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Embryos of *Anacridium Aegyptium*, L. (Orthoptera-  
Acrididae) I- The Ventral Head Glands**

by

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# Some Observations on the Endocrine Centers in the Embryos of *Anacridium Aegyptium*, L. (Orthoptera-Acrididae) I- The Ventral Head Glands

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## ABSTRACT

The ventral head glands of the embryos of *Anacridium aegyptium* L. were studied in subsequent stages histologically and beginning with the development of the glands, the morphological properties of the embryonic stages were considered.

1- These glands are a pair of structures and first appearing in embryos which have completed their rotation in the micropilar end of the egg (at Katatrepsis). They are situated one on either side of the head and extend from a point on the epidermis just posterior to the dorsal primary head muscles to the suboesophageal ganglion and the adjacent epidermis.

2- The glands are syncytial and have deeply staining nuclei of varying size and shape. At the active stage the nuclei increase in size and some become lobulated. Some vacuoles appear in the cytoplasm which seems to form a network.

3- Throughout the embryonic stages one maximal period of activity appears. This coincides with the separation of the epidermis from the cuticle (embryonic moulting). Then the glands also seem to continue their function which is possibly connected with late embryonic developmental phenomena such as sclerotization and melanin deposition.

4- In the first hatchlings the glands are smaller in size.

## INTRODUCTION

The importance of endocrine centers in insects (Invertebrate) as well as in vertebrates has become increasingly evident in recent years. The term, prothoracic gland, was first used for one of them by Ke [14] in several lepidopteran larvae. Variations

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in the name, such as prothoracic glands, thoracic glands, ventral head glands, tentorial glands have been used according to their location (in the thorax or in the head).

Considering the evidence obtained from the work of Williams [24,25] with lepidoptera, a brain factor released by the neurosecretory cells of the pars intercerebralis stimulates the thoracic glands to secrete their substances (moulting and metamorphosis hormone, ecdysone, and growth and differentiation - GD - hormone). Since then the various structures which have been known for a long time have been considered to be homologous with the thoracic glands of lepidoptera. Because of one of their functions, the glands were also named ecdysial glands [6].

The glands are also responsible for normal oxidatif metabolism and all protein synthesis (essential to growth [27], and the sclerotization of the cuticle [1]2 and for colour variations and the maturing of the gonads [1].

There are many studies of these glands especially on the larval, nymphal and adult stages of insects [23, 24, 25, 26]; [22]; [21]; [28, 29]; [3] but only a few on the embryo [9, 10, 11]; [15].

The main aim of this study is to observe the histology of the ventral head gland in the embryos of *Anacridium aegyptium* L. and to see whether there are variations in their histological structure in the successive embryonic stages. The main rational behind this approach is to reach some conclusive idea of the function of the ventral head gland.

#### MATERIAL AND METHODS

The locust *Anacridium aegyptium* L. used in the present work was collected in the field around Bornova - İzmir in 1969, 1970 1971. The adult males and females were kept under crowded conditions in glass-fronted cages. The cages were kept in a room maintained at 30°C-32°C. and a relative humidity of 60-65 % and under long day (24 hours) light. These insects have an adult diapause that can be broken by keeping them at a regime

of a long day light [4]. The locusts were fed on the leaves of *Ligustrum vulgare* L. and dry bran.

The females were permitted to oviposit in a zinc cup containing sterilized and moist sand. The egg-pods were collected daily. Thus a series of egg-pods were obtained which were known to be not than 24 hours old.

The pods in the petri dishes that contained moist and sterilized sand were exposed to a constant temperature in an incubator from the first day until they hatched. Hatching period was between 20 and 21 days at 32°C. At 24 hour intervals, the eggs were dissected from the egg-pods. The eggs were cleaned of sand grains with a brush and were washed in water and dried with filter paper. The younger eggs were fixed in hot alcoholic Bouin and the older ones with aqueous Bouin [8], [18] and Helly's solution. In order to secure complete fixation, it was necessary to puncture the eggs in several places (on the side which did not contain the embryo) whilst in the fixative. After fixation, they were kept in 70 % alcohol until needed.

As is known, locust eggs of the same age, even from the same pod, tend to develop at different rates. For this reason, the embryos were dissected from the brown coloured chorion layers of the egg-shells. The developmental stages of embryos were determined morphologically. For the serial sections the embryos were cut in half and the halves that contained the head regions were used for the other treatment.

The presence of a thick and almost impermeable vitelline membrane made the sectioning of the yolky locust eggs more difficult [18]. Because of this Slifer's and King's methods [20] were applied.

The embryos or the eggs which had been kept in 70 % alcohol were exposed for 24 hours to 4 percent phenol in 80 % alcohol, dehydrated in 95 % alcohol and cleared in methyl bezoate + 1 % celloidin, -Peterfi's celloidin paraffin method- [17] and were washed in benzene. They were then infiltrated and embedded in paraffin wax (50°C - 55°C and 55°C - 60°C) in the usual way.

The paraffin wax was trimmed away from the face of the block until the yolk was just exposed and then the block was soaked in water for one to two days.

The principal features of this process consisted of the treatment with phenol and soaking in water [20]. In order to secure a good ribbon of sections, both these steps were applied simultaneously and these serial cross sections were cut at  $8\ \mu$  thickness.

Gomori's chrome haematoxylin phloxin [2], [5], [7] and Mayer's haemalum were employed to stain the sections. Both stains gave approximately the same results for the ventral head glands.

The very early hatchlings (less than 24 hours old) were also treated in the same way as indicated above.

A micrometer eye piece was used for measurement of the nuclear diameter. Two diameters of the nuclei (width and length) were measured in from 100 to 400 nuclei belonging to 8 different specimens.

#### OBSERVATIONS

The eggs which were exposed to  $32^{\circ}\text{C}$  in an incubator hatch in 21 to 22 days.

Twenty-four embryonic stages of *Anacridium aegyptium* are recognized upon the external morphological differentiation. During the fifteenth stage the head of embryo reaches the posterior micropylar end of the egg (Anatrepsis). After this the embryo begins to move around the micropylar end of the egg. The movement of the embryo occupies two stages, the sixteenth and the seventeenth.

The embryo completes the turning, at the eighteenth stage.

The ventral head glands in the embryo can be observed for the first time at the eighteenth stage just after revolution is completed (Katatrepsis).

*The general properties of the ventral head glands* : The two glands are situated on each side of the head, and arise from the epidermis

just anterior to, and on either side of the second maxillary segment. They are located in a dorso - lateral position posterior to the dorsal primary head muscles (Fig. 1) and extend backwards and downwards to connect with the sub-oesophageal ganglion, and the muscles and the body wall epidermis in the same region.

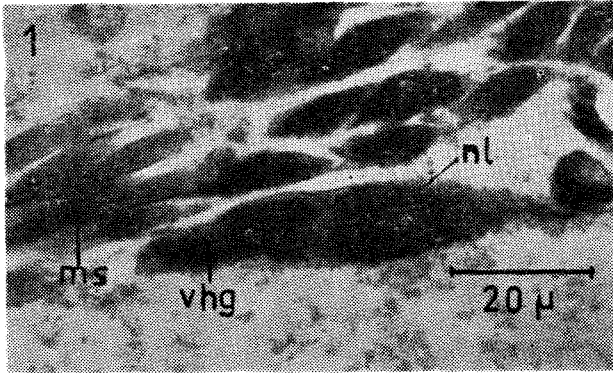


Fig. 1. Connection of ventral head gland (v.h.g.) to the head muscles. At the 21 st stage. Cross section.

The glands resemble a rope with small branches at some places (Fig. 2). They are tapered towards each end are therefore largest at the middle. They are surrounded by a thin, connective tissue wich does not appear to penetrate inside the gland. They

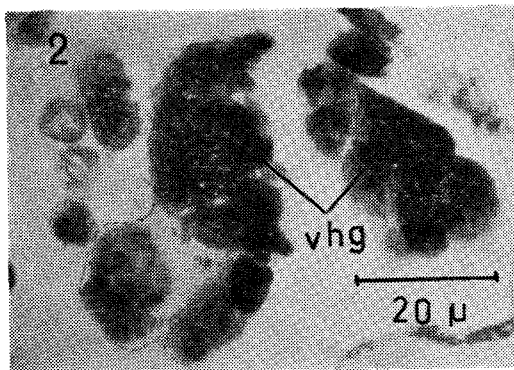


Fig. 2. Two small branches of v.h.g. At the 21 st stage. Cross section.

appear usually near tracheae. Sometimes it is possible to see some branches of nerves and tracheoles extending to the glands (Fig. 3). Some of the tracheoles, but never nerves can be distinguished inside the glands.

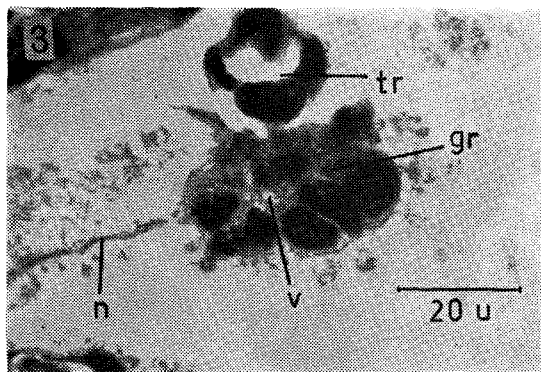


Fig. 3. v.h.g. near trachea. At the 21 st stage. Cross section.

The glands are syncytial and have deeply stained darker blue nuclei and sometimes, it is possible to distinguish a nucleolus inside them. The nuclei are in different shapes and sizes, generally elliptic, sometimes round. For that reason two diameters of the nuclei (width and length) are taken in measurement for the statistical analysis.

The boundary and size of the nuclei change according to the developmental stage. The regular and lobulated nuclei can be considered as the signs of inactivation and activation situation of the gland respectively [22], [16]. The cytoplasm also shows variations according to the embryonic stage. The vacuoles and the dark blue small granules in the cytoplasm indicate the activation of the glands [1], [16].

The external significant properties of the embryonic stages (after the turning of the embryo around the micropylar end of the egg) and the histological features of the ventral head glands can be described as follows.

**At the 18 th stage:** The turning of the embryo is completed. The embryo fills about half the length of the egg and almost the



whole of its width. In the hind leg the femur forms a right angle with the tibia. There is no pigment in the eyes.

The ventral head glands have a large ribbon like shape. They have small and tightly packed nuclei (Fig. 4). The nuclei are not lobulated.

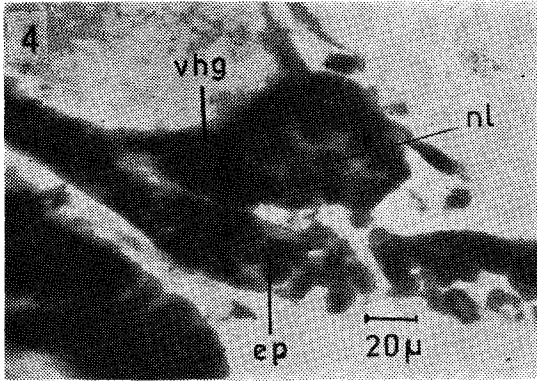


Fig. 4. v.h.g. At the 18 th stage. Cross section.

**At the 19 th stage:** The embryo occupies nearly the whole length of the egg. The dorsal side of the embryo is associated with the vitellus except for the last few abdominal segments. Red pigment is present in the dorsal edge of the eye. The hind femur extends to the fourth abdominal segment.

The nuclei of the ventral head glands are different in size and circular or elliptical in shape. Both diameters of the nuclei are a little larger than the previous embryonic stage, but there is no differentiation in the cytoplasm.

**At the 20 th stage:** The embryo occupies the whole length of the egg. Pigmentation of the eyes is more developed, and they are becoming dark red in colour. The hind femur extends to the sixth abdominal segment. Pleuropodia are dark brown in colour. The teeth of the mandibles, and the spurs and claws of the legs are not yet developed. The hind femur extends to the sixth abdominal segment.

Both diameters of the nuclei of the glands are larger. Lobulated nuclei appear in the cytoplasm.

**At the 21 st stage:** The hind femur extends to the eight abdominal segment. The whole eyes are deep red and pleuropodia become developed and blackish-brown in colour. At this stage the epidermis of the embryo begins to separate from the provisional laid cuticle (embryonal cuticle).

The nuclei of the ventral head glands become larger and more lobulated than before. The cytoplasm also shows differentiation. In some places, it resembles a network of tiny vacuoles and granules (Fig. 5). These granules stain dark blue and light violet-blusih colour with Gomori's chrome haematoxylin phloxin. The condition of nuclei and cytoplasm of the glands can lead one to the conclusion that the glands are active.

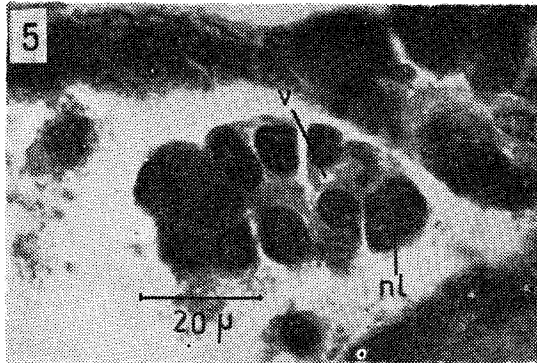


Fig. 5. v.h.g. near tentorium.

The tapering of the glands at both ends at this stage is much more noticeable than before and their inwardly curved shape becomes more pronounced.

**At the 22 nd stage:** The teeth of the mandibles are conspicuous and light brown in colour. The epidermis is completely separated from the cuticle of the embryo (Fig. 6). At this stage the nuclei of the ventral head glands are more lobulated than before and

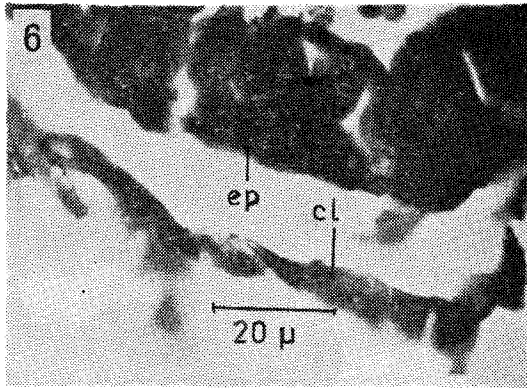


Fig. 6. Separation of cuticle from epidermis. At the 22 nd stage. Cross section.

they reach their maximum size (Graph 1 and 2). The cytoplasm resembles a network of vacuoles, small tracheoles and granules (Fig. 7). The glands appear to be in their maximal activation period.

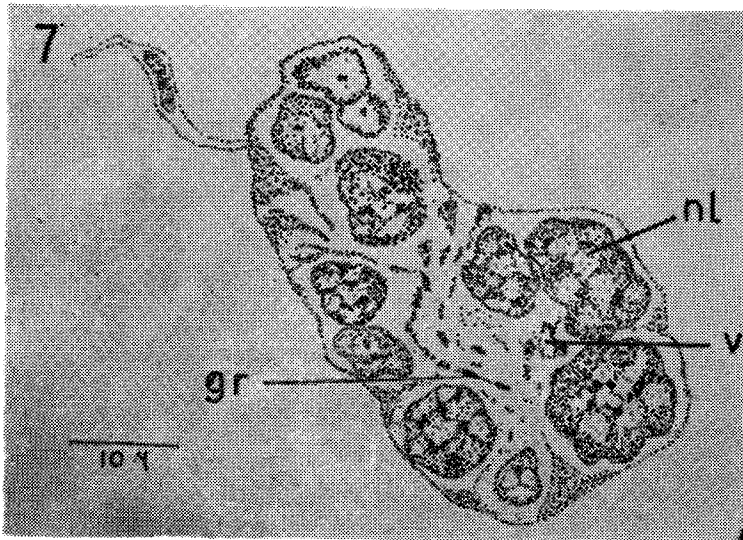


Fig. 7. v.h.g. At the 22 nd stage. With camera lucida. Cross section.

Cl-cuticle, ep-epidermis, gr-granule, n-nerve, nl-nucleus, tr-trachea v.h.g.-ventral head gland.

**At the 23 rd stage:** The mandibular teeth of the embryo are brown. The dorsal surface of the head, the labrum, the antennae, the mandibles and the maxillae are light brown in colour with very small pigmented spots.

The hind tibia has a longitudinal row of spines. The distal spurs of the hind tibia and the claws of the hind tarsus are developed. The light brown crescent is apparent on the distal end of the hind femur.

The nuclei of the ventral head glands become smaller, the number of lobulated nuclei is much reduced. The differentiation of the cytoplasm decreases, in other words, the vacuoles and the tracheoles are fewer.

**At the 24 th stage:** The teeth of the mandibles, the claws of the tarsi and the spines of the tibia are black - brown. The spines on the hind legs are much thicker. The antennae and anal appendages have small tiny spines. The head, the thorax and the segments of the appendages have light brown pigmented spots. At the end of this stage small or bigger dark brown spots appear on the dorsal surface of the whole body.

The sizes of the nuclei in the ventral head glands resemble those of the 23 rd stage, but they are arranged side by side in a chain and sometimes their shape is a little lobulated. There is no differentiation in the cytoplasm.

**In the hatchlings:** After 21 days at 32° C many eggs of the egg - pods hatch. Many of early hatchlings are covered with dark brown melanin pigmented spots and become darker in 24 hours. Only a few green nymphs are seen.

The ventral head glands of the darker early hatchlings only were studied. As in the embryo, they are located at either side of the head extending from the dorso-lateral epidermis and muscles of the head to the sub-oesophageal ganglion and epidermis and muscles at the same region. Their nuclei are located in a few rows through the sections, The nuclei of the ventral head glands are smaller and their shape is not lobulated. The cytoplasm is very sparse between the nuclei.

Measurements of the diameters of the nuclei and the mean and standard errors referring to the each embryonic stage are given at the table I. Variations in the size of the nuclei were also shown in two graphs.

TABLE I

Two diameters (width and length) of nuclei of the v.h.g. in the embryos of *Anacridium aegyptium*, L.

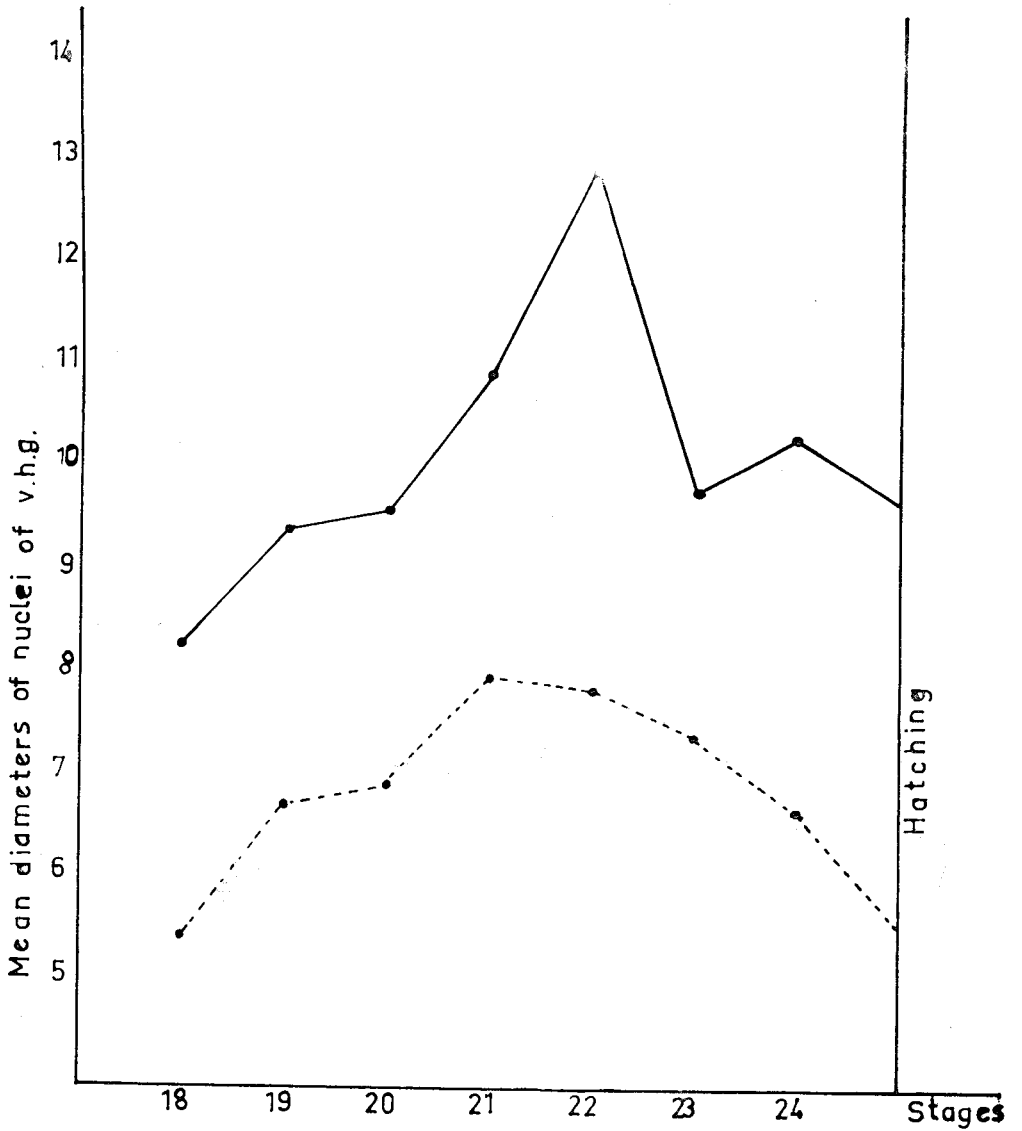
| Stages                    | Range of width of nuclei (micron) | Mean and S.e. of width (micron) | Range of length of nuclei (micron) | Mean and S.e. of length (micron) |
|---------------------------|-----------------------------------|---------------------------------|------------------------------------|----------------------------------|
| 18                        | 3,3 — 8,3                         | 5,45 ± 0,18                     | 6,6 — 11,6                         | 8,33 ± 0,21                      |
| 19                        | 3,3 — 11,6                        | 6,76 ± 0,10                     | 3,3 — 16,6                         | 9,46 ± 0,11                      |
| 20                        | 3,3 — 18,3                        | 6,96 ± 0,10                     | 5 — 20                             | 9,68 ± 0,15                      |
| 21                        | 3,3 — 18,3                        | 8,03 ± 2,10                     | 5 — 21,6                           | 10,95 ± 0,15                     |
| 22                        | 3,3 — 18,3                        | 7,81 ± 0,11                     | 6,6 — 23                           | 12,98 ± 0,21                     |
| 23                        | 3,3 — 15                          | 7,43 ± 0,18                     | 5 — 20                             | 9,86 ± 0,26                      |
| 24                        | 3,3 — 11,6                        | 6,72 ± 0,35                     | 6,6 — 16,6                         | 10,38 ± 0,41                     |
| in 24 hrs. after hatching | 3,3 — 8,3                         | 5,63 ± 0,20                     | 6,6 — 15                           | 9,73 ± 0,30                      |

## DISCUSSION

The pair of ventral head glands which have been observed in locust embryos (*Locusta pardalina* and *Locusta migratoria*) were first determined to be homologous with the thoracic glands by Jones [9, 10, 11].

These glands were first studied in the embryos of the gregarious and solitary phases of *Schistocerca gregaria* after completion of their rotation in the mycropilar end of the egg (Katatrepsis) [15]).

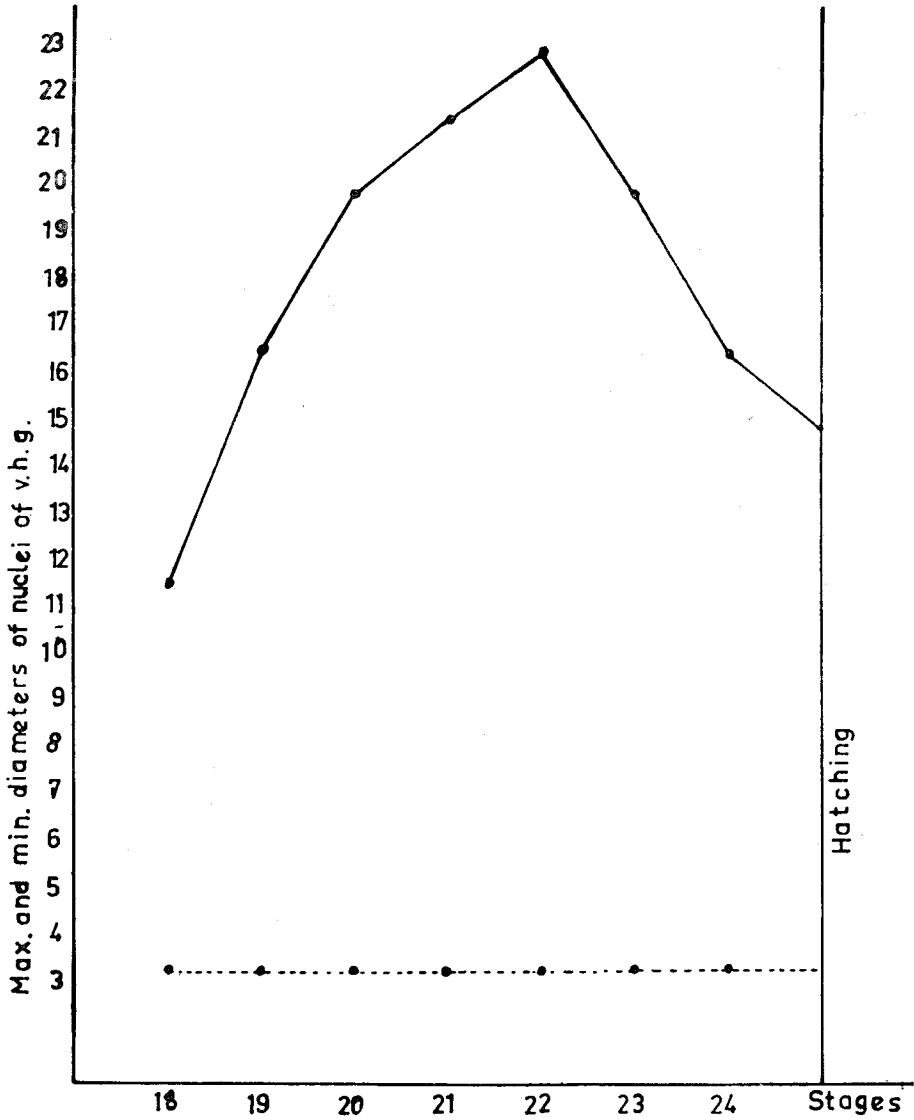
In the present work, the glands were differentiated after rotation during the time when the embryo occupied only the posterior half of the egg, as in the previous work the embryo of *Schistocerca gregaria*.



Graph:1—Mean diameters of nuclei of v.h.g. of embryos and first hatchlings of *Anacridium aegyptium* L.

— Length

--- Width



Graph: 2- Max. and min. diameters of nuclei of v.h.g. of embryos and first hatchlings of *Anacridium*

*aegyptium*, L.  
 ——— Max.  
 ..... Min.

The location of the ventral head glands in the embryos of *Anacridium aegyptium* is the same as in *Schistocerca gregaria*, [15], *Locusta migratoria* and *Locusta pardalina* [10, 11].

They are located in the posterior region of the head and are connected to the dorso-lateral muscles and epidermis at the dorso-anterior end and extend downward to connect with suboesophageal ganglion, muscles and epidermis at the ventro-posterior end. In the embryos of *Anacridium aegyptium*, these organs seem to taper at each end as in the embryos of *Schistocerca* [15] and *Locusta migratoria*, in contrast to *Locusta pardalina* where does not seem to be any tapering [10].

The ventral head glands of the embryos of *Anacridium aegyptium* are located very close to the tracheae. The small tracheoles can be seen in some sections of the glands of the older stages as in the embryos of *Schistocerca gregaria*. However, as already reported by **Schrarrer** [19], it is not possible to trace any thin connective tissue fibres of striated muscle, or nerves originating in the prothoracic ganglion, in sections of the gland in its longitudinal axis.

The cytoplasm of the ventral head glands is syncytial and contains deeply stained nuclei of various shapes and sizes depending upon the embryonic stages. The active period of the glands can be determined by the changes in the size of their nuclei [22], and in the differentiation of the cytoplasm [1], and [16].

At the active stage the nuclei become larger and lobulated and the cytoplasm resembles a network with small vacuoles and tracheoles and contains small granules which stain dark and light violet-blue.

The changes in the appearance of the nuclei and cytoplasm observed during this study indicate that the maximal activity of the glands occurs during the 21<sup>st</sup> and 22<sup>nd</sup> embryonic stages. This activity appears to coincide with the separation of the epidermis from the cuticle and the development of pleuropodia in the embryo of *Anacridium aegyptium* as it does in *Locusta migratoria* and *Locusta pardalina* [10, 11], and in *Schistocerca gregaria* [15].



At the twenty third and twenty fourth embryonic stages, the mean and the maximum diameters of the nuclei and the differentiation of the cytoplasm decrease. The lobulated nuclei are fewer though some lobules are still apparent.

After the moult in the embryos of *Anacridium*, the glands may continue their secretory activity for some period. This finding is also supported by the studies briefly mentioned below. Jones [10, 11] showed that the activity of the ventral head glands is necessary for stimulating the moult and controlling the subsequent events in the late embryonic development. This author uses the term of the "development hormone" instead of ecdysone (hormone of prothoracic gland). The differentiation of the mouth parts, the appendages (the development of teeth, spines, claws etc.), the sclerotization of the cuticle [12, 13], the beginning of the colouration of the body parts and the formation of melanin spots on the body of the embryo of *Anacridium* take place during late development. These late developmental events may depend upon the function of the ventral head glands. This situation agrees with Jones' results with the embryos of *Locusta migratoria* and *Locusta pardalina* and Wigglesworth's suggestion that ecdyson is connected with the synthesis of protein [27]. Karlson [13] implies that ecdyson is necessary for the metabolism of tyrosine which is eventually incorporated into the structure of the cuticle.

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### Ö Z E T

*Anacridium aegyptium*, L. un embriyonik gelişme safhalarında ventral baş bezinin histolojik yapısı incelendi. Bezin teşekkülünden itibaren embriyoların morfolojik özellikleri nazarı itibare alınarak gelişme safhaları saptandı. Çekirgelerde aynı yaşta olan yumurtalarda (aynı kokon içerisinde bulunanlarda bile) farklı derecelerde gelişme temayülü olduğu için ventral baş bezinin yapısı yaşa göre değil embriyonik gelişme safhalarına göre incelenmiş ve karşılaştırılmıştır.

1- Ventral baş bezi ilk defa, embriyo yumurtanın mikropil bulunan ucu etrafında dönüşünü tamamladıktan sonra gözlenebilir. Bu bezler başın her iki yanında ilkel baş kaslarının dorso-lateralinde epidermisten başlıyarak içeriye ve geriye doğru şerit halinde uzanarak sub-oesophagus gangliyonu ve o bölgedeki kas ve epidermisle brileşirler.

2- Bez sinsityal bir özellik gösterir, nukleusleri yuvarlak veya elips şeklinde olup **Gomori**'nin "chrome haemotoxylin phloxin" i ile koyu boyanırlar. Bezin aktif safhasında nukleusler büyüyerek loplul bir şekil alır ve sitoplazmada vakuoller ve granüller belirerek sitoplazma ağ şeklinde görülebilir.

3- Embriyo devreleri boyunca bezde bir maksimum periyot saptanır ve bu durum kutikulanın epidermisten ayrılması ile ilgili görünür (embriyonik kutikula). Maksimum aktif devreden sonra da bez görevine devam eder. Bezin bu görevinin embriyonun ileri gelişme safhalarındaki farklılaşmalar ve bilhassa melanin pigmentlerinin teşekkülü, kutikular farklılaşmalar ile ilgili olduğu zannedilir.

4- Yumurtadan çıkan çok genç ninflerde bezler başın gerisinde ve her iki yanında çok ince birkaç nukleus sırasından ibaret birkaç şerit halinde uzanır. Nukleuslar küçülmüştür ve sitoplazmada farklılaşma yoktur, bundan dolayı bezin aktif olmadığı düşünülür.

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