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RELATIONSHIP BETWEEN BOVINE PAPILLOMA AND EQUINE SARCOID: EXPERIMENTAL INOCULATION OF DONKEYS WITH BOVINE PAPILLOMA EXTRACT

(With 13 Figs.)

By

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العلاقة بين الحليمات الجلدية في البقر وساركويد الخيول

حقن تجريبي للحمير بمستخلص الحليمات الجلديـة

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تمت بنجاح محاولات لعمل الساركويد في الحمير ونقلت العدوى من ثور بقرى مصاب بالحليمات الجلدية الى حمير سليمة بطريقتين ، احدهما : بزراعة أجزاء صغيرة من هذه الحليمات ، والأخرى: بحقن مستخلص منها داخل الجلد وبعد ٩ الى ١٦ يوم من نقل العدوى ظهرت أورام في أربعة من خمسة حمير وكذلك تم دراسة الخواص الأكلينيكية والباثولوجية لهذه الأورام خلال فترات نموها المختلفة . ولقد وجد تشابه كبير في الخواص الأكلينيكية والباثولوجية بين الأورام الناتجة في الحمير والساركويد الخيلي. ويستخلص من هذه الدراسة أنه يحتمل أن يكون سبب الساركويد في الحمير هو فيروس الحليمات الجلدية في الأبقار.

SUMMARY

Attempts to produce equine sarcoid in donkeys were successful. Bovine papilloma was transferred between affected bulls and nonaffected donkeys by transplanting whole bovine papilloma tissue or by inoculating the skin with extract of bovine papilloma. Tumours were produced in 4 of 5 donkeys 9 to 16 days after infection. The clinical course of the developing tumours were studied. Surgical removal, macromorphological as well as histopathological studies were done. All clinical, macromorphological and histopathological features of the developing tumours were equivelant to those of equine sarcoid.

It was concluded that bovine papilloma virus might be an etiologic agent of equine sarcoid in donkeys.

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INTRODUCTION

Equine sarcoids are spontaneous, locally invasive, fibroblastic connective tissue tumors of the skin reported in horses, mules and donkeys (JACKSON, 1936). Sarcoids appear to be more frequently reported in donkeys than in horse (JONES and HUNT, 1983). There appears to be no sex, age, color, geographical or seasonal predilection are usually located on the head. legs and ventral abdomen, but they can appear anywhere on the animals body (STAFUSS, et al. 1973; STANNARD and PULLEY, 1978 and BROWN, 1983). Sarcoids are frequently multiple, rarely pruritic or painful, and have been reported to occur on previous wounds or site of trauma (RAGLAND, et al. 1970; LANE, 1977; STANNARD and PULLEY, 1978 and MeMULLAN, 1982). Metastasis to internal organs or invasion of blood vessels and lymphatic has not been reported (STANNARD and PULLEY, 1978; McMULLAN, 1982 and BROWN, 1983). Recurrence following treatment has been extremely common (OLSON and COOK, 1951; SMITH, 1972 and WYMAN, et al. 1977).

Many authors have speculated that equine sarcoids have an infectious cause (RAGLAND, et al. 1966; VOSS, 1969; RAGLAND, et al. 1970; STAFUSS, et al. 1973; WYMAN, et al. 1977 and STANNARD and PULLEY, 1978). OLSON and COOK (1959); RAGLAND and SPENCER (1969) and VOSS (1969) reported that in horses intradermal and subcutaneous injection of bovine papilloma virus produced localized, fibroblastic growths similar to equine sarcoid which suggests the interresting possibility of a causal relationship to equine sarcoids.

The present study was undertaken in an attempt to produce equine sarcoids in donkeys by interadermal inoculation and surgical transplantation of bovine warts extract and wart tissue containing bovine papilloma (BP) Virus.

MATERIAL and METHODS

Bovine papilloma extract:

The bovine papilloma extract used in this study was wart tissue removed from an infected bull (Fig. 1) and homogenized in phosphate buffered saline solutin (pH, 7.5), 10% suspension of ground bovine warts tissue, centrifuged at (6,000 r.p.m. at OC) for 20 minutes to obtain a supernatant solution which contained BP virus. Penicillin (200 units/ml) and streptomycine (200 mg/ml) were added to the supernatant solution (VOSS, 1969).

Extract of normal bovine skin, for use as control material, was prepared by the same procedure used to obtain bovine papilloma extract. Each of five donkeys was inoculated in 8 sites with BP extract. In the vicinity of these inoculated sites another 8 sites were inoculated with normal bovine skin extracts. Four sites were

on the left or right side of the neck (two sites on the anterior one third and two sites on the posterior third), two sites on the medial side of the left or right leg and two sites on the base of the left or right ear. All of the inoculations were given intradermally with 0.5 ml of inoculum per site. In each of the same donkeys surgical transplantation was also attempted. Two small pieces of bovine wart tissue from the same infected bull (Fig. 1) were sutured between the skin in two sites, one was on the middle third of the neck and the other was on the medial side of the leg.

The donkeys were observed daily for signs of tumour growth and regression for 6 months following inoculation and transplantation. Surgical removal and histopathological study of some visible growths were made on 3, 5, 7, 9 weeks later. At the end of 6 months all visible growths and transplantation sites were removed for histopathological study.

RESULTS

One of the five donkeys had not developed any response to inoculated wart extract or surgical implantation of wart tissues in 6 months. Four donkeys developed growths in 32 of 40 inoculation sites, i.e. 80%. Growths developed at 6 of 8 transplantation sites and 26 of 32 inoculation sites. The donkeys showed growths on both sides of the neck (anterior, middle and posterior third fig. 2 & 3), medial side of the leg (Fig. 4) and at the base of the ear (Fig. 5). 6 of 32 growths were completely excised, but none of them recurred. Growths did not develope in any of the sites inoculated with extracts of normal bovine skin.

Clinical observation:

Several clinical features were noted during the course of experiments, some of the surgical transplant sites showed swelling and oedema within three or foru days followed by heavy suppuration which stopped about 7 to 10 days after the time of transplantation. The same sites however began to show growth as early as forteen days. The response was first detected by palpation of small, firm nodules in the skin. these nodules gradually enlarged and reached maximal size 60 to 90 days after their first appearance. Tumours at the sites of inoculation grew rapidly whereas the crosponding tumours at the site of surgical transplantion grew slowly.

In donkeys which showed growths, the time of tumour regression was much more variable than that of tumour induction. Donkeys number one and two showed growth at all inoculation and transplantation sites by 9 days. These growths seemed to be growing well and then were seem to stop and actually begin to shrink, so that by 115 days there were definite sings of regression. At about 150 days nearly all tumours disappeared. Donkeys number three developed only 7 tumours, which showed growth at about 16 days and regression at 130 days. By six months nearly all tumours

disappeared and only the healed scar were visible. Donkey number four developed only five growths, which began at 12 days and showed regression at 160 but had not disappeared completely at 6 months.

Pathological observations:

The response of inoculated animals was firstly detected by palpation of small firm nodules in the skin. These nodules gradually enlarge and were frequently visible several days after being detected by palpation. Size of the tuomurs varies among the different individuals and in the different sites of inoculation in the same individual. It was 5 to 10 cm (Fig. 6) in their greatest dimension with the average being aproximately 8 cm.

Grossly, the tumours were restricted to the dermis and developed singly. On cut section the tuomurs appeared as pale yellow hard in consistancy and homogenous firm tissue which was limited to the dermis. The tumours were quit uniform in appearance (Fig. 6).

Micromorphological studies:

The early lesions were composed of proliferating, small uniform fibroblastic cells in the deep dermis. The collagen bundles of the dermis and the subcutaneous straiated muscle present in some areas were heavly encercled by an abundant population of these cells (Fig. 7). At an early stage (one month post inoculation), the amount of fibroblastic tissue was small in proportion to the amount of traped dermal fibers, but this ratio was the opposite in older lesions (two months post experimental inoculation). In a relatively recent tumour (one month post inoculation), the fibroblastic proliferation was accompanied by a mild, diffuse infilteration of eosinophil cells (Fig. 8). The degree of infilteration with eosinophils was variable and was more pronounced in the tumours which resulted from transplantation of small pieces of bovine warts. Later on (two months post infection) lymphocytes appeared in the area. However, in a relatively more older lesion (70 days post infection) the lymphoid and eosinophil cells were only observed perivascularly (Fig. 9). In older tumours collagenation was complete and the tumours were highly fibrous. At all stages of development all parts of the tumours were constantly of uniform development (Fig. 10). Long fraunds of proliferating epithelial cells constantly pentrating deep into the tumour tissue (Reta pigs). These fraunds were sometimes simple or branched (Fig. 11). The fibroblasts and collagen of the tumours were always perpendicular to the reta pigs (Fig. 12). Few sebaceous glands sometimes developed in the deeper parts of the tumour (Fig. 13). The superficial surface of the tumour showed necrotic and/or ulcerative changes and a heavy inflammatory cellular infiltration.

DISCUSSION

Histopathological studies of all tumours removed from our experimental animals at different periods during the course of the experiments revealed all features of equine sarcoid (RAGLAND and SPENCER, 1969; RAGLAND, et al. 1970; JONES and HUNT, 1983; JUPP and KENNEDY, 1985 and MAKADY, et al. 1987). However, we must underline the fact that participation of the epidermis in the proliferative response was a major deference between the tumour developed in our experimental animals and that of equine sarcoid. This was evident by the appearance of some epithelial structure of the epidermis here and there in the tumours. This was also obtained by RAGLAND and SPENCER (1969) and RAGLAND, et al. (1970).

The appearance of eosinophil and lymphocytes was more prominent in the advanced stages of the tumour development, this may have been the cellular evidence of immunity or hypersensitivity.

Collagenous maturation with regression of the lesion was marked in the more advanced stages of tumour development, this occure diffusely through the lesions and progressed slowly. The appearance of collagenous maturation in the tumours was propably early evidence of regression.

Whereas the tumours produced in donkeys were indistinguishable from equine sarcoid, they either had undergone complete regression within 6 months or were histologically in the late stage of regression. Although regression with apparent recovery has occurred in equine sarcoid (BROWN, 1983), it does not occur often and recurrence following treatment has been extremely common (SMITH, 1972; CHEEVERS, 1982 and GINETZKY, et al. 1983).

In the present study the donkeys developed detectable growths at the inoculation and transplantation sites at 9 to 16 days post infection. This result was in agreement with those obtained by RAGLAND and SPENCER (1969) in horses and ponies. One donkey had not developed any response, this may be due to individual variation in susceptibility to bovine papilloma virus.

It was noted that surgical transplantation and inoculation of bovine papilloma extract produced growths in four of five donkeys, suggesting that perhaps the etiology of equine sarcoid migth be the virus of bovine papillomatosis (RAGLAND and SPENCER, 1969; VOSS, 1969 and GENETZKY, et al. 1983).

From this study we could conculde that the extract of BP as well as small pieces of it could produce equine sarcoid in 80% of inoculated donkeys. The short incubation period and the higher percentage of affected animals observed during this study indicated that the donkeys were highly susceptable for BPV. Further investigation is required to study whether or not the virus could be transmitted from infected

cows to contact. Donkeys under natural condition and the role of blood sucking insect in this transmission.

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LEGEND OF FIGURES

Fig. (1): Cutaneous papillomas below the left eye and on the forehead of bull and from which materials for inoculation and transplantation have been obtained.

Fig. (2): Donkey showing bovine papilloma virus-induced tumour on the side of the neck, 30 days after infection. One tumour produced at the transplant site on the middle third, two tumours produced at the inoculation sites on the anterior third and two tumours produced on the posterior third.

Fig. (3): Showing development of induced tumours on the neck, 70 days follwing inoculation and transplantation.

Fig. (4): Showing tumour developed on the medial side of the leg, 120 days following transplantation. This site exhibited swelling and suppuration before growth occurred.

Fig. (5): Showing tumour developed at the base of the ear, at the end of six-month period.

Fig. (6): Cross-section of 60-day-old tumour, excised from the side of the neck.

Fig. (7): Showing fibroplastic proliferation around the subcutaneous straited muscle, H & E (10 X 12,5 X).

Fig. (8): Showing mild diffuse eosinophil cells infiltration (one month post infection). H & E (10 X 40 X).

Fig. (9): Showing eosinophil along with mild lymphoid cells reaction (two months post infection). H & E (10 X 40 X).

Fig. (10): The tumour was developed uniformally at a different stages. H & E (10 X 40 X).

Fig. (11): Long and branched fraunds of proliferating epithelial cells pentrating deep into the tumours. H & E (10 X 40 X).

Fig. (12): Fibroblasts and collagen fibres were always runing perpendicular to the reta pigs. H & E (10 X 10 X).

Fig. (13): Development of some of the sabecous glands in the deeper parts of the tumour. H & E (10 X 10 X).

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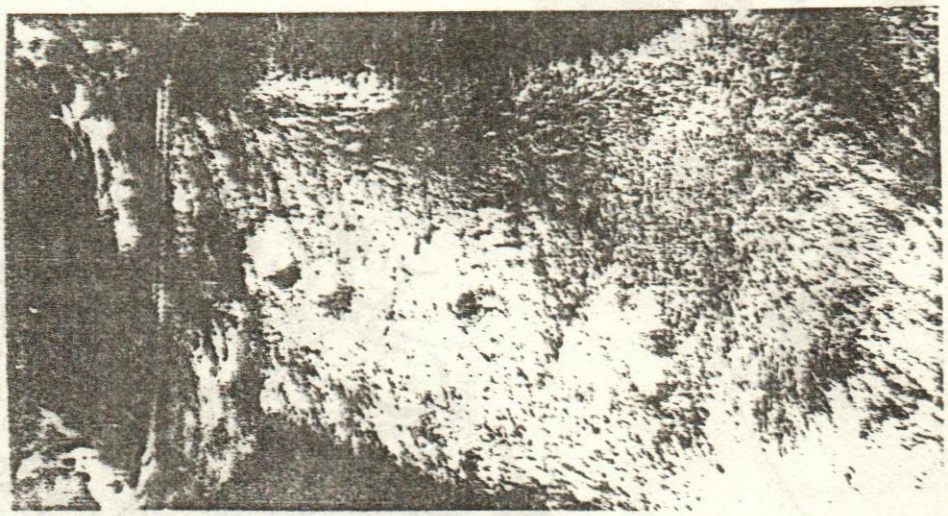

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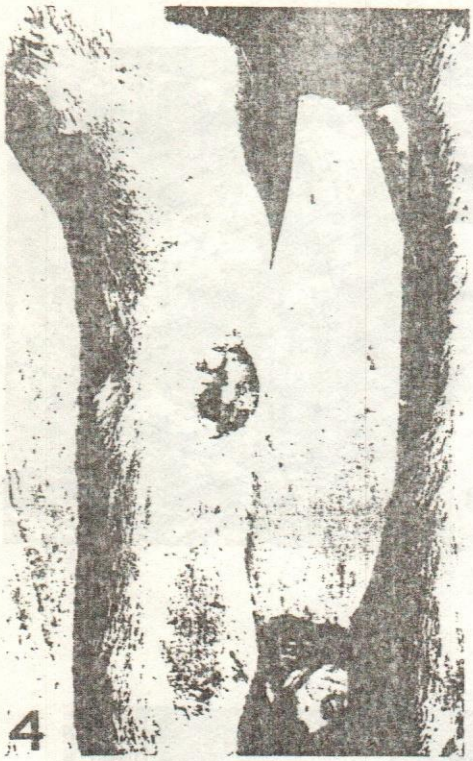

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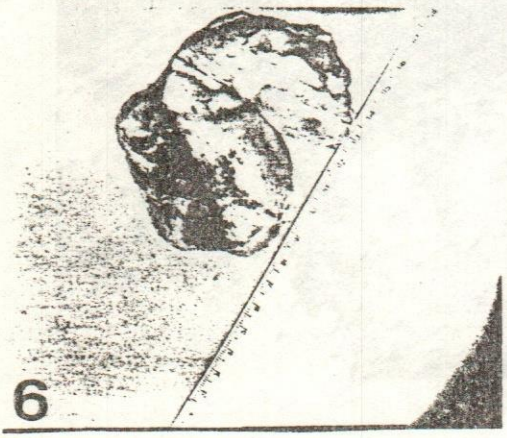

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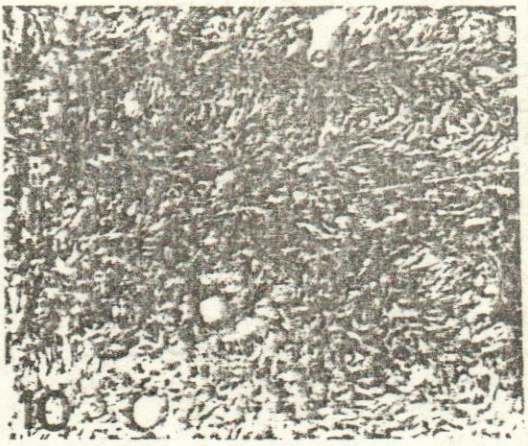

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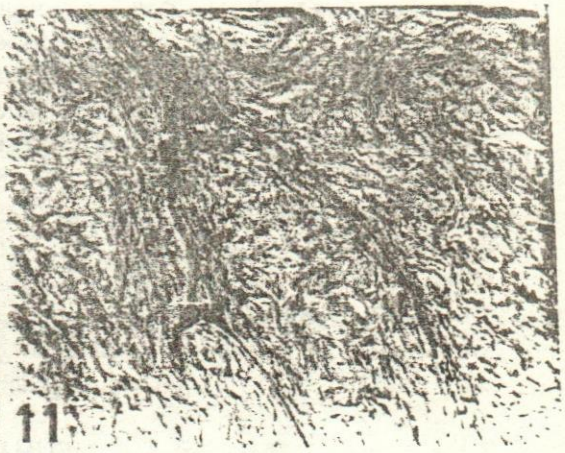

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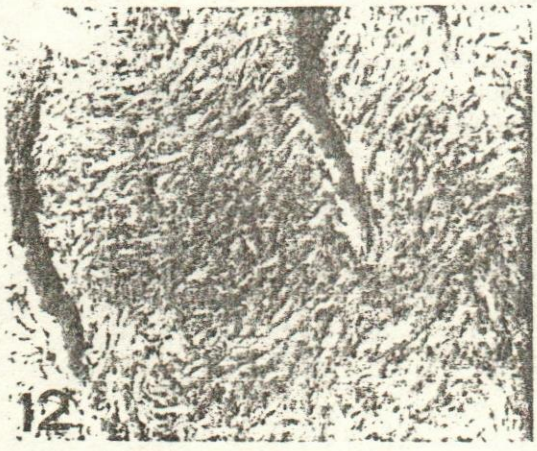

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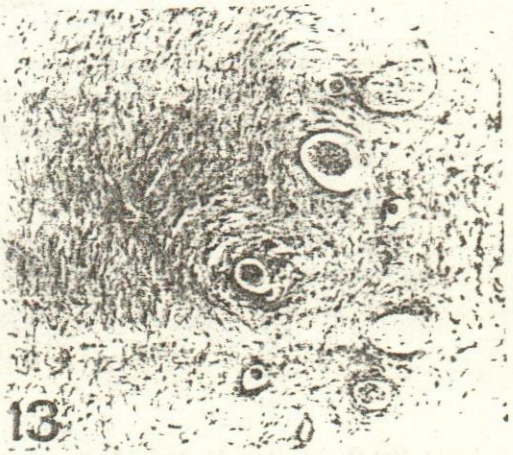

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