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EFFECT OF PRESERVATION TEMPERATURE ON HATCHABILITY AND EMBRYGENESIS OF *BOMBYX MORI* L., EGGS

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ABSTRACT

The effect of preservation temperature during aestivation period on embryonic development and hatchability of two univoltine strains of the silkworm *Bombyx mori*; the pure race Novi and the hybrid 157 k X 38 A had been studied. The obtained results indicated the following :

1. The highest hatchability rate resulted in case of eggs kept at 20°C.
2. 30°C was very harmful to egg vitality and the percentage of hatched eggs did not exceed 8%.
3. The hybrid was less sensible for changes of aestivation temperature than the pure race.
4. The embryo in a ventral position immersed in the yolk with L or C shapes and appeared with a layer of columnar cells; the ectoderm, a layer of spindle shaped cells and the mesoderm.
5. The amniotic membrane appeared in a primitive form and lied in proximity with the cytoplasm of yolk globules.
6. The serosa cells showed numerous large vacuoles full of brown pigments.

Key Words: Rearing temperature, *Bombyx mori*, Hatchability, Embryogenesis.

INTRODUCTION

Keeping silkworm eggs during aestivation period was one of the problems needed ot be solved in Egypt due to the usually high temperature during summer-time, which ranges indoors from 27-30°C. **Yu *et al* (1990), Roychoudhury *et al* (1992), Shamsuddin *et al* (1993), Hurkadli *et al* (1998) and Vijayalakshmi *et al* (1998)** recorded different ways with different ranges of temperatures for keeping aestivated eggs. Factors affecting rate of hatchability were studied by **Rahman and Ahmed (1989), Chaturvedi and**

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Upadhyay (1990), and Kamble (1998), (Cold storage) and **Reddy *et al* (1998)** (Light intensity). Embryogenesis in *B. mori* eggs was studied by **Indrasith *et al* (1987), Takahashi *et al* (1992), Amit *et al* (1997), Nagy *et al* (1994) and Nirmala *et al* (1999).** Accordingly, it was believed necessary to conduct investigations to determine the most suitable range of temperature during aestivation period of the silkworm, *Bombyx mori* L., at local circumstances.

MATERIAL AND METHODS

Two univoltine strains; Novi (race) and 157 K X 48 A (hybrid), were chosen for investigation with three treatments of temperature (10, 20 and 30°C). Histological studies were conducted to determine the thermal effect on the components of the egg.

Freshly emerged adults of both sexes were kept together, to couple and oviposit in adjusted rooms of approximately 25°C and relative humidity of 60-70%. Deposited eggs were washed, dried and counted. For each treatment, 100 eggs of each strain were replicated ten times in paper bags with stock batches for histological studies. The eggs were preseved in adjusted incubators (at 10, 20 and 30°C) during the aestivation period about 6 months (from June till December). Temperature was then decreased to 5-6°C within 15 days, and eggs were transferred to refrigerators adjusted at the same temperature and kept for the hibernation season (about 3 months). As soon as breeding season began, the temperature was raised gradually to 18°C and eggs were transferred to incubators where hatching took place at 22-23°C. Following **Cubells (1955),** hatched larvae were kept for a long time before counting or judging the effect of the treatment. This helped in obtaining the right numbers of hatched larvae. Experiments were repeated twice in 1999 and 2000.

Histological studies of aestivated eggs were conducted on samples of one month-intervals. The double embedded method of collodion and wax was adopted. The procedure was the same used by **Tawfik (1958).** Sections were stained in 0.5 percent Harris iron haematoxylin, counter stained with one per cent eosin and mounted in Canada Balsam.

RESULTS AND DISCUSSION

A. Hatchability rates

Data summarized in Table (1) represent the hatchability rates of silkworm eggs of the two tested strains kept at different temperatures during the aestivation period. The analyses of variance indicated significant differences among treatments. Aestivated eggs kept at 20°C were found to have the highest rates of hatchability, followed by eggs kept at 15°C. Eggs kept at 30°C resulted in very low rates in both years of experimentation. It was noticed that the increase of 10 degrees of aestivation temperature (from 20°C to 30°C) affected the eggs viability and resulted in very low percentage (8%) of hatchability, in case of both treated strains. This result goes in line with the conclusions of

Table 1. Hatchability rates of the two silkworm strains kept during aestivation period at various temperatures.

Temperature During Aestivation	Percentage of hatched eggs			
	Race Novi		Cross 157 K X 48 A	
	1999	2000	1999	2000
15°C	60.74% ±3.70	71.06%±1.40	81.81%±2.04	87.30%±1.96
20°C	91.01%±2.25	93.75%±0.70	82.57%±4.43	93.58%±1.06
30°C	8.70%±0.68	6.83%±0.88	8.22%±1.28	7.38%±0.80

L.S.D. at 1% level of probability = 0.04%.

Rahman *et al* (1989), Chaturvedi and Upadhyay (1990) and Hurkadli *et al* (1998) who stated that the hatching percentage was affected by egg preservation at low temperature.

The obtained reults indicate that both the pure race Novi and the hybrid 157 K X 48 A behaved in the same trend in case of exposure to 20°C resulting in the highest percentages of hatched eggs in both years. It was noticed that in 1999 and 2000 the hatched eggs of the pure race dropped in the two years, respectively, from 91.01% and 93.75% in the treatment of 20°C to 60.74% and 71.06% in the treatment of 15°C. On the other hand, hatchability of the hybrid eggs was 82.57% and 93.58% in the treatment of 20°C and decreased only to 81.81% and 87.30% in the treatment of 15°C in the two years, respectively. This means that the reduction from 20°C to 15°C caused a decrease in the rates of hatched eggs averaging 0.76%, 6.28% in the hybrid and 30.27% and 22.69% in the race in the two years respectively. These results may indicate that hybrids are less sensible to changes of aestivation temperature than pure races. However, it has to be noted that even races differ in their sensibility **Roychoudhury *et al* (1992) and Vijayalakshmi *et al* (1998)** as reported by the treatment of 30°C had the same lethal effect in both race and hybrid.

B. Embryological studies

The external shape of the egg remained elliptic or oval during the aestivation period. The egg contained protoplasm compacted with masses of yolk or vitellus. Each mass was spherical in shape and binucleated with meshes full of yolk. The vitelline membrane appeared as a fine envelope surrounding the egg protoplasm. The embryo was immersed in the yolk in a ventral position with its cephalic and caudal ends protrading deeply in it. The serosal membrane extended around the vitelline

globutes in the form of a sheath of flattened cells oviginating from the embryo. The amniotic membrane took the shape of a connecting link of rounded cells between the serosa and the embryo. The changes in size and shape of a developing embryo of *B. mori* were monitored by **Nagy *et al* (1994).**

a. Position and limits of the embryo

The embryo lied at its lateral grooved position on its longitudinal axis with the cephalic extremity correspondent to the pole including the micropyle while the far caudal end directed towards the pole of antimicropyle (Fig. 1). This evidence is in full agreement with that of **Nagy *et al* (1994).** During dispause, the embryo (Fig. 2) lost its regular structure and appeared in the form of a curved body L or C shaped. The central cytoplasmic nourishing part became in contact with the internal border of the embryo. The yolk or vitelline globules adjacent to the embryo were seen as few granules scattered on the periphery as described by **Amit *et al* (1997).** The amnion became loose and contact with the embryo at its extreme edges (Fig. 3).

b. The aspect of mesoderm

Sections in the embryo showed a layer of ectoderm cells characterized by its columnar shape and large nuclei. These elements were very often interrupted by intercellular gaps which remained stable in the examined plates. The 18 segments previously formed early in the embryogenesis were not well discerned during the first period of diapause (Fig. 4), and appeared contact with the basal cells of nearer masses inducing fusions of at least 2-3 segments (Fig. 5). Cross sections indicated clearly over growth of the two mesodermal lateral plates lacking any groove or invagination and extending mid dorsally towards any of the two sides (Fig. 6). These cells were not easily differentiated and appeared as irregular bud shaped masses. They stretched gradually towards the two wings covering the internal border of ectoderm with exception of the medium space. The same procedure of mesoderm formation was described by **Nirmala *et al* (1999)** in case of *B. mori*. However, they referred to the segmentation of ectoderm before the formation of somites; a phenomenon which does not occur during diapause of silkworm embryo. The cells at the chephalic and caudal ends of the different plates became more considerable than the other intermediate parts of the embryo (Fig. 7). At the end of the aestivation period the two mesodermal grooves of extreme ends of embryo became much obvious and more developed to form the stomodaeum and proctodaeum (Fig. 8).

c. Folds of the ectodermal border

The amniotic membrane was present in an irregular form during dispause of embryo (Fig. 9). Consequently, its cells appeared in a primitive and delicate form (Fig. 10). The amnion cells were noticed sometimes in direct contact with the granulated yolk spheres scattering between amnion and serosa (Fig. 11). As discribed by **Inrasith *et al* (1987),** the yolk globules or vitellophags adjacent to

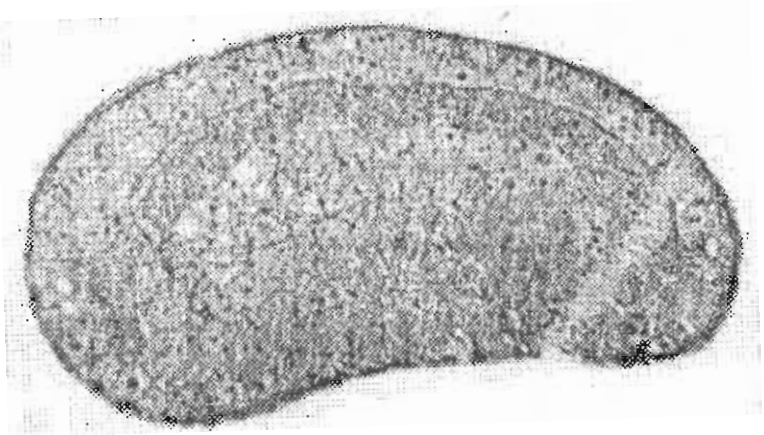

Fig. 1. Medium longitudinal section of Novi race showing the embryo invaginated in the yolk in a ventral position (X27C).

Fig. 2. Section in medium part of Novi race during aestivation period to show granulated central zone and yolk spheres adjacent to the embryo. The twisting and folds of the embryo are obvious with the fuses of mesoderm (X 100).

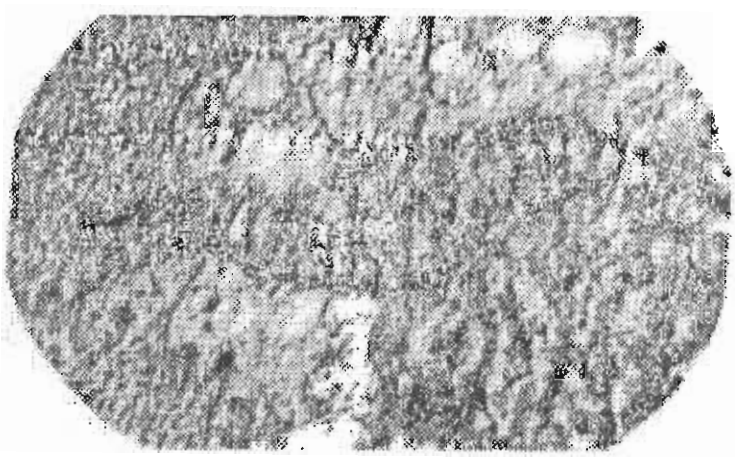

Fig. 3. Part of transverse section of Novi embryo showing the deep and apparent invagination of estoderm and the loose amnion (X 100)

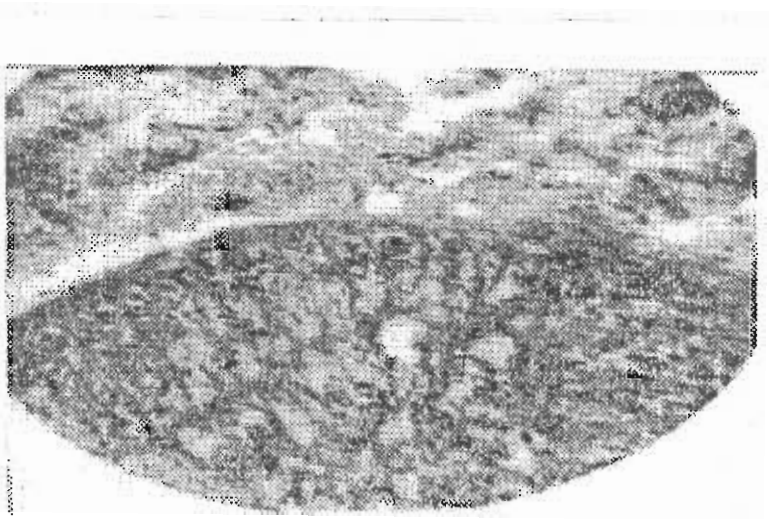

Fig. 4. Transverse section of 157 X 48A embryo showing the columnar ectodermal cells and the spindle mesodermal cells (X 1000)

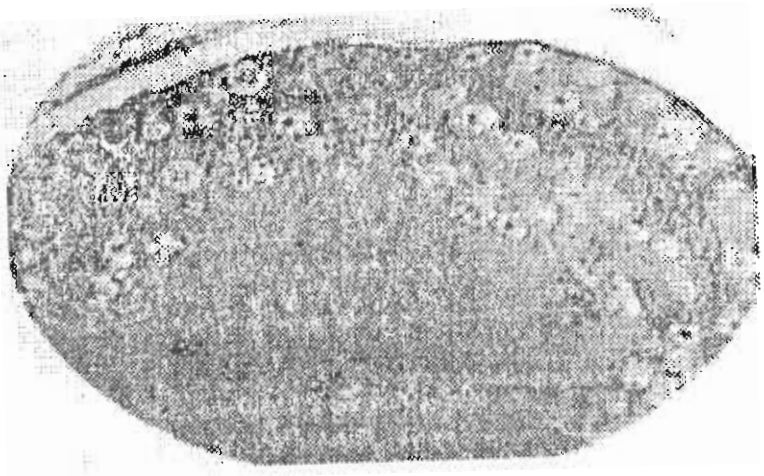

Fig. 5. Transverse section in Novi embryo during aestivaation period showing the mesodermal buds of 2-3 segments (X 200).

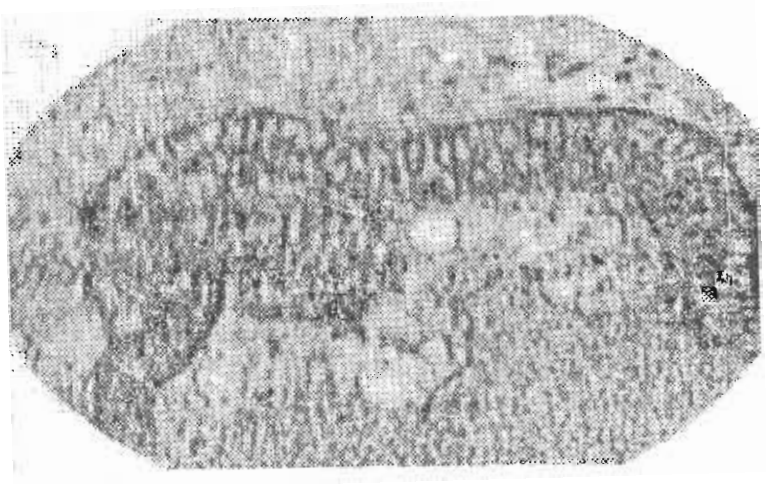

Fig. 6. Transvers section of 157K X 48A embryo showing the two mesodermal wings (X675).

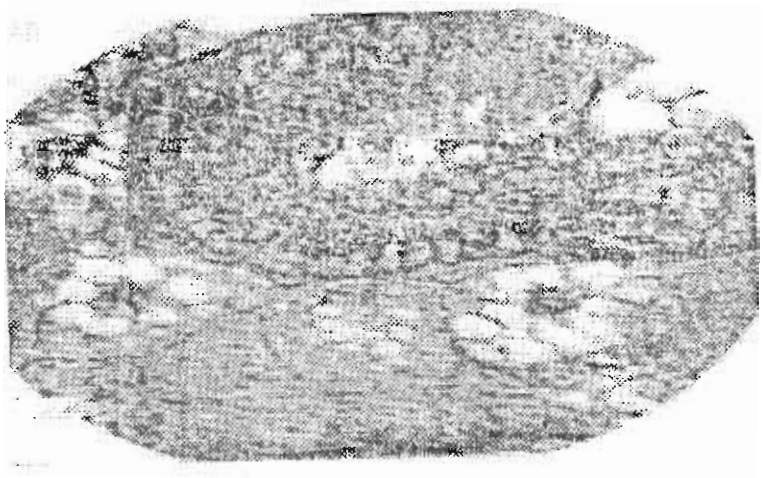

Fig. 7. The extreme cephalic part connected with the central nourishing zone in a transverse section of the 157K X 48A embryo (X1000).

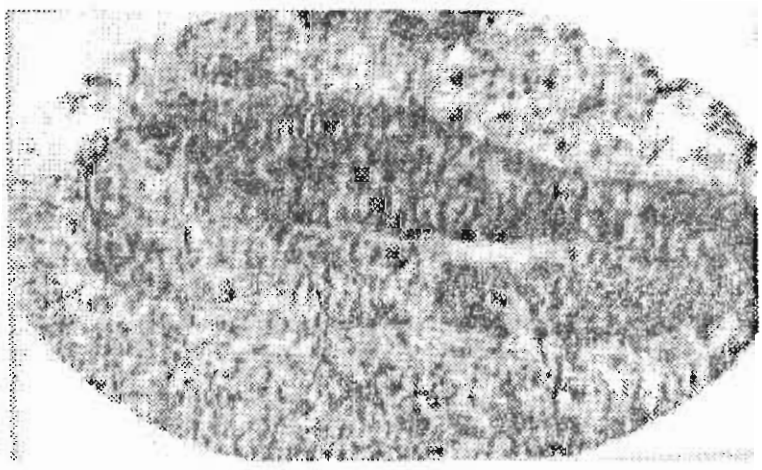

Fig. 8. The stomodaeum invagination of Novi embryo during aestivation period (not well developed) (X1000).

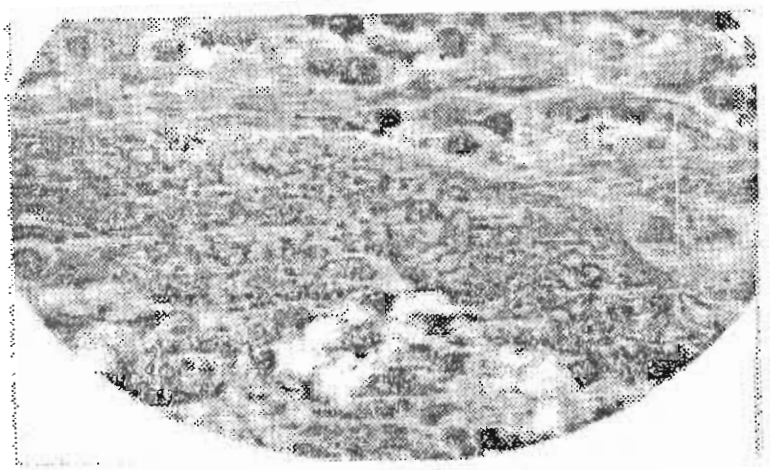

Fig. 9. Part of transverse section of Novi embryo during aestivation to show amniotic cavity (X 1000).

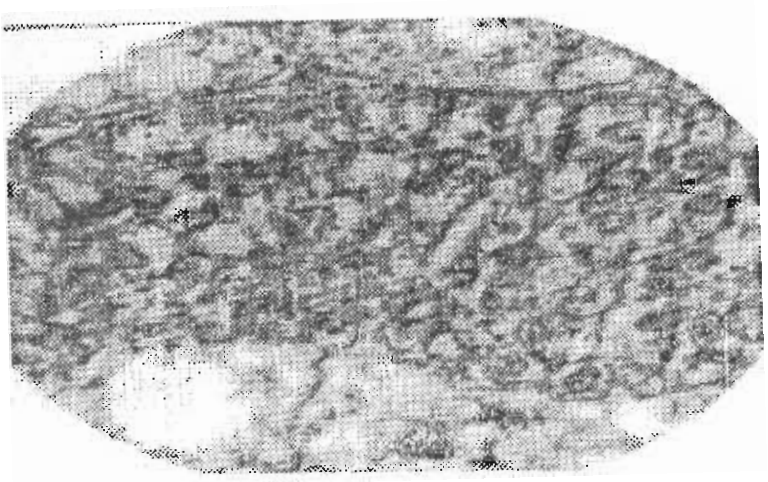

Fig. 10. The primitive amniotic cells of 157K X 48A embryo during aestivation period (X 1000).

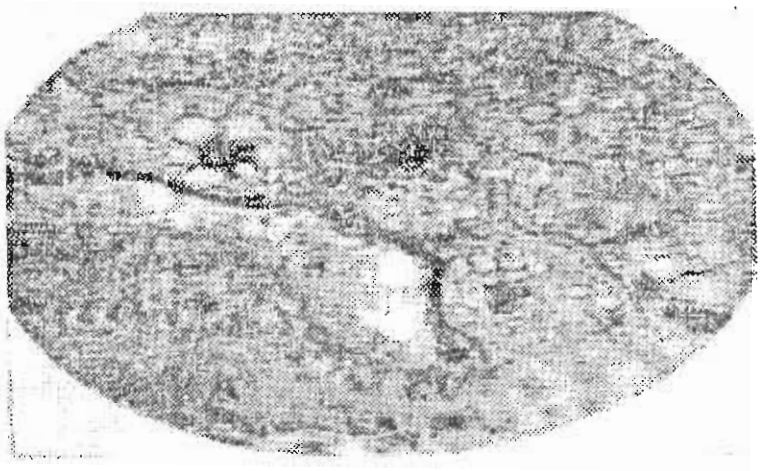

Fig. 11. Transverse section of Novi embryo to show the amnion apart from the embryo. The grating cells are in the amniotic cavity (X1000).

Fig. 12. The shape of yolk spheres with apparent granules and binucleated (X15000).

the embryo were minute and contained a single periphery of perfect granulated vitelline (Fig. 12). Consequently, the nourishing central area remained with its network granules and unique nucleus in contact with the embryo (Fig. 2).

d. Vaculation of the serosa

Permenant preparations at intervals during aestivation period, showed numerous large vacuoles manifesting in the cells of serosa causing move thickness of the layer towards the internal cavity of the egg. The vacuole was produced between the nucleus and the external margin of the cell, pushing the nucleus backwards towards the internal part similar evidence were shown by **Takahashi *et al* (1992) and Amit *et al*** (1997). The brown pigments of the serosa were found with irregular distribution in the cavity of vacuoles. The description of the embryo, position, shape and contents during aestivation period was in full agreement of what **Nagy *et al*** (1994) had mentioned before.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٤٧ ، ع(٣) ،١٠٦٣-١٠٧٥ ، ٢٠٠٢

تأثير درجة حرارة الحفظ على فقس البيض والنمو الجنينى فى بيض دودة القز [٦٨]

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تم دراسة تأثير درجة حـــرارة الحفـظ خلال فترة البيات الصيفى على فقس بيـــض سلالتين من دودة القز ثنائية الجيل ، الأولـى هى السلالة نوفى والثانية هجيـــن ١٥٧ك × ٣٨ أ، مع إجراء دراسة جنينية خلال فـــترة البيات الشتوى . ويمكن تلخيص النتـــائج المتحصل عليها فيما يلى :

١-تم الحصول على أعلـــى معـــدل لفقـــس البيض عند الحفظ على درجة حـــرارة ٢٠°م .

٢-تعتبر درجة حرارة ٣٠°م ضـــارة جـــداً لحيوية البيض ، حيث لم تتعد نسبة الفقس فى هذه الحالة ٨% .

٣-كان الهجين أقل حساسية للتغيرات فـــى درجة حرارة الحفظ عن السلالة النقية .

٤-أظهرت القطاعات أن الجنين فى الوضـع البطنى يكون مغموراً فى المح على هيئـة شكل (L) أو (C) .

٥-يظهر الجنين على هيئة طبقة من الخلايـا العمادية (الاكتودرم) وطبقة من الخلايـــا المغزلية (الميزودرم) .

٦-يظهر الغشاء الأمنيونى علـــى هيئـة بدائية .

٧-تظهر بالخلايا المصابه فجـــوات كبـيرة مليئة بالصبغات البنية .

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