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HISTOLOGICAL STUDY OF GENERAL STRUCTURE AND MECHANISMS OF SECRETION OF THE PEDAL MUCUS GLANDS IN THE SNAIL *Lymnaea auricularia*(L.).

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ABSTRACT

General histological structure and mechanisms of secretion of the pedal mucus glands in the snail *Lymnaea auricularia* (L.) are studied. The glands are composed of mucigenous units which include : mucigenous cells, mucigenous unions and mucigenous segments . These units are arranged in two zones : aggregation zone and distribution zone .The aggregation zone is mainly composed of mucigenous cells and mucigenous unions which are densly packed , while the distribution zone comprises loosely distributed units which are mainly formed of mucigenous segments . The mucigenous segments are motile parts separated either from mucigenous unions or from mucigenous cells .They move towards the surface epithelium of the foot. During this movement the segments undergo continuous changes in their structure and size. They, then, insert themselves between cells of the foot epithelium and finally discharge their components on the foot surface.

INTRODUCTION

The Pond snail *Lymnaea auricularia* belongs to Gastropoda which is a class of the phylum Mollusca .Pedal mucus glands of the gastropod snails produce slime for locomotion and food-collecting strings (Hickman, 1973). These glands are conspicuous features in both gastropods and bivalves. (Moor, 1983).

In most gastropods, pedal mucus glands have enlarged flask shaped cells with narrow necks within the epithelium (Lane and Nott, 1975).

As a rule the origin of these glands is an ectodermal invagination. the distal part forms the secreting portion while the place of invagination remains as gland orifice (Raven, 1958). In other cases, however, pedal mucus glands are formed from the foot mesenchyme as ductless glands. They discharge their secretion between the epithelial cells of fools sole e.g. *Patella* (Smith, 1935). and *Haliotis* (Crofts, 1938).

With regard to histological and histophysiological work on these glands, detailed information were, at best, incomplete in literature. (Campion, 1961; Hickman, 1973; Ruppert and Barnes, 1994).

Lymnaeae are regarded as particularly important gastropods . These snails are intermediate hosts for a number of parasite species that affect both humen and animals producing costly health and economic difficulties (Lee, 1993; Hillyer and Apt, 1997).

Despite the importance of these snails, previous histological studies on their organ systems were almost insufficient. As for their pedal mucus glands, information about detailed structure of their components and about mechanisms of their function were virtually absent.

The aim of the present study is to provide such information.

MATERIALS AND METHODS

Adult snails were obtained from Alassafya creek of shatt Al-Arab in Garmat Ali. Specimens used in the present study (3mm snails) were derived from these snails. They were reared in laboratory under a temperature ranging between 25-30°C.

The method used for histological processing was slightly modified from that of Mollenhauer (1964) for resin thin sections. It can be used for light microscopy, as well as electron microscopy (Humason, 1972). This method gives much better sections than those of paraffin method allowing investigators to obtain more details in light microscopy.

The specimens were fixed in 10 % neutral formalin for 24 hours, washed in running water for 12 hours and progressively dehydrated in 30%, 50%, 70%, 90%, 95%, and 100% ethanol. They were cleared in propylene oxide and embedded in epon 812. Sections were cut at 3 µm and stained with toluidin blue.

RESULTS

Pedal mucus glands in *Lymnaea auricularia* are composed of specific units (mucigenous units). The glands discharge their product on the outer surface of the foot. The mucigenous units include: Mucigenous cells, mucigenous unions and mucigenous segments. These units are localized

within the connective tissue of the foot forming two distinctive zones aggregation zone and distribution zone (Fig. 1).

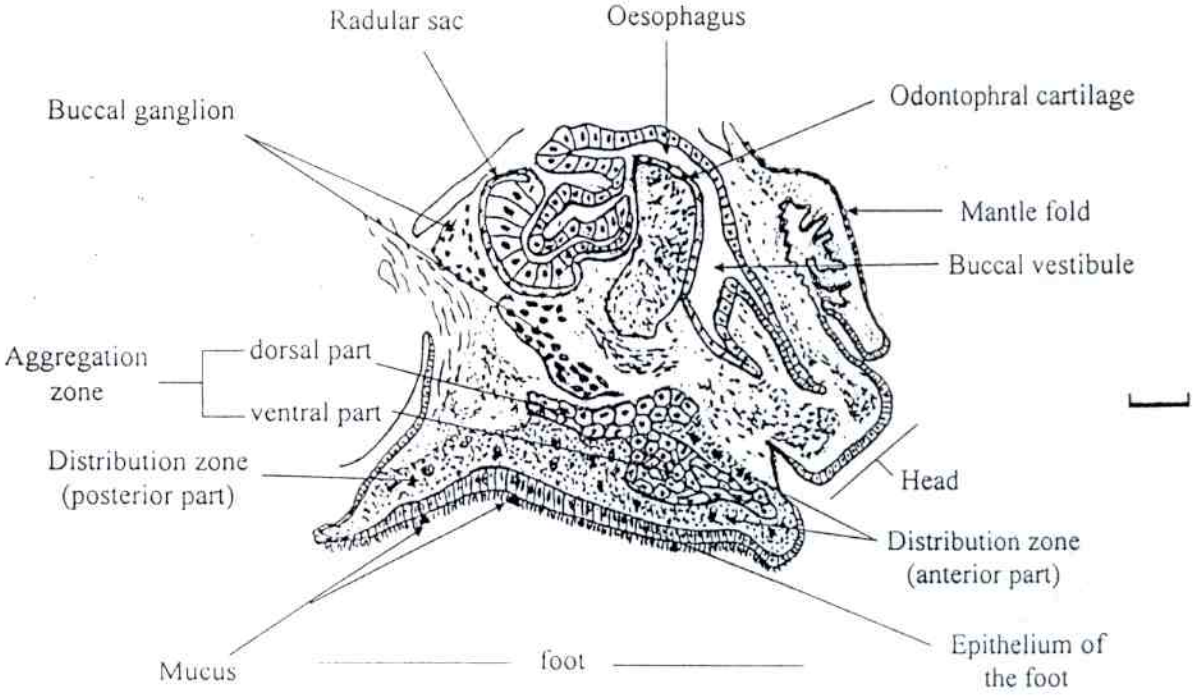

Fig -1- Semidiagramatic sagittal section of the headfoot showing its main Parts. Note the positions of aggregation and distribution zones.

Scale bar: 20 μm.

MUCIGENOUS UNITS:

1-Mucigenous cells:

The typical shape of these cells is regular polygone with a centrally located nucleus (Fig.2-A). The cells may assume, however, other different shapes. The nuclei, in regularly shaped cells, are located at the center of the cell, while those of irregularly shaped cells may be located at any place of these cells. The chromatin material is darkly stained. The cell is surrounded by a thin plasma membrane. The cytoplasm forms a thin layer around the nucleus. This layer is darkly stained making it difficult to be distinguished from the slightly darker nucleus. From the cytoplasmic layer several branching threads of cytoplasm extend dividing the cell into several spaces within which mucigenous grains are synthesized (Fig.2).

2-Mucigenous unions :(plates 2,4 and 6 , large arrows)

This structure is formed from a fusion of two or more mucigenous cells .It is characterized by a large size, irregular shape, acquisition of two or more nuclei and their mucigenous grains are usually larger than those of mucigenous cells.

3- Mucigenous segments:

The origin of these units is parts separated from either mucigenous cells (fig. 2-B2 and plate 6) or mucigenous unions (Fig. 2-B1, plates: 4 and 5, small arrows). The segments have different shapes and sizes.Their components usually split away gradually producing smaller segments and, occationally, individual grains.

Mucigenous segments have some cytoplasm. They are surrounded by a plasma membrane and contain larger mucigenous grains in comparison with those of other mucigenous units.

The structure of these zones may be well understood by studying median longitudinal sections. Although these zones are morphologically distinctive, they are complementary with regard to development of the gland units i.e. the mucigenous units start their development, in shape and function, within the aggregation zone and continue this process as they move towards the edges of this zone and then to the distribution zone.

1-Aggregation zone (Fig. 1 and plates 1-5):

This zone occupies the anterior half of the foot ventral region, behind a groove in which the mouth is located, and extends a little through the posterior half.

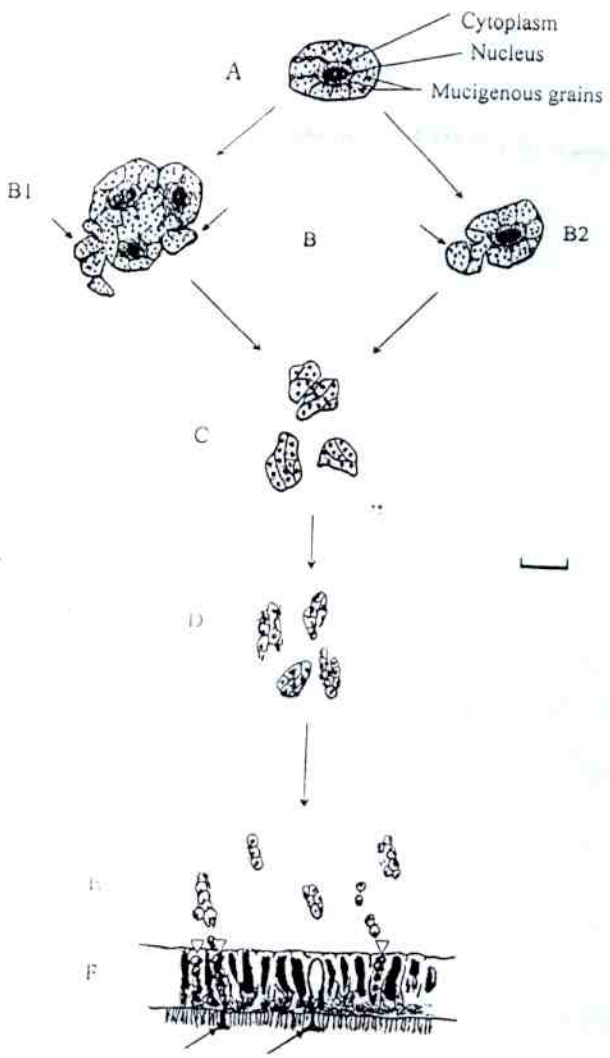

Fig. 2- Secretion pattern of the mucigenous glands.

A – A mucigenous cell

B – The process of segments separation (arrows) from:

B1- A mucigenous union, which is formed by fusion of two or more mucigenous cells (three fused cells are shown)

B2- single mucigenous cell.

C- Mucigenous segments. Note that their components start to enlarge.

D - and E- Splitting off of the segment components .

This process successively produces smaller and narrower segments and even single grains. The components of these segments usually grow to larger sizes.

F- Insertion of mucigenous segments and grains between epithelial cells of the foot (arrowheads) . Their components merge to form mucus which is eventually secreted on the outer surface of the epithelium (arrows)

Scale bar: 5 μm

Plate 1- Sagittal section of the headfoot showing it's main parts. Note the positions of : aggregation zone (az) and distribution zone (dz) . Scale bar:20 µm .

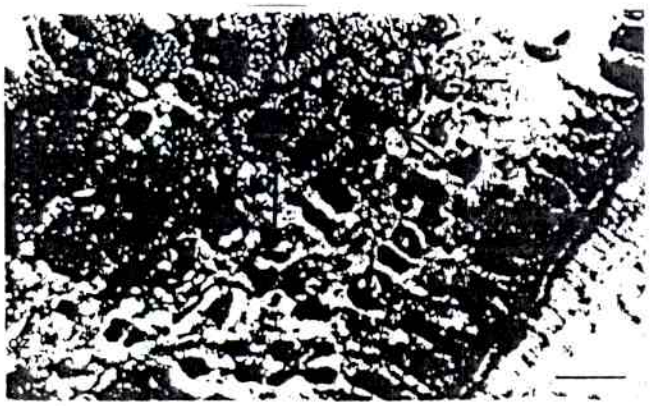

Plate-2

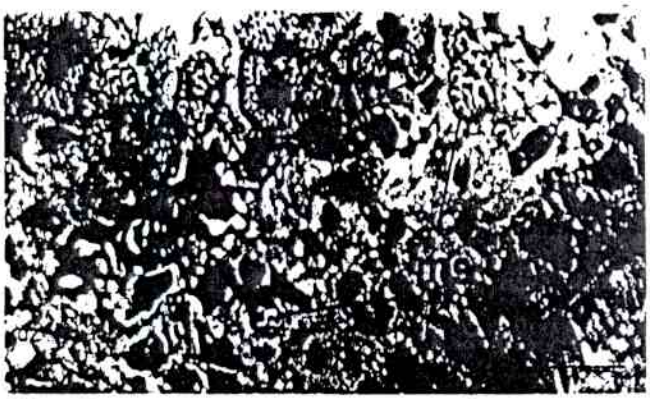

Plate-3

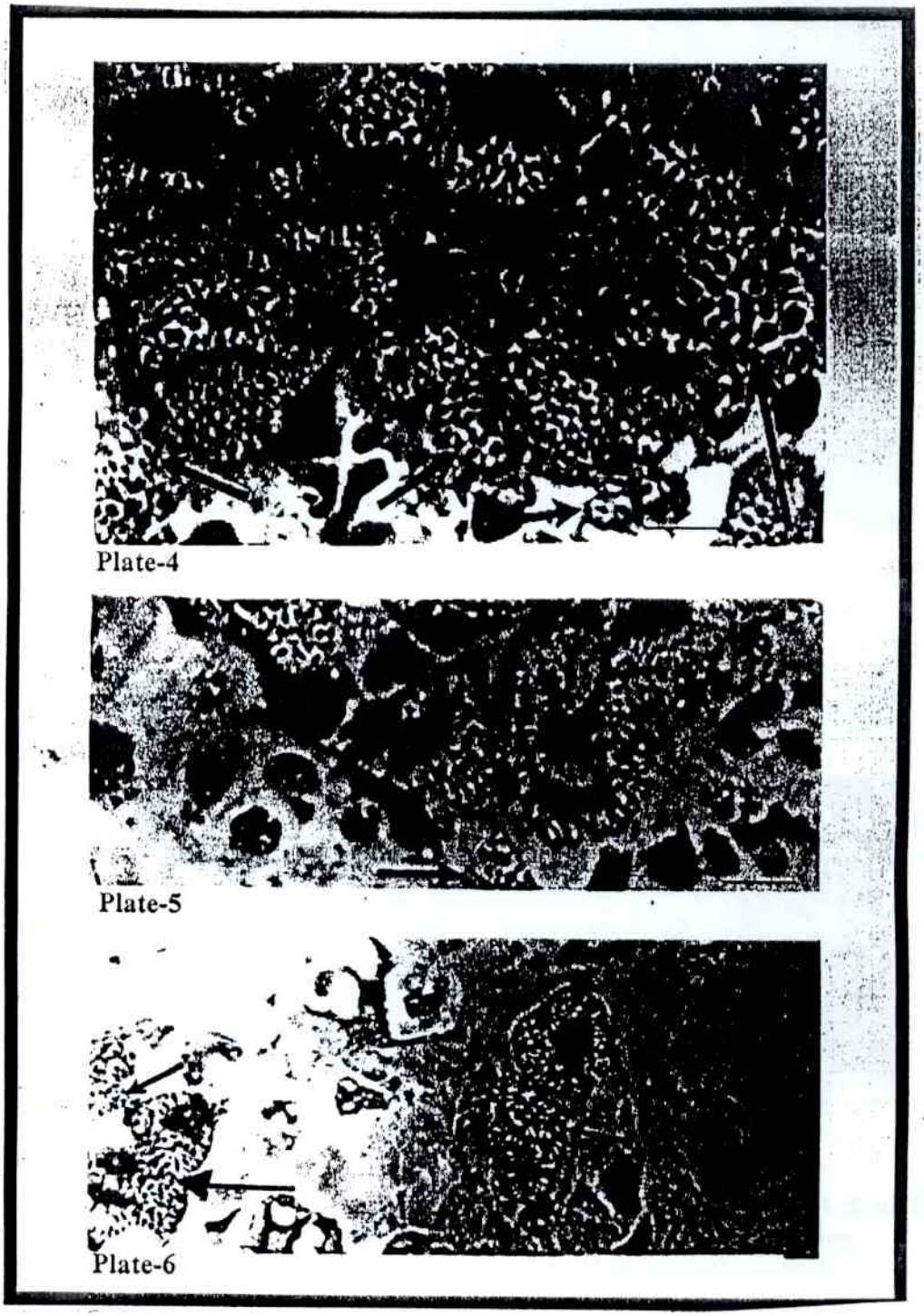

Plate-4

Plate-5

Plate-6

Plates2-6: a- Segments separation (small arrows) :
Most segments separation take place inside aggregation zone (Plates 2-5) . However, some of them occure within distribution zone (plate 6) .
b - Mucigenous unions (Plates 2,4,5 and 6, large arrows):
These unions can be recognized by the disappearance of parts or all of the plasma membranes between the united mucigenous cells.
az: aggregation zone , dz : distribution zone

Plate7: Mucigenous segments (arrows) moving within the distribution zone towards the surface epithelium of the foot.

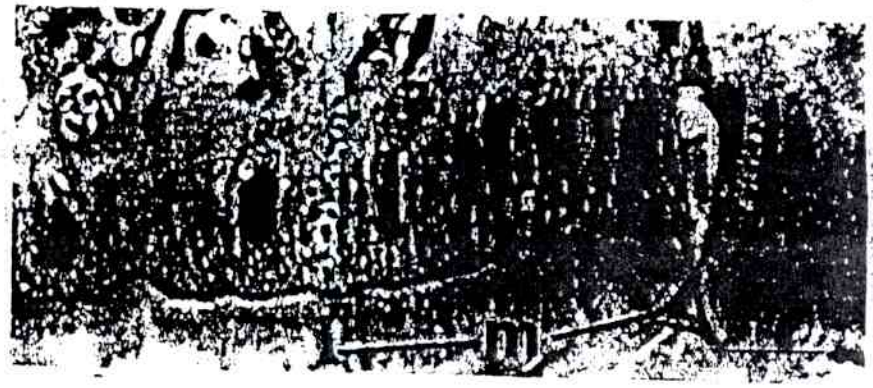

Plate 8: Insertion of mucigenous segments and grains (arrows) between the epithelial cells of the foot and subsequent discharging of their component (mucus) on the external surface of the foot epithelium.
m: mucus.
The bars in plates 2&3 represent 10 μm and in plates 4-8 represent 5 μ

The aggregation zone have a peculiar shape. Its border is situated at different distances from the surface epithelium of the foot depending on its position against this epithelium i.e. the distance between the anterior border and the surface epithelium is relatively short but as one moves posteriorly , the distance increases till it becomes remarkably large against the posterior border.

Two main parts can be distinguished in this zone: ventral and dorsal . The dorsal part incorporates with the ventral part at the posterodorsal end of the latter and from this end it extends both anteriorly and posteriorly (Fig.1 and plate 1). The ventral part has a thick posterior end but becomes gradually thinner towards its anterior end. The dorsal part is less thick than the ventral part. It thins out, moderately, in the posterior direction.

The aggregation zone is densely occupied by the mucigenous units. These units are located very close to each others. The mucigenous cells and mucigenous unions being much more frequent than the mucigenous segments within this zone.

2-Distribution zone (Fig. 1 and plates 1,2,3,6 and 7):

This zone is represented by the region that situated between the border of the aggregation zone and the epithelial tissue that the secretion is poured on its outer surface.

This zone contains much less number of mucigenous units than those in the aggregation zone. These units are separated from each others by relatively wide distances. Most of these units are mucigenous segments . There may be few mucigenous cells but the presence of mucigenous unions are very rare.

The mucigenous units undergo changes during their movement towards the surface epithelium. Changes include increase in size of the units components leading to increase in the units' total size. Usually, the components of mucigenous units split into two or more smaller parts which, mostly, undergo mergence of their contents before they are discharged onto the outer surface.

DISCUSSION

The Process of glands secretion is marked by cytological changes associated with biochemical synthesis of the secretory products, and influenced by environmental signals (Bloom and Fawcett, 1975).

It should be noted that, as in the above statement on glands secretion, there was frequent emphasis, in morphological (including developmental) published work, on two basic themes: 1- relationship between structure and function and 2-environmental influence on structural changes and activities (Sobel, 1990; Alvarez and Schoenwolf, 1992; Seely, *et al*. 1998).

However information on these two themes provided by studies on gastropods pedal mucus glands, were scant (Campion, 1961; Hickman, 1973; Ruppert and Barnes.1994).

The present investigation focuses on: the structural organization of the gland units and interactions with their environments. Such interactions are essential for morphogenesis as well as for differentiation and are accounted for the relationship between structure and function.(Wolpert, *et al.* 1998).

The mechanisms illustrating environmental influence on structure were extensively studied in developmental processes. The main category that is concerned by these studies is cell interactions with its environment. In several studied animal cell types, environments influence cell development leading to changes in their shapes and to their gradual differentiation (Ham and Veomett, 1980). Signals that activate a genomic program of morphogenesis and differentiation may arise within a population of one cell type (homotypic), arrive from other cell types (heterotypic) or from extracellular environments (Grant, 1978).

It is obvious that similar signals can influence cells of the aggregation zone, during their development, to differentiate and change in form, since these cells form a closely packed population surrounded by extracellular matrix and other cell populations.

Environmental influence continue after differentiation. This is important in maintaining cell function, which in turn is reflected in cell shape e-g muscle cells, which is responsible for locomotion of the body, look different from epithelial cells which protect, secrete, or absorb (Seely, *et al.* 1998).

In order to be discharged on the outer surface, the secretory product, synthesized by the mucigenous cells within the aggregation zone, must be carried through the distribution zone. This is accomplished by the mucigenous segments.

During their migration, the mucigenous segments become exposed to different new environments and undergo changes in their structure.

The mechanisms involved in cell migration are related to two main properties of the cell: adhesiveness and motility .Adhesiveness is the result of interactions between cell surface proteins and extracellular molecules. Motility is determined, as responds to these interactions, by rearrangements in internal cytoskeleton of the cell. For cell movement to occure, part of the cell surface must be anchored to a substratum. Movement is due to the generated force exerted by the anchored part of the cell which pulls the cell along (Grant, 1979; Wolpert, *et al.* 1998).

Although mucigenous segments lack nuclei they may, to certain extent, follow mechanisms similar to those of true cells (cells having nuclei).

This may be supported by the fact that cell fragments, which also devoid of nuclei "like blood platelets" can interact with their surrounding environments through their plasma membrane molecules and by having the contractile proteins: actin and myosin (Seely, *et al.* 1998).

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دراسة نسيجية للعلاقة بين التركيب العام وآلية الافراز للغدد المخاطية للقدم في القوقع(L.) *Lymnaea auricularia*

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الخلاصة

تمت دراسة التركيب النسيجي العام مع الية الافراز للغدد المخاطية للقدم في القوقع ***Lymnaea auricularia*** **(L.)** . تتكون الغدد من وحدات مخاطية تشمل : خلايا مخاطية ومدمجات مخاطية وقطع مخاطية . وتنتظم هذه الوحدات ضمن منطقتين هما : منطقة التجمع ومنطقة الانتشار . وتتكون منطقة التجمع ، بشكل رئيسي ، من خلايا مخاطية ومدمجات مخاطية مزدحمة بكثافة ، بينما تحتوي منطقة الانتشار على وحدات متفرقة عن بعضها تتكون اساساً من قطع مخاطية . والقطع المخاطية هي عبارة عن اجزاء متحركة انفصلت اما من مدمجات مخاطية او من خلايا مخاطية . وهي تتحرك باتجاه النسيج الطلائي السطحي للقدم . وفي خلال هذه الحركة تعاني هذه القطع من تغيرات مستمرة في تركيبها وحجمها . ثم تحشر نفسها بين خلايا النسيج الطلائي للقدم وفي النهاية تطلق مكوناتها على سطح القدم .