Peristaltic Constrictions in Fertilized Barnacle Eggs (*Pollicipes polymerus*)

During the maturation divisions of the fertilized barnacle eggs, an unusual event occurs: a series of constriction rings move slowly from the animal to the vegetal pole (Figure 1). This process, here called peristaltic constriction, was noted at the turn of the last century[1–3] but has been overlooked by a number of contemporary barnacle embryologists[4–6]. As far as we know, no comparable process has been reported in other animals except perhaps in the eggs of a salamander[7], an insect[8], and a shrimp[9]. This study is designed to measure some dynamic parameters of peristaltic constriction in eggs of a gooseneck barnacle, *Pollicipes polymerus*, by using time-lapse cinematography, and to obtain some evidence of its causal mechanism by using different chemical inhibitors.

Materials and methods. Freshly fertilized eggs were dissected out from the mantle cavity of adults and were pipetted into filtered and UV-light treated sea water in watch glasses at the ambient sea water temperature (13–15 °C).

Time-lapse films were made with a Bolex H16M camera and cinephotomicrographic apparatus (Sage Instruments) coupled to a Zeiss Universal microscope with Nomarski differential interference contrast optics. Ambient sea water temperature was maintained with a thermoelectric cooling stage[10]. An L and W photo-optical data analyzer was used for film analysis.

Batches of eggs were treated with: 0.1–20 μg/cm³ Cytochalasin B (dissolved in dimethylsulfoxide) for up to 140 min and 0.4–400 μg/cm³ colchicine, 10–20 μg/cm³ cycloheximide, 5–20 μg/cm³ actinomycin D, or 550 μg/cm³ antimycin A (dissolved in 0.95% ethanol) for up to 90 min. Appropriate controls were carried out by using sea water, 0.2% v/v dimethylsulfoxide and 0.95% ethanol.

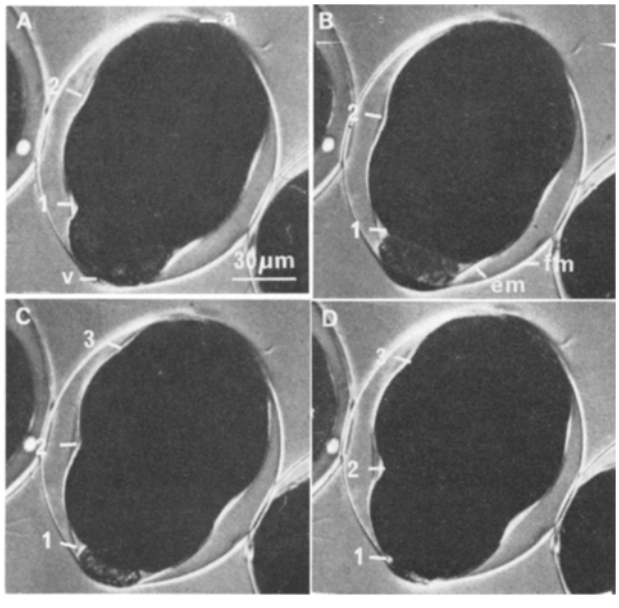

Fig. 1. Stage III egg showing 3 constriction rings moving from animal to vegetal pole in a 120 sec sequence (A–D). *A*, animal pole; *V*, vegetal pole; *FM*, fertilization membrane; *EM*, egg membrane.

Results and discussion. Although variability in the rate of embryonic development in different eggs is considerable, normal development from the beginning to the completion of peristaltic constriction is divided here into 5 stages. Stage I: 1.5 h after insemination; egg spherical; 1, then 2 constriction rings; ooplam visibly homogeneous. Stage II: 2 h after insemination; egg still spherical or beginning to elongate; 2 constriction rings; coplasm visibly homogeneous; first polar body forms. Stage III: 2.2 h after insemination; elongation of the egg into an ovoid shape; 3 to 5 constriction rings; beginning of ooplasmic segregation (shifting of large yolk platelets to the vegetal half); initial elevation of egg membrane from the egg surface. Stage IV: 4–6 h after insemination; further elongation of the egg; 1 to 2 constriction rings; further segregation of ooplasm; further lifting of the egg membrane. Stage V: 4–6 h after insemination; further elongation of the egg; peristaltic constriction stops; ooplasmic segregation nearly complete; egg membrane elevated; second polar body forms.

The results of the microcinematographic film analysis, as shown in Figures 2 and 3, illustrate a number of points of peristaltic constriction. One sees in Figure 2 that it takes about 8.5 min for a constriction ring to travel about 85% of the egg's length (total length is 140 μm). Figure 2 shows also that the constriction rings travel faster at the animal half than at the vegetal half and the velocity of the peristaltic motion decreases with embryo age; constriction rings at Stages I and II travel right to the vegetal tip, whereas rings at Stages III-V stop at increasing distances from the vegetal tip.

The amplitude of the constriction changes in relation to developmental stage as well as the position of the constriction in the egg (Figure 3). This figure shows that the amplitudes of the constriction are greater in older embryos (III–V) than that of younger ones (I–II). Moreover, the amplitude of constriction of all stages increases generally from animal to vegetal pole but drops sharply at the vegetal pole in older stages (III–V).

The rates of peristaltic motion in different embryos seem to be independent of the number of rings present. For example, the interval between constriction rings reaching the vegetal pole is 3 min in an egg with 3 rings, and the same amount of time is required in an egg with 4 rings.

Chemical inhibitors used in this study which may affect the peristaltic constrictions include: Cytochalasin B in dimethylsulfoxide, colchicine, actinomycin D, cycloheximide and antimycin A. Cytochalasin B is known to inhibit some cellular contraction[11–14] although the mechanism of its action is controversial. Some experiments indicate Cytochalasin B disrupts contractile

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microfilaments[15–18], whereas others show it appears to affect membrane phenomena[14,19,20]. In our study we find that 0.25 to 20 μg/cm³ Cytochalasin B in 0.025 to 0.2% dimethylsulfoxide effectively stopped and dampened all peristaltic constrictions within 2 to 30 min. Colchicine, a known microtubule inhibitor[21], produced no effect on peristaltic constriction at concentrations up to 400 μg/cm³ for 80 min but both ooplasmic segregation and meiotic division were inhibited; 4 μg/cm³ did not affect ooplasmic segregation, but apparently arrested mitosis, for cleavage failed to occur. Actinomycin D, an inhibitor known to block amino acid transport[22], stimulate mRNA degradation[23], and inhibit protein[24] and phospholipid synthesis[25], slowed the rate of peristaltic motion slightly at concentrations of 5–20 μg/cm³, but did not stop it. Ooplasmic segregation was unaffected, but development ceased at the 2 to 4 cell stage. Cycloheximide, an inhibitor of protein synthesis[26], slowed the peristaltic motion slightly at concentrations of 10–20 μg/cm³ but did not affect ooplasmic segregation. Antimycin A, an inhibitor of the cytochrome oxidase cycle in mitochondria, and therefore of energy release[27,28], did not inhibit the constrictions at 550 μg/cm³ but arrested ooplasmic segregation.

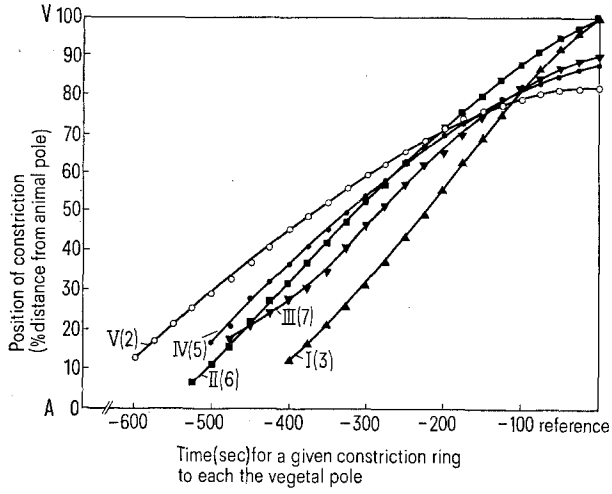

Fig. 2. Peristaltic movement of constriction rings measured in different developmental stages (I–V) as a function of time. The number in parentheses following the developmental stage refers to the number of constriction rings measured for that curve. The time the constriction ring ends vegetally was chosen as the reference time.

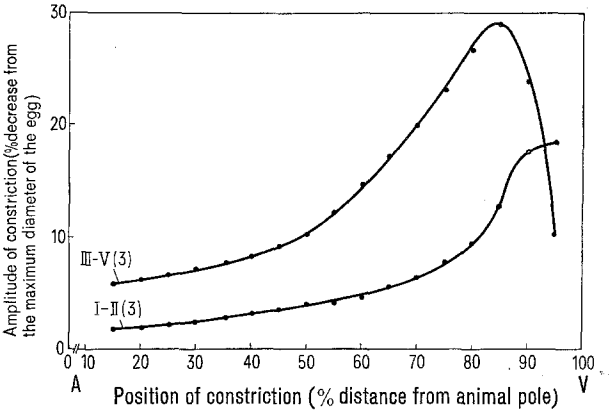

Fig. 3. Amplitude (average) of constriction in eggs of different developmental stages (I–V) as a function of the constriction position on the animal-vegetal axis. The number in parentheses following the developmental stage refers to the number of eggs measured for that curve.

Our experimental evidence indicates that the peristaltic constrictions in this barnacle egg are probably caused by microfilaments not by microtubules. Protein synthesis may be necessary for the constrictions, but oxidative phosphorylation is not. As for the relationship between the peristaltic constrictions and the concurrent morphogenetic changes, such as ooplasmic segregation, egg elongation, membrane lifting and polar body formation, our evidence is only suggestive. Ooplasmic segregation appears to be independent of the peristaltic constrictions, as no segregation was observed at Stages I and II when the rates of constriction movement were highest; moreover, when the peristaltic constrictions are stopped by Cytochalasin B, ooplasmic segregation continues. One must be aware, however, that some cytoplasmic constituents which cannot be seen under a light microscope may have segregated because of the peristaltic constrictions. Egg elongation is probably related to the peristaltic constrictions as the increase of the peristaltic velocity at the middle of the egg during Stage III corresponds to the time of greatest observed elongation; the gradual slowing of the constriction movement corresponds to the completion of elongation; and when the peristaltic constrictions are stopped by Cytochalasin B at Stage I or II, the elongation also stops. Lifting of the egg membrane may be related to contractile action as well, as the membrane first becomes visible along the constriction ring in Stage III embryos (Figure 1). Although our experimental results did not elucidate the relationship between the polar body formation and the peristaltic constrictions, it should be stressed that the site of polar body formation (animal pole) is also the area where the constriction rings are generated. Can this area be regarded as a 'pace-maker' in relation to the peristaltic constrictions?[29]

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[29] We thank Dr. R. L. FERNALD, Director, Friday Harbor Laboratories, and Dr. CADET HAND, Director, Bodega Bay Laboratories, for providing research facilities. This research was supported by an NRC grant to F. S. CHIA and American Cancer Society grant No. 633 to T. E. SCHROEDER.

Résumé. La vitesse et l'amplitude des resserrements péristaltiques dans les œufs fertilisés de balane (*Pollicipes polymerus*) dépend de l'âge de l'embryon et de la position du resserrement dans celui-ci. Les resserrements péristaltiques sont probablement réglés par des microfilaments et non par des microtubules et il se peut que la synthèse de protéine soit nécessaire, mais la phosphorylation oxydative ne l'est pas. Le rapport possible entre les resserrements et les événements morphogénétiques des œufs fertilisés est discuté.

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Die Mitosehäufigkeit in der normalen Epidermis der Maus nach einmaliger DMSO-Einwirkung

Genaue Kenntnisse über die Einwirkung des DMSO (Dimethylsulfoxyd) auf die Mitoseaktivität der epidermalen Zellen liegen bisher nicht vor. Es wurde lediglich eine erhöhte Zahl der Teilungsfiguren nach Einwirkung des DMSO auf die Meerschweinchenhaut beobachtet[1]. In diesem Zusammenhang sind auch die Befunde von Korfsmeier[2] zu nennen, der in Kulturen von Hautepithel und Fibroblasten nach DMSO-Zugabe eine Stimulation der DNS-Synthese erreichen konnte.

Material und Methode. Die Versuche wurden an 5 Gruppen zu je 5 Albino-Mäusen im Alter von 21 bis 28 Tagen vorgenommen. Bei allen Tieren wurden auf einem 1,5 cm^2 grossen Feld der Rückenhaut beiderseits je 2 Tropfen einer 90%igen DMSO-Lösung[3] nach mechanischer Depilation aufgetragen. Je 5 Tiere wurden nach einem Zeitintervall von 12, 24, 36, 53 und 72 h jeweils nachmittags getötet, nachdem ihnen 5 h vorher 0,1 mg Colcemid s.c. injiziert worden war.

Um einen eventuellen Einfluss der Rasur auf die Mitoserate zu prüfen, wurden 2 weitere Gruppen nach Rasur, ohne DMSO-Anwendung, zu den Intervallzeiten 0 und 29 h getötet. Als Vergleichsgruppe dienten 5 Tiere, die weder rasiert noch mit DMSO vorbehandelt waren. Sie wurden ebenfalls 5 h nach Colcemid-Applikation geopfert. Die 5 h nach Colcemid-Anwendung entnommene Rückenhaut wurde in Bouinscher Lösung fixiert, in Paraffin eingebettet und die ca. 3–5 µm dicken Schnitte wurden mit Haemalaun-Eosin gefärbt.

Bei jedem Tier wurde die relative Häufigkeit der Mitosen basaler und suprabasaler Zellen, jeweils auf 1000 Basalzellen bezogen, für beide Körperseiten getrennt bestimmt.

Der Vergleich der Häufigkeiten beider Körperseiten erfolgte mittels des *t*-Tests für abhängige Stichproben, der Vergleich der Ergebnisse mit dem Ausgangswert mittels des Vierfeldertests. Alle Wahrscheinlichkeitsangaben beziehen sich auf zweiseitige Fragestellungen.

Untersuchungsergebnisse und Besprechung. Die Darstellung (Figur 1) der relativen Häufigkeit der Mitosen in Abhängigkeit von der Zeit zeigte keine Änderung der arithmetischen Mittel der Mitosehäufigkeit bei den unbehandelten, rasierten Tieren im Vergleich zu den unbehandelten, nichtrasierten Tieren.

Dagegen zeigt sich 12 h nach DMSO-Anwendung eine deutliche Reduktion der Streuung der relativen Mitosehäufigkeit bei nicht zu sicherndem Unterschied der mittleren Häufigkeit vom entsprechenden Wert der nicht behandelten Tiere. 24 h nach der Behandlung mit DMSO kommt es zu einer signifikanten Zunahme der relativen Mitosehäufigkeit – im Mittel 55,7. Mitosen – gegenüber

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Häufigkeit der Mitosen basaler und suprabasaler Zellen in der Haut der Maus (bezogen auf 1000 Basalzellen)

Material	Mitosen/1000 Z.					$\bar{X}_d$ (*t*-Wert)[a]	$\bar{X}$	χ^2	$P <$
Ausgangswert:									
unrasierte Haut ohne DMSO	rechts 26	28	38	12	25	1.8 (0.615)	26.7	–	–
	links 39	27	40	9	23				
Vergleich:									
rasierte Haut ohne DMSO	rechts 42	15	30	24	20	3.4 (0.787)	27.9	0.0271	0.8
	links 62	17	29	19	21				
rasierte Haut 29 h nach Rasur ohne DMSO	rechts 23	37	34	27	33	0.4 (0.150)	30.6	0.2732	0.7
	links 25	27	39	30	31				
Test:									
rasierte Haut 12 h nach Rasur, mit DMSO	rechts 32	25	38	39	15	3.2 (0.958)	28.2	0.0421	0.9
	links 28	23	35	25	22				
rasierte Haut 24 h nach Rasur, mit DMSO	rechts 48	48	39	68	79	1.4 (0.285)	55.7	10.64	0.005
	links 41	43	44	54	93				
rasierte Haut 36 h nach Rasur, mit DMSO	rechts 23	9	18	20	18	3.2 (1.372)	19.2	1.254	0.3
	links 22	8	29	26	19				
rasierte Haut 53 h nach Rasur, mit DMSO	rechts 22	18	15	12	18	1.0 (0.512)	16.5	2.461	0.2
	links 20	14	15	18	13				
rasierte Haut 72 h nach Rasur, mit DMSO	rechts 15	29	30	38	26	7.2 (1.770)	29.2	0.1150	0.85
	links 12	41	50	29	32				

[a] Für zweiseitiges $P = 0.1$ beträgt $t = 2.81$ für 4 Freiheitsgrade.