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Melvin A. Cassady
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To the Graduate Council:

I am submitting herewith a thesis written by Melvin A. Cassady entitled "A Study of the Factors Influencing the Artificial Hatching of the Eggs of *Macracanthorhynchus hirudinaceus*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Helen L. Ward, Major Professor

We have read this thesis and recommend its acceptance:

Arthur C. Cole, A. W. Jones

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

January 13, 1950

To the Committee on Graduate Study:

I am submitting to you a thesis written by Melvin A. Cassady entitled "A Study of the Factors Influencing the Artificial Hatching of the Eggs of Macracanthorhynchus hirudinaceus." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

Helen L. Ward
Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Committee

E. G. Waters
Dean of the Graduate School

A STUDY OF THE FACTORS INFLUENCING THE ARTIFICIAL HATCHING OF THE EGGS OF *MACRACANTHORHYNCHUS HIRUDINACEUS*

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

---

by

Melvin A. Cassady

March 1950

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INTRODUCTION

Until recently the Acanthocephala have been regarded as a group of uncertain taxonomic position. Some parasitologists have considered them an appendix to the phylum Nemathelminthes while others have thought they should be classified with the Platyhelminthes. Van Cleave (1948) proposed a new classification for the Acanthocephala in which they were regarded as a separate phylum with two recognizable classes. The basis for his proposal was the distinct characteristics of the worms. The class Metacanthocephala includes the orders Palaeacanthocephala and Archiacanthocephala. The worms in this class are small to large in size and their hosts have either aquatic or terrestrial habitats. The second class, the Eoacanthocephala, includes the orders Gyracanthocephala and Neoacanthocephala. These are small worms having aquatic hosts. This classification is being accepted more widely by recent workers and authors of textbooks in the field.

The Acanthocephala are common parasites of vertebrates. Distinctive characteristics of the phylum include the absence of a digestive tract, a more or less flattened, unsegmented body and a spinous, retractile proboscis. The worms are mostly small although some reach a length of more than sixty centimeters. The sexes are separate. The body wall consists of a thin cuticula, a syncytial hypoderm, and circular and longitudinal muscles. Paired elongations of the hypoderm, the lemnisci, extend posteriorly from the base of the neck. The genital pore in both sexes is near the posterior end. The fore body, as differentiated from the trunk, is divided more or less distinctly into the

retractile proboscis and the neck. In the various species, the proboscis changes its form from a short half globule to an elongate-cylindrical form. Without exception it is supplied with posteriorly curved hooks. They occur in longitudinal and transverse rows, usually alternating, or in a spiral arrangement. The form and size of the proboscis and the number, shape and arrangement of the hooks are useful in species differentiation.

The proboscis sheath, into which the proboscis can be withdrawn, is a muscular hollow cylinder closed at the posterior end. It arises at the base of the proboscis. The contraction of the muscular sheath causes the protrusion of the proboscis through the pressure exerted on its liquid contents. By this action, it can be thrust into the tissues of the host, where the curved spines anchor the worm securely. With the relaxation of the sheath, the proboscis is retracted and its fluid streams into the lemnisci (Rauther, 1931).

The male worm is easily distinguished from the female by its smaller size. The male sex organs consist of two oval or cylindrical testes, the cement glands and a bell-shaped invaginated bursa. The hardened secretion of the cement glands serves to clasp the female sex opening during copulation. The bursa, whose walls have the same structure as the outer body wall, can be protruded during copulation by special muscle arrangements and again retracted. A central ligament extends the length of the body cavity of the female and male. In the immature stage the females have a pair of ovaries which give rise to free-floating ovarian balls. They eventually fill the body cavity with numerous ova which are surrounded with embryonic membranes after

fertilization. Ova with well developed embryos are discharged from the body cavity by a selective apparatus, the uterine bell. This is connected to the uterus by the uterine tract. The vagina, which joins the uterus, opens to the outside at the posterior end of the body. Macracanthorhynchus hirudinaceus, the giant thorny-headed worm of hogs, belongs to the class METACANTHOCEPHALA, order ARCHIACANTHOCEPHALA, family OLIGACANTHORHYNCHIDAE.

Van Cleave (1947) summarizes very clearly the life cycle of the Macracanthorhynchus hirudinaceus. The fertilized egg within the body cavity of the female worm develops into an embryo surrounded by a series of membranes or shells. The embryo is known as the acanthor, which normally hatches only when the egg is swallowed by the intermediate host, a beetle larva. In the gut of the host the rostellar spines of the acanthor bore through the wall of the digestive tube into the body cavity. This stage is referred to by Kates (1943) as the second stage of the acanthor. Within the body cavity of the intermediate host the acanthor undergoes metamorphosis. It transforms into a simple ball of embryonic cells. This is the initial stage in a succession of larval forms called the acanthellas in which the rudiments of the structures of the adult worm begin to appear. The final step in development within the body of the intermediate host is the continued growth of these structures into a form characteristic of the adult worm. This is the end of the larval existence, and the individual is considered an immature acanthocephalan to which the term "juvenile" is often applied. Further development to sexual maturity follows the ingestion of the infected arthropod by the definitive host in whose intestine growth

of the juvenile continues.

The mature eggs of the swine thorny-headed worm are 80 to 100 microns long by 46 to 65 microns wide (Kates, 1943) and appear brown in transmitted light and almost white in reflected light. The shell has four layers, a thin and membranous outer layer, a thick granular second layer, and two thin transparent inner layers which swell in contact with water after being dried. Von Brand (1940) found that the innermost egg membrane consists of chitin and that large amounts of polysaccharide are present in the embryonalkern of the embryo. At the anterior end of the second layer is a thin area, the raphe. The shell splits at the raphe when the egg hatches. The outer shell is heavily sculptured and pitted giving an uneven appearance when viewed with the microscope slightly out of focus. Inside is the larva covered with numerous small spines. At its anterior end is a group of larval hooks. The larva is infective to white grubs and the hooks and spines aid in the penetration of the gut tissue. The immature eggs of Macracanthorhynchus hirudinaceus may be of the same size as the area surrounded by the innermost membrane or they may be the normal size of a mature egg. The features of the enclosed embryo are indistinguishable and the entire egg is light colored and quite transparent.

In this study the writer has attempted to determine the effects of certain environmental factors on the artificial hatching of the eggs of Macracanthorhynchus hirudinaceus. The factors, pH of rewetting solution, temperature, and length of storage, were chosen because of their possible relationship to conditions involved in the natural hatching of the eggs.

The eggs of the thorny-headed worm can be induced to hatch in

large numbers by the drying and rewetting process. This makes available the interesting first larval form for study or for laboratory demonstration. If a suitable culture medium for these larval forms could be found, the embryology of the Acanthocephala could be studied more easily. Research involving the grub worms is complicated and time consuming.

MATERIALS AND METHODS

The giant thorny-headed worms, Macracanthorhynchus hirudinaceus, were collected post mortem from the intestines of infected hogs at a local meat packing company. The eggs used in the tests were obtained from gravid female worms. They were removed by making an incision near the posterior end of the worm and pressing them out onto clean three by one glass slides. About one drop of egg concentrate was placed on each slide, smeared to a one layer thickness and allowed to dry overnight at room temperature. The preparations were then stored in slide boxes until examined. This technique was one used by Moore (1942) and is a modification of Manter's method. A system of numbering was devised which designated the slides having eggs coming from the same worm. For example, A-1, A-2, A-3; II-1, II-2, II-3, etc.

In order to study the effects of pH on artificial hatching, buffer mixtures, having a pH range from 6.5 to 10.0 with gradations of 0.2 and 0.4, were prepared from formulas listed by Clark and Lubs in the Handbook of Chemistry. The following solutions were used: 0.1 N NaOH (sodium hydroxide), 0.1 M KH_2PO_4 (monopotassium phosphate), and 0.1 M boric acid in 0.1 M HCl (hydrochloric acid). The procedure employed in testing the effects of pH of the solutions used in rewetting the dried eggs to induce artificial hatching was as follows: a few drops of the mixture of known pH were placed on the smear of eggs. A definite number of microscopic fields (5) was chosen in which to approximate the number of unhatched mature eggs and the hatched acanthors present. The counts were made through the use of a compound microscope and a low-

power objective. At first, the observations were made at intervals of twenty, forty and sixty minutes. The same areas were covered in each count, and were located by noting accurately the scale reading on the mechanical stage of the microscope at the initial observation. Later it was found to be more practical to observe a greater number (20) of fields at the end of a one hour period after rewetting the smear. Slides which had been stored at room temperature for periods from one to 21 days were rewetted with solutions of various pH. The ratio of hatched acanthors to unhatched mature eggs was the basis for determining the percentage of hatchings. Determinations were not made until the total number of mature eggs and the total number of hatched acanthors on a slide had been counted.

For experiments on the effects of temperature an equal number of slide preparations were stored at each of three temperatures, room temperature (18°-32°C.), 39°C., and 5°C. After definite periods of time had elapsed, the eggs were moistened with a solution having a pH of 8.8. This pH had been found to be more or less the optimum in previous tests involving only the effects of the pH factor.

The effects of the storage time were observed by rewetting groups of slides which had been stored for different periods from one to 21 days with solutions of various pH. Three sets of preparations were kept at room temperature, 39°C. and 5°C. respectively for a period of 40 days before being rewetted with a solution having a pH of 8.8.

Following some experiments performed by Moore (1942), in which neutral red was used, eight slides were rewetted with a solution of

Bismark brown diluted to 1:1000 parts and allowed to stand for thirty minutes before observations were made. Methylene blue was used for rewetting six slides. Four drops of methylene blue diluted to 1:1000 in 15 cc. of solution having a pH of 8.8 provided the moistening agent for four slides. All of the above slide preparations had been stored for a period of one day at room temperature (18°-20°C.).

OBSERVATIONS

The results of the major part of this study are recorded in Tables I through XII. The data reported in Tables I through VII show the percentage of hatchings induced by rewetting eggs which had been stored at room temperature with solutions having a pH of 7.0, 7.4, 7.8, 8.0, 8.4 and 9.0. From the results obtained, it was found that the rewetting solution with a pH of 8.8 provided the highest hatching percentage on slides which had been stored for different periods from one to fourteen days. See Table VI. Five solutions with a pH of 6.5, 6.7, 9.4 and 10.0 respectively were used as moistening agents for five groups of ten slides each. No hatchings occurred. These are not given in tabular form.

Tables VIII, IX and X present the percentage of hatchings observed on slide preparations which had been stored at room temperature (18°-32°), 39°C. and 5°C. for periods of 1, 2, 3, 4, 5, 7 and 11 days before being rewetted with a solution having a pH of 8.8. The highest percentage of hatching, 4.7, occurred on a slide which had been stored at 39°C. for two days. The maximum percentage of hatching of eggs stored at room temperature for the same length of time was 3.2. Eggs stored at 5°C. for two days had a maximum hatching percentage of 0.96. See Table XI.

The effect of time of exposure upon artificial hatching may be seen in Table XII. Slides which had been stored at room temperature were tested at various intervals of time with a solution having a pH of 8.8. The results given in Table XII show that a greater percentage

of hatching occurs for a time interval of one day than for seven or 15 days. Generally speaking, the number of eggs that was hatched by the drying and rewetting process decreased as the storage time was increased. There were occasional exceptions to this, however. As shown in Table V, both maximum and minimum percentages were higher for slides stored three days than for those stored one day. Both were rewetted with a solution having pH of 8.4. Three groups of ten slides each of which had been stored at room temperature, 39°C. and 5°C. respectively for a period of 40 days were rewetted with a solution having a pH of 8.8. No hatchings were observed.

Ten slides were tested with dilute solutions of methylene blue and eight with Bismark brown. No hatchings were observed.

The artificial hatching of the eggs of Macracanthorhynchus hirudinaceus, as observed under the microscope, appears to be associated with a noticeable and sudden increase in size of the larvae. After hatching these acanthors are sluggish. Occasionally they may be seen undergoing a characteristic movement in which the anterior end of the body is drawn in, proboscis-like, and then suddenly thrust forward. This action rarely was observed beyond a period of 30 minutes after the acanthor hatched. The body outline of the acanthor is somewhat torpedo-shaped. Circular rows of minute spines, which become more irregular toward the anterior end, cover the entire outer surface. Two pairs of larger spines occur at the anterior tip, or rostellum. Internally the acanthor consists of a translucent jelly-like substance containing numerous nuclei. A mass of small nuclei are visible in the center surrounded by a smaller number of larger nuclei. The latter are only

faintly visible in living specimans and remain constant in number during development (Kates, 1943).

TABLE I

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 7.0

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
8*	1	154	0	0.00	115	2	1.70
1**	2				113	1	0.08
2	3	1090	0	0.00	1425	9	0.63
10	5	840	0	0.00	915	3	0.32
1**	6				1796	2	0.11
1**	7				1094	5	0.45
3	9	1563	0	0.00	1149	3	0.26
2	10	1229	0	0.00	884	1	0.18
3	21	1221	0	0.00	1210	0	0.00

*The percentages for five of these slides were calculated with observations from five fields; others with observations from twenty fields.

**When one slide only was examined, the results are given in the right hand column.

TABLE II

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 7.4

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
4	1	1052	2	0.18	1158	9	0.78
5*	2	52	0	0.00	44	1	2.20
1**	3				1058	2	0.17
4	7	1023	0	0.00	1078	3	0.27
2	9	1415	0	0.00	1332	1	0.07
2	10	717	2	0.27	627	2	0.31
2	14	959	0	0.00	1187	0	0.00

*These percentages were calculated with observations from five fields; others with observations from twenty fields.

**When only one slide was examined, the results are given in the right hand column.

TABLE III

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 7.8

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
5	1	1710	3	1.70	1074	7	0.65
1**	2				995	4	0.42
2	3	1092	3	0.27	1061	4	0.37
2	4	1372	11	0.82	955	8	0.83
10*	6	178	0	0.00	82	1	1.20
2	7	1080	2	0.18	908	2	0.22
1**	9				1157	3	0.25
2	14	1285	1	0.07	1052	1	0.09
1**	22				1033	0	0.00

*The percentages for five of these slides were calculated with observations from five fields; others with observations from twenty fields.

**When only one slide was examined, the results are given in the right hand column.

TABLE IV

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 8.0

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
3	1	1474	21	1.40	1536	29	1.80
2	2	536	8	1.40	499	9	1.80
1**	5				1005	6	0.50
2	6	1098	8	0.72	630	6	0.81
6*	7	112	0	0.00	185	2	1.10
1**	9				1151	9	0.78
18	21	934	0	0.00	1020	0	0.00

*The percentages for five of these slides were calculated with observations from five fields; others with observations from twenty fields.

**When only one slide was examined, the results are given in the right hand column.

TABLE V

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 8.4

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
4	1	1042	19	1.80	695	21	3.00
2	2	1133	23	2.00	1117	28	2.50
4	3	1175	30	2.50	1140	40	3.60
2	5	1483	18	1.20	810	12	1.40
2	6	710	10	1.30	1105	15	1.30
1**	7				1385	15	1.30
5*	8	138	1	0.72	99	2	2.20
2	10	543	6	1.10	523	7	1.30
1**	14				1107	7	0.63
5	21	626	0	0.00	1080	4	0.40

*These percentages were calculated with observations from five fields; others with observations from twenty fields.

**When only one slide was examined, the results are given in the right hand column.

TABLE VI

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 8.8

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
3	1	761	27	3.50	898	38	4.20
5	2	1186	30	2.60	1368	44	3.80
2	5	743	21	2.90	1098	50	4.30
2	6	679	17	2.50	859	23	2.60
6	7	1080	8	0.74	1108	32	2.80
4	8	1092	20	1.60	992	21	2.10
6*	9	110	1	0.91	142	3	2.60
5	12	1050	0	0.00	912	23	2.40
2	14	1007	10	0.99	954	13	1.30

*The percentages for five of these slides were calculated with observations from five fields; others with observations from twenty fields.

TABLE VII

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING EGGS WITH SOLUTION HAVING pH OF 9.0

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
1**	1				426	9	2.70
1**	2				1273	19	1.40
2	3	1408	20	1.40	1485	22	1.50
1**	5				866	20	2.10
1**	6				969	12	1.20
1**	7				1036	8	0.77
2	8	1331	4	0.30	1397	5	0.35
6	10	978	2	0.22	946	8	0.84
5*	14	251	0	0.00	224	1	0.40
2	21	1205	2	0.16	1097	3	0.27

*These percentages were calculated with observations from five fields; others with observations from twenty fields.

**When only one slide was examined, the results are given in the right hand column.

TABLE VIII

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING SOLUTIONS HAVING A pH OF 8.8 WHEN STORED AT ROOM TEMPERATURE

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
5	1	1069	21	2.10	1068	36	3.30
2	2	848	21	2.50	940	31	3.20
4	3	891	25	2.80	554	22	4.10
11	4	1148	14	1.20	1071	33	3.00
12	5	1020	16	1.50	882	26	2.90
6	7	910	8	0.87	859	14	1.60
4	11	1038	11	1.10	1008	21	2.00

TABLE IX

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING SOLUTIONS HAVING A pH OF 8.8 WHEN STORED AT 39°C.

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
5	1	1095	22	2.00	1034	39	3.60
2	2	999	21	2.10	777	37	4.70
4	3	1197	14	1.20	735	28	3.90
14	4	1152	17	1.40	962	37	3.80
4	5	1195	18	1.40	1047	30	2.80
10	7	1022	13	1.20	653	21	3.30
4	11	978	8	0.87	895	12	1.30

TABLE X

THE PERCENTAGE OF HATCHINGS INDUCED BY REWETTING SOLUTIONS HAVING A pH OF 8.8 WHEN STORED AT 5°C.

Number of Slides Used	Period of Storage (days)	Slide Showing Minimum Hatchings			Slide Showing Maximum Hatchings		
		Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings	Number of Eggs Counted	Number of Hatchings	Percentage of Hatchings
3	1	894	0	0.00	870	0	0.00
6	2	803	0	0.37	1055	9	0.96
4	3	904	0	0.00	1075	5	0.46
8	4	1144	0	0.00	1141	8	0.71
13	5	976	0	0.00	1149	5	0.43
7	7	919	0	0.00	925	8	0.86
2	11	865	0	0.00	1106	2	0.18

TABLE XI

EXAMPLE OF THE EFFECT OF TEMPERATURE ON ARTIFICIAL HATCHING OF EGGS OF MACRACANTHORHYNCHUS HIRUDINACEUS WHEN RE-WETTING WITH SOLUTION HAVING pH OF 8.8*

Number of Slides Used	Period of Exposure To Drying (days)	Exposure Temperature	Percentage of the Hatchings Min.	Max.
4	3	Room	2.80	4.10
4	3	39°C.	1.20	3.90
4	3	5°C.	0.00	0.46

*Taken from Tables VIII, IX and X.

TABLE XII

EXAMPLE OF THE EFFECT OF DRYING ON ARTIFICIAL HATCHING OF EGGS OF *MACRACANTHORHYNCHUS HIRUDINACEUS* WHEN REWETTING WITH SOLUTION HAVING pH OF 8.8 AT ROOM TEMPERATURE*

Number of Slides	Period of Exposure to Drying (days)	Percentage of Hatchings	
		Min.	Max.
3	1	3.50	4.20
6	7	0.74	2.80
2	14	0.99	1.30

*Taken from Table VI.

DISCUSSION

Results obtained from tests on the effects of pH on artificial hatching of the eggs of Macracanthorhynchus hirudinaceus show that the largest percentages of the hatchings occurred in moderately alkaline media. Eggs rewetted with a solution having a pH of 8.4 had a maximum hatching percentage of 3.0. A maximum hatching percentage of 4.2 was obtained when slides were rewetted with a solution having a pH of 8.8. A solution with a pH of 9.0 was used for moistening eggs and the maximum percentage of hatchings was 2.7. These results are concerning slides stored for one day at room temperature. It was noted that when egg smears were treated with solutions having pH of 7.4 or 7.8, some of the egg shells split and the acanthors began to emerge. At this point, however, they appeared to die suddenly. No explanation was readily available. As previously stated, when solutions with a pH of 6.5, 6.7, 9.4 or 10.0 were used, no indication of possible emergence of the acanthors from the eggs was observed. The minimum pH at which any hatchings occurred was 7.0 and the maximum was 9.0. More hatchings occurred when slides were rewetted with a solution having a pH of 9.0 than when a solution with a pH of 7.0 was used. According to Wigglesworth (1947), the intestinal contents of the larvae of some herbivorous Coleoptera are always strongly alkaline. The common grub worm, which is the intermediate host of Macracanthorhynchus hirudinaceus, is a member of this group. It might be assumed, therefore, that the natural hatching of the eggs and the subsequent development of the larvae takes place in a highly alkaline environment. This fact provides us with a plausible explanation for the higher percentage of

hatchings when strongly alkaline solutions are used as rewetting agents. The writer knows of no previous studies in which the pH of the rewetting solutions was considered as a factor affecting the artificial hatching of the eggs of Macracanthorhynchus hirudinaceus.

In the tests where slide preparations were stored at 5°C., the explanation for the low hatching percentages could be the fact that the eggs did not become completely dry because of the moisture present in the refrigerator. This factor might have greater effect than the low temperature though it has not been proved. The differences in the percentage of hatchings between the eggs kept at room temperature (18°-32°C.) and those kept at 39°C., might be explained in part in that the latter temperature was constant. Also the room temperature sometimes remained at an approximate low of 18°C. for several hours. Data obtained from slides stored at 5°C. indicate that extreme cold, continuously applied, might inhibit artificial hatching. See Table X. Kates (1943) discussed the importance of temperature in the development of the larva in the body of the intermediate host under experimental conditions and under natural conditions. Higher temperatures increased the activity of the grub worm and speeded up the development of the parasite. Lower temperatures slowed or stopped the activity of the grub worm and the development of the parasite. He found that the normal period of development under experimental conditions at a mean temperature of 24°C. varied from sixty to ninety days while the minimum developmental period under natural soil conditions in summer is about three months. Kates (1942) also found that dried eggs kept for periods of four to forty-two days at temperatures of

-10° to -16° were viable when fed to grub worms. The temperatures at which the experiments on artificial hatchings and natural hatchings were conducted are similar to those found in natural soil conditions. Since a wide range of temperatures under experimental conditions did not destroy completely the viability of the eggs, it might be supposed that the natural hatching of the eggs is not seriously affected by temperature changes.

In the tests made where the eggs were stored for a period from one to 21 days, it was found that the percentage of hatchings usually dropped after a storage of seven days. A further decrease was apparent at 14 and 21 days. As stated previously, slides stored for 40 days at room temperature, 39°C. and 5°C. showed no hatchings. Kates (1942) dried the eggs of Macracanthorhynchus hirudinaceus on glass slides for approximately 10 minutes in a current of air at room temperature (26°C.). Some of the preparations were kept at temperatures of 5° to 9°, others at 21° to 26°, and still others at 37° to 39°C. At intervals of 16, 25, 35 and 50 days, eggs from one slide from each temperature group were mixed with moist sterile soil, and the number of viable eggs present in each sample estimated by grub-feeding tests. One slide was stored for 265 days at temperature of 21° to 26°C. before the eggs from it were fed to a grub worm. His results indicated that continuous drying at temperatures of 21° to 26°C., for a period of 265 days and at 5° to 9°C., and 37 to 39°C., for 50 days failed to destroy the viability of the eggs. No appreciable reduction in the number of viable eggs was observed in the dry preparations exposed for the different intervals of time. The results obtained by the writer from similar experiments but with artificial hatching

only, do not agree entirely with the data obtained by Kates. A decided decrease in the number of acanthors hatched artificially was observed as the length of the storage period was increased. Apparently prolonged storage has greater inhibitory effects on the number of artificial hatchings than upon the number of eggs which will hatch naturally. It might be assumed that there are factors existing in natural hatching which cannot be duplicated with artificial hatching methods. Since the dried eggs used by Kates were fed to grub worms to test their viability, hatchings should occur readily. In induced artificial hatching the eggs may not respond as easily to their unnatural environment as they would to the natural. Spindler and Kates (1940) found that eggs which had been placed on soil plots and exposed to natural weather and temperature conditions remained viable for as long as three and one-half years. The ability of the contained larvae to infect grubs of the June beetle was the criterion used to test the viability of the eggs. The writer obtained no hatchings from eggs which had been kept at room temperature for a period of 40 days before being rewetted with a solution having a pH of 8.8.

From the data presented in this study, it is seen that the eggs of Macracanthorhynchus hirudinaceus can be induced to hatch artificially only when rewetting solutions having a pH within the narrow range of 7.0 to 9.0 were used. The ranges of temperature and storage time at which hatchings were observed were rather wide. Upon being rewetted with a solution having the optimum pH of 8.8, hatchings occurred on slides which had been stored at temperatures from 5° to 39°C. for periods of one to

21 days. It seems probable that the eggs are more sensitive to changes in pH than they are to temperature or storage time.

Both Manter (1928) and Moore (1942) were successful in inducing artificial hatching of acanthocephalan eggs by the drying and rewetting process. In place of the plain water used by Manter for rewetting desiccated eggs, Moore used 10 drops of a saturated solution of neutral red in 20 cc of neutral distilled water. They found that only eggs from the body cavity of a mature female worm may be induced to hatch in this manner. The writer rewetted dried egg smears with dilute solutions of methylene blue and bismark brown, but no hatchings were observed. Methylene blue penetrated the egg shell readily and stained the enclosed larva. Bismark brown stained the outer shell of the egg but did not show any indication of having passed through the other layers or to the larva.

In all of the numerous cases observed, hatching seemed to be an entirely passive process on the part of the larva. Apparently one of the chief factors involved was the marked swelling of the body due, possibly, to the absorption of the water. This phenomenon is an example of a form of diffusion known as imbibition. While in the shell, the larva is 56 to 65 microns long by 26 to 27 microns wide. After the eggs have been in water a few minutes, the heavy outer shell tends to soften and split at the raphe allowing the inner shell containing the acanthor to slide out. At this point the acanthor measures 85 to 135 microns in length by 27 to 36 microns in width (Kates, 1943). Abnormal hatchings were rather numerous. One of the most common abnormalities was the failure of the

larva to escape from the membrane which immediately surrounds the inner egg shell. A definite constriction in the anterior part of the body of the acanthor was observed several times.

SUMMARY

A brief discussion of the phylum Acanthocephala is given in the introduction to this study.

Tests were conducted on the effects of the pH of the rewetting solution, temperature, and storage time on the artificial hatching of the eggs of Macracanthorhynchus hirudinaceus, the swine thorny-headed worm.

In order to test the effects of the pH of the rewetting solution, eggs which had been stored at room temperature for periods from one to 21 days were rewetted with solutions of various pH. For experiments on effects of temperature, an equal number of slide preparations were stored at each of three temperatures, room temperature (18°-32°C.), 39°C. and 5°C. They were rewetted with solutions having a pH of 8.8 after definite numbers of days had elapsed. The effects of storage time were observed by rewetting groups of slides, which had been stored from one to 21 days, with solutions having the same pH.

A solution with a pH of 8.8 was found to induce the highest percentage of hatching when used as a rewetting agent on desiccated eggs which had been stored at room temperature.

When a solution having the optimum pH of 8.8 was used to treat dried eggs, those stored for different periods of time at 39°C. showed the largest percentage of hatching. Percentages of hatchings only slightly lower than those determined at 39°C. were recorded for slides kept at room temperature. Eggs stored at 5°C. showed low viability when rewetted with a solution having a pH of 8.8.

Results obtained indicate that the number of hatchings decreases as the period of storage increases, after one day. Slides kept at room temperature for a period of 14 days showed fewer hatchings than the ones stored for seven days. The slides stored for 21 days had a much lower percentage of hatching than those kept for 14 days. No hatchings were observed after a storage period of 40 days at room temperature.

A discussion of the relationship of the experimental factors to conditions involved in natural hatching of eggs is given.

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