SOME VIROLOGICAL AND PATHOLOGICAL STUDIES ON CAMEL POX

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Abstract

In private camels farm at Marsamatroh Governorate, Egypt, 5 out of 100 dromedary camels showed clinical manifestation referred to camel pox. The lesions were confined to the skin of the head, nose, eyelids and neck. Five from skin biopsies were taken for virological and pathological studies. The characterization of the viral isolate revealed its multiplication on the corioallantoic membrane (CAM) of chicken embryos at the age of 11 days with the formation of minute white pock lesions 5 days post-inoculation. Histopathological investigation showed eosinophilic cytoplasmic inclusion bodies in the cells of CAM. The virus was identified by agar gel immunodiffusion test using specific hyperimmune serum of camel pox virus. Electron microscopy was done on two skin biopsies and one sample from CAM, as well as 3 other CAM samples for negative stain. Pox virions were detected in the cytoplasm of CAM cells and in the cells of tunica intima of dermal blood vessels. Histopathological examination of skin biopsies showed degeneration and necrosis of prickle cells, as well as, vascular deamage in the dermis with mononuclear cell infiltration around the blood vessels. Intracytoplamic inclusion bodies were seen in some prickle cells which appeared as homogenous round eosinophilic bodies.

INTRODUCTION

Camel pox is a proliferative skin disease affecting camel and caused by specific Orthopox virus cameli related to family Poxviridae. Pox is species specific disease distinct from vaccinia, sheep pox and cow pox viruses (Tantawi *et al*, 1974 and Alhendi *et al*, 1994).

Camel pox was first described by Lesse (1909) in Pengab, India. The disease has a wide distribution among camel population in India, Pakistan, Afghanistan, Iran, USSR, The Middle East, as well as, North and East Africa (Munz, 1992).

In Egypt, the causative virus was isolated by Tantawi *et al.* (1974) in Fayoum, and by Kenawy *et al.* (1989).

Munz (1992) stated that the incubation period of the disease ranges from 10 to 15 days in natural cases. The onset of the disease is indicated by fever and weakness, followed by swelling of the skin and mucosa in affected parts of the body. Around the lips, nose and eyelids, lesions may become visible in the form of papules, which quickly pass through vesicular and pustular stages. Lesions also, develop on the mucous membranes of the oral cavity resulting in difficulty in eating. Associated lymph nodes are often swollen and facial oedema is apparent. These clinical signs as mentioned by Pfahler (1986) and Kaaden *et al.* (1992) may be confused with contagious camel ecthema caused by parapoxvirus and papillomatosis caused by camel pappiloma virus.

Diagnosis of camel pox as noticed by Tantawi *et al.* (1974), Kenawy *et al.* (1989) and Kaaden *et al.* (1992) was based on the history, clinical signs and the course of the disease. Confirmation was obtained through isolation of the virus on the chorioallantoic membrane of developing chick embryo (CAM), and identification of the virus by agar diffusion test using specific hyperimmune serum. In addition, Andrews (1978) noted that the electron microscopical detection of the virus particles in pox lesions was one of the diagnostic tools of the disease. Munz *et al.* (1992) recorded the differentiation between pox and pox like diseases in camel (camel pox, camel contagious ecthyma and papillomatosis) by the use of electron microscopy. The orthopox virus causing camel pox, is brick-shaped, while, parpox virus causing camel contagious ecthyma shows criss-cross pattern, and papilloma virus has an icosa hedric arrangement.

Donny (1988) and Jubb and Kenedy (1985) stated that camel pox lesions began with balloning degeneration of stratum spiosum of the epidermis, reticular degeneration and acanthosis resulting in intraepidermal microviscles. The dermal lesions included oedema and perivascular dermatitis; mononuclear cells and neutrophils were present in varying proportions. Pox lesions contained characteristic eosinophilic, intracytoplasmic inclusion bodies which are single of 3-7 μm in diameter and of type A. (Cowdrey type A).

The present investigation deals with virological and pathological studies on camels that suffered from skin lesions similar to pox in one private farm at Marsamatroh Governorate, Egypt. The optical and electron microscopical pictures were described as an aid for diagnosis.

MATERIALS AND METHODS

In a private farm at Marsamatroh Governorate, Egypt, skin lesions similar to pox on 5 out of 100 camels have appeared. Camels were inspected for other clinical signs. Five skin biopsies were surgically removed from the upper part of the neck for virological and pathological examinations.

I. Virological Examinations

Skin biopsies were kept in minimal essential media as maintenance media containing 1% foetal calf serum for virus isolation.

For virus isolation, skin biopsies were pooled and ground in a sterile morter according to the method described by Kenawy *et al.* (1989). Isolation procedure on chrioallantoic memebrane of 11-12 days old embryonated chicken eggs were used for virus isolation. From the prepared fluid, 0.2 ml was inoculated for each egg via chorioallantoic membrane route (CAM) according to the technique described by McCarthy and Dunbell (1961). After five days post-inoculation, the chorioallantoic membranes were investigated, and the isolated virus was passaged 7 successive times.

The isolated virus was identified using the agar gel immunodiffusion test which was carried out according to Mansi (1958) by testing the isolate against hyperimmune sera of camel pox virus obtained from Vaccine and Serum Production Research Institute (Abbasia).

2. Pathological Examinations

1. The gross appearance of skin lesions that appeared on the neck of five dromedaries camels was recorded.

2. **Electron microscopy**

Two skin biopsies and one sample from CAM pock lesions were fixed in 3% glutaraldehyde, processed and sectioned for transmission electron microscopy in E.M. Center Specialist Ein Shams Hospital. In addition, negative stain was adopted in

3 samples from CAM bock lesions to detect the virus in clear form.

3. Light microscopy

A) Number of 5 skin biopsies from infected camels were fixed in 10% formol saline, and were prepared for paraffin embedding; sections were stained with haematoxylin and eosin (H & E) according to Carleton *et al.* (1967). In addition, staining with phloxine tartrazine and toluidin blue, basic fuchcin for inclusion bodies were applied (Clyden *et al.*, 1971).

b) Proper samples from the chorioallantoic membrane (pock lesions after the 7th passage) was collected from 15 eggs and fixed in 10% formalin. Thin paraffin sections were prepared, and stained with H & E and Man's stain for microscopical detection of inclusions.

RESULTS

I. Clinical Signs

In Autumn 1995 at Marsamaroh Governorate, total of 5 out of 100 drormedary camels with an age ranging from 3-5 years and bleonging to a private farm, were suffereing from skin lesions in the form of circumscribed rounded papules, and vesicles of 0.5-1.5 cm in diameter. These lesions were found in the nose, eye lids and lips and in the upper part of the neck.

2. Virological Results

The virus was isolated on the CAM of the embryonated chicken egg and produced pock lesions which appeared very clear on the 5th day postinoculation (Table 1).

Table 1. Propagation of the virus on CAM.

No. of passage	Changes of CAM	AGP
1	Oedematous	-
2	Oedematous	-
3	Oedematous	-
4	Pock lesions at the site of inoculation	+
5	Pock lesions at the site of inoculation	+
6	Pock lesions at the site of inoculation	+
7	Pock lesions at the site of inoculation	+

Five days post-inoculation of 11-12 days old embryonated chicken eggs with 0.2ml of prepared fluid from the skin lesions of camels, the gross appearance of CAM contained diffuse opaque thickening with slight heamorrhagic spots, as well as, round individual white opaque pock lesions 0.7-1.0 cm in diameter with smooth surface raised on CAM. (Fig. 1).

The identification of the virus isolate was done using the agar gel diffusion test with standard hyperimmune sera of orthopox of camel. The test showed clear precipitation line which started to appear from the forth passage.

3. Electron Microscopical Findings

a. Skin

Changes of the skin consisted of absence of the intercellular spines inbetween the prickle cells with the presence of vacules in between. Some of the nuclei showed indulation with disarrangement of chromatin and an increase of euchromatin on the expense of heterochromatin. (Fig. 2). Blood vessels showed swelling of the endothelial lining, as well, pericytes appeared swollen also. Pox virions were detected in the cells of tunica intima that showed exocytosis. (Fig 3, 4).

b. Chorioallantoic membrane (CAM)

In the epithelial cells of ectoderm, pox virions were detected in the cysternae of rough endoplasmic reticulum which appeared swollen (Fig. 5). In addition, there was marked increase in the number of lysosomes in the cells examined; virions were also detected free in the cytoplasm of the cells. Lipoblastic cells showed virions scattered in the cytoplasm besides fat vacuoles and phagocytic ones. (Fig. 6). The endodermal epithelial cells showed virion scattered in the cytoplasm, by use of negative staining technique, orthopox virus appeared as brick shaped particles measuring 260-280 nm. and the superficial protein filaments were arranged irregularly. (Fig. 7).

4. Pathological Findings

The histopathological examination of skin lesions showed coagulative necrosis, epidermal erosion with some prickle cells (Fig. 8), in addition to areas of epidermal vacuolation (Fig. 9). The vacuoles were mostly found near and around the nuclei of some brickle cells, These vacuoles had different sizes and sometimes occupied nearly all the entire cell area. The nuclei shrank in size, shriveled, or were marked

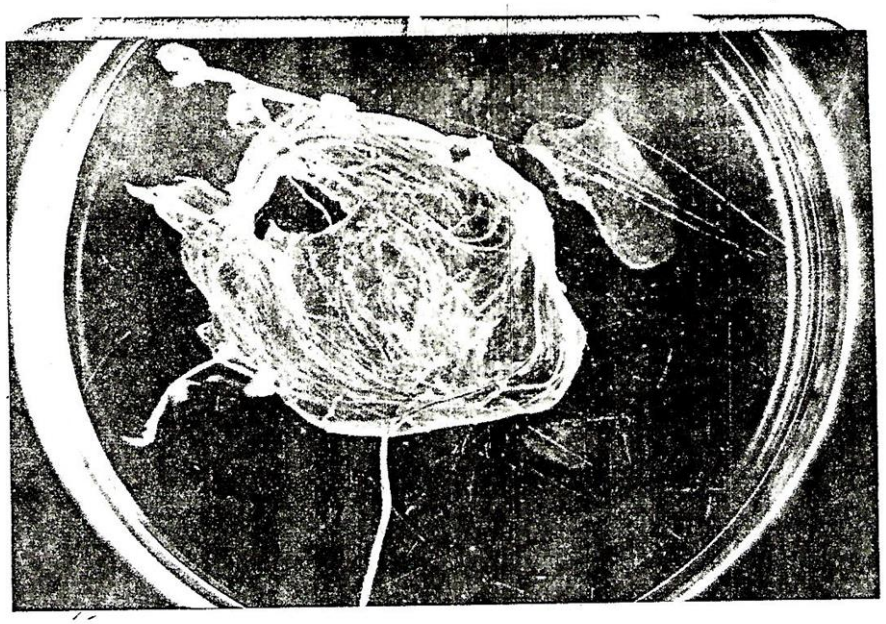

Fig. 1. Chorioallantoic membrane showing pock lesions with slight heamorrhages.

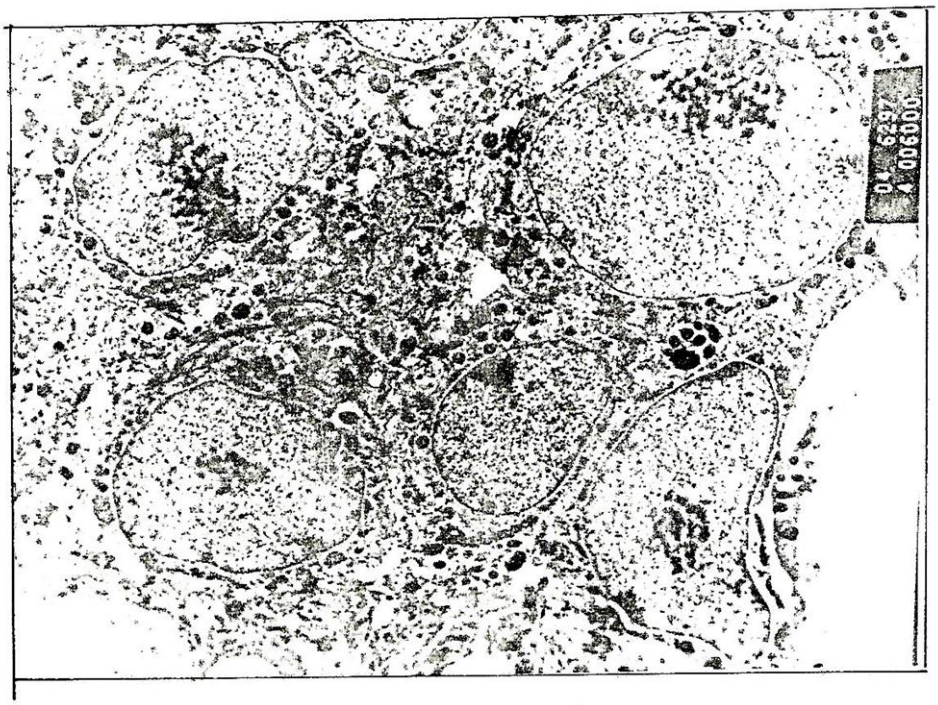

Fig. 2. T.E. of the skin showing stratum spionsum, notice.

1. Absence of intercellular spines
2. Some nuclear andulation.
3. Increase of euchromatin to heterochromatin ratio.
4. Vacuolation. (x 11, 000)

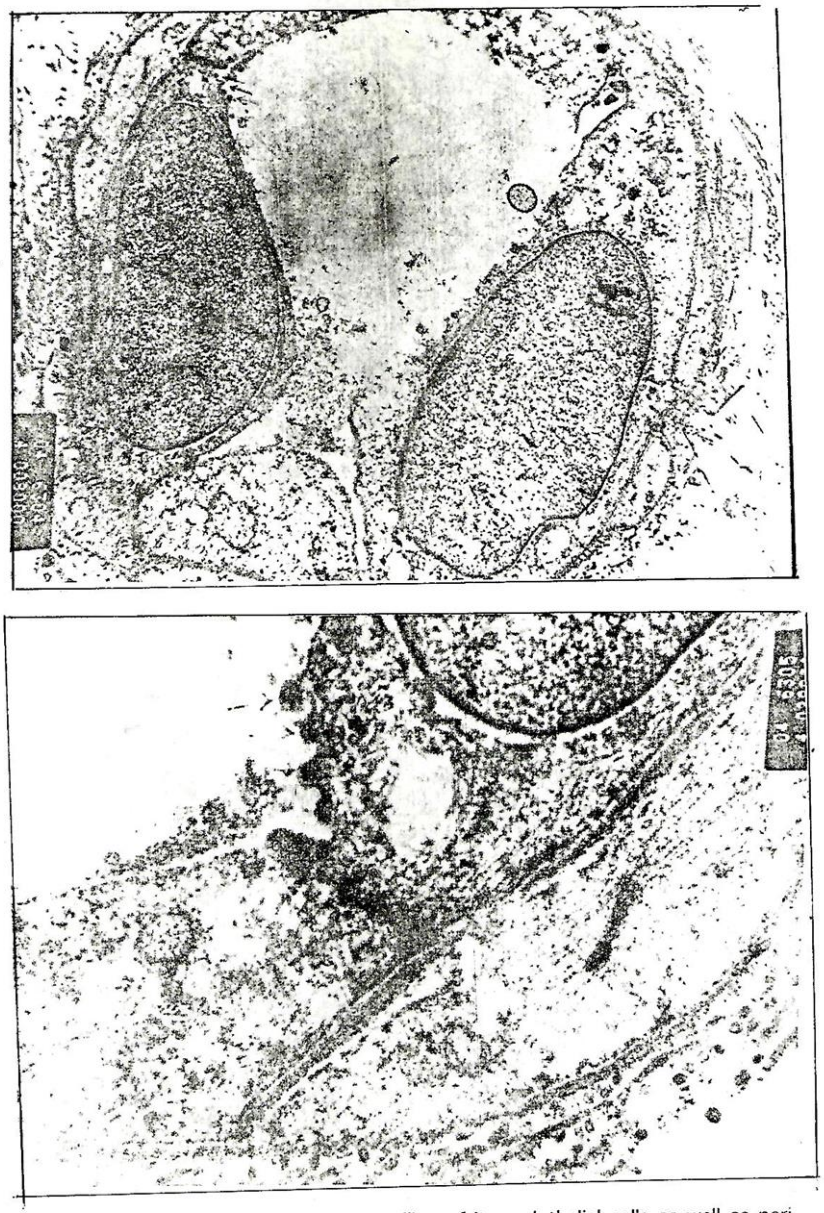

Fig. 3, 4. T.E. Dermal B.V. showing swelling of its endothelial cells as well as pericytes and exocytosis. of pox virus through blood vessel wall x 5.000 - 7.000.

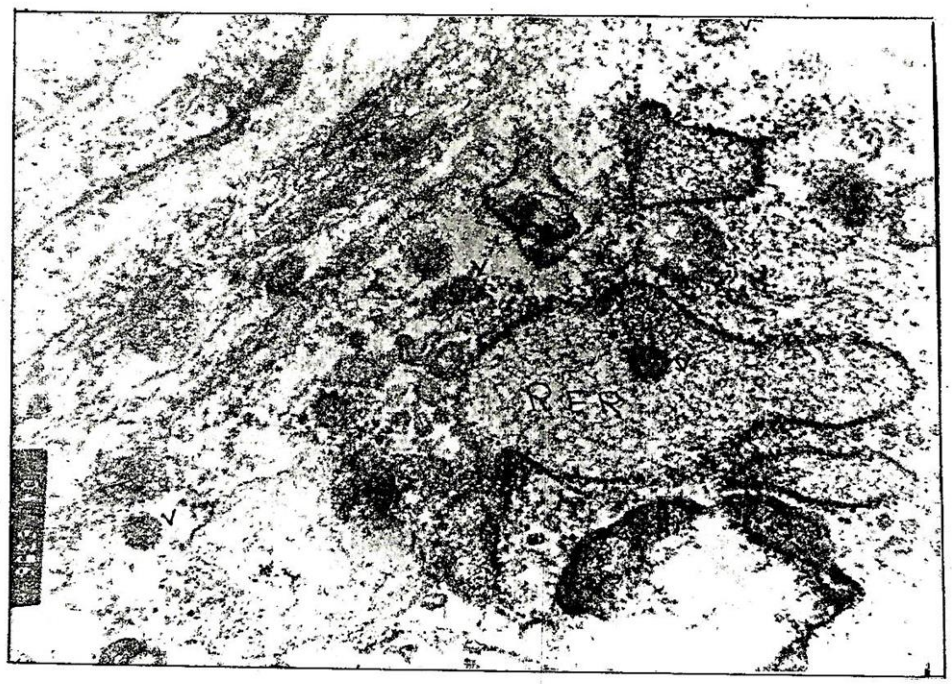

Fig. 5. T.E. Cysterna of rought endoplasmic reticulum showing virion replications. x 60.000.

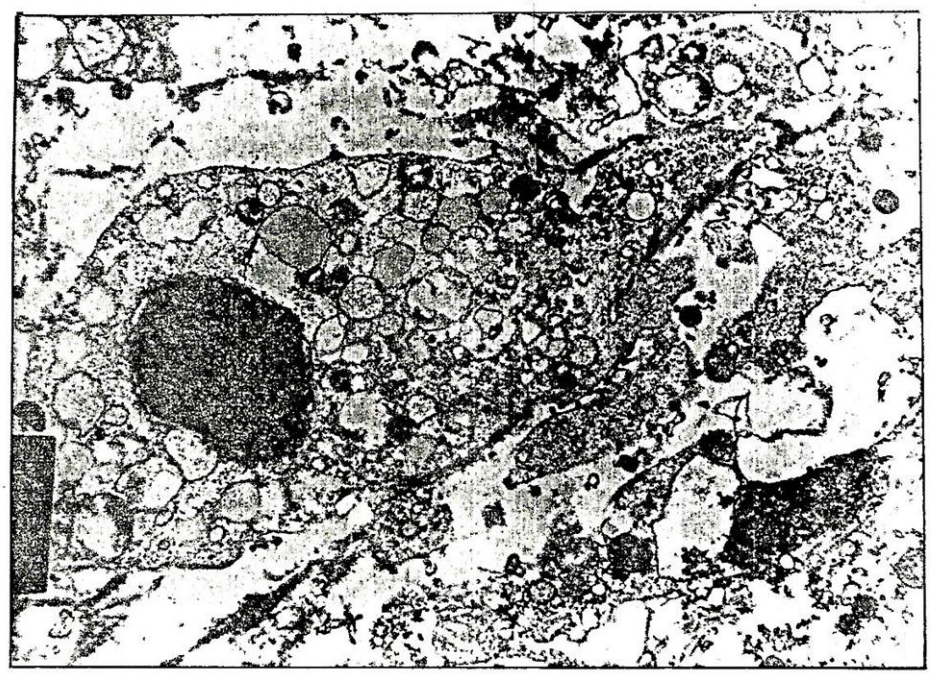

Fig. 6. T.E. Lipoblast in the mesoderm of CAM showing virion of camel pox in the cytoplasm. x 14.000.

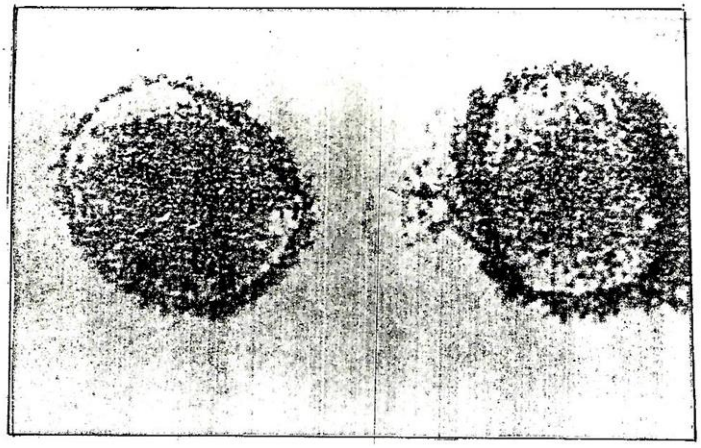

Fig. 7. T.E. Negative stained virus (mature) of the 7th stage of propagation on CAM. x 180.000.

Fig. 8. Skin showing epidermal erosion erosion with coagulative necrosis of some prickle cells. H & E, X 400.

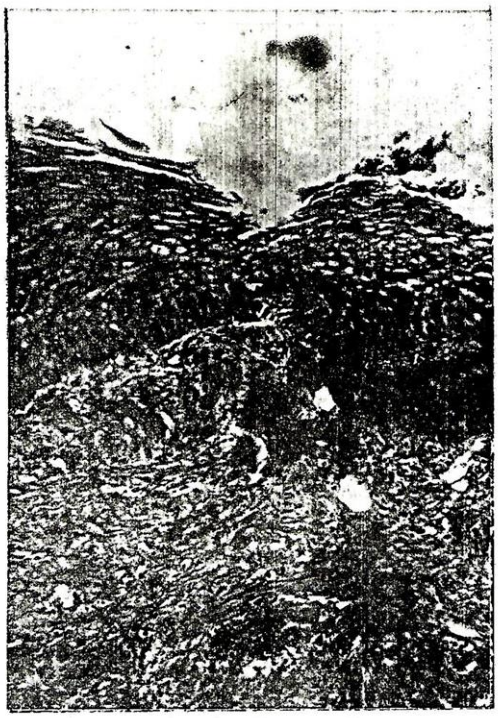

Fig. 9. Skin showing epidermal vacuolar degeneration of most of the cells of stratum spinosium. H. & E., x 100.

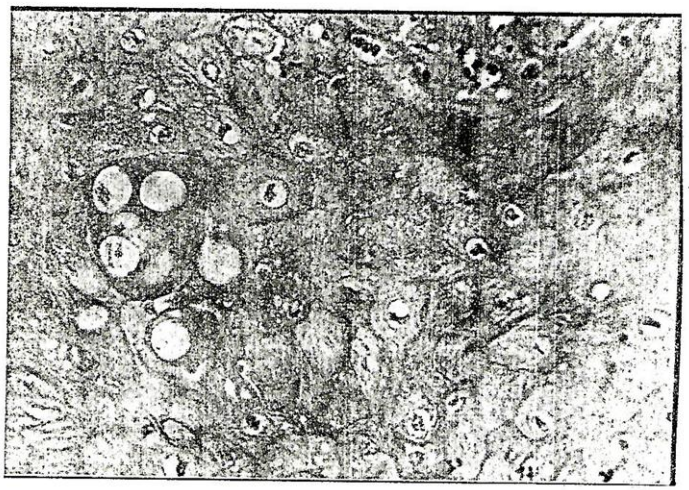

Fig. 10. Sking showing epidermal vacuolations and some leucocytic cell infiltration.

only by small particles of chromatin, or totally disappeared leaving only borded empty areas (Fig. 10).

Most of the prickle cells were swollen and gave the appearance of balloning state, some of them burst, coalesced with each other to form large vesicles which appeared in different forms and sizes (Fig, 11). Some leucocytic infiltrations occurred in the lumen of some vesicles. Just under stratum cornium, severe degeneration and necrosis of some prickle cells were seen, as well as, multinuclear leucocytic infiltration. Involvement of stratum basalis was not uncommon, but only small areas were affected in the form of ulcer (Fig. 12).

Intracytoplasmic inclusion bodies were seen in some of prickle cells in the form of homogenous round eosinophilic bodies (Figs. 13, 14, 15).

All skin lesions showed focal infiltration with mononuclear cells in the dermis (Fig. 10), specially, around small blood vessels and associated with oedema and fibrinous exudate (Fig. 17). Some blood vessels showed thrombus formation with swelling of the endothelium and its protrusion into the lumen.

The histopathological findings of CAM showed severe hyperplastic proliferation of ectodermal epithelial cells with swelling and ballooning features. Intracytoplasmic eosinophilic inclusions were seen in both ectodermal and endodermal epithelial cells (Fig. 18, 19). The mesoderm showed dilatation of blood vessels, congestion and mild heamorrhages, with slight proliferation of its cells. The endoderm showed clear proliferation of its epithelium, but to a lesser degree than ectoderm (Fig. 18, 19).

DISCUSSION

The clinical findings showed that, 5 out of 100 dromedary suffered from skin lesions in the form of papules and vesicales on the head and neck, nose and ey lids. Similar mild picture of pox was previously recorded by Alhendi *et al.* (1994). The gross appearance of skin lesions was similar to that mentioned by Kaaden *et al.* (1992).

The reported results indicated that the isolated virus was camel pox virus. The virus was found to be able to replicate on CAM of chicken embryos producing pock lesions which were completely different from cow pox and vaccinia viruses (Downie 1949, Fenner and Burnet 1959) and somewhat resmbling buffalo pox virus isolated by Tantawi *et al.* (1967).

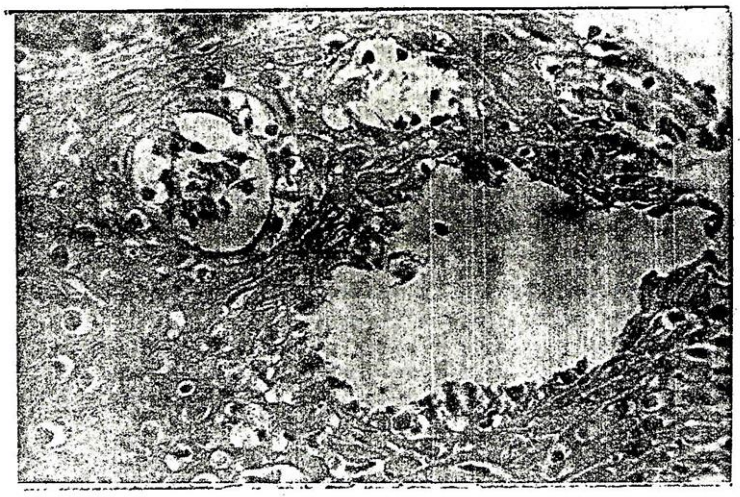

Fig. 11. Skin showing epidermal vesicles of different forms and sizes. H & E X 650.

Fig. 12. Skin showing ulcer formation with lymphocytic infiltration of dermis . H & E, X 100.

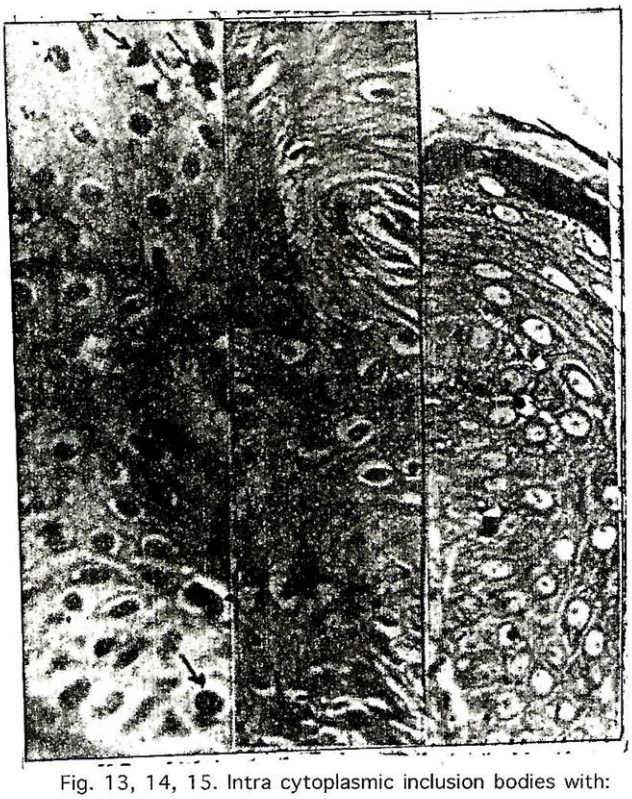

Fig. 13, 14, 15. Intra cytoplasmic inclusion bodies with:
13. Fluxin tartrazin stain.
14. H & E STAIN.
15 Toluidin blue basic fuchin. (x 650).

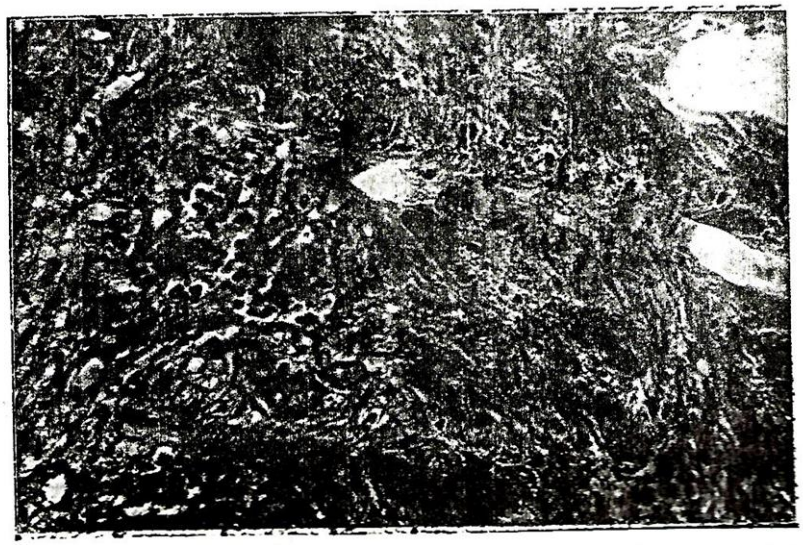

Fig. 16. Skin showing dermal focal infiltrtion with mononuclear cells. H. & E. x 250.

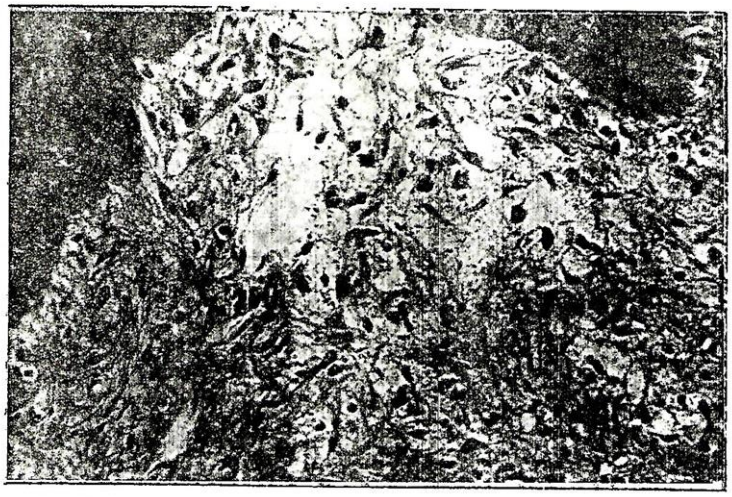

Fig. 17. Skin showing dermal oedema and fibrinous exudate. H. & E. X 250.

Fig. 18, 19. Chorioallantoic membrane showing:

a. Endodermal, and b. Ectodemal proliferation of epithelial cells with intra cytoplasmic inclusions. Man; s stain x 650.

The viral isolate was tested by standard hyperimmune sera of orthopox of camel that showed clear precipitation line starting from the forth passage of the isolate on CAM of chicken embryo. These results were similar to those obtained by Kenawy (1989).

The electron microscopical findings of the skin samples proved that orthopox viral infection was the probable cause of pox in our camels. The distribution and the morphological features of the virus particles were similar to orthopox viruses detected by Kaaden *et al.* (1992). The size of virus particles observed by use of negative stain in CAM coincided with the findings of Munz (1992).

The results of examination of skin lesions lesions by electron microscopy were similar to those obtained by Munz *et al.* (1986 c).

The replication of the virion in the tunica intima of blood vessels of skin epidermis, and also, the presence of virions in the mesoderm of CAM chicken embryo emphasized the tropism of the virus on the cells of the blood vessels, skin and on the mesodermal cells which are known to be able to give rise to blood vessels of the embryonated chicken (Munz *et al.* 1986 c).

Histopathological examination of the skin lesions showed proliferative and degenerative changes in stratum spinosum with typical intracytoplasmic eosinophilic inclusions. Coagulative necrosis and vascular damage detected in the skin lesions emphasized that camel pox virus has pathological effect similar to the other pox viruses (Cheville 1966, Gibbs and Johnson 1970). The necrotic changes observed in stratum spinosum could be attributed to the thrombosis of dermal blood vessels and not to the direct effect of the orthopox virus. The lymphocytic infiltration at the site of lesions may be related to the toxic products of the necrotic tissues.

Intracytoplasmic eosinophilic inclusion bodies were commonly seen in the cells of ectoderm and endoderm of CAM of chicken embryos. These findings were similar to those recorded for cow pox inclusions by Jezek *et al.* (1983).

The haemorrhages found in the mesoderm proved the greater mesodermotropic affinities of this virus and its predilection seats for endothelial cells linning blood vessels. These results resembled those obtained by Yasuo *et al.* (1971) in vaccinia.

As reported by Alhendi *et al.* (1994), the hyperplastic proliferation in both ectodermal and endodermal cells proved that the virus is an epitheliortropic one.

The previous and the present studies revealed the appearance of camel pox in camel populations in an Egyptian governorate. This problem needs more studies to adopt the proper prophylactic and control measures of the disease.

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بعض الدراسات الفيرولوجية والباثولوجية على جدرى الجمال

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ظهرت فى إحدى المزارع الخاصة بالجمال بمحافظة مرسى مطروح أعراض مرض الجدرى على خمسة جمال من بين مائة جمل وحيد السنام. ظهرت بثرات جلدية مميزه للمرض فى منطقة الرأس والرقبة. تم اخذ خمس عينات من الجلد المصاب وذلك للدراسات الفيرولوجية والباثولوجية.

تم عزل الفيروس المسبب للمرض على أغشية الكوريو آلانتويس لاجنه كنا كيت عمر ١١ يوما بعد تمريره ٧ مرات. وبعد ٥ ايام من الحقنه الرابعة ظهرت بثرات على الغشاء الكوريو آلانتويس والذى ثبت باجراء الفحوص الهستوباثولجية عليها عدم وجود اجسام احتوائية، بها بينما ظهرت هذه الاجسام الاحتوائية الادينوفيلية فى سيتوبلازم الخلايا (الكوريو آلانتويس) فى الحقنة السابعة.

أجرى اختبار الاجار الترسيبى باستخدام مصل مناعى مرجعى نوعى ضد فيروس جدرى الجمال.

أجريت فحوص الميكروسكوب الالكترونى على عدد ٢ من عينات الجلد المصابة وعدد عينة واحدة من اغشية الكوريو آلانتويس المحقون، وتم صباغتها بالصبغة السلبية للفيروس لفحصها على الميكروسكوب الالكترونى.

اظهرت الفحوص الهستوباثولوجية لعدد ٥ عينات من الجلد المصاب ان بها استحالة وتنكرس بالطبقة الشوكية لمنطقة البشره بالاضافة الى اصابة الاوعية الدموية لمنطقة الأدمه ووجود تجمعات ارتشاحية من خلال وحيدة النواه حولها. وقد لوحظ وجود اجسام احتوائية ادينوفيليه بسيتوبلازم بعض خلايا الطبقة الشوكية لمنطقة البشره.

هذا وقد تم توضيح النتائج فى عدد ١٩ صورة ومناقشتها.