THE USE OF FORMALDEHYDE AS A DISINFECTANT IN THE CONTROL OF INCUBATOR TRANSMITTED PULLORUM DISEASE

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THE USE OF FORMALDEHYDE AS A DISINFECTANT IN THE CONTROL OF INCUBATOR TRANSMITTED PULLORUM DISEASE

by

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INTRODUCTION

Pullorum disease is still a perplexing problem to the poultry industry. It is difficult to control due to its many modes of transmission. This disease is passed from the infected hen through the egg to the chick and the chick may transmit the disease to non-infected stock during the hatching period or the first few weeks of brooding. The spread of the disease and the mortality of chicks is directly influenced by conditions during the first two weeks of brooding.

The specific cause of pullorum disease is a bacillus known as Salmonella pullorum. This organism enters the body through the respiratory or alimentary tract through the agency of contaminated air, feed or water; or it may have been in the egg from which the chick hatched. The organism is found in the blood, in the unabsorbed yolk, in the bone marrow, and in the internal organs of the chick following death from the disease.

The control of pullorum disease depends largely upon two factors: first, the detection of the infected breeding fowls by means of agglutination tests and their immediate removal from the breeding flock and second, the sanitary protection of healthy stock against infection in incubators, brooder houses and runways.

The value of formaldehyde in the control of pullorum disease infection in the incubators is the basis for this thesis.

REVIEW OF LITERATURE

Since it was discovered that pullorum disease was spread via the incubator, the incubator manufacturers have been looking for methods of combating this mode of dissemination. T. S. Townsley (1), in 1930, research director of the Smith Incubator Company was one of the incubator men to work upon incubator hygiene as a control of pullorum disease. However, Moore, Upp and Hinshaw (2) in 1926 were the first to discover that pullorum disease could be disseminated in the forced draft incubators. The work of Hinshaw and others was confirmed by Bunyea and Hall (3).

Many types of disinfectants were tested without apparent success until the workers tried formaldehyde (HCHO).

The discovery of formaldehyde is usually attributed to Hoffmann (4) who in 1867 succeeded in preparing the aldehyde of the methyl group, by the partial oxidation of methyl alcohol. Almost as soon as it became commercially available, formaldehyde was hailed as an effective disinfectant. In 1888, Low (5) recognized the strong antiseptic properties of formaldehyde. In 1892, Trillat (6) discovered that a bouillon containing 1 : 50,000 of formaldehyde was not suitable for the growth of the anthrax germ. E. A. de Schweinitz, (7) Chief of the Biochemistry Division of the United States Department of Agriculture in 1896 states: "Formaldehyde possesses most of the properties of a good disinfectant". Other early experiments with formaldehyde were carried out by Miguel, Bandet, and Trillat (8) for the disinfection of rooms. The summary in the 1896 Yearbook of Agriculture is as follows:

1. "Formalin in concentration of 1 : 10,000 makes growth of tuberculosis, anthrax, cholera, typhus pus, and diphtheria germs impossible.

2. "In gaseous form, a weak dilution is sufficient to check the growth of the above.

3. "A one per cent solution will kill pathogenic organisms in an hour.

4. "Spraying with formalin solution and subsequent inclosure of the articles in a closed space will easily sterilize them.

5. "Feces are deodorized by a one per cent solution and in 13 minutes are germ-free. Buildings are readily disinfected by a one per cent to one and one-half per cent volume of gas in 6 to 13 hours.

6. "Formaldehyde is useful as an etching material and preservative.

7. "Odor can be readily dissipated by the use of a dilute solution of ammonia, which readily absorbs the gas."

In the U. S. D. A. Bulletin (9) the following advantages and disadvantages of formaldehyde are listed.

The advantages summarized:

1. It is a powerful germicide.
2. Its action is not hindered greatly by albuminous substances or organic matter.
3. It is relatively non-poisonous.
4. It is not injurious to delicate fabrics, to paint, or to metals.
5. It is the only known gaseous disinfectant which can be used effectively and safely in the household.

The disadvantages summarized:

1. The gas has a strong tendency to condense in cold weather and it is not reliable as a disinfectant when the temperature of the air is much below 65° F.
2. It has a very penetrating odor and the gas is irritating to eyes and nose.
3. To accomplish disinfection by the gas a long period of exposure is necessary and considerable work is required in the proper sealing of rooms which are to be disinfected.

Many of the workers on this problem agree that the germicidal efficiency of formaldehyde gas is greatly influenced by the relative humidity. When the amount of moisture is decreased, the germicidal efficiency of formaldehyde is decreased.

Two methods of releasing the formaldehyde have been employed, namely: the cheesecloth method and the potassium permanganate method. It is with the latter method that we are concerned.

Graham (13) of the Illinois Experimental Station reports that formaldehyde in the amounts recommended by that station destroyed S. pullorum in 43 minutes. Graham states, "There is no known incubator fumigant as efficient as formaldehyde in forced draft incubators for the suppression of S. pullorum and not-with-standing claims to the contrary, formaldehyde possesses a superior efficiency in incubator disinfection and costs much less". Doctor Graham (13) reports that chicks are not injured by exposure to formaldehyde and recommends three distinct fumigations twelve hours apart.

Some workers have recommended the practice of fumigating the chicks at the time of hatching so as to destroy organisms that may be liberated by the chicks at this time. Dakin and Speer (11) recommend three fumigations during the process of hatching. The first fumigation was to take place before ten per cent of the chicks are hatched, a second treatment twelve hours later with the immediate removal of all dry chicks, and the third treatment at the end of 48 hours. They also recommended the dosage of 0.2 gm. potassium permanganate and 0.4 cc. formalin per cubic foot of air space for a period of ten minutes.

Graham and Michael (13) have done considerable work on pullorum disease and their work shows that "Formaldehyde fumigation of forced draft incubators by either the cheesecloth method or the potassium permanganate method is of definite value in the suppression of pullorum disease". These men recommend fumigation during and before the hatch comes off.

Bushnell and Payne (14) report: "With a temperature of 99 to 100 degrees and a wet bulb reading of 90 degrees, treatment with 0.35 cc. formalin and 0.175 gm. potassium permanganate per cubic foot of space kills practically all exposed pullorum organisms within five minutes after the formaldehyde has been liberated". "Chicks subjected to a ten minute exposure of formaldehyde liberated from 0.35 cc. formalin and 0.175 gm. of potassium permanganate per cubic foot of air space with a wet bulb reading of 90°F are apparently not injured".

Winter (15) reports, "fumigation between hatches or before hatches is preferable to fumigation during the hatch. There is no danger of hurting hatchability if fumigation is used during the seventeenth

or eighteenth day of incubation, while fumigation during the hatch may injure the chicks". However, Winter gives directions for the fumigation of chicks during the hatch in this same bulletin, but he says that fumigation during hatching period is not recommended. "Chicks of low vitality may be injured by routine fumigation and good chicks are certainly not improved by it. Chicks more than 48 hours old should not be subjected to formaldehyde fumigation".

Bushnell and Brandly (16) declare that fumigation during the hatching period is desirable and recommend three periods of fumigation eight hours apart. "Neither the very young chicks nor the eggs seem to be injured if the formaldehyde fumigation is carried out according to recommended directions". They also recommend 0.35 cc. formalin and 0.175 gm. $KMnO_4$ for each cubic foot of air space for ten minutes.

In checking with the incubator manufacturers, it was found that two companies, the Buckeye and Smith both recommend fumigation before the hatch comes off and during the hatch. Smith Incubator Company (17) recommends that the first fumigation should be used when 15 to 20 per cent of the chicks are hatched, the second when 50 to 60 per cent are hatched and the third fumigation when the hatch is about complete.

The Buckeye Company (18) states: "To be effective the wet bulb reading in the incubator should be at least 85°F and preferably 90°F. The higher the humidity the more effective the gas and the less danger of injuring the chicks". The Buckeye Company states: "Fumigation of eggs is effective in preventing 'Mushy Chicks'". They recommend fumigating the chicks once when about two-thirds are hatched or six to eight hours before the hatch is completed.

Both companies recommend neutralizing the formaldehyde gas with 26 per cent ammonia hydrate for a ten minute period.

Carus Chemical Company (21) at LaSalle, Illinois, one of the manufacturers of potassium permanganate crystals, states in one of its advertising leaflets: "Fumigation during the hatching period, while the chicks are still damp or not completely dry and fluffed out, will prevent the spread of this disease from the diseased to the disease free chicks hatching at the same time. It will not cure the chick that hatches from the infected egg". They further state: "Do not expose eggs to fumigation until they have been in the incubator at least for four days. Fumigation during the first four days of incubation may prove injurious to the chick embryo in its early stage of development".

Scott (22) in 1928 compared the germicidal powers of phenol and formaldehyde and found that a 1 to 200 formaldehyde solution killed all of the aerobic bacteria both the spore forming and the non-spore forming in six to twelve hours. He states, "Phenol in a strength up to five per cent acts very slowly on the anaerobic organisms while formaldehyde in dilutions of 0.5 to 0.75 per cent sterilizes anaerobic cultures rapidly".

According to McCulloch (23) the phenol coefficient of formalin (40 per cent formaldehyde) is 1.3. I found that with Eberthella typhosa and S. pullorum the phenol coefficient was 1.3 in each case, thus confirming his findings.

Tilley and Schaffer (24) in 1928, using E. typhosa as the test organism, obtained a phenol coefficient of 1.05 for formaldehyde.

Mallmann (12) and staff did extensive work on this subject but their results showed that fumigation with formalin with the amounts recommended and even with greater amounts had very little effect on the incidence of S. pullorum in the chamber employed (unpublished work). They were unable to kill all the bacteria upon either the dry or the wet chicks. "Chicks exposed for ten minutes to the formalin gas showed marked respiratory disturbances and died 36 hours later".

Weisner (20) states: "Every setting of eggs should be subjected to the fumigation but care should be exercised not to fumigate after the eggs have begun to hatch. We do not recommend the fumigation of chicks". However, in his recommendations for fumigation of eggs the time of exposure to the formaldehyde gas has been raised from ten minutes to three hours.

Gwatkin (10) demonstrated conclusively that S. pullorum will live upon pieces of egg shell in an incubator for twelve days and at room temperature for at least 47 days. The fact that this organism will persist in an incubator justifies the use of fumigation to rid the machine of the infection. He recommends the use of 0.5 gm. of formalin and 0.25 gm. of potassium permanganate per cubic foot of air space with an exposure of two hours.

According to Bushnell and Payne (14), the lethal dose of formalin for S. pullorum is between 55 and 60 cc. of formalin per hour for eight and one-half hours.

PURPOSE

It is the purpose of this study to further the knowledge of the value of formaldehyde in the control of pullorum disease infection in the incubator.

EFFECT OF FORMALDEHYDE GAS UPON CHICK

In the preliminary experiments, it was found that chicks subjected to formaldehyde gas showed marked reactions. It was then decided to autopsy each chick, after subjecting it to definite amounts of formaldehyde gas for a definite period of time, to learn the action of the gas upon the flesh, internal organs, and eyes. The following results were secured after placing day old White Leghorn chicks in an air tight box and fumigating with potassium permanganate crystals and 40 per cent formalin. The box was built in such a manner that the actions of the chicks could be clearly seen.

Fig. 1. Photograph of Chicks in Fumigation Chamber Through Top Window.

Table I

The Condition of Chicks at Autopsy After Fumigation

Dosage Time Examined	Controls Immediately	Single 10 minutes Immediately	Single 10 minutes 10 min. after.	Double 10 minutes Immediately	Single 20 minutes Immediately	Double 20 minutes Immediately
	1	2	3	4	5	6
Flesh color	Normal tan	Cooked Brownish tan	Cooked Mahogany	Cooked Mahogany	Dark brown	Very dark brown
Condition of mouth and throat	Barely moist	Very moist	Saliva running out of mouth	Full of foamy sticky saliva	Full of sticky,foamy saliva	Foamy, sticky saliva running out
Condition of liver and lungs	Normal red	Cooked, dark brown	Mahogany color	Dark brown, friable and hard	Lungs yellow and very friable	Liver yellow and friable, lungs tan
Condition of eyes	Normal wide open	Closed	Closed	Closed	Closed	Closed almost sealed
Odor	None	Faint formalin	Slight formalin	Slight formalin	Faint formalin	Strong formalin
Condition of head	Normal	Shook, loud cheeping, gasping for air	Shook side to side, bent forward, gasping for air	Shook side to side, distressed cheep, difficult breathing	Shook side to side	Dead upon removal *

*Chick took bath in formalin.

AMOUNT OF FORMALDEHYDE GAS INHALED BY CHICKS DURING FUMIGATION

Due to the fact that formaldehyde is made by the partial oxidation of methyl alcohol, a question is raised as to whether the death loss, in chicks from fumigation, is due to the effect of the formaldehyde or whether it is due to methyl alcohol poisoning. Tests were conducted to determine just how much formaldehyde gas is actually taken up by the chick during the fumigation process. A process, by which the amount of formaldehyde gas that a chick actually inhaled during the fumigation period, was developed. One school of thought believes that the loss of chicks, due to mortality, from the day of hatching until four weeks of age, may be due to fumes of formaldehyde inhaled during the fumigation period. In the first experiments, an attempt was made to determine the amount of gas actually taken into the lungs. It is also thought that death might be due to methyl alcohol poisoning. Attempts to kill chicks with methyl alcohol fumes caused the chicks to stagger as if in a drunk stupor but they did not die. After a short period of time these chicks seemed to recover their normal balance. The writer was unable to find any literature dealing with chick mortality due to methyl alcohol poisoning.

Using a bell jar as an inclosure, a chick was exposed to fumes of formaldehyde gas liberated by adding formalin to finely ground crystals of potassium permanganate. The chicks' actions could be closely observed. Each chick had great difficulty in breathing, and

attempted to clear its nose and mouth by shaking its head. As the chick found it more difficult to breathe, it was noticed that a thick, sticky, mucous secretion issued from its mouth. This secretion was tested for presence of formaldehyde. First a color indicator was set up using a certain percentage of the indicator, Phloroglucinol (Merck's Reagent), and straight formaldehyde. The indicator, Phloroglucinol turns red upon coming in contact with formaldehyde. According to the reference book, the indicator shows a red color in dilutions up to 1 : 30,000, but in our tests it would show red only up to 1 : 10,000. No formaldehyde could be detected in the mucous secretion. The lungs were then removed and macerated with fine sand and alcohol and tested without detecting any trace of formaldehyde using the indicator. I also macerated the lungs by pressing them between two pieces of filter paper without finding any traces of formaldehyde. The windpipe was removed and alcohol was passed through it. This fluid was then tested after it ran out of the chick's mouth. This procedure showed faint signs of formaldehyde. A repetition of this procedure, using distilled water instead of alcohol, was unsuccessful.

To check under a more practical condition, twenty day-old chicks were fumigated in the recommended manner and upon removal from the hatching compartment were tested in all of the above mentioned ways without any signs of formaldehyde. At the conclusion of this work, it was decided that it was impossible to determine the amount of formaldehyde taken in by the chicks during fumigation period, using the procedures discussed.

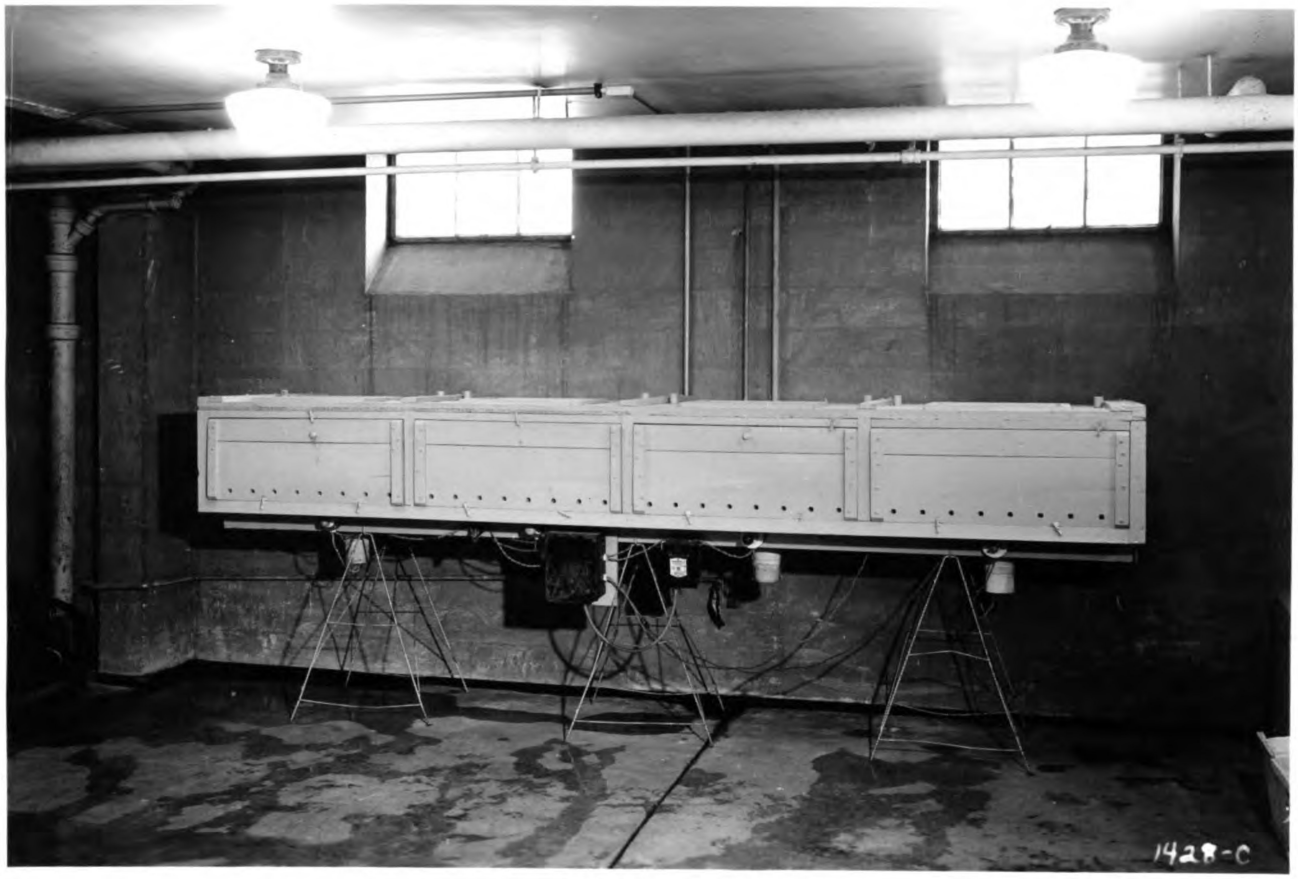

Fig. 2. Photograph of Experimental Brooder Showing Front View with Doors in Place.

THE LETHAL ACTION OF FORMALDEHYDE

In the next tests, it was decided to determine the lethal action of formaldehyde upon S. pullorum artificially infected chicks in both moist and dry condition.

To be assured that a known type of chick was being used, all chicks were hatched from a flock of Single Comb White Leghorns. These birds were blood tested and in some cases retested to be sure that all adult stock was pullorum-free. In these experiments the same section of a Jamesway incubator was used for hatching in order to prevent concurrent contamination. The hatching compartment of this section of the incubator had 7.35 cubic feet of air space with the following dimensions: 28.75 in. by 27.25 in. by 13.5 in.

One half of these chicks had the web on the right foot between the first and second toes cut for purpose of identification. These birds were called controls and were not inoculated with S. pullorum. The remaining 80 chicks were then inoculated with S. pullorum by swabbing the mouth and throat of each chick with a swab moistened with S. pullorum. After inoculation all birds were placed in separate hatching compartments on the same tray and fumigated. The amounts of formalin and potassium permanganate, the exposure periods, wet bulb and dry bulb readings varied in each experiment. After exposure all birds were placed in the experimental brooder in the following order:

Pen 1 - Twenty infected and twenty clean chicks

Pen 2 - Forty clean chicks

Pen 3 - Twenty infected chicks and twenty clean chicks

Pen 4 - Forty infected chicks

The brooder temperature for the first two days was set at 95°F. and then lowered to 85°F. for the remaining five days. Each brooder unit had an individual thermostatic control which regulated the temperature for each compartment. Each pen contained a 32 inch feeding tray and a water fountain. The floor was covered with about two inches of wood shavings. Prior to use, the brooders were cleaned and disinfected with iodine suspensoid.

After remaining in this brooder for seven days each group of chicks was placed in a battery brooder. These chicks were fed the Spartan Starter for the first three weeks and then gradually changed to the Spartan Grower for the remainder of the experiment.

Each chick, upon death, was checked bacteriologically by smearing portions of the heart, liver, and lung upon plates of brilliant green liver infusion agar and Eosin-methylene-blue (E.M.B.) agar. After 24 hours of incubation, colonies, appearing to be S. pullorum, were transferred to agar slants and later were planted in sucrose, lactose, dextrose, maltose, and mannite broth.

Until the cultures were made, the dead chicks were stored in a refrigerator. All cultures showing acid and gas in dextrose and mannite and no change in sucrose, lactose and maltose were reported as S. pullorum.

The first two trays containing 321 eggs were set in the Jamesway incubator on May 28, 1940. The first hatch of chicks was taken off on June 19. One hundred and sixty good strong chicks were selected for this series of experiments. During the fumigation period,

the temperature was set at 100°F. and the wet bulb reading was 85°F. giving a relative humidity of 54 per cent. On June 20, all the chicks in Pen 1 were found dead due to overheating. The thermostat became stuck allowing the heat to rise to 120°F. It was decided to carry on the remaining pens as if nothing had happened to the number one pen.

For brevity, fumigation will be referred to by dosages used. For example, a single, double, or triple dose of formalin and potassium permanganate was used for a certain time period. In each case the following is meant:

Single dose - 0.175 gm.* $KMnO_4$ and 0.35 cc.* formalin per cubic foot
or 1.35 gm. $KMnO_4$ and 2.7 cc. formalin for 7.74 cubic feet.

Double dose - 2.70 gm. $KMnO_4$ and 5.4 cc. formalin for 7.74 cubic feet.

Triple dose - 4.05 gm. $KMnO_4$ and 8.1 cc. formalin for 7.74 cubic feet.

*Amounts recommended by Robert Graham of University of Illinois, Circular No. 403.

Table II

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - June 19, 1940

Fumigation - Single dose for 10 minutes

Dry bulb reading - 100°F.)) Relative humidity 54 per cent
Wet bulb reading - 85°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		20 infected 20 uninfected		40 uninfected		20 infected 20 uninfected		40 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
6	Died			0	0	1	1	0	0
10	Died					1	0		
11	Died	Chicks all died from overheating				1	1		
16	Died							2	1
18	Died							2	2
20	Died					1	0	1	1
21	Died							1	0
22	Died			1	0				
27	Killed			39	0	36	0	34	0
Total				40	0	40	2	40	4

Table III

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - July 2, 1940

Fumigation - Single dose for 10 minutes

Dry bulb reading - 100°F.)
)Relative humidity 68 per cent
Wet bulb reading - 90°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		20 infected 20 uninfected		40 uninfected		20 infected 20 uninfected		40 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
9	Died					2	2		
10	Died							4	4
11	Died	Chicks all died from overheating				1	0		
12	Died			1	0	1	1	1	1
31	Killed			39	0	36	3	35	5
Total				40	0	40	6	40	10

Table IV

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - July 10, 1940

Fumigation - Single dose for 10 minutes

Dry bulb reading - 98°F.)
)Relative humidity 99+ per cent
Wet bulb reading - 98°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		20 infected 20 uninfected		40 uninfected		20 infected 20 uninfected		40 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
1	Died	1	1						
2	Died					1	0	1	1
3	Died	1	1					1	1
6	Died					2	1	2	2
9	Died					1	0		
29	Killed	38	0	40	0	36	0	36	0
Total		40	2	40	0	40	1	40	4

Table V

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - July 24, 1940

Fumigation - Single dose for 10 minutes

Dry bulb reading - 100°F.)
)Relative humidity 59 per cent
Wet bulb reading - 87°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		20 infected 20 uninfected		40 uninfected		20 infected 20 uninfected		40 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
1	Died					1	1	1	1
5	Died							1	1
6	Died					1	1		
8	Died							1	0
10	Died	3	3						
12	Died			1	0	1	1	2	2
13	Died			1	0	2	2	1	1
14	Died	1	1	1	0			1	1
21	Died					1	1		
22	Died					1	1	1	0
26	Died	1	0	1	0	1	0	1	0
55	Killed	35	0	36	0	32	0	31	0
Total		40	4	40	0	40	7	40	6

Table VI

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - August 1, 1940

Fumigation - Single dose for 20 minutes

Dry bulb reading - 100°F.)
)Relative humidity 83 per cent
Wet bulb reading - 95°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		20 infected 20 uninfected		40 uninfected		20 infected 20 uninfected		40 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
2	Died					2	2	4	3
4	Died	2	2			4	4	3	3
5	Died	1	0					1	1
6	Died	1	1						
8	Died	1	1						
9	Died					1	0	2	1
14	Died			1	0				
20	Died					1	1	1	0
21	Died							2	1
25	Died	1	0			2	0	3	0
34	Killed	34	1	39	0	30	0	24	0
Total		40	5	40	0	40	7	40	9

Table VII

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - September 12, 1940

Fumigation - Double dose for 10 minutes

Dry bulb reading - 99°F.)
)Relative humidity 96 per cent
Wet bulb reading - 97°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		15 infected 15 uninfected		30 uninfected		15 infected 15 uninfected		30 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
3	Died	1	1	1	0				
4	Died			1	0	5	5	2	2
5	Died	3	3					4	4
6	Died	1	1			2	2	1	1
7	Died							1	1
8	Died	2	2	1	0			2	2
12	Killed	23	3	27	0	23	7	20	4
Total		30	10	30	0	30	14	30	14

Table VIII

The Isolation of S. pullorum at Autopsy of Chicks Examined

Date of hatch - September 19, 1940

Fumigation - Double dose for 20 minutes

Dry bulb reading - 100°F.)
)Relative humidity 93 per cent
Wet bulb reading - 98°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks: Pen 1 (15 infected 15 uninfected) No.	Pen 1 No. infected	Pen 2 (30 uninfected) No.	Pen 2 No. infected	Pen 3 (15 infected 15 uninfected) No.	Pen 3 No. infected	Pen 4 (30 infected) No.	Pen 4 No. infected
4	Died					1	1	1	1
5	Died	4	4			6	6	4	4
8	Died	1	1			2	0		
9	Died							2	1
10	Died					2	2	2	2
16	Killed	25	0	30	0	19	0	21	1
Total		30	5	30	0	30	9	30	9

Table IX

The Isolation of *S. pullorum* at Autopsy of Chicks Examined

Date of hatch - September 26, 1940

Fumigation - Single dose for 20 minutes

Dry bulb reading - 98°F.)
)Relative humidity 73 per cent
Wet bulb reading - 90°F.)

Age of chick (days)	Mortality	Distribution of clean and infected chicks							
		Pen 1		Pen 2		Pen 3		Pen 4	
		10 infected 10 uninfected		20 uninfected		10 infected 10 uninfected		20 infected	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
4	Died					1	1	1	1
5	Died	3	3			1	1	4	4
6	Died					2	1	2	2
7	Died			1	0	1	0	1	1
9	Died	1	1	1	0	1	1		
10	Died	1	1						
11	Died	1	1	1	0	1	1	1	1
13	Died	1	0						
15	Killed	13	0	17	0	13	0	11	0
Total		20	6	20	0	20	5	20	9

To be more certain of the virulence of the culture, six strains of S. pullorum from autopsied birds were combined into a 12 hour culture. Ten cc. of this combination were injected into a White Rock male. Two days later this bird was bled and a pour plate made with citrated blood to recover the organism. Eight cc. of the recovered organism was injected intravenously and ten cc. injected subcutaneously into a second bird. Four days later this bird died and the organism was again recovered from the liver and spleen.

To check the virulence of this organism again, 24 day-old White Leghorn chicks were selected. Various amounts of culture were injected intraperitoneally.

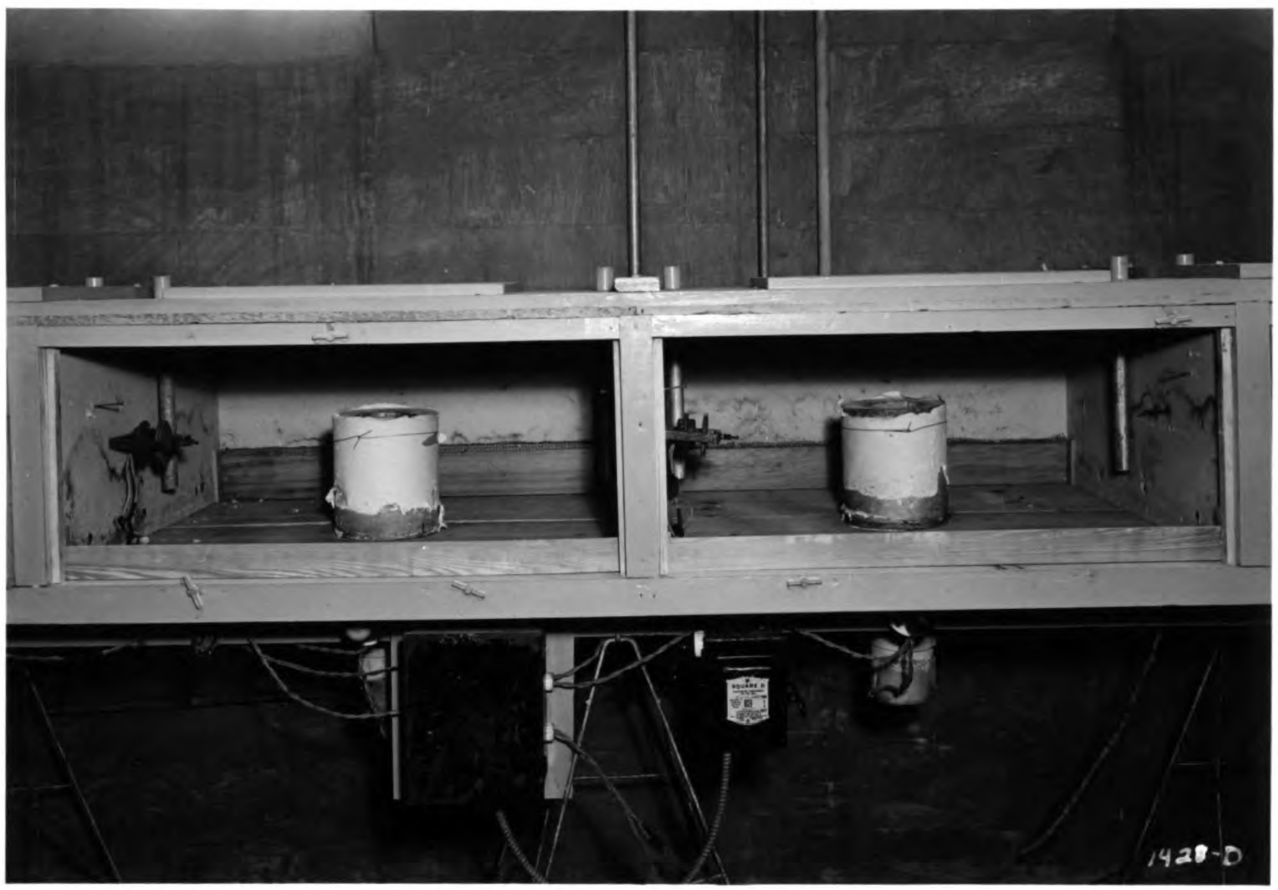

Fig. 3. Photograph of Experimental Brooder Showing Interior

Table X

Virulence of Culture as Determined by Injection into Day Old Chicks

Date of Experiment - September 5, 1940

Each chick injected intraperitoneally

Age of chick (Days)	Mortality	Group 1 Controls		Group 2 0.1 cc.		Group 3 0.5 cc.		Group 4 0.25 cc.	
		No.	No. infected	No.	No. infected	No.	No. infected	No.	No. infected
1	Died					1	1		
2	Died			2	2	3	3	2	2
3	Died			1	1	2	2	2	2
4	Died			1	1			2	2
5	Died			1	1				
6	Died			1	1				
7	Killed	6	0						
Total		6	0	6	6	6	6	6	6

Table XI

The Isolation of S. pullorum from Infected Chicks by Streaking Tissues on Plain Agar and E.M.B. Agar Plates After Fumigation

Date of Experiment - September 17, 1940

Dry bulb reading - 100°F.)
)Relative humidity 62 per cent
Wet bulb reading - 88°F.)

Group	Medium used	Dosage	Time minutes	Results
1	Plain agar	Single	10	- *
1 A	Plain agar	Single	10	+ **
2	E.M.B.	Double	10	-
2 A	E.M.B.	Double	10	+
3	Plain agar	Single	20	-
3 A	Plain agar	Single	20	+
4	E.M.B.	Double	20	-
4 A	E.M.B.	Double	20	+
5	Plain agar	Double	30	-
5 A	Plain agar	Double	30	+
6	E.M.B.	Triple	10	-
6 A	E.M.B.	Triple	10	+

* - S. pullorum not isolated.

** - S. pullorum isolated.

A - Controls

This experiment, the isolation of S. pullorum from infected chicks by streaking the tissues on plain and E.M.B. agar plates after fumigation, was repeated with the relative humidity readings of 66 and 68 per cents, in which results identical with those recorded in the preceding table were obtained.

Fig. 4. Photograph Showing Special Chamber Used in Fumigation Experiments.

Table XII

Germicidal Efficiency of Formaldehyde in Destroying
S. pullorum Streaked on E.M.B. Plates
From Broth and Slant Cultures

Date of Experiment - October 4, 1940

Dry bulb reading - 100°F.)
)Relative humidity 54 per cent
Wet bulb reading - 85°F.)

Group	Dosage	Time Minutes	Exposed			Controls		
			Plate	Broth	Slant	Plate	Broth	Slant
1	Single	10	1	N *	N	1	G**	G
			2	G	N	2	G	G
			3	N	N	3	G	G
			4	N	N	4	G	G
2	Double	10	1	N	G	1	G	G
			2	N	N	2	G	G
			3	N	N	3	G	G
			4	G	N	4	G	G
3	Single	20	1	N	N	1	G	G
			2	N	N	2	G	G
			3	N	N	3	G	G
			4	N	N	4	G	G
4	Double	20	1	N	N	1	G	G
			2	N	N	2	G	G
			3	N	N	3	G	G
			4	N	N	4	G	G
5	Double	30	1	N	N	1	G	G
			2	N	N	2	G	G
			3	N	N	3	G	G
			4	N	N	4	G	G
6	Triple	10	1	N	N	1	G	G
			2	N	N	2	G	G
			3	N	N	3	G	G
			4	N	N	4	G	G

* N - no pullorum growth

** G - pullorum growth

A fumigation chamber with the following inside dimensions was made: 24 inches wide, 23½ inches long, and 23 3/4 inches deep. This box was made of one-half inch plywood, fitted carefully and glued so as to make it air tight. See photograph Figure 4, page 30. The opening to this chamber was four inches square, fitted so that when clamped the chamber was air tight. This opening was on the front side located near the base of the box. The top of this chamber was made with a glass, four inches square, so that one could look into the box and observe the reaction of the chicks. This box was also equipped with an electric light bulb. The air space of this box was 7.74 cubic feet and it was on this figure that the amounts of formalin and potassium permanganate were calculated.

Beginning June 25, 1940, eggs from Single Comb White Leghorn hens were placed in the Jamesway incubator each week. The number of chicks varied each week depending on the hatchability of the eggs. Beginning October 7, 1940, the chicks on livability test were kept in a battery brooder. The fecal material was removed from the trays daily. The water troughs were scrubbed daily. In every case, precautions were taken to avoid the spread of the S. pullorum from one section of the brooder to another. The temperature, of each section, was kept constant in order to obtain the best growth possible. Every effort was made to maintain ideal conditions at all times.

The following experiments are quantitative and were performed in an attempt to prove that not all the organisms on the chicks are killed.

For brevity, fumigation will be referred by dosages used. For example, a single, double, or triple dose of formalin and potassium permanganate was used for a certain time period. In each case the following is meant:

Single dose - 0.175 gm.* $KMnO_4$ and 0.35 cc.* formalin per cubic foot
or 1.35 gm. $KMnO_4$ and 2.7 cc. formalin for 7.74 cubic feet

Double dose - 2.70 gm. $KMnO_4$ and 5.4 cc. formalin for 7.74 cubic feet

Triple dose - 4.05 gm. $KMnO_4$ and 8.1 cc. formalin for 7.74 cubic feet

* Amounts recommended by Robert Graham of University of Illinois, Circular No. 403.

EFFECT OF FUMIGATION UPON CHICKS IN INCUBATOR

This experiment was to determine the number of S. pullorum found on chicks after subjecting them to formaldehyde. The amounts of formaldehyde and exposure periods varied.

Eleven chicks were dipped into a suspension of S. pullorum. Chicks were dried and then fumigated in groups. The chicks were then dipped in sterile water after fumigation. The rinse waters were plated in brilliant green agar using one and five cc. amounts. All plates were incubated at 37° C. for 24 hours.

Table XIII

The Exposure of Dried Chicks to Formaldehyde

Date of Experiment - October 11, 1940

Group	Dosage	Time minutes	Chick	Dilutions	
				1 cc.	5 cc.
1	Single	10	1	P *	P
			2	P	P
			3	P	N **
			4	P	P
2	Double	20	1	N	P
			2	N	N
			3	P	P
			4	N	N
3	Control		1	P	P

* P - S. pullorum present.

** N - S. pullorum not present

Twenty chicks were dipped into a 24 hour culture of S. pullorum and without drying were then fumigated. After fumigation each chick was dipped into sterile water to wash off any organisms present and the usual dilutions were made. Plain agar was added to the plates and incubated at 37°C. for 24 hours.

Figures quoted refer to number of S. pullorum organisms isolated.

Table XIV

Number of Bacteria Removed from Formaldehyde Treated Chicks. Wet Before Exposure.

Date of Experiment - October 30, 1940

Group	Dosage	Time minutes	Chick	No. of bacteria per ml. of rinse water	Average Number of bacteria per chick
1	Single	10	1	0	
			2	7,500	
			3	37,500	
			4	37,500	20,625
2	Double	10	1	322,500	
			2	37,500	
			3	180,000	
			4	7,500	136,875
3	Single	20	1	15,000	
			2	7,500	
			3	45,000	
			4	37,500	26,250
4	Double	20	1	15,000	
			2	0	
			3	0	
			4	0	3,750
5	Controls		1	405,000	
			2	383,500	
			3	457,500	
			4	405,000	412,750

Twenty chicks were dipped, dried, fumigated, and the colonies on plates were counted at the end of 24 hours incubation.

Table XV

Number of Bacteria Removed from Formaldehyde Treated Chicks. Dried Before Exposure

Date of Experiment - November 6, 1940

Group	Dosage	Time minutes	Chick	No. of bacteria per ml. of rinse water	Average Number of bacteria per chick
1	Single	10	1	12,075,000	
			2	10,800,000	
			3	14,025,000	
			4	5,625,000	10,633,750
2	Single	20	1	9,075,000	
			2	8,775,000	
			3	11,325,000	
			4	1,200,000	7,593,750
3	Double	10	1	4,200,000	
			2	9,000,000	
			3	2,325,000	
			4	300,000	3,956,250
4	Double	20	1	30,750,000	
			2	8,130,000	
			3	13,275,000	
			4	13,950,000	16,526,250
5	Controls		1	607,500,000	
			2	618,975,000	
			3	19,050,000	
			4	125,550,000	342,768,750

Ten chicks were dipped in a suspension of S. pullorum, dried, and fumigated. After fumigation the chicks were rinsed in sterile water and then put into brooder to check the livability of the two groups.

Fig. 5. Photograph Showing Method of Dipping and Rinsing Chicks.

Table XVI

Number of Bacteria Removed from Formaldehyde Treated Chicks
Dried Before Exposure and the Viability of
Resulting Treated Chicks

Date of Experiment - November 8, 1940

Group	Dosage	Time minutes	Chick	Number of bacteria per ml. of rinse water	Average number of bacteria per chick	Viability Age of Chick (days)	
1	Single	10	1	517,000		Died	11
			2	285,000		Died*	4
			3	870,000	557,333	Died*	5
2	Single	20	1	1,402,500		Died*	1
			2	472,500		Died*	5
			3	690,000	855,000	Died*	5
3	Double	10	1	345,000		Died*	1
			2	892,500		Died*	2
			3	165,000	467,500	Died*	3
4	Controls		1	3,307,500	3,307,500	Died	4

* S. pullorum isolated at autopsy

Fifteen one-day old chicks were fumigated. After first dipping into a S. pullorum culture and drying, these chicks were then fumigated in groups.

Fig. 6. Photograph Showing Section of Jamesway Incubator Used for Experiments.

Table XVII

Number of Bacteria Removed from Formaldehyde Treated Chicks
Dried Before Exposure and the Viability of
Resulting Treated Chicks

Date of Experiment - November 25, 1940

Group	Dosage	Time minutes	Chick	Number of bacteria per ml. of rinse water	Average number of bacteria per chick	Viability Age of Chick	(days)
1	Single	10	1	877,500		Died	1
			2	907,500		Died*	2
			3	825,000	870,000	Died*	2
2	Single	20	1	1,342,500		Died*	2
			2	1,725,000		Died*	2
			3	1,222,500	1,430,000	Died*	3
3	Double	10	1	2,025,000		Died*	2
			2	592,500		Died*	2
			3	1,417,500	1,345,000	Died*	3
4	Double	20	1	975,000		Died*	2
			2	0		Died*	2
			3	9,075,000	3,350,000	Died*	2
5	Controls		1	13,500,000		Killed	5
			2	6,225,000		Killed	5
			3	1,095,000	6,940,000	Killed	5

* S. pullorum isolated at autopsy

Eighteen day-old chicks were dipped in a suspension of S. pullorum, dried thoroughly and then fumigated in groups of one to six. An additional group six was added in this experiment whereby the chicks were exposed to a triple dose of formalin for a time limit of ten minutes.

Fig. 7. Photograph Demonstrating Method of Streaking Chick Organs.

Table XVIII

Number of Bacteria Removed from Formaldehyde Treated Chicks Dried Before Exposure and the Viability of Resulting Treated Chicks

Date of Experiment - November 27, 1940

Group	Dosage	Time minutes	Chick	Number of bacteria per ml. of rinse water	Average number of bacteria per chick	Viability Age of Chick	(days)
1	Single	10	1	1,537,500		Died*	6
			2	1,305,000		Died*	7
			3	412,500	1,085,000	Killed	8
2	Single	20	1	3,630,000		Died*	1
			2	1,545,000		Died*	2
			3	1,470,000	2,215,000	Died*	2
3	Double	10	1	2,055,000		Died*	2
			2	2,565,000		Died*	2
			3	3,510,000	2,710,000	Died*	2
4	Double	20	1	1,057,500		Died*	2
			2	240,000		Died (Drowned)	5
			3	967,000	754,833	Died*	2
5	Controls		1	1,050,000		Killed	8
			2	1,252,500		Killed	8
			3	982,500	1,095,000	Killed	8
6	Triple	10	1	7,500		Died*	2
			2	165,000		Died*	2
			3	105,000	92,500	Died*	2

* S. pullorum isolated upon autopsy

Five groups of chicks were again fumigated. However, groups four and five had only two chicks each as this week's hatch was very small. Livability was again checked for each group.

Fig. 8. Photograph Before Battery Brooder Used in Experiments.

Table XVIX

Number of Bacteria Removed from Formaldehyde Treated Chicks
Dried Before Exposure and the Viability of
Resulting Treated Chicks

Date of Experiment - December 5, 1940

Group	Dosage	Time minutes	Chick	Number of bacteria per ml. of rinse water	Average number of bacteria per chick	Viability Age of Chick (days)	
1	Single	10	1	1,882,500		Died*	7
			2	2,392,500		Died*	5
			3	1,342,500	1,872,500	Died*	8
2	Single	20	1	810,000		Died*	3
			2	1,027,500		Died*	3
			3	435,000	757,500	Died*	3
3	Double	10	1	907,500		Died*	2
			2	517,500		Died*	2
			3	510,000	645,000	Died*	2
4	Double	20	1	472,000		Died*	1
			2	390,000	431,000	Died*	1
5	Controls		1	1,605,000		Died	4
			2	1,417,000	1,511,000	Killed	10

* S. pullorum isolated upon autopsy

Table XX

Number of Bacteria Removed from Formaldehyde Treated Chicks. Dried Before Exposure.

Summary of Tables XV to XIX inclusive

Group	Dosage	Time minutes	Chicks	No. of bacteria per ml. of rinse water	Average Number of bacteria per chick
1	Single	10	16	55,679,500	3,479,968
3	Double	10	16	31,327,500	1,957,968
2	Single	20	16	46,147,000	2,884,187
4	Double	20	12	79,281,500	6,606,791
5	Triple	10	3	277,500	92,500
6	Controls		13	1,401,506,500	107,808,190

Table XXI

Number of Bacteria Removed from Formaldehyde Treated Chicks. Wet Before Exposure.

Summary of Table XIV

Group	Dosage	Time minutes	Chicks	No. of bacteria per ml. of rinse water	Average Number of bacteria per chick
1	Single	10	4	82,500	20,625
3	Double	10	4	547,500	136,875
2	Single	20	4	105,000	26,250
4	Double	20	4	15,000	3,750
5	Controls		4	1,651,000	412,750

Summary

In every instance the treated chicks carried high counts on both the wet and dry chicks. Data show that although some organisms are removed, there are still enough organisms left to cause the disease.

Because only four chicks are used for each part of the experiment, it is very difficult to secure results which are very compatible on the quantitative basis. The results obtained from the difference in time period and dosages were variable but the trend shows that there is a slight diminution for the longer periods and stronger dosages as there is a decrease in the number of organisms over the controls. However, there are still enough organisms left on the chick to reinfect the chicks as shown by the death loss.

RESULTING VIABILITY OF FUMIGATED CHICKS

This experiment was carried on to determine resulting livability of chicks after exposing them to certain fumigation periods. At the beginning of these particular experiments, it was decided to fumigate these chicks in the specially built fumigation chamber, but it was finally decided to finish the series by fumigating in the hatching compartment of the Jamesway incubator. It was thought that the formaldehyde fumes were not as completely removed from the fumigation chamber after each fumigation period as readily as from the Jamesway incubator. Furthermore, the experiments run in this manner were more comparable to the conditions found in the field.

Each group of chicks was fumigated in the hatching compartment and subjected to various doses of formalin and potassium permanganate for various periods of time. All chicks were then put into the battery brooder and checked for livability.

Table XXII

Resulting Viability of Fumigated Chicks

Date of Experiment - October 30, 1940

Fumigation - Single dose for 10 minutes

Relative humidity - 77 per cent (Temp. 100°F. / Wet bulb 93°F.

Number of chicks used - 32

Age of chicks (days)	Group 1 Controls	Group 2 Fumigated
1	0 dead	4 dead
2	0 dead	2 dead
3	0 dead	1 dead
4	1 dead	0 dead
Totals	1 dead	7 dead
26	15 killed	9 killed

The remaining birds were killed November 25. Upon autopsy the nine birds fumigated showed congested lungs and unabsorbed egg yolks, while the controls appeared normal.

Thirty-two day-old chicks were divided into two groups of sixteen chicks each.

Table XXIII

Resulting Viability of Fumigated Chicks

Date of Experiment - November 13, 1940

Fumigation - Double dose for 10 minutes

Relative humidity - 54 per cent (Temp. 100°F. (Wet bulb 85°F.

Number of chicks used - 32

Age of chicks (days)	Group 1 Controls	Group 2 Fumigated
1	0 dead	1 dead
7	0 dead	1 dead
11	0 dead	1 dead
12	0 dead	1 dead
12	0 dead	4 dead
13	0 dead	2 dead
14	0 dead	1 dead
15	0 dead	0 dead
Totals	0 dead	11 dead
31	16 killed	5 killed

The remaining birds were killed on December 5. Sixteen controls and five fumigated chicks were autopsied. In each case the fumigated chicks showed congested lungs and unabsorbed egg yolks.

Table XXIV

Resulting Viability of Fumigated Chicks

Date of Experiment - November 20, 1940

Fumigation - Double dose for 20 minutes

Relative humidity - 78 per cent (Temp. 100°F. / Wet bulb 90°F.)

Number of chicks used - 26

Age of chicks (days)	Group 1 Controls	Group 2 Fumigated
1	0 dead	0 dead
2	0 dead	0 dead
3	0 dead	1 dead
4	0 dead	1 dead
5 - 20	0 dead	0 dead
Totals	0 dead	2 dead
30	13 killed	11 killed

The two chicks which died appeared normal when autopsied. The remaining chicks were killed and autopsied on December 24 and they appeared normal.

Table XXV

Resulting Viability of Fumigated Chicks

Date of Experiment - November 27, 1940

Fumigation - Triple dose for 10 minutes

Relative humidity - 83 per cent (Temp. 100°F. / Wet bulb 95°F.

Number of chicks used - 30

Age of chicks (days)	Group 1 Controls	Group 2 Fumigated
1	0 dead	0 dead
2	1 dead	2 dead
3	1 dead	0 dead
4	1 dead	3 dead
5	1 dead	1 dead
6	0 dead	1 dead
8	0 dead	1 dead
9	0 dead	1 dead
10	0 dead	2 dead
13	0 dead	1 dead
Totals	4 dead	12 dead
25	11 killed	3 killed

Upon autopsy, the three fumigated chicks showed lung congestion and unabsorbed egg yolk in each case while the control chicks appeared normal.

Another hatch of 20 chicks was divided into two groups of ten chicks each. Ten chicks were subjected to a single dose of formalin for twenty minutes. These chicks were put in the battery brooder to check comparative behavior.

Table XXVI

Resulting Viability of Fumigated Chicks

Date of Experiment - December 5, 1940

Fumigation - Single dose for 20 minutes

Relative humidity - 96 per cent (Temp. 100°F. (Wet bulb 99°F.

Number of chicks used - 20

Age of chicks (days)	Group 1 Controls	Group 2 Fumigated
1	0 dead	0 dead
2	0 dead	1 dead
3	1 dead	1 dead
4	0 dead	0 dead
5	0 dead	1 dead
6	0 dead	1 dead
7	0 dead	1 dead
Totals	1 dead	5 dead
26	9 killed	5 killed

The remaining chicks were killed and autopsied on December 30. All chicks appeared normal.

EFFECT OF FUMIGATION UPON EGG SHELLS, CHEESE CLOTH SQUARES AND COVER GLASSES

This experiment was carried on to study the effect of fumigation upon egg shells, cheese cloth squares and cover glasses. These experiments were similar to those in which chicks were used for fumigation trials, except that in this case egg shells, cheese cloth squares, and cover glasses were impregnated with S. pullorum after first being sterilized.

In this test, the cheese cloth squares, shells and glasses were dipped in the culture of S. pullorum and while still wet were fumigated in groups and then immediately washed in 100 cc. of sterile water. Appropriate dilutions were made and plain agar was poured onto the plates after which they were incubated at 37° C. for twenty-four hours.

All figures quoted refer to number of S. pullorum organisms isolated.

Table XXVII

The Number of Bacteria Removed from Formaldehyde Treated Egg Shells, Cheese Cloth Squares and Cover Glasses. Wet Before Exposure.

Date of Experiment - November 11, 1940

Group	Dosage	Time minutes	Medium	No. of bacteria per ml. of rinse water	Average
1	Single	10	Cloth	9,300,000	
			Shells	12,000,000	
			Glass	17,250,000	12,850,000
2	Double	10	Cloth	23,750,000	
			Shells	10,575,000	
			Glass	19,275,000	17,866,666
3	Single	20	Cloth	8,175,000	
			Shells	2,625,000	
			Glass	300,000	3,700,000
4	Double	20	Cloth	11,925,000	
			Shells	15,375,000	
			Glass	18,075,000	15,125,000
5	Controls		Cloth	31,800,000	
			Shells	65,325,000	
			Glass	12,900,000	36,675,000

In this test the egg shells, cheese cloth squares and cover glasses were dried after dipping into the S. pullorum culture.

Table XXVIII

The Number of Bacteria Removed from Formaldehyde Treated Egg Shells. Dried Before Exposure.

Date of Experiment - November 11, 1940

Group	Dosage	Time minutes	Egg shells	No. of bacteria per ml. of rinse water	Average
1	Single	10	1	877,500	
			2	75,000	
			3	225,000	392,500
2	Single	20	1	1,950,000	
			2	1,650,000	
			3	900,000	1,500,000
3	Double	10	1	8,925,000	
			2	7,200,000	
			3	1,425,000	5,800,000
4	Control		1	31,575,000	31,575,000

Table XXIX

The Number of Bacteria Removed from Formaldehyde Treated Cheese Cloth Squares. Dried Before Exposure.

Date of Experiment - November 11, 1940

Group	Dosage	Time minutes	Cloth squares	No. of bacteria per ml. of rinse water	Average
1	Single	10	1	0	
			2	255,000	
			3	975,000	410,000
2	Single	20	1	23,625,000	
			2	45,000,000	
			3	975,000	22,866,666
3	Double	10	1	1,102,500	
			2	127,000	
			3	67,500	423,333
4	Control		1	61,425,000	61,425,000

Table XXX

The Number of Bacteria Removed from Formaldehyde Treated Cover Glasses. Dried Before Exposure

Date of Experiment - November 11, 1940

Group	Dosage	Time minutes	Cover glasses	No. of bacteria per ml. of rinse water	Average
3	Double	10	1	1,417,500	
			2	3,307,500	
			3	1,400,000	2,041,666
4	Control		1	9,157,500	9,157,500

Table XXXI

The Number of Bacteria Removed from Formaldehyde Treated Egg Shells, Cheese Cloth Squares, and Cover Glasses. Dried Before Exposure

Summary of Tables XXVIII to XXX inclusive.

Group	Dosage	Time minutes	Medium	No. of bacteria per ml. of rinse water	Average
			Egg Shells		
1	Single	10	3	1,177,500	392,500
2	Single	20	3	4,500,000	1,500,000
3	Double	10	3	17,650,000	5,883,333
4	Control		1	31,575,000	31,575,000
			Cloth Squares		
1	Single	10	3	1,230,000	409,999
2	Single	20	3	79,600,000	26,533,333
3	Double	10	3	1,297,000	432,333
4	Control		1	61,425,000	61,425,000
			Cover Glasses		
3	Double	10	3	6,125,000	2,041,666
4	Control		1	9,157,500	9,157,500

Table XXXII

The Number of Bacteria Removed from Formaldehyde Treated Egg Shells, Cheese Cloth Squares and Cover Glasses. Wet Before Exposure.

Summary of Table XXVII.

Group	Dosage	Time minutes	Medium	No. of bacteria per ml. of rinse water
			Shells	
1	Single	10	1	12,000,000
2	Double	10	1	10,575,000
3	Single	20	1	2,625,000
4	Double	20	1	15,375,000
5	Controls		1	65,325,000
			Cloth Squares	
1	Single	10	1	9,300,000
2	Double	10	1	23,750,000
3	Single	20	1	8,175,000
4	Double	20	1	11,925,000
5	Controls		1	31,800,000
			Cover Glasses	
1	Single	10	1	17,250,000
2	Double	10	1	19,275,000
3	Single	20	1	300,000
4	Double	20	1	18,075,000
5	Controls		1	12,900,000

Summary

In this series, egg shells, cheese cloth squares, and glass cover slips were used instead of baby chicks. These data show that the fumigation lowers the bacterial count when compared with the controls. However, enough organisms are left to act as a possible source of danger to other chicks. The trend shows a diminution for the longer periods of time and stronger doses.

THE INCIDENCE OF S. PULLORUM IN FECAL MATERIAL AFTER FUMIGATION

The tetrathionate broth acts as an inhibitor for Escherichia coli. The feces were first weighed and then a suspension of S. pullorum was thoroughly mixed into the feces. A very thin film of the feces was spread over the bottom of Petri dishes and fumigated. After fumigation, the feces were removed and introduced into ten cc. of tetrathionate broth and allowed to incubate for eighteen hours at 37° C. Then a smear was made from the broth and streaked on freshly prepared MacConkey agar plates and again incubated at 37° C. for twenty-four hours. The plates were then read.

At the end of the first eighteen hours, the tetrathionate broth seemed to be very cloudy indicating that there was growth in every test tube. This broth was then transferred to MacConkey agar plates and at the end of a twenty-four hour incubation period, the fumigated plates showed no growth, while all plates had growth at the end of forty-eight hours. This growth was later demonstrated to be due to S. pullorum.

Table XXXIII

Incidence of S. pullorum in Fecal Material After Fumigation

Date of Experiment - December 3, 1940

Group	Dosage	Time minutes	Tube	Readings made from tetrathionate broth 24 hours	Readings made from MacConkey agar 48 hours
1	Single	10	1	No growth	+ *
			2	No growth	+
			3	No growth	+
2	Single	20	1	No growth	+
			2	No growth	+
			3	No growth	+
3	Double	10	1	No growth	+
			2	No growth	+
			3	No growth	+
4	Double	20	1	No growth	+
			2	No growth	+
			3	No growth	+
5	Controls		1	S. pullorum	+
			2	S. pullorum	+
			3	S. pullorum	+

* This growth later (December 5) was demonstrated to be due to S. pullorum.

Before completing the above series of experiments with the tetrathionate broth, another experiment was made testing the effect of formaldehyde gas upon the organism E. coli. I concluded that E. coli was so thoroughly dispersed in the fecal material that in order to kill this organism the fumigant had to be potent. It proved impossible to obtain a suspension of S. pullorum which was exactly comparable to that existing naturally as in the case of E. coli.

On November 18, fifteen one-gram samples of fresh chicken fecal material were carefully weighed out. Each sample was carefully spread over the bottom of sterile Petri dishes. These plates were then fumigated. Upon removal from the fumigation chamber, nine cc. of 0.85 per cent physiological salt solution was poured into the dish and the fecal material was mixed into the salt solution. From this mixture, dilutions of 1 : 10, 1 : 100, 1 : 1000 were made into a new medium, single strength lauryl sulphate tryptose lactose broth. This new broth is being used by Doctor Mallmann in checking water samples. E. coli is definitely shown to be present if gas is produced in this broth.

The tubes were examined at the end of twenty-three hours of incubation at 37° C.

Table XXXIV

Colon Index After Fumigation

Date of Experiment - November 18, 1940

Group	Dosage	Time minutes	Sample	Colon Index per cc.
1	Single	10	1	1000
			2	1000
			3	1000
2	Single	20	1	1000
			2	1000
			3	1000
3	Double	10	1	1000
			2	10
			3	1000
4	Double	20	1	1000
			2	1000
			3	1000
5	Control		1	1000
			2	1000
			3	1000

Only three tubes showed absence of gas production.

DISCUSSION

It has been demonstrated in the data submitted that fumigation with formaldehyde is definitely a marginal disinfection. One must remember that the dosages used must be exact and the type of material used must be constant, otherwise, ineffective fumigation would result. Fumigation as practiced by the hatcherymen is definitely a marginal disinfection practice.

Inasmuch as the organism (S. pullorum) emanates from the intestinal tract of the chick, discharges, after fumigation, will reinfect any area traveled by the chick. Thus, fumigation will only give temporary freedom from disease organisms in this area even though the fumigation process were 100 per cent effective. To effect the elimination of the organisms throughout the entire hatching period, it would be necessary to have continuous disinfection which is neither feasible nor practicable.

Even though some of the organisms are destroyed during the fumigation period, not all of the organisms are destroyed. Therefore, the value of fumigation with formaldehyde is limited.

It has been established that a dry formaldehyde gas has no germicidal action on dry bacteria. To become effective, the gas must be in solution. The data presented in this paper substantiate this theory.

Any fecal matter found in the hatching trays is in a dry state and the S. pullorum exists imbedded in the dry fecal matter. Because

formaldehyde has little penetrability, it is impossible to destroy these organisms by fumigation.

Chicks may be injured by the process of fumigation with formaldehyde even though the fumigation is carried out according to the recommended manner. This damage to the chicks may offset any benefit gained by destroying a few pullorum organisms. The damage to the chicks is clearly demonstrated by the mortality suffered during these experiments.

SUMMARY

The following results were secured when fumigation with formaldehyde was practiced in doses recommended by various authorities:

1. Fumigation with formaldehyde caused marked injury to the chicks as demonstrated by the mortality.

2. Fumigation with formaldehyde did not destroy S. pullorum on the infected chicks.

3. Fumigation with formaldehyde did not destroy S. pullorum on the infected chicks and allowed the disease to spread to the clean stock.

4. Fumigation with formaldehyde did not destroy S. pullorum in fecal matter.

5. Fumigation with formaldehyde did not destroy S. pullorum on infected egg shells, cheese cloth squares, and glass cover slips.

6. The greatest mortality from S. pullorum occurred about the sixth day although death may result any time between one and twenty-eight days.

CONCLUSION

1. Pullorum disease in incubators cannot be controlled merely by fumigation with formaldehyde.

2. If the eggs introduced into the incubator are free from pullorum disease and the incubator is free of any contamination, then fumigation with formaldehyde is unnecessary.

RECOMMENDATIONS

1. It is recommended that clean stock be used, that incubators be kept disease free by exercising care in preventing the introduction of diseased stock.

2. As a safety factor, terminal disinfection of the incubator should be practiced.

LITERATURE CITED

1. Townley, T. S.: Humidity in Incubators. Proc. 21st Ann. Meeting Poultry Sci. Assoc., 1929, 25, 56-60.
2. Moore, J. M., C. W. Upp, and W. P. Hinshaw: Studies in the Transmission of Bacillary White Diarrhea in Incubators. Jour. Amer. Vet. Med. Assoc. (N.S.), 1926, 21, 631-641.
3. Bunyea, H., and W. J. Hall: Transmission of Pullorum Disease in Incubators. Amer. Vet. Med. Assoc., 1930, 30, 245-255.
4. Hoffman: As cited by E. C. McCulloch in Disinfection and Sterilization. Lee and Febiger, 1936.
5. Low: As cited in the 1896 U. S. D. A. Yearbook of Agriculture.
6. Trillat: As cited in the 1896 U. S. D. A. Yearbook of Agriculture.
7. De Schweinitz, Chief of the U. S. D. A. in 1896: As cited in the 1896 U. S. D. A. Yearbook of Agriculture.
8. Miquel, Bandet, Trillat: As cited in the 1896 U. S. D. A. Yearbook of Agriculture.
9. Dorset, M.: Some Common Disinfectants. U. S. D. A. Farmers' Bull. 926.
10. Gwatkin, R.: Some Experiments on the Disinfection of Eggs and Incubators. Ontario Vet. Col., 1926, 58-65.

    _____ . The Disinfection of Incubators with Formaldehyde During Hatching. Biol. Absts., 1929, 5, 11221.
11. Dakan, E. L., and F. Speer: Sanitation in the Hatchery. Ohio State Univ. Ext. Bull. 90. 1929.

12. Mallmann, W. L., and J. M. Moore: Studies of Pullorum Disease. II. The Incidence of Salmonella Pullorum in Eggs from Infected Hens. Jour. Amer. Vet. Med. Assoc., 1936, LXXXIX (N.S.), 42, 1, 35-52.

———————— : Studies of Pullorum Disease. I. The Influence of Different Temperatures in Brooding. Jour. Amer. Vet. Med. Assoc., 1934, LXXXIV (N.S.), 37, 3, 525-536.

13. Graham, R., and W. M. Michael: Studies in Incubator Hygiene. Poultry Sci., 1932, XI, 2, 110-116.

———————— : Incubator Hygiene in Control of Pullorum Disease. Univ. Ill. Circ. 403.

———————— : Studies on Incubator Hygiene. III. Germicidal Properties of Formaldehyde. Poultry Sci., XV, 6, 492-493.

14. Bushnell, L. D., and L. F. Payne: Fumigation of Forced Draft Incubators. Jour. Amer. Vet. Med. Assoc., 1929, (N.S.), 28, 611-625.

———————— : Dissemination of Pullorum Disease in the Incubator. Kansas State Exper. Sta. Tech. Bull. 29. August 1931.

15. Winter, A. R.: Prevention and Control of Poultry Diseases. Ohio State Univ. Bull. 115. July 1936.

16. Bushnell, L. D., and C. A. Brandly: Poultry Diseases, Their Prevention and Control. Kansas State Agric. Exper. Sta. Bull. 247. June 1929.

17. Smith Incubator Company. T. S. Townley: Fumigation Directions. Incubator Operating Instructions.

18. Buckeye Incubator Company. P. W. Muth: Fumigation. Operation Instructions for Incubators.

20. Weisner, E. S.: Incubator Sanitation and Fumigation. Michigan State Col. Ext. Mimeograph Brief. 1940.

21. Carus Chemical Company, LaSalle, Illinois: Manufacturers of Potassium Permanganate. 1940.

22. Scott, H. M., and L. F. Payne: Further Studies on Dissemination of Salmonella Pullorum Infection in Incubators. Jour. Amer. Vet. Med. Assoc., 1928, (N.S.), 25, 599-610.

23. McCulloch, E. C.: Disinfection and Sterilization. Lee and Febiger, 1936.

24. Tilley and Schaeffer: As cited in McCulloch's Disinfection and Sterilization.

REFERENCES

1. Alp, H. H.: Keeping the Farm Flock Healthy. Univ. Illinois Circ. 374.

2. Barger, E. H., and L. E. Card: Diseases and Parasites of Poultry. Lee and Febiger, Phil., 1935, 98-104.

3. Blount, W. P. - Chanticleer: Disinfection of Incubators. Int. Rev. Poultry Sci., 1933, Tome 6, 67.

4. Boyd, J. L.: Find Disinfectants Slightly Affect Eggs. Michigan Agric. Exper. Sta. Quart. Bull. 10, 175-178. 1928.

5. Braune, H. and R. Lenke: The Viscosity of Gases and Vapors. A. Physic. Chem. Abts. A 148, 195-215.

6. Buckley, J. S., H. Bunyea, and E. B. Cram: Diseases and Parasites of Poultry. U. S. D. A. Farmers' Bull. 1652. Revised May 1939.

7. Bunyea, H.: Use of the Rapid Whole-Blood Test for Pullorum Disease. U. S. D. A. Misc. Publ. 349. June 1939.

8. Chandler, S. C.: Fumigation. Textbook, Animal Parasites, 1918, 383.

9. Christianson, H.: Fumigation of Hen Houses. Farm and Dairy, 1919, 38, 1427.

10. Coon, C. J.: Some Experiments in Disinfecting Incubators with Formaldehyde. Jour. Amer. Vet. Med. Assoc., 1928, (N.S.), 25, 627-630.

11. Dorset, M.: Some Common Disinfectants. U. S. D. A. Farmers' Bull. 926.

12. Dougherty, J. E.: Artificial Incubation of Eggs. California Agric. Ext. Serv. Circ. 19. November 1930.

13. Durant, A. J.: White Diarrhea in Chickens. Univ. Missouri Col. Agric. Ext. Circ. 85, 4. 1920.

14. Effect of Composition of Air on the Growth and Mortality of Chick Embryos. Colorado Bull. 257 A. November 1925.

15. Graham, R., and W. M. Michael: Studies on Incubator Hygiene 7. Results of Fumigation of Salmonella Pullorum in Incubators at Full Capacity. Poultry Sci., 1936, XV, 6, 492-493.

16. Graham, R., and W. M. Michael: Studies on Incubator Hygiene 8. The Resistance of Salmonella Aertycke, Salmonella Anatum, and Salmonella Pullorum from Quail to Formaldehyde Fumigation. Poultry Sci., 1936, XV, 6, 494-495.

17. Graham, R.: Incubator Fumigation Lessons Pullorum Disease. The Baby Chick, February 1932. Int. Rev. Poultry Sci. Tome 5, 89.

18. Graham, R.: Pullorum Disease of Chicks. Univ. Illinois Circ. 432. March 1935.

19. Graham, R.: Pullorum Disease Control with Special Reference to Incubator Disseminated Diseases. Vet. Alumni Quart. Ohio State Univ. 20, 1933, 177-188, No. 4. Int. Rev. Poultry Sci. Tome 6, 1932, 111.

20. Graham, R., and E. H. Barger: Studies in Incubator Hygiene 4. Note on Germicidial Effect of Formaldehyde on Fowl Pox Virus. Poultry Sci., 1936, XV, 1, 48-52.

21. Graham, R., and V. M. Michael: Studies in Incubator Fumigation. Poultry Sci., 1931, X, 7, 388.

22. Graham, R., and V. M. Michael: Studies in Incubator Hygiene 1. Poultry Sci., 1932, XI, 2, 110-116.

23. Graham, R.: Pullorum Disease in Chicks. Univ. Illinois, Circ. 432.

24. Graham, R., and V. M. Michael: Incubator Hygiene in Control of Pullorum Disease. Univ. Illinois Circ. 403.

25. Jull, M. A.: Mortality Problem; Embryo Mortality. U. S. Egg and Poultry Mag., 40, 28-31. July 1934.

26. Hinshaw, W. R.: Studies in Transmission of Bacillary White Diarrhea in Incubators. Amer. Vet. Med. Assoc. Jour., 1926, 5, 631-641.

27. King, D. F.: The Prevention of the Dissemination of Salmonella Pullorum in Forced Draft Incubators. Thesis for Master of Science degree, Kansas State College, 1929.

28. Koch, H. J.: Disinfectants and Fumigants. Chem. Engineer. Mining Rev., 1931, 23, 270-273.

29. Marcellus, F. N.: Incubator Disinfection in Control of Salmonella Pullorum. Proc. 4th World's Poultry Congress, 1930, 401-407.

30. McClintic, T. B.: The Limitations of Formaldehyde Gas as a Disinfectant. Hygienic Lab. Bull. 27, 112. 1906.

31. Murphy, R. R.: Study of the Control of the Dissemination of Salmonella Pullorum in Forced Draft Incubators. Thesis for Master of Science degree, Kansas State College, 1930.

32. Posen, N. A., and L. V. Dieter: Disinfection with Formaldehyde. Jour. Ind. and Engineer. Chem., 1919, 11, 448-451.

33. Purdue Univ. Ext. Bull. 101: Incubation. February 1927.

34. Townsley, T. S.: Humidity in Incubators. Proc. 21st Ann. Meeting Poultry Sci. Assoc., 1929, 25, 56-60.

35. Shepard, H. H.: The Chemistry and Toxicology of Insecticides. Formaldehyde. Burgess Publishing Company. 344-358.

36. Tennessee Bull. 136: Incubation. February 1926.

37. Vickers, G.: Hatchery Management. Ohio State Ext. Bull.

38. Wehner, C.: Disinfection of Incubators. Int. Rev. Poultry Sci., 1932, 4, 4, 13.