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MORPHOGENESIS AND VIABILITY OF LARVAE IN THE EGGS OF ENTEROBIUS VERMICULARIS

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Abstract. When comparing the morphology of eggs dissected from the uterus of mature female with that of eggs deposited into the perianal region of the host, different stages of development were observed. The first embryonal stage is of cellular character and possesses a base of tail. The fully differentiated second stage is tadpole-shaped and requires oxygen for further development. The third and fourth stage develop within the eggs in the anal region; the fifth stage is the infective larva. It has a cuticle on its body surface and a differentiated digestive tube. The infective larva hatches from the egg-shell only after exposure to digestive enzymes. The preinfective stages are more susceptible to drying than the infective stage. However, even this stage may be damaged by long-lasting drying. The mature eggs can remain viable for 2-3 days at the temperature of 22 °C and relative humidity of 34—44 % and 15.6—22.9 % of larvae hatch from them after exposure to the digestive solution. The larvae are able to survive for 20 min.

The resistance and viability of eggs of pin-worms in different media and at different temperatures and pressures was investigated by many previous authors. Leuckart (1876) studied the effect of oxygen, Philpot (1924), Lentze (1935) and Sondak (1935) the role of temperature and Jones and Jacobs (1940) the humidity of environment. Wilhelmi and Quast (1925), Oleinikov (1929) and Lentze (1935) discovered the eggs in nasal secretion of children and estimated therefore the number of eggs contained in the dust of the room. They assumed that the children became infected by inhaling it. Also Nolan and Reardon (1939) and Schüffner and Swellengrebel (1949) admitted the possibility of inhaling the eggs together with the dust, but this assumption was not verified experimentally. Sandars (1963), on the contrary, found only a small number of infective eggs in the dust of the room. The majority of the undeveloped eggs were desiccated and only some eggs containing infective larva survived.

In our experiments, we studied the resistance of eggs to desiccation under natural conditions of the laboratory. For evaluation of the experimental material the exact knowledge of the morphogenesis of larvae is necessary, because the resistance of eggs depends also on the stage of their development. We have therefore compared the morphology of embrya inside the eggs dissected from the uterus of mature female to the morphology of embrya in eggs deposited into perianal region of the host.

MATERIAL AND METHODS

Living females of *Enterobius vermicularis* and free eggs were obtained by swabbing the anal folds of children at the Clinic of Internal Diseases of the Thomayer's Hospital and in Regional Department of Hygiene and Epidemiology in Prague, where the children with massive oxyuriasis were treated.

Schüffner's tubes with swabs were washed into a drop of tap water on slides with a pit or in test tubes. Living females were also introduced into test tubes containing a smal quantity of water. The material was transferred to the laboratory in a thermos flask with ice and stored at 4 °C. The eggs were dissected from the uterus of females and put on a glass slide containing a drop of water. The released eggs were then transferred with a pipette into centrifuge tubes. A large number of eggs for experimental purpose were assembled by centrifugation. The eggs were cultured to the infective stage in Petri dishes with a small quantity of water, placed in a moist incubation chamber and incubated at 37 °C for 6 hours. The chambers were prepared from glass boxes with moist cellulose wadding on the bottom, covered with perforated tin foil for aeration. The warming of the chamber in water bath was controlled by Vertex. For examination of the development of embrya the eggs were removed from Petri dishes at regular intervals and put in slides framed by varnish and containing a drop of alum gelatine. The slides were covered with cover-glass supported by a glass splinter fixed by a mixture of vaseline, paraffine and wax. After mild drying the glasses with eggs were placed into 1N HCl and then transferred into fixation solution of absolute alcohol and acetic acid (3:1) and stained with 2 % orcein in propionic acid (according to Pearse 1961). Other eggs and females were fixed in Baker's fixation solution, or in 10 % neutral formalin and processed by histological methods. Paraffine sections were stained by common histological and histochemical methods.

Infective eggs were tested for viability after drying for several periods of time. Since the eggs from perianal swabs were in various stages of development and the undeveloped eggs were more susceptible, they were incubated to the infective stage in incubation chambers by the same method as the eggs isolated from females.

A certain number of infective eggs were then transferred to glass slides and exposed to drying at 22 °C and relative humidity of 34—44 % for 6, 12 and 24 hours and 3 and 5 days. The viability of dried eggs was then determined according to hatching of infective larvae after previous exposure to digestive solution (0.7 % pepsin and 0.2 % HCl or 0.5 % pepsin with veronal acetate buffer) at the temperatures of 22 °C and 37 °C. We regarded as viable those eggs from which the larvae hatched and moved for 20 min after transfer from digestive solution into physiological saline in slides (according to Fairbairn 1960). The percentage of viability in individual experiments was calculated from the ratio of hatched and moving larvae after 6-hr exposure to digestive solution and number of eggs with infective larvae at the beginning of experiment.

RESULTS

A. MORPHOGENESIS OF EGGS IN THE UTERUS OF FEMALE

The sac-like uterus of mature female is filled with a large number of eggs with the definitive egg-shell consisting of three principal layers. The fertilized eggs are in different stage of cleavage. A large round nucleus can be seen in the centre of plasma at the beginning of cleavage, later on also two nuclei and animal pole is marked by a lighter bordered plasma (Plate I, Fig. 1). Yolke granules containing tyrosine are dispersed in the plasma. The first division runs in meridional direction to the longitudinal axis of the egg, giving rise to two blastomeres — the anterior, smaller blastomere (S_1) and the larger, posterior one (P_1) (Plate I, Fig. 2). First the anterior and then the posterior cell divides equally to form the four-celled stage of rhombic shape. The first anterior cell then becomes more active and divides twice in succession, while the second anterior cell divides once, making seven cells in all (Plate I, Fig. 3). The first three cleavages are equal, but thereafter they are unequal, the daughter cells never reaching the size of the parent cells (Plate I, Fig. 4). The embryo in the stage of morula occupies nearly the whole egg. A split-like cavity of blastocoel is sometimes visible in the centre of embryo. Simultaneously with the formation of gastrula the embryo extends towards the egg poles. A band of smaller lighter ectodermal cells is formed arround the outside and the large darker cells form the endoderm in the centre. During further development the ectodermal cells divide actively, forming numerous small cells with large basophilic nuclei at the anterior end, later giving rise to the head of embryo. In the centre of this clear area is a small depression, the base of stomodaeum. At the opposite end the body

of embryo is tapered and the base of tail attenuation is formed by the division of lighter cells of ectoderm (Plate II, Fig. 1). The base of tail continues growing and the moving second-stage embryo is formed. It is spindle-shaped, with long tail, and Leuckart (1876) likened it to a tadpole (Plate II, Fig. 2). This is the last stage of the development inside the uterus of mature female.

B. MORPHOLOGY OF EGGS DEPOSITED INTO PERIANAL REGION

In the moist medium of anal region (88—99 % humidity), at the admission of oxygen and body temperature the second embryonal stage develops and grows. The endodermal region of this spindle-shaped embryo increases in size and extends posteriorly along the egg axis. The tail end is growing and head end acquires the elongated form. The tail end of the third-stage embryo reaches nearly to its head (Plate III, Fig. 1). The body is filled with numerous granules containing tyrosine and lipids. Glycogen is present in posterior end of body. The fourth-stage embryo gets a worm-like shape. It is coiled inside the egg and its head end reaches the apical pole of the egg. The tail is ventrally coiled and its end lies close to the head (Plate II, Fig. 2). The embryo moves inside the egg by contracting the body wall and shifting the secretory granules. The developing oesophageal region can be seen at the anterior end and the intestine is filled with large embryonal cells. The digestive tube, however, is not yet luminized and a fine cuticle covers the surface of body (Plate IV, Fig. 1). The hatched infective larva is twice as long as the preinfective stage (Plate IV, Fig.2) and the digestive tube is luminized. Our experiments revealed that the development from the second to third stage lasts 2 – 3 hours, from the third to fourth stage 1/2 to 1 hour and to the infective stage 1 1/2 hour on the average.

Hatching of the infective larvae from the egg-shell starts after exposure to digestive enzymes. Treatment of eggs with digestive solutions affects the outer layer only. The egg-shell ruptures at the thinned margin of the "operculum" (Hulínská, Hulínský 1973), through which the larva emerges from the egg-shell head- or tail-end first (Plate IV, Fig. 3).

C. EFFECT OF DRYING ON THE VIABILITY OF INFECTIVE EGGS

The results of our experiments concerning viability and survival of eggs after drying are given in Tables 1 and 2. Each experiment was repeated several times and the mean percentage of viability was then calculated. A short drying (for 6 hr) causes a slight damage of the eggs and 66 % of larvae on the average hatch from 50 infective eggs at the temperature of 22 °C. They are able to move in the solution for more than 20 minutes. The results obtained were the same with eggs isolated both from anal swabs and from living females, under condition that only infective eggs were applied. The preinfective stages are very susceptible to drying and although some embrya may emerge from the egg-shell in the digestive solution, they are deformed and die in the solution. More larvae hatch at higher temperature (37 °C), from 55 eggs 75.9 % on the average. If the eggs were not exposed to drying, the percentage hatch from 55 eggs was 81.2 % at the temperature of 22 °C and 92.9 % at the temperature of 37 °C on the average. The longer the period of drying, the lower the number of surviving eggs and hatched larvae, as shown in Tables 1 and 2. At the temperature of 22 °C most larvae hatched during the second and third hour of experiment, whereas at the temperature of 37 °C most larvae hatched during the first hour (Figs. 1 and 2). After 24-hr drying the number of hatched larvae decreased and at the temperature of 22 °C only 33.2 % of eggs survived. At the temperature of 37 °C the mean percentage hatch was somewhat higher,

Table 1. Tests of viability of dried infective eggs (from anal swabs) using digestive solution of 0.7 % pepsin and 0.5 % HCl at 22 °C.

No. of infective eggs	Period of drying	Living larvae released from shells in %						Viability in %
		1/2	1	2	3	4	5	
		hours						
50	6 hr	—	12	18	12	18	4	64
50	6 hr	—	14	17	18	18	2	69
60	12 hr	—	11.6	8	8.3	8.3	16.6	53.2
60	12 hr	—	3.3	20	15	13.3	1.6	53.2
60	24 hr	—	—	16.6	13.3	3.3	1.6	34
55	24 hr		—	9	9	10.9	3.6	32.5
60	2 days		6.6	8.3	10	—	—	24.2
55	2 days	—	9	3.6	7.2	1.8	—	21.6
55	3 days	—	—	12.7	—	—	3.6	16.3
60	3 days	—	—	3.3	11.6	—		14.9
50	5 days	—	—	—	—	—	—	0
60	5 days	—	—	—		—	—	0
55	without drying	3.3	12.7	18.1	25.4	12.7	9	81.2

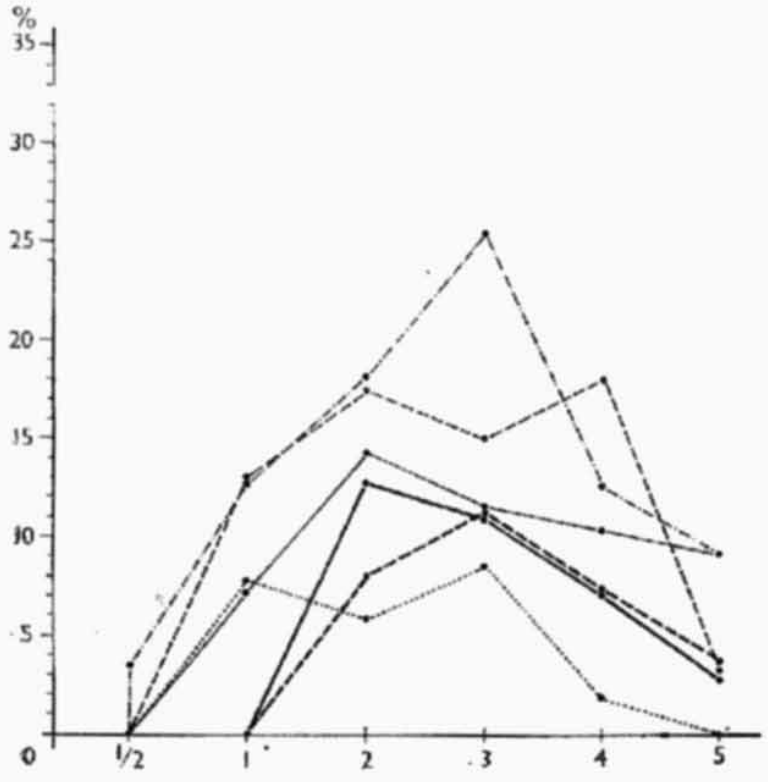

Fig. 1. The relationship between the percentage hatch of infective viable larvae and the time period of drying and exposure to digestive solution (1/2, 1, 2, 3, 4 and 5 hr) at the temperature of 22 °C.

Explanations: –.–.– eggs without drying, – – – – – eggs dried for 6 hr, —— eggs dried for 12 hr, – – – eggs dried for 24 hr, ... eggs dried for 2 days, – – – – eggs dried for 3 days.

Table 2. Tests of viability of dried infective eggs (from anal swabs) using digestive solution of 0.7 % pepsin and 0.5 % HCl at 37 °C

No. of infective eggs	Period of drying	Living larvae released from shells in %						Viability in %
		1/2	1	2	3	4	5	
		hours						
55	6 hr	10.9	23.6	32.7	9	3.4	—	79
55	6 hr	12.7	20	34.5	7.2	3.6	—	78
55	6 hr	12.7	18.1	25.4	10.9	3.6	—	70.7
60	12 hr	6.6	16.6	18.3	11.6	3.3	—	56.4
55	12 hr	12.7	10.9	3.6	12.7	10.9	—	50.8
55	24 hr	7.2	9	18.1	7.2	—	—	41.5
60	24 hr	3.3	11.6	16.6	6.6	—	—	38.1
55	2 days	12.7	3.6	3.6	—	—	—	19.9
60	2 days	13.3	6.6	3.3	—	—	—	23.2
55	2 days	7.2	9.0	3.6	—	—	—	19.8
55	3 days	—	3.6	1.8	—	—	—	5.4
55	3 days	—	12.7	—	—	—	—	12.7
55	3 days	—	9.0	—	—	—	—	9.0
55	without drying	9	18.1	12.7	23.6	9	18.1	91.5
55	without drying	10.9	12.7	10.9	32.7	18.1	9	94.3
55	5 days	no larva survived						0

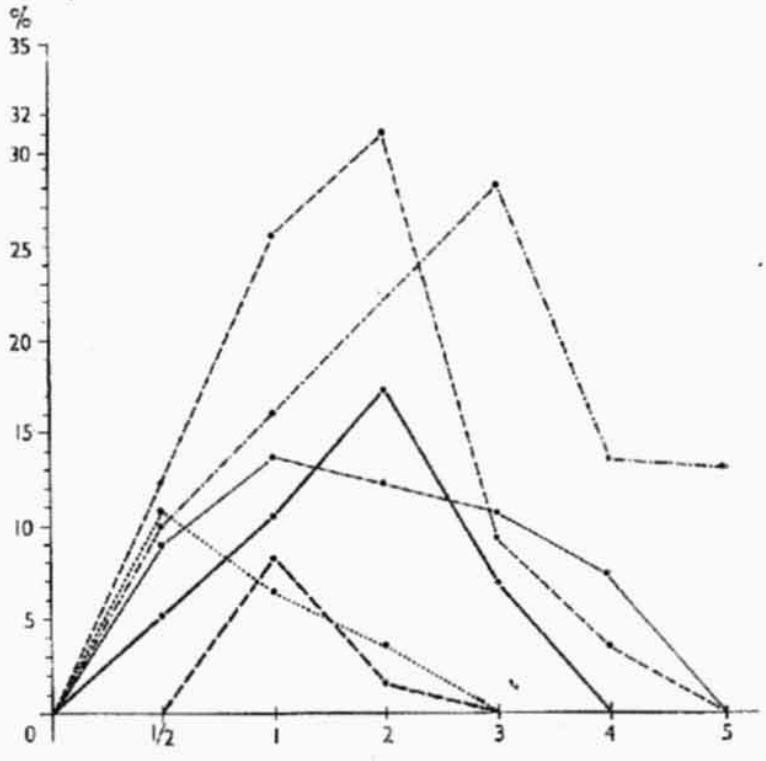

Fig. 2. The relationship between the percentage hatch of infective viable larvae and the time period of drying and exposure to digestive solution (1/2, 1, 2, 3, 4 and 5 hr) at the temperature of 37 °C. For explanations see Fig. 1.

39.8 %. Maximum period of drying was 2—3 days, when the mean percentage hatch at 22 °C was 22.9 % after two days and only 15.6 % after three days. At the temperature of 37 °C the effect of drying was still higher, and the mean percentage hatch was 20.9 % after two days and 9 % after three days. The composition of the digestive solution had no effect on the results of experiments, as it is seen in Table 3 showing the solution of 0.5 % pepsin with veronal-acetate buffer.

Table 3. Tests of viability of eggs (from females) using digestive solution of 0.5 % pepsin buffered with veronal acetate (pH 7.2) at 37 °C

No. of infective eggs	Period of drying	Living larvae released from shells in %							Viability in %
		1	2	3	4	5	6	7	
		hours							
55	without drying	9	18.1	12.7	23.6	6.9	18.1		91.5
55	without drying	—	12.7	10.9	32.7	18.1	9	10.9	94.3
55	6 hr	—	7.2	12.7	10.9	23.6	1.8	12.7	78.9
55	6 hr	—	3.6	18.1	25.4	10.9	3.6	12.7	74.3
60	12 hr	—	11.6	8.3	3.3	3.3	16.6	11.6	54.7
55	12 hr	—	12.7	10.9	3.6	12.7	—	10.9	50.8
55	24 hr	3.6	12.7	—	—	10.9	—	3.6	30.8
55	24 hr	—	3.6	—	12.7	3.6	—	14.5	34
55	2 days	—	3.6	—	—	10.9	—	3.6	18.1
55	2 days	—	—	—	—	12.7	—	—	12.7
55	3 days	—	—	1.8	—	3.6	3.6	1.8	10.8
55	3 days	—	—	1.8	—	3.6	—	3.6	9.0
50	5 days	6 dead larvae							

DISCUSSION

In our experiments, we have compared the morphology of the eggs dissected from the uterus of females with those obtained from perianal region of host. To complete our knowledge we cultured experimentally the eggs to the infective stage. In this way we obtained a picture of all developmental stages.

We have found that the eggs contained in the uterus of females are in different stage of cleavage up to differentiation of the first spindle-shaped embryonal stage. Immediately before the female starts to deposit eggs into perianal region, some second-stage embrya develop. Therefore this stage can be found mostly in swabs from anal region and only occasionally in ovijector and uterus of mature females. In the anal region, the eggs containing second-, third- and fourth-stage embrya can be found. The embrya of third and fourth stage move within the egg-shell and grow at both tail and head end. They are vermiform and the digestive tube is differentiating. More than the morphology of embrya, which was partly described by Leuckart (1876), Heller (1903), Philpot

(1924) and Ergardt and Wigan (1949), the factors influencing the embryonal metabolism have been dealt with. Engelbrecht (1963) studied the consumption of lipids and glycogens during embryogenesis of eggs and deposition of glycogen in the embryo, which he called, similarly as Leuckart (1876), a "tadpole". Leuckart's knowledge of the development of pin-worm is not complete and the morphology of the stages described and figured is not sufficiently explained. On the other hand, Lentze (1935) was engaged rather in the biology of this parasite .The knowledge of morphogenesis of eggs was necessary for our experimental studies of the resistance of eggs in the environment. We have investigated the effect of drying on infective stages under laboratory conditions (temperature of 22 °C, humidity up to 44 %). The infective larvae hatch from the eggs only after exposure to digestive enzymes of the host, as it was confirmed by Leuckart (1865), Philpot (1924) and experimentally also by Zawadowsky and Schalimov (1929). The physiology of hatching, however, has not yet been elucidated. Our experiments revealed the dependence of hatching of larvae on the period of drying, during which the physical and probably also chemical conditions inside the shells (permeability) are changed. For this reason the infective larva subjected previously to longer drying does not hatch or after rupture of the shell is so damaged that it dies in the solution. The developed eggs can withstand at most two- to three-days' drying. Still higher effect of drying was observed in the preinfective stages. Only slight drying leads to the destruction of the egg-shell and to the death of embrya. Since the eggs in anal swabs are in various stages of development, it may be supposed that some eggs contaminate the objects of the environment without reaching infective stage. These eggs are of no significance for peroral infection or infection by inhalation of dust. This fact was not considered either by Oleinikov (1929) or by Lentze (1935) when they determined the contents of eggs in the dust of room and they assumed that its inhalation resulted in the infection of children examined. Also Jírovec (1946, 1948) was of the same opinion. Sandars (1963), on the contrary, found only few infective eggs in the room dust and he was therefore of different opinion; he considered the infection caused by inhalation to occur only exceptionally. The results obtained by Jones and Jacobs (1940) support Sanders' opinion. In our experiments, the eggs survived two- to three-days' drying, but none of them could withstand the drying for five days.

Considering that only dried eggs may be raised with the dust and that their viability decreases with the length of drying, it may be assumed that pin-worm infection caused by inhalation of dust is of importance in larger groups of children only (e.g. kindergarten). In the infected children the life cycles of the parasite run differently and egg-laying occurs during the last two days of the life cycle. Each female lays 12—16 thousand of eggs into the anal region of the host. Geller (1946) and Podyapolskaya and Kapustin (1958) observed up to 3 000 worms in a child. These child institutions can thus easily be infected with fresh eggs. Our experiments revealed that on the average 53.2 % of eggs can survive for 12 hours and 18.7 % for two days. Our results could not be compared with those of Lentze (1935) and Jones and Jacobs (1940) due to different methods applied in the experiments. Lentze (1935), moreover, did not give the developmental stage and number of eggs and the hatching of larvae regarded as a criterion of viability. However, he included in the number of hatched larvae also the damaged ones. In our experiments, we found that even damaged larvae may hatch, but that they die soon in the solution. Of the same opinion were also Jones and Jacobs (1940), who considered viable those eggs, which contained the larvae capable of surviving for 40 min.

МОРФОГЕНЕЗ И ЖИЗНЕСПОСОБНОСТЬ ЛИЧИНОК В ЯЙЦАХ *ENTEROBIUS VERMICULARIS*

Д. Гулинска

Резюме. Сравнивая морфологию яиц полученных из матки половозрелой самки с яйцами отложенными в перианальную область хозяина, мы наблюдали разные стадии развития. Первая эмбриональная стадия имеет клеточный характер и содержит основу хвоста. Вполне дифференцированная вторая стадия имеет форму головастика и для дальнейшего развития требует кислород. Третья и четвертая стадии развиваются внутри яйца в анальной области; пятая стадия — инвазионная личинка. У нее кутикула на поверхности тела и дифференцированная пищеварительная трубка. Инвазионная личинка вылупляется из оболочки только после действия пищеварительных ферментов. Прединвазионные стадии более чувствительны к высушиванию, чем инвазионная стадия. Но даже эта стадия может погибнуть вследствие долговременного высушивания. Зрелые яйца жизнеспособны в течение 2—3 дней при температуре 22 °C и относительной влажности 34—44 %; после действия пищеварительного раствора из них вылупляется 15,6—22,9 % личинок, которые выживают 20 минут.

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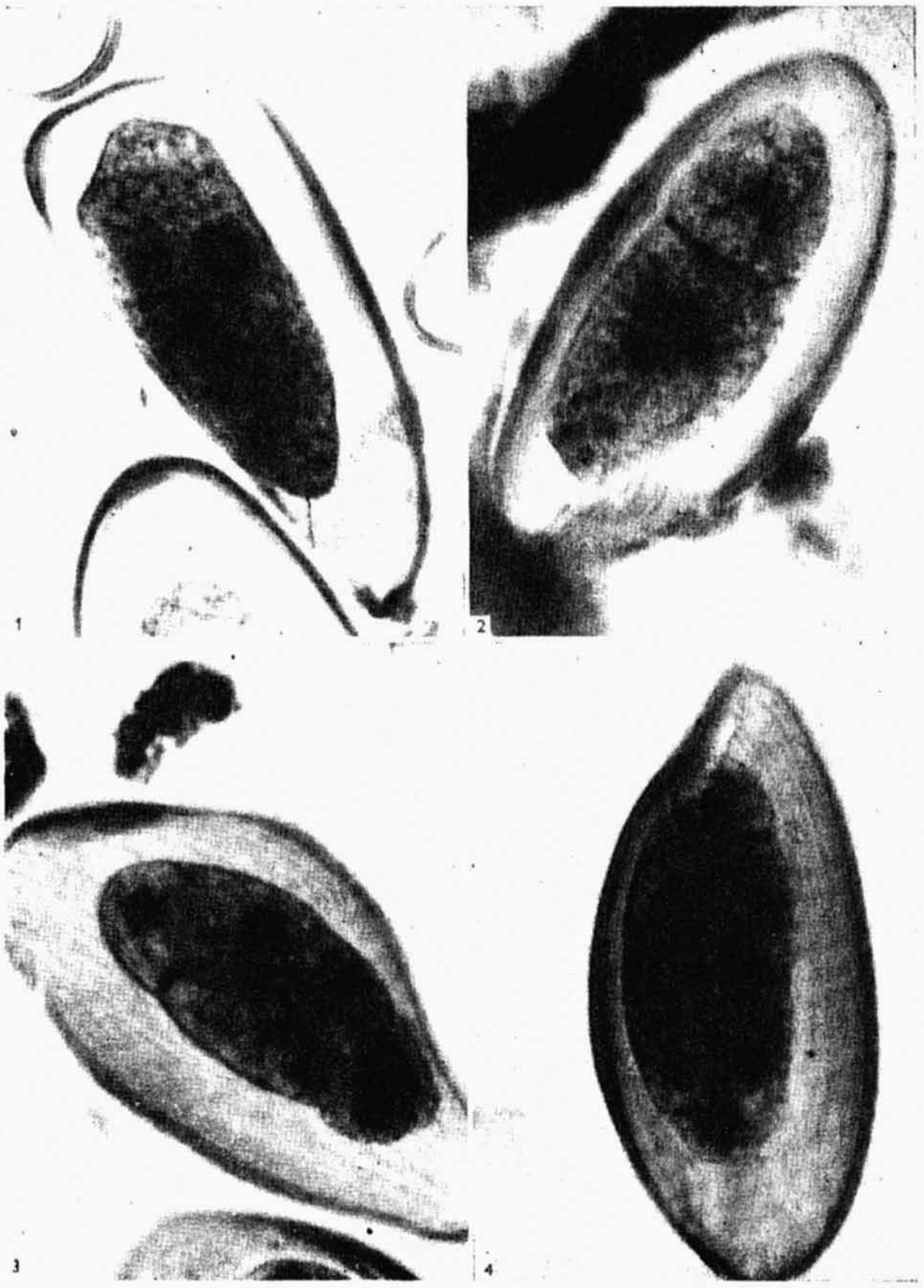

Plate I.

Fig. 1. Longitudinal section through mature egg. Large bladder-like nucleus and numerous granules of lipoprotein are contained in the plasma at the beginning of caryokinetic division. The surface of plasma is bordered with a vitelline membrane. (Coupled tetrazonium reaction for tyrosine, ×400)

Fig. 2. Longitudinal section through the egg. The first cleavage at meridional direction, separating larger blastomere form the posterior smaller one. (Coupled tetrazonium reaction combined with Sudan III B reaction, ×400)

Fig. 3. Longitudinal section through the egg with 7 blastomeres. During further cleavage first posterior blastomere divided twice in succession, while the second anterior cell divided once making seven cells in all. (Coupled tetrazonium reaction, ×400)

Fig. 4. Longitudinal section through the egg with 8 blastomeres. During further cleavage smaller and smaller blastomeres are formed at posterior end. (Coupled tetrazonium reaction, ×400)

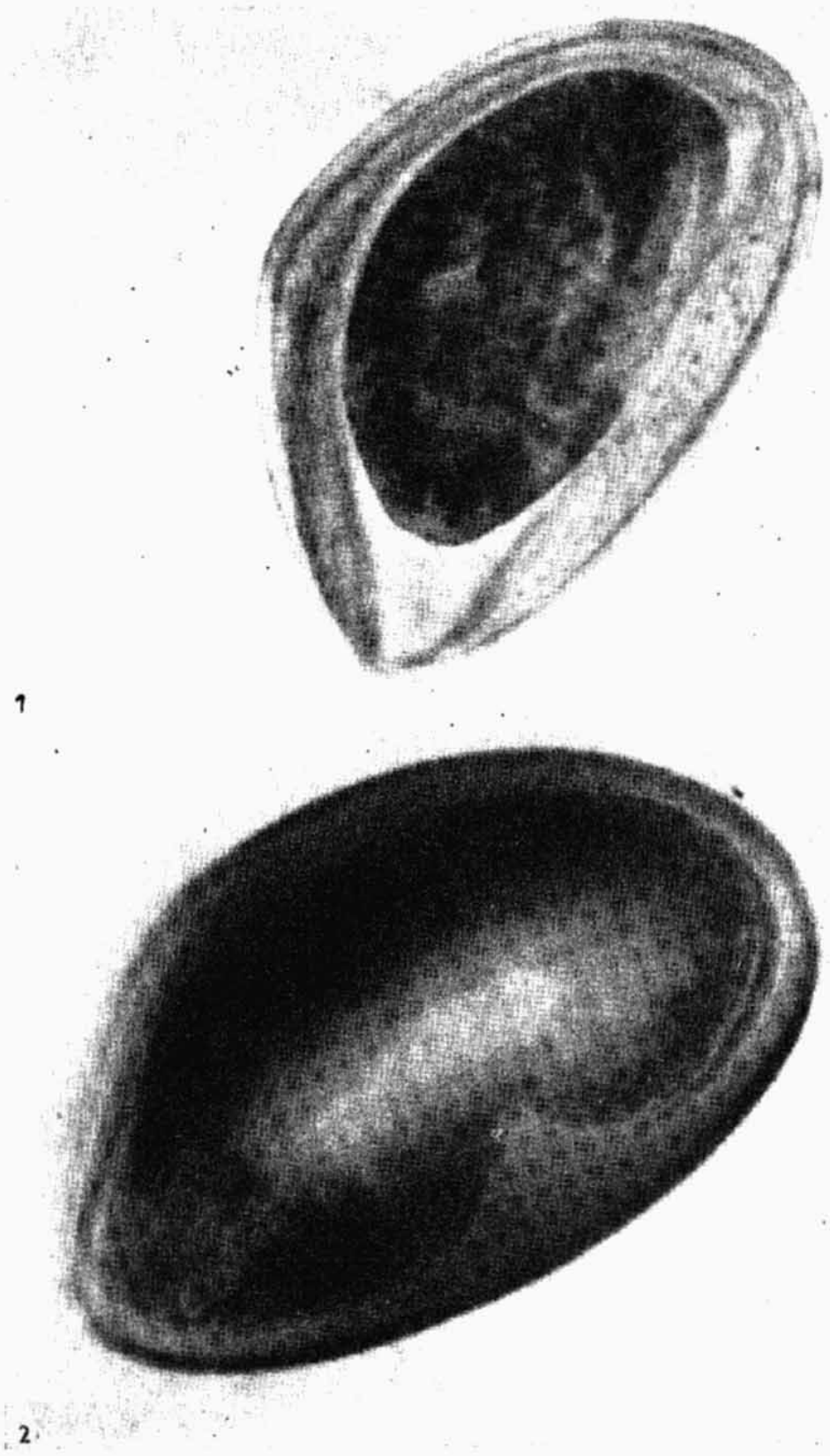

Plate II.

Fig. 1. First-stage embryo. Body spindle-shaped, filled with embryonal cells with large basophilic nuclei. Tail at posterior end of embryo. The egg-shell slightly pressed. (2 % orcein, toluidine blue, ×260)

Fig. 2. Second-stage embryo occupying nearly the whole egg. Body of embryo attenuating in a tail at posterior end. (Bromphenol blue, ×450)

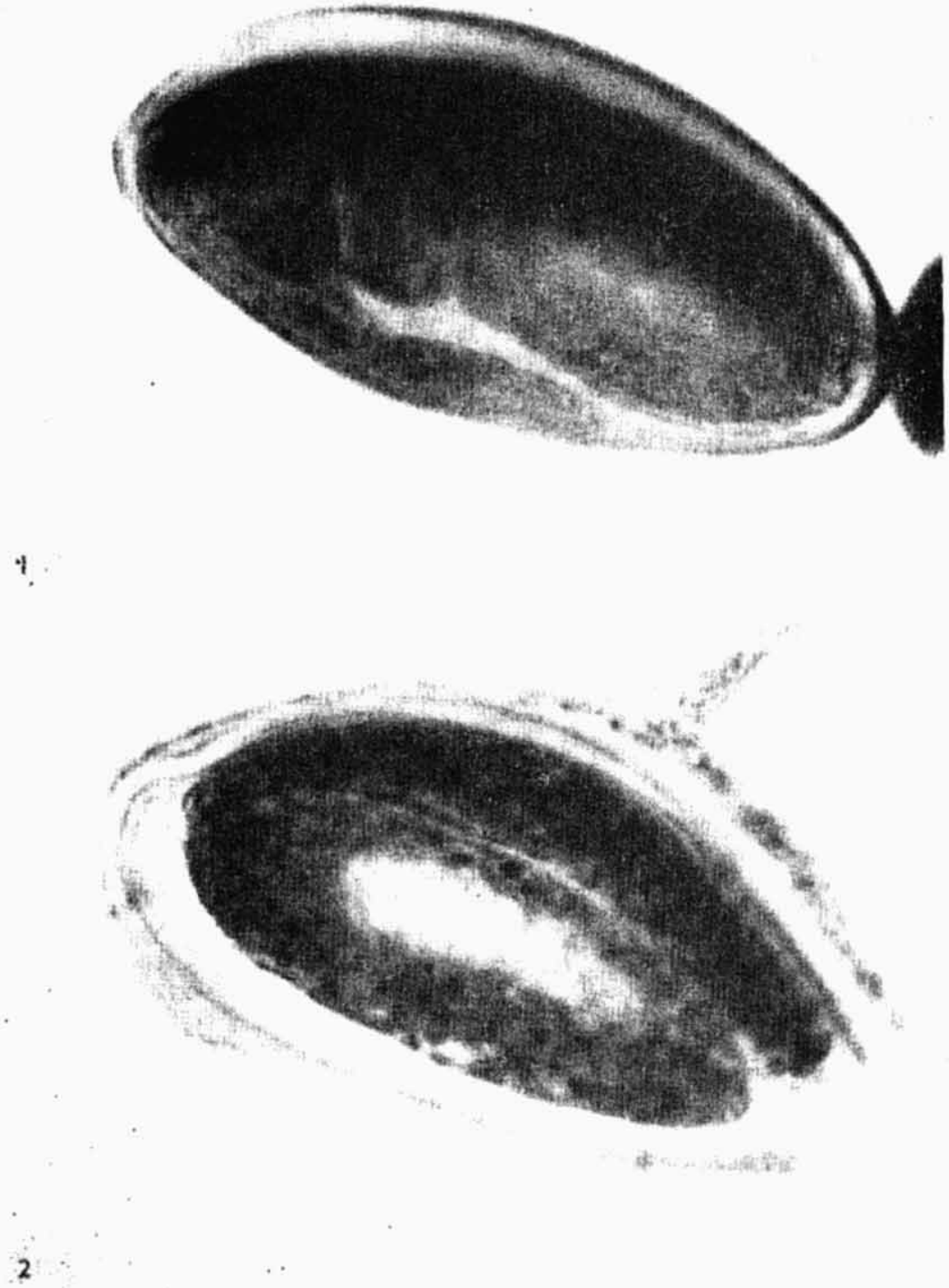

Plate III.

Fig. 1. At the third stage the head end attenuates and elongated tail starts to form. Head end is ventrally coiled and situated near the middle part of body. (Bromphenol blue, coupled tetrazonium reaction, ×400)

Fig. 2. Fourth-stage larva. Body vermiform, long tail coiled ventrally. Base of oesophagus and intestine filled with embryonal cells. (Coupled tetrazonium reaction, ×400)

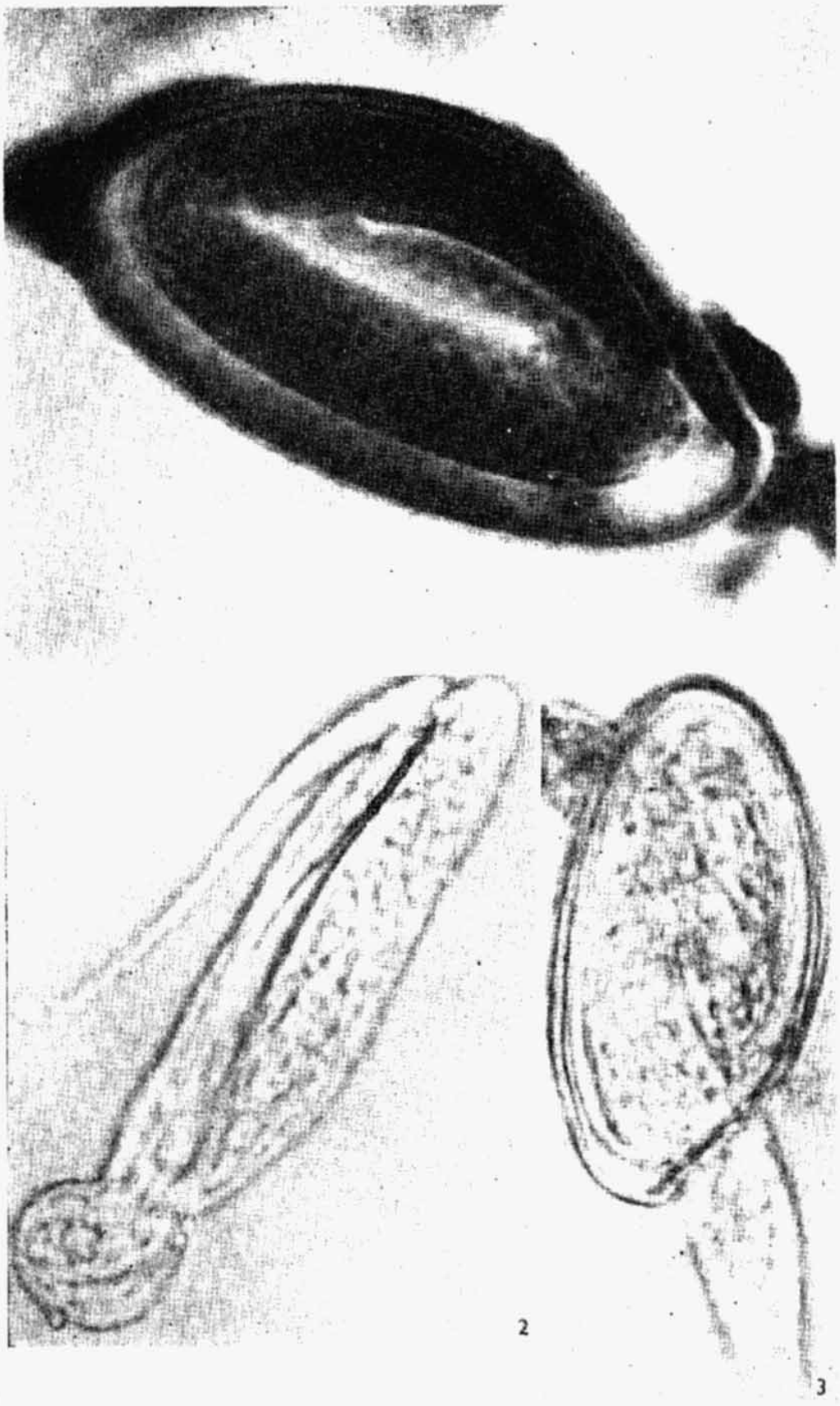

Plate IV.

Fig. 1. Fourth-stage larva with distinct cuticle on body surface. (Peracetic acid-aldehyde fuchsin for proteins (cystine) ×400)

Fig. 2. The hatched infective larva is twice as long as the preceding stage. It moves by contracting the body wall as seen in the native photograph. (×100)

Fig. 3. The infective larva emerging from the egg its head end first. (Native, ×100)