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DISSEMINATION OF PULLORUM DISEASE IN THE INCUBATOR

SUMMARY

Formaldehyde fumigation, regardless of whether all ports are closed or left open, will satisfactorily sterilize incubators of the forced-draft type. With a temperature of 99 to 100°F. and a wet-bulb reading of approximately 90° F., 0.35 c.c. formalin liberated by 0.175 gm. potassium permanganate per cubic foot of space, kills practically all exposed *S. pullorum* organisms within five minutes after the formaldehyde has been liberated. Other writers find that it is necessary to increase this to 0.40 c.c. of formalin and 0.20 gm. of potassium permanganate for uniformly successful results.

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. In addition, a relative humidity above 50 per cent largely prevents the formation of paraformaldehyde when the incubator is operated at 99 to 100° F.

Three fumigations of hatching eggs with 0.35 c.c. formalin per cubic foot for one hour each at weekly intervals, with all ports closed did not injure the hatchability of the eggs.

Continuous fumigation with the pan method of evaporating the formaldehyde was found impractical. The minimum lethal dose of formaldehyde liberated by the hot-plate method was found to be between 60 and 70 c.c. formalin per hour in an incubator with 100 cubic feet of air capacity.

Experiments dealing with formaldehyde fumigation show that a forced-draft incubator can be sterilized successfully without injuring the hatchability of the eggs. Other experiments, however, reveal that when the chicks are left in the incubator for 36 hours, it is not feasible to keep the machine sterilized either by continuous fumigation or too frequent discontinuous fumigation without injuring the hatching chicks.

Chicks subjected to one 10-minute exposure of formaldehyde liberated from 0.35 C.C. of formalin added to 0.175 gm. potassium permanganate per cubic foot air space with a wet-bulb reading of 90°F., are apparently not injured. Under field conditions, with incubators not so tightly constructed, it is recommended that 0.40 c.c. of formalin and 0.20 gm. of potassium permanganate be used per cubic foot of air space.

Chicks in a Buckeye No. 9 incubator fumigated with formaldehyde liberated by the hot-plate method at the rate of 60 c.c. of formalin per hour for 8½ hours, and 55 c.c. formalin per hour for 36 hours were severely injured. Likewise, chicks fumigated by means of the formalin-potassium permanganate method with 40 c.c. of formalin every 1½ hours for 36 hours and with 40 c.c. of formalin every three hours for 36 hours were either killed or mere seriously injured.

In determining the minimum lethal dose of formaldehyde gas by the formalin-potassium permanganate method in the Smith incubator, it was found that the dose per volume was approximately the same as for the Buckeye machine.

Relative humidity was found to have a very decided effect upon checking the circulation of chick down. Maintaining a wet-bulb reading of 90 to 95° F. in the incubator resulted in a decided decrease in the number of circulating particles of material as compared to a wet-bulb reading of 83° F. or below.

From the results obtained by other laboratories the use of formaldehyde fumigation at intervals of 12 hours is effective in reducing the spread of pullorum disease in the incubator. This amount of treatment does not appear to injure the chicks for short periods of exposure. This should be applied at a wet-bulb reading of 88 to 90° F. Most of the gas escapes in a few minutes and the incubator will be freed of gas in an hour, or the formaldehyde may be removed in a few minutes by ventilation or with ammonia. It is necessary to mix the gas thoroughly with the air of the incubator before it reaches the chicks.

When proper precautions are used to remove most of the circulating down and dust from the incubator very satisfactory results will be obtained by fumigation.

The question of humidity alone is also of great practical importance. The exact mode of operation to obtain the best results will differ in different incubators. The satisfactory temperature for the wet- and dry-bulb thermometers must be determined by experiment. Evidently a wet-bulb reading of between 88 and 90° F. and a dry-bulb reading of 98 to 100° F. will cover the extreme ranges which should be used during the hatch.

High humidity reduces the amount of chick down, dust, and bacteria floating in the air of the machine. The nearer saturated the atmosphere the smaller the amount of such circulation. The highest relative humidity consistent with the production of the maximum hatch of high-quality chicks, without injury to the incubator, should be used during the hatching period or from the nineteenth to twenty-first days of incubation. A wet-bulb reading of 80 to 85° F. gives good results for the remainder of the incubation period.

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DISSEMINATION OF PULLORUM DISEASE IN THE INCUBATOR[1]

L. D. Bushnell and L. F. Payne

INTRODUCTION

Experiments to determine the spread and control of pullorum disease through the incubator were started at this station in 1926 and continued to 1930. The purpose of this bulletin is to review the work that has been done on this subject here and elsewhere and to draw such conclusions as seem justified from the results. It should be stated in the beginning that it is the opinion of the authors that the only way to completely control the disease is to eliminate the reactor birds from the flock by means of the agglutination test. However, the data which follow show that the spread of infection from diseased to healthy chicks within the incubator can be materially reduced by fumigation and high humidity during the hatch and such operations may be justified until other control measures can be instituted.

INCUBATORS AS A SOURCE OF INFECTION

In 1926 Hinshaw, Upp, and Moore (9) began a study of the bacteriology of incubator air. Up to the time of the publication of their report (1926), there was no published information on that point, although Weaver (30) reported similar results the same year. Rettger, Kirkpatrick, and Stoneburn (23), Durant (6), Steiner (26), and others had suggested the incubator as a possible source of the pullorum disease, but had advanced no experimental proof that this was an important factor in disease dissemination. Hinshaw *et al.* (9) conducted experimental investigations on this point and found that after thoroughly cleaning and disinfecting an incubator, very few organisms were present in the air. However, as the chicks began to hatch, the number of organisms increased rapidly until the chicks were removed. The organisms responsible for this increase during hatching were largely of two fecal types, For this reason, it seemed logical to assume that *Salmonella pullorum,* the cause of pullorum disease (bacillary white diarrhea) might also increase in numbers and hence be spread from chick to chick in the incubator. Attempts to isolate this or-

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ganism from the air of machines in which infected chicks were hatching failed. Later attempts to isolate it from artificially contaminated machines were successful.

To prove definitely that the *Salmonella pullorum* organism could be disseminated through the air of the incubator, some chick down was obtained, sterilized, and artificially contaminated with broth cultures of the pullorum organism. This material was air-dried and placed on one of the lower trays of a forced-draft type incubator. There were about 100 chicks beginning to hatch in trays below where the down was placed. There were also nine chicks in the compartment in the opposite end of the incubator. The chicks were allowed to remain in the hatching tray for 38 hours after the down was introduced. They were then placed in brooders and all that died were subjected to careful autopsy and cultures were made from the heart's blood, liver, and yolk. The experiment was continued for 14 days after which the remaining chicks were killed and blood samples taken for agglutination tests. Experiments were also made on the dissemination of the disease by artificially infecting the eggs as they were pipped by injecting cultures around the chick in the shell. In the above experiments 16.6 per cent of the exposed chicks died of pullorum disease.

In a later series of experiments by Hinshaw, Scott, and Payne (10), chicks were used from a flock of birds containing carriers of the pullorum organism as determined by the agglutination reaction. The summary of the results obtained are shown in Table I.

TABLE I.—SUMMARY OF MORTALITY OF EXPOSED CHICKS.

Group	Hatches	Number of chicks	Total mortality	Died, pullorum disease		Died, other causes	
				Number	Per cent	Number	Per cent
I	8	400	175	147	36.8	28	7.0
II	8	441	114	82	18.6	32	7.3
III	8	637	48	0	0	48	7.5
IV	8	603	216	181	30.0	36	6.0

Group I. Chicks hatched from eggs of normal birds incubated on trays adjacent to those from reacting birds.

Group II. Chicks hatched from normal eggs incubated on trays in the opposite end of the incubator from those of reacting hens.

Group III. Chicks hatched from the eggs of non-reactor hens. A separate incubator was used for these chicks. (Controls.)

Group IV. Chicks hatched from eggs of a reactor flock. Hatched in same machine as groups I and II.

It is quite evident that there is a marked difference between the number of deaths from pullorum disease among the chicks hatched from the eggs of reactor birds and exposed chicks as compared to those from eggs of a flock which was free from the disease and not exposed (Group III). In this instance, no losses whatever occurred in two of the eight hatches. There was an average loss from the disease of 36.8 per cent of the hatch when adjoining trays contained infected eggs, and 18.5

per cent in the chicks hatched when infected eggs were hatched in the opposite end of the machine. The control hatch showed no losses from pullorum disease, but an average mortality from all other causes of about 7.5 per cent. Only chicks from which cultures of *S. pullorum* were isolated and identified were listed as having died of the disease.

All unhatched eggs and dead embryos from this work were subjected to bacteriological examination with results shown in Table II.

TABLE II.—SUMMARY OF BACTERIOLOGICAL EXAMINATION OF EGGS.

Group	Hatches	Eggs examined	Eggs infected with *S. pollorum*	
			Number	Per cent
I	8	131	0	0.0
II	8	130	0	.0
III	8	168	0	.0
IV	8	871	91	10.4

These results indicate that the flock of hens supplying the eggs from Groups I, II, and III were entirely free of pullorum disease.

An examination was made of 354 eggs laid by 21 infected hens and 23.1 per cent were found to contain the organism. The infected eggs laid over this period varied from 3.6 to 100 per cent for different birds. These results emphasize the point that the removal of 99 per cent of all reactors in a flock may not prove effective in eliminating the disease.

Scott, Hinshaw, and Payne (25) found that the average mortality in a 14-day brooding period for 18 groups (1,162 chicks) infected with pullorum disease was 42.6 per cent. The average for 14 groups (919 chicks) not infected with the disease was 6.3 per cent. The mortality varied from 17.3 to 91.5 per cent in the diseased groups. Mortality commenced earlier, reached a higher peak, and continued for a greater length of time in diseased than in normal chicks.

Bunyea and Hall (2) presented a review of their work on pullorum disease in 1929. They studied the transmission of the disease in four types of incubators, two of the still-air type and two of the agitated or forced-draft type. These machines were operated under the best known conditions. The results obtained with a few hatches under hens were also included. The hatchability with incubators was 82 per cent for the normal eggs and 75 per cent for the eggs from bacillary white diarrhea infected stock, compared with a hatchability under hens of 72 per cent and 61 per cent, respectively.

The chicks were brooded for two weeks and all chicks that died during that time were examined and studied bacteriologically. The results are included in Table III.

The mortality from pullorum disease among the exposed,

compared to the non-exposed chicks, is quite evident and substantiates the earlier experiments of Hinshaw and his associates.

Bunyea and Hall report that there was transmission of the disease in all types of incubators. The table shows a much higher transfer in the agitated-air machines than in the still-

TABLE III.—TRANSMISSION OF PULLORUM DISEASE IN AGITATED AIR AND IN STILL AIR TYPES OF INCUBATORS.
(Adapted from Bunyea & Hall.)

	Per cent hatch			Per cent mortality			Per cent pullorum transmission		
	N.C.	N.E.	B.W.D.	N.C.	N.E.	B.W.D.	N.C.	N.E.	B.W.D.
Agitated air..	73	69	67	5	26	53	0	78	87
Still air.....	69	62	53	11	17	63	0	49	88

N. C. designates normal eggs or chicks used as controls;
N. E., normal eggs or chicks exposed to the disease;
B. W. D., eggs or chicks from parents infected with pullorum disease.

air machines. This is to be expected due to the rapid and complete circulation of the air in the former. The losses in chicks from the pullorum diseased stock is about the same in both types of incubators.

These writers made a bacteriological examination of chicks dead in the shell, and report the isolation of *S. pullorum* from 1.5 per cent of the controls and from 51 per cent of the eggs from the diseased stock. Hinshaw, Upp, and Moore (9) used 111 control chicks and lost one in their first experiment. Hinshaw, Scott, and Payne (10) examined 429 eggs from their controls and did not find one to be infected with the pullorum organism. It is to be remembered that these eggs were from a flock of hens which contained no reactors to the agglutination test in the 1 to 20 dilution. A total of more than 2,000 eggs and chicks were examined from this flock during a period of six years and in but one case was the pullorum organism isolated from an egg and that was during the first test made. This is fairly conclusive evidence that a flock may be made free of this disease and kept free if certain conditions are observed.

All writers are now agreed that the disease-producing organism is easily transferred from infected to healthy chicks in the incubator at the time of hatching.

SUSCEPTIBLE AGE OF CHICKS TO PULLORUM INFECTION

The importance of the incubator in the dissemination of pullorum disease is due to the great susceptibility of newly hatched chicks. Rettger, Kirkpatrick, and Stoneburn (23) present Table IV to illustrate this point.

For many years the mode of transfer of pullorum disease

was not adequately explained. Jones (12) was able to isolate the pullorum organism from the inner shell membrane of eggs containing dead embryos and suggested incubator infection of chicks following the picking at shells, infected droppings, etc. The organism placed on the shell of eggs in the incubator did not survive for three weeks. However, the present day practice

TABLE IV.—COMPARATIVE SUSCEPTIBILITY TO PULLORUM DISEASE OF CHICKS INFECTED AT DIFFERENT AGES.

Pen No.	Description	Per cent mortality
1 (26 chicks)	Not infected	11.5
2 (26 chicks)	Infected when 36 hours old	33.5
3 (26 chicks)	Infected when 60 hours old	23.3
4 (26 chicks)	Infected when 84 hours old	19.2
5 (26 chicks)	Infected when 108 hours old	7.7

of hatching twice a week or oftener does not allow sufficient time for the organisms to die between hatches. At the same time the picking at infected shell is of little importance compared to the distribution of this organism through the air.

Although the question of incubator contamination is of some importance, the most important factor is the contami-

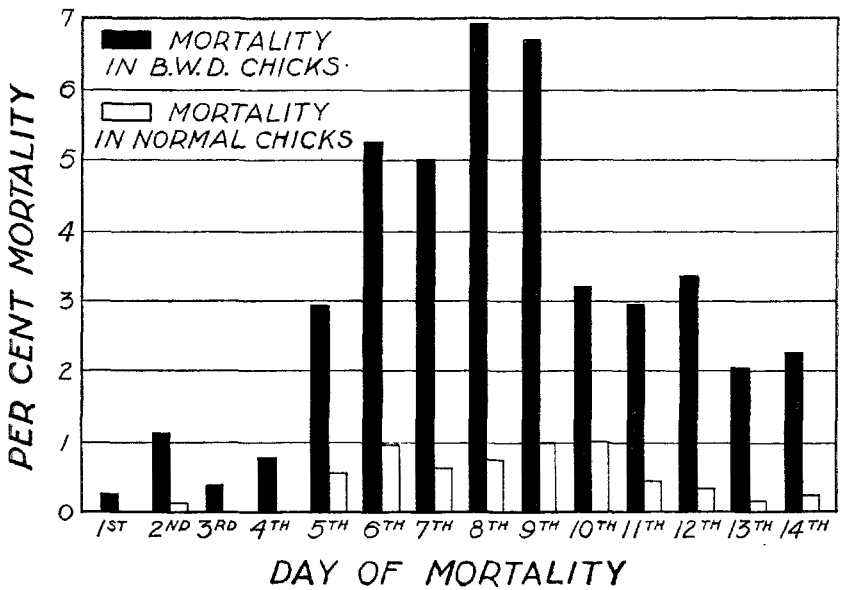

FIG.—Graph showing distribution of mortality over a 14-day period for chicks infected with pullorum disease in the incubator, compared with the same distribution of mortality for normal chicks.

nation arising from freshly hatched chicks from infected eggs. Such chicks are bathed in a suspension of the pullorum organism. As the chick dries and the down is thrown into the air,

it is inhaled by healthy chicks, and these in turn become diseased. Organisms have been isolated from the mouth, back, breast, and intestinal tract of such chicks. Fortunately, such chicks are usually not numerous but the few which do hatch are exceedingly dangerous to the remaining chicks. A conservative estimate indicates that one such chick may infect from 30 to 50 other chicks in the same incubator. These in turn will infect others by contaminating the feed and drinking utensils, so that it is quite possible for one infected chick to cause infection in from 50 to 100 other chicks during the first month of life.

Scott, Hinshaw, and Payne (25) present data from which the distribution of mortality of normal chicks compared to chicks infected with pullorum disease was obtained. (Fig. 1.)

It will be noted that the peak of the deaths is between the sixth and ninth day. It is a common observation that chicks may die of this disease until about three or four weeks of age. Many chicks which continue to live are in a weakened and unthrifty condition for long periods and some of these mature and become carriers of the organism.

A review of the literature indicated, beyond a doubt, that pullorum disease might be transmitted through the air of the incubator. Such being the case, it was felt that some steps should be taken to devise means of controlling transmission of the disease in the incubator.

OUTLINE OF EXPERIMENTS TO CONTROL THE SPREAD OF PUG LORUM DISEASE IN THE INCUBATOR

When the mode of dissemination of the disease became known, experiments were started to find methods for its control. It is a well recognized fact that the ultimate complete eradication of the disease will not be accomplished by preventing its spread through the incubator. The eradication will depend on the destruction of the disease at its source, that is, the carrier hen. However, until such time as a comprehensive testing campaign can be instituted, any methods which will reduce its spread to healthy chicks will be of value.

FUMIGATION OF INCUBATORS

The first experimental work in this connection was with formaldehyde. Various products had been recommended for spraying incubators but these were not considered satisfactory for treating machines containing eggs or chicks.

Pernot (21) investigated the mortality of incubator chicks in 1908 and recommended the use of formaldehyde gas for fumigation, using 0.465 c.c. of formalin and 0.189 gram of potassium permanganate crystals for each cubic foot of space. This method proved highly effective in killing organisms, but injured the hatchability in some cases.

Gwatkin (7) (8) reported on a series of experiments in which he was able to successfully fumigate an incubator six or eight times during hatches without apparent injury to the hatchability of the eggs. He found that 2 c.c. of formalin and 0.666 gram of potassium permanganate for each cubic foot of space were necessary to kill *S. pullorum*. In addition, he found it necessary to seal the still-air type of incubator and leave it closed for an hour.

Coon (4) found that 0.25 c.c. of formalin and 0.125 gram of potassium permanganate crystals per cubic foot of a forced-draft incubator killed *S. pullorum* cultures in one hour. He did not find it necessary to seal the machine during the operation.

Dakan and Speer (5) reported on the use of formaldehyde in the incubator. They recommended 0.40 c.c. of commercial formalin and 0.20 gram potassium permanganate crystals for each cubic foot of incubator space. They also recommended a wet-bulb reading of 90° F. The first fumigation was applied when about 10 per cent of the chicks were out of the shell. Fumigation was applied again after 12 hours. Chicks were removed as soon as possible after the second treatment. Again after 12-hour intervals, the remaining chicks were treated and removed from the incubator as soon as they were dry.

Bushnell, Payne, and Coon (3) reported experiments on the control of the infection of chicks in the incubator. Their first work was to attempt, to find a satisfactory method of applying formaldehyde. With a temperature of 99 to 100° F. and a wet-bulb reading of about 90° F., it was found that 0.35 c.c. of formalin and 0.175 gram of potassium permanganate for each cubic foot of space killed all exposed *S. pullorum* organisms within five minutes. It was not found necessary to close the machine during the operation. Chicks fumigated for long periods of time with large doses were severely injured. This treatment was not successful in incubators having a low wet-bulb reading.

Marcellus, Gwatkin, and Glover (18) conducted a series of experiments using sterilized egg shells contaminated with a fresh culture suspension of *A. pullorum*. This material was placed in wide-mouthed two-ounce sterilized glass bottles and covered with a "moderately thick" layer of absorbent cotton. They found that the 1.5 c.c. of formalin and 1 gram of potassium permanganate per cubic foot of space in 15 minutes exposure with ports open were effective in killing the organisms. Smaller amounts were not entirely effective. The temperature by the dry-bulb thermometer was increased 2° F. and that of the wet-bulb from 4 to 14° F. depending on the amount of ingredients used. This soon returned to normal after the fumigation. If disinfection were applied between the 24 and 96 hours of incubation embryonic mortality was increased even with the lowest concentration which proved effective with an exposure of one hour. No harmful results were observed in

chicks gassed while pipping the shell. Later gassing from three to five hours produced heavy mortality. They recommend that disinfection of incubators should not be practiced during the period of embryonic life between the twenty-fourth and ninety-sixth hours.

Townsley (27) found that with a high degree of humidity in the air, forced-draft type incubators can be effectively fumigated with formaldehyde gas. He found a wet-bulb reading of 90° F. to be most satisfactory for this purpose. In recent literature sent to the users of the Smith incubators he recommends fumigation of incubators each 12 hours during the time of the hatch. The first fumigation should be given within 12 hours after the first chick hatches and subsequent treatments should be administered each 12 hours until the hatch is finished.

When the chicks hatch rapidly, so that only two fumigations are necessary, it is not advisable to remove the early-hatched chicks before the second fumigation is applied. In case of delayed hatches where three fumigations are necessary it may pay to remove the dry chicks just before the second and third application of gas.

For the Senior Smith 250 c.c. of formalin and 140 grams of potassium Permanganate and for the Junior Smith 150 c.c. of formalin and 85 grams of potassium permanganate are recommended.

Wildman and Freiberg (31) conducted a long series of fumigation experiments with the Buckeye incubator. A part of their summary and conclusions are given here.

They found that 40 c.c. of formalin and 20 grams of potassium permanganate per 100 cu. ft. was the least amount which could be relied upon to consistently kill dried out *S. pullorum* cultures in an incubator with a wet-bulb reading of 85° F. and a 10-minute exposure. Test cultures were not killed by 50 c.c. formalin and 25 grams of permanganate in 10 minutes with a wet-bulb reading of less than 80° F. Hatching chicks could not be subjected to these or even two fumigations with 40 c.c. of formalin and 20 grams of permanganate at 12-hour intervals with a wet bulb of 85° F., without appreciable loss. An increase of humidity above a wet-bulb reading of 85° F. rendered the fumigant more effective in killing the bacteria and also prevented rapid drying of chicks, thereby enabling the fumigant to kill more of the bacteria on the chick's down before it is released to circulate in the air. Among hatching chicks subjected to a single fumigation of 10 minutes, with 40 c.c. of formalin and 20 grams of permanganate, at a wet-bulb temperature of 83 to 85° F., there was a slight dissemination of the infection. When the amount of formalin was increased to 45 c.c. and the permanganate to 22.5 grams, there was slight injury to the chicks but no dissemination of the infection. With the latter amount of fumigant and a wet-bulb reading of

91 to 95° F. the chicks were not injured and there was no dissemination of infection through the air of the incubator. Although chicks were not injured by using 20 c.c. of formalin and 10 grams of permanganate at wet-bulb readings of 93 to 95° F. for seven exposures at six-hour intervals, there was some dissemination of the infection and the method is not recommended.

These writers concluded that when the proper conditions are maintained the loss from pullorum disease due to its dissemination through the air of the incubator can be reduced so low as to be almost negligible. They recommend that the humidity should be kept as high as is consistent with good hatching practice during the hatching and fumigating period. The best results can be obtained with a wet bulb about 90° F. For the No. 9 machine, they recommend 40 c.c. (1.35 fluid ounces) of formalin and 20 grams (0.7 ounce) of potassium permanganate crystals. This mixture is placed on the fan board near the fan. After fumigation for 10 minutes, the machine should be aired for five minutes. The chicks should then be removed from the incubator.

Data are also included on the effect of the amount of moisture on the organisms when they are exposed to the fumigant. They were not able to duplicate, exactly, the results of the Kansas laboratory with the air-dried material. They were able to destroy *A. pullorum* cultures when exposed in a moist condition with the amount of fumigant which the Kansas station recommended, but were unable to do so when the material was dried to a point at which moisture was no longer visible. Such irregular results are probably to be expected when the experimental conditions are so difficult to control and may be due to a number of factors such as the amount of moisture on the organisms and in the air, the irregular distribution of the fumigant, the number of organisms exposed, etc. Cultures of the colon and streptococcus types gave results quite similar to those obtained with *A. pullorum.*

Wildman (32) reported on the use of 40 c.c. of formalin and 20 grams of potassium permanganate per 100 cubic feet of incubator space with a wet-bulb reading of 84 to 85° F. Chicks were exposed to 10 minutes at three different periods: the first when about 10 per cent of the chicks were out of the shell and the next two exposures at 12-hour intervals thereafter. Practically all the chicks were hatched at the time of the third fumigation. There was a mortality of 15.1 per cent among 2,535 chicks from non-reacting hens at the end of the second week, while of 2,428 chicks from reactor hens, 23.6 per cent had died at that time. This treatment was considered to be too severe since an examination of the trachea of the dead chicks showed a swollen and inflamed condition of the larynx, trachea, and bronchi.

The writer also gives data on the results obtained by removing the chicks after each fumigation. Of the 2,391 chicks from non-reactors so treated, there was a mortality of 3.5 per cent as compared to a mortality of 15.9 per cent from the reactor flock. It is reported that this treatment did not completely prevent the crossing over of the disease in the incubator. This conclusion seems scarcely justified, since the control flock was not entirely free of pullorum disease. The flock had been tested and the reactors removed but there were still some losses from pullorum disease among control chicks as shown by the data.

A comparison was made between chick losses in tested and in non-tested flocks with the results shown in Table V.

TABLE V.—LOSSES FROM TESTED AND FROM NON-TESTED FLOCKS.

	Number of chicks	Percent mortality at the end of		
		First week	Second week	Third week
Tested hens.	4,089	7.8	2.6	3.0
Non-tested hens. . .	5,480	8.8	19.4	25.1

There was a difference of 21.6 per cent livability in favor of the tested flock. The hatchability of the eggs was not affected by fumigation since egg-for-egg settings of non-fumigated eggs gave no higher per cent hatch than those subjected to repeated fumigations. Chicks subjected to one exposure did not appear to suffer permanent injury.

Graham and Michaels report that 20 c.c. of formalin per 100 cubic feet of incubator space evaporated from cheesecloth killed *S. pullorum* in an agitated-air incubator in less than one hour when the wet-bulb reading was 90° F. or more. This was as effective as the potassium permanganate-formalin method using 35 c.c. formalin and 17.5 grams of potassium permanganate crystals. The fumigation caused temporary distress, but the mortality traceable to fumigation was not significant. Newly-hatched chicks fumigated by the cheesecloth method (two releases at 12-hour intervals) appeared less affected by the fumigant than chicks fumigated three times by the potassium permanganate method. Less than 50 per cent of the amount of formalin was required in two releases by the cheesecloth method compared to the three of the potassium permanganate method.[2]

Although the cheesecloth method of applying formaldehyde requires less material, it requires a longer period of exposure to be effective. Earlier experimental results led the authors to conclude that a short intense fumigation was less injurious to

2. Graham, Robert, and Michaels, V. M, Studies in Incubator Fumigation. Abstracts and notes of papers presented at the 23rd Annual Meeting of Poultry Science Association. University of Kentucky (Lexington), August 10 to 12, 1931.

chicks than a continuous method. However, considerably larger amounts of material were used.

EXPERIMENTAL DATA

The points considered in the experimental work are: (1) The type of disinfectant and its methods of application; (2) the organisms used and the condition under which they were exposed; (3) the influence of relative humidity on disinfection.

The Disinfectant (Formaldehyde)

It was realized at the outset that to obtain successful results under practical conditions it would be necessary to employ a gaseous disinfectant since the presence of eggs in the incubator from the beginning to the end of the hatching season excluded the use of a liquid disinfectant. Likewise, the very rapid exchange of air in the forced-draft machine makes it necessary to use rather large doses in order to kill the organism quickly. Of all the known gaseous disinfectants, formaldehyde seemed to be the most promising. It is highly germicidal to microorganisms and comparatively non-poisonous to higher forms of animal life. It is easily applied in the form of a gas and the high temperature and high humidity of the incubator atmosphere greatly increases its toxic action. Formaldehyde was first obtained by Hoffman (11) in 1866. He made it by passing vapors of methyl alcohol, laden with air, over a heated platinum coil. In 1886, Loew and Fischer (16) discovered that formaldehyde possessed powerful antiseptic properties. Since then, the observations on the germicidal action of formaldehyde have been investigated by many others. As a result, this substance is used on a large scale as a disinfectant. Walter (29) in 1896 called attention to the confusion which had arisen in the literature of that time concerning the use of the terms formaldehyde and formalin, the latter being a mixture of formaldehyde gas in water (approximately a 40 per cent solution).

Formalin may be vaporized by applying heat. The early methods consisted in boiling over a flame, later other methods of freeing the gaseous formaldehyde were developed. Among the latter is the use of potassium permanganate. The chemical reaction resulting from a mixing of these compounds causes the formaldehyde to be given off in the form of a gas. Some formic acid and methyl alcohol may also be formed. It is estimated that under the most favorable conditions 80 to 90 per cent of the formaldehyde present is liberated by this method. In the presence of moisture the gas is a highly germicidal product. In a dry atmosphere it is relatively ineffective.

The relative efficiency of mixing formalin and potassium permanganate in varying proportions has been studied by numerous writers. McClintic (17) recommended about the proportion of 1 c.c. of the former to 0.5 gm. of the latter. Rideal

and Rideal (24) found 40 gm. of permanganate to 100 c.c. of formalin to be effective. Several writers quoted by Rideal and Rideal expressed the opinion that 1 c.c. of formalin to 0.5 gm. of permanganate is best. Posen and Dieter (22) submitted a large number of chemical methods to an exhaustive bacteriological study and came to the conclusion that under the proper conditions of operation the formaldehyde-permanganate method was effective, and recommended the following quantities for sterilizing each 1,000 cubic feet of room space: Formalin, 1 pint; potassium permangante, 0.5 pound.

Coon (4) found a slight excess of permanganate over that recommended by McClintic to give a slightly more rapid and complete vaporization of the formalin, unless the vessel containing the mixture was placed in a large volume of cold water, in which case the mixture was cooled so that complete vaporization of all formalin was not attained.

The superiority of formaldehyde depends upon its high value as a germicide and the ease with which it may be applied. The secret of successful fumigation of the incubator, especially while chicks are present, lies in the freeing of large volumes of gas in a short time. This is necessary because of the highly irritating action of the fumes on mucous membranes and the rapid exchange of air in the incubator. An attempt is thus made to supply an amount of gas which will be highly germicidal and at the same time relatively non-poisonous to the chicks. With the formalin-permanganate method, the reaction is over in about five minutes, or about the time required for successful fumigation.

Formaldehyde exists in three well recognized isomeric states:

1. Formaldehyde (HCOH) is a gas at ordinary temperatures. It is colorless and possesses slight odor, but is very irritating to the mucous membranes of the nose and conjunctiva.

2. Paraformaldehyde $(CHOH)_2$ is a white substance soluble in both warm water and alcohol, and consists chemically of two molecules of formaldehyde. It is this substance which is supposed to be present in formalin, formol, etc.

3. Metaformaldehyde "trioxymethylene" $(CHOH)_3$ is formed by the union of three or more molecules of formaldehyde. It is a white powder giving off a strong odor of the gas, and is but slightly soluble in alcohol and water.

Formaldehyde gas is of the same specific gravity as air, hence they do not mix rapidly. Formaldehyde combines chemically with nitrogenous organic compounds in such a way as to change their chemical nature. Added to egg albuminum it will prevent its coagulation by heat and will render gelatin and glue insoluble in water.

The commercial solutions of formaldehyde known as formalin, formol, etc., are supposed to contain 40 per cent of for-

TABLE VI.—AGGLUTINATION REACTION AND AUTOPSY FINDINGS.

Leg band No.	Reaction					B.W.D. lesions	*S. pullorum* isolated
	Test 2-1	Test 3-2	Test 4-3	Test 6-28	Test 9-27		
1	160	320	160	(*a*) 25R	160	+	(*b*) Gall.
2	160	160—	160	25R	160	—	—
3	40	Slight	25R	25R	20	+	+
4	160	160	320	(*c*) —	—	—	—
5	640	320	320	—	640	+	+
6	640	640	1280	25R	—	—	—
7	160	160	160	25R	160	—	—
8	40	40	160	25R	80	?	(*d*) Colon
9	320	160+	320	25R	40	+	+
10	160	160	320	25R	640	+	0
11	160	80+	Died 3-11	—	—	—	—
12	640+	320+	Died 3-13	—	—	—	—
13	160	320	40	25R	80	+	Gall.
14	160	80+	320	25R	160	—	—
15	—	—	25R	—	—	—	—
16	40	80	25R	25R	80	+	+
17	320+	160+	160	—	—	—	—
18	320	320	640	25R	160	+	+
19	320	640	320+	25R	160	+	Colon
20	160	160	320	25R	320	+	+
21	160	160	320	25R	160	+	+
22	80	160	320	25R	640	+	+
23	80	80	80	—	—	—	—
24	40	40	25R	25R	—	—	—
25	—	—	25R	25R	0	0	0
26	640	640	Died 4-16	—	—	—	—
27	80	80	—	—	—	—	—
28	40	80	25R	—	—	—	—
29	160	320	160	25R	320	—	+
30	320	160	80	—	—	—	—
31	160	320	40	25R	20	+	(*e*) Cont.
32	640	640	1280	Died 8-30	—	—	—
33	160	160	160	25R	320	+	Colon
34	40	40	25R	25R	0	+	0
35	80	40+	Died 3-21	—	—	—	—
36	80	80	25R	25R	0	0	0
37	320	320	80	25R	160	+	0
38	40	Slight	Died 3-22	—	—	—	—
39	40	40	80	—	—	—	—
40	80	80	Died 7-28	—	—	—	—
41	40	40	Died 3-23	—	—	—	—
42	—	—	1280	Died 9-5	—	—	—
43	80	80	40	25R	40	0	0
44	320	320+	640	25R	640	+	+
45	80	80	—	25R	80	+	+
46	80	40+	40	—	160	+	+
47	80	80	320	—	—	—	—
48	—	—	40	—	—	—	—
49	80	160	80	—	—	—	—
50	40	160	—	—	—	—	—
51	40	40	—	—	—	—	—
52	20	80	80	25R	160	+	+
53	160	320	1280	—	—	—	—
54	80	80	25R	25R	40	+	+
55	160+	320	320	25R	40	?	0
56	640	320+	160	25R	320	+	Cont.
57	320	320	80	25R	80	+	+
58	160	160	80	25R	40	+	+
59	640	1280	—	25R	320	+	+
60	640	640	640	Died	—	—	—
61	80	40	—	—	20	0	0
62	320	320	1280	25R	160	+	0
63	—	—	40	25R	80	+	Colon
64	80	160	40	25R	320	+	+
65	320	320	320	25R	160	?	0
66	160	160	40	25R	160	+	+
67	640	320	Died 3-17	—	—	—	—
68	—	—	25R	25R	20	?	0
69	640	640	80	Died	—	—	—
70	80	160	80	Died 6-4	—	—	—
71	160	160	Died 8-15	—	—	—	—
72	—	—	640	25R	—	—	—
73	160	160	80	25R	320	+	+
74	80	40+	40	25R	80	?	0
75	160	320	80	25R	80	+	+
76	160	160	80	25R	80	+	+
77	40	80	25R	25R	40	+	+

TABLE VI.—(Concluded.)

Leg band No.	Reaction					B.W.D. lesions	*S. pullorum* isolated
	Test 2-1	Test 3-2	Test 4-3	Test 6-28	Test 9-27		
78	80	40+	40	25R	160	+	+
79	40	40	40	25R	40	+	Colon
80	160	160	160	25R	80	+	0
81	80	80	160	25R	320	—	—
82	640	640+	1280	25R	320	+	+
83	320	320	640	Died Aug.	—	—	—
84	160	80	25R	25R	40	0	0
85	40	80	40	25R	40	0	0
86	40	40	25R	25R	20	?	0
87	80	160	40	Died	—	—	—
88	160	160	80	25R	40	+	+
89	640	640	1280	25R	640	+	0
90	320	320	160	25R	640	—	—
91	80	80	40	25R	40	+	—
92	320	320	Died 3-21	—	—	—	—
93	160	160	40	25R	160	—	—
94	320	640	160	—	160	?	Colon
95	160	160	Died 3-17	—	—	—	—
96	320	320	80	—	—	—	—
97	160	320	160	25R	640	—	+
98	160	160	25R	25R	40	?	Cont.
99	—	—	25R	25R	0	0	0

(a) 1:25 dilution rapid method agglutination test.
(b) Gall.= *S. gallinarum.*
(c) Several birds were removed from the flock by persons unknown.
(d) Colon, some member of the colon group.
(e) Cont. Contaminated.

maldehyde gas. The gas content, however, is not constant, especially after exposure to the air. When the solution becomes cold there is a strong polymerization and precipitation of insoluble "trioxymethylene." Commercial solutions of formalin are usually acid in reaction due to the presence of formic acid. They usually contain some methyl alcohol which has been added to increase the solubility and stability. Commercial formalin is usually regarded as a solution of the formaldehyde gas in water. However, the gas is condensed in order to dissolve it and is believed to exist in one of its polymeric states, so that the solution known as formalin is probably principally dissolved paraformaldehyde. For this reason, the simple heating of a solution does not always result in driving off the gas, but may simply evaporate the water and deposit solid paraform, thereby not accomplishing the desired end.

The temperature of the room is an important factor in the disinfecting action of formaldehyde. The gas condenses to solid paraform at low temperatures. Therefore, fumigation should never be attempted below 50° F. and the maximum efficiency is not reached until above 80° F. An increase in temperature greatly aids its disinfecting action.

A certain amount of moisture is essential to obtain a successful gaseous disinfection, and although there is no such thing as a dry formaldehyde gas, the higher the moisture content the greater the germicidal action. Efforts made to produce a dry gas result in its conversion to a solid polymer. In atmospheres below 75 per cent moisture, formaldehyde has little

effect and it is only in atmospheres completely saturated that the maximum effect is obtained.

Formaldehyde gas cannot be depended upon to accomplish much more than surface disinfection except on long periods of exposure. In the fumigation of fabrics it has been noted that the fibers around the meshes tend to polymerize the gas and deposit it as paraform on the surface. Also, large amounts of the gas are lost by uniting chemically with organic matter and thus preventing its penetration.

Bacteria exposed directly to the action of formaldehyde gas are killed almost immediately. Even spores are killed by exposure for an hour or more. Circulating formaldehyde gas is much more effective in its penetrating action than gas not in circulation. The ready solubility of the gas in water causes the moisture in a room to become saturated within a short period of time. In the forced-draft type of incubator the gas reaches all parts of the equipment in a few seconds.

The action of formaldehyde may be neutralized by addition of ammonia. Half the molecular equivalent of aqua ammonia driven into the enclosure will neutralize the formaldehyde present by the formation of hexamethylene tetramine.

Exposing the Organism to Formaldehyde

The organisnis in the incubator were exposed by replacing the hatching door with a strip of beaver board through which some three-fourths inch holes had been bored. These holes were fitted with rubber stoppers carrying stiff wire loops about a foot in length. Wire screen was stretched across the stiff wires of the loops, thus forming small wire basket carriers in which the contaminated material could be exposed inside the machine. The tight-fitting stoppers prevented the escape of the fumigant and the wires held the contaminated material in such a position as to be exposed on all sides to the action of the gas. At the same time, sampling was easy without loss of large amounts of the disinfectant.

The cultures used throughout were typical for *Salmonella pullorum* and *Pasteurella avicida*. They were grown on standard meat infusion agar for 24 hours, suspended in physiological salt solution and poured over small bits of sterile gauze, filter paper, egg shells, or pads of chick down wrapped in a single layer of gauze. All contaminated material was air dried in an incubator before being used. At intervals during fumigation some of the contaminated material was removed from the machine and placed in tubes of nutrient broth. In order to avoid any antiseptic action on the samples by disinfectant which may have been transferred to the broth tubes, a small amount of the broth was transferred to another tube. All tubes were incubated at 37° C. for several days. If no growth occurred the sample

TABLE VII.—FREQUENCY WITH WHICH REACTOR HENS LAID EGGS INFECTED WITH S. PULLORUM.

Hen No.	Days non-infected eggs were laid	Days infected eggs were laid	Total examined	Total infected
1	2-26, 28; 3-9		3	0
2	2-15, 17, 19, 22, 24, 26, 28; 3-1, 8, 12		10	0
3	3-8, 10		2	0
5	3-10		1	0
6	2-25; 3-12	2-24, 26; 3-1, 9, 10, 15	8	6
7	2-22, 25, 27; 3-7, 9, 11	3-15	7	1
8	3-24		1	0
9	2-15, 17, 20, 21, 24, 26; 3-3, 6, 14	2-27	10	1
13	2-19, 20; 3-9	2-12	4	1
14	2-15, 18, 19, 20, 21, 24, 26; 3-1, 6, 11, 13	2-16, 23; 3-4, 10, 15	16	5
17	3-7, 13	3-8, 10	4	2
18	2-28; 3-2, 3, 5, 6, 13	3-12	7	1
20	3-4, 8		2	0
21	2-21, 23, 25, 26, 28; 3-1, 13	3-8	8	1
22	2-25, 28; 3-2, 5, 6, 11, 12, 15		8	0
23	2-25, 27, 28; 3-2, 6, 11, 13		7	0
24	2-25, 26, 27; 3-1, 6, 12, 13, 15		8	0
26	2-18, 20, 25, 26, 28		5	0
27	2-15, 17, 18, 19, 23, 24, 26, 27, 28		9	0
29	2-20, 23, 24, 26, 27; 3-6, 10	3-1, 9, 14	10	3
31	2-21, 24, 26, 28; 3-3, 5, 9, 11		8	0
32	3-14	3-15	2	1
33	2-15, 17, 20, 21, 24, 26, 27; 3-1, 6, 8, 12	2-23	12	1
34	3-2, 3, 11	2-28; 3-12	5	2
35	2-23, 25; 3-2, 7, 9	2-24, 26, 27; 3-1, 12, 13	11	6
36	2-27; 3-4, 12		3	0
37	3-15	2-27	2	1
39	2-21, 23, 25, 27; 3-3	3-10	6	1
41	2-28; 3-2, 5, 7, 11		5	0
48	2-26; 3-1, 3, 9, 11	2-25	6	1
49	2-24, 25, 27, 28; 3-11		5	0
50	2-16, 18, 19, 21, 23, 24, 25, 26, 27; 3-3, 5, 9		12	0
51	2-25, 26; 3-2	2-28	4	1
52	2-25, 27, 28; 3-11, 13		5	0
53	2-21, 23, 24, 25; 3-4, 5, 6	2-28; 3-10, 11	10	3
55	3-7, 13, 14	2-11, 14	5	2
57	2-15, 17, 18, 20, 21, 23, 24, 26, 27, 28; 3-1, 2, 5, 6, 7, 12, 14, 15	3-3	19	1
60	2-21, 24, 25, 26, 27; 3-13	2-22; 3-8, 14	9	3
61	2-18, 20, 24, 25, 27, 28; 3-1		7	0
64	2-24, 26, 28; 3-2		4	0
65	2-24, 25, 26, 27, 28; 3-1, 8, 9	3-13	9	1
66	2-16, 17, 21, 24, 25, 27, 28; 3-2, 3, 5, 6		11	0
68	3-1	3-10	2	1
70	2-20, 24, 26, 27; 3-1, 8, 12	2-21, 28	9	2
71	2-15, 18, 19, 20, 23, 24, 25, 27; 3-2, 6	2-28; 3-8, 14	13	3
77	3-3, 10	3-9	3	1
78	2-22, 25		2	0
79	2-25, 26; 3-11		3	0
80	2-23, 25, 26, 27; 3-2, 3, 4, 9, 11, 12	3-8	11	1
82	2-20, 22, 23, 25, 27; 3-12, 13		7	0
83	2-17, 18, 20, 21, 22, 25, 27, 28; 3-1, 4, 9, 11, 13	3-15	14	1
84	2-15, 18, 20, 21, 22, 25, 26, 27, 28; 3-1, 2		11	0
85	2-15, 17, 18, 20, 24, 25, 27, 28; 3-1		9	0
86	2-16, 17, 20, 24, 25, 28; 3-1, 3		8	0
87	3-6	3-23, 24	3	2
88	2-15, 18, 20, 24, 25, 26, 28; 3-1, 5		9	0
89	2-21, 23, 25, 27; 3-1	2-28; 3-10	7	2
97		2-24, 28; 3-10	3	3
		Grand total.	404	60

was considered to be sterile. Control cultures were always made on unfumigated material to see that the organisms were still viable when exposed.

Experimental Flocks

Eggs for this work were from two sources. A flock of 83 birds on the college farm which had been carefully tested for

several years and from which no chicks had died of pullorum disease furnished the controls. All the birds of this flock reacted negative to the agglutination test and were considered to be entirely free of the disease. None of the eggs which were examined contained *S. pullorum* and none of the chicks died of pullorum disease during the experiment unless exposed after hatching. The flock was blood tested annually for four seasons before eggs were used.

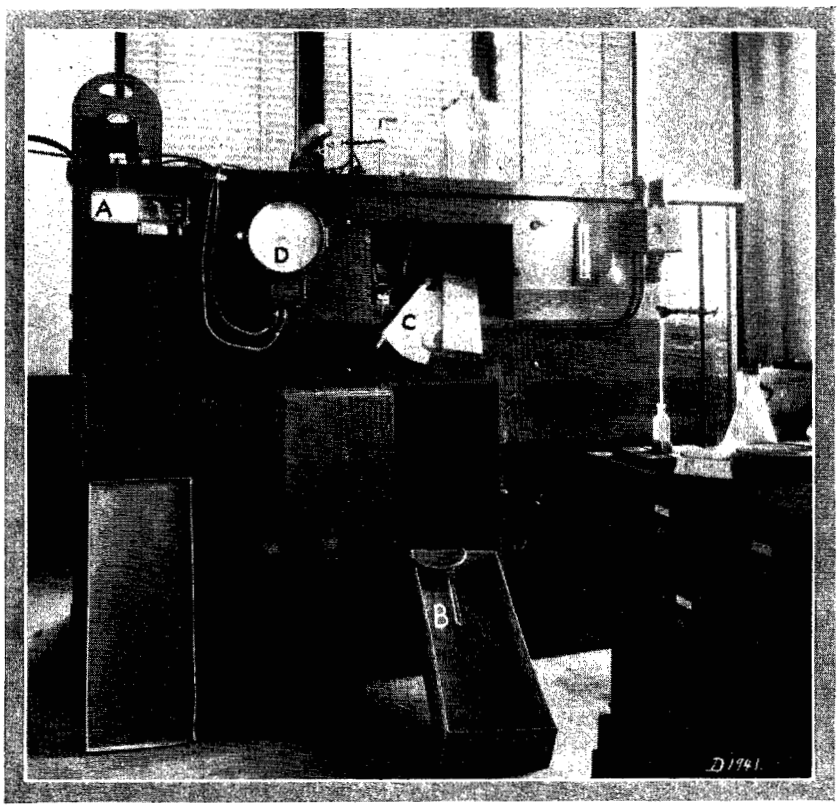

FIG. 2.—Experimental Buckeye incubator. (A) Mercoid switch for controlling humidity. (B) The electric water heater. (C) Wet bulb for regulating humidity. (D) Bi-record recording thermometer. (This is not standard equipment.)

A flock of Rhode Island Reds all of which reacted positively to the agglutination test in dilutions of 1 to 40 or above were used for carriers. They were trapnested for about a month. Practically all the unincubated eggs and all incubated eggs which did not hatch were examined bacteriologically for *S. pullorum*. This was done in order to find if the reactors were actually carriers of the organism. Table VI shows the reactions and post mortem findings of the reactor flock.

To begin with there were 99 birds in this flock. From February to September, 16 of them died, two were removed because of injury, and 18 were removed for other reasons. Table VI shows the agglutination titre over a period of several weeks. One of the points to be noted especially is that the agglutina-

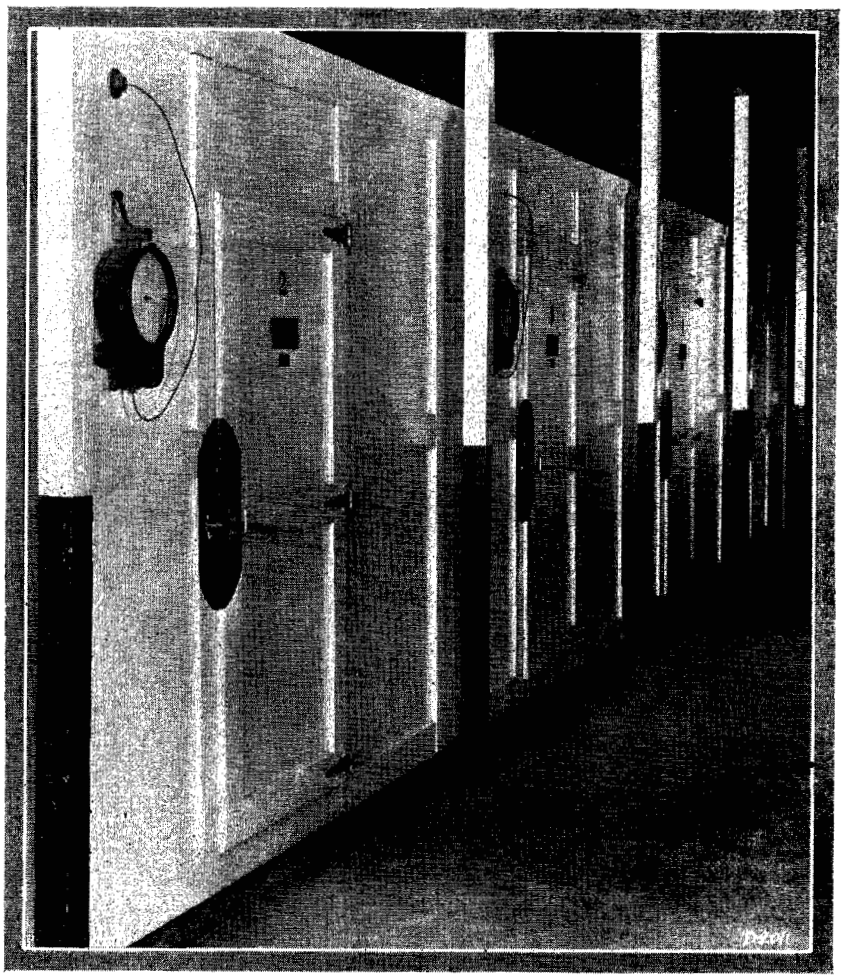

FIG. 3.—Smith incubators used to study formaldehyde fumigation.

tion titre did not fluctuate so greatly as has been reported by several other writers. It did fluctuate in the higher ranges but usually did not go to zero in the lower dilutions.

At the close of the experiment 56 of these birds were subjected to autopsy and records were made of the lesions and bacteriological findings. Of the reactors, 76.8 per cent showed

visible lesions of the disease and *S. pullorum* was isolated from 51.8 per cent of the cases.

Examination of Eggs

After incubating the eggs for a few days they were examined bacteriologically by a technic similar to that used by Maurer (19). Briefly, this consisted of soaking the eggs in 5 per cent phenol for 15 minutes; removing the excess phenol with alcohol; flaming the small pole of the shell with a Bunsen burner flame until the shell was brown; removing a small portion of the shell with sterile forceps; discarding the white and collecting the yolk in about 200 c.c. of sterile nutrient broth. The yolk was forced from the shell by placing the broken end of the egg on the mouth of the flask containing the broth and flaming the opposite end of the shell. The flasks were incubated at 37° C. for 72 hours and loops of the material streaked on nutrient agar plates. Only when there was obtained a typical growth, with typical staining reactions and fermentations, was a culture said to be *S. pullorum*.

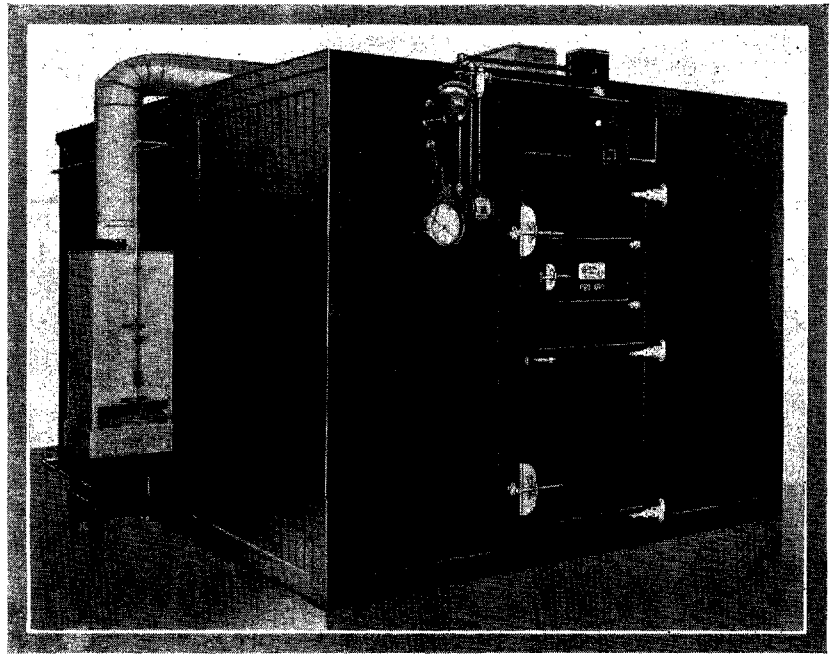

FIG. 4.—Smith incubator with attachment for fumigation. The formalin-permanganate mixture is placed in the apparatus at the side of the machine. Steam is likewise introduced at this point and the gas is drawn into the interior by means of the fans.

The criterion on which the writers based identification was the presence of a gram negative rod of the characteristic size

which fermented dextrose with gas and acid production, or acid only, and which did not ferment lactose, sucrose, or maltose. Agglutination tests on part of these cultures showed that they were agglutinated by known positive serum. (All of the cultures were not tested in this manner.) As reported above the titre of the serum of these birds was 1 to 40 or higher at the time these tests were made.

There has been much criticism of the agglutination test as a means of detecting the presence of actual carriers of the pullorum disease organism. Hence a point of interest was to ascertain whether there is a direct relation between a positive reaction to the agglutination test and the carrier condition. Table VII shows the relation for certain birds which laid eggs during the period of this experiment.

Of the 58 birds here listed, which laid eggs during the short period under investigation, 31 or 53.4 per cent laid one or more eggs from which the organism of pullorum disease was isolated. Of the 404 eggs examined bacteriologically, 60 or 14.8 per cent were found to be infected.

Livability of Chicks from Reactor Flock

Chicks hatched from the same lot of eggs which were examined were placed in "Liv-an-Gro" brooders and kept for 14 days. Table VIII shows their livability.

TABLE VIII.—LIVABILITY OF CHICKS FROM REACTOR FLOCK.

Date of incubation	Num. of eggs		Chicks dead after		*S. pollorum*		Per cent of chicks infected
	Incubated	Hatched	7 days	14 days	Isolated	Not isolated	
3-9	96	51	7	30	28	2	54.9
3-9	95	56	18	29	28	1	50.0
3-9	90	45	7	15	15	0	33.3
3-15.....	96	60	8	35	35	0	58.3
3-15.....	75	40	9	32	28	4	70.0
3-15.....	75	35	7	20	15	5	42.8
3-22.....	96	48	4	22	22	0	45.9
3-22.....	96	43	6	25	23	2	53.5
3-22	96	45	2	14	11	3	24.4
3-29.....	95	52	5	23	22	1	42.3
3-29.....	96	42	9	27	23	4	54.7
3-29.....	96	54	9	27	25	2	46.5
4-5	96	40	6	25	25	0	62.2
4-5	96	44	9	26	20	6	45.4
Total.....	1,294	655	106	350	320	30	Av., 48.9

It will be observed that 48.9 per cent of the chicks from the reactor flock died of bacillary white diarrhea by the fourteenth day after hatching. The dead chicks were subjected to autopsy and cultures were taken from the heart and liver. Only when a typical culture was isolated and tested was the chick considered to have died of the disease.

It will be noted from an examination of Tables VII and VIII that there is a great variation in the frequency with which hens lay infected eggs, and with which infected eggs hatch into chicks which die of the disease. This is a factor the sig-

nificance of which is entirely unknown and even unrecognized by most hatcherymen. This also explains why results with reactor flocks are so variable; some hatches giving high hatchability and livability, while other hatches from the same birds give low hatchability and heavy chick mortality.

TABLE IX.—OCCURRENCE OF S. PULLORUM ON THE BODY SURFACE AND IN THE NASAL CLEFTS OF NEWLY HATCHED CHICKS.

Wing band No.	Chick fluff from body		Nasal cleft swab	
	Growth	*S. pullorum*	Growth	*S. pullorum*
1	+	−	+	−
2	+	−	−	−
3	+	−	+	−
4	−	−	....	−
5	−	−	−	−
6	−	−	+	−
7	−	−	−	−
8	+	−	−	−
9	+	−	+	−
10	−	−	−	−
11	+	−	−	−
12	−	−	−	−
13	−	−	−	−
14	+	+	+	(a) +
15	−	−	+	−
16	−	−	−	−
17	+	−	−	−
18	+	−	+	−
19	−	−	+	−
20	−	−	−	−
21	+	−	−	−
22	+	−	−	−
23	+	−	+	−
24	−	−	+	−
25	−	−	+	−
26	+	−	+	−
27	−	−	−	−
28	−	−	−	−
29	−	−	−	−
30	+	−	−	−
31	+	−	+	−
32	+	−	−	−
33	−	−	−	−
34	+	−	+	(a) +
35	+	−	−	−
36	+	−	+	−
37	+	−	−	−
38	−	−	−	−
39	−	−	−	−
40	−	−	−	−
41	+	−	+	−
42	+	−	−	−
43	−	−	−	−
44	+	−	+	−
45	−	−	−	−

(a) *S. pullorum* isolated on autopsy.

Table IX shows the results of examining some of the living chicks from the reactor flock for the presence of the organism of pullorum disease. It is believed that enough data are presented to show what will usually be found from this type of examination. The examination was made by culturing, and examining later for *S. Pullorum* some fluff clipped from the breast and back and, also, a swab of the nasal cleft of each of the chicks as they emerged.

These data show that two of the 45 chicks were hatched

from eggs which must have been infected with *S. pullorum*. Both the body and the nasal cleft were contaminated with *S. pullorum*. Other observations have shown that the organisms will live for several hours when air dried on chick down. Also, that many organisms may be carried by the particles of the dried material which is commonly, but incorrectly, called chick

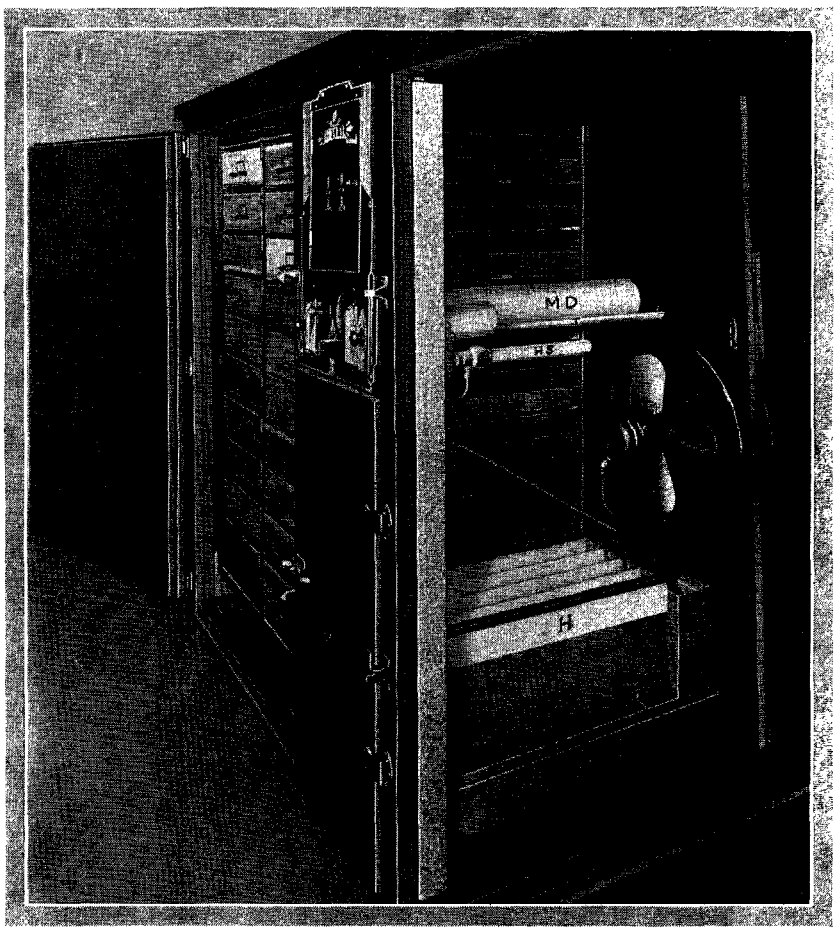

FIG. 5.—Sectional view of the Buckeye separate hatcher with the front door open. (H) Humidifiers or more properly the vaporizer. (HS) The humidistat which controls the vaporizer. (T) Thermostat for temperature control. (MD) Motor damper to control ventilation.

The function of the motor damper is to automatically introduce fresh air and at the same time expel the exhausted air, thereby maintaining a constant air condition balanced with correct temperature and humidity. Under each hatching tray there is a baffle or pan to catch down, droppings, and other refuse, and prevent them from being carried from one tray to another.

The air movement is lateral, therefore, the advancing animal heat is not transmitted directly from one tray to another. The vaporizer does not "spray" water in the machine but creates and throws off a water vapor which is immediately carried throughout the hatcher by the air movement.

down; incorrectly, because practically none of it is actual "down" from the chick.

Fumigation Experiments with S. Pullorum

Action of Formaldehyde on S. Pullorum and P. Avicida with Ports Closed

For reasons already stated formaldehyde was selected as a fumigant for the first experiments. Two No. 9 Buckeye and four 47,000-egg Smith machines were used in determining the

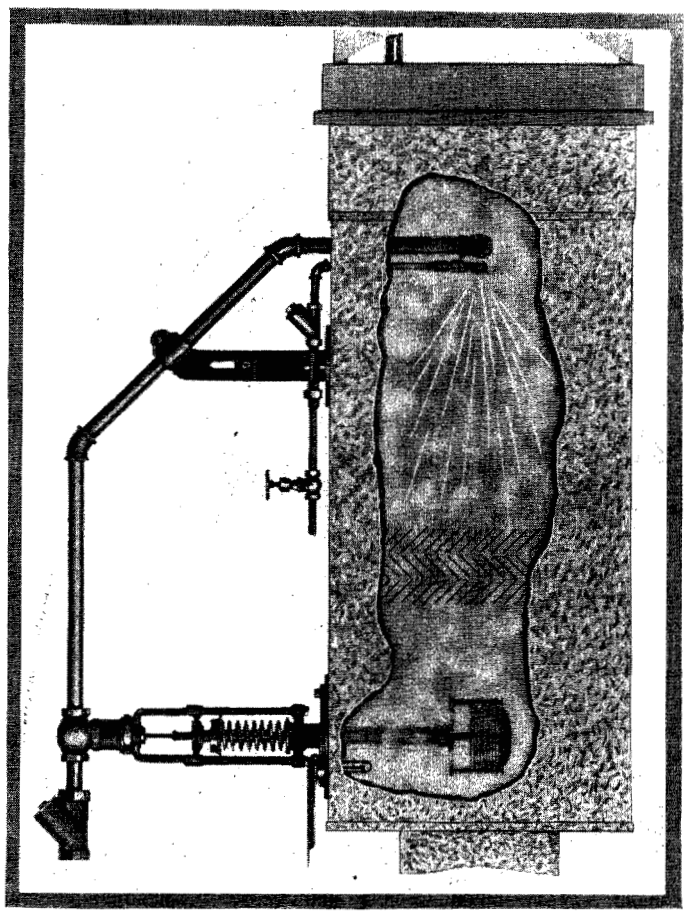

FIG. 6.—Smith incubator showing attachment for automatic control of moisture.

minimum lethal dose of formalin for the *S. pullorum* and *P. avicida* organisms in incubators. Each Buckeye incubator was in a separate room while the four Smith incubators were all in one room. One of the Buckeye machines was two years old and heated by means of a hot water system. The other was new and electrically heated.

TABLE X.—GERMICIDAL ACTION OF FORMALDEHYDE ON S. PULLORUM AND P. AVICIDA WITH ALL PORTS CLOSED.

Date fumigated	3-5			3-7			3-9			3-12			3-14			3-19			3-19			3-20			3-20			3-21			3-23			3-23			3-31			6-18 (a)			6-18 (b)			6-29			6-30			Organism
Hrs. (c)	24	48	72	24	48	72	24	48	72	24	48	72	24	48	7	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	
5 min. (d)													−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+				S
													−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−							+	+	+										P
																																																	A
10 min.													−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+				S
													−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+							+	+	+										P
																																																	A
15 min.										−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	+	+	S
										−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−							+	+	+										P
																																																	A
20 min.													−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+							+	+	+	+	+	+	+	+	−	−	−	S
													−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−																			P
																																																	A
25 min.													−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−							+	+	+	+	+	+						S
													−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−																			P
																																																	A
30 min.										−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−				−	−	−	+	+	+	+	+	+						S
										−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−							−	−	−										P
																																																	A
45 min.										−	−	−	−	−	−	−	−	−																						+	+	+	+	+	+	−	−	−				S
										−	−	−	−	−	−	−	−	−																															P
																																																	A
60 min.	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+										−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	S
	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−																−	−	−								P
																																																	A
20 min.	+	+	+	−	−	−	−	−	−																																								S
	+	+	+	−	−	−	−	−	−																																								P
																																																	S
180 min.	+	+	+	−	−	−	−	−	−																																								P
	+	+	+	−	−	−	−	−	−																																								A
HCHO (e)	2,000			70			25			35			35			35			35			25			10			20			10			35			10			(f)10			(f)20			30			40			.....
KMnO4 (g)	—			35			17½			17½			17½			17½			17½			12½			5			10			5			17½			5			—			—			15			30			....
Dry bulb (h)	100			100			100			100			100			100			100						100			100			100			100			100			100			100			100			.....
Wet bulb (i)	68			85			87			90			92			90			92						91			92			93			94			91			90			90			90			.....

(a) Formalin dripped on hot plate, 10 c.c. per hour.
(b) Formalin dripped on hot plate, 20 c.c. per hour.
(c) Hours of incubation in broth.
(d) Number of minutes cultures were fumigated with formaldehyde gas.
(e) Formalin: Number of c.c. used in each experiment.
(f) Number of c.c. per hour.
(g) Potassium permanganate: Number of grams used in each experiment.
(h) Dry-bulb thermometer reading.
(i) Wet-bulb thermometer reading.
-|- Indicates growth.
— Indicates no growth.
SP *Salmonella pullorum.*
PA *Pastuerella avicida.*

The incubators used are illustrated in figures 2 and 3. The devices for controlling humidity and recording the temperature in figure 2 are not standard equipment but were installed for this particular experiment. The latest equipment for controlling humidity in both the Buckeye and Smith incubators is illustrated in figures 4, 5, and 6.

In the experiments carried on by Gwatkin (7) in 1926 it was found necessary to close and tightly seal the door and ports of the incubator for one hour. Guided partly by these experiments, the M.L.D. (minimum lethal dose) or minimum amount, of formaldehyde necessary to kill *S. pullorum* and *P. avicida* with all ports closed was first determined.

For this work small sterile cheese-cloth packets of chick down were contaminated and exposed to varying amounts of formalin and for various periods of time on 17 different occasions. Likewise, the relative humidity was varied. The results are presented in Table X.

In the first experiment, on March 5, formalin alone was used but since growth occurred after exposure for three hours, this method was discontinued and the formalin-permanganate method was used in all subsequent experiments in which the ports were kept closed.

Thirty-five c.c. of formalin from which the formaldehyde gas was liberated by 17.5 grams of potassium permanganate apparently gave the best results when the dry-bulb reading was maintained at 100° F. and the wet-bulb reading at approximately 90° F. When smaller amounts of formalin were used, inconsistent results were obtained. Thirty-five c.c. of formalin may, therefore, be taken to represent the M.L.D. for *S. pullorum* under the conditions of this experiment. The M.L.D. varies with different conditions. It is influenced especially by the humidity, temperature, and rate of diffusion of gas out of the machine. Thus, it will be noticed that there is a close relation between the disinfecting value of formaldehyde and the amount of moisture in the incubator air. By increasing the relative humidity it was possible to kill the exposed organisms with a smaller amount of formaldehyde.

ACTION OF FORMALDHYDE ON S. PULLORUM WITH PORTS OPEN.

It seemed desirable to determine the M.L.D. of formaldehyde with all ports open, since it was not known just what effect the closing of the ports for one hour would have on the normal operation of the incubator. It is reasonable to expect that the control of the temperature relative humidity, and supply of fresh air would be somewhat influenced by opening and closing ports. The experiments were conducted as before except that the incubator was only partly filled with eggs. The results are shown in Table XI.

Six experiments were conducted. In four cases the formaldehyde was liberated by adding formalin to potassium perman-

ganate, and in two cases formaldehyde was allowed to evaporate from a pan containing formalin placed on the bottom of the machine.

When formaldehyde was liberated by adding formalin to potassium permanganate it was observed that practically the same results were obtained as when the incubator was fumigated with all ports closed. This is due to the fact that in most

TABLE XI.—GERMICIDAL ACTION OF FORMALDEHYDE ON S. PULLORUM AND P. AVICIDA WITH ALL PORTS OPEN.

Date	4-14			4-16			4-20			4-20			4-24			4-28			Organism	
Hrs. (a)	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72		
5 min. (b)	−	−	−	−	−	−	+	+	+	−	−	−							S P	P A
	−	−	−	−	−															
10 min.	−	−	−	−	−	−	−	−	−	−	−	−							S P	P A
	−	−	−	−	−															
15 min.	−	−	−	−	−	−	−	−	−	−	−	−							S P	P A
	−	−	−	−	−															
20 min.	−	−	−	−	−	−	−	−	−	−	−	−							S P	P A
	−	−	−	−	−															
25 min.	−	−	−	−	−	−				−	−	−							S P	P A
	−	−	−	−	−															
30 min.	−	−	−				+	+	+	−	−	−				−	−	−	S P	P A
	−	−	−													−	−	−		
45 min.	−	−	−	−	−	−	−	−	−										S P	P A
	−	−																		
60 min.	−	−	−	−	−	−			−	−			−	−	−				S P	P A
	−	−	−	−	−								−	−	−					
HCHO (c)	50			25			12.5			20			2000 c.c. pan (d)			1000 c.c. pan (e)				
$KMnO_4$ (f)	25			12.5			6.25			10			None			None				
Dry bulb (g)	100.5			100.5			100			100			100			100				
Wet bulb (h)	89			88			89			88			75–80			81				

(a) Hours of incubation in broth.
(b) Number of minutes cultures were fumigated with formaldehyde gas.
(c) Formalin: Number of c.c. used in each experiment.
(d) 2,000 c.c. formalin placed in pan 14" x 19" in 100 cubic feet of incubator space. No growth in 1, 2, or 18 hours.
(e) 1,000 c.c. formalin placed in pan 7½" diameter in 100 cubic feet of incubator space. No growth in 1-2, 1-12, or 2-14 hours.
(f) Potassium permanganate: Number of grams used in each experimnet.
(g) Dry-bulb thermometer reading.
(h) Wet-bulb thermometer reading.
+ Indicates growth.
— Indicates no growth.
SP *Salmonella pullorum.*
PA *Pasteurella avicida.*

cases the exposed organisms were killed in a very short time, usually in less than 10 minutes in either case.

According to McClintic's (17) work on formaldehyde fumigation carried on for the Public Health Service in 1906, the majority of the organisms were killed within the first five minutes. The formaldehyde is immediately liberated by the heat of the reaction between the formalin and the potassium permanganate, thus bringing the concentration in the incubator up to its maximum in less than five minutes. The forced draft immediately mixes the air in the incubator so that the concen-

tration is very soon the same in all parts of the machine. As a result the formaldehyde exerts its maximum germicidal properties in a shorter time in a forced-draft incubator than it would in a still-air room, such as McClintic used, where there is a diffusion of gases rather than a forced mixture of the rapidly circulating air.

FUMIGATION BY THE PAN METHOD

Two experiments in fumigating the incubator with ports open were tried by placing 2,000 c.c. of formalin in a pan 14 x 19 inches in one case, and 1,000 c.c. of formalin in a beaker

TABLE XII.—FORMALDEHYDE FUMIGATION BY VAPORIZATION FROM A PAN OF FORMALIN.

Date	5-7			5-8			5-9			5-14			5-16			5-16			5-23			5-25		
Dry bulb	100°F.			100°F.			100°F.			100°F.			100°F.			100°F.			100°F.			100°F		
Wet bulb	83°F.			84°F.			86°F.			86°F.			86°F.			86°F.			86°F.			86°F		
Number of beakers	1			2			2			2			1			2			2			2		
Diameter of beakers	7¼ in.			7¼ in.			7¼ in.			7¼ in.			7¼ in.			7¼ in.			7¼ in.			7¼ in.		
Amount of formalin added to beakers	500 c.c.			500 c.c.			500 c.c.			500 c.c.			500 c.c.			500 c.c.			500 c.c.			500 c.c.		
Hours of incubation in broth	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Exposure																								
5 min.	+	+	+	+	+	+																		
10 min.	+	+	+	+	+	+																		
15 min	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+
20 min.	+	+	+	+	+	+																		
25 min.	+	+	+	+	+	+																		
30 min.	+	+	+	+	+	+				−	−	−	+	+	+	−	−	−	+	+	+	+	+	+
45 min.	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+
60 min.	+	+	+	+	+	+				−	−	−	+	+	+	−	−	−	−	−	−	−	−	−
15 min.	+	+	+	+	+	+				−	−	−	+	+	+	−	−	−	+	+	+	+	+	+
30 min.	+	+	+	+	+	+				−	−	−	+	+	+	−	−	−	−	−	−	−	−	−
45 min.	+	+	+	+	+	+				−	−	−	+	+	+	−	−	−	−	−	−	+	+	+
60 min.	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	+	−	−	−
15 min.	+	+	+	+	+	+	+	+	+															
30 min.	+	+	+	+	+	+																		
45 min.	+	+	+	+	+	+	−	−	−															
60 min.	+	+	+	+	+	+	−	−	−															
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\+ Indicates growth. — Indicates no growth.

7¼ inches in diameter in another case. In the first experiment all the exposed organisms were killed in one-, two-, and 18-hour exposures. In the latter experiment no growth occurred in tubes of sterile broth to which had been added the contaminated pledgets of cheese cloth fumigated for ½, 1½, and 2¼ hours.

The formalin in the pan and beaker underwent changes which were not fully understood. After standing in the incubator for a number of hours a white precipitate, which apparently was paraformaldehyde, formed. The length of time required for this precipitate to form varied with different conditions. It appeared that the water in the formalin, having a

lower vapor pressure, was vaporized at a greater rate than the formaldehyde; consequently, not only the boiling point of the remaining solution was raised, but the formalin became much more concentrated. Due to the variable rate of evaporation brought about as a result of the increased vapor pressure and concentration of the formalin there seemed to be no practical means of maintaining a constant concentration of formalin, and therefore this method of introducing formaldehyde was abandoned.

Table XII shows the results with this method.

It may be noted that there is considerable variation in the M.L.D. under the same conditions, This is partly due to the fact that the organisms were not always introduced at the same time as the formalin, and also because the formaldehyde evaporated more rapidly at first than later. This point was not recognized until after these experiments had been completed, but in each case the formalin had been in the incubator for at least one hour and in some cases several hours before the organisms were introduced so that the atmosphere was well saturated with formaldehyde gas. However, similar conditions would exist under practical hatching conditions.

TABLE XIII.—FORMALDEHYDE FUMIGATION BY THE HOT-PLATE METHOD.
(All ports open.)

Date	6-12			6-13			6-14			6-15			6-16			6-20			6-22		
Dry bulb	100°F.			100°F.			100°F.			100°F.			100°F.			100°F.			100°F.		
Wet bulb	90°F.			89°F.			90°F.			89-90°F.			89-90°F.			90°F.			90°F.		
Number c. c. formalin per hour	120			90			80			75			70			25			30		
Hours of incubation in broth	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Exposure:																					
15 min	—	—	—	—	+	+	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+
30 min	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
45 min	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
60 min	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
75 min	—	—	—	—	—	—										+	+	+			
90 min	—	—	—	—	—	—										—	—	+			
105 min	—	—	—																		
120 min	—	—	—																		

+ Indicates growth. — Indicates no growth.

FUMIGATION BY THE HOT-PLATE METHOD

Having found that the pan method of evaporating formaldehyde was unsatisfactory it was next decided to drip formalin on a "hot plate" and a technic for introducing formaldehyde by this method was worked out. The attempt was then made to determine the M.L.D. by this method. The results are presented in Tables XIII and XIV.

These data indicate that the M.L.D. for *S. pullorum* by this method is about 70 c.c. per hour. Fifty-five c.c. per hour greatly

reduced the vigor of *S. pullorum* as indicated by the fact that growth did not occur in all cases in 24 hours after exposure to fumigation. When smaller amounts were used the results were not constant. In order to successfully fumigate an incubator of this type and size it appears that it will be necessary to introduce at least 70 c.c. of formalin on a hot plate every hour chicks are hatching.

DETERMINATION OF THE M.L.D. OF FORMALDEHYDE IN SMITH INCUBATORS WITH ALL PORTS OPEN

A Smith incubator owned by a commercial hatchery was used to determine the M.L.D. of formaldehyde for this type. S. pullorum was the only organism considered in these experiments. The results are shown in Table XV.

TABLE XIV.—FORMALDEHYDE FUMIGATION BY THE HOT-PLATE METHOD.

Date	6-23			6-25			6-26			6-27			6-28			7-6		
Dry bulb	100°F.			100°F.			100°F.			100°F.			100°F.			100°F.		
Wet bulb	90°F.			90°F.			89.5F.			90°F.			88-91°F.			89-90°F.		
Number c.c. formalin per hour	40			50			60			65			60			55		
Hours of incubation in broth	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Exposure:																		
15 min	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20 min	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45 min	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+
60 min	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+		+	+
75 min	—	+	+	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+
90 min	+	+	+				—	—	—	—	—	—				—	—	+
105 min	+	+	+				+	+	+									
120 min	—	—	—				—	—	—									

+ Indicates growth. — Indicates no growth.

The results indicate that 200 c.c. of formalin liberated by 115 gm. of potassium permanganate, when the wet-bulb thermometer had a reading of 89 to 92° F., is not sufficient to secure consistently satisfactory sterilization. Three hundred and 400 c.c. of formalin liberated by 165 gm. and 220 gm., respectively, of potassium permanganate with the dry-bulb reading at 99° F. and the wet-bulb reading at 90 and 91° F., killed the *S. pullorum* on the exposed contaminated material in a sufficient number of cases so that either dose could be recommended for a machine of this size.

The Smith machine with approximately eight times the cubic capacity of the No. 9 Buckeye required approximately 10 times as much formalin. The air in the Buckeye machine is completely displaced in about 20 minutes and in the old model Smith machine in about four minutes. In the late model Smith electric incubators a damper in the intake pipe can be adjusted to reduce the complete change of air in the machine to three or four times per hour. This reduces the amount of current

TABLE XV.—RESULTS OF FUMIGATING SMITH INCUBATORS.

Number of minutes	Filter paper	Filter paper trans.(a)	Cheese-cloth	Cheese-cloth trans.	Filter paper	Filter paper trans.	Cheese-cloth	Cheese-cloth trans.	Filter paper	Filter paper trans.	Cheese-cloth	Cheese-cloth trans.
	200 c.c. formalin — 115 gm. $KMnO_4$				300 c.c. formalin — 165 gm. $KMnO_4$				400 c.c. formalin — 220 gm. $KMnO_4$			
5	(b)+		(c)—	—	—		+	+	—	—	+	+
10	+		+	+	+		—	—	+	+	—	—
15	+		—	—	—		+	+	—	—	—	—
20	—		+	+	—		—	—	—	—	—	—
25	—		—	—	—		—	—			—	—
30	+		—	—	—		—	—	—	—	—	—
35												
40									—	—	—	—
45									—	—	—	—
Dry bulb ..	99	99	99	99	99	99	99	99	98	98	98	98
Wet bulb .	(d)89–92	89–92	89–92	89–92	91	91	91	91	90	90	90	90
Control ...	+	+	+	+	+	+	+	+	+	+	+	+
	500 c.c. formalin — 280 gm. $KMnO_4$				400 c.c. formalin — 200 gm. $KMnO_4$				400 c.c. formalin — 220 gm. $KMnO_4$			
5	+		—	—	+	+	—	—	—	—	+	+
10	+		+	+	+	+	+	+	—	—	—	—
15	—		—	—	(e)0	0	0	0	0	0	0	0
20	—		—	—	—	—	—	—	—	—	—	—
25	—		—	—	0	0	0	0	0	0	0	0
30	—		—	—	—	—	—	—	—	—	—	—
35	—		—	—	0	0	0	0	0	0	0	0
40	—		—	—	—	—	—	—	0	0	0	0
45	—		—	—	0	0	0	0	—	—	—	—
Dry bulb ..	99	99	99	99	99	99	99	99	99	99	99	99
Wet bulb .	89–91	89–91	88–91	89–91	85	85	85	85	89–90	89–90	89–90	89–90
Control ...	+	+	+	+	+	+	+	+	+	+	+	+

(a) Transfer from tube containing sample to a second tube.
(b) Growth in broth to which had been added contaminated material.
(c) No growth in broth to which had been added contaminated material.
(d) The temperature of the wet bulb increased two or three degrees during the operation but soon returned to normal.
(e) Not examined at that time.
+ Indicates growth. — Indicates no growth.

needed to heat the machine. The formaldehyde exerts most of its germicidal action in the first five minutes after liberation, so that the maximum action has been exerted before the air of the incubator has been changed.

Formaldehyde Fumigation and the Hatchability of Incubated Eggs

Having determined the minimum amount of formaldehyde gas necessary to destroy *S. pullorum* the next step was to determine its effect upon the hatchability of incubating eggs and hatching chicks. The results obtained on hatchability will be reported first.

It is reasonable to expect that tests with the ports closed would be more severe than with the ports open and since the

TABLE XVI.—EFFECT OF FORMALDEHYDE FUMIGATION ON INCUBATING EGGS.

Flock	Date set	Fumigated			Non-fumigated		
		Number of eggs	Number of chicks	Per cent hatch	Number of eggs	Number of chicks	Per cent hatch
Reactor...	3-9	95	56	58.9	96	51	53.1
Control ...	3-9	96	50	52.1	96	53	55.3
Reactor...	3-15	96	55	59.4	96	60	62.5
Reactor...	3-15	75	37	49.3	75	40	53.3
Control ...	3-15	96	59	61.7	96	66	68.7
Reactor...	3-22	96	45	46.8	96	48	50.0
Control ...	3-22	96	59	61.7	96	56	58.9
Reactor...	3-29	96	56	58.9	96	42	43.8
Reactor...	3-29	96	54	56.2	95	52	54.7
Control ...	3-29	96	58	60.4	96	59	61.7
Reactor...	4-5	96	40	41.7	96	40	41.7
Control ...	4-5	96	58	60.4	96	58	60.4
Total reactor ...	——	650	343	52.8	650	333	51.2
Total control....	——	480	284	59.2	480	292	60.9

M.L.D. for formaldehyde was the same in each case the tests on the eggs were all conducted with the ports closed.

The eggs experimented with were from both the control and the reactor flocks and the experiments covered a period of approximately one month, so that the seasonal factor did not play an important part.

Each setting of eggs was fumigated three times: the first usually on the first day of incubation with subsequent fumigations at weekly intervals. Thirty-five c.c. of formalin and 17.5 gm. of potassium permanganate were used in each case. The results of these experiments are shown in Table XVI.

The data indicate that the hatchability of eggs fumigated three different times with formaldehyde liberated by adding 35 c.c. of formalin to 17.5 gm. of potassium permanganate, with all ports closed, is not noticeably affected.

Marcellus, Gwatkin, and Glover (18) used approximately four and one-half times this amount and found some injury

to embryos between the ages of 24 and 96 hours. They recommended that fumigation be discontinued during these periods.

The data in Table XVI also show that the eggs from the reactor flock give slightly lower hatchability than those from the control flocks and that the fumigated eggs possessed about the same hatchability as the non-fumigated eggs. However, the differences are not significant and this amount of formaldehyde can be used to fumigate between hatches. With hatches coming on twice each week it will be difficult to avoid fumigating during the period suggested by the above writers, but the amount used in these experiments does not appear to injure the embryos at any period of development.

Effect of Fumigation on S. Pullorum in Eggs

Observing that formaldehyde gas was very effective in killing *S. pullorum* in the incubator air without injuring the hatchability of the eggs, the question naturally arose as to whether the gas either killed or attenuated the organisms inside the eggs. As a result, 650 eggs from the reactor flock were subjected to three treatments of one hour each at weekly intervals. Also, 932 eggs from the same flock were not fumigated in order to serve as a control. The results of this experiment are shown in Table XVII.

TABLE XVII.—EFFECT OF FUMIGATION ON S. PULLORUM IN EGGS.

Date of incubation	Fumigated eggs					Non-fumigated eggs				
	Num. of eggs	Num. of chicks	Per cent hatch	Num. of chicks infected	Per cent of chicks infected	Num. of eggs	Num. of chicks	Per cent hatch	Num. of chicks infected	Per cent of chicks infected
3-9	95	56	58.9	28	50.0	96	51	53.1	28	54.9
3-9	–	–	–	–	–	90	46	51.1	15	32.6
3-15	96	55	59.4	30	54.5	96	60	62.5	35	58.3
3-15	75	37	49.3	15	40.5	75	40	53.3	28	70.0
3-22	96	45	46.8	11	24.4	96	48	50.0	22	45.8
3-22	–	–	–	–	–	96	43	44.8	23	53.5
3-29	96	56	58.9	33	58.9	96	42	43.8	23	54.7
3-29	96	54	56.2	25	46.3	95	52	54.7	22	41.3
4-5	96	40	41.7	17	42.5	96	40	41.7	25	62.5
4-5	–	–	–	–	–	96	44	45.8	20	45.4
Total	650	343	52.8	159	46.3	932	466	50.0	241	51.8

Table XVII shows that three formaldehyde fumigations with 35 c.c. of formalin liberated by 17.5 grams potassium permanganate acting for one hour at weekly intervals did not injure the hatchability of eggs obtained from the reactor flock. The hatchability of the fumigated eggs was 2.8 per cent better

than the unfumigated eggs. This is not enough to be significant. On the other hand, *S. pullorum* was isolated from 51.8 per cent of the chicks from non-fumigated eggs as compared to 46.3 per cent from those eggs which had been fumigated.

With the relatively small number of eggs used in the experiment and the fact that a lack of equipment made it necessary to hatch the fumigated and non-fumigated eggs in the same incubator, one cannot state definitely that the fumigations through actually killing or attenuating the organisms in the eggs, was responsible for the 5.5 per cent lower death rate from *S. pullorum* infection. In dealing with experiments of this type in which pathogenic microorganisms are a factor, variable results are generally obtained. This is quite clearly shown by the rather wide variation in the per cents of chicks dying from *S. pullorum* infection in each individual hatch, regardless of whether the eggs were fumigated or non-fumigated. These limited results indicate that there is probably no influence exerted by the formaldehyde on the organisms within the egg.

Formaldehyde Fumigation of Chicks

Complete sterilization of an incubator would seem to involve sterilization of the chick down of the freshly hatched chicks as well as of the air and the incubator itself. This involves several difficulties since there must be a lethal dose at all times and the ports must be left open to allow for the proper exchange of air with the outside. The hatch usually extends over a period of from 18 to 36 hours. Thus the early-hatched chicks would be exposed to the action of the gas over long periods of time.

Considerable time was used in attempts to develop a method suitable for continuous fumigation. Experiments were also conducted with ports open and closed as well as with different relative humidities in the incubator air. In a few cases chicks several days old were used to determine the influence of age upon susceptibility to the action of the gas. The results of these experiments are reported in the following pages.

In the first experiment baby chicks, varying in age from 1 to 10 days, were obtained from a local custom hatchery and subjected to formaldehyde fumigation to determine the effect of the gas.

A No. 9 Buckeye incubator with a capacity of approximately 100 cubic feet of air was used in the experiments. It was found that exposure for one hour to 70 c.c. formalin killed 15 out of 18 chicks. In other experiments in which 35 c.c. of formalin were used, variable results were obtained. Several chicks died but some of the deaths were due to *S. pullorum* infection. In two experiments not complicated with *S. pullorum* infection, and in which chicks were exposed for one hour to 35 c.c. of formalin, no apparent harmful effects were noticed. In another ex-

periment, 10 out of 30 chicks died in three days. In the last case the chicks were 10 days old while in the other two cases, one lot was 24 hours and the other four days of age. Aside from a difference of one degree in the wet-bulb reading, all other factors were the same.

In the experiments conducted with all the ports open, the livability of the exposed chicks was not affected where 20 and 50 c.c. of formalin were used. In another experiment, eight out of 20 chicks died as a result of too severe fumigation. In this experiment, a pan 14 x 19 inches, containing 2,000 c.c. of formalin, was placed in the bottom of the incubator. Approxi-

TABLE XVIII.—RESULTS OF FORMALDEHYDE FUMIGATION ON LIVABILITY OF CHICKS.

	All ports closed for time of exposure												All ports open for time of exposure					
Date	3-7-28		3-9-28		3-12	3-14-28		3-17-28		3-23-28		3-31	4-14		4-16	4-24		4-28	
Hrs. fumigated	1	2	1	1	1	½	1	½	1	¼	1	¼	½	1	1	1	18	1	18
Wet-bulb reading	85	85	87	87	90	92	92	90	90	93	93	91	89	89	88	80	75	81	81
Dry-bulb reading	100	100	100	100	99	100	100	100	100	100	100	100	100	100	100.5	100	100	100	100
C.c. of formalin	70	70	35	35	35	35	35	35	35	35	35	10	50	50	25	Pan		Pan	
Gm. of pot. permanganate	35	35	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	5	25	25	12.5	0	0	0	0
Age of chicks (days)	2	2	4	4	7	1	1	4	4	10	10	1	1	1	3	1	1	1	1
Number of chicks	18	18	10	9	20	20	20	20	20	15	15	20	20	20	20	20	20	20	20
Dead chicks, 24 hrs.	11	13	0	0	1	0	0	0	0	1	5	0	0	0	0	1	5	1	0
Dead chicks, 48 hrs.	15	17	1	2	5	0	0	0	0	3	6	0	0	0	0	1	7	1	0
Dead chicks, 72 hrs.	15	17	2	3	5	0	1	0	1	4	6	0	0	0	2	2	8	1	2
Dead chicks, 5 days	16	17	4	4	7	1	1	0	1	4	7	0	0	0	2	3	8	1	2
Per cent mortality	88.8	94.4	40.0	44.4	35.0	5.0	5.0	0	5.0	26.6	46.6	0	0	0	10.0	15.0	40.0	5.0	10.0
S. pullorum isolated	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—		

mately 70 c.c. of formalin were evaporated every hour. This amount seemed to be very irritating to the chicks. Later a beaker with a diameter of 7¼ inches was partly filled with formalin and placed in the heating compartment of the incubator. From 10 to 15 c.c. of formalin evaporated every hour. Chicks fumigated with this amount for 18 hours were not injured. The results of these experiments are shown in Table XVIII.

The results of these experiments indicate that the use of 35 c.c. of formalin liberated by 17.5 grams of permanganate will injure chicks if exposed for periods of one hour. Shorter periods of exposure are less injurious. The chicks which are already affected with *S. pullorum* are more severely injured than normal chicks. It is evident that this method of fumigation will

not cure chicks already diseased and that the "pan" method of fumigation is not satisfactory.

Continuous Fumigation by the Hot-plate Method

Since it was found necessary to drip 60 to 70 c.c. of formalin per hour upon a hot plate in the No. 9 incubator in order to keep the concentration of formaldehyde gas high enough to kill *S. pullorum* in the length of time it takes a newly hatched chick to dry off, it seemed desirable to determine the effect of such fumigation on the chicks,

In the first experiment 60 c.c. of formalin were allowed to drip on a hot plate each hour for 8½ hours. Because of an ac-

TABLE XIX.—RESULTS OF CONTINUOUS FUMIGATION OF CHICKS WITH FORMALDEHYDE FOR 8½ HOURS.

Pen	Number of chicks	Number dead at end of						Per cent dead
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	5 days	7 days	
Control, fumigated	15	3	4	5	6	6	7	46.6
Reactor, fumigated	26	5	8	8	12	14	15	57.7
Control, unfumigated	58	2	3	3	4	4	4	6.9

cident which interrupted the operation of the incubator, the experiment was discontinued after the preliminary work. The dry bulb registered 100° F. and the wet bulb from 89 to 90° F. during the experiment. All ports were left open. The results of this experiment are shown in Table XIX.

Of the fumigated reactor chicks, 57.7 per cent, and of the fumigated control chicks, 46.6 per cent were dead by the seventh day. During the same period only 6.9 per cent of the unfumigated control chicks died. Since the fumigated chicks remained in the incubator for at least 30 minutes after the fans were idle, it was thought that perhaps this might have had some influence on the high mortality.

In the second case, 55 c.c. formalin per hour were used for a period of 36 hours. The dry- and wet-bulb thermometers were maintained at the same temperatures as in the previous case. (Table XX.) As a result, 79.6 per cent of the fumigated control chicks and 96.6 per cent of the fumigated reactor chicks died in seven days following the fumigations. Only 6.7 per cent of the unfumigated control chicks died in the same period.

An experiment was then conducted in which the chicks were fumigated every 1½ hours for 10 minutes with 40 c.c. of formalin. The formaldehyde gas was liberated by pouring the formalin on 25 grams of potassium permanganate, and was neutralized with ammonium hydroxide 10 minutes after the introduction of the formalin into the machine. It was thought

that by this method the chicks might not be injured by formaldehyde gas, while at the same time any organism on the chick down would be killed. The dry-bulb thermometer was maintained at 100° F. and the wet bulb at 88 to 90° F. The results are given in Table XXI.

It may be seen that four days after the chicks were fumigated, 30, or 61.2 per cent, of the fumigated control chicks and 26, or 92.9 per cent, of the fumigated reactor chicks were dead. The results were, therefore, somewhat discouraging. On autopsy the dead chicks showed lesions not generally seen in cases of formaldehyde fumigation poisoning. The larynx was very edematous and there was an edematous infiltration of the

TABLE XX.—CONTINUOUS FUMIGATION OF CHICKS WITH FORMALDEHYDE FOR 36 HOURS.

Pen	Number of chicks	Number dead at end of						Per cent dead
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	5 days	7 days	
Control, fumigated	44	12	26	29	29	30	35	79.6
Reactor, fumigated	59	20	27	30	35	50	57	96.6
Control, unfumigated	59	3	3	3	4	Placed in brooder		6.7

submucosa in the pharyngeal region. Even the mucosa of the trachea was somewhat infiltrated with serum and markedly congested. In practically all cases the lungs were congested while the beak was cyanotic, indicating that the chicks died as a result of suffocation brought about by an edema of the larynx. In all probability, the edematous condition was brought about by inhalation of ammonia rather than the formaldehyde.

Another experiment was then conducted in which the length of time between fumigations was extended to three hours and the relative humidity was increased in order to lengthen the time it would take the chick down to dry. No ammonium hydroxide was used. The dry-bulb reading was maintained at 100° F. and the wet bulb at 90 to 92° F. The weather was very humid with practically no wind during the experiment. The results of this experiment are shown in Table XXII.

Five days after hatching, 37.7 per cent of the chicks from the control flock and 54.5 per cent of the chicks from the reactor flock were dead. Practically none of the unfumigated chicks from the control flock died. In addition, the fumigated chicks still alive were somewhat undersized and in poor condition.

The heavy mortality recorded in these experiments indicates that formaldehyde fumigation of sufficient strength and frequency to be continuously effective has a harmful effect on

the chicks. It may be used periodically with beneficial results but the continuous process is not to be recommended.

Action of Formaldehyde on Chicks

When chicks are subjected to prolonged fumigation they appear greatly distressed. At first, there is gasping for breath and excessive lachrymation. The eyes appear to be highly irritated, for the chicks constantly open and close the eyelids. Quite often they attempt to scratch the eye with the toes, as if to remove an irritating substance. Likewise, the head may be thrown around and the eye rubbed against the body, in order to find some relief from the irritating action of the gas on the conjunctiva. At first, they chirp, appear excited, and run more or less blindly around in the hatching tray. Later, the amount of chirping decreases and they may crowd together, gasping for

TABLE XXI.—RESULTS OF FORMALIN-POTASSIUM PERMANGANATE FUMIGATION EVERY 1½ HOURS FOR 36 HOURS ON BABY CHICKS.

(Formalin neutralized with ammonium hydroxide with all ports open.)

Pen	Number of chicks	Number dead at end of 24 hours	48 hours	72 hours	96 hours	Per cent dead
Control, fumigated	49	12	26	29	30	61.2
Reactor, fumigated	28	18	26	26	26	92.9
Control, unfumigated	58	0	0	0	0	0.0

air. At the time of inhalation the head is thrown forward from the body with the mouth opened. In those exposed for a long time, a hoarse, inspiratory wheeze may be heard. It was also observed that chicks have a tendency to pass more droppings than normal. The droppings are somewhat watery in consistency. In addition there seems to be more mucus present in the mouth, indicating that the secretory glands are somewhat stimulated. This is especially evident at first.

After several hours of fumigation, the oral cavity becomes dry and severely congested, with the tip of the tongue completely dried and hardened, having the appearance of dried beef. The external nares are highly reddened or congested in most cases. There is also present a conjunctivitis in which the more prominent blood vessels are well injected. In more severe cases cyanosis of the beak may be observed.

Autopsy generally shows a cyanosis of the beak and a dry oral cavity with the tip of the tongue brown or reddish brown and completely dried. The larynx may be slightly edematous while the trachea is congested. The lungs are greatly congested and in many cases quite hardened. Pneumonia was observed

in a few cases, especially where the chicks did not die until at least the third day after the fumigation. The liver was somewhat ochre colored or streaked with yellowish areas. The kidneys showed signs of degeneration, as evidenced by their light, and in some cases, mottled appearance.

According to some authors, guinea pigs and rabbits are not seriously injured by fumigation for a period of 12 hours with formaldehyde. It has also been known for a long time that ordinary formaldehyde fumigation will not kill insects, mice, or rats. For this reason its use for disinfecting purposes in ship holds has been discontinued even though it has very high germicidal properties. These facts suggest that the high mortality in chicks is due not to a difference in tolerance but rather to a difference in anatomy and physiology.

In the class Aves is found one significant difference in the anatomy of the body from that found in the Mammalia. All

TABLE XXII.—RESULTS OF FORMALIN-POTASSIUM PERMANGANATE FUMIGATION EVERY THREE HOURS FOR 36 HOURS ON BABY CHICKS.

Pen	C.c. of formalin	Gm. of pot. per. manganate	Number of chicks	Number dead at end of				Per cent of mortality
				24 hrs.	48 hrs.	72 hrs.	5 days	
Control, fumigated	40	25	61	13	19	21	23	37.7
Reactor, fumigated	40	25	44	17	20	24	24	54.5
Control, unfumigated.....	...	...	51	...	Placed in storage brooder. Practically no losses.			

members of the former, to which belong the common fowl, have air sacs. These are respiratory appendages with openings into the lungs, and are, in reality, large sacs which are used in breathing. The rate of change of air in the air sacs is quite slow and since they may contain a large amount of air, when filled, it is readily seen that when chicks are fumigated with formaldehyde, a much larger amount of the gas would be absorbed by the blood stream than in the case of the Mammalia which have no air sacs. Thus, the peculiar anatomical structure of the chick may make it unusually susceptible to the action of toxic gases and make the successful fumigation of incubators containing chicks a difficult procedure. The much more severe injury in the older chicks than in day-old chicks is explained on the basis of the more highly developed air sacs in the latter.

Influence of Relative Humidity on the Circulation of Microorganisms in the Air of Forced-draft Incubators

Realizing that very little added weight would overcome the buoyancy of the chick down suspended in the incubators, experiments were conducted to see what effect an increase in

relative humidity would have on the circulation of chick down.

The effect of an increased relative humidity on the circulation of suspended chick down was determined by exposing agar plates at intervals of one and five minutes at various wet-bulb readings. The colonies of bacteria and molds appearing on the exposed agar plates give a fair index as to the number of circulating organisms in the incubator air.

The humidity has a very marked influence as indicated by the figures given in Table XXIII. The machines in which the

TABLE XXIII.—EFFECT OF RELATIVE HUMIDITY ON THE CIRCULATION OF MICRO-ORGANISMS IN THE BUCKEYE INCUBATOR.

		Number of colonies of bacteria and molds appearing on plates							
		One-minute exposure				Five-minute exposure			
Wet-bulb reading (F.)	Dry-bulb reading (F.)	Plate No. 1	Plate No. 2	Plate No. 3	Total	Plate No. 1	Plate No. 2	Plate No. 3	Total
71	99.5	30	22	21	73	79	64	67	210
80	100.0	15	14	20	49	50	47	45	142
85	100.0	6	10	4	20	18	17	30	65
90	100.0	3	2	5	10	10	9	4	23
94	100.0	1	1	2	4	4	8	6	18

Wet-bulb readings (F.)	Per cent decrease in number of colonies between wet-bulb readings	Wet-bulb readings (F.)	Per cent decrease in number of colonies between wet-bulb readings
94-90	40	94-90	27
94-85	80	94-85	72
94-80	91	94-80	87
94-71	94	94-71	96
90-85	20	90-85	64
90-80	30	90-80	83
90-71	78	90-71	41
85-80	80	85-80	54
85-71	81	85-71	73
80-71	5	80-71	32

plates were exposed were fairly free from dust and had not contained eggs or chicks for nearly three months.

A comparison of the results obtained in the different incubators shows quite a variation in the colony count on the agar plates. However, this is no more than is to be expected, for the least disturbance in the normal circulation of the air in the incubators, such as might be brought about by a fluctuating electric current supplying the fans, a flapping canvas curtain, or a person walking in the incubator, might stir up chick down or dirt that had settled out of the air. Consequently, only a general average of the total bacterial count in all the incubators should be considered.

The results of the experimental work are shown in Table XXIII. Counts were made of both molds and bacteria. Since the molds were probably in the spore stage at the time of the exposure each colony of mold represents one spore in most cases.

A comparison of the decreases in the number of colonies of bacteria and molds when the humidity is increased, indicates that the more thoroughly the air in the incubator is saturated the greater is the decrease in the number of circulating micro-organisms. Thus when the wet-bulb reading is 94° F., there is a decrease of 27, 72, 87, and 96 per cent in the number of colonies when exposed for five minutes, compared with the number when the readings are 90, 85, 80, and 71° F., respectively. With exposures for one minute and a wet-bulb reading of 94° F.,

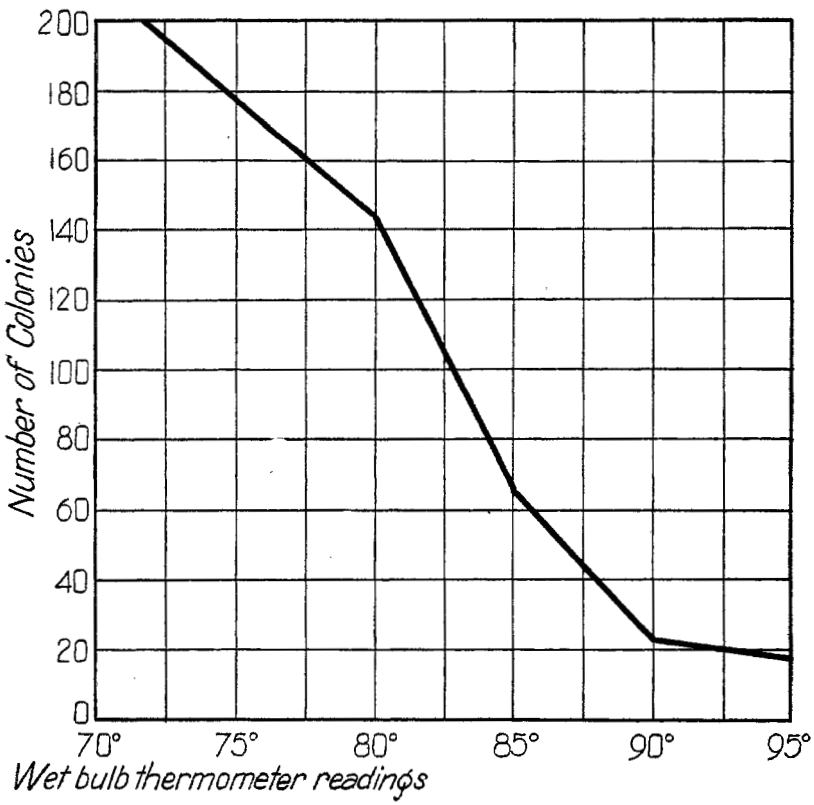

FIG. 7.—Graph showing average decrease in number of colonies of bacteria and molds as the wet-bulb thermometer reading was increased.

there is a decrease of 40, 80, 91, and 94 per cent compared with readings of 90, 85, 80, and 71° F., respectively.

The average of the total number of colonies which appeared on the agar plates exposed in all the incubators for one minute shows that increasing the reading of the wet-bulb thermometer from 82 to 90.5° F. reduced the number of colonies 62.5 per cent. Increasing the wet-bulb reading from 82 to 83° F. to 85° F. resulted in a decrease of 20.2 per cent, while increasing

the wet-bulb reading from 85 to 90.5° F. showed a decrease of 52.9 per cent in the number of colonies.

On agar plates exposed for five minutes, an increase in the wet-bulb reading from 83 to 85° F. decreased the colonies 13.9 per cent; an increase from 85 to 90° F. resulted in a decrease of 21.7 per cent; and an increase of 83 to 90° F. decreased the number of colonies 48.2 per cent. Increasing the wet-bulb reading from 85 to 90° F. and from 83 to 85° F. decreased the number of colonies 21.7 per cent and 13.9 per cent, respectively,

In both cases, where the plates are exposed for one and five minutes and the wet-bulb reading increased from 83 to 90.5° F., a very decided reduction in the number of colonies was noticed.

TABLE XXIV.—EFFECT OF RELATIVE HUMIDITY ON THE CIRCULATION OF MICROORGANISMS IN SMITH INCUBATORS.

Temperature		Incubator No. 1 (a)		Incubator No. 2		Incubator No. 3		Incubator No. 4	
		Number of minutes plates were exposed							
Dry bulb (F.)	Wet bulb (F.)	1	5	1	5	1	5	1	5
99	82–83	- -	- -	- -	- -	(b) 52	193	76	139
99	85	- -	- -	81	220	20	105	56	103
99	89–90.5	1	2	58	166	15	90	21	81
				Per cent					
Difference between 82–83 and 85°F.				-	-	61.5	45.6	26.3	25.9
Difference between 85 and 89–90.5°F.				28.4	24.6	25.0	14.3	62.5	21.3
Difference between 82–83 and 89–90.5°F.				-	-	71.1	53.6	72.3	41.7

(a) Incubator No. 1 had been fumigated the day before the plates were exposed.

(b) Average number of colonies of bacteria and molds appearing on four agar plates.

The difference in the decrease in the number of colonies between the wet-bulb readings from 83 to 85° F. and 85 to 90.5° F. is quite marked. In general a greater decrease occurred between 83 and 85° F. than between 85 and 90.5° F. This difference is well shown in figure 7 which shows results obtained in the Buckeye machines.

The decrease in the number of colonies appearing on agar plates when the wet-bulb readings are increased is probably due to a more thorough saturation of the air with moisture. This results in the suspended particles becoming overweighted with droplets of water, thus settling out of the air. Also, the chances of droplets of moisture attaching themselves to the suspended particles become increasingly greater. These settle to the floor and walls where they remain out of circulation. The results with the Smith incubators are shown in Table XXIV.

Figure 8 shows a series of agar plates exposed to the air of a forced-draft incubator at various degrees of relative hu-

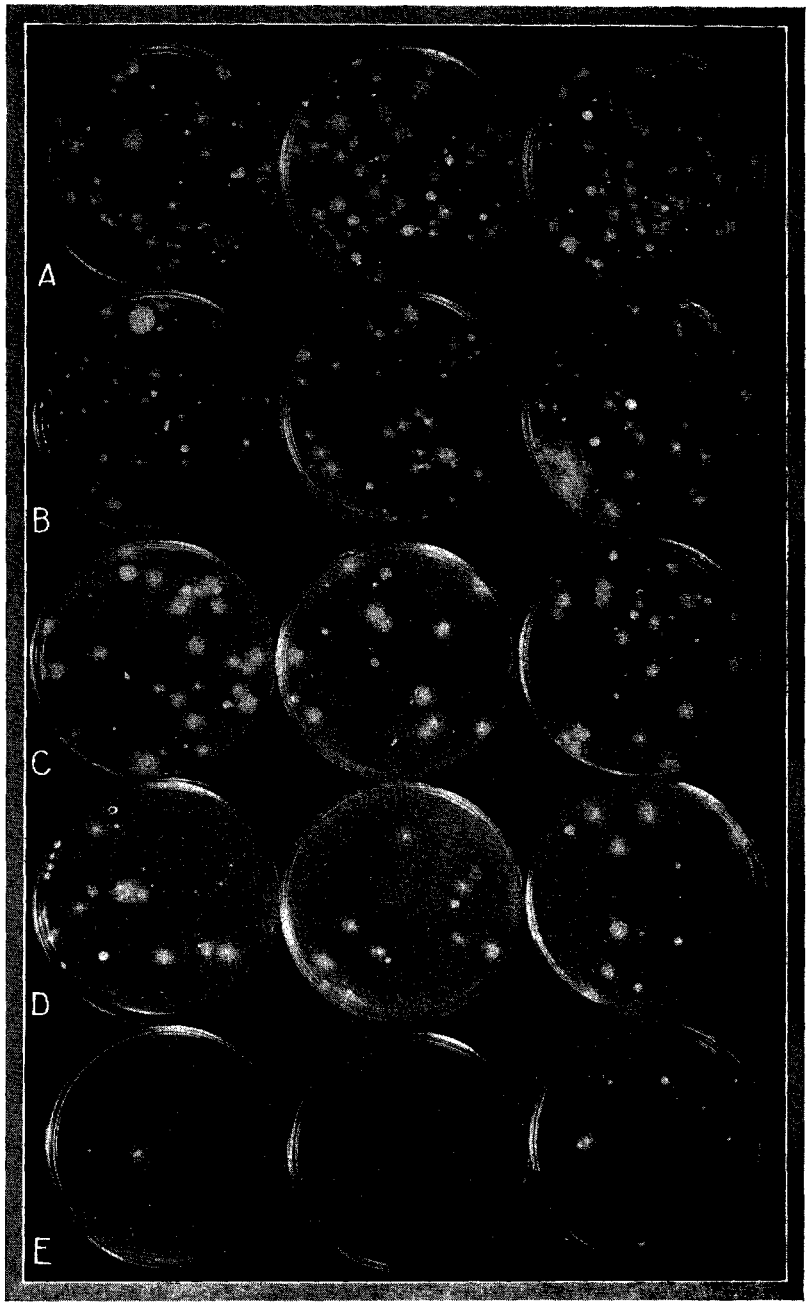

FIG. 8.—A series of agar plates obtained by exposure for 5 minutes to the air of a forced-draft incubator with wet-bulb readings as follows: (A) At 71°F.; (B) at 80°F.; (C) at 85°F.; (D) at 90°F.; and (E) at 94°F.

midity. It will be noted that there is a very marked decrease in the amount of chick down floating in the air as the humidity increases. It will also be noted that there is a marked decrease in thenumber of bacteria and molds as indicated by the decrease in the number of colonies on the plates. These illustrations agree with the curves of the colony counts. (Fig. 7.) It thus appears that an increase in the humidity not only greatly aids the action of formaldehyde, but also greatly reduces the number of microorganisms floating in the air of the machine. The degree of humidity which can be used and still not affect

TABLE XXV.—INFLUENCE OF HUMIDITY ON SPREAD OF PULLORUM DISEASE IN INCUBATORS.

(Summary of mortality results.)

Wet-bulb reading (F.)	Per cent hatchability	Number of chicks	Mortality		Mortality due to B. W. D.	
			Number	Per cent	Number	Per cent
Group I						
75	57.2	410	123	30.00	91	22.19
85	59.4	168	25	14.88	21	12.50
95	42.1	271	47	17.34	31	11.43
Group II						
75	57.0	202	47	23.26	40	19.80
85	60.9	117	15	12.82	15	12.82
95	53.5	249	14	5.62	3	1.20
Group III						
75	55.0	195	68	34.87	48	24.61
85	54.7	105	18	17.14	15	14.28
95	50.8	248	17	6.85	1	.40
Group IV						
Control	- - - -	464	23	4.95	0	0.00

Group I. Chicks from the reactor flock.

Group II. Chicks from a normal flock exposed in the same end of the machine with the Group I chicks.

Group III. Normal chicks exposed in the opposite end of the machine from Groups I and II.

Group IV. Control chicks from a normal flock hatched in a separate machine.

the livability of the chicks is reported on later. It appears clear that the greater the humidity consistent with good hatching practices the better will be the results.

King, Payne, and Bushnell (14) made a more extensive report on the influence of high humidity on the control of pullorum disease spread in incubators. In their experiments, 12 hatches were taken off, six at a wet-bulb reading of 95° F., two at a wet-bulb reading of 85° F., and four at a wet-bulb reading of 75° F. In each hatch at least one tray of eggs from reactor hens was hatched in the same incubator with eggs from hens known to be free of the disease. All chicks were removed from the incubator 24 hours after the peak of the hatch, and placed in wire-bottom brooders where they were reared for two weeks.

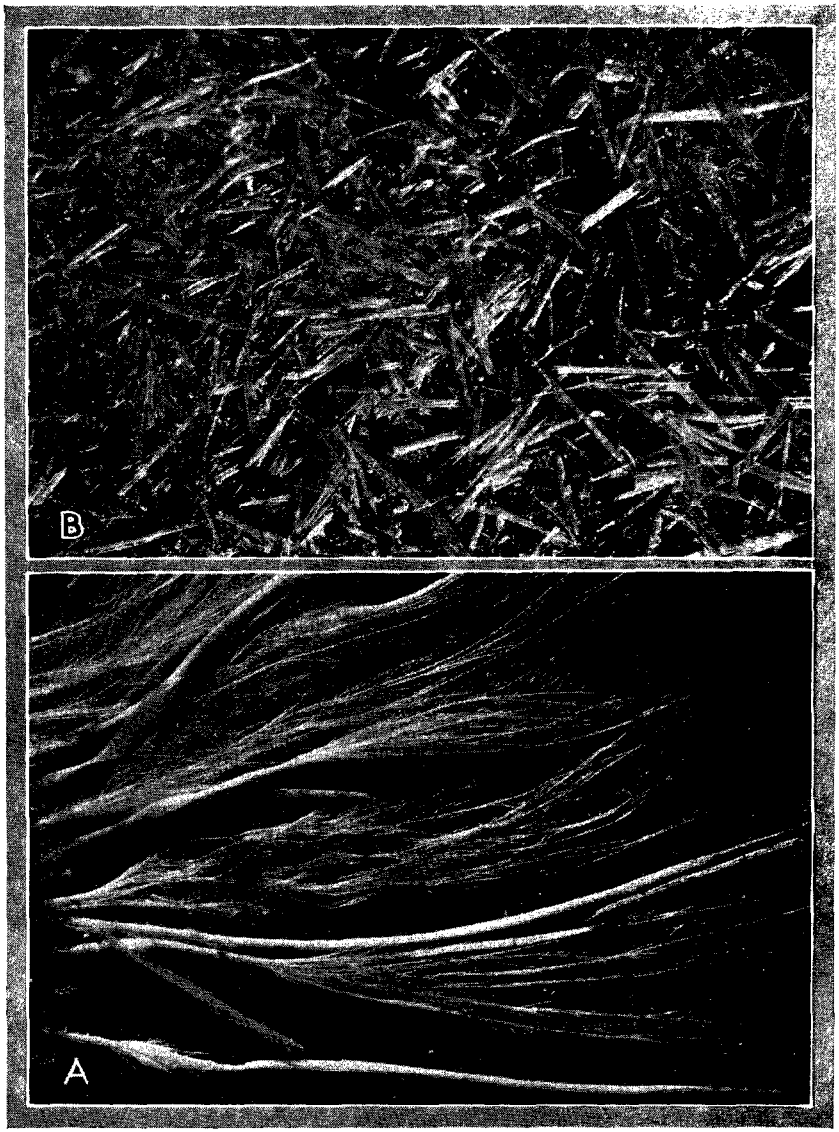

FIG. 9.—(A) Chick down surrounded by the material which dries and becomes free as shown in (B). "Chick down" is not, strictly speaking, "down" at all, but is the covering of the down.

The chicks hatched from the eggs laid by reactor hens were brooded in a separate room from those from non-reactor hens. Control chicks from non-reactor hens were hatched and brooded in a separate room. The moisture was controlled by a mercoid switch which was connected with an immersion heat-

er placed in a water bath of the incubator. (Fig. 2.)

Cultures were made from the chicks which died. When *S. pullorum* was found, the death was considered as being due to that organism. The results of these experiments are shown in Table XXV.

Not only did this experiment show that a wet-bulb reading of 95° F. at hatching time held the dissemination of infection below 1 per cent, but it also gave an explanation of the reason. Close examination of the down of a chick just hatched will show a number of barbs protruding from one follicle. (Fig. 9.) Each group of barbs is surrounded by a capsule or sheath much like that of a new feather. As the chick dries and brushes itself against objects, this capsule or sheath is broken and in the form of irregular rod shaped pieces makes up a large part of the chick down found in the incubator after the hatch has been taken off. A chick hatched from an egg containing *S. pullorum* organisms would be well covered with them and when these are blown through the air, as was shown by Hinshaw *et al.* (9), they will infect healthy chicks. If a wet-bulb reading of 95° F. is maintained while the chicks are hatching, they do not fluff out so rapidly and the capsule surrounding the barbs does not break until the chicks have reached the brooder where the environment is less conducive to the spread of the disease-producing organisms. It is plain that chicks hatched at a wet-bulb reading of 75° F. would leave a maximum amount of chick down in the incubator, while those hatched at 95° F. would leave a minimum amount. The chicks hatched at the high wet-bulb reading were not so large, and the hatchability was not quite so good at the lower wet-bulb reading.

King (13) reported that there was a decrease in hatchability with very high humidity. Of 829 eggs hatched at 85° F. there was a hatchability of 57 per cent of total eggs set. Of 1,597 eggs hatched at a wet-bulb reading of 95° F. there was a hatchability of 48 per cent of the total eggs set. This is a difference of 9 per cent 12.77, a significant difference. Townsley (28) succeeded in getting better hatches and larger chicks when the wet-bulb reading was 90° F. during the hatch than at any other humidity. (A wet bulb of 85° F. is recommended except at the time of hatching.) Lamson and Kirkpatrick (15) showed that the per cent of hatch of fertile eggs was not affected by high humidity until the relative humidity in the incubator throughout the hatch was over 60 per cent (a wet-bulb reading of 87° F.).

Murphy (20) continued the study of humidity initiated by King, Payne, and Bushnell (14) the previous year. His experiments were conducted as described above, except that the temperature of the incubator was held at the recommended point except during the twentieth and twenty-first days (the time of hatching) when it was decreased, as indicated in the

TABLE XXVI—INFLUENCE OF THE RELATIVE HUMIDITY DURING THE HATCH ON HATCHABILITY AND LIVABILITY OF CHICKS.

	Temperature (F.)	Wet bulb (F.)	Per cent relative humidity	Eggs set	Per cent fertile	Per cent hatch of fertile eggs	Time of hatch	Difference in hours	Per cent "sticky" chicks	Per cent imperfectly healed navels	Av. weight of chicks at 2 weeks (gm.)	Average per cent mortality at 2 weeks
Group I												
Experimental	96	95	99.5	287	85.4	58.8	20d. 10h.	17	- -	- -	61.8	6.8
Control	100	85	56.5	287	84.3	59.5	19d. 17h.		- -	- -	61.9	7.2
Group II												
Experimental	98	95	91.5	264	85.0	76.2	20d. 9h.	12	1.2	8.1	68.6	2.6
Control	100	85	56.5	264	84.8	76.3	19d. 21h.		2.3	6.4	66.3	.7
Group III												
Experimental	100	95	84.5	270	84.8	79.9	20d. 3h.	3	13.1	23.5	66.8	4.2
Control	100	85	56.5	270	85.6	71.0	20d.		.6	7.9	64.4	3.6

table, in order to change the relative humidities. In Table XXVI, adapted from Murphy's report, are shown the results of the influence of humidity on hatchability.

There were three groups of eggs hatched at different degrees of humidity in each instance, also a control group was hatched in separate incubators run according to the procedure recommended by the manufacturers. Under the column "Time of Hatch" is recorded the time of the appearance of the first chick. There is included a list of the "sticky" chicks and those with improperly healed navels obtained from three of the nine hatches.

TABLE XXVII.—INFLUENCE OF HUMIDITY ON DISSEMINATION OF PULLORUM DISEASE THROUGH THE INCUBATOR.

(Summary of mortality results.)

Number of hatches	Wet-bulb reading (F.)	Per cent hatch of fertile eggs	Number of chicks	Mortality		Mortality due to *S. pullorum*	
				Number	Per cent	Number	Per cent
Group I							
4	95	70.2	374	90	24.1	80	21.4
3	90	71.2	358	44	12.3	41	11.5
Group II							
4	95	73.5	239	33	13.8	12	5.0
3	90	76.4	194	12	6.2	1	.
Group III							
4	95	76.4	252	19	7.5	5	2.1
3	90	75.2	195	19	9.8	1	.5
Group IV							
4	85	72.2	235	13	5.5	0	0.0
3	85	78.0	206	9	4.4	0	.0

Group I. Chicks from the reactor flock.

Group II. Chicks from a normal flock exposed in the same end of the machine with the Group I chicks.

Group III. Normal chicks exposed in the opposite end of the machine from Groups I and II.

Group IV. Control chicks from a normal flock hatched in a separate machine.

The term "sticky" chicks refers to chicks which have not fluffed out. The down adheres to the body and sometimes bits of shell or shell membrane are stuck to the down. These chicks appeared normal in all other respects.

Murphy continued the observations of the influence of humidity on the dissemination of pullorum disease through the incubator. The method of procedure was about the same as described above. Chicks from a disease-free flock were exposed at different degrees of humidity in the same incubator with chicks from a diseased flock. Only those chicks from which the pullorum organism was isolated were considered as having died of pullorum disease. Table XXVII includes results on seven hatches.

The results shown in Table XXVII vary slightly from those reported by King, who found a decrease in mortality with the higher relative humidity. King reported less mortality at a wet-bulb reading of 95° F. than at 85° F. while Murphy secured lower mortality at a wet-bulb reading of 90° F. than at 95° F.

In discussing his results, Murphy states that the hatch in Group I (Table XXVI) was low in both incubators because of the season. There was no difference in hatchability or livability in either Group I or II due to the difference in relative humidity during incubation. The Group III chicks with a dry-bulb reading of 100° F. and a wet-bulb reading of 95° F. gave a decrease in hatchability of 8.9 per cent below that of the controls. There were 12.5 per cent more sticky chicks and 15.6 per cent more chicks with improperly healed navels although the mortality at two weeks of age was approximately the same for both groups. Although such results may appear to be significant it is doubtful if they should be so considered because

TABLE XXVIII.—EFFECT OF LOW TEMPERATURE ON TIME OF HATCHING.

(Group I. Chicks from reactor flock.)

Pen	Hatch 1	Hatch 2	Hatch 3	Average
Experiments.....	20 days 0 hours	20 days 8 hours	20 days 22 hours	20 days 10 hours
Control	19 days 20 hours	19 days 14 hours	19 days 18 hours	19 days 17 hours

of the small number of experiments and the normal variability to be expected in such work. Murphy reported an increase of 15.7 per cent of fertile eggs hatched due to high humidity in one experiment, and a decrease of 12.3 per cent due to the same factor in another experiment under the same conditions. It is evident that averages of even a small number of experiments should be interpreted with caution.

There was also considerable difference in the time of hatching. When the temperature was lowered to 96° F. during the hatching period, the hatches averaged 17 hours later than the controls. The influence of this is illustrated by the effect on the three hatches which make up Group I. (Table XXVIII.)

Hatch 1 had been exposed to a temperature of 96° F. once; hatch 2, twice; and hatch 3, three times. There appeared to be a significant difference between the number of "sticky" chicks and chicks with improperly healed navels among those held at low, compared to those held at high relative humidity. Townsley (28) states that "cleaner chicks" are produced, with fewer "sticky" chicks when using a relatively high humidity in the incubator, provided the temperature is correct. According to his work with a Smith machine, when the humidity was increased the temperature should be reduced. Similar results are shown by Murphy. However, this might not apply under

different conditions or to all makes of incubators. In all of the hatches except two, the chicks from the high-humidity incubator were heavier than those from the control incubator. This was not due to difference in the weight of eggs set, since there was less than a gram difference in the average weights of the two groups.

King (13) observed that the "fluffing out" process was retarded at high humidities. Murphy collected the down during the first 24 hours of brooding of 150 chicks held at different degrees of humidity. The results are shown in figure 10. The

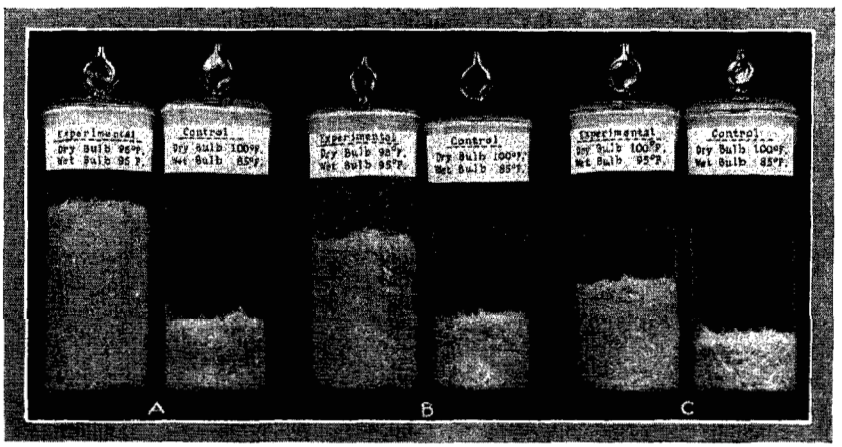

FIG. 10.—Vials showing the amount of "chick down" collected during the first 24 hours of brooding from groups of 150 chicks hatched at different wet-bulb readings. The down in the vial on the left in series (A) came from chicks hatched at a dry-bulb reading of 96°F. and a wet-bulb reading of 95°F. In series (B) the down in the vial on the left came from chicks hatched at a dry-bulb reading of 98°F. and a wet-bulb reading of 95°F. In series (C) the down in the left-hand vial came from chicks hatched at a dry-bulb reading of 100 and a wet-bullb reading of 95. In each series the down in the vial on the right came from chicks hatched at a dry-bulb reading of 100°F. and a wet-bulb reading of 85°F.

containers on the left in each pair of jars were held at dry-bulb readings of 96, 98, and 100° F., respectively. Those on the right were held at a uniform reading of 100° F.

Townsley (28) conducted experiments with three degrees of relative humidity, 33 per cent, 56 per cent, and 70 per cent. and wet-bulb thermometer temperatures of 75, 85, and 90° F., respectively. These incubators were operated with the same dry-bulb temperature reading (99° F.). The eggs in the machine with the high humidity hatched a full day earlier than those in the machine with medium humidity, and the eggs in the dry machine required a day longer than those in the medium-humidity incubator. It was also noted that the chicks from the high-humidity machine were smaller in size than those in the medium-humidity incubator and exhibited a characteristic type of stickiness, being smeared with the contents

of the egg. Many showed traces of blood in the shell. The chicks from the dry machine were also small in size and exhibited a different kind of stickiness. In this case the shell or shell membrane usually stuck to the chicks.

In Townsley's second series of experiments, the incubator with the low humidity was adjusted to 100° F. and the one with the high humidity reduced to 98° F. The machine with the medium humidity was continued at 99° F. This adjustment in temperature evened up the time required to complete the hatches. With the reduced temperature, the high-humidity incubator gave practically as good hatches and about the same viability as obtained from the medium-humidity incubator operated at the standard temperature. Also the chicks were larger and the stickiness mentioned above disappeared. In the low temperature machine, the hatch was poor throughout.

Townsley states that the relative degree of humidity in the incubator is not only important in its effect on the size and quality of the chicks, but that it also has a very important influence on the temperature at which the machine must be operated for maximum results. As the per cent of humidity is increased, the operating temperature needs to be decreased, and when the humidity is very low, the temperature needs to be run somewhat above normal.

The following directions (1) have been circulated among the users of the Buckeye incubator. They are included in some detail, since they are in conformity with good hatching practices.

The incubator should be fumigated three times during the period while the chicks are hatching, at such intervals that the greatest number of chicks will be exposed to the formaldehyde gas while they are still moist or before they are entirely fluffed out.

1. The first fumigation should be given when 15 to 20 per cent of the chicks are hatched. Chicks will not withstand repeated fumigation without some injury, therefore, all chicks which are exposed to each fumigation should be removed from the incubator whether they are wet or dry. They should be placed in chick boxes in a warm room. They will not be chilled if the regular number (25) is placed in each compartment as the number of wet ones will be small in proportion to the total number taken off after each fumigation.

2. The second fumigation should be given when 50 to 60 per cent of the chicks are hatched.

3. The third fumigation should be given when the hatch is about complete.

It has been determined by comparative tests that this fumigation when conducted according to instructions does not affect hatchability.

To be effective the wet-bulb reading in the incubator should be at least 86 and preferably 90° F. The higher the humidity the more effective the gas and the less danger of injuring the chicks.

The fumigation is carried out by putting the specified amount of formalin into a deep enamelware, glass, or porcelain bowl or dish with a rounded bottom, placing the dish on the fan board near the fan and then adding the required amount of potassium permanganate. The door should be closed at once and kept closed for from eight to 10 minutes, but not

longer, after which time the gas should be eliminated from the incubator. The old practice was to ventilate the machine for two or three minutes. We have found that by sprinkling a solution of ammonia on the walls of the air chamber below or above the fan boards, thus assuring rapid removal of the ammonia gas, the formaldehyde gas is neutralized at the end of the fumigation period and there is no need of ventilating the incubator. Temperature and humidity are conserved and the whole procedure is rendered less disagreeable to the operator.

The ammonia to be used should be a 26 per cent solution, the strongest grade obtainable, known as stronger ammonia water. It is carried by practically all druggists.

For all Buckeye Mammoth incubators use about one-half as much ammonia as formalin.

The incubator is kept closed for about eight to ten minutes after introducing the ammonia. If at the end of this time a strong odor of formaldehyde remains, increase slightly the amount of ammonia used or if the ammonia odor is strong, reduce the amount used.

If ammonia is not used the incubator should be thoroughly ventilated by opening all doors for two or three minutes after which the chicks should be removed as the chicks will not withstand repeated or prolonged exposure to fumigation.

APPENDIX

TABLE OF EQUIVALENTS

With the following wet- and dry-bulb temperatures the relative humidity will be the amount indicated in the table below.

DRY-BULB THERMOMETER

WET-BULB THERMOMETER Degrees F.	80°F.	85°F.	90°F.	95°F.	100°F.
	Relative Humidity*				
100					100
99					96
98					93
97					89
96					86
95				100	83
94				96	80
93				93	77
92				89	73
91				85	70
90			100	82	**68**
89			96	79	65
88			92	76	62
87			89	72	59
86			85	69	56
85		100	81	66	54
84		96	78	63	51
83		92	74	60	49
82		88	71	57	46
81		84	68	54	44
80	100	80	65	51	41
79	96	77	61	49	39
78	91	73	58	46	37
77	87	70	55	43	35
76	83	66	52	41	33
75	79	63	49	49	28

*Adapted from Kent's Mech. Engineer's book, p. 660, Tenth Edition.

DRY AND LIQUID MEASURE EQUIVALENTS

One ounce	=	28.35 grams
One gram	=	.03527 ounces
One fluid ounce	=	30 cubic centimeters
Sixteen fluid ounces or 1 pound	=	473 cubic centimeters
One quart	=	946 cubic centimeters

METHOD OR CALCULATING AMOUNT OF FUMIGANT TO USE

The cubical content of an incubator is determined by multiplying the width times the height times the depth. The total volume multiplied by the dosage per cubic foot will give the amount of material required for one fumigation.

Example: The incubator measures 6' wide, 5'8" high, and 8' deep and the recommended dosage is 0.4 cubic centimeter of formalin and 0.2 gram of potassium permanganate per cubic foot of air space.

6 x 5⅔ x 8	=	272 cubic feet
272 x 0.4	=	108.8 c.c. formalin
272 x 0.2	=	54.4 gms. potassium permanganate

To be effective, formaldehyde should not be used in temperatures below 60°F. and the relative humidity should be high. A wet-bulb temperature of 55°F. or higher should exist with a room temperature of 60°F. or a wet-bulb of 90°F. or above for incubators operating at 99 to 100°F.

Length of time exposed = 10 minutes.

Whenever it is advisable to add ammonia to neutralize formalin, use one-half as much ammonia as formalin. In the above example 55 c.c. would suffice.

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