

التغيرات البنكرياسية في الحيوانات المصابة
تجريبيا بمرض الحمى القلاعية

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- تم حقن ٣ عدد عجول أعمارها تتراوح بين سنة وسنة ونصف وكذا ٢ اثنان من عدد النعاج بفيروس الحمى القلاعية من العترة ٥ وذلك باستخدام جرعة تركيزها (10^{7.5} MLD 50/ml) وقد حقنت العجول بكمية مقدارها واحد مل في اللسان بينما حقنت النعاج بجرعة مقدارها واحد مل في كل من اللسان وكذا بين الظلفين وأيضا عن طريق الأنف ثم ذبحت العجول بعد الحقن بمدة ٣ ، ٥ ، ٣٠ يوم بينما ذبحت النعاج بعد ٤ ، ٦ يوم من الحقن.
- وأخذ عينات دم من جميع الحيوانات قبل العدوى وعند الذبح
 - وباجراء الفحص الظاهري للحيوانات المذبوحة أمكن رؤية الصورة الباثولوجية المميزة للمرض في العجول بينما تمثلت هذه الصورة بشكل أخف من النعاج
 - وقد أظهر الفحص الميكروسكوبي للبنكرياس في العجلين المذبوحين بعد ٣ ، ٥ أيام من الحقن وجود مناطق من التحلل والتكزز في الانسجة المفترزة للعصارة البنكرياسية بينما لم يحدث تغير يذكر في جزر لينجرهانز
 - وبتحليل مصل الدم في العجلان المذكوران لوحظ زيادة في نشاط أنزيمي الأميليز والليباز وكذلك انخفاض في مستوى السكر بالدم
 - هذا ملم تظهر الدراسة أي تغير في شكل ووظيفة البنكرياس في العجل المذبوح بعد ٣٠ يوم من العدوى وكذا في النعاج

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PANCREATIC CHANGES IN ANIMALS EXPERIMENTALLY INFECTED WITH FOOT AND MOUTH DISEASE VIRUS

(With One Table & 4 Figs.)

By

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SUMMARY

Three calves, 1-1 1/2 year old, and two adult sheep were used in the present study. The animals were experimentally infected by inoculation of FMD virus (subtype O, strain 2/72 Egypt) using a dose of $10^{7.5}$ MLD₅₀/ml. The cattle were given only a single dose of one ml by intradermolingual route while sheep were inoculated through intradermolingual, intradigital and intranasal routes with one ml each. Blood was collected before infection and at the time of slaughter. The experimental calves were slaughtered at 3, 5 and 30 days after infection while sheep were slaughtered 4 and 6 days postinoculation. Gross examination of slaughtered animals revealed typical pathological lesions of the disease in calves while in sheep, only mild changes were found.

The microscopical picture of the pancreas of two calves slaughtered 3 and 5 days post-infection showed areas of degeneration and necrosis in the acinar tissue while the islets of langerhans were apparently normal.

Sera from these calves revealed an elevated activity of amylase and lipase and drop of glucose level. On the other hand, no changes were observed in amylase or lipase activity or glucose level in infected sheep and the calf slaughtered after 30 days.

INTRODUCTION

Foot and mouth disease virus (FMDV) is an epitheliotropic virus known to induce lesions in the gastrointestinal tract. In this disease, the pancreas was reported to be involved in the infection. In cattle suffering from diabetes after experimental infection with FMDV, BARBONI and MANOCCHIO (1962) and BARBONI, *et al.* (1966) reported that the pancreatic islets may disappear almost completely and in this organ there were also signs of acinar necrosis and regeneration in the form of regeneration of tubular structures. In acute FMD in guinea

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pig, IVANOV and AKULOV (1934) mentioned that the pancreatic blood vessels were congested and the acinar cells showed vacuolation or granular degeneration. Similar changes of vacuolation and granulation were also found by these authors in the ducts and in individual cells of the islets of Langerhans. Moreover, the authors reported that in a localized areas, there were deformed very pale cell groups appearing only as boundaries limited with a thin protoplasmic membrane and having a severely degenerating nuclei. These areas were commonly seen in the exocrine part and occasionally in the islets of Langerhans. Platt (1956 & 1958) also found pancreatic damage with involvement of the duct system or islets of Langerhans in mice and guinea pigs.

In the present work, the pancreas of cattle and sheep experimentally infected with FMDV was studied histopathologically. In addition, some parameters of the functional activity of the pancreas in the form of enzymatic activity of amylase and lipase, as well as the glucose level in the blood serum, were determined.

MATERIAL and METHODS

The experimental animals consisted of 3 Friesian calves, 1-11/2 years old, and 2 sheep of local breed about one year old. The animals were inoculated by a virulent FMDV (subtype O, strain 2/72 Egypt) at a dose level of $10^{7.5}$ MLD₅₀/ml of tissue culture medium. For cattle, one ml was inoculated intradermolingual, while for sheep, the virus was given intradermolingual in the interdigital tissue and intranasal using a dose of one ml in each route. The animals were put under observation and the body temperature was recorded twice daily. The calves were slaughtered after 4, 5 and 30 days after infection while sheep were slaughtered after 5 and 6 days.

Blood samples for clear serum were collected before infection and at the time of slaughter from all investigated animals.

The evacuated animals were subjected to gross pathological examination and tissue specimens from different organs, including the pancreas, were taken and fixed in 10% formalin solution. The tissue specimens were processed for embedding in paraffin. Sections 5-6 μ thick were stained with haematoxylin and eosin, as well as with Gomori's aldehyde fuchsin (CULLING, 1974).

The sera collected were individually analysed for estimation of α -amylase, lipase and glucose using test kits supplied by BioMerieux (France).

RESULTS

Gross pathological findings:

Primary lesions in the form of erosions in the middle third of the dorsum of the tongue at and around the site of inoculation occurred in calf No. 1 and 2. In calf No. 3, relatively small area covered with glistening slightly shrunken mucosa was seen in the tongue. Lesion in the tongue was observed in only one of the two sheep. The affected area showed no defect in the mucosa, and in cross section, it appeared as a circumscribed cavity filled with suppurative greenish yellow material. Erosions were also observed in the lower gum, lower lip, and dental pad in calf No. 1 and 2, and in one sheep. No similar lesions were found in calf No. 3 or the other sheep.

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Defect of the skin with bleeding occurred in calf No. 1 and 2 in the interdigital space. Other lesions consisted of congestion and swelling of the lymph nodes of the head, reddening of the mucosa of the abomasum and mild to severe enteritis in calf No. 1 and 2. In addition, the pancreas was reddened. In calf No. 3, the heart showed pale degenerative areas in the myocardium.

Microscopical findings of the pancreas:

Lesions were demonstrated in the pancreas of calf No. 1 and 2 only. The capillary network of the organ were dilated and engorged with blood. In the parenchyma, a well demarcated areas of degenerating and necrotic acinar cells and tissue fragments were observed (Fig. 1). The affected cells either lost their granulation and characteristic staining affinity, appearing pale and cytolytic, or were shrunken, smaller and darkly stained. The tubular and acinar arrangement was disrupted and the affected cells were disappeared and widely separated from each others and as a result, no differentiation could be made between acini, ducts, or islets of Langerhans. The cells of the border areas appeared shrunken and darkly basophilic. No infiltration of inflammatory cells was seen in/or around the necrotic areas. However, mononuclear cells in few numbers were distributed in the surrounding parenchyma and occasionally around interlobular blood vessels.

In less-severely affected areas, early changes could be recognized in the form of individual cells that undergo hydropic degeneration lying within the acini (Fig. 2). In widely distributed areas, the acinar cells were devoid of eosinophilic apical zymogen granulation and appear faintly basophilic. Homogenously acidophilic pyramidal cells were scattered in and between the acini. The intertubular duct epithelium showed no apparent changes.

The degenerative and necrotic changes in the exocrine acini of the pancreas were more severe in calf No. 2 than that in calf No. 1.

The islets of Langerhans were clearly demonstrated in all animals and apart from those that lie directly in the degenerating and necrotic areas of the parenchyma, they rarely showed any abnormalities (Fig. 3). Occasionally, cells suffering from hydropic degeneration or even necrosis may be observed (Fig. 4). The greater bulk of islets of both cattle and sheep consisted of polygonal cells with lightly basophilic cytoplasm and a nucleus having relatively large chromatin granules and clear nucleoli. These cells were supposed to be B-cells. The other types of cells seen in the islets were polygonal cells with marked cell boundaries and clear cytoplasm, and another triangular or pyramidal-shaped acidophilic closely packed cells having a small darkly stained oval nucleus. These were considered to be A_1 (D-cells) and A_2 types of cells.

No abnormal changes could be observed in calf No. 3 or in the two sheep infected with FMDV.

Results of serum analysis:

As shown in table 1, the enzymatic activity of the serum of calf No. 1 and 2 after infection revealed twice or three-time more activity than that of serum collected before infection.

The glucose level dropped after infection in these two animals. In calf No. 3 as well as in the two sheep, there were no obvious changes in serum amylase, lipase or glucose level; this, however, was still within the normal physiological range.

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As revealed by our results, changes consisting of scattered areas of degeneration and necrosis, mainly of the exocrine acinar structures were found in the pancreas of the two calves slaughtered at the viraemic stage with more or less typical lesions of FMD. In these animals, the infiltrating inflammatory cells were very few in number, the islets of Langerhans were more or less intact, and the duct system did not show any abnormal changes. These are the main differences from the results reported by BARBONI and MANOCCHIO (1962) and BARBONI, *et al.* (1966) in cattle. Our results, that the pancreatic necrosis affecting exclusively the acinar tissue, are in agreement with the findings of VANOV and AKULOV (1956) in guinea pigs, as well as Platt (1958) in mice experimentally infected with FMDV.

The histopathological picture of the pancreas of calf No. 1 and 2 was consistent with biochemical analysis of amylase, lipase and glucose in the serum of these animals. The rise in the activity of both amylase and lipase is considered, generally, indicative of pancreatic cell damage. The activity of both enzymes was found to increase above normal in the blood in cases of acute pancreatitis and pancreatic necrosis (BROBST and BRESTER, 1967; FINCO and STEVENS, 1969; BROBST, *et al.* 1970; ANDERSON and STRAFUSS, 1971; MIA, *et al.* 1978; HASSAN, 1980). The rise of amylase and lipase could also be related to stress (FINCO and STEVENS, 1969); ETTINGER, 1975). However, slaughter as a stress factor could be excluded as the main cause of elevated enzymatic activity in calf No. 1 and 2 since no similar rise had been occurred in calf No. 3 or in sheep in the present experiment.

The drop of glucose level in the serum of calf No. 1 and 2 could be attributed to fever and diarrhoea which accompanied FMD infection. The fevered animals have no appetite and do not ruminate leading to drop in glucose level (HUTYRA, *et al.* 1959).

The effect of malnutrition on the pancreas must be taken in consideration. MILLER and RIGDON (1952) found that protein deficiency can result in degeneration of the acinar cells with the occurrence of strongly acidophilic blending of the zymogen granules, vacuolar formation in the cytoplasm, pyknosis and karyorrhexis; the islets of Langerhans remain unchanged. Lowered food intake in our experimental animals, as a result of mouth lesions, might have a similar effect on the pancreas. It appears, however, that the effect of FMDV infection on the pancreas is transient since no changes could be detected in calf No. 3 slaughtered 30 days after infection.

The clinical picture of FMD in sheep is variable, and this animal is known to be slightly resistant to infection (ROHRER, 1967). No structural or functional disturbances could be found in the pancreas of sheep in the present study.

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Table (1)

Serum amylase, lipase activity, and glucose level in cattle and sheep before and after experimental infection with foot and mouth disease virus.

Exp. animals		Duration of infection (days)	AMYLASE u/100		LIPASE u/100		GLUCOSE mg/100	
No.	Species		B	A	B	A	B	A
1	Cattle	3	75	250	59	177	80	25
2	Cattle	5	70	124	177	236	40	20
3	Cattle	30	90	120	59	89	40	25
4	Sheep	4	100	100	236	177	60	40
5	Sheep	6	106	118	80	69	50	45

B = Before.

A = After.

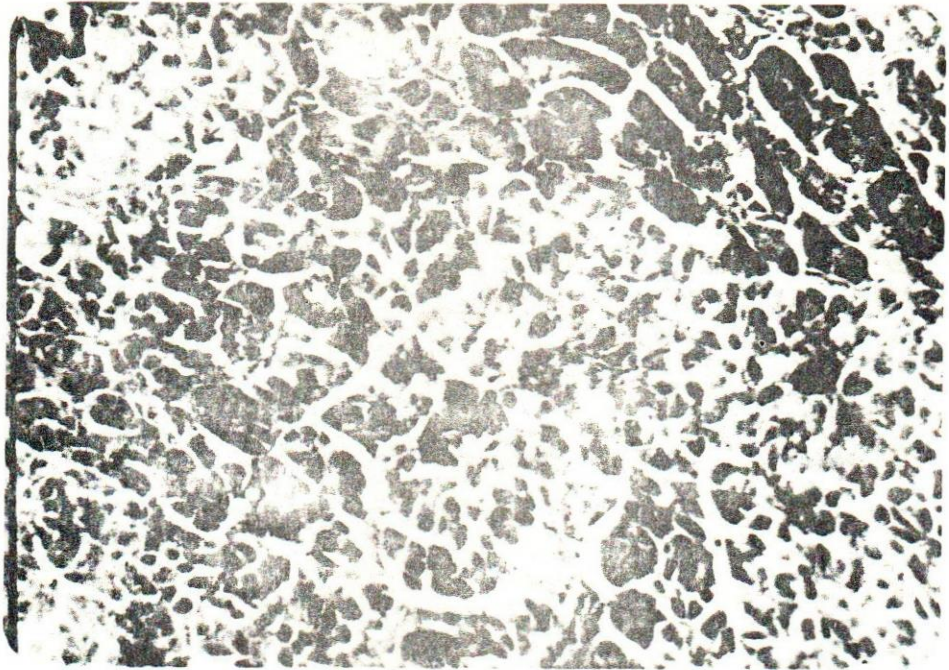


Fig. (1): Section of the pancreas showing areas of degeneration and necrosis in the exocrine acinar tissue (H&E, 100x)



Fig. (2): Pancreas showing degeneration of individual cells of the acinar tissue (H&E, 400x)

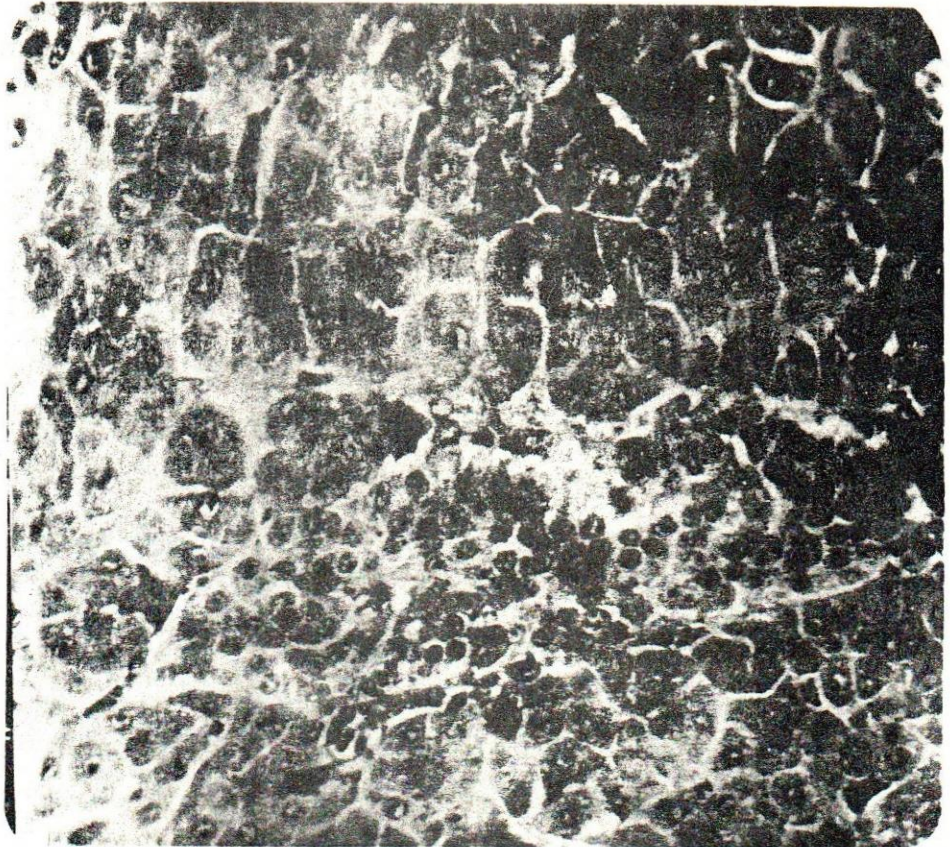


Fig. (3): Pancreas of infected calf; normal islets of Langerhans. (H&E, 200x)

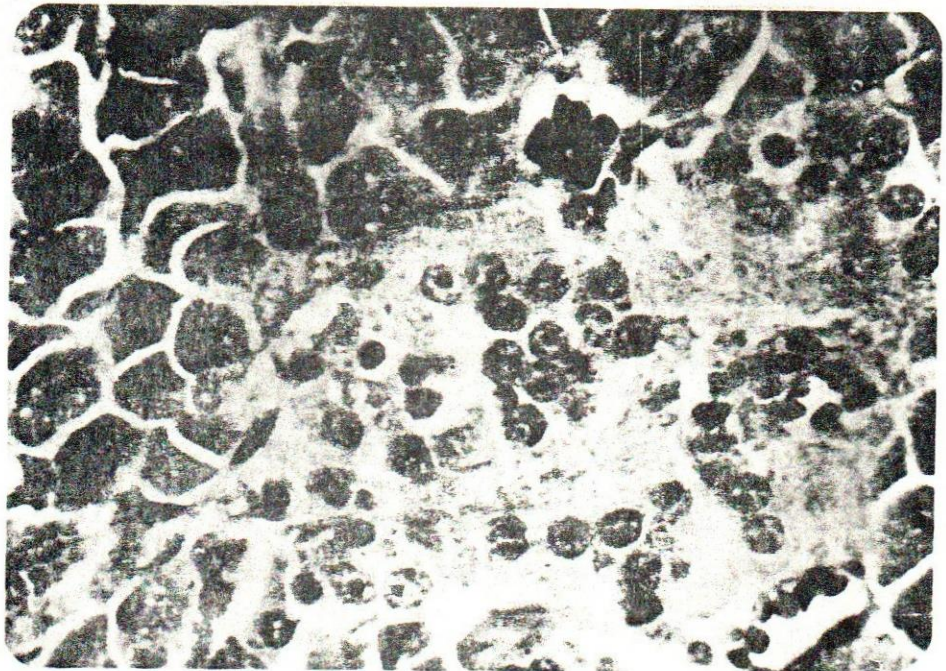


Fig. (4): Pancreas showing mild degeneration and nuclear pyknosis in a few number of cells of an islet. (H&E, 400x)