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Egg Transport in the Coelom of the Newt *Cynops pyrrhogaster* . I. The Ovulated Egg is Transported to the Ostium by the Ciliary Movement of Coelomic Epithelial Cells

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ABSTRACT—We investigated the mechanism of egg transport in the newt not only by inserting various conditioned eggs into the recipient's body but also by placing them on the coelomic epithelia of the opened body cavity in the adult female newt. Most of the inserted coelomic eggs were oviposited, while 4 of 14 inserted de-jellied uterine eggs and 3 of 10 inserted de-jellied fertilized eggs were oviposited. The coelomic eggs placed on the coelomic epithelia were transported toward the ostium and entered the ostium. The de-jellied uterine eggs and the de-jellied fertilized eggs were transported to the ostium as well. Of all the eggs examined, the coelomic egg was transported the fastest. The transport speeds of coelomic eggs treated with periodic acid and the speed of boiled coelomic eggs were less than those of untreated coelomic eggs. In contrast, the transport speeds of coelomic eggs treated with trypsin and the speed of coelomic eggs removed from their vitelline envelopes (naked eggs) were faster than those of untreated coelomic eggs. Other experiments were carried out in order to ascertain the dependence of sexual activity on egg transport. The speed of coelomic egg transport in artificially sexually activated females was faster than in sexually inactive females, although the ciliary movement could always be observed in both sexually active females and sexually inactive females. This suggests that the speed of egg transport on the coelomic epithelia is controlled by the sexual activity of the female.

Key words: egg tansport, coelom, cilia, hormone, newt

INTRODUCTION

In vertebrates, gametes released from the gonads are transported to the site of fertilization via gonoducts such as the uterus or the ductus deferens. Gamete transport is one of the most important roles of the gonoduct.

In most species, spermatozoa are released directly from the testis into the ductus deferens. In regards to egg transport from the ovary to the uterus, little difference is seen among species. In fish, eggs are released into the ovarian cavity and enter the oviduct directly. In reptiles, avians, and mammals, eggs released into the coelom are caught by the ostium immediately after ovulation, since the ostium opens near the ovary. Particularly in mammals, the ostium is referred to as the fimbriae, and egg transport on the fimbriae has been thoroughly studied by Odor and Blandau (1973). The egg, which is caught on the fimbriae, is transported to the ampullae by ciliary movement. Based on *in vivo* observations of egg transport in rabbits, it is thought that the cilia of the fimbrial epithelia are of primary importance to egg transport. If the fimbriae are surgically removed, the ovulated mass remains attached to the ovarian surface for many hours (Odor and Blandau, 1973).

In amphibia, many ovulated eggs can be seen in the coelom because the ostium is far from the ovary (Fig.1). Eggs seen in the coelom are referred to as coelomic eggs. Rugh (1935) demonstrated that coelomic eggs are transported to the ostium by means of the ciliary action of the coelomic epithelia.

In this paper, we confirm the mode of egg transport described by Rugh (1935) and demonstrate the characteristics of the mechanism of egg transport via the coelomic epithelia of female newts in detail.

MATERIAL AND METHODS

Animals

Adult newts, *Cynops pyrrhogaster*, were collected in Yamagata, Japan, and were kept in a refrigerator at 4°C until use.

Eggs

Ovulation was induced by two subcutaneous injections of

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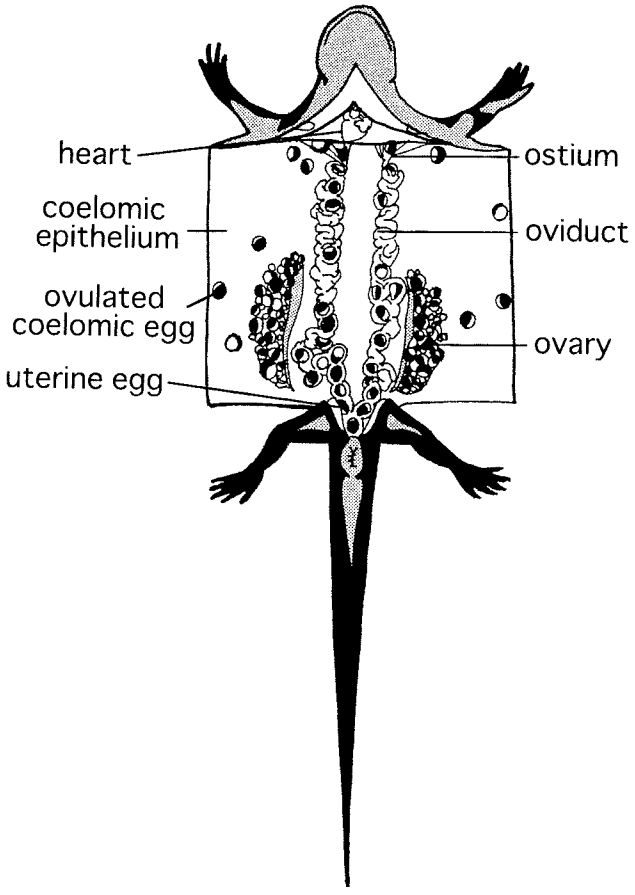

Fig. 1. Schematic arrangement of the reproductive organs in the female newt. Many ovulated eggs are seen in the coelom because the ostium is located relatively far from the ovary.

human chorionic gonadotropins (hCG, Teikoku-zoki Co., Japan) at 100 IU every other day. The coelomic eggs were collected 10 to 12 hr after the second injection, after which female newts were pithed and their body cavities opened. These eggs were kept in Steinberg's solution at room temperature until use (within 2 hr). The oviposition began 30 hr after the second injection. These oviposited eggs were determined to be already fertilized because the females had stored the spermatophore in their cloacae from the last mating season. To obtain unfertilized eggs, these females were pithed and their body cavities opened, and the oviducts were then removed. Eggs were collected from the lower portion of the oviducts and were immersed in 1/10 Steinberg's solution. These unfertilized eggs are referred to hereafter as "uterine eggs." The jelly, which surrounded the fertilized egg and the uterine egg, was removed with scissors. These de-jellied eggs were kept in Steinberg's solution at room temperature until use.

Treatments of the eggs

One set of eggs was prepared as follows: coelomic eggs and de-jellied uterine eggs were boiled in Steinberg's solution for 10 min at 75°C. Eggs damaged during boiling were rejected. A second set of eggs were prepared as follows: coelomic eggs were treated with 0.05% trypsin-Steinberg's solution (pH 7.8) for 5 min at 28°C in a shaken water bath and rinsed well in Steinberg's solution. A third set of eggs were prepared as follows: coelomic eggs and de-jellied uterine eggs were treated with 1% periodic acid-Steinberg's solution (pH 6.0) for 10 min or 20 min at 25°C in a shaken water bath and rinsed 8 times at 10 min intervals in Steinberg's solution. Naked eggs were prepared as follows: the vitelline envelope was gently removed from the coelomic egg using watchmaker forceps in Steinberg's solution under a stereomicroscope.

Anesthesia

The newts were deeply anesthetized with 0.05% phenylurethane solution. The surgery was performed within 1 hr of anesthesia administration.

Insertion of various materials into the coelom

Various materials were inserted into the coelom of the hormonally activated females. Coelomic eggs, de-jellied uterine eggs, and de-jellied fertilized eggs (2–4 cell stage) were respectively inserted into the female body, as were the boiled eggs, glass beads, and agar beads. The inserted eggs were marked with Nile blue on the surface of the vegetal hemisphere in order to distinguish them from eggs native to each newt. The diameters of all inserted

Table 1. Insertion of various eggs or other transplants into coelomic cavity of the female newt

transplants	No. of examined newts	oviposited[a]	not oviposited[b]		
			in oviduct[c]	in coelom[d]	N.D.[e]
coelomic egg*	5	4	1		
uterine egg*	14	4	3	5	2
fertilized egg*	10	3	1	5	1
boiled coelomic egg**	5			5	
boiled uterine egg**	8			8	
agar bead**	4			4	
glass bead**	3			3	

[a] Values given are the numbers of newt in which at least one transplant was identified among all oviposited eggs.
[b] Values given are those of incised newts that died before oviposition or in which no transplants were identified.
[c] Values given are those numbers of newts in which at least one transplant was identified in the oviducts.
[d] Values given are the numbers of newts in which all transplants were identified in the coelom.
[e] Undetermined transplants in the oviposited eggs, oviduct, or coelom.
* Two eggs per female were inserted.
** Four transplants per female were inserted.

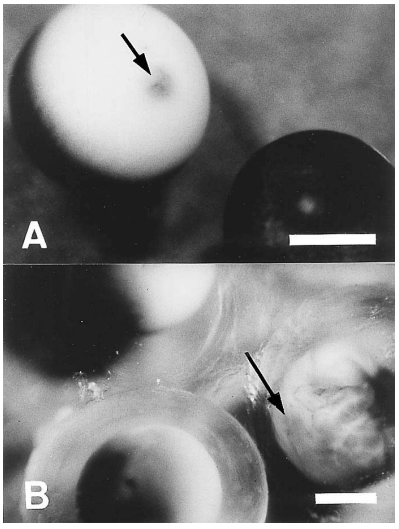

Fig. 2. The ovulated eggs are marked with Nile blue. A: The inserted coelomic eggs were marked with a Nile blue dot on the surface of the vegetal hemisphere (arrow). B: The marked egg (arrow) was oviposited and enveloped in jelly. Scale bar, 1 mm

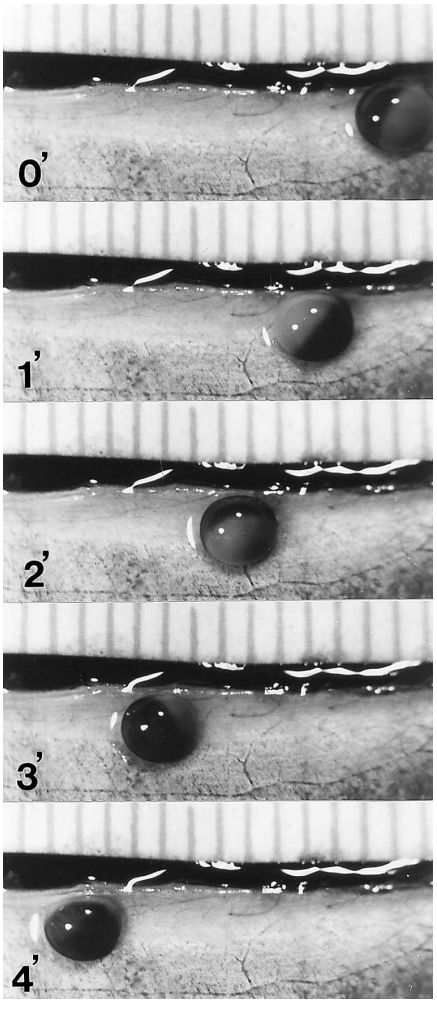

Fig. 3. Transportation of coelomic egg on the coelomic epithelia in a sexually activated female. Time-lapse photographs were taken at 0, 1, 2, 3, and 4 min. The egg was transported 11 mm over a period of 4 min, with a calculated transport speed of 45.8 μm /s.

egg were approximately 2 mm. An incision approximately 3 to 4 mm long was made in the middle part of the abdomen of each recipient, and the materials were inserted into the coelom. Two coelomic eggs were inserted into each female, as were de-jellied uterine eggs or de-jellied fertilized eggs. Each female newt received 4 boiled eggs, 4 glass beads and 4 agar beads respectively. The abdominal wounds were glued with surgical bonds (Biobond: Yoshitomi-seiyaku Co., Japan), and the recipients were then kept quiet in a moist chamber. Most of the recipients could oviposit normally. All oviposited eggs were observed under a stereomicroscope regardless of the presence or absence of insertions. If the recipient died or the insertions were not observed among their oviposited eggs, the body was opened and examined for the location of the insertion.

Egg transport on the coelomic epithelia

In order to observe egg transport on the coelomic epithelia, the abdomen was opened at the midline, and the coelomic epithelia were extended to both sides of an anatomical dish. The coelomic epithelia could be kept in a horizontal position because this dish provided a hollow which conformed to the body shape of the newt. At first, the movement of the coelomic egg on the coelomic epithelia was recorded on a video recorder or by means of time-lapse photography. Subsequently, the movements of other eggs were recorded in the following order: the de-jellied uterine egg, the de-jellied fertilized egg, and the variously treated coelomic egg. Finally, the movement of the coelomic egg was re-recorded in order to ver-

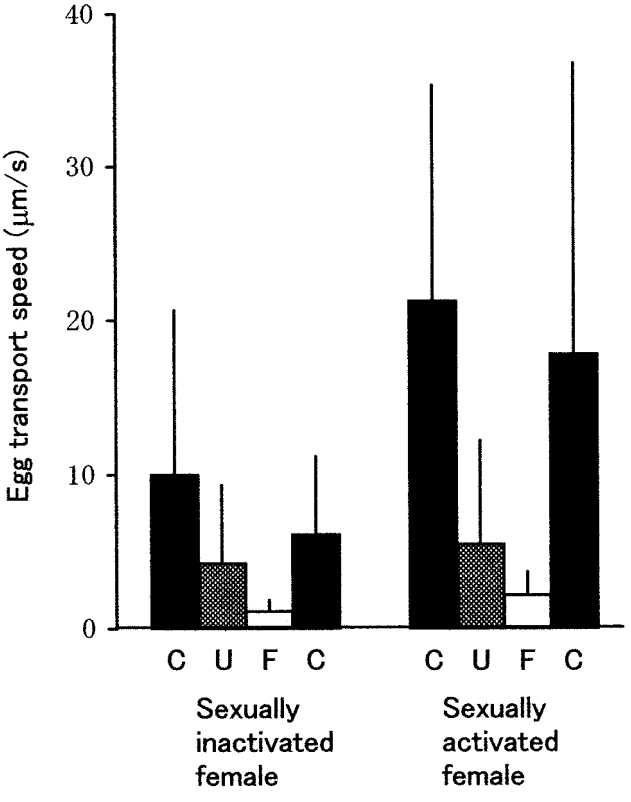

Fig. 4. The speeds of transport for 3 types of eggs on the coelomic epithelia of sexually inactivated or sexually activated female newts. The sexually inactivated female was not injected with hCG. The sexually activated female was ovulation-induced 24 hr after the second injections of hCG. The coelomic egg (C), de-jellied uterine egg (U) and de-jellied fertilized egg (F) were examined in sequence on the coelomic epithelia. The speeds of egg transport were calculated. Each value represents the average plus the standard deviation.

ify that the coelomic epithelia had been active after the experiments. These records were used to determine the speed of the egg transport. Data were expressed as the average and the standard deviations for 5 females. The values were analyzed using Student's t-test. P values<0.05 were judged as significant.

Scanning electron microscopy of eggs and coelomic epithelia

The eggs and the coelomic epithelia were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. After dehydration in graded concentrations of an ethanol series, the specimens were critical-point dried with carbon dioxide. Finally, they were coated with carbon and gold in a vacuum evaporator. These specimens were observed by means of a scanning electron microscope (JSM-5400, JEOL Co., Japan).

Ciliary current of coelomic epithelia

A piece of coelomic epithelium was stripped off from the opened abdomen of the females using a razor and was immersed in sterilized Steinberg's solution. This specimen was mounted on a glass slide and was observed under a light microscope (BH-2, Olympus, Japan).

RESULTS

Ovipositions of various materials inserted into the coelom

The results of these experiments are summarized in Table 1. The inserted coelomic eggs were observed within a jelly layer among the oviposited eggs or in the oviduct (Fig. 2). The inserted uterine eggs or fertilized eggs were also observed among the oviposited eggs or in the oviduct. Most of the inserted coelomic eggs were oviposited, while 4 of 14 inserted de-jellied uterine eggs and 3 of 10 inserted de-jellied fertilized eggs were oviposited. The boiled eggs or artificial materials (glass beads or agar beads), however, remained in the coelom.

Egg transport on the coelomic epithelia

The coelomic egg was transported on the coelomic epithelia slowly and with a backward rolling action toward the

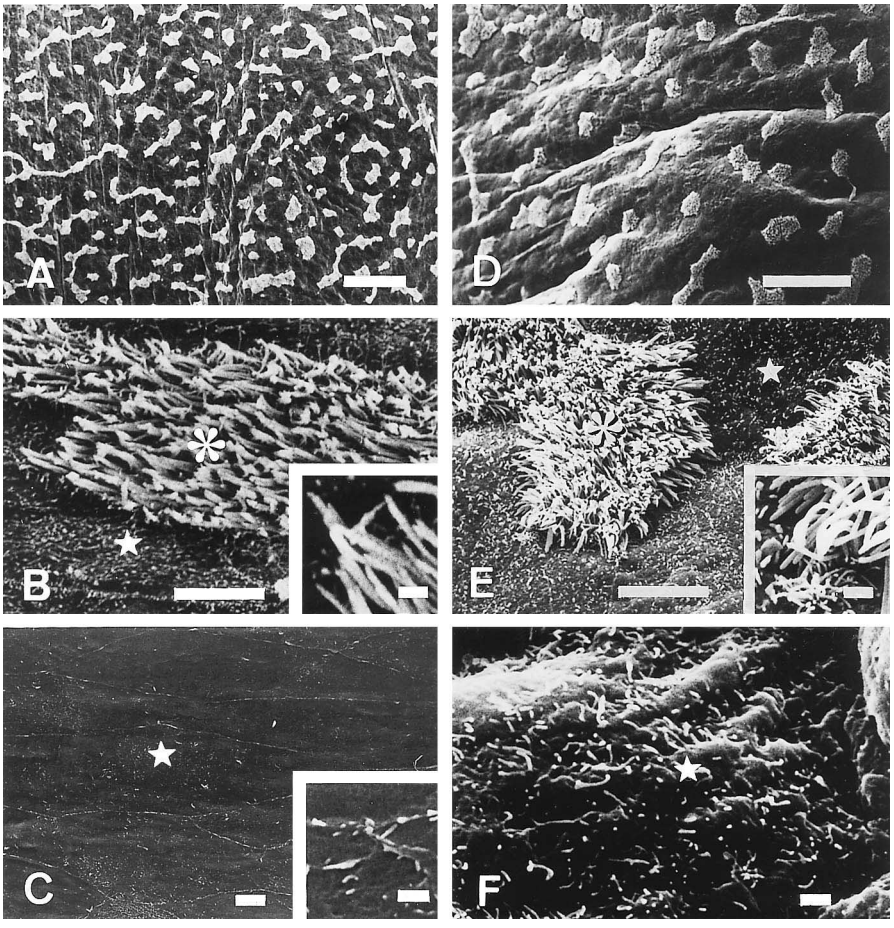

Fig. 5. Scanning electron microscope observations of the surface of the coelomic epithelia of the adult newts. Neither females nor males were injected with hCG. A and B show the surface of the coelomic epithelia in the female. There were 2 types of cells: one was a ciliated cell (*) and the other was a non-ciliated cell (★). C shows the surface of the coelomic epithelia in a male. Only non-ciliated cells (★) were observed. D and E show the surface of the liver in a female. Ciliated cells (*) and non-ciliated cells (★) were observed. F shows the surface of the liver in a male. Only non-ciliated cells (★) were observed. The insets in B, C, and E show the images at high magnification. Scale bars, A: 200 μm B, C, E: 10 μm D: 100 μm F, and insets B, C, E: 1 μm

ostium (Fig.3). The uterine egg and the fertilized egg were also transported on the coelomic epithelia. Fig. 4 shows the transport speeds of the coelomic egg, de-jellied uterine egg, and de-jellied fertilized egg on the coelomic epithelia. The coelomic egg was fastest of all examined eggs whatever the hormonal state of the recipients. The speed of de-jellied uterine eggs and the speed of de-jellied fertilized eggs were almost the same in all sexual conditions. But the speed of

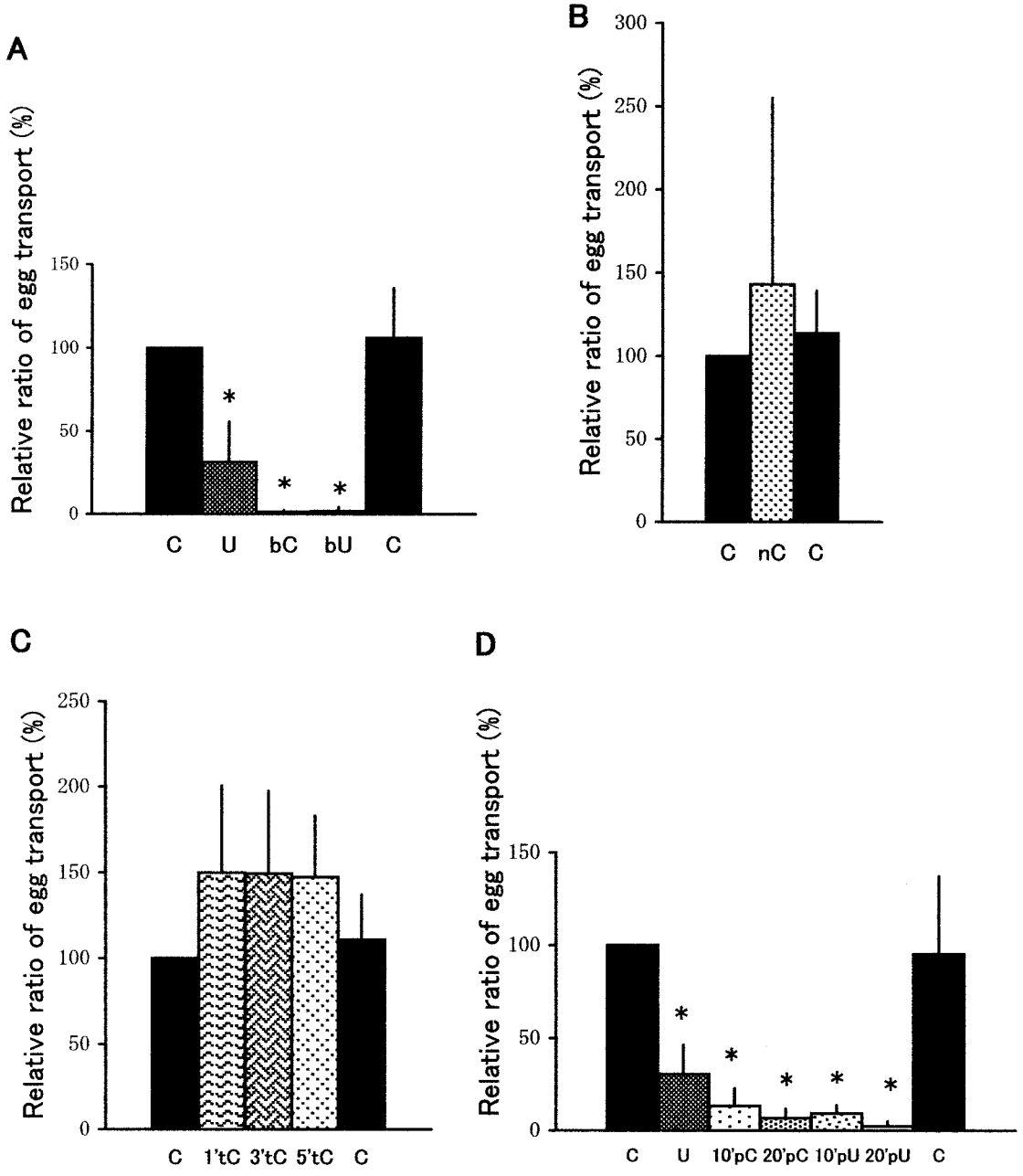

Fig. 6. Comparison of the speeds of egg transport at various treated eggs. A, Relative ratios of the speeds of egg transport of boiled egg on the coelomic epithelia of sexually activated newts. Coelomic eggs (C), de-jellied uterine eggs (U), boiled coelomic eggs (bC), and boiled uterine eggs (bU) were examined on the coelomic epithelia and recorded in sequence. The speeds of transport were calculated. The speed of egg transport of the first coelomic egg was considered as control, and the speed of egg transport of each egg was expressed with the relative ratio to control. These conventions also apply in the following figure. B, Relative ratios of the speeds of egg transport of naked coelomic egg on the coelomic epithelia of sexually activated newts. Coelomic eggs (C) and naked coelomic eggs (nC) were examined on the coelomic epithelia in sequence. C, Relative ratios of the speeds of egg transport of trypsin-treated egg on the coelomic epithelia of sexually activated newts. Coelomic eggs (C) and coelomic eggs treated with 0.05% trypsin for 1, 3, or 5 min (1'tC, 3'tC, 5'tC, respectively) were examined on the coelomic epithelia in sequence. D, Relative ratios of the speeds of egg transport of periodic acid-treated egg on the coelomic epithelia of sexually activated newts. Coelomic eggs (C), uterine eggs (U), coelomic eggs treated with 1% periodic acid for 10 or 20 min (10'pC, 20'pC, respectively), and uterine eggs treated with 1% periodic acid for 10 or 20 min (10'pU, 20'pU, respectively) were examined on the coelomic epithelia in sequence. Each value represents the average plus the standard deviation. Asterisks indicate significant differences compared to the control.

coelomic eggs in sexually activated females was higher than that in inactivated females.

Scanning electron microscopic observation of the coelomic epithelial surface and liver surface

Fig. 5 shows the epithelial surfaces of the coelom and the liver of the adult female and male newts. In females, both the coelomic epithelia and liver surface were composed of ciliated cells and non-ciliated cells. The non-ciliated cells often showed some microvilli. The ciliated cells were concentrated on the ventral side of the coelom but were few in number on the dorsal side. The ciliated cells gathered and appeared as a wavy mosaic pattern. The ciliated cells on the liver existed independently of each other. In males, only non-ciliated cells were present on the coelomic epithelia and the surface of the liver.

Ciliary movement

In the adult female newt, ciliary movements were observed on the surface of the coelomic epithelia by a light microscope (data not shown). The cilia were beating, irrespective of the hormonal states of the female, but their beating activity was higher in hormonally activated females than in inactive females. The current of the ciliary beating ran from the dorsal side to the ventral side.

The transport speed of variously conditioned coelomic eggs on the coelomic epithelia of the female

The speeds of egg transport differed in terms of how the eggs were treated (Fig. 6). In the boiled coelomic eggs,

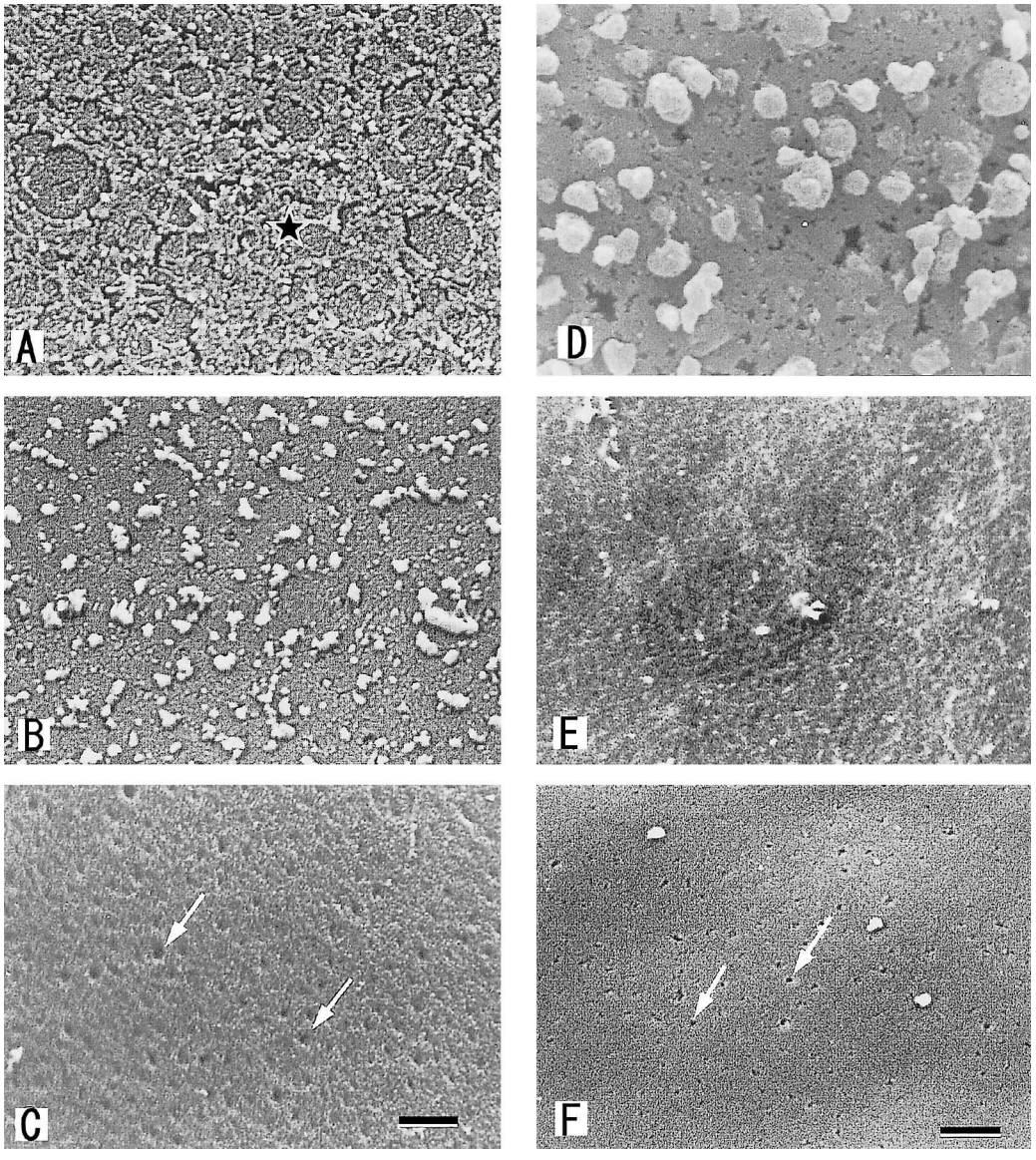

Fig. 7. Scanning electron micrographs of vitelline envelopes (VEs) in 3 types of newt eggs and coelomic egg VEs modified by various treatments. The VE of coelomic eggs (A) had meshwork structures (★) , but the VE of uterine eggs (B) did not. The VE of fertilized eggs (C) had smooth surfaces with many micropores (arrow). Coelomic eggs were boiled (D), treated with 0.05% trypsin for 5 min (E), and treated with 1% periodic acid for 10 min (F). The VE treated with periodic acid had smooth surfaces with many micropores (arrow). Scale bar, 1 μm

the speed was significantly reduced compared with that of untreated coelomic eggs (Fig. 6A). The naked egg was transported faster than the untreated egg (Fig. 6B). The trypsin-treated eggs also had a tendency to be transported faster than the untreated eggs (Fig. 6C). In contrast, the speed of the periodic acid-treated eggs was significantly reduced compared with that of untreated eggs (Fig. 6D).

Scanning electron microscopic observation of the surface of variously treated egg envelopes

Fig. 7A, B, C illustrates the surface structure of the vitelline envelope (VE) in coelomic eggs, uterine eggs and fertilized eggs, respectively. The surface of the VE of the coelomic eggs had a reticular structure, while that of the uterine egg's VE had no reticular structure. The fertilized egg's VE surface was smooth and had many micropores, which, in contrast, were not observed on the VEs of the coelomic eggs and uterine eggs. Fig. 7D, E, F illustrates the surface of the coelomic egg VEs after various treatments. In each treated VE, the reticular structure disappeared. In particular, the surface of the VE treated with periodic acid became quite smooth, and numerous micropores could be seen on the surface of the VE as well as on the surfaces of the fertilized eggs.

DISCUSSION

In amphibians, eggs are ovulated in the coelom, transported through the oviduct, and finally oviposited. The ostium is far from the ovary, because the oviduct must be very long in order to secrete jellies. For this reason, the ovulated eggs must be transported on the coelomic epithelia from the ovary to the ostium (Fig.1). It is necessary for successful fertilization that the eggs be transported efficiently and smoothly from the coelom.

In frogs, when eggs or other artificial materials (cf. buckshot or glass beads) were inserted into the coelom of the female, they were carried into and through the oviduct and accumulated jelly (Rugh, 1935; Arnold and Shaver, 1962). In addition, it has been demonstrated that the adult female frog has many cilia on the surface of the coelomic epithelia and liver, so the eggs from the ovary must therefore be transported on the coelomic epithelia by means of ciliary movement. In our study, most of the coelomic eggs inserted into the coelom as well as some of the inserted de-jellied uterine eggs and de-jellied fertilized eggs were oviposited. Boiled eggs and artificial materials, in contrast, stayed in the coelom and were never oviposited. In general, it is expected that a lot of insertion may bring about a high probability of discovered insertions in their oviposited eggs. But it was difficult to insert many raw eggs, such as coelomic egg, into the coelomic cavity experimentally, since the raw eggs were very soft and fragile. In spite of many insertions, all artificial materials and boiled eggs had remained in the coelomic cavity. In a female which inserted the glass beads, especially, it was observed that the beads inserted had been enclosed by the coelomic epithelia several months after the operation (data not shown). In the cases of raw eggs, some de-jellied uterine eggs and de-jellied fertilized eggs were seen in the outside of the coelomic cavity, the other remained in the coelomic cavity, although most of the coelomic egg were seen in the outside of the coelomic cavity. Thus, even in the cases of raw eggs, there were the differences in the tendency of the ovipositions among the kind of eggs inserted. These results indicate that not all materials can be transported in the coelom of newt and strongly suggest that the transport of an egg to the oviduct on the coelomic epithelia is indispensable to the oviposition.

The coelomic eggs were transported on the coelomic epithelia of the opened body cavity in the adult female newts, and ciliary movement on the coelomic epithelia was seen in the female newts. In contrast, cilia and ciliary movement were never observed on the coelomic epithelia of the adult male newt, and the eggs placed on its epithelia were never transported (data not shown). It is suggested that the egg transport on the coelomic epithelia is female specific phenomenon. These results agree with those of Rugh (1935). The present study indicated that the coelomic epithelia in the female were composed of two types of cells, ciliated cells or non-ciliated cells. The ciliated cells formed a wavy mosaic pattern on the coelomic epithelia of the female newts. In addition to the above results, it was demonstrated that the direction of the beating current was from the dorsal side to the ventral side. It is understandable that more ciliated cells are located on the ventral side than on the dorsal side. Since they always take the crawl posture, an egg automatically gathers on the ventral side due to gravity. Therefore, it is apparent that the ovulated eggs gathered on the ventral side from the dorsal or lateral side and were transported to the ostium, passing through the liver and coelomic epithelia by means of actively beating cilia. The question is raised as to why the coelomic epithelia and liver of amphibians possess the capability of egg transport. At a breeding season, the ovaries become very large and occupy the greater portion of the coelomic cavity, and many eggs are ovulated into the coelomic cavity for a short time. In order to adapt to the variation of ovarian size and a lot of ovulated eggs flexibly, it might be considered that the coelomic epithelia and the liver surface gained the capability of egg transport as the fimbria surrounding the ovaries.

The speed of egg transport was twice as high in sexually activated females than in inactive females. And the ciliary movement was more pronounced in sexually activated females than in inactive females as demonstrated by a video-recorded analysis. These findings suggested that egg transport is closely related to the action of ciliary movement. Ciliated cells are seen in the epithelia of respiratory organs such as the trachea, sensory organs, and in transport organs such as the oviduct. It is known that cilia play a more important role than smooth muscle in egg transport through the oviductal ampullae of the rabbit (Halbert *et al*., 1976). It is evident that, in addition to the oviduct, the coelomic epi-

thelia and the liver surface of adult female newts are one of the organs of egg transport. It is also known that egg transport on the fimbrae and through the ampullae of the oviduct is activated in rabbits in estrus (Odor and Blandau, 1973; Boling and Blandau, 1971). When marked coelomic eggs were inserted into the coelom of female newts not induced to ovulate by hCG, they never oviposited (data not shown). This suggests that the functional state of the coelomic epithelia is closely related to the sexually activated state in the newt, as well as to the fimbrae and ampullae of oviducts in mammals (Odor and Blandau, 1973; Boling and Blandau, 1971). In contrast, Rugh showed that the female frog could oviposit inserted eggs or buckshot, regardless of the frog's state of sexual activity (1935). The reason for the difference in Rugh's observations and those of our report is not apparent, though it may be related to the difference between the species. It is suggested that the egg transport in the coelomic cavity of newt is quite selective and is dependent on the state of sexual activity, as compared with the case of the frog.

Usually, only the coelomic egg is transported on the coelomic epithelia. The present study indicates that the speed of egg transport on the coelomic epithelia differs according to the type of egg examined. It is believed that the difference in speeds of egg transport is related to the nature of the vitelline envelope (VE). In the anuran, it is well known that the VEs of coelomic eggs, uterine eggs and fertilized eggs differ chemically (Gerton and Hedrick, 1986a, 1986b; Takamune *et al*., 1987; Lindsay *et al*., 1988; Lindsay and Hedrick, 1989) and microstructurally (Larabell and Chandler, 1988, 1989, 1990). In the newt, *Cynops pyrrhogaster*, it has been reported that the structural proteins of the coelomic egg VE and the uterine egg VE are different, as demonstrated by analysis of SDS-PAGE electrophoresis (Onitake *et al*., 1988). In *Xenopus laevis* eggs, it is known that the coelomic egg VE and uterine egg VE have large meshwork structures but that the VE of the fertilized egg does not (Larabell, 1988). In the present study, in *Cynops pyrrhogaster*, similar results were obtained. The VE of the fertilized egg had a smooth surface compared with the VEs of coelomic eggs and uterine eggs. It is believed that the cause of the reduction in the speed of egg transport was related to the modification of the surface structure of the VE. When the coelomic egg was treated with periodic acid, the surface of the VE had a strong resemblance to the surface of the fertilized egg VE. And the speed of egg transport was also remarkably reduced as was that of the fertilized egg. These observations may aid in elucidating the mechanism of egg transport on the coelomic epithelium. Periodic acid cuts sugars by means of periodate oxidation, while boiling causes the denaturation of protein. It is clear that the sugar and protein components of the VE determines the speed of egg transport on the coelomic epithelia. On the other hand, it was shown that the speed of egg transport increased when the coelomic egg VE was physically removed (naked egg) or was chemically digested by trypsin. The shape of the naked egg became flat because its container, the VE, was removed. Increased contact with a broader area of the coelomic epithelia might be considered the reason for the high speed of the naked egg relative to that of the coelomic egg. Most of the naked eggs, however, ruptured during their transport on the coelomic epithelia. This suggests that the VE protects the egg cell from damage during transport in the coelom. In addition, extended treatment with or high concentrations of trypsin caused the egg to rupture, while extended treatment with or high concentrations of periodic acid did not break down the egg (data not shown). It is therefore probable that protein is the main component of the VE and that sugar is included in the surface structure of the VE. A reason for the relatively high transport speed of the trypsin-treated egg was that it became flat and had a large contact area with the coelomic epithelia, as did the naked egg. It is certain that the glycoproteins (or sugars and proteins) of the VE play an important role both chemically and structurally in egg transport on the coelomic epithelia.

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