of treatment, and of these, eight were subsequently found to be free from *S. pullorum* infection.

In our hands treatment with the drug was not followed by a significant reduction in titre, and *S. pullorum* was isolated from all the treated birds. Salmonella were not, however, isolated from any of the eggs laid by treated birds. The present paper describes a continuation of this work.

Materials and Methods

The carriers of *S. pullorum* were naturally affected birds detected at the routine blood testing of a flock participating in the Accredited Poultry Scheme. Carriers of *S. gallinarum* were survivors of an artificially induced outbreak of fowl typhoid. All birds were kept under observation for from two to three months before dosing, and were subjected to weekly agglutination tests by the tube and rapid whole-blood methods. It can be assumed that in practice carriers detected by the agglutination test which were to be treated would be segregated and held in a pen during treatment and pending retesting. Accordingly, the earlier experiments were conducted on these lines so that the results obtained might be regarded as valid under field conditions. Carriers were kept in small groups and after the period of observation described, were dosed by administering furazolidone* 0.04 per cent. in the mash for 12 consecutive days. During the period of treatment all other foods were withheld, but the birds had constant access to the medicated mash which was fed dry. In view of the disappointing results of treatment, to obviate the risk of reinfection or cross-infection which it was considered might be responsible, in later experiments all birds were kept in individual cages on wire floors, within an isolation unit. This had no effect on the results of treatment.

During the period of observation, in addition to weekly blood testing, the yolks of all eggs laid were cultured in Selenite F medium (Hobbs & Allison, 1945) and examined for the presence of salmonella by the usual procedures. A number of untreated carriers were kept as controls. At the end of the period of observation, all birds were destroyed, autopsied, and subjected to bacteriological examination. In birds in which there were obvious lesions, *e.g.* distorted ovules, pericarditis, or myocarditis, direct cultures were made on blood agar and MacConkey medium, and the affected organ or organs, normal ovules, liver, spleen, gall bladder, and intestines cultured in Selenite F. To obviate any possibility of bias, blood samples and eggs were examined by different workers who were kept unaware of their respective sources, whether from treated or untreated birds, and neither worker was ever informed of the date of commencement and cessation of treatment. To still further ensure impartiality from time to time a control bird was treated. A similar procedure was adopted for the bacteriological examination of the birds and it was only when the experiment was over that the respective findings were correlated.

The effect of treatment on the agglutination titre of carriers of *S. pullorum* and *S. gallinarum*

Eight carriers of *S. pullorum* and 18 carriers of *S. gallinarum* were treated. The results are in Table I.

As the period of observation before treatment varied from two to three months or longer, and interval between dosing and final bleeding, just before the bird was destroyed for bacteriological examination varied from 2 to 32 weeks, it is impossible to include all the titres. A selection of 15 has been made; those for the three weeks preceding dosing, the 11 weeks following dosing, and the final titre. Over all there was no significant reduction in titre in the treated

* Neftin.

TABLE I

EFFECT OF TREATMENT WITH FURAZOLIDONE 0·04 PER CENT. FOR 12 DAYS ON THE AGGLUTINATION TITRE OF CARRIERS OF *S. pullorum* AND *S. gallinarum*

Bird No.	Before treatment (weeks) 3	2	1	After treatment (weeks) 1	2	3	4	5	6	7	8	9	10	11	Titre when killed	Interval between dosing and killing	Bact. exam.	Organism
1727	320	320	320	320	80	160	160	160	160	320	320	320	640	320	160	32	+	*S. pullorum*
780	80	20	20	80	40	40	80	80	40	80	80	160	160	160	160	32	+	„
1705	80	160	160	320	160	80	80	160	160	160	160	320	160	160	160	21	+	„
1226	80	80	80	160	80	80	40	80	20	40	40	40	40	40	40	18	+	„
1229	80	80	80	80	80	80	80	80	20	80	20				20	8	+	„
1711	80	80	80	80	80	40	20	40	20	80	80				80	8	+	„
1223	80	160	80	160	80	40	40	40	20	40					40	7	+	„
1221	20	20	20	40	40	40	20	20	10	10					10	7	+	„
149	40	40	40	40	80	80	160	80	40	80	80	80	160	160	40	32	+	*S. gallinarum*
1605	40	80	320	640	320	160	320	320	320	160	320	320	320	320	40	32	—	„
1486	80	80	80	80	40	40	80	80	80	40	80	80	80	80	40	19	+	„
108	80	80	80	80	40	40	40	40	20	40	20	40	20	40	40	18	—	„
1719	40	40	40	20	20	20	20	40	20	20	40	40	40	40	20	18	+	„
1702	20	20	40	80	10	10	10	10	10	10	80	40	40	40	40	14	+	„
1969	40	40	80	80	80	20	20	80	640	640	640	640	1,280	640	640	14	+	„
1920	40	40	80	80	80	80	20	80	80	160	320	160	160	160	320	14	+	„
1493	10	10	10	20	10	10	10	10	10	10	10	—	10	—	20	14	—	„
1715	20	20	20	40	40	20	20	20	20	20	20	20	20	20	20	14	—	„
133	40	40	20	20	40	20	40	40	20	40	40	40	20	40	40	7	+	„
2760	10	40	40	80	80	40	20	10	20						20	6	—	„
2762	80	160	80	80	80	80	40	40	40						40	6	+	„
2785	320	320	80	160	160	160	80	80	160						160	6	—	„
2748	20	10	40	20	40	20	20								20	4	+	„
2751	160	160	320	160	320	80	160								160	4	+	„
2781	160	160	320	320	160	80	160								160	4	+	„
1511	20	20	40	40	40										40	2	+	„
									Untreated birds									
1469	80	80	80	80	40	40	80	40	40	40	40	20	20	20			+	„
2780	320	80	40	40	40	20	20	40	20	10	20	40	20	20			+	„
2795	160	80	80	40	80	80	80	160	160	160	160	80	160	160			+	„
2231	80	80	80	40	80	80	80	160	80	160	160	80	160	80			+	„
2133	40	40	20	20	40	20	40	40	20	40	40	40	40	40			+	„
2711	80	80	80	80	80	80	80	160	160	160	320	160	80	80			+	„
2721	20	20	10	40	40	40	40	40	10	20	20	20	20	20			+	„
2732	40	40	40	20	20	40	40	20	40	20	40	20	20	40			+	„
2769	80	80	80	80	160	160	320	320	160	160	160	160	160	160			+	„
1706	160	160	160	160	160	160	160	160	160	160	80	160					+	„
2752	160	320	160	40	40	80	80	80									+	„
1720	80	80	80	80	80												+	„

All the above birds were female except 1486

birds. In several there was a temporary lowering of the agglutinin level but after a few weeks in most cases the titre had returned to its former level. In a few birds the titre rose after dosing. Although all treated birds continued to react to the end of the experiment, as salmonella were not isolated from six birds, it must be assumed that if the period of observation had been lengthened the titre would have disappeared in these cases and possibly in some others in which it was very low.

Effect of treatment on persistence of *S. pullorum* and *S. gallinarum*

Bacteriological examination of the birds at the end of the experiment showed the presence of *S. pullorum* in all the eight treated birds previously infected with this organism and of *S. gallinarum* in 12 of the 18 " carriers " of this germ.

Detailed results of the isolations from the various organs of individual birds are in Table II in which are also included for comparison the results of bacteriological examination of six non-treated fowls.

In 10 of the 20 carriers in which salmonella persisted after treatment, the organism was isolated from the distorted ovules only; the other organs, including normal ovules present in the ovary, appeared to have been sterilised. Salmonella were isolated from three sites or more in only 4 of 26 treated birds, whereas positive cultures were obtained from three or more sites in four of the six control fowls. In the only male bird treated, *S. gallinarum* persisted in the heart lesions.

Effect of treatment on egg transmission of *S. pullorum* and *S. gallinarum*

Several birds in which the ovary was subsequently found to be completely degenerate did not lay an egg during the period of observation, and a few of the caged birds developed the vice of egg-eating. This limited the number of eggs available for bacteriological examination, nevertheless sufficient were obtained to show that before treatment with furazolidone at least 10 of the 25 experimental hens were laying eggs containing either *S. pullorum* or *S. gallinarum*,

TABLE II

RESULTS OF BACTERIOLOGICAL EXAMINATIONS OF FOWLS TREATED WITH FURAZOLIDONE

Bird		Distorted ovules	Normal ovules	Liver spleen gall bladder	Oviduct	Heart	Intestines	Result	Organism
Treated	1727	+						+	*S. pullorum*
	780	+						+	"
	1705	+		+			+	+	"
	1226	+						+	"
	1229	+	+	+				+	"
	1711	+						+	"
	1223	+						+	"
	1221	+			+			+	"
	149	+						+	*S. gallinarum*
	1605							−	"
	1486					+		+	"
	108							−	"
	1719			+				−	"
	1702	+						+	"
	1969	+						+	"
	1920	+						+	"
	1493							−	"
	1715							−	"
	133	+						+	"
	2760							−	"
	2785	+		+		+		+	"
	2762	+						+	"
	2748							−	"
	2751	+				+		+	"
	2781	+						+	"
	1511	+		+		+	+	+	"
Control	1469	+				+	+	+	"
	2780	+		+			+	+	"
	2795	+		+		+		+	"
	2752	+		+		+		+	"
	1706	+						+	"
	1720	+				+		+	"

and that after treatment only one continued to do so, and that on a greatly reduced scale. Figures for individual birds are in Table III.

Discussion

The results of treatment with furazolidone of " carriers " of *S. pullorum* and *S. gallinarum* differ from those of Gordon & Tucker (1955). It is difficult to suggest a reason for this. It might be considered that the types of fowls used are not strictly comparable in that the " carriers " of *S. gallinarum* used here were products of artificially induced infection whereas those quoted by the former authors were field cases. This does not provide an explanation, however, as treatment was more effective in carriers of *S. gallinarum* than in carriers of *S. pullorum*, which are strictly comparable with the birds used by Gordon & Tucker.

While there was a distinct drop in titre in many of the treated birds, in most cases it was of a temporary nature and after a few weeks the titre resumed its former level and sometimes exceeded it. Had the period of observation after dosing been extended, however, at least six of the treated birds from which salmonella were not isolated, would have ceased to react. In five of these birds the titres were low even before dosing; in two it was 80, in two it was 40, and in one it was 20. In the remaining bird (1605) a titre of 320 before dosing was more or less maintained during the period covered by Table I. As long as 19 weeks after dosing it was 640, after which time it steadily fell over a period of five weeks to 80. By

TABLE III

BACTERIOLOGICAL EXAMINATION OF EGGS FROM CARRIERS OF *S. pullorum* AND *S. gallinarum* BEFORE AND AFTER TREATMENT WITH FURAZOLIDONE

	Before treatment		After treatment		
Birds	eggs laid	+ eggs	eggs laid	+ eggs	Organism
1711	33	16	34	0	*S. pullorum*
1223	26	8	28	0	"
1221	10	0	15	0	"
1226	4	0	29	0	"
133	20	0	30	0	*S. gallinarum*
1969	19	12	0	0	"
108	7	0	58	0	"
1719	1	0	33	0	"
149	8	0	67	0	"
1605	0	0	35	0	"
2781	14	4	6	0	"
2748	30	4	11	0	"
2762	15	13	22	2	"
2760	16	8	24	0	"
2751	2	1	5	0	"
2779	9	2	26	0	"
2732	5	0	25	0	"
2743	4	1	18	0	"
	223	69 30·9%	466	2 0·4%	

the 28th week it had risen again to 320 after which it gradually declined to 40 at the end of the 32nd week when the bird was destroyed. It is considered that the fall in titre and failure to isolate *S. gallinarum* from this bird is associated with natural recovery rather than the result of treatment nearly eight months earlier. The persistence of agglutinins after treatment with furazolidone has also been noted in the field; 19 birds from three flocks in which reactors were still positive after treatment were subjected to bacteriological examination and salmonella were isolated from 18.

That furazolidone exerts a considerable bactericidal effect on *S. pullorum* and *S. gallinarum* in "carriers" is seen by a comparison of the results of bacteriological examinations of treated and untreated fowls (see Table II). The six negative cases have been discussed earlier. In the others the distribution of the surviving salmonella is more localised; in 10 cases infection was confined to the distorted ovules in the ovary. In only two treated birds could persisting infection be termed widespread whereas in half the untreated controls this was so. It would appear either that the salmonella in the distorted ovules are too numerous to be completely overcome by the action of furazolidone or that possibly as a result of a diminished blood supply to the diseased ovules the drug may not be available in sufficient concentration. Its effect on the organism elsewhere in the ovary is profound. After a course of treatment salmonella were rarely recovered from apparently normal ovules and were isolated from only two of over 450 eggs laid by the treated birds. Both positive eggs were from the same bird, which during the three weeks prior to dosing with furazolidone laid 15 eggs from 13 of which *S. gallinarum* was isolated. During the four weeks following treatment it laid 22 eggs of which the two positive were the 20th and 21st of the sequence. It seems probable that if this bird had lived it again would have become a regular producer of positive eggs.

The proportion of positive eggs laid by reactors varies. Moore (1946) isolated *S. gallinarum* from 9 per cent. of eggs from a pen of 21 fowl typhoid reactors. Simms (1946) reported that 10 per cent. of eggs laid by fowls artificially infected with fowl typhoid and 4 per cent. of eggs laid by natural carriers contained *S. gallinarum*, and Hall, Legenhausen & Macdonald (1949) recorded an average of 6 per cent. of infected eggs from a reactor flock. Wilson (1955b) found 108 positive eggs out of 1,843 eggs laid by 21 carrier fowls. Individual production of infected eggs varied from 5 to almost 40 per cent. with an average of 18 per cent. Bird 2762 must therefore be regarded as an abnormally high producer of infected eggs; this may account for its exceptional response to treatment.

The results of the laboratory tests and the field experience quoted, reveal the usefulness and limitations of furazolidone in the treatment of carriers of *S. pullorum* and *S. gallinarum*. Its administration will result in a proportion of birds being sterilised of salmonella and ultimately becoming negative to the agglutination test; a larger percentage will continue to harbour the organism, mostly in the distorted ovules, and will remain reactors. It is likely, except in exceptional cases, that treated birds will cease laying eggs containing salmonella. This may well prove the most valuable property of the drug in the treatment of carriers. It would be useful in an infected flock from which reactors had been removed, in which it became necessary to incubate eggs before retesting was completed; this can be quite a lengthy period as two clear tests are often desirable. Furazolidone may prove even more valuable in the prevention of salmonellosis (disease caused by other salmonella than *S. pullorum* and *S. gallinarum*) in turkeys and ducks, which often presents a difficult problem as in these species direct egg transmission, rare in the domestic fowl, is not uncommon. It must be borne in mind, however, that furazolidone is not as effective in chicks infected with *S. typhi-murium* as with *S. pullorum* or *S. gallinarum* (Wilson, 1955), so that proof of its usefulness in other salmonella must await the result of critical experimentation.

Conclusions

1. The feeding of furazolidone 0.04 per cent. in the mash continuously for 12 days to birds which gave positive results to agglutination tests for *S. pullorum* by both tube and rapid methods, failed in most cases to cause a significant or permanent lowering of the agglutination titre. None of the birds ceased to react as a result of treatment.

2. In 20 of 26 treated carriers, salmonella persisted after treatment. Distorted ovules were the commonest site of remaining infection.

3. Treatment had a marked preventive effect on the laying of infected eggs; only two of 466 eggs laid after treatment contained salmonella. On this account it is suggested that the drug may be particularly useful in the prevention of salmonellosis in turkeys and ducks.

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Fowl Typhoid—The Effect of Vaccination on the Natural and Experimental Disease

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DURING the last decade there has been a striking increase in the incidence of fowl typhoid in many regions where for long it has been considered endemic, and the disease has become prevalent in areas in which previously it was unknown. An exception to this general statement is provided by its almost complete disappearance from the Outer Hebrides, where it has been a serious source of loss for more than 50 years (Wilson, 1940).

In the past, the customary recommendations for dealing with an outbreak of the disease have been the immediate slaughter of all ailing birds followed by the application of the agglutination test, formerly by the tube method and latterly by the rapid whole blood test for the detection of carriers ; the vaccination of the remainder of the flock, and the direction of the farmer's attention to the importance of disinfection and improved methods of hygiene, sanitation and general management.

In general, this procedure achieved a reasonable degree of success in controlling the disease, but more recently a change in the attitude of the poultry farmer has become evident in a growing reluctance to make use of the agglutination test, and to carry out adequate disinfection. Greater reliance has been placed on vaccination and/or chemotherapy, and suggestions have been advanced that the disease should be made notifiable.

History of Vaccination

For many years a stock vaccine has been available to poultry farmers from the Ministry of Agriculture's Veterinary Laboratory at Weybridge. It is unnecessary here to describe its preparation, but briefly it consists of broth cultures of *Salm. gallinarum* killed by the addition of formalin and phenol. It has been difficult to assess its real value for, while good results apparently followed its use, in many cases preliminary testing leading to the removal of carriers and improved hygienic procedures must have played an important part in reducing infection, and some of the success ascribed to vaccination may have been largely attributable to these measures.

Small-scale experiments (Wilson, 1946) suggested that a dead vaccine, either stock or autogenous, was of little value and later work confirmed this (Wilson, 1947). While good results were obtained with an attenuated live vaccine there are practical difficulties attendant on its use in the field. Apart from the dangers inherent in the administration of a potentially pathogenic micro-organism, birds vaccinated with live vaccine react to the agglutination test for bacillary white diarrhoea ; this would preclude its use in accredited flocks.

In Northern Ireland some success has been claimed for a dead autogenous vaccine which appeared to give protection for six to eight weeks (Luke, Gordon & Gracey, 1951). According to Hall, MacDonald & Legenhausen (1949) vaccination against fowl typhoid with killed cultures of *Salm. gallinarum* is carried out to a limited extent in the United States of America but there is a dearth of evidence to substantiate its efficacy. These authors reported the results of trials with 15 different dead vaccines, none of which was successful. When the interval between vaccination and exposure was short (two to three weeks) mortality was less than when the interval was over three weeks. After 30 days there was no indication of protection. The present paper chiefly describes trials carried out during 1953 and 1954 which, although unsuccessful, are perhaps worth recording.

Materials and Methods

Vaccine No. 1

This vaccine was prepared from a virulent smooth strain of *Salm. gallinarum* (Fewson) by the method described by Felix (1941) for antityphoid and paratyphoid vaccines in man in which treatment of the organism with 75 per cent. alcohol replaced the earlier method of killing by heat and preserving with phenol. It was standardised so that 1 ml. contained 2,500 by 10^6 organisms.

Vaccine No. 2

Prepared in the same way as No. 1 except that the organisms were killed by heating for one hour at 53° C.

Vaccine No. 3

Prepared by the technique of Freund, Thomson, Hough, Sommer & Pisani (1948). The adjuvant was liquid paraffin and falba but acid-fast bacilli were not added. The vaccine was standardised so that 1 ml. contained 2,000 by 10^6 organisms and the dose used was 0·2 ml.

Vaccines Nos. 4 and 5

Prepared by the same technique as No. 3, using *Salm. enteritidis* var. Jena and *Salm. enteritidis* var. Essen respectively.

Vaccine No. 6

Consisted of a suspension of live *Salm. gallinarum* of the strain used in vaccines 1, 2 and 3, after 70 sub-cultivations on agar at three-day intervals, and was standardised so that 1 ml. contained approximately 1,600 by 10^6 organisms.

Method of Testing

Growing chickens and adult fowls were vaccinated and later challenged by the subcutaneous injection of a standard dose of the homologous strain of *Salm. gallinarum* (1,600 by 10^6). In order to make the conditions of challenge more comparable to those obtaining in a field outbreak of the disease, other vaccinated birds were exposed to infection by contact with recovered birds, or with fowls infected by inoculation with virulent culture. In the case of vaccine 6, birds were also subjected to challenge by oral dosage. Unless otherwise stated, birds were kept in pens on concrete floors. Feeding consisted of dry mash in hoppers and a daily feed of grain scattered in the litter which was peat moss. To ensure maximum exposure to infection droppings were infrequently removed. Vaccine No. 1 was also subjected to a large-scale field trial.

Results

Killed Vaccines

Vaccines Nos. 1 and 2

Several small-scale trials showed that the above vaccines gave no protection against fowl typhoid experimentally induced by the injection of *Salm. gallinarum* even when the dose of vaccine was increased to five times the normal strength or a boosting dose of vaccine was given.

Vaccine 1 was tested under conditions more closely approximating those of a natural outbreak of fowl typhoid by keeping four groups of vaccinated and non-vaccinated fowls for one year in adjacent loose-boxes each having a floor area of 100 sq. ft. Droppings were allowed to accumulate and the litter was replaced infrequently. The individual groups were treated as follows :—

In the first group 17 vaccinated birds and nine controls were placed with six birds, which were injected with a lethal dose of *Salm. gallinarum*. All six injected fowls, three vaccinated fowls and one control fowl died of acute fowl typhoid.

In the second group 24 vaccinated fowls and six controls were placed on litter which had been contaminated by the addition of droppings from affected fowls. Three deaths from fowl typhoid occurred in the vaccinated group and one in the controls. No cases of fowl typhoid developed in the remaining groups which comprised vaccinated birds, controls and natural carriers, and vaccinated and control birds respectively.

It is rather remarkable that under the conditions of the experiment the incidence of fowl typhoid remained so low. It is well recognised, however, that many carriers cease eliminating *Salm. gallinarum* in the faeces and in others elimination is scanty and sporadic but a build-up of infection might have been expected. The presence of a number of actively sick birds provides a more favourable method of spread of the disease.

The number of birds that died is too small to be significant but there is little difference proportionally in the incidence of the disease in vaccinated and non-vaccinated birds in the two groups in which the disease occurred.

Field Trial.—During 1953 almost 45,000 ml. of vaccine 1 were used in a series of field trials. An analysis of the results showed that vaccination provided no significant protection against naturally occurring fowl typhoid, thus confirming the results obtained in the laboratory in the case of the artificially produced disease.

Vaccines Nos. 3, 4 and 5

Following a preliminary trial with vaccine 3 in which 70 per cent. of vaccinated birds survived a challenge 21 days later which killed 90 per cent. of control birds, 57 pullets were vaccinated with 0·2 ml. of vaccine 3, 28 pullets with vaccine 4, and 29 pullets with vaccine 5. Forty-three birds of the same strain were left unvaccinated to act as controls. The birds were divided into two comparable groups, and 16 weeks after vaccination a proportion of each group was inoculated, and the remaining birds left exposed to the infection resulting from contact with the inoculated birds.

The results are shown in Table I.

Not only had none of the vaccines any significant protective effect against the disease produced in the individual bird by the inoculation of virulent culture which probably represented a severer challenge than might be expected under natural conditions, but they also failed to induce sufficient immunity in the remaining birds, to withstand the weight of infection produced by contact with actively diseased birds under circumstances similar to those which might be expected in a severe natural outbreak of the disease.

Immunisation of Mice against Salm. gallinarum

The failure of killed vaccines to protect fowls against fowl typhoid and the absence of any potentiating effect on antibody formation by the falba vaccine suggested inability of the fowl to respond to the stimulus provided by a dead vaccine.

It was decided to test the protective value of vaccine 3 in mice. *Salm. gallinarum* appears to be pathogenic for this species by parenteral injection only ; it was not possible to produce symptoms of illness by giving drinking water heavily contaminated with *Salm. gallinarum* but the subcutaneous inoculation of 800 by 10^6 organisms was almost invariably lethal. In a pre-

TABLE I

THE EFFECT OF CHALLENGE 112 DAYS AFTER VACCINATION WITH VACCINES 3, 4 AND 5

	Vaccine 3 *S. gallinarum*		Vaccine 4 *S. enteritidis* (Jena)		Vaccine 5 *S. enteritidis* (Essen)		Controls	
	Challenged	Exposed	Challenged	Exposed	Challenged	Exposed	Challenged	Exposed
Number of birds	42	15	18	10	18	11	30	13
Deaths from fowl typhoid	21	10	15	5	12	10	19	10
Mortality (per cent.)	50	66·6	83·3	50	66·6	90·9	63·3	76·9

liminary experiment 30 mice were vaccinated each with 0·2 ml. of vaccine 3 and 34 days later were challenged with varying doses of *Salm. gallinarum*. The results are seen in Table II.

TABLE II

IMMUNISING EFFECT OF VACCINE 3 FOR MICE AGAINST A VARYING CHALLENGE WITH *S. gallinarum*

	Vaccinated		Controls	
Challenge dose	Number injected	Deaths	Number injected	Deaths
S. gallinarum				
200 × 10^6 ...	10	0	10	5
400 × 10^6 ...	10	1	10	2
800 × 10^6 ...	10	2	10	9
Total	30	3	30	16

The vaccine produced a protection of 80 per cent. against a weight of infection which caused a mortality of 90 per cent. in unvaccinated mice and an average protection of 90 per cent. over the three vaccinated groups compared with 50 per cent. mortality in the control groups.

Results of a trial on a larger scale are shown in Table III.

TABLE III

IMMUNISING EFFECT OF VACCINE 3 FOR MICE AGAINST A STANDARD CHALLENGE OF *S. gallinarum* 78 DAYS AFTER VACCINATION

	Vaccinated		Controls	
Challenge	Number of mice	Died fowl typhoid	Number of mice	Died fowl typhoid
S. gallinarum 800 × 10^6 Subcutaneous injection	20	1	20	18
Suspension in lieu of drinking water for 21 days	20	0	12	0
1 ml. = 400 × 10^6				

The vaccine provided 95 per cent. protection against a weight of infection causing 90 per cent. mortality in unvaccinated mice.

Both vaccinated and control groups of mice remained healthy when given drinking water heavily contaminated with *Salm. gallinarum* (1 ml. = 400 by 10^6 organisms) for 21 days.

Live Vaccine

Twenty-four fowls 10 to 12 months old were inoculated with vaccine 6 which it will be recalled was prepared from the same strain of *Salm. gallinarum* as vaccines 1, 2 and 3, after 70 subcultivations on agar at three-day intervals. Half of the birds received 1 ml. of vaccine and the remainder 0·05 ml. One-half of each group was housed in pairs in coops and the remainder, singly, in battery cages with wire-mesh floors.

The culture proved to be still virulent and all the vaccinated birds showed typical symptoms of fowl typhoid of varying severity. There was complete cessation of egg production and eight birds died from the acute disease. Deaths were equally divided between the groups receiving the larger or smaller doses of vaccine, and those kept in coops or cages.

All except one of the surviving birds appeared to make a complete recovery ; one bird resumed laying 37 days after vaccination and another seven birds between the seventh and ninth weeks after vaccination. The time of year, which was November, may have lengthened the period of non-productivity. The ailing bird died from chronic fowl typhoid 10 weeks after vaccination.

During the 70 days following vaccination 48 eggs were laid from 10 of which *Salm. gallinarum* was isolated. One bird laid six infected eggs, another two and two others a single infected egg each. *Salm. gallinarum* was not isolated from any of the eggs from the remaining four birds which were laying at this time.

In our hands the feeding of furazolidone to adult carriers has not caused them to become negative to the agglutination test or to be sterilised of salmonella as reported by Gordon & Tucker (1955), but it has been noted that they invariably cease to lay infected eggs and seldom continue to excrete the organisms in the faeces (Wilson, 1955). Accordingly, to determine the fate of the *Salm. gallinarum* in the challenging dose to be given later, the 15 vaccinated birds received furazolidone 0·04 per cent. in the food for 10 days and remained under observation for a period of six weeks before challenge. During this time *Salm. gallinarum* was not isolated from any of 153 eggs laid or from samples of faeces from each bird examined daily for eight days immediately prior to challenge. For this purpose nine vaccinated and nine control birds each received 800 by 10^6 organisms by subcutaneous injection and six vaccinated and six control birds were given 3,200 by 10^6 *Salm. gallinarum* by pipette directly into the crop.

The results are in Table IV.

TABLE IV

EFFECT OF CHALLENGE OF FOWLS IMMUNISED WITH LIVE VACCINE 6

	Vaccinated		Controls	
Challenge	Number injected	Deaths fowl typhoid	Number injected	Deaths fowl typhoid
S. gallinarum 800 × 10^6 Subcutaneous inoculation	9	1	9	7
S. gallinarum 3,200 × 10^6 into crop	6	0	6	6
Total mortality	15	1 (6·6 %)	15	13 (86·6 %)

All the unvaccinated birds showed typical symptoms of fowl typhoid with profuse sulphur-yellow diarrhoea, and 13 out of 15 died from the acute disease.

Except for one bird which developed typical symptoms of fowl typhoid and died from the acute disease six days after challenge all the vaccinated birds remained apparently normal. Egg production, however, ceased two to three days after challenge. In nine of the 12

hens which had been in lay, production was resumed in four to five days and in three birds in 16, 18 and 19 days. *Salm. gallinarum* was isolated from the eggs laid by four birds in 10 of 12, eight of 21 and two single eggs respectively.

Three of these birds had not previously laid positive eggs but *Salm. gallinarum* had been isolated from six of 15 eggs from the remaining bird prior to dosing with furazolidone. There was a sequence of 29 negative eggs and a lapse of 53 days between the laying of the last positive egg before dosing and the first positive egg after challenge. It seems probable that this represents a case of re-infection and not a carry-over from the earlier infection. The results of the bacteriological examination of eggs are summarised in Table V.

TABLE V

BACTERIOLOGICAL EXAMINATION OF EGGS LAID BY A GROUP OF 15 HENS AFTER (A) VACCINATION WITH ATTENUATED CULTURE ; (B) TREATMENT WITH FURAZOLIDONE (0·04 PER CENT.) ; AND (C) CHALLENGE WITH *S. gallinarum*

	Period of observation (days)	Number of hens in lay	Number of hens laying + eggs	Total eggs laid	+ eggs
(A)	70	7	4	48	10
(B)	42	12	0	153	0
(C)	31	12	4	178	20

Individual faecal samples were collected from all birds, daily for 31 days following challenge. In the vaccinated group *Salm. gallinarum* was isolated from the faeces of one bird on three occasions, of one bird twice and of eight birds once. The earliest positive isolation was three days after challenge and the latest 14 days. In the remaining four birds the faeces were negative.

In the control group one faecal sample was positive 24 hours after challenge and within a few days *Salm. gallinarum* had been isolated from the faeces of every bird. One of the two survivors showed intermittent excretion of the organism throughout the period of observation, the other became negative 15 days after challenge and remained so.

Discussion

It is apparent that dead vaccines are incapable of stimulating an effective immunity against fowl typhoid.

The promise of the falba vaccine, which protected 70 per cent. of vaccinated birds against a challenge 21 days later sufficiently strong to kill almost 90 per cent. of untreated controls, was not maintained when the interval between vaccination and challenge was increased to 50 days.

The production of a solid immunity in mice against subcutaneous inoculation of *Salm. gallinarum* by this vaccine is of interest. In view of the report of McLeod (1954) that vaccination of mice with dead cultures of *Salm. dublin* and *Salm. typhi-murium* produced a moderate to a high survival rate against intraperitoneal injection of doses of the respective organisms which killed most of the controls but gave only a small degree of protection against infection by ingestion, attempts were made to produce infection in mice with *Salm. gallinarum* by prolonged administration of the organism in the drinking water, but without success.

The failure of a dead vaccine to induce immunity in fowls against a strong challenge with *Salm. gallinarum* is perhaps not surprising. Although killed vaccines are widely used in protecting man against typhoid, it has been pointed out (Topley & Wilson, 1955) that while T.A.B. vaccination probably affords some degree of protection against slight and occasional exposure to infection it has little or no effect when exposure is severe and frequent ; against paratyphoid it appears to be even less efficacious. It is considered that vaccination would form a poor substitute for sanitary control in a population exposed to a severe and continuous risk of infection.

No conclusions of practical value can be drawn from the experiment with live vaccine, although previous work has shown that an attenuated live vaccine produces a strong immunity (Wilson, 1946, 1947). In the present case the organism comprising vaccine 6 was still sufficiently virulent to cause a mortality of 37·5 per cent. in inoculated fowls (before attenuation a similar dose would have caused 100 per cent. mortality) ; the solid immunity still present in all except one of the birds 112 days after inoculation, must be considered to be associated with recovery from active disease.

Smith (1955) reported that 20 chickens which had recovered from infection nine weeks previously, and 11 chickens infected 10 to 17 weeks previously, remained healthy when exposed to massive challenge. It is the general experience that most recovered birds possess a useful immunity but in some it is of a temporary nature ; when a number of recovered birds are retained for periods of one to two years it is not uncommon for deaths to occur from time to time from virulent fowl typhoid (Wilson, 1954). This has also been observed by Hall *et al.* (1949) who reported that of 55 birds which had reacted strongly for an average period of one year, 14 eventually died of the acute disease.

The absence of symptoms of illness, apart from temporary cessation of egg laying in all except one of the birds inoculated with attenuated culture, when challenged with a lethal dose of *Salm. gallinarum* suggests the possible prophylactic value of a living vaccine in natural outbreaks of fowl typhoid. For the reasons already discussed such a vaccine must have a limited application. In addition the present work suggests that following vaccination there might be a transient excretion of the vaccinal strain in the faeces and eggs. This would not be of great practical importance, however, for the organism would be of low virulence ; moreover, excretion either in eggs or faeces could readily be controlled by routine dosing with furazolidone at a fixed interval after vaccination.

Conclusions

1. Killed vaccines described in the paper provided no protection against experimentally induced fowl typhoid and a large-scale field trial with one of the vaccines showed it to be ineffective in preventing the natural disease.

2. Inoculation with a partly attenuated culture caused the deaths of about one-third of the birds and induced a solid immunity in the remainder. When the latter were challenged, symptoms of illness did not develop but there was a temporary cessation of egg production. In several birds there was a transient excretion of *Salm. gallinarum* in the faeces and two birds laid a proportion of positive eggs throughout the period of observation.

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