SALMONELLA PULLORUM INFECTION IN ADULT TURKEYS

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THESIS

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SALMONELLA PULLORUM INFECTION IN ADULT TURKEYS

I COMPARATIVE STUDY OF TETRATHIONATE BROTH MANDELIC ACID BROTH AND SELENITE F BROTH ENRICHMENT MEDIA IN THE ISOLATION OF SALMONELLA PULLORUM

II RELATIONSHIP BETWEEN THE AGGLUTINATION REACTION AND THE OPEN CARRIER STATE

By

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TABLE OF CONTENTS

	Page
Introduction	1
Previous Work	2
Formulas of the Media	7
Materials and Methods	8
Table I	12
Table II	13
Table III	14
Table IV	15
Table V	16
Table VI	17
Table VII	18
Discussion	21
Conclusions	24
Part II - Materials and Methods	25
Table 1	27
Table 2	28
Table 3	29
Table 4	30
Discussion	31
Summary	32
Bibliography	34

INTRODUCTION

The question as to whether pullorum disease spreads more or less readily among adult turkeys is often brought up by turkey raisers. This question has not been answered yet by adequate experimental work. Obviously, as is the case with other infectious diseases, pullorum disease could not spread from bird to bird unless open carriers were present, disseminating a sufficient number of organisms to infect other birds. This thesis contains the records of a study of Salmonella pullorum infection in 24 adult turkeys, which came from an infected flock. These birds were brought in for observation in June 1946. S. pullorum was isolated from them at that time.

In the course of the work three other turkeys were infected artificially, and the period during which the germ was found in the feces as well as the serological reactions were recorded and compared.

The purpose of this work was: 1. To determine whether growing and adult infected birds discharge S. pullorum with their excreta; 2. whether the dissemination of this organism takes place continuously or intermittently, and 3. what relation may exist between positive agglutination reaction and the open carrier state.

As a preliminary step in our attempts to detect the presence of S. pullorum in the living bird, a study was made of the comparative effectiveness of three enrichment media for the isolation of Salmonella. The comparison of the three media, tetrathionate broth, selenite F broth, and mandelic acid broth was made to determine their inhibiting power for the coliform organisms, and their adequacy for demonstrating the presence of Salmonella.

PART I

PREVIOUS WORK

Since 1923, when Miller first recommended tetrathionate broth for an enrichment medium in the isolation of members of the Salmonella, Shigella and Eberthella groups, systematic studies of the different media recommended, have appeared. Studies have been made on the effectiveness of the enrichment medium over plain media, as well as on the role played by the different constituents of the medium. An abundant amount of literature exists on tetrathionate which has been studied principally by three men, Pollok, Know, and Gell.

Liefson (1936) and Gahar (1943) reported a new medium for the isolation of Salmonella, Shigella and Eberthella. Both workers used selenite salts and reported a great selective power of the new medium as well as a specific range of inhibiting power that permitted them to isolate with great ease, the Salmonella present.

The Digestive Ferments Company, Difco, put out another medium, mandelic acid broth, that was reported as highly inhibitory by Teninga (1947).

Transfers were made from the enrichment media to McConkey and SS agar plates. The relation between the enrichment media and the secondary plating media is very important and has been pointed out by the majority of workers.

Miller (1923) found that B. parathyphosum, B.,Salmonella and Proteus reduced tetrathionate while B. coli, B. dysenteriae and many others lacked that power.

Jones, (1936), working with thyphoid bacilli, stated that one million bacilli were necessary to produce growth on McConkey agar plates whereas B. coli and B. paratyphosus grew very well from small inocula. He pointed out the importance of the pH and the standardization of McConkey's medium as necessary to obtain good results. Looking for a selective medium which would permit easy recognition of the parathyphoid group, he found that in tetrathionate the growth of the B. dysenteriae and B. coli was entirely suppressed after an inoculation of one million organisms, whereas other members of the same group grew satisfactorily.

Knox (1942), working with paracolon bacilli and B. paratyphosum on tetrathionate, found that with B. paratyphosum the period of rapid increase in cells was prolonged so that in five hours the population was two and one half times the number reached without tetrathionate. At the same time he reported that the growth of paracolon bacilli on the same medium was fairly slow. Attempting to explain this physiological effect, Knox found that similar results were obtained when broth cultures in an ordinary medium were subjected to free airation. Thus he confirmed the belief of Wilson and Topley (1936) that tetrathionate acts as an alternative hydrogen acceptor to oxygen and in that way it acts as oxygen although not in the same degree. The practical conclusion was that in mixed cultures the salmonella group has an advantage over the coli group, since this is unable to reduce tetrathionate.

Pollack and Gell (1945) reported the results of their studies concerning the efficiency of McConkey medium when used for direct

and indirect plating. The report showed that the number of positives increased slightly but definitely when this medium was used in direct plating, and in comparison with routine media. Using tetrathionate he found that indirect plating on McConkey medium gave about three times as many positives as direct plating on McConkey's agar and two times as many as direct plating on other solid selective media.

Nassey (1942) reported that tetrathionate gave a pure, heavy growth of Salmonella when the number of infecting organisms was 15 per cc., and found that in order to obtain the same growth by direct plating on McConkey's agar he needed five to ten times more organisms. He stated that the smallest number of organisms necessary to be detected in a feces suspension varied from 15 to 255 per cc. Mayfield and Sober (1940) had begun to use SS agar because of unsatisfactory results with McConkey's agar. They found SS agar very efficient in the isolation of Salmonella and reported that the morphology of the colonies was more characteristic in this medium.

Cooper, Keller and Glesive (1945) also reported a superiority of SS agar over McConkey's and desoxycholate citrate agar in the isolation of Salmonella. Mallok (1942) reported his experiments with SS agar and noted that from 40 positive cases, 34 isolations of Salmonella were successful on SS agar only, 27 in tetrathionate followed by McConkey's agar and 7 on McConkey's. In eight cases SS agar only was positive and in five instances only tetrathionate picked up the pathogens. In two of these cases SS agar and McConkey's medium were overgrown by colon bacilli because too large an

inoculum was used. They reported that McConkey's medium did not give good results and that the combination of tetrathionate and SS agar allowed successful isolation in 97.5% of the cases studied. When tetrathionate was used followed by McConkey's agar only 72% of positives were successful.

In respect to the selenite F medium, Liefson (1936) began to work on selenite salts for the purpose of isolating Ebertella, Shigella and Salmonella. The toxicity of the selenium salts has been noted previously and thought to be useful because of its inhibiting effects on certain groups of microbes. Liefson reported that selenite F medium exhibits differential inhibiting effects that permit one to differentiate very sharply among closely related bacteria. He found that selenite F was highly toxic for colon bacilli, but it was not sufficiently toxic to inhibit the growth of typhoid bacilli and enterococci. Attempting to explain the usefulness of the medium in the isolation of Salmonella, he found that during the first 10 to 12 hours the growth of the colon bacilli decreased followed by a rapid increase of the typhoid bacilli which soon outnumbered the colon bacilli. He reported that even when a few tpyhoid bacilli were present in a large amount of feces, they may be recovered with great ease and made to multiply to one million in 24 hours of incubation. He concluded that the sodium selenite F medium was superior to other existing media for the isolation of Salmonella and the typhoid bacilli.

Gohar (1943) using selenite solutions in concentrations of 1/100 to 1/100,000 found that selenite was highly inhibitive for

B. dysenteriae, Vibrio cholerae, and B. coli. On the other hand the salmonella group and Streptococcus fecalis were more resistant to the toxic effects of selenite salts. He also recommended the use of selenite F medium and McConkey's agar together in the isolation of Salmonella.

Liefson (1936) observed, however, that the usefulness of selenite medium is conditioned by the pH, by the presence of phosphates and that the use of the autoclave on selenite F medium, destroyed its effectiveness.

In regard to mandelic acid medium I have not found any specific report aside from that presented by Teninga (1947). While the formulae of the tetrathionate broth and selenite F medium are well known, the formula for the mandelic acid medium has not been published.

FORMULAS

- Difco tetrathionate

Proteose peptone No. 2		5 gm.
Bacto bile salts		1 gm.
Calcium carbonate		10 gm.
Sodium thiosulfate		30 gm.
Iodine solution	2ml/100ml	

Iodine solution is prepared by dissolving 6 gm. of iodine crystals and 5 gm. potassium iodide in 20 ml of water.

- Difco selenite F.

Sodium selenite	5 gm.
Bacto tryptone	4 gm.
Bacto lactose	4 gm.
Disodium phosphate	10 gm.

- Difco mandelic acid media

Formula unknown

SS Agar.

Bacto beef extract	5	gm.
Proteose peptone Difco	5	gm.
Bacto lactose	10	gm.
Bacto bile salts No. 3	8.5	gm.
Sodium citrate	8.5	gm.
Sodium thiosulfate	8.5	gm.
Ferric citrate	1	gm.
Bacto brilliant green	0.33	mg.
Bacto neutral red	0.025	gm.

McConkey's Agar.

Bacto peptone	17	gm.
Proteose peptone	3	gm.
Bacto lactose	10	gm.
Bacto bile salts No. 3	1.5	gm.
Sodium chloride	5	gm.
Bacto agar	13.5	gm.
Bacto neutral red	0.03	gm.
Bacto crystal violet	0.001	gm.

MATERIALS AND METHODS

In the spring of 1946 the Poultry Clinic of the Department of Bacteriology and Public Health, Michigan State College, received 200 turkeys from a flock infected with S. pullorum. One hundred and fifty of these died in two weeks.

In October 1946, 28 turkeys remained alive and were found to be pullorum negative by bacteriological examination. However, the kidneys and intestines of 24 turkeys, that died during the summer, yielded S. pullorum. The feces of these turkeys contained lactose fermenters, Esch. coli, Aurobacter aerogenes, and Proteus. These feces were used in testing the inhibiting power of the three media under investigation.

The media used were all dehydrated products (Difco).

- Tetrathionate broth.

Tetrathionate Difco	4.6	gm
Distilled water	100.	ml

This solution was heated to 95° C. and cooled to 60° C. At this point 2 ml of iodine solution was added to each 100 ml of medium. The medium must be used shortly after the iodine is added.

- Mandelic acid broth.

Mandelic acid Difco	2.6	gm
Distilled water	100.	ml

The solution was heated to 85° C. to avoid a possible precipitation of its components due to heat.

Selenite broth.

Selenite Difco	2.3	gm.
Distilled water	100.	ml

The solution was heated to 85° C, following the precautions recommended for mandelic acid medium.

As secondary solid media, SS agar and McConkey's agar were used.

SS Agar.

SS agar Difco	60	gm.
Distilled water	1000	ml

The agar was dissolved by steaming for 45 minutes and rapidly poured into regular Petri dishes. No sterilization is required. Autoclaving injures SS agar.

McConkey's Agar

McConkey's agar Difco	50	gm.
Distilled water	1.	ml

The method of preparation was the same as for SS agar.

Bacteriological examination of live turkeys for S. pullorum.

1. Rectal swabs were taken from each turkey. These were put into 5 ml of sterile peptone broth for half an hour.

2. One ml of the inoculated broth was placed into 10 ml of each of the three media using sterile pippettes.

3. The tubes were incubated for 17-20 hours at 37° C. The period of incubation is very important since after the twentieth hour the growth of Salmonella begins to decrease.

4. Single loopfuls of the three media were smeared on SS agar plates marked off into two halves so that each plate received a sample from the same feces through two different enrichment media.

5. The plates were incubated 48 hours at 37° C and the colonies were then counted.

The three media were tested four times with the same sample of feces. The results are shown in Tables I, II, III, and IV.

Tables V and VI compare the efficiency of the three media, where used in examining feces which contain Salmonella.

The material used was feces of those same birds to which was added a pure 24-hour culture of S. pullorum. The strain was isolated from the same flock.

Procedure:

1. One gm. of feces was emulcified in 3 ml of sterile saline solution.
2. One tenth of a 24 hour culture of S. pullorum was added.
3. A loopful of the emulsion was placed in 10 ml of each of the three media, and the tubes incubated for 17-20 hours at 37° C.
4. Plates of SS agar and McConkey agar were used for plating and the SS agar plates were incubated for 48 hours and the McConkey plates 20 hours.
5. The colonies on the three media were counted.
6. The identification of the Salmonella was made by sugar fermentation. The experiment was repeated twice each time with 10 different samples. The results are tabulated in Tables V and VI.

Tables VII shows the comparative ability of the three media in giving evidence of the presence S. pullorum in the intestinal tract of artificially infected turkeys. Three turkeys were infected orally, each with 10 ml of a 24-hour pure culture of the same strain used in the second experiment. The procedure followed was the same as in the first experiment. In this part new lots of selenite F and mandelic acid media were used, and, perhaps, this accounts for the results obtained.

The tests began 5 days after the turkeys were infected and were continued until one died and the others were killed for post mortem examination for evidence of pullorum infection. The results are shown in Table VII.

TABLE I

Growth of Esch. coli on SS Agar following cultivation in selective enrichment media.

Plate No.	Mandelic	Selenite	Tetrathionate
12	++	+	-
13	-	++	++
14	-	+	++
16	-	-	-
17	-	++	++
20	-	+	+++
21	-	+	++
22	-	++	-
23	-	+	+
25	-	+	-
26	-	-	+
27	-	-	+
28	-	-	-
29	-	-	-
30	-	+	++
31	-	-	+
32	+	+	-
33	+	-	++
35	+	++	+++
37	-	-	++
38	-	-	-
41	-	-	-
42	-	+	-
43	-	-	+
51	+	-	++
88	-	-	+
89	+	+	++
90	-	+	-

\- negative
\+ 1-4 colonies
++ 5-20 colonies
+++ more than 20 colonies

TABLE II

Growth of Esch. coli on SS Agar following cultivation in selective enrichment media.

Plate No.	Mandelic	Selenite	Tetrathionate
12	+-	+	+
13	-	+	++
14	-	-	++
16	-	+	++
17	+	-	++
20	-	-	+
21	-	+	+
22	-	-	++
23	+	-	+
25	-	-	+
26	-	-	+
27	-	+	+
28	-	-	+
29	-	-	+
30	+	-	+
31	-	-	-
32	+	-	+
33	-	-	+
35	-	-	+
37	+++	+++	+
38	—	-	+
41	-	-	+
42	+	-	-
43	-	++	-
51	-	-	++
88	+	-	++
89	-	-	+
90	+	-	+++

TABLE III

Growth of Esch. coli on SS Agar following cultivation in selective enrichment media.

Plate No.	Mandelic	Selenite	Tetrathionate
12	-		
13	-	+	+
14	-	+	-
16	+	-	+
20	-	-	-
21	+	+++	-
23	+	+	++
25	-	+	+
26	+	-	-
27	+	--	+
28	-	-	+
29	-	-	-
30	-	+	++
31	-	-	-
32	-	-	++
35	+	—	++
37	-	-	-
38	-	+	-
41	-	-	+
42	-	-	+
43	-	-	+++
44	-	-	+++
51	+	+	+++
87	-	+	+++
88	-	+	-
89	-	-	+
90	+	-	-
91	-	+	+

Note: A direct streaking from the suspension of feces on agar was made, obtaining good growth of different types of colonies.

TABLE IV

Growth of Esch. coli on SS agar following cultivation in selective enrichment media.

Plate No.	Mandelic	Selenite	Tetrathionate
12	-	-	-
13	-	-	+
14	-	-	++
16	-	+	++
20	-	-	+
21	-	-	-
23	+	-	+
25	-	-	++
26	-	-	-
27	-	-	+
28	-	+	-
29	+	-	+
30	+	-	-
31	-	-	-
33	-	-	+
35	-	-	+
37	-	-	-
41	-	-	-
42	-	-	-
43	-	-	-
44	+	+	-

Note: Mandelic acid and selenite F media became darker, coinciding with an increase in inhibiting power.

TABLE V

Growth of S. pullorum following cultivation in selective enrichment media when 0.1 ml of pure culture was inoculated into 5 ml of emulsion of feces.

	SS Agar			McConkey's Agar		
Plate No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
1	+	-	+++	+	-	++
2	-	-	+++	-	-	++
3	-	-	++	-	-	++
4	-	-	++	-	-	++
5	+	-	+++	-	-	++
6	+	-	+++	-	-	++
7	+	+	+++	+	-	++
8	+	-	++	-	-	++
9	+	-	++	-	-	++
10	+	-	+	+	-	++

Note: 1. S. pullorum was recovered only from the 10th tube in the series in which mendalic acid medium was used.

2. On McConkey's agar, as expected, Esch. coli colonies as well as Salmonella colonies developed.

3. Selenite medium and mandelic acid medium appeared to be too inhibitory for Salmonella, the original sample had 0.1 of pure culture of S. pullorum, and it was recovered through tetrathionate.

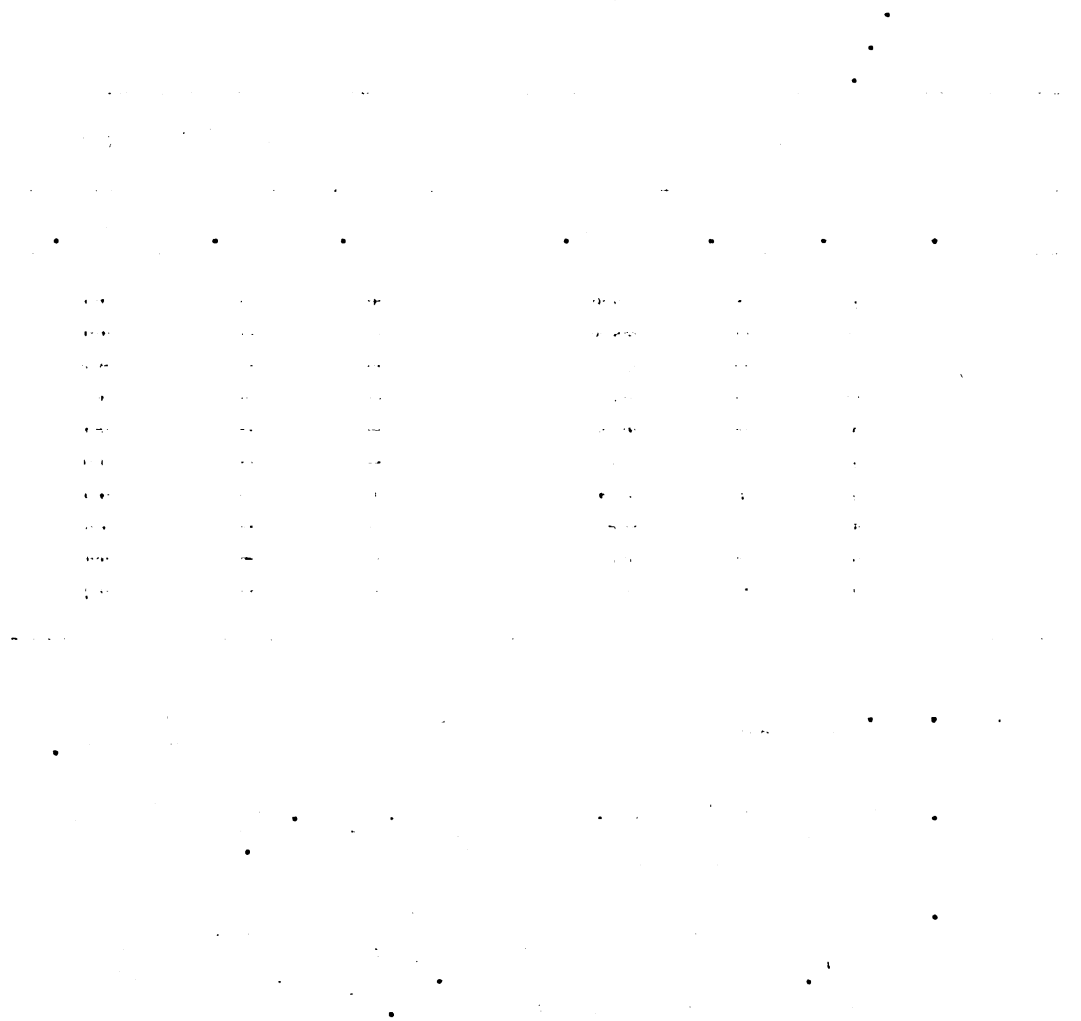

TABLE VI

Growth of S. pullorum following cultivation in selective enrichment media when 0.1 ml of pure culture was inoculated into 5 ml of emulsion of feces.

	SS Agar			McConkey's Agar		
Plate No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11	-	-	++	-	-	++
12	-	-	++	-	-	++
13	-	-	++	-	-	+
14	-	-	++	-	-	++
15	-	-	++	-	-	+
16	-	-	++	-	-	+
17	-	-	++	-	-	+
18	-	-	++	-	-	++
19	-	-	++	-	-	++
20	-	-	++	-	+	++

Note: After the second experiment with mandelic acid and selenite F media, two new samples of these media were requested. These media are very hygroscopic and must always be kept in tightly stoppered bottles, if not the media will oxidize and become excessively inhibitory.

TABLE VII

Growth of S. pullorum following cultivation in selective enrichment media when feces of artificially infected birds were used.

(#1)

	SS Agar			McConkey's Agar		
No. of Bird	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	-	+	+	E. coli	E. coli	E. coli ++
11932	-	-	-	-	-	-
11950	+	+	+	E. coli proteus	E. coli	Proteus -

(#2)

	SS Agar			McConkey's Agar		
No. of Bird	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	-	+	+++	- E. coli	-	+
11932	E. coli -	E. coli -	-	E. coli		
11950	-	+	+	E. coli +		+

Controls

Emulsion of S. pullorum 5 ml of saline solution from pure slant culture

0.1 ml	-	—	-	-	-	--
0.6 ml	-	-	---	-	-	---

\+ Growth of S. pullorum
\- No growth of S. pullorum

TABLE VII (Continued)

(#3)

	SS Agar			McConkey's		
Bird No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	+	-	++	+	-	++
11932	E. coli	E. coli	-	-	- E. coli	- E. coli
11950	-	+	+		-	-

(#4)

	SS Agar			McConkey's		
Bird No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	++	+	+	-	+	+
11932	-	-	-	- E. coli	-	-
11950	+	-	+	E. coli -	E. coli +	- +

(#5)

	SS Agar			McConkey's		
Bird No. Turkey	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	++	++	+	E. coli	+	+
11932	E. coli	E. coli	-	E. coli	E. coli	-
11950	+ E. coli	-	-	E. coli -	E. coli -	+

TABLE VII (Continued)

(#6)

	SS Agar			McConkey's		
Bird No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	-	++	++	E. coli	+	+
11932	E. coli	E. coli	-	E. coli	E. coli	-
11950	E. coli	-	-	E. coli	E. coli	-

(#7)

	SS Agar			McConkey's		
Bird No.	Man.	Sel.	Tetra.	Man.	Sel.	Tetra.
11929	-	-	-	-	-	-
11932	-	-	-	E. c o l i		
11950	-	-	-	-	E. coli	-

Controls

Emulsion of S. pullorum in 5ml of saline solution from pure slant culture

S. pullorum	-	+	++	-	-	+

Note: The results shown in Table VII (7) indicate that the birds had become negative.

The results obtained with the new samples of selenite F medium are very different from those shown in the Tables I, II, III, IV, V, VI.

DISCUSSION

From the work done it was concluded that tetrathionate broth is superior to mandelic acid broth and selenite F broth for the isolation of S. pullorum. This conclusion was based on their comparative ability to inhibit organisms other than S. pullorums and their adequacy for detecting S. pullorum.

When S. pullorum was present, it was proved that tetrathionate broth was the only medium that consistently allowed the growth of this organism. In regard to the other two media it was observed that an increasing inhibitory power accompanied a change of color in the broth, which, in both cases, was much lighter when the medium was prepared from newly received, bottled samples. This aroused the suspicion that some chemical change had occurred in the lots of selenite F, and mandelic acid media first received. In the case of selenite F broth the results shown in Table VII differ completely from the result obtained when an old sample of the medium was used. One instance was recorded in which S. pullorum was recovered from a tube containing 7 ml of the medium instead of the 10 ml used before. S. pullorum was isolated on McConkey's Agar in the case mentioned but on SS agar the combined inhibitory effect of the two media evidently suppressed the growth of S. pullorum.

When 0.1 ml of pure culture of S. pullorum was inoculated into 5 ml of feces emulsion, S. pullorum was recovered in few cases, but the medium did not seem to be adequate for detection of S. pullorum in a high percentage of cases. When a new sample of selenite F

medium was used, S. pullorum was recovered in all but one case. On SS agar pure cultures of S. pullorum were obtained but on McConkey's agar occasionally mixed cultures were obtained. This serves to indicate the high selective action obtained when subcultures from selenite F broth cultures were made on SS agar.

Through personal information, during the writing of this discussion, I learned that Dr. Bevins found selenite F to be the best medium for the isolation of S. pullorum. My own observations on the change in Selenite F medium with increased inhibitory action and the importance of the amount of inoculum in relation to the amount of medium indicate that selenite F broth offers possibilities of effectiveness provided the condition of the medium and every phase of the procedure are definitely determined and properly controlled. Since these factors have not yet been definitely established the use of tetrathionate enrichment broth is recommended for the isolation of S. pullorum.

Mandelic acid medium showed a relative effectiveness only the first time it was used. It was thought that the presence of oxygen, even in small quantities, altered the medium. This opens a field for a biochemical study of this medium. An understanding of the results obtained will be possible only when the medium has been tested under different conditions. These conditions should include quantity of the medium in relation to: 1. the size of the sample; 2. the number of organisms present in concentrated and diluted suspensions.

Another thing to be studied is the effect of air on these two media. Until this has been done no definite evaluation of mandelic acid medium can be made.

CONCLUSIONS

1. Tetrathionate broth inhibits coliform organisms and allows the growth of S. pullorum in the first 17 hours of incubation. This medium was most effective in the isolation of S. pullorum from feces of turkeys. Tetrathionate broth followed by subcultures on SS agar is recommended for the isolation of S. pullorum. When McConkey's agar is used for subcultures the growth of coliform organisms is not completely inhibited.
2. Selenite F broth has a higher inhibitory action on coliforms than tetrathionate broth, which allows the growth of Proteus. Under certain conditions Selenite F broth proved too inhibitory and suppressed growth of S. pullorum. The combined inhibitory effects of Selenite F medium and SS agar are too great to insure growth of Salmonella. When Selenite F medium is used the subcultures should be made on McConkey's agar.
3. Mandelic acid medium inhibited all kinds of organisms present in feces of turkeys.

PART II

This part represents some preliminary studies on the pullorum disease carrier state.

MATERIALS AND METHODS

The feces of twenty five turkeys from a flock infected with S. pullorum contained S. pullorum in June 1946, when they were two months old. At that time the blood serum of several of these birds was positive to the agglutination test with pullorum antigen.

When this work began these twenty five turkeys were thought to be suitable subjects for a study of the pullorum infection carrier state. Periodic examinations were made of droppings from these birds to see if the birds discharged S. pullorum. At the same time agglutination tests were made in order to determine whether there might be any relation between the agglutination titer and positive fecal cultures.

The procedure for the bacteriological examination was the same as that employed in Part I of this work.

For the agglutination test, pullorum antigen prepared by Dr. J. A. Bevins of the Department of Bacteriology and Public Health of Michigan State College.

The dilution of the serum used was of 1/25. The tubes were incubated twenty hours at 50° C. in a water bath, and two hours at room temperature.

In view of the negative results obtained during the fall and winter 1946-47, (Table 1, page 27) three turkeys (not from the original 25) were infected orally with a pure culture of S. pullorum.

The length of time during which they discharged the organism in the feces and the agglutination titer of these birds were observed. The results are shown in Tables II, III, and IV.

During the Spring 1947, 100 eggs laid by these twenty five turkeys and 50 laid by the three artificially infected birds were examined for S. pullorum. The whole egg was broken in 100 ml of tetrathionate broth, incubated for 17 hours at 37° C, and subcultures were made on SS agar.

One of the 100 and five of the 50 eggs yielded S. pullorum.

TABLE 1

Table showing the results of the bacteriological examination for S. pullorum in the droppings of 28 birds from a flock naturally infected with S. pullorum five months earlier and results of the agglutination test.

Bird No.	Bacteriological Exam	Agglutination Exam
12	-	-
13	-	-
14	-	-
16	-	-
17	-	-
20	-	-
21	-	-
22	-	-
23	-	-
25	-	-
26	-	-
27	-	-
28	-	-
29	-	-
30	-	-
31	-	-
32	-	-
33	-	-
35	-	-
37	-	-
38	-	-
41	-	-
42	-	-
43	-	-
51	-	-
88	-	-
89	-	-
90	-	-

Note: These birds were tested in the same way every two weeks during the fall and winter (1946-1947) with identical results. One hundred eggs laid during spring (1947) were negative for S. pullorum with one single exception.

TABLE 2

Comparative results of the bacteriological examination and agglutination test for pullorum infection in bird No. 11929, infected orally on February 22, 1947, with 10 ml of a 24 hour agar culture of S. pullorum suspended in saline.

Date		Bacteriological Examination	Agglutination Test
Feb.	26	+	++
March	6	+	+
	14	+	++
	20	+	++
	28	+	++
April	2	-	++
	10	-	+
	18	-	+
	23	-	+
May	3	-	+
	10	-	+
	15	-	+
	23	-	+
	31	-	+

Note: Bird No. 11929 was killed on June 30th. It was found apparently normal on autopsy and S. pullorum was not isolated from its organs.

Key: - negative agglutination test and negative culture
+ weakly positive agglutination test and positive culture
++ marked positive agglutination test
+++ strong, positive agglutination test

TABLE 3

Comparative results of the bacteriological examination and the agglutination test for pullorum infection in bird No. 11932 infected in the same way as bird No. 11929 with S. pullorum on February 22nd.

Date		Bacteriological Examination	Agglutination Test
Feb.	26	-	-
March	6	-	-
	14	-	-
	20	-	-
	28	-	++
April	2	-	++
	10	-	+
	18	-	+
	23	-	+
May	3	-	+
	10	-	+
	15	-	+
	23	-	+
	31	-	+

Note: Bird No. 11932 died on June 18th. The autopsy did not show visible sign of pullorum infection. S. pullorum was isolated from the ovary and liver but not from other organs.

TABLE 4

Comparative results of the bacteriological examination and agglutination test for pullorum infection in bird No. 11950 infected in the same way as bird No. 11929 with S. pullorum on February 22nd.

Date		Bacteriological Examination	Agglutination Test
Feb.	26	+	≠
March	6	+	≠ ≠
	14	+	≠ ≠
	20	+	≠
	28	+	-
April	2	-	-
	10	-	-
	18	-	≠ ≠
	23	-	≠
May	3	-	≠
	10	-	-
	15	+	≠
	23	-	≠
	31	-	≠

Note: On June 17th bird No. 11950 died. On autopsy the following changes were found: hydropericardium, necrotic foci in the spleen. The kidneys were found enlarged. Not a pathological change. The vitelene membrane of the ovary was thickened. There was severe peritonitis. S. pullorum was isolated from the heart, liver, lungs, intestines and ovary.

DISCUSSION

Due to the limited scope of the work no definite conclusions can be made.

Each of the three birds reacted differently. Two of the birds discharged the organism through the intestine during the first four weeks following infection, while the other did not discharge S. pullorum at all. However, the organism was isolated from its liver and ovary and its serum was reactive after the fourth week of infection. The organism was not recovered by taking rectal swabs and subjecting them to careful bacteriological examinations as described.

When five months of age the original 28 turkeys did not discharge S. pullorum with their droppings as far as could be ascertained by cultures made from rectal swabs. They were also negative to the pullorum agglutination test. When they were two months old one of them discharged S. pullorum and the serum of five of them reacted to the agglutination test. S. pullorum was isolated from one of 100 eggs examined, showing that S. pullorum can be transmitted through eggs while no dissemination through droppings was evident.

SUMMARY

1. Twenty five turkeys from a flock infected with S. pullorum were found to be consistently negative on bacteriological examination and to the agglutination test for pullorum infection when they were five months old. At two months of age one had been found to discharge S. pullorum in the feces and five were positive to the agglutination test. These turkeys were possible carriers since they had been in contact with the disease as poults.
2. The feces and sera of three turkeys artificially infected orally with S. pullorum were also tested for pullorum infection. Two of them discharged the organism regularly for five weeks following the infection. After this time Bird No. 11929 remained negative, the other one, No. 11950, again discharged S. pullorum, twelve weeks following infection, and died two weeks after showing clinical symptoms. The third one, No. 11929 was killed for autopsy. S. pullorum was not isolated from its organs. The serum of Bird No. 11950 was always positive to the agglutination test, with the exception of a period of two weeks following the dissapearance of the S. pullorum from the feces. The serum of Bird No. 11929 was positive during the entire period of this study.
3. Bird No. 11932 did not discharge S. pullorum through the intestine, but the serum became highly reactive the fourth week following the infection and remained positive for the remainder of the experimental period. When it was killed S. pullorum was isolated from the liver.

4. One of 100 eggs (1%) laid by 25 turkeys from a naturally infected flock yielded S. pullorum. Five of 50 eggs (10%) laid by three artificially infected turkeys yielded S. pullorum.

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