A STUDY OF THE TECHNIQUES OF INFECTING CHICKENS WITH THE LARGE INTESTINAL ROUNDWORM, ASCARIDIA GALLI (SCHRANK, 1788)

by

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INTRODUCTION AND REVIEW OF LITERATURE

In the past 20 years or more, the large intestinal roundworm of fowls, Ascaridia galli (Schrank), has been utilized in laboratory experimentation. A careful examination of the studies reported by workers who have used this parasite and its host reveals a striking variation of the numbers of worms recovered from chicken to chicken even though a standardized number of ova were fed each chicken. Some studies which have utilized total worm number recoveries have revealed factors influencing the mean numbers and lengths of worms recovered as well as factors influencing their survival.

One of the factors involved in variations in worm numbers has been shown by Ackert (1931) to be the number of worm eggs administered to experimental chicks. He noted that variation in per cent of survival, mean length, and mean number of Ascaridia galli worms varied with the number of embryonated eggs used in the infection. Optimum survival and growth was obtained when 50 to 100 ova were given to each experimental chick. In the same paper, Ackert speculated that the following factors might influence numbers of worms in experimental infections: hatching rate of eggs, avian peristalsis, and a possible immunological factor.

Ackert, Cooper, and Dewhirst (1947) found that viability of A. galli eggs was a factor in worm number recoveries from chickens. Using the criterion of host infection, they found that the viability of the ova decreased as their age increased. The

eggs used were 36 and 120 days old.

Todd, Hansen, Kelly, and Wyant (1950) found that cultures incubated for 14-21 days at 30° - 33° C. gave the greatest virulence or host injury as measured by weight gains of the chicken and that a decrease in virulence resulted with greater age of A. galli cultures.

Todd (1952) also found that an optimum age exists with respect to viability of eggs. He found a corresponding decrease in the mean lengths and numbers of worms as the age of the culture increased.

Increased age of the embryonated eggs with a concomitant decrease in the amount of stored food material within the embryo has been shown by Ackert et al (1947) as a reason for the decreased viability of older A. galli cultures.

Elliot (1950) showed dimunition of reserve fat supplies with age was accompanied by a decrease in infectivity. Younger cultures were shown to be most infective.

Hansen, Oonyawangyse, and Ackert (1953) and Mahan (1952) in studying the effect of culturing ascarid ova in air or in water found no statistically significant differences between the number of worms recovered or weight gains of the infected and control chickens fed the two types of culture. However, the mean lengths of the worms recovered from the chickens fed ova cultured in air were greater than the mean lengths of worms recovered from chickens fed ova cultured in water. It appears from the report of Todd, Insko, Kelly, and Hansen (1949) that the numbers of species of nematodes present in chickens impedes the

growth of chicks in a progressive manner.

Symptoms of parasitosis produced by A. galli are most pronounced during a period of from 14 to 17 days subsequent to ingestion of infective ova. This period coincides with the tissue phase stage of the larva of this parasite (Ackert, 1931; Ackert and Tugwell, 1948).

Even though the previously reported studies utilized standard cultures of A. galli and standardized host conditions, the experimental chicks still yielded varying numbers of worms from chick to chick. Therefore, the present study was initiated to determine if infection techniques might be a factor in such variation. Current techniques as well as new techniques were studied to ascertain whether or not infection techniques could influence these observed variations in the number of worms recovered from a given lot of experimentally infected chicks.

MATERIALS AND METHODS

Source and Culture of A. galli Eggs

The eggs used throughout the study were obtained from living adult gravid females removed from freshly killed chickens at a commercial poultry dressing plant in Manhattan, Kansas. The females were washed in tap water and the posterior ends of the worms severed at the level of the anus. The uteri were pressed out into a Petri dish for rinsing in water and were then transferred to another Petri dish where the eggs were teased out of the uteri into the water. One part of 2 per cent formalin in 75

parts of water constituted the final culture media. The formalin was added to inhibit mold formation and occasional ciliate protozoan contamination both of which tend to delay or stop normal embryonation of the eggs. In each of the cultures prepared, uteri of four to five females were used. The cultures were incubated in 2 to 3 mm of water at 30° to 33° C. for 14 to 21 days before they were used in the infection studies.

It was found that, by using only the portion of the uterus closest to the vulva, higher percentages of embryonation could be obtained in the cultures. Ackert (1931) showed a progressive increase in the per cent of fertile eggs as the sampling of the uterus approached the vulva. However, in several uteri examined, the percentage of fertile eggs was not always the same in each branch of the uterus. One branch would exhibit a normal gradation in the numbers of fertile eggs, and the other branch would have only infertile eggs. Fertility can be determined by the presence of a clear equatorial spot in the center of the cytoplasm of the egg.

In an attempt to speed up the process of preparing A. galli egg cultures, the use of an artificial digestion solution acting on the uterine wall was tried and found to be practical. The uteri were removed, as previously described, and placed in a watch glass containing 2 to 3 ml of the digestive solution. This digestive solution has been used by Ackert and Tugwell (1948) to recover tissue phase A. galli larva from the intestinal wall of the chicken. This solution contains 0.5 per cent hydrochloric

acid and 1.0 per cent pepsin. Within two to three minutes the uterine wall was completely digested, leaving the fully developed eggs freely dispersed in the solution. Immature eggs were digested due possibly to an incomplete shell which afforded inadequate protection. No effect upon the rate of embryonation or percentage of the ova developed was observed.

The eggs dispersed in the digestion solution were pipetted into a thin micro-porous wax paper cup which was pressed around a cork and wedged into the upper end of a centrifuge tube. This arrangement allowed removal of the digestive solution which adhered to the eggs. The removal or washing of the digestive solution was done by adding water into the wax cup and centrifuging the water through the paper, leaving the eggs free of the digestive solution. Water was added through a hole in the cork between each centrifuging. The washing was found to be necessary as the digestive solution made an excellent medium for mold growth after uterine digestion. Another method of washing the eggs after digestion of the uterus, tried with some success, was the use of two thicknesses of number 20 silk bolting cloth as a screen during the addition of water. This method required no centrifuging as the water passed through the silk screen easily. After washing, the eggs were removed and placed in Petri dishes for incubation.

Chickens and Methods of Feeding *A. galli* Eggs

All chickens used in the three experiments were straight run White Rocks purchased from a single commercial hatchery. They

were received as day-old chicks and raised in electric brooders and battery cages. Standard commercial rations were used to feed them. Prior to experimental infections the chicks used in each of the three experiments were weighed, banded, and then separated into two groups of approximately equal weights. When the chicks were 14 days of age, each chick was fed 100 ± 10 embryonated ova of A. galli by means of a calibrated pipette inserted into its esophagus. A record was kept on the order of infection of each chick by means of the wing band number. The first chick randomly selected for infection was designated number 1 and so on through the entire infected group.

The chicks were infected by using a water suspension of ova and the standard procedures of other workers (Riedel, 1947). A small amount of washed fine sand was added to the vial containing the water and eggs. The sand served to break up the egg masses commonly found in such suspensions. The contents of the vial were then shaken gently because vigorous shaking may cause mechanical hatching, particularly with the older egg cultures. The infectivity of hatched larvae is not well known (Ackert, 1931). The same calibrated pipette was used for standardizing the infection solution at 100 ± 10 A. galli eggs per unit dosage as was used to infect the chicks. Microscopic counts of eggs per unit dose were made, and the standardization involved either addition of eggs or water until several microscopic counts gave a mean count of 100 ± 10 eggs per unit volume dose. Prior to infecting each chick, the vial was gently agitated. Eggs of A. galli tend to settle rather rapidly to the bottom of the vial;

hence, agitation between the infections of each chick insured more even distribution of the infective ova, thus each chick was more likely to receive the predetermined dose of 100 ± 10 eggs.

In one series of the experiments a sugar solution was used in place of the water medium for infecting chicks with A. galli ova. The proper specific gravity of the sugar solution needed to suspend the ova so that they neither rose nor settled in the solution was accomplished in two ways. A drop of sugar solution of known molarity and containing ova was placed under a microscope. An egg approximately near the center of the drop was focused upon and then observed for a period of five minutes to observe its movements. A second method for determining the proper specific gravity to suspend these eggs utilized an electrophotometer transmitting a wave length of 600 A. While not as accurate as the first-described method, it would record differences in light transmission due to settling or rising of the eggs over a time interval. Both methods established the fact that a 1.25 M sucrose solution suspended the ova of A. galli in a satisfactory manner. To prepare a standardized sugar solution suspension of eggs, a water-egg mixture was first made up with a unit number of eggs per dose in excess of the required number, 100 ± 10. The volume was determined and a proportionate weight of sugar added to obtain a 1.25 M solution. After several microscopic counts to estimate the number of eggs per unit volume, an additional amount of 1.25 M solution was added to adjust the egg count to 100 ± 10 eggs per unit dose.

Twenty-one days after infection with A. galli ova, the chicks were killed. A 21-day infection has been shown by Ackert and Tugwell (1948) and others to allow enough time for worms to leave the mucosal tissue of the intestine and to reside in the lumen of the host's intestine. At autopsy the intestine from the duodenal loop to the yolk sac diverticulum was removed from each chick and identified by placing with it the appropriate wing band. The intestinal contents were flushed into a glass jar using the hydraulic method of Ackert and Nolf (1929). The worms present were allowed to relax for several hours to straighten, and then were placed in 10 per cent formalin for later counting and measuring.

The worms recovered from the experimental chicks were measured with the aid of a view-camera. The image of each worm was projected on a ground glass plate of the view-camera and then traced on onionskin paper. All of the tracings were measured with a milled wheel which recorded their lengths in millimeters. These lengths were divided by the magnification factor of six in order to obtain actual lengths.

RESULTS

Counts of Eggs in Standardized Water and Sugar Suspensions

From Culture 1, was prepared Suspensions I and II, each standardized to 100 ± 10 A. galli ova per unit dose volume of infection solution. The average numbers of A. galli ova per count in Suspension I and in Suspension II were 129.45 ± 35.20 and

98.13 ± 15.06, respectively (Table 1). A regression coefficient was calculated using the numbers of A. galli ova per count as the variable and the order of count as the independent. The regression coefficients for egg numbers were 4.16 and 0.77, respectively. Only the regression coefficient of 4.16 was significant at the 5 per cent level. The difference in variance about the means of Suspension I and Suspension II was found to be significant at the 2 per cent level. The standard deviation of Suspension I was approximately twice that of Suspension II (Table 1). Figure 1 shows the series of counts made with the water suspension of eggs and with the sugar suspension of eggs. The regression lines are also shown. It can be seen by inspection that more of the sugar suspension egg counts stay within the desired deviation of ± 10 ova than do the water suspension counts.

From Culture 2, Suspension I and Suspension II were each standardized at 100 ± 10 ova per unit dose volume. The average numbers of ova per count for Suspension I and Suspension II were 96.8 ± 32.50 and 95.16 ± 13.10, respectively. The regression coefficient on egg numbers were 1.81 and 0.39, respectively. The regression coefficient of 1.81 was found to be significant at the 5 per cent level. The difference in variance about the means of Suspension I and Suspension II was found to be significant at the 2 per cent level. The standard deviation of Suspension I was approximately twice that of Suspension II (Table 1). Figure 2 shows the series of egg counts and the regression lines for the water and sugar suspensions of eggs. The number of egg counts within the desired limits of ± 10 ova was greatest in the

Table 1. Results of egg counts using the entire contents of the vials containing eggs suspended in water or in sugar solution.

Culture	Suspension*	Eggs: Initial number per unit dose	Eggs: Av. number and std. dev.	Statistics: Regression coefficient	Statistics: Significance of reg. coff. T test (%)	Statistics: Significance of variance F test (%)
1	I	100 ± 10	129.45 ± 35.20	4.16	1	
	II	100 ± 10	98.13 ± 15.06	0.77	-	2
2	I	100 ± 10	96.80 ± 32.50	1.81	5	
	II	100 ± 10	95.16 ± 13.10	0.39	-	2
3	I	50 ± 5	61.60 ± 14.53	1.86	1	
	II	50 ± 5	51.60 ± 5.42	0.29	-	2
4	I	50 ± 5	53.08 ± 13.00	0.67	1	
	II	50 ± 5	48.00 ± 9.38	0.38	-	10

* I = Water. II = Sugar solution.

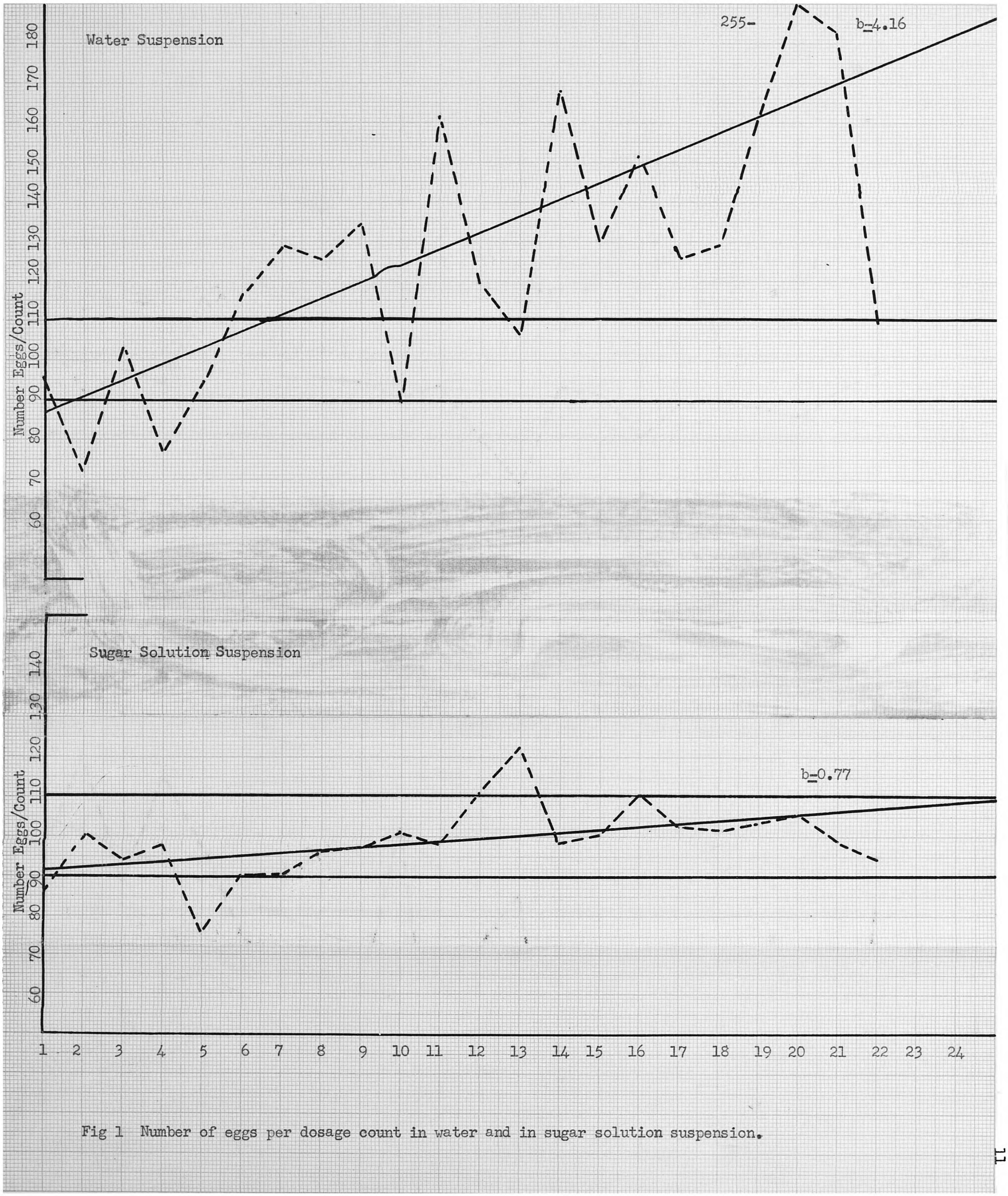

Fig 1 Number of eggs per dosage count in water and in sugar solution suspension.

Water Suspension

Number Eggs/Count

60 70 80 90 100 110 120 130 140 150 160 170 180

b=1.81

Sugar Solution Suspension

Number Eggs/Count

60 70 80 90 100 110 120 130 140

b=0.39

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Fig 2 Number of eggs per dosage count in water and in sugar solution suspension.

series of sugar suspension counts.

From Culture 3, Suspension I and Suspension II were each standardized at 50 ± 5 ova per unit dose (Table 1). The average numbers of ova per count in Suspension I and in Suspension II were 61.60 ± 14.53 and 51.60 ± 5.42, respectively. The regression coefficient of 1.86 was significant at the 1 per cent level. The difference in variance between Suspension I and Suspension II was found to be significant at the 2 per cent level. The standard deviation of Suspension I was approximately three times as large as that of Suspension II. Figure 3 shows the series of egg counts made from Culture 3 using the water suspension and the sugar suspension media. Regression lines are also shown. The greater number of counts found within the desired deviation of ± 5 ova is found in the sugar series of counts.

From Culture 4, Suspension I and Suspension II were each standardized at 50 ± 5 ova per unit of dose volume (Table 1). The average numbers of ova per count for Suspension I and Suspension II were 53.08 ± 13.00 and 48.00 ± 9.38, respectively. The regression coefficients on egg numbers per count were 0.67 and 0.38, respectively. The coefficient of 0.67 was significant at the 1 per cent level. The difference in variance between Suspension I and Suspension II was significant at the 10 per cent level. The standard deviation about the mean in Suspension I was larger than that of Suspension II. Figure 4 shows the series of counts made from Culture 4 using both the water and sugar suspension of eggs. The number of counts found within the desired limits of + 5 ova is greatest in the sugar suspension

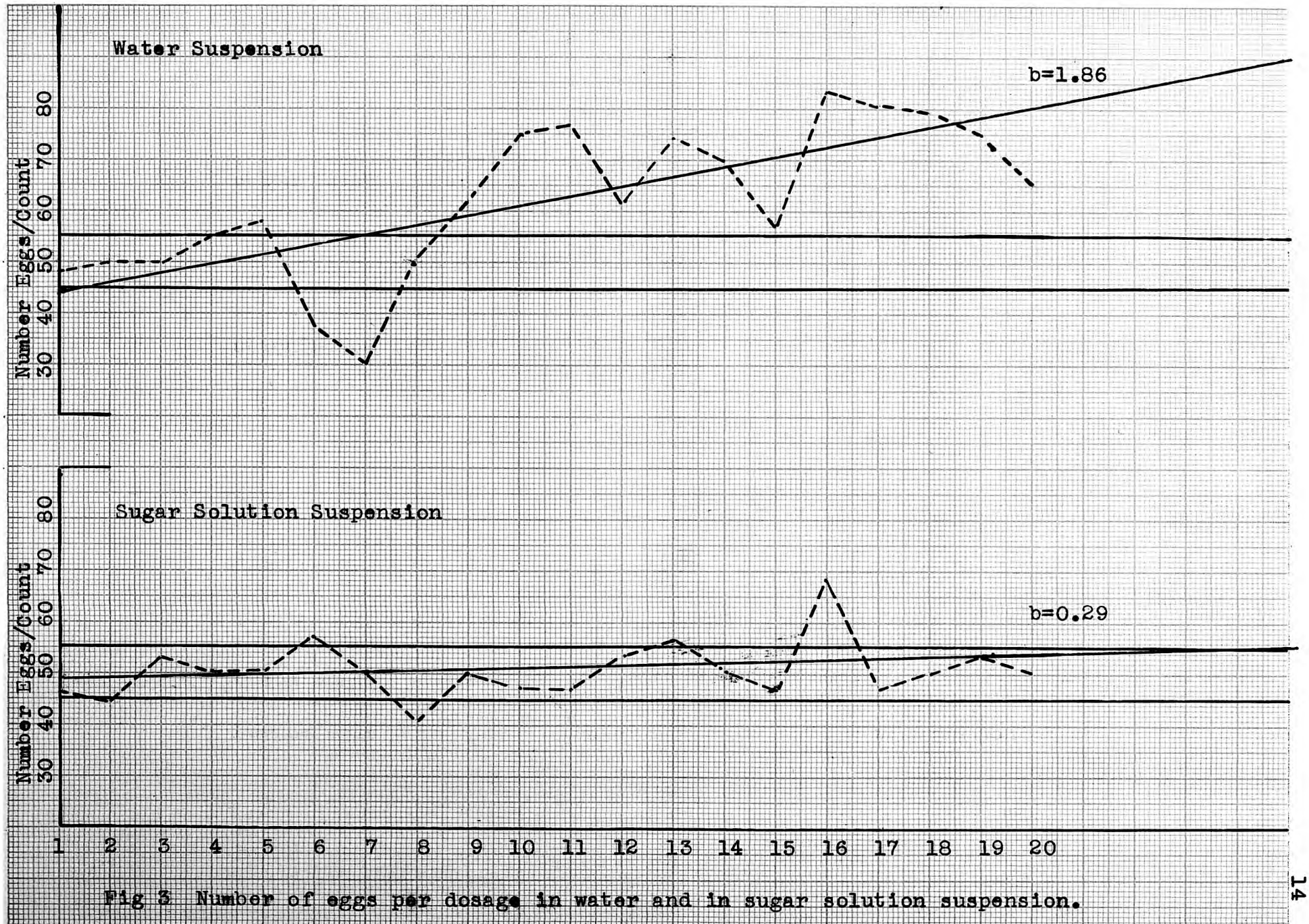

Fig 3 Number of eggs per dosage in water and in sugar solution suspension.

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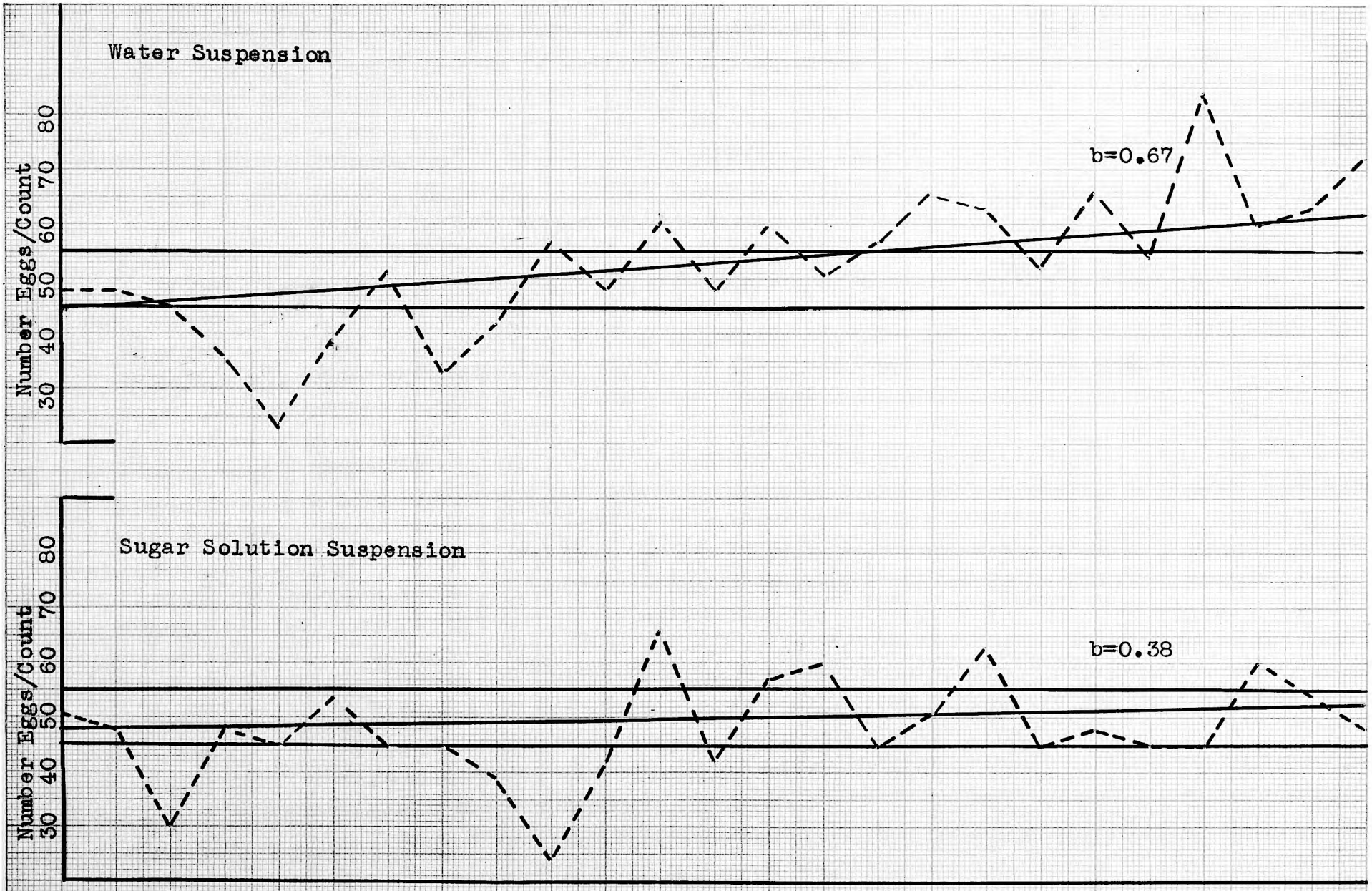

Fig 4 Number of eggs per dosage in water and in sugar solution suspension.

series of counts.

The combined results of counts of eggs in water and in sugar suspensions showed significant differences. In all water suspension counts there were significant increases in the numbers of ova per count as sampling progresses through the entire contents of the vial. As the volume of the water solution was decreased by removal of each unit dose, the number of eggs per count became progressively greater with a significant positive regression slope. No such significant positive regression slope occurred in any of the sugar suspension counts. The variation of counts about the mean was significantly higher in all counts of eggs suspended in water as compared with counts of eggs suspended in sugar solution. Therefore, in the water suspension, two significant results were shown: (1) the increase in the number of eggs per count as the volume decreases, and (2) the greater variation from count to count irrespective of an increase in the mean number.

The greater variation encountered in water suspensions may also influence initial standardizations at the desired level prior to infection of experimental chickens. Comparison of average egg counts to initial standardization showed that the sugar average counts were within the desired plus or minus limits in all cases; whereas, the water count averages in Cultures 1 and 3 were outside the desired limits (Table 1).

Egg counts taken from remaining infection solutions of A. galli eggs suspended in water used by several other workers showed higher counts in the remaining solution than in the in-

itial standardization, as well as a variation exceeding the desired plus or minus 10 ova. Egg counts recorded from experiments where sugar was used in solution to suspend the eggs showed less variation and an average count which is approximately that of the initial standardization. The egg counts taken from remaining infection solutions served to support the conclusion that sugar provides more constancy in numbers of ova per unit dose and less variation about the desired mean number of ova.

Experiment 1

Because the previously reported studies revealed the marked superiority of the sugar medium over the water medium in maintaining a consistency in the numbers of ova per dose, three experiments were conducted to test whether or not the sugar medium method would likewise yield less variation in the numbers of worms recovered from chick to chick. Each of these three experiments utilized two groups of chicks of equal weights. The chicks in Group I were fed 100 $\pm$ 10 ova in the water medium; whereas, those in Group II received the same numbers of eggs suspended in a 1.25 M sugar solution. The eggs were obtained from the same cultures. Identical techniques and equipment were used to infect the fowls in both groups.

The results of Experiment 1 showed that the chicks in Group I and Group II made average weight gains of 151.86 gm and 136.02, respectively (Table 2). Statistical analysis of the differences in weight gains between Groups I and II revealed no significant

Table 2. Comparison of weight gains of chicks and the numbers and lengths of worms recovered from chicks fed eggs suspended in water or in sugar solution.

Experiment number	Group* number	Chicks: Number	Chicks: Av. wt. gain (gm)	Worms: Av. number and std. dev.	Worms: Av. length and std. dev. (mm)
1	I	38	151.86	16.0 ± 8.06	25.0 ± 0.60
	II	37	136.02	5.2 ± 3.61	24.2 ± 0.63
2	I	20	173.45	4.7 ± 0.57	20.1 ± 0.45
	II	20	193.70	7.8 ± 0.55	20.3 ± 0.88
3	I	15	207.00	6.6 ± 2.30	17.2 ± 0.76
	II	14	217.70	3.0 ± 2.35	16.4 ± 0.66

* Group I = Chicks fed ova suspended in water.

Group II= Chicks fed ova suspended in sugar solution.

difference in average weight gains. The regression coefficients of weight gains computed on the order of infection for chicks in Group I and Group II were -1.660 and -0.950, respectively. A significant coefficient on weight gains was found only in Group I (Table 3).

An average of 16.0 worms per chicks were recovered from the chicks in Group I; whereas, an average of 5.2 worms were recovered from the chicks in Group II (Table 2). The difference in mean numbers of worms recovered from the chicks proved to be statistically significant. The regression coefficients on worm numbers recovered by order of infection of the chicks were + 0.832 and + 0.118. The regression coefficient of + 0.832 was found to be significant (Table 3).

The average lengths of the worms recovered from Group I and Group II were 25.0 mm and 24.2 mm, respectively. No significant difference was found between the worm lengths of the two groups.

The results of this experiment showed that the regression coefficient of Group I was significantly negative and exceeded the coefficient of weight gains of Group II (Table 3). This means that the drop in weight gains from the first chicks infected to the weight gains made by the last chicks to be infected was greater from bird to bird in the water-infected chicks than it was in the sugar-infected chicks. This drop was accompanied by a corresponding increase in the number of worms recovered from chicks first infected to chicks infected at the end of the order of infection. The increase in worm numbers recovered per chick was the greatest in Group I.

Table 3. Comparison of regression coefficients of worm numbers and weight gains of chicks by order of infection with ova suspended in water or in sugar solution.

Experiment number	Group* number	Regression coefficient	
		Worm number	Weight gain
1	I	0.832**	-1.660**
	II	0.118	-0.950
2	I	0.165	-0.392
	II	-0.066	0.143
3	I	0.573	-1.230
	II	-0.413	-0.087

* Group I = Chicks fed ova suspended in water.
Group II = Chicks fed ova suspended in sugar solution.

** Significant at the 5 per cent level.

There has been a question as to whether or not chicks lose A. galli ova immediately following experimental oral infections, so this experiment was designed to obtain additional data relative to that problem. The possibility of the chicks losing A. galli ova by placing their beaks in the drinking water shortly after an infection could be a factor in the variation of worm number recoveries from chick to chick. A study on the possible loss of A. galli ova following an experimental oral infection was also included in this experiment. The fecal samples were collected from the chicks 24 hours subsequent to exposure to ova. The samples of feces were screened to eliminate the larger particles and washed with repeated additions of water and centrifuging until a concentrated fecal sample was obtained. A saturated sodium nitrate solution was added to the concentrated fecal sample until a meniscus formed at the top of the vial. A glass slide was placed upon the meniscus. Any A. galli eggs present would be displaced from the bottom of the vial, rising to the top and adhering to the glass slide. Microscopic examination of the glass slide revealed the absence of any eggs from either Group I or Group II. Guberlet (1924) reported both free larvae and unhatched larvae from the feces of chicks following an experimental infection of 750 A. galli ova per chick. Possibly the greater number of ova per chick used by Guberlet explains his findings.

The drinking water recovered during the 24-hour period following infection was examined for the presence of A. galli ova. The sediment in the bottom of the drinking water pans was also

examined using the flotation technique. There were no A. galli ova found in the drinking water or in the sediment from the pans in either Group I or Group II.

Experiment 2

The results of Experiment 2 showed that the chicks in Group I made average weight gains of 173.45 gm; whereas, the chicks in Group II made average weight gains of 193.70 gm (Table 2). Statistical analysis of the differences in weight gains between Group I and Group II gave no significant differences in average weight gains. The regression coefficients of weight gains by infection order of the chicks in Group I and Group II were -0.392 and +0.143, respectively. The regression coefficients on weight gains showed no significant slope in either group (Table 3).

An average of 4.7 worms per chick were recovered from the chickens in Group I; whereas, an average of 7.8 worms were recovered from the chicks in Group II. The difference in mean numbers of worms recovered was statistically significant. The regression coefficients on worm numbers recovered by infection order were +0.165 and -0.066. These regression coefficients showed no significant slope.

The average lengths of the worms recovered from Group I and Group II were 20.1 mm and 20.3 mm, respectively. There was no significant difference between the mean lengths of the worms from Group I and Group II.

The results of Experiment 2 support the trends evident in

Experiment 1. The regression coefficient on weight gains of chicks of Group I is again negative and of a greater absolute value than the weight gain regression coefficient of the chicks in Group II. The average weight gains are not significantly different. The correlation between worm number regression and weight gain regression again, as in Experiment 1, presents an inverse relationship. As the worm number increases or decreases there is a corresponding respective decrease or increase in weight gains. This relationship is demonstrated more sharply in the water-infected chicks.

Experiment 3

The results of Experiment 3 revealed that the chicks in Group I and Group II made average weight gains of 207.00 gm and 217.70 gm, respectively. Statistical analysis of the difference in average weight gains between Group I and Group II were not significant. The regression coefficients of weight gains by infection order were -1.230 and -0.087 for Groups I and II, respectively. There was no significant slope in either of the two weight gain regression coefficients.

An average number of 6.6 worms per chick was recovered from the chicks in Group I; whereas, an average number of 3.0 worms was recovered in Group II. The difference in the average number of worms recovered was found to be significant. The regression coefficients of worm numbers recovered by infection order of the chicks were +0.573 and -0.413 for Groups I and II, respectively. There was no significant slope in either of the two worm number

regression coefficients.

The average lengths of the worms recovered from Group I and Group II were 17.2 mm and 16.4 mm, respectively. No significant difference was found to exist between the worm lengths of these two groups.

The results of Experiment 3 showed that in Group I, along with an increase in worm numbers recovered as the infection order progresses, there was a concomitant decrease in the weight gain regression coefficient. Again, as in the preceding experiments, the coefficients in Group I have greater slope than those in Group II. Group II showed negative regression coefficients for numbers of worms recovered as well as for weight gains. The negative regression coefficient for weight gain has the least slope of any of the weight gain coefficients in the three experiments.

Combined Results of Experiments

There were no statistically significant differences in average weight gains made by the chicks of Groups I and II in the three experiments. In Experiments 2 and 3, the sugar-infected groups made the higher average weight gains; whereas, in Experiment 1, the sugar-infected group made the smaller comparative weight gain during the three weeks of infection. The weight gain regression coefficients of the water-infected chicks were all negative, and within each experiment exhibited greater slope than the weight gain regression coefficients of the sugar-infected groups. With the exception of Group I, Experiment 1, the weight

gain regression coefficients were not statistically significant.

The average number of worms recovered per chick was higher in the water-infected groups of Experiments 1 and 3 than among the sugar-infected groups in the same experiments. In Experiment 2, the reverse was true with a higher number of worms recovered in the sugar-infected group. However, in an analysis of variance on average worm numbers recovered, combining the three experiments, it was found that a significant difference does exist between the numbers of worms recovered from sugar-infected chicks and from water-infected chicks. The grand means of worm numbers recovered from the water groups and the sugar groups were 9.1 and 5.3 worms per bird for Groups I and Groups II, respectively. It was also found by the analysis of variance of worm numbers that the time interval between the three experiments did not introduce a significant variation in the numbers of worms recovered between each experiment. The lack of significance in time interval between each experiment would support the standardization of each experiment in all respects with the exception of variation in infection techniques.

The regression coefficients on worm number recoveries in all water-infected groups were positive and within each experiment were of greater value than the worm number regression coefficients of the sugar-infected chicks. A significant regression coefficient on worm numbers was found in Group I, Experiment 1. Other coefficients were not significant, but did approach significance in the water-infected groups of Experiments 2 and 3.

The average worm lengths of Group I and Group II in each

experiment were not of significant difference.

Out of a total of six experimental groups in the three experiments, five of them showed an inverse relationship between worm number and weight gain regressions. As the numbers of worms recovered increased from chick to chick in the order of infection, there was a corresponding decrease in the weight gains among the same chicks. Within each experiment this relationship was of greater negative correlation in the water-infected groups than in the sugar-infected groups.

DISCUSSION

Variation in worm numbers recovered from experimentally infected chicks may be due to many factors. Elimination of variables leaving only, as far as could be determined, host variation, parasite variation, and infection techniques, as was done in this study, showed that techniques have been a variable factor. In the actual egg counts of water suspensions it can be shown that the plus or minus 10 eggs deviation does not provide adequate limits by which to demarcate egg doses fed to chicks; nor does the dosage stay at the desired mean level of 100 or 50 eggs per dose. Water counts show a significant rise in eggs per unit dose, which correlates with worm numbers recovered by order of infection of chicks. It was also noted that, with such increase in worm numbers, there is a corresponding decrease in weight gains of the chicks.

The use of eggs in a sugar suspension provides less variation from bird to bird as to numbers of eggs given. Thus, re-

gardless of the variation of the host or of the parasite, one may be reasonably sure that the initial infection is fairly constant and would generally stay within the desired limits of the potential infection. The general decrease in significance of regression coefficients of the sugar-infected worm number recoveries and the constancy of the microscopic counts of eggs suspended in sugar solution would support this point.

The removal of the increased numbers of eggs by order of infection with the use of a sugar suspension solution may allow an experimental variable to assert its effect more strongly. The increase of eggs per unit dose with the water infection may supersede or mask such variables.

A comparison of regression coefficients of weight gains shows greater slope in the water-infected groups as compared to sugar-infected groups within each experiment. Egg counts and worm number regressions show increase as the order of sampling of the infection solution progresses. Thus, with more larvae in birds at the end of the order of infection and less larvae in the birds found near the beginning of the infection order, the difference in weight gain regressions between the water-infected groups and the sugar-infected groups may be explained as the effect of larval numbers during the tissue phase of the A. galli life cycle.

No significant difference in the mean length of worms can be shown. This fact shows that the sugar suspension used in infection of chicks does not inhibit worm growth after infection.

It should be noted that the increase in eggs per unit dosage in infection of chickens does not invalidate previous results of other workers who utilized the water suspension of ova for infection of chicks. The standard practice has been to alternate between the control and the experimental groups in the infection of chicks. Thus, any increase in eggs per dose would be evenly distributed between the two groups as would any of the effects of such increase. However, in any infection which did not alternate from control to experimental in administering the infective dose, there would exist a variable between the two groups in the number of ova given to each chick. With the use of a sugar suspension in infection, infection of an entire group without alternating to the control group could be done with little variation in numbers of ova given to each chick.

The past study showed that infection techniques have been a variable and that sugar infection will reduce variation in worm number recoveries from experimentally infected chicks. However, it is felt that techniques are not the entire answer to variation in worm number recoveries from chicks and that other data must be obtained to explain this variation more fully. It has also been shown that the sugar solution suspension will not insure an exact number of eggs per bird in an experimental infection even though it is a definite improvement over the water suspension technique.

It has been demonstrated by various workers that the symptoms of parasitism produced by A. galli are most pronounced at the time when they are in the tissue phase stage of their life

cycle (Ackert, 1931; Ackert and Tugwell, 1948; Todd et al, 1949; and Todd and Hansen, 1951). The principal symptoms of parasitosis at this time are manifested in retarded growth rates of the infected host. Todd and Hansen (1951) reported evidence suggesting two types of host parasite relationships between A. galli and the chick. In the first type, the chick regarded the parasite as intolerable and made an attempt to or did eliminate the worms. This reaction retarded the growth rate of the chick. In the second type of relationship, the chick regarded the parasite with tolerance and made efficient weight gains, while the parasite likewise grew more rapidly. This work does not mean that worm numbers are not important, for in every group of chicks infected there may be several of the birds which regard the parasite as intolerable and so would be affected by varying numbers of A. galli present in an infection. These chicks would have weight gains varying quite possibly with the initial number of A. galli ova used to give the potential larval infection. For those chicks which regard the A. galli as intolerable, a uniform initial infection as results when eggs are suspended in sugar solution would be necessary for experimental uniformity.

As previously stated, there were significantly more worms recovered from the chicks fed the water-egg suspensions than were recovered from the chicks fed eggs suspended in sugar solution in Experiment 1. Likewise, there was a significant trend in this experiment for the numbers of worms to increase in the chicks fed the water-egg suspension as the order in which they were infected progressed. No similar relationships between the

worm numbers recovered and worm number increase by infection order were noted in Experiments 2 and 3. In Experiment 1, the same volume, approximately 10 to 12 ml, of infecting solution was used for 80 chicks as was used in Experiments 2 and 3 for infecting 40 chicks. In Experiment 1, all but a few millimeters of the infection solution was used; whereas, in the latter experiments use was made of approximately one-half of the infection solution. The difference in volume of infection solution used in Experiment 1 and in Experiments 2 and 3 offers a plausible explanation for the observed results in Experiment 1. It has been shown that, where a water suspension of eggs is used, the last third of a suspension yields numbers of eggs per dose far in excess of the initial standardized limits. The first two-thirds of the volume yield numbers of eggs per dose which are relatively close to the initial standardization. Thus, in Experiment 1, which utilized the entire amount of the suspension to infect the 80 chicks, the chicks receiving the terminal third of the total egg-water suspension received doses of eggs far in excess of those chicks which were infected with the initial two-thirds of the suspension. The larger dose of eggs fed the former chicks accordingly developed into more worms. It is evident, therefore, that one should use only about one-half the volume of a water-egg suspension when experimentally infecting chicks with A. galli. This would tend to eliminate dosages with the higher egg count occurring at the bottom portion of the infection vial contents in a water suspension. In a sugar suspension of eggs this would not be a factor as there is no settling out of eggs

causing the cumulative increase of eggs per unit volume in the last portion of the infection solution.

SUMMARY

A study was made of the current as well as new techniques employed in experimentally infecting chicks with embryonated eggs of Ascaridia galli to ascertain whether or not these techniques were responsible for the variation in the numbers of worms recovered from chick to chick. Three experiments were conducted utilizing 144 White Rock chicks separated, in each experiment, into two equal weight groups. In each group, each chick was fed 100 ± 10 A. galli embryonated eggs. The chicks of Group I of each experiment were fed eggs suspended in water; whereas, the chicks of Group II were fed eggs suspended in a 1.25 M sugar solution. A record of the order of infection was kept of each chick in both groups. The order of infection was recorded to determine if the position of the chick in the infection order influenced the numbers of worms recovered from the chick at autopsy. The results of the study on infection techniques are as follows:

1. Microscopic counts of eggs suspended in water showed a significant increase in the numbers of eggs per unit volume as the volume of the infection mixture was decreased. These counts also showed that the desired deviation of plus or minus 10 eggs was not accurately describing the actual deviation occurring in egg-water infection mixtures.

2. Microscopic counts of eggs suspended in a 1.25 M sugar

solution showed that the number of eggs per unit dose did not increase significantly as the volume of the infection solution decreased. These counts showed less variation in numbers of eggs per unit dose between samples and were significantly of less variation than the water variation in egg counts.

3. In all of the experiments there were no significant differences in weight gains between the chicks in Group I and Group II.

4. With the exception of Group I, Experiment 1, there were no significant regression coefficients of weight gains by order of infection of chicks.

5. The results of all of the experiments show that the chicks in Group I harbored significantly more worms than did the chicks in Group II.

6. The regression coefficients of numbers of worms recovered from chicks by order of infection in Groups I and II showed no significant slope except in Group I of Experiment 1. However, there was a consistent trend for the regression coefficient of worms recovered from the chicks in Group I to follow a positive slope.

7. In five out of six of the groups of chicks there was an inverse relationship between the regression coefficients of weight gains and the numbers of worms recovered.

8. There were no significant differences between the lengths of the worms recovered from the two groups of chicks in all of the experiments.

9. Group I, Experiment 1, showed the most significant in-

crease of worm number recoveries and weight gain decreases of any of the other groups. This was due to the use of the last third of the water-egg infection mixture with its increased number of eggs per unit dose, resulting in increased worm numbers toward the end of the infection order.

10. The use of an artificial digestive solution completely digested the uteri of the A. galli females; thus, a more complete liberation of the eggs from the uterus was effected in preparation of cultures. This reduced the numbers of egg masses in the infection mixture when experimentally infecting chicks.

11. The use of an electrophotometer for standardizing dosages of A. galli eggs for infection purposes was found not to be practical. The inability of the electrophotometer to distinguish the embryonated eggs from the unembryonated eggs at high levels of embryonation made it necessary to make special counts to determine percentage of embryonation.

12. The variation in worm numbers recovered from chick to chick following an experimental infection can be explained only in part as resulting from infection techniques. Apparently, other factors such as physiological variations of the chick and/or the parasite influence the numbers of worms surviving.

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A STUDY OF THE TECHNIQUES OF INFECTING CHICKENS WITH THE LARGE INTESTINAL ROUNDWORM, ASCARIDIA GALLI (SCHRANK, 1788)

by

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It has been observed that considerable variation has existed in the numbers of Ascaridia galli recovered from chick to chick following an experimental infection. Therefore, the present study was initiated to determine if present infection techniques were a factor in explaining this observed variation in worm number recoveries from chicks.

All A. galli eggs used in the study were obtained from living adult gravid females removed from freshly killed chickens. The eggs were cultured in 2 to 3 mm of water at 30° C. to 33° C. for 14 to 21 days prior to use in the infection studies. The use of an artificial digestion solution aided in preparing egg cultures due to complete breakdown of the uterine wall.

Microscopic counts of embryonated eggs were taken of standardized infection mixtures utilizing the entire contents of the vial. The infection mixtures were both the water-egg mixture and the 1.25 M sugar solution suspension of eggs. Both mixtures were standardized at either 100 ± 10 or at 50 ± 5 embryonated eggs per unit volume. The counts were recorded by order of sampling from the vial as it would occur in an actual infection of chicks. These counts were made to determine the actual numbers of eggs and variation per unit dose occurring in infection after the initial standardization.

All chicks used in the three experiments were straight run White Rocks received as one-day-old from a single commercial hatchery. In each experiment the chicks were separated into two groups of equal weight. Group I was infected with 100 ± 10 em-

bryonated eggs suspended in water, whereas Group II received a like number of eggs suspended in a 1.25 M sucrose solution. A record of the order of infection was kept by means of wing band numbers. The chicks were killed at the age of five weeks, after three weeks of infection, and the worms recovered from the intestine. The worms were preserved in 10 per cent formalin and measured by projecting the images of the worms on a ground glass plate of a view camera. A milled wheel recorded lengths of worms in millimeters.

The results of the study of infection techniques are as follows:

1. Microscopic counts of eggs suspended in water showed a significant increase in the numbers of eggs per unit volume as the volume of the infection mixture was decreased. These counts also showed that the desired deviation of plus or minus 10 eggs about the mean of 100 eggs per unit dose was not accurately describing the actual deviation occurring in the egg-water infection mixtures.

2. Microscopic counts of eggs suspended in a 1.25 M sucrose solution showed that the numbers of eggs per unit volume did not increase significantly as the volume of the infection solution decreased. These counts showed less variation in numbers of eggs per unit dose between samples, and were significantly of less variation than the water variation in egg counts.

3. In all of the experiments there were no significant differences in weight gains between the chicks in Group I and Group II.

4. With the exception of Group I, Experiment 1, there were no significant regression coefficients of weight gains by order of infection of chicks.

5. The results of all of the experiments show that the chicks in Group I harbored significantly more worms than did the chicks in Group II.

6. The regression coefficients of numbers of worms recovered from chicks by order of infection in Groups I and II showed no significant slope except in Group I of Experiment 1. However, there was a consistent trend for the regression coefficient of worms recovered from the chicks in Group I to follow a positive slope of greater value than the respective slope of Group II.

7. In five out of six of the groups of chicks there was an inverse relationship between the regression coefficients of weight gains and numbers of worms recovered.

8. There were no significant differences between the lengths of the worms recovered from the two groups of chicks in all of the experiments.

9. Group I, Experiment 1, showed the most significant increase of worm number recoveries and weight gain decreases of any of the other groups. This was due to the use of the last third of the water-egg infection mixture with its increased number of eggs per unit dose, resulting in increased worm numbers toward the end of the infection order.

10. The use of an artificial digestive solution completely digested the uteri of the A. galli females; thus, a more complete

liberation of the eggs from the uterus was effected in preparation of cultures. This reduced the numbers of egg masses in the infection mixture when experimentally infecting chicks.

11. The use of an electrophotometer for standardizing dosages of A. galli eggs for infection purposes was found not to be practical. The inability of the electrophotometer to differentiate the embryonated eggs from the unembryonated eggs at high levels of embryonation made it necessary to make special counts to determine percentage of embryonation.

12. The variation in worm numbers recovered from chick to chick following an experimental infection can be explained only in part as resulting from infection techniques. Apparently, other factors such as physiological variations of the chick and/or the parasite influence the numbers of worms surviving.