COMPARATIVE RATE OF DEVELOPMENT AND VIABILITY OF *ASCARIDIA GALLI* EGGS CULTURED RESPECTIVELY IN AIR AND WATER

by

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INTRODUCTION AND REVIEW OF LITERATURE

Knowledge of the rate of development, resistance, and viability of eggs of nematodes has been accumulating for nearly a century. Leuckart (1879), working with the ova of Ascaris lumbricoides, Parascaris equorum (Syn. Ascaris megalocephala), Toxocara canis and several free living nematodes, found that a damp environment was necessary for normal development of the ova. He later pointed out that while moist air was a favorable medium for their growth, they were nevertheless capable of enduring dryness for weeks and even months. During the years, various other investigators have contributed to this field of study. Most of the work has been confined largely to the Ascaris of man and pig, and comparatively little work has been done on the rate of development and viability of the eggs of Ascaridia galli (Schrank, 1788).

Otto (1929) found that the ova of the human and pig Ascaris did not develop to the embryo stage in an atmosphere less than 80 per cent saturated, although they remained viable for more than 28 days. McRae (1935), working with the ova of A. galli, reported that only an occasional ovum developed beyond the morula stage, and none beyond the tadpole stage when kept in an atmosphere 40 to 50 per cent saturated. Ova kept in a relative humidity of 77 to 82 per cent at 22° C. remained viable from 2 to 4 weeks but rarely developed beyond the morula

stage. Ova kept in 82 to 86 per cent relative humidity at 22° C. developed more slowly than those in water. At this relative humidity only 4 per cent of the ova disintegrated. Ova kept in 100 per cent relative humidity at 30° C. developed at the same rate as did those in water.

Ackert (1931) reported the optimum developmental temperatures for A. galli eggs to be 30° to 33° C. At early cleavage stages, ova survived temperatures of -12 to -8° C. for 15 hours, but not for 22 hours. Twelve hours' exposure to 43° C. proved to be lethal for eggs in all stages of development. Ackert and Cauthen (1931) reported that the ova of A. galli (syn. A. lineata) were destroyed in 3 weeks when exposed in less than ½ inch of unshaded soil during the summer, but ova in shaded places remained viable from spring to autumn. At depths of 2 to 6 inches viability was markedly increased. In 2 inches or less of soil ova failed to survive normal winter (subzero) weather in Manhattan, Kansas.

Levine (1937) reported that temperatures ranging from 0° C. to subzero affected the degree of destruction of embryonated A. galli ova. Embryonated ova in soil exposed to weathering in the shade were viable for 242 days (March to November, 1935) but were not viable when tested the following January after having been subjected to freezing weather Embryonated ova thoroughly dried in feces survived for 21 days but they were not viable after 51 days. Nonembryonated ova exposed to weathering in shade survived the winter and were

embryonated when tested after exposure of 279 days. Non-embryonated ova exposed to sun also survived the winter and were viable after 186 days but not after 245 days. Nonembryonated ova in soil survived for 45 days but not 52 days at -1° C.

Ackert, Cooper, and Dewhirst (1947) reported that egg cultures of A. galli incubated in water at 30° to 33° C. for a period of 36 days proved to be more viable than eggs of a 120-day old culture. Embryonated eggs from both the 36-day old culture and 120-day old culture were fed to the chickens. The average worm length of 26.3 mm was obtained from the 36-day old culture as compared with the average worm length of 15.5 mm from the 120-day old culture.

Knowledge of the rate of development and the viability of the ascarid eggs cultured in air and in water has been presented in the above review of literature. But information on the comparative rates of development of such nematode eggs, day by day, in air and in water appeared to be lacking.

Investigations of ascarid parasitism are more readily carried out by culturing the ova in water than by culturing in air. The objectives of the present study are to determine the rate of development of chicken ascarid eggs in water and in air cultures, as well as the viability of the embryos developing from the ova in the two types of cultures.

MATERIALS AND METHODS

Source of Eggs

Eggs of Ascaridia galli used in the experiments were obtained from the bodies of 10 living gravid female worms. The uteri from each of the gravid worms were removed by severing the anterior end of each of the worms with a razor blade and gently pressing out the uteri and other internal organs with a small spatula. Approximately 4 to 5 mm lengths of the uteri were removed from the region of the vagina and placed in a Syracuse dish containing 3 to 5 drops of distilled water. This portion of the uteri contained approximately 98 per cent of the fertilized eggs (Ackert, 1931). The eggs were gently teased from the uteri with a dissecting needle and examined under the low power of a compound microscope to determine the fertility of the eggs. A fertilized egg may be detected by a clear equatorial area in the cytoplasm (Ackert, 1931).

Egg Cultures

Two hundred eggs were placed on each of 8 glass slides, the slides were then placed in either 90 per cent relative humidity or in water for 30 days at 30° C. The slide cultures

of eggs were prepared in the following manner. A square measuring 6 x 6 mm was scratched on each of the 8 glass slides with the aid of a diamond point pencil. A thin layer of Meyer's egg albumen was smeared on 4 of the slides which were to be placed in water. The albumen acted as an adhesive and prevented the eggs from floating off the slides. A platinum wire loop was used to transfer 200 eggs to each of the 8 slides. The number of eggs per slide was ascertained by an actual count made with the aid of a compound microscope. Excess eggs were removed with a small piece of moist filter paper. The egg masses on the 4 slides to be placed in 90 per cent relative humidity was permitted to dry, care being taken not to allow the eggs to become too dry. Such preliminary drying of the egg masses resulted in their firmly sticking to the surface of the slide.

The 4 slides containing the eggs to be cultured in water were placed in Petri dishes and covered with tap-water to a depth of 1 to 2 mm. The Petri dishes were then placed in an incubator, the temperature of which was maintained at 30° C.

The 4 slides containing the eggs to be cultured in air were placed in humidity chambers in which the air was maintained at 90 per cent relative humidity by chemical means. The humidity chambers were glass jars 5 inches in diameter by 5½ inches high and covered by screw tops. Inside the jars were glass supports made by fusing 4 right-angle glass rods. These rods supported a wire screen on which were placed the slides

containing the eggs. Each jar contained about 300 cc of a sulfuric acid solution which according to Buxton and Mellanby (1934) would maintain a relative humidity of 90 per cent. The sulfuric acid solution was prepared in the following manner. The stock solution of sulfuric acid consisted of equal volumes of concentrated sulfuric acid and distilled water. To 161 cc of stock solution was added 712 cc of distilled water and the desired quantity of this solution was then poured into each jar.

The egg development was observed with a compound microscope at intervals of 48 hours for a period of 30 days. To facilitate examination, a drop of water was placed on the egg mass at every examination and subsequently dried by exposure to an air current produced by an electric fan. When the slides were dry, they were returned to the humidity chamber.

The various stages of egg development were determined by comparison with the illustrations of Ackert (1931). Because of the irregularity in the rate of egg development as well as the numerous developmental stages, it was thought best to use the following developmental classification of eggs: 1-cell, 2-cell, 4-cell, 5-8 cell, 9-32 cell, early morula, late morula, tadpole, vermiform, and coiled embryo stage.

The number of eggs developing from the 1-cell stage to the coiled embryo stage during the 30-day incubation period was used as the criterion for measuring the rate of egg development in both types of egg cultures.

The mortality rate among the ova was determined by establishing a ratio of dead ova to the total number of ova observed. Dead ova are characterized by the presence of granulation, vacuolation or a clearing of the cytoplasm.

Viability Tests

At the end of 30 days of incubation, the viability of the A. galli ova cultured in air and in water was determined by feeding 200±10 embryonated eggs to each of 20 chicks. Ova to be fed to the chicks were removed from the culture slides by putting a drop of water on the slides and then wiping the ova off with a small piece of bread. This bread was then force-fed to the chicks used in the test. Each slide was then examined under a compound microscope in order to determine whether or not the ova had been completely removed from the slides. If any ova were found on the slide, a second wiping was made with another piece of bread. One group of 10 chicks received 200±10 embryonated ova which had been cultured in 90 per cent relative humidity for 30 days and each of 10 chicks in the second group was fed 200±10 embryonated ova which had been cultured in water for the same length of time.

In an effort to maintain uniformity, all of the chickens used in this study were Single Comb White Leghorns purchased from an approved commercial hatchery. They were received as day-old chicks and raised in electric brooders and battery

cages. The chicks used in Experiment 1 were 19 days old and those in Experiment 2 were 26 days old when they were parasitized. The 20 chicks used in each of the experiments were weighed and divided into two groups of approximately equal weights.

A. galli Recovered from Chicks

The parasitized experimental chicks were sacrificed 21 days following exposure to embryonated A. galli eggs. At autopsy, the intestine from the gizzard to the yolk sac diverticulum was removed and the contents flushed into a glass jar with water under pressure by the hydraulic method of Ackert and Nolf (1929). In addition, in the second experiment each intestine was slit open after it had been flushed out, and the mucosa was scraped off and kept in a separate jar for further examination.

All of the worms that were collected were left in the jars for about 6 hours at which time they became relaxed and could be fixed in an extended condition. Fixation was accomplished by adding commercial formaldehyde to the jars in quantity sufficient to make a 10 per cent concentration. The formalized intestinal contents were poured into a large bottom glass container held over a dark background, and the macroscopic worms were removed with the aid of a small curved teasing needle to vials containing 10 per cent formalin. The

worms from each chick were placed in separate vials. The remaining intestinal contents were poured, a little at a time, into a 10 cm Petri dish cover which had been marked with a series of parallel lines 10 mm apart. The contents were then systematically examined under a wide field binocular microscope and all of the remaining worms were removed and placed in the vials with the macroscopic worms. The number, length, and sex of the worms were determined later.

Measurement of the larger worms was accomplished with the aid of a large view back camera. The camera was adjusted so that the image of the worm on the ground glass plate was enlarged six times. A tracing of the enlarged image of the worm was made on tissue paper, then the length of the tracing was measured in millimeters with a milled wheel which reduced by six times the length of the tracings (Ackert et al., 1935; Ackert, Whitlock, Freeman, 1940). Measurement of the smaller worms was performed by means of tracings made with the aid of a camera lucida. The tracings were measured with the graduated milled wheel and the length of each worm was recorded.

EXPERIMENTAL RESULTS

Experiment 1

On October 12, 1950, egg cultures as previously described were prepared using female A. galli worms designated as A, B,

C, D, and E (Table 1).

Rate of Development. As mentioned previously, the criterion used for comparing the rate of development of the ova in air and in water cultures was the stage of egg development (Table 1). On the 2nd day of incubation, 837 ova in the water culture were in the early morula stage whereas only 128 ova in the air culture had reached the early morula stage. On the 4th day of incubation, 780 ova in the water culture were in the tadpole stage as compared to 366 ova in the tadpole stage in the air culture. This trend prevailed through the 6th and the 8th day of incubation and was strikingly illustrated on the 10th day of incubation when 37 of the ova in the water culture had reached the coiled embryo stage of development whereas none of the ova in the air culture had gone beyond the vermiform stage. From the 12th to the 30th day of incubation, the total number of the ova which had reached the coiled embryo stage in the water culture exceeded the total number of ova in the same stage of development in the air culture even though the change in the total number of coiled embryos in the water culture was not appreciable after the 14th day. As indicated by the total number of ova attaining the coiled embryo stage, a tendency toward slowing of the developmental rate can be noted in the water culture at about the 14th day, but is not present to any great extent in the air culture until about the 16th day. These results showed that the ova cultured in water underwent a more rapid embryogeny

than ova cultured in 90 per cent relative humidity.

The mortality rate of the ova in the air culture exceeded that of the water culture (Table 1). A total of 60 ova were found dead in the air culture on the 30th day of observation as compared with a total of 4 dead ova in the water culture.

Viability. The test of viability of the coiled embryos developing in both types of culture was initiated on November 11, 1950 and was terminated on December 2, 1950.

The results of the viability test are given in Table 2. The average number of worms recovered at autopsy from 10 chicks fed 200±10 embryonated ova which had been cultured in 90 per cent relative humidity was 4.3 and the average length of these worms was 24.2 mm. The total number of worms recovered from the 10 chicks was 43. The average number of worms recovered from the 10 chicks fed 200±10 embryonated ova which had been cultured in water was 2.9 with an average length of 4.7 mm. The total number of worms recovered was 29.

Table 1. Rate of development of A. galli ova in 90 per cent relative humidity at 30° C. as compared with ova in water culture. Two hundred ova from each of five worms were used in the preparation of cultures, *Group I.

Days of incubation	Worm culture no.	Egg development: Cell stage: 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
2	A	2	-	7	-	10	-	18	-	19	2	107	1	33	195	-	-	-	-	-	-	-	-	4	2	-	-	-	-
	B	4	-	10	-	3	-	3	-	3	1	138	-	34	199	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	C	6	5	6	6	3	5	3	2	21	7	132	8	18	161	-	-	-	-	-	-	-	-	6	6	5	-	-	-
	D	8	3	12	-	-	-	7	2	18	3	104	11	43	179	-	-	-	-	-	-	-	-	4	2	4	-	-	-
	E	25	12	17	-	32	-	28	7	31	24	63	46	-	103	-	-	-	-	-	-	-	-	-	6	4	2	-	-
Total		45	20	52	6	48	5	59	11	92	37	544	66	128	837	-	-	-	-	-	-	-	-	14	16	18	2	-	-
4	A	-	-	4	-	6	-	17	-	4	1	5	-	8	-	12	-	138	197	-	-	-	-	4	2	2	-	-	-
	B	-	-	-	-	1	-	4	-	1	-	4	1	18	-	31	4	133	195	-	-	-	-	-	-	8	-	-	-
	C	1	4	4	4	2	2	2	4	3	3	-	4	62	7	45	12	67	154	-	-	-	-	6	6	8	-	-	-
	D	-	2	9	-	-	-	5	1	14	4	25	7	46	13	63	37	28	134	-	-	-	-	4	2	6	-	-	-
	E	5	3	18	-	4	2	12	3	5	2	28	7	94	12	30	63	-	100	-	-	-	-	-	6	4	2	-	-
Total		6	9	35	4	13	4	40	8	27	10	62	19	228	32	181	116	366	780	-	-	-	-	14	16	28	2	-	-
6	A	-	-	3	-	6	-	12	-	3	1	6	-	10	-	6	-	93	-	54	197	-	-	4	2	3	-	-	-
	B	-	-	-	-	1	-	2	-	2	-	4	1	7	-	14	-	12	-	148	199	-	-	-	-	10	-	-	-
	C	1	4	-	4	-	-	3	3	4	2	2	8	51	6	17	29	48	24	60	114	-	-	6	6	8	-	-	-
	D	-	2	8	-	-	-	4	1	12	3	11	7	22	10	53	3	58	6	21	166	-	-	4	2	7	-	-	-
	E	4	2	3	-	5	2	3	1	16	2	18	6	83	4	51	-	13	4	-	171	-	-	-	6	4	2	-	-
Total		5	8	14	4	12	2	24	5	37	8	41	22	173	20	141	32	224	34	283	847	-	-	14	16	32	2	-	-
8	A	-	-	3	-	6	-	4	-	3	1	8	-	12	-	4	-	21	-	131	197	-	-	4	2	4	-	-	-
	B	-	-	-	-	1	-	2	-	2	-	3	1	6	-	10	-	2	-	160	199	-	-	-	-	11	-	3	-
	C	1	4	-	-	-	-	2	2	3	4	2	5	38	6	21	12	26	8	91	153	-	-	6	6	10	-	-	-
	D	-	2	7	-	-	-	2	1	12	3	4	7	11	10	17	3	52	6	84	166	-	-	4	2	7	-	-	-
	E	4	2	2	-	3	2	3	1	6	2	15	6	32	4	63	-	42	4	26	171	-	-	-	6	4	2	-	-
Total		5	8	12	-	10	2	13	4	26	10	32	19	99	20	115	15	143	18	492	886	-	-	14	16	36	2	3	-
10	A	-	-	1	-	2	-	-	-	4	1	7	-	9	-	10	-	14	-	139	186	-	11	4	2	6	-	4	-
	B	-	-	-	-	1	-	2	-	2	-	3	1	6	-	8	-	4	-	157	183	-	16	-	-	11	-	6	-
	C	1	4	-	-	-	-	1	2	2	4	3	5	36	6	18	12	22	4	93	157	-	-	6	6	14	-	4	-
	D	-	2	6	-	-	-	2	1	12	3	-	7	14	8	8	4	3	3	140	162	-	6	4	2	7	2	4	-
	E	4	2	2	-	3	2	3	1	4	2	9	6	24	4	43	-	41	4	63	167	-	4	-	6	4	2	-	-
Total		5	8	9	-	6	2	8	4	24	10	22	19	89	18	87	16	84	11	592	855	-	37	14	16	42	4	18	-
12	A	-	-	1	-	2	-	-	-	2	1	4	-	6	-	8	-	4	-	122	165	32	32	4	2	8	-	7	-
	B	-	-	-	-	1	-	2	-	2	-	3	1	6	-	4	-	4	-	119	158	36	41	-	-	11	-	12	-
	C	1	4	-	-	-	-	1	2	2	4	2	5	31	6	15	8	14	6	95	147	10	12	6	6	16	-	7	-
	D	-	2	6	-	-	-	2	1	12	3	-	7	14	8	6	4	5	3	108	140	26	28	4	2	9	2	8	-
	E	4	2	2	-	3	2	3	1	4	2	9	6	18	4	35	-	36	4	70	144	12	27	-	6	4	2	-	-
Total		5	8	9	-	6	2	8	4	22	10	18	19	75	18	68	12	63	13	514	754	116	140	14	16	48	4	34	-

Table 1. (cont.)

Days of incubation	Worm culture no.	Cell stage 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Egg development: Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
14	A	-	-	1	-	2	-	-	-	2	1	4	-	3	-	6	-	4	-	81	7	73	190	4	2	9	-	11	-
	B	-	-	-	-	1	-	2	-	2	-	3	1	6	-	4	-	4	-	79	3	71	196	-	-	11	-	17	-
	C	1	4	-	-	-	-	1	2	1	4	2	5	29	6	13	8	6	6	75	18	43	141	6	6	16	-	7	-
	D	-	2	6	-	-	-	1	1	12	3	-	7	14	8	5	4	6	3	67	20	67	148	4	2	10	2	8	-
	E	4	2	2	-	3	2	2	1	4	2	7	6	12	4	28	-	18	4	61	10	52	161	-	6	4	2	3	-
Total		5	8	9	-	6	2	6	4	21	10	16	19	64	18	56	12	38	13	363	58	306	836	14	16	50	4	46	-
16	A	-	-	1	-	2	-	-	-	2	1	4	-	2	-	5	-	3	-	11	4	141	193	4	2	10	-	15	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	5	-	7	-	2	-	8	3	141	196	-	-	11	-	19	-
	C	1	4	-	-	-	-	1	2	1	4	2	5	29	6	11	8	5	6	12	18	106	141	6	6	17	-	9	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	7	18	12	108	159	4	2	13	2	14	-
	E	4	2	2	-	3	2	2	1	4	2	6	6	12	4	25	-	18	4	26	10	90	161	-	6	4	2	4	-
Total		5	8	9	-	6	2	6	4	21	10	14	18	62	14	51	10	35	17	75	47	586	850	14	16	55	4	61	-
18	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	4	-	7	4	144	193	4	2	10	-	17	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	5	-	4	-	3	-	9	3	139	196	-	-	11	-	22	-
	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	11	8	4	6	10	15	109	144	6	6	18	-	10	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	4	12	8	111	166	4	2	15	2	15	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	19	1	16	4	23	6	99	165	-	6	4	2	8	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	60	15	41	11	34	14	61	36	602	844	14	16	58	4	72	-
20	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	4	-	7	4	140	193	4	2	12	-	19	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	4	-	3	-	8	3	136	196	-	-	11	-	27	-
	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	10	15	108	144	6	6	18	-	13	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	110	168	4	2	15	2	16	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	17	1	14	4	17	6	109	165	-	6	4	2	8	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	38	11	31	12	54	36	603	866	14	16	60	4	83	-
22	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	2	-	6	4	139	193	4	2	12	-	23	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	4	-	3	-	6	3	136	195	-	-	11	-	29	1
	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	8	15	107	144	6	6	18	-	16	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	108	168	4	2	15	2	18	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	15	1	12	4	17	6	108	165	-	6	4	2	13	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	36	11	27	12	49	36	598	865	14	16	60	4	99	1
24	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	2	-	6	4	138	193	4	2	12	-	24	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	4	-	3	-	6	3	135	195	-	-	11	-	30	1
	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	8	12	104	147	6	6	18	-	19	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	106	168	4	2	15	2	20	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	15	1	12	4	17	6	103	165	-	6	4	2	18	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	36	11	27	12	49	33	586	868	14	16	60	4	111	1

Table 1. (concl.)

Days of incubation	Worm culture no.	Egg development: Cell stage 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	2	-	6	4	138	193	4	2	12	-	24	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	3	-	2	-	7	3	134	195	-	-	11	-	32	1
26	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	8	12	102	147	6	6	18	-	21	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	104	168	4	2	15	2	22	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	15	1	12	4	17	6	101	165	-	6	4	2	20	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	35	11	26	12	50	33	579	868	14	16	60	4	119	1
	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	2	-	6	4	135	193	4	2	12	-	27	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	3	-	2	-	4	3	135	194	-	-	11	-	34	2
28	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	8	12	101	147	6	6	18	-	22	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	104	168	4	2	15	2	22	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	15	1	12	4	17	6	101	165	-	6	4	2	20	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	35	11	26	12	47	33	576	867	14	16	60	4	125	2
	A	-	-	1	-	2	-	-	-	2	1	2	-	3	-	4	-	2	-	6	4	134	193	4	2	12	-	28	-
	B	-	-	-	-	1	-	2	-	2	-	2	1	4	-	3	-	2	-	4	3	133	194	-	-	11	-	36	2
30	C	1	4	-	-	-	-	1	2	1	4	1	5	28	6	10	8	3	6	8	12	97	147	6	6	18	-	26	-
	D	-	2	6	-	-	-	1	1	12	3	-	6	14	4	3	2	7	2	12	8	101	168	4	2	15	2	25	-
	E	4	2	2	-	3	2	2	1	4	2	6	4	10	5	15	1	12	4	17	6	101	165	-	6	4	2	20	-
Total		5	8	9	-	6	2	6	4	21	10	11	16	59	15	35	11	26	12	47	33	566	867	14	16	60	4	135	2

* A = Air culture (90 per cent relative humidity).
W = Water culture.

Table 2. Results of viability tests. Two hundred embryonated A. galli ova from the 90 per cent relative humidity culture and the water culture, respectively, were fed to 19-day-old chicks, Group I.

Chicken number	Worm culture letter	Ova cultured in 90% rel. humidity: No. of worms: Male	Female	Av. length of worms, mm: Male	Female	Chicken number	Worm culture letter	Ova cultured in water: No. of worms: Male	Female	Av. length of worms, mm: Male	Female
A4234	AI	7	11	25.4	28.8	A4192	AI	0	0	0	0
A4224	AII	1	1	30.0	15.0	A4217	AII	0	3	0	2.8
A4235	BI	0	0	0	0	A4219	BI	0	0	0	0
A4191	BII	0	0	0	0	A4223	BII	1	2	16.6	3.5
A4190	CI	1	3	25.0	30.0	A4215	CI	0	0	0	0
A4213	CII	0	0	0	0	A4229	CII	1	0	3.3	0
A4194	DI	3	2	20.3	28.3	A4230	DI	5	6	2.7	2.8
A4208	DII	3	2	19.4	25.0	A4218	DII	0	2	0	2.0
A4210	EI	2	2	21.6	25.0	A4197	EI	1	1	3.3	3.3
A4204	EII	4	1	16.4	28.3	A4225	EII	3	4	7.4	2.4
Total		21	22	158.1	180.4			11	18	33.3	16.8
Average		2.1	2.2	22.6	25.8			1.1	1.8	6.7	2.8

Experiment 2

Experiment 2 was initiated on October 19, 1950 and was terminated on November 18, 1950. Eggs of A. galli to be cultured in air and in water were collected from another 5 female worms designated as A, B, C, D, and E (Table 3). All methods and procedures used in Experiment 1 were repeated in Experiment 2.

Rate of Development. The embryogeny of the ova of A. galli cultured in air and in water is given in Table 3. On the 2nd day of incubation 937 ova in the water culture developed to an early morula stage as compared with 87 ova at the same developmental stage in the air culture. On the 4th day of observation 14 ova in the water culture developed to the vermiform stage, whereas none of the ova in the air culture had gone beyond the vermiform stage. Further evidence which indicated that the ova in the water culture developed more rapidly than the ova in the air culture was demonstrated by the greater number of ova in the coiled embryo stage as compared to those in the water culture from the 10th to the 30th day of incubation.

The mortality rate of the ova in the water culture was lower than that of the air culture as demonstrated by the presence of only one dead ovum in the water culture on the 30th day of observation as compared with a total of 47 dead

ova in the air culture.

Viability. The test of the viability of the coiled embryos developing in the above cultures was initiated on November 18, 1950 and was terminated on December 8, 1950.

The results of the viability test are shown in Table 4. The average number of worms recovered from the 10 chicks exposed to 200±10 embryonated ova from the air culture was 3.8 with an average length of 25.9 mm. The total number of worms recovered was 38. The average number of worms recovered from the 10 chicks exposed to the ova cultured in water was 12.5 with an average length of 6.2 mm. The total number of worms recovered was 125.

Table 3. Rate of development of *A. galli* ova in 90 per cent relative humidity at 30° C. as compared with ova in water culture. Two hundred ova from each of five worms were used in the preparation of cultures, Group II.

Days of incubation	Worm culture no.	Egg development: Cell stage 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
	A	11	-	16	-	4	-	25	2	33	-	86	3	21	194	-	-	-	-	-	-	-	-	-	1	4	-	-	-
	B	-	-	-	-	-	-	-	-	13	1	158	1	29	198	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	C	22	5	32	3	16	-	12	-	23	7	87	18	-	164	-	-	-	-	-	-	-	-	5	3	3	-	-	-
	D	21	4	14	-	-	-	3	1	15	6	119	2	24	185	-	-	-	-	-	-	-	-	-	2	4	-	-	-
	E	3	-	-	-	-	-	3	-	14	1	163	2	13	196	-	-	-	-	-	-	-	-	-	1	4	-	-	-
Total		57	9	62	3	20	-	43	3	98	15	613	26	87	937	-	-	-	-	-	-	-	-	5	7	15	-	-	-
	A	2	2	4	0	2	-	13	1	10	1	11	1	37	-	32	2	80	190	-	2	-	-	-	1	9	-	-	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	15	-	4	-	181	190	-	8	-	-	-	-	-	-	-	-
4	C	4	2	2	2	7	-	6	-	4	1	17	4	42	-	76	11	31	177	-	-	-	-	5	3	6	-	-	-
	D	9	2	3	-	4	-	6	-	8	4	45	3	34	3	18	3	65	179	-	4	-	-	-	2	8	-	-	-
	E	2	-	-	-	-	-	-	-	2	1	-	-	23	-	8	2	161	196	-	-	-	-	-	1	4	-	-	-
Total		17	6	9	2	13	-	25	1	24	8	73	9	151	3	138	18	518	932	-	14	-	-	5	7	27	-	-	-
	A	2	2	2	-	-	-	7	1	8	1	14	2	24	-	18	-	46	2	69	191	-	-	-	1	10	-	-	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	7	-	6	-	43	2	142	196	-	-	-	-	2	-	-	-
6	C	1	2	-	1	3	-	2	-	2	2	3	3	8	-	32	-	35	1	101	188	-	-	5	3	8	-	-	-
	D	7	2	3	-	2	-	-	-	5	3	28	2	19	3	48	2	24	1	53	185	-	-	-	2	11	-	-	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	9	2	57	1	120	197	-	-	-	1	4	-	-	-
Total		12	6	5	1	5	-	9	1	17	7	45	9	64	3	113	2	205	7	485	957	-	-	5	7	35	-	-	-
	A	2	2	2	-	-	-	2	1	4	1	12	1	15	1	16	-	54	2	78	191	-	-	-	1	15	-	-	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	6	1	186	197	-	-	-	-	2	-	-	-
8	C	1	2	-	1	3	-	2	-	2	2	3	3	7	-	11	-	21	1	134	188	-	-	5	3	11	-	-	-
	D	7	2	2	-	1	-	-	-	4	3	18	2	13	3	38	2	17	1	88	185	-	-	-	2	12	-	-	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	3	-	11	1	172	197	-	-	-	1	4	-	-	-
Total		12	6	4	1	4	-	4	1	12	7	33	8	45	4	70	2	109	6	658	958	-	-	5	7	44	-	-	-
	A	2	2	2	-	-	-	2	1	4	1	12	1	15	1	16	-	54	2	78	178	-	13	-	1	15	-	-	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	3	1	184	179	-	18	-	-	2	-	5	-
10	C	1	2	-	1	1	-	-	-	3	2	3	3	4	-	10	-	17	1	144	177	-	11	5	3	11	-	1	-
	D	7	2	2	-	1	-	-	-	4	3	17	2	9	1	19	1	18	2	111	173	-	14	-	2	12	-	-	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	3	-	10	1	173	191	-	6	-	1	4	-	-	-
Total		12	6	4	1	2	-	2	1	13	7	32	8	38	2	50	1	102	7	690	898	-	62	5	7	44	-	6	-
	A	2	2	2	-	-	-	2	1	4	1	6	-	12	2	7	-	24	2	79	113	43	78	-	1	16	-	3	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	3	1	141	104	36	93	-	-	2	-	12	-
12	C	1	2	-	1	1	-	-	-	3	2	3	3	4	-	6	-	11	1	132	126	17	62	5	3	11	-	6	-
	D	7	2	2	-	1	-	-	-	3	3	12	2	7	1	18	1	15	2	89	113	32	74	-	2	14	-	-	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	3	-	10	1	132	114	36	83	-	1	4	-	5	-
Total		12	6	4	1	2	-	2	1	12	7	21	7	33	3	36	1	63	7	573	570	164	390	5	7	47	-	26	-

Table 3. (cont.)

Days of incubation	Worm culture no.	Egg development: Cell stage 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	7	-	12	2	58	11	79	180	-	1	16	-	4	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	88	4	87	193	-	-	2	-	15	-
14	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	8	1	89	12	64	176	5	3	11	-	9	-
	D	7	2	2	-	1	-	-	-	3	3	9	2	8	1	15	1	16	2	76	5	49	182	-	2	14	-	-	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	3	-	6	1	92	7	73	190	-	1	4	-	12	-
Total		12	6	4	1	2	-	2	1	12	7	18	6	28	4	31	1	44	7	403	39	352	921	5	7	47	-	40	-
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	7	-	6	2	13	11	125	180	-	1	16	-	9	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	14	4	157	192	-	-	2	-	19	1
16	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	4	1	14	12	142	176	5	3	11	-	10	-
	D	7	2	2	-	1	-	-	-	3	3	9	2	8	1	13	1	14	2	26	5	97	182	-	2	14	-	6	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	6	-	2	-	4	1	14	4	148	193	-	1	4	-	18	-
Total		12	6	4	1	2	-	2	1	12	7	18	6	28	4	28	1	30	7	81	36	669	923	5	7	47	-	62	1
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	7	-	6	2	8	11	126	180	-	1	16	-	13	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	14	4	154	192	-	-	2	-	22	1
18	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	4	1	8	10	141	178	5	3	11	-	17	-
	D	7	2	2	-	1	-	-	-	3	3	9	2	8	1	10	1	14	2	24	5	99	182	-	2	14	-	9	-
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	9	4	148	193	-	1	4	-	25	-
Total		12	6	4	1	2	-	2	1	12	7	18	6	26	4	24	1	31	7	63	34	668	925	5	7	47	-	86	1
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	12	8	126	183	-	1	16	-	13	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	156	192	-	-	2	-	26	1
20	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	4	1	8	4	141	184	5	3	11	-	17	-
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	9	1	7	2	19	5	114	181	-	2	14	-	12	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	9	4	148	193	-	1	4	-	25	-
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	21	1	22	7	56	25	685	933	5	7	47	-	93	2
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	6	8	125	183	-	1	16	-	20	-
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	153	192	-	-	2	-	29	1
22	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	2	1	6	4	141	184	5	3	11	-	21	-
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	5	1	6	2	19	3	114	183	-	2	14	-	17	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	9	4	146	193	-	1	4	-	27	-
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	17	1	19	7	48	23	679	935	5	7	47	-	114	2
	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	6	8	122	181	-	1	16	-	23	2
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	153	192	-	-	2	-	29	1
24	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	2	1	6	4	141	184	5	3	11	-	21	-
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	5	1	6	2	19	3	114	183	-	2	14	-	17	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	9	3	143	194	-	1	4	-	30	-
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	17	1	19	7	48	22	673	934	5	7	47	-	120	4

Table 3. (concl.)

Days of incubation	Worm culture no.	Egg development: Cell stage 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile cell A	Non-fertile cell W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
26	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	6	8	118	181	-	1	16	-	27	2
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	151	192	-	-	2	-	31	1
	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	2	1	6	4	141	183	5	3	11	-	21	1
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	5	1	4	2	17	3	115	183	-	2	14	-	20	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	6	3	145	192	-	1	4	-	31	2
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	17	1	17	7	43	22	670	931	5	7	47	-	130	7
28	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	6	8	118	181	-	1	16	-	27	2
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	151	192	-	-	2	-	31	1
	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	2	1	6	4	139	183	5	3	11	-	23	1
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	5	1	4	2	17	3	111	183	-	2	14	-	24	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	6	3	145	192	-	1	4	-	31	2
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	17	1	17	7	43	22	664	931	5	7	47	-	136	7
30	A	2	2	2	-	-	-	2	1	4	1	6	-	8	2	5	-	4	2	6	8	116	181	-	1	16	-	29	2
	B	-	-	-	-	-	-	-	-	-	1	-	1	4	-	2	-	2	1	8	4	151	192	-	-	2	-	31	1
	C	1	2	-	1	1	-	-	-	3	2	3	2	2	1	4	-	2	1	6	4	139	183	5	3	11	-	23	1
	D	7	2	2	-	1	-	-	-	3	3	6	2	6	1	5	1	4	2	17	3	108	183	-	2	14	-	27	1
	E	2	-	-	-	-	-	-	-	2	-	-	1	4	-	1	-	5	1	6	2	141	192	-	1	4	1	35	2
Total		12	6	4	1	2	-	2	1	12	7	15	6	24	4	17	1	17	7	43	21	655	931	5	7	47	1	145	7

* A = Air culture (90 per cent relative humidity).
W = Water culture.

Table 4. Results of viability tests. Two hundred embryonated *A. galli* ova from the 90 per cent relative humidity culture and the water culture, respectively, were fed to 26-day-old chicks, Group II.

Chicken number	Worm culture letter	Ova cultured in 90% rel. humidity: No. of worms: Male	No. of worms: Female	Av. length of worms, mm: Male	Av. length of worms, mm: Female	Chicken number	Worm culture letter	Ova cultured in water: No. of worms: Male	No. of worms: Female	Av. length of worms, mm: Male	Av. length of worms, mm: Female
A4231	AI	0	0	0	0	A4202	AI	4	10	7.1	4.3
A4207	AII	3	4	19.1	24.3	A4232	AII	1	1	13.0	3.3
A4214	BI	1	2	25.1	26.6	A4220	BI	4	13	2.3	3.1
A4205	BII	0	0	0	0	A4206	BII	9	9	7.1	4.8
A4198	CI	1	2	19.1	24.1	A4196	CI	0	2	0	25.8
A4236	CII	4	8	43.0	34.1	A4199	CII	0	21	0	3.4
A4226	DI	1	0	16.6	0	A4201	DI	6	6	14.4	13.3
A4227	DII	2	2	15.4	20.0	A4228	DII	2	4	2.3	2.2
A4211	EI	2	5	27.9	35.8	A4195	EI	14	10	8.6	10.5
A4221	EII	1	0	29.1	0	A4203	EII	4	5	5.2	4.0
Total		15	23	195.3	164.9			44	81	40.0	74.7
Average		1.5	2.3	24.4	27.5			4.4	8.1	5.0	7.4

Combined Results

The combined results of Experiments 1 and 2 concerning the rate of development of A. galli ova cultured in air and in water are shown in Table 5. A total of 20 cultures was used in these studies, 10 cultures were exposed to 90 per cent relative humidity and 10 cultures were kept in water. These cultures were prepared from 4000 ova taken from 10 gravid female A. galli as previously described in the section on Materials and Methods. Concerning the terminology in Table 5, the term "embryonated ova" is defined as those ova which developed to the coiled embryo and vermiform stages as well as those ova classified as ruptured cells. The rupturing of some of the ova when in the coiled embryo stage in the air culture was probably due to the wetting of the ova when they were examined under the compound microscope and by drying of the ova before they were returned to the humidity chamber.

From the 2nd day through the 30th day of incubation the ova in the water cultures had reached a more advanced stage of development than the ova in the air cultures. At the end of the 30th day of incubation, 1861 ova were embryonated, 23 were nonfertile, 5 were dead, and 111 ova failed to develop beyond the tadpole stage. The egg cultures exposed to 90 per cent relative humidity for 30 days contained 1591 embryonated ova, 19 nonfertile ova, 107 dead ova and 283 ova failed to develop beyond the tadpole stage.

Table 5. Rate of development of A. galli ova in 90 per cent relative humidity at 30° C. as compared with ova in water culture.* Combined results of Groups I and II (based on a study of 2000 ova in each group).

Days of incubation	Egg development: Cell stage: 1 A	1 W	2 A	2 W	3 A	3 W	4 A	4 W	5-8 A	5-8 W	9-32 A	9-32 W	Early morula A	Early morula W	Late morula A	Late morula W	Tadpole A	Tadpole W	Vermiform A	Vermiform W	Coiled embryo A	Coiled embryo W	Non-fertile egg A	Non-fertile egg W	Dead cell A	Dead cell W	Ruptured cell A	Ruptured cell W
2	102	29	114	9	68	5	102	14	190	52	1157	92	215	1174	-	-	-	-	-	-	-	-	19	23	33	2	-	-
4	23	15	44	6	26	4	65	9	51	18	135	28	379	35	319	134	884	1712	-	14	-	-	19	23	55	2	-	-
6	17	14	19	5	17	2	33	6	54	15	86	31	237	23	254	34	429	41	768	1804	-	-	19	23	67	2	-	-
8	17	14	16	1	14	2	17	5	38	17	65	27	144	24	185	17	252	24	1150	1844	-	-	19	23	80	2	3	-
10	17	14	13	1	8	2	10	5	37	17	54	27	127	20	137	17	186	18	1282	1753	-	99	19	23	86	4	24	-
12	17	14	13	1	8	2	10	5	34	17	39	26	108	21	104	13	126	20	1087	1324	280	530	19	23	95	4	60	-
14	17	14	13	1	8	2	8	5	33	17	34	25	92	22	87	13	82	20	766	97	658	1757	19	23	97	4	86	-
16	17	14	13	1	8	2	8	5	33	17	32	24	90	18	79	11	65	24	156	83	1255	1773	19	23	102	4	123	1
18	17	14	13	1	8	2	8	5	33	17	29	22	86	19	65	12	65	21	124	70	1270	1789	19	23	105	4	158	1
20	17	14	13	1	8	2	8	5	33	17	26	22	83	19	59	12	53	19	110	61	1288	1799	19	23	107	4	176	2
22	17	14	13	1	8	2	8	5	33	17	26	22	83	19	53	12	46	19	97	59	1277	1800	19	23	107	4	213	3
24	17	14	13	1	8	2	8	5	33	17	26	22	83	19	53	12	46	19	97	55	1259	1802	19	23	107	4	231	5
26	17	14	13	1	8	2	8	5	33	17	26	22	83	19	52	12	43	19	93	55	1249	1799	19	23	107	4	249	8
28	17	14	13	1	8	2	8	5	33	17	26	22	83	19	52	12	43	19	90	55	1240	1798	19	23	107	4	261	9
30	17	14	13	1	8	2	8	5	33	17	26	22	83	19	52	12	43	19	90	54	1221	1798	19	23	107	5	280	9

* A = Air culture (90 per cent relative humidity).
W = Water culture.

From a consideration of the data presented in Table 6, it can be seen that on the 30th day of incubation 93 per cent of the ova cultured in water developed to embryonated ova as compared with 80 per cent embryonated ova in the air culture. The mortality rate among the ova in the air culture was 5 per cent in contrast with no deaths among the ova cultured in water. It is interesting to note that about 3 to 4 per cent of the ova in the air culture appeared to become dormant in the morula stage. The ova in the dormant stage while not showing any signs of granulation or vacuolation, characteristic of dead ova, nevertheless did not complete their embryogeny.

The data given in Table 6 are shown graphically in Fig. 1. The graph not only delineates the comparative rates of development but also the degree of development of the ova in the two types of cultures. On the 6th day of incubation 90 per cent of the ova cultured in water had reached embryonation whereas only 38 per cent of the ova cultured in 90 per cent relative humidity had reached the same stage of embryogeny. At the end of the 30th day of incubation, 93 per cent of the ova cultured in water were embryonated as compared with 80 per cent embryonated ova in the air culture.

The combined results on the viability test of the coiled embryos developing in 90 per cent relative humidity showed a total number of 81 worms from 20 parasitized chicks with an average of 4.0 worms. The length of the worms ranged from 7.5

Table 6 . Comparative rate of development of A. galli ova cultured in 90 per cent relative humidity and in water (based on the study of 2000 ova in each of the cultures*), Groups I and II.

Days of incubation	Percentage of various stages of egg development																						Non-fertile egg		Dead cell		Ruptured cell	
	Cell stages												Early morula		Late morula		Tad pole		Vermiform		Coiled embryo							
	1		2		3		4		5-8		9-32																	
	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W	A	W
2	5	1	6	-	3	-	5	1	9	3	58	5	11	89	-	-	-	-	-	-	-	-	1	1	2	-	-	-
4	1	1	2	-	1	-	3	-	3	1	7	1	19	2	16	7	44	86	-	1	-	-	1	1	3	-	-	-
6	1	1	1	-	1	-	2	-	3	1	4	1	12	2	13	2	21	2	38	90	-	-	1	1	3	-	-	-
8	1	1	1	-	1	-	1	-	2	1	3	1	7	2	9	1	13	1	57	92	-	-	1	1	4	-	-	-
10	1	1	1	-	-	-	1	-	2	1	3	1	6	1	7	1	9	1	64	88	-	5	1	1	4	-	1	-
12	1	1	1	-	-	-	1	-	2	1	2	1	5	1	5	1	6	1	54	66	14	27	1	1	5	-	3	-
14	1	1	1	-	-	-	-	-	2	1	2	1	5	1	4	1	4	1	38	5	33	88	1	1	5	-	4	-
16	1	1	1	-	-	-	-	-	2	1	2	1	4	1	4	1	3	1	8	4	63	89	1	1	5	-	6	-
18	1	1	1	-	-	-	-	-	2	1	2	1	4	1	3	1	3	1	6	3	64	90	1	1	5	-	8	-
20	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	3	1	6	3	64	90	1	1	5	-	9	-
22	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	2	1	5	3	64	90	1	1	5	-	11	-
24	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	2	1	5	3	63	90	1	1	5	-	12	-
26	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	2	1	5	3	63	90	1	1	5	-	12	-
28	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	2	1	5	3	62	90	1	1	5	-	13	-
30	1	1	1	-	-	-	-	-	2	1	1	1	4	1	3	1	2	1	5	3	61	90	1	1	5	-	14	-

* A = Air culture (90 per cent relative humidity).
W = Water culture.

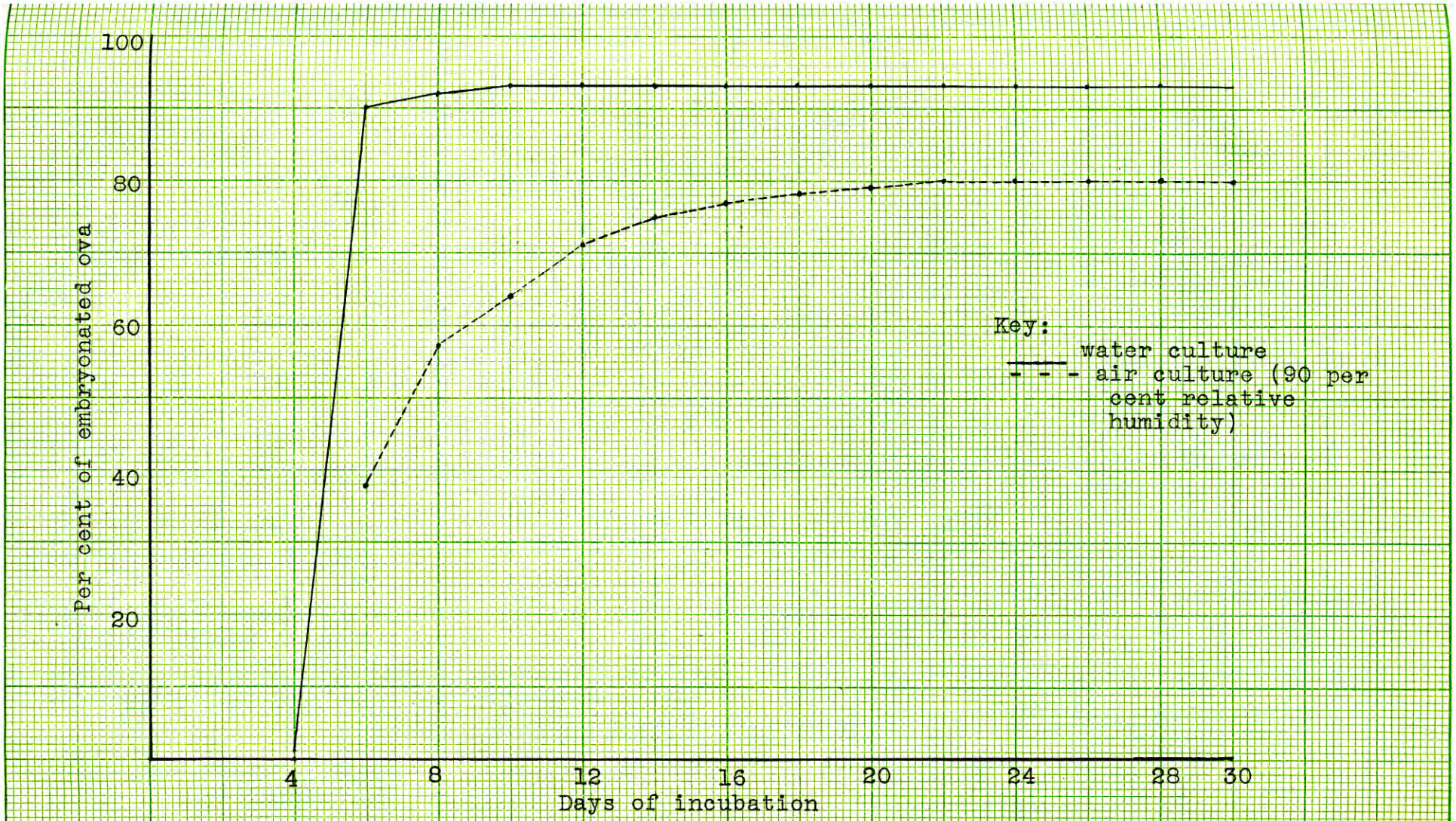

Fig. 1. Per cent of A. galli ova becoming embryonated in 4 to 30 days of incubation in water and in 90 per cent relative humidity at 30° C.

to 38.3 mm and averaged 25.5 mm. The ova cultured in water, when fed to chicks, produced a greater number but smaller worms than the ova cultured in 90 per cent relative humidity. A total of 154 or an average of 7.7 worms was recovered from 20 chicks exposed to embryonated ova which had been cultured in water. The length of the worms ranged from 1.3 to 26.6 mm and averaged 5.4 mm.

The combined results of the viability test indicate that the ova cultured in 90 per cent relative humidity produced in the chicks worms which were more than 4 times longer than the worms produced by ova cultured in water. However, the number of worms recovered from the chicks exposed to the water culture ova was almost twice as great as the number of the worms recovered from the chicks exposed to the ova cultured in 90 per cent relative humidity.

In order to establish the ratio of mucosa larvae to lumen larvae among the chicks fed the ova cultured in air and in water in Experiment 2, the flushed contents and the mucosal scrapings from the intestines of all the parasitized chicks were collected in separate glass jars. The worms from each jar were collected, counted, measured and sexed (Table 7). Forty-seven worms were recovered from the intestinal mucosa of the 10 chicks fed ova which had been cultured in water. No worms were recovered from the intestinal mucosa of the 10 chicks fed ova which had been cultured in 90 per cent relative humidity.

Table 7. Results of a study of the tissue phase, Group II.

Chicken number	Worm culture number	Ova cultured in 90% rel. humidity: Worms collected: Flushing* M	Flushing* F	Mucosal scraping M	Mucosal scraping F	Av. length, mm: Lumen larvae M	Lumen larvae F	Mucosa larvae M	Mucosa larvae F	Chicken number	Worm culture number	Ova cultured in water: Worms collected: Flushing M	Flushing F	Mucosal scraping M	Mucosal scraping F	Av. length, mm: Lumen larvae M	Lumen larvae F	Mucosa larvae M	Mucosa larvae F
A4231	AI	0	0	0	0	0	0	0	0	A4202	AI	4	7	0	3	7.1	5.5	0	3.2
A4207	AII	3	4	0	0	19.1	24.3	0	0	A4232	AII	1	0	0	1	13.3	0	0	3.3
A4214	BI	1	2	0	0	25.0	26.6	0	0	A4220	BI	2	7	2	6	2.2	3.4	2.4	2.8
A4205	BII	0	0	0	0	0	0	0	0	A4206	BII	7	7	2	2	6.9	5.6	7.3	4.0
A4198	CI	1	2	0	0	19.1	24.1	0	0	A4196	CI	0	2	0	0	0	25.8	0	0
A4236	CII	4	8	0	0	43.0	34.1	0	0	A4199	CII	0	5	0	16	0	3.6	0	3.3
A4226	DI	1	0	0	0	16.6	0	0	0	A4201	DI	6	6	0	0	14.4	13.3	0	0
A4227	DII	2	2	0	0	15.4	20.0	0	0	A4228	DII	2	4	0	0	2.3	2.2	0	0
A4211	EI	2	5	0	0	27.9	35.8	0	0	A4195	EI	6	3	8	7	14.0	17.7	3.3	3.3
A4221	EII	1	0	0	0	29.1	0	0	0	A4203	EII	4	5	0	0	5.2	4.0	0	0
Total		15	23	0	0	195.2	164.9	0	0			32	46	12	35	65.4	81.1	13.0	199
Average		1.5	2.3	0	0	24.4	27.5	0	0			3.2	4.6	1.2	3.5	8.1	9.0	4.3	3.3

* M = Male.
F = Female.

DISCUSSION

The study presented in this thesis has demonstrated that the ova of the A. galli kept in 90 per cent relative humidity at 30° C. for a period of 30 days developed more slowly than the ova cultured in water under the same conditions of temperature and time. Similar results were reported by McRae (1935) who, working with the ova of A. galli, demonstrated that the ova cultured in 82 to 86 per cent relative humidity at 22° C. developed more slowly than the ova cultured in water at the same temperature. Only 4 per cent of the ova failed to become embryonated in the cultures kept in 82 to 86 per cent relative humidity.

In the present study a slightly higher mortality rate and a lower percentage of embryonated ova were found in the egg cultures kept in 90 per cent relative humidity. The small discrepancies in the rate of development and deaths of the ova in the two studies can be related to differences in temperatures used to culture the ova. The temperature used in the present study was 8° C. higher than the temperature used by McRae (1935).

The ova cultured in water developed uniformly throughout the incubation period which corresponded with the findings of Ackert (1931).

No studies have been reported in the literature testing

the viability of ascarid ova cultured in air by feeding such embryonated ova to chicks. Such viability tests of the ova cultured in 90 per cent relative humidity were conducted in this study.

The criteria for judging the viability of the ova cultured in air and in water were the numbers and lengths of the worms harbored by the two groups of chicks 21 days subsequent to exposure to the ova from the two types of cultures. In Experiment 1, nearly twice as many worms were recovered from chicks exposed to embryonated ova cultured in air as were recovered from the chickens exposed to embryonated ova cultured in water. However, in Experiment 2, only one-third as many worms were recovered from chickens exposed to the ova cultured in air as were recovered from chicks exposed to ova cultured in water. In both experiments a considerable fluctuation occurred in the number of worms recovered from birds within each group. Such variations in numbers of worms recovered are inherent in all experiments with A. galli since this worm is continuously eliminated in small numbers before and after the tissue phase of its life cycle. The criterion of numbers of worms recovered in judging viability of ova in this study, therefore, is of no value. Since the present study is based upon only 20 chicks in each group, no general conclusion can be made regarding this criterion until the variable of experimental error can be ascertained more accurately. The criterion of length of worms recovered from the infected chicks in this

study as a basis for judging the comparative viability of ova cultured in air and in water appears to be valid in view of the fact that the worms developing from ova cultured in air were much longer than the worms developing from ova cultured in water in both Experiments 1 and 2. Apparently the retarded embryogeny of the ova cultured in air had conditioned the viability of the developing larvae as was shown by the very rapid growth of the larvae within the host.

SUMMARY

A study was made to ascertain the rate of development and viability of A. galli ova cultured in 90 per cent relative humidity and in water kept at 30° C. for a period of 30 days. Two experiments were performed utilizing 8000 ova collected from 10 gravid female A. galli worms. These ova were later used in the viability test and were fed to 40 Single Comb White Leghorn chicks. The results were as follows:

1. The ova of A. galli cultured in 90 per cent relative humidity at 30° C. developed at a slower and at a more irregular rate than the ova cultured in water at the same temperature.

2. Eighty per cent of the A. galli ova cultured in 90 per cent relative humidity at 30° C. became embryonated on the 30th day of incubation with only a 5 per cent mortality.

3. Ninety-three per cent of the A. galli ova cultured in water at 30° C. became embryonated on the 30th day of incubation with no deaths.

4. The average number of worms recovered from 20 chicks each of which was exposed to 200±10 embryonated ova cultured in 90 per cent relative humidity was 4.0. The average length of these worms was 25.5 mm.

5. The average number of worms recovered from 20 chicks each of which was exposed to 200±10 embryonated ova cultured in water was 7.7. The average length of these worms was 5.4 mm.

6. Forty-seven tissue phase larvae were recovered from 10 chicks 21 days subsequent to feeding each with 200±10 embryonated ova of A. galli from a 30-day-old water culture incubated at 30° C.

7. No tissue phase larvae were recovered from 10 chicks 21 days subsequent to feeding each with 200±10 embryonated ova of A. galli from a 30-day-old culture kept in 90 per cent relative humidity at a temperature of 30° C.

8. In this study the retarded embryogeny of the ova cultured in air had conditioned the viability of the developing larvae as was shown by the very rapid growth of the larvae within the host.

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COMPARATIVE RATES OF DEVELOPMENT AND VIABILITY OF *ASCARIDIA GALLI* EGGS CULTURED RESPECTIVELY IN AIR AND IN WATER

by

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D. V. M., University of the Philippines, 1936

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The purpose of the study presented in this thesis was to determine the rate of development and the viability of A. galli ova cultured in 90 per cent relative humidity and in water at 30° C. for a period of 30 days. Two experiments were performed utilizing a total of 8,000 ova collected from 10 gravid female A. galli worms. Two hundred ova were placed on each of 40 glass slides, half of these slides were placed in 90 per cent relative humidity and the other half were kept in water. Microscopical examination of the egg cultures was made every 48 hours, and the stages of egg development were recorded.

At the end of 30 days' incubation, the viability of the coiled embryos in both types of egg cultures was determined. A dose of 200±10 embryonated eggs from each of the two types of cultures was fed to each of 40 straight run Single Comb White Leghorn chicks. The chicks used in Experiment 1 were 19 days old and those used in Experiment 2 were 26 days old.

The chicks were sacrificed 21 days after exposure to the embryonated eggs from both types of cultures. The worms from each exposed chick were collected and placed in separate vials; later the worms were counted, measured, and sexed.

The results obtained from the two experiments were as follows:

1. The ova of A. galli cultured in 90 per cent relative humidity at 30° C. developed at a slower and at a more irregular rate than the ova cultured in water at the same tempera-

ture.

2. Eighty per cent of the A. galli ova cultured in 90 per cent relative humidity at 30° C. became embryonated eggs on the 30th day of incubation with only a 5 per cent mortality.

3. Ninety-three per cent of the A. galli ova cultured in water at 30° C. became embryonated on the 30th day of incubation with no deaths.

4. The average number of worms recovered from 20 chicks each of which was exposed to 200±10 embryonated ova cultured in 90 per cent relative humidity was 4.0. The average length of these worms was 25.5 mm.

5. The average number of worms recovered from 20 chicks each of which was exposed to 200±10 embryonated ova cultured in water was 7.7. The average length of these worms was 5.4 mm.

6. No tissue phase larvae were recovered from the 10 chicks each of which was fed 200±10 embryonated ova cultured in 90 per cent relative humidity.

7. Forty-seven tissue phase larvae were recovered from 10 chicks 21 days subsequent to feeding each chick with 200±10 embryonated ova cultured in water.

8. Apparently the retarded embryogeny of the ova cultured in 90 per cent relative humidity had conditioned the viability of the developing larvae as was shown by the very rapid growth of the larvae within the host.