Dept. of Pathology,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.I. El-Sherry

INFECTIOUS STUNTING SYNDROME OF BROILER CHICKENS IN EGYPT. HISTOPATHOLOGICAL AND SCANNING ELECTRON MICROSCOPICAL INVESTIGATION

(With 13 Fig.)

By

S.S. EL-BALLAL and K. EL-ZANATY*

(Received at 30/4/1994)

ظاهرة أعاقة النمو في بداري التسمين في مصر فحوص باثولوجيه بالميكروسكوب الضوئي والالكتروني الماسح

صلاح البلال ، كمال الزناتي

تم دراسة ظاهرة أعاقة النمو فى بدارى التسمين فى كل من الكتاكيت المصابه حقلياً والكتاكيت التى أحدثت فيها العدوى تجريبياً . أخذت العينات من الأمعاء والمعده الغديه والبنكرياس وغدة السيمس وحوصله فبريشياس والطحال عند عمر ١٤ ، ٢١ ، ٢٨ ، ٣٥ يوماً من الكتاكيت المصابه حقلياً والكتاكيت التى أحدثت فيها العدوى الصناعيه . كما تم أخذ عينات من الأمعاء للفحص بأستعمال الميكروسكوب الألكترونى الماسح . لوحظ أنخفاض شديد فى وزن الكتاكيت المصابه عند عمر ٢١ ، ٢٨ ، ٣٥ يوماً عن مثيلاتها فى المجموعه الضابطه . كما لوحظ كذلك أنخفاض شديد فى أوزان الأمعاء والبنكرياس والسيمس وحوصلة فبريشياس والطحال اذا ما قورنت بمثيلاتها فى المجموعه الضابطه . كانت التغيرات الباثولوجيه فى الأمعاء على هيئة تنكرز فى الخلايا الطلائيه المبطنه للأمعاء ولكهوف ليبركيين وتخلل الخلايا الليمفاويه والأكوله فى الطبقه تحت الطلائيه . كما لوحظ تكوين الخراريج الكهفيه فى الأمعاء أعتباراً من اليوم الرابع عشر . أوضح الميكروسكوب الألكترونى الماسح ضمور وألتصاق الخملات وتساقط الخلايا الطلائيه المبطنه للأمعاء . كانت التغيرات الباثولوجيه فى البنكرياس على هيئة تنكرز فى الخلايا فى الكتاكيت الصغيره ثم تطورت الى ضمور وتليف البنكرياس فى الأعمار المتقدمه . كما لوحظ ألتهاب فى الأوعيه الدمويه للبنكرياس فقط فى الكتاكيت المصابه حقلياً . أما ألتهاب المعده الغديه فكان من الظواهر الثابته فى جميع الأعمار ولقد عانت الأعضاء الليمفاويه (غدة السيمس ، الطحال ، حوصلة فريشيس) من ضمور شديد خاصة فى الأعمار المتقدمه فى كل من الكتاكيت المصابه حقلياً والكتكيت المصابه تجريبياً . تم عزل وتصنيف عدد من عترات فيروس الريو وتم مناقشة دورها المحتمل فى أحداث ظاهرة أعاقة النمو فى بدارى التسمين . أمكن من هذه الدراسه أستنتاج أن ظاهرة أعاقة النمو تؤدى الى تغيرات باثولوجياً شديدة فى كل من الأمعاء والبنكرياس مما يعوق هضم وأمتصاص المواد الغذائيه وبالتالى أنخفاض معدل النمو فى البدارى.

---

*: Dept. of Avian Diseases, Fac. of Vet. Med., Assiut University.

SUMMARY

Field cases of infectious stunting syndrome, and experimental cases produced by inoculating one day old chicks with intestinal homogenates obtained from field cases, were studied. Samples for histopathological investigation were taken from the intestine, pancreas, proventriculus, thymus, bursa of Fabricius, spleen, liver and heart at 14, 21, 28 and 35 days of age from field and experimental cases. Intestinal segments were fixed in 5% cacodylate buffered glutaraldehyde for scanning electron microscopy. Marked reduction in the live weight was observed in the affected chickens at 21, 28 and 35 days. Similarly, the mean weight of the intestine, pancreas, thymus, bursa of Fabricius and spleen of the stunted chickens was severely reduced than the control at all age groups. Degeneration and necrosis of the enterocytes in the crypts of Lieberkuhn and infiltration of the lamina propria of the villi with lymphocytes and macrophages were the prominent intestinal lesions, crypt abscesses were observed from 14 days of age. SEM demonstrated atrophy and fusion of the intestinal villi, desquamation of the microvilli from the brush border of the villous enterocytes and finally loss of the enterocytes from the tips of the villi. Pancreatic lesions were degenerative in young birds and proceeds to atrophy and fibrosis in older ones. Proliferative vasculitis was observed at 14 days of age only in field cases. Focal pancreatitis was evident in field cases at 35 days. Proventriculitis was a constant finding in most cases. There was marked atrophy of the thymus, bursa of Fabricius and spleen. Several reovirus isolates were isolated from gastrointestinal homogenate and visceral organs of the affected chicks and their possible role in the production of the stunting syndrome was discussed. As a result of this study, it is apparent that ISS causes significant intestinal and pancreatic lesions which affects the process of digestion and absorption of food with subsequent reduction of the growth rate.

Keywords: ISS, broilers,histopathology, SEM.

INTRODUCTION

Infectious stunting syndrom (ISS) which was first described by *OLSEN (1977) and KOUWENHOVEN et al. (1978)* in broilers, is now recorded in private and governmental farms in Assiut and Sohage provinces by *EL-ZANATY (1993)*. A number of descriptive terms have been given to this disease; brittle bone disease *(HEIDE et al. 1981)*, malabsorption syndrome *PAGE et al., 1982)* and helicopter disease *(KOUWENHOVEN et al., 1978)*. Field and experimental studies of ISS have been described by *REECE and FRAZIER (1990)*. Pathologically, the disease was characterized by degeneration of the intestinal crypts *(REECE and FRAZIER, 1990)*, pancreatic degeneration and atrophy *(PASS et al., 1982)* and atrophy of the thymus and bursa of Fabricius *(BRACEWELL and WYETH, 1981)*. However, *BRACEWELL and RANDALL (1984)* emphasized that certain striking differences in the clinical signs and lesions were observed in different countries or even in different parts of the country. This paper aimed to describe the pathological changes associated with ISS in field and experimental cases in Assiut province.

MATERIALS and METHODS

1- Chicks :

60 one day old chicks (Hubbard) from private farm proved to be free from reovirus antibodies were used in this study. The chicks were reared in isolators, fed add libidum on commercial broiler chicken ration.

2- Preparation of the inoculum:

Tissue samples (2 cm sections) from the proventriculus, duodenum, jejunum and ileum with their contents were collected from five chickens naturally affected with the stunting syndrome. 10% suspension was made using physiological buffer saline (PBS) containing 10.000 IU pencillin, 10.000 ug streptomycin and 250 ug amphotericin/1 ml. The suspension was left for one hour at -4°C. homogenized, centrifuged and the supernatant was stored at -20°C. Loopfull from heart blood, liver and spleen were taken from the same cases for bacteriological examination.

3- Virus isolation:

Virus isolation was accomplished by inoculating 0.1 ml of the sample supernatant into four 10 day old embryonated chicken eggs (obtained from poultry farm, Faculty of Agriculture, Assiut University) via chorio-allantoic membrane (CAM). Virus identification was performed by testing the affected CAM

material in an agar gel precipitation (AGP) test against reovirus (S-1133 strain) antiserum, hemagglutinating (HA) activity and chloroform sensitivity as previously described by *EL-ZANATY (1993).*

4- Experimental infection:

60 one day old chicks were divided into two groups (30 chicks/group). The chicks in the experimental group were orally inoculated with 0.5 ml/chick antibiotic treated gastrointestinal homogenate supernatant. The chicks in the control group were orally inoculated with 0.5 ml/chick sterile PBS. All chicks were observed for clinical signs, mortality and post mortem lesions. At 14, 21, 28 and 35 days post, infection, three chicks from each group were weighted and then sacrificed for post mortem and histopathological examination. The whole intestine, pancreas, spleen, bursa and thymus (one lobe) were weighted.

5- Histopathology:

Samples for histopathological investigation were taken from the proventriculus, small intestine (doudenum, jejunum and ileum), pancreas, liver, heart, spleen, bursa and thymus from experimentally infected as well as naturally diseased chicks at 14, 21, 28 and 35 days of age, Tissue samples were fixed in 10% buffered formalin. The intestinal samples were fixed by intraluminal injection of 10% buffered formalin into the ligated segments of small intestine until a mild distension was obtained and then immersed in the same solution. Fixed specimens were processed routinely, embedded in parablast, sectioned at 6 um and stained with hematoxylin and eosin.

6- Scanning electron microscopy:

Intestinal segments were fixed by intraluminal injection of 5% cacodylate, buffered glutaraldehyde into ligated segments of the small intestine. Segments were then immersed in the same fixative solution. After two hours, the ends of the ligated segments were cut to remove the intestinal contents and were transfered to freshly prepared fixative solution. The samples were then cut lengthwise, and sectios 5x5 mm were obtained. Intestinal samples were washed several times in cacodylate buffer and processed for SEM according to the modified OTOTO method of *MALICK and WILSON (1975).* Samples were examined and photographed with SEM (JSM T200) at 25 KV.

RESULTS

1-Virus isolation:

Several reoviruses were isolated from the gastrointestinal homogenate and visceral organs of field cases. All reovirus isolates produced characteristic pock lesions on CAM of embryonated chicken eggs. The isolates were insensitive to chloroform treatment, did not hemagglutinate chicken erythrocytes and reacted positively in agar gel precipitation test against reference reovirus antiserum.

2-Gross lesions:

Stunted chickens had their intestines dilated with poorly digested food. Their live weight was markedly lower than the control at 21, 28 and 35 days of age figure (1). The weights of the intestine, pancreas, thymus, bursa of Fabricius, spleen were markedly lower than that of the control at all age groups fig. 2-6. Severe atrophy of the thymus, bursa of Fabricius and spleen was observed (fig. 7a).

3-Histopathology:

Intestine:

Intestinal lesions were observed from 14 days of age. There was degeneration of the enterocytes in the crypts of Lieberkuhn (Fig 7b). The degenerated crypts were replaced by lymphoid cells (Fig.7c). In older birds (28 days) there was frequent pyknosis and apoptosis of enterocytes in the crypts. The degenerated crypts were replaced by lymphocytes and heterophils. Cystic crypts were observed from 14 days of age and increased in frequency in older chickens. They were lined by squamous or cuboidal enterocytes and contained degenerating cells, mucin and heterophils (So. called crypt abscesses) fig. (7d). The crypts of Lieberkuhn were hyperplastic and contained many mitotic figures. Starting from 21 days of age there was atrophy and fusion of the intestinal villi, infiltration of the lamina propria by lymphocytes and macrophages. Heterophils were observed frequently at 35 days of age infiltrating the lamina propria of the villi. At 28 days of age, there was loss of the enterocytes from the tips of the villi. The number of intraepithelial lymphocytes was increased at this age.

Scanning electron microscopy of the intestinal mucosa of the control chickens revealed that the normal villi of chicken intestine were finger like in shape (fig. 8a, 8b). In stunted chickens there was severe atrophy and fusion of the intestinal villi and exposure of the crypts (fig. 9a). Fusion of the villi occurred through the formation of the interepithelial bridge

between two adjacent villi (fig. 9b). The surface of the enterocytes was covered by microvilli (fig. 10a). In stunted chicken there was desquamation of the microvilli from the enterocytes and loss of the enterocytes at the tips of the villi (Fig. 10b).

The intestinal lesions in naturally affected birds were nearly similar to those described in experimentally infected ckickens.

Pancreas:

The pancreatic lesions were largely confined to the exocrine part of pancreas and consisted of degeneration, atrophy and fibroplasia. The early changes noticed were partial or complete loss of zymogen granules from the apical cytoplasm and lack of basophilic material in the perinuclear cytoplasm of the acinocytes (fig. 11a). The acini contained vacuolated acinocytes which contained paler and larger nuclei than normal cells (fig. 11a). There were foci of lymphocytic aggregations in the stroma and surrounding the pancreatic ductus. In older birds (28 days of age) the affected acini appear to remain in a atrophied state with subsequent stromal fibropbsia. There was marked stromal fibroplasia (Fig. 11b). The pancreatic ducts were dilated and filled with acidophilic material and lined with squamous epithelial cells.

Proliferative vasculitis and thrombosis were observed at 14 days of age only in naturally affected birds (fig. 11c). At 35 days of age there was infiltration of the stroma and the tissue surrounnding the ducts with heterophils (fig. 11d, 11e).

Proventriculus:

Proventriculitis was observed in most cases both in experimental and naturally affected chickens. There was marked lymphoid and heterophilic infiltration in the lamina propria (fig. 12a) and between the proventricular glands.

Liver and Heart:

No lesions were observed in the liver or heart of experimentally or naturally affected chickens.

Lymphoid organs:

The bursa of Fabricius had narrow plica and the bursal follicles were small in size. In young birds (14 days of age) the cortex of the bursal follicles were narrow and had single cell necrosis while the medulla showed slight depletion of the medullary lymphocytes (fig. 12b). In some follicles the cortex

was completely absent and replaced by heterophilic infiltration (fig. 12c). At 21 days of age there was increase in the amount of the interfollicular connective tissue (fig. 12d). In older birds these changes proceeds to severe lymphoid depletion of both the cortical and medullary lymphocytes and obvious increase in the amount of the interfollicular connective tissue (fig. 13a).

The thymic lobes in young chickens showed lack of corticomedullary differentiation. They were composed of reticular cells. epithelial elements, vascular channels and diffuse infiltration of lymphocytes (fig. 13b). In older birds, the thymic lobes had normal architecture but the cortices were thinner. There was focal areas of lymphocyte loss in the cortex (fig. 13c).

The spleen showed no distinction between the white and red pulp. There was very few secondary white pulp nodules (1-2/ spleen). In older chickens (35 days of age) there was lymphoid depletion of the periarteriolar lymphoid sheath and proliferation of the endothelial cells of the sheathed arterioles (fig. 13d). The lymphoid organs in naturally affected chickens showed the same changes described in experimentally diseased birds.

DISCUSSION

In this study, ISS has been reproduced by oral inoculation of day old chicks with the intestinal homogenates obtained from field cases. In the experimental and field cases, the lesions were observed in the intestine, pancreas, proventriculus, bursa of Fabricius, thymus and spleen. The intestinal lesions were observed from 14 days of age and include degenerative and necrotic changes in the enterocytes in the crypts of Lieberkuhn and on the tips of the villi. There was inflammatory cellular infiltration in the lamina propria and crypt abscesses. Similar intestinal lesions in young chickens with stunting syndrome were reported *(FARMER, 1986; REECE and FRAZIER, 1990). KOUWENHOVEN* et al. *(1978)* considered that the intestines were the primary site of infection in ISS and this was substantiated by the finding of increased alkaline phosphatase of intestinal origin in acutely infected chickens by *VERTOMMEN* et al. *(1980).* Membranc bound cytoplasmic inclusions containing picornavirus like particles were demonstrated by *FRAZIER and REECE (1990)* in the macrophages and mesenchymal cells in the lamina propria and occasionally in the villous enterocytes. They considered that the entery of virus particles into the lamina propria could occur by initial infection of enterocytes, phagocytosis by macrophages and passage of these cells through the basement

membrane into the lamine propria. The infiltration of heterophils and lymphoid cells into the lamina propria and subsequent formation of cystic crypts is a common reaction to infection and injury *(BARKER and VAN DREUMEL, 1985)*. Damage to the lamina propria could interfere with the differentiation of the enterocytes and subsequently reduced the absorptive capacity of the gut *(FRAZIER and REECE, 1990)*. The hyperplasia and increased number of mitotic figures in the crypts of Lieberkuhn observed in this study represents a regenerative phenomena subsequent to the loss of enterocytes from the tips of the villi. Atrophy of the villi, was demonstrated by SEM, may be due to imbalance between cell proliferation in the crypts and cell loss in the extrusion zone, villus fusion and progressive distension of the villi by inflammatory cells.

Pancreatic degeneration, atrophy and fibrosis was evident in this study. Similar changes were described *(BRACEWELL and RANDALL, 1984; PASS et al., 1982)*. *PASS et al., 1982,* suggested that pancreatic degeneration is a secondary phenomena following from earlier damage to and obstruction of the pancreatic ducts. In our study, proliferative vasculitis and thrombosis was observed in field cases and we suggest that pancreatic degeneration may be of vascular origin following occlusion of the main artery by inflammation and thrombosis.

Atrophy of the lymphoid organs was evident in this study in field and experimental cases. Similar findings were reported in ISS affected chickens *(PASS et al., 1982 and BRACEWELL and RANDALL, 1984)*.

Many viruses have been implicated in the aetiology of stunting syndrome, including reoviruses, caliciviruses, rotaviruses, parvoviruses, and enteroviruses *(FRAZIER and REECE, 1990)*. In the present study several avian reoviruses were isolated from the affected chickens. Reoviruses were frequently isolated from the intestines of chickens with ISS, and they may play a role in the etiology of this condition *(MORADIAN et al., 1990)*. Howevere, *FRAZIER and REECE (1990)*, suggested that ISS has a multifactorial aetiology, and that an enterovirus is the initial stimulus for the further expression of the disease.

REFERENCES

Barker, I.K. and Van Dreumel, A.A. (1985): The alimentary system. In: Pathology of Domestic Animals, Volume 2, 3rd Edition, pp. 51-90. Edited by Jubb, K.V.F., Kennedy, P.C. and Palmer, N. London: Academic Press.

Bracewell, C.D. and Randall, C.J. (1984): The infectious stunting syndrome. World's Poultry Science Journal, 40: 31-37.

Bracewell, C.D. and Randall, C.J. (1981): Infectious stunting of chickens. Veterinary Record, 109: 64.

El-Zanaty, K. (1993): Stunting syndrome in broiler chickens: Clinical, field and experimental investigation. Assiut Veterinary Medical, Journal 29: 254-269.

Farmer, H. (1986): Research progress on infectious stunting syndrome, Poultry International, 25: 12-13.

Frazier, J.A. and Reece, R.C. (190): Infectious stunting syndrome of chickens in Geat Britain: Intestinal ultrastructural pathology. Avian Pathology, 19: 759-777.

Heide, L. Van Der, Lutticken, D. and Horzinek, M. (1981): Isolation of a reovirus as a possible etiological agent of osteoporosis ("brittle bone disease", "femoral head necrosis") in broiler chickens. Avian Diseases, 25: 847-856.

Kouwenhoven, B.; Vertommen, M.H. and Eck, J.H.H. Van (1978): Runting and leg weakness in broilers: Involvement of infectious factors. Veterinary Science Communications, 2: 253-259.

Malick, L.E. and Wilson, R.B. (1975): Modified thiocarbohydrazide procedure for scanning electron microscopy: Routine use for normal, pathological, or experimental tissue. Stain technology, 50: 265-269.

Moradian, A.; Thorsen, J.S. and Julian, R.J. (1990): Single and combined infections of specific pathogen free chickens with infectious bursal disease virus and an intestinal isolate of reovirus. Avian Diseases, 34: 63-72.

Olsen, D.E. (1977): Isolation of a reovirus-like agent from broiler chicks with diarrhoea and stunting. Proceedings of the 26th Western Poulry Diseases Conference, Davis, California, pp. 131-139.

Page, R.K.; Fletcher, O.J.; Rowland, G.N.; Gaudry, D. and Villegas, P. (1982): Malabsorption syndrome in broiler chicks. Avian Diseases. 26: 618-624.

Pass, D.A.; Robertson, M.D. and Wilcox, G.E. (1982): Runting syndrome in broiler chicken in Australia Veterinary Record, 110: 386-387.

Reece, R.L. and Frazier, J.A. (1990): Infectious stunting syndrome of chickens in Great Britain: field and experimental studies. Avian Pathology, 19: 723-758.

Vertommen, M.; Eck, J.H.H. Van; Kouwenhoven, B. and Nel Van Kol (1980): Infectious stunting and leg weakness in broilers: 1- Pathology and biochemical changes in blood plasma. Avian Pathology, 9: 133-142.

LEGENDS

Fig. 7a: Severe atrophy of the thymus, bursa of Fabricius and spleen in ISS affected chicken. b: LM showing degeneration and necrosis of the enterocytes in the crypts of Lieberkuhn H. & E., 560X. c: LM showing replacement of the crypts by lymphoid cells, intraepithelial lymphocytes in the crypts (TL) H. & E. 560X. d: LM showing cystic crypts formation (C), they contain degenerating cells, heterophils and mucin H. & E. 140X.

Fig. 8a: SEM of the intestinal mucosa of a control chicken, note the crypt (C) villous (V) ratio, 250X. b: SEM demonstrating the normal finger shape intestinal villi of the chicken, extrusion zone (E), 1250X.

Fig. 9a: SEM of the intestinal mucosa in ISS affected chicken showing sever atrophy and fusion of the intestinal villi, exposure of the crypts (X), 250X. b: Higher magnification SEM demonstrating the fusion of two intestinal villi through the formation of inter epithelial bridge ( ), 1250X. The inset is a LM showing villous fusion H. & E. 140X.

Fig. 10a: Higher magnification SEM of the intestinal mucosa in a normal chicken showing the microvilli (brush border) of the enterocytes (M) and the opening of goblet cells (G), 8750X. b: SEM of the intestinal muscosa in a stunted chicken showing desquamation of the microvilli (M), loss of enterocytes from the tips of the villi (L) 8750. The inset is a LM demonstrating necrosis of enterocytes in the tips of the villi H. & E. 140X.

Fig. 11a: LM of the pancreas in ISS affected chicken showing loss of zymogen granules and perinuclear basophilia of the acinocytes H. & E. 560X. The inset showing the normal struture of the acinocytes H. & E. 560X. b: LM showing atrophy of the acini and stromal fibroplasia, H. & E. 560X. c: LM showing proliferative vasculitis. H. & E. 560X. d: LM showing heterophilic infiltration of the pancreatic stroma, H. & E. 560X. e: LM showing periductal fibroplasia and heterophilic infiltration, H. & E. 560X.

Fig. 12a: LM showing proventriculitis in ISS affected chicken, note massive lymphoid and heterophilic infiltration, H. & E. 140X. b: Bursa of Fabricius in ISS affected chicken showing narrow cortex and lymphoid depletion of the medulla (D), H. E. 140X. c: LM showing heterophilic infiltration of the cortex of the bursal follicles, H. & E. 560X. d: LM showing prominent Fibroplasia in the bursa of Fabricius, H. E. 560X.

Fig. 13a: LM showing severe lympyhoid depletion in the bursal follicles and fibroplasia, H. & E. 560X. b: LM showing lack of cortico-medullary differentiation in the thymus gland of ISS affected chicken H. & E. 140X. c: LM of thymus gland showing focal loss of cortical lymphocyes H. & E. 140X. d: LM of the spleen showing lymphoid depletion of the periarteriolar lymphoid sheath and proliferation of the endothelial cells of sheathed arteriole (A). H. & E. 140X.

Fig.(1) showing:
Changes in the body weight

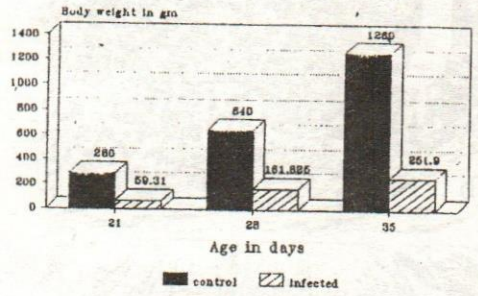


Fig.(2) showing:
Changes in the intestine weight

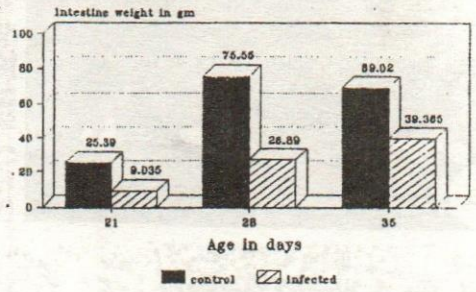


Fig.(3) showing:
Changes in the Pancrease weight

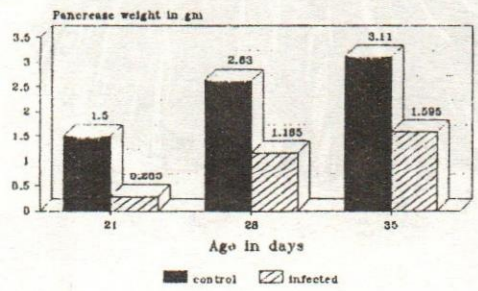


Fig.(4) shwoing:
Changes in the thymus weight

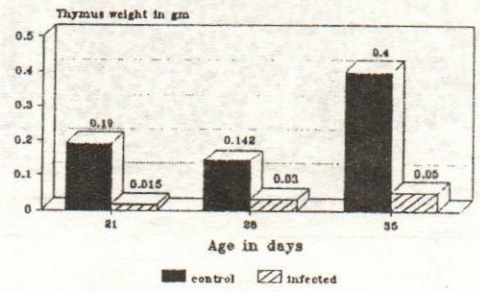


Fig.(5) showing:
Changes in the bursa weight

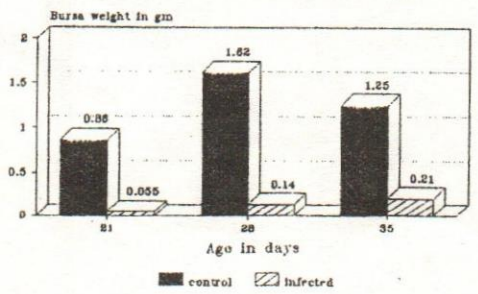
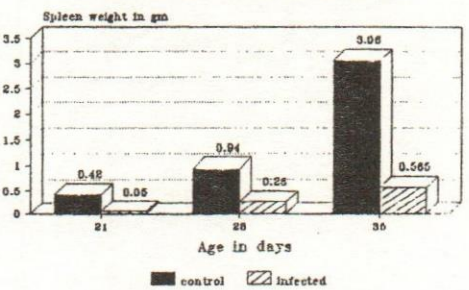
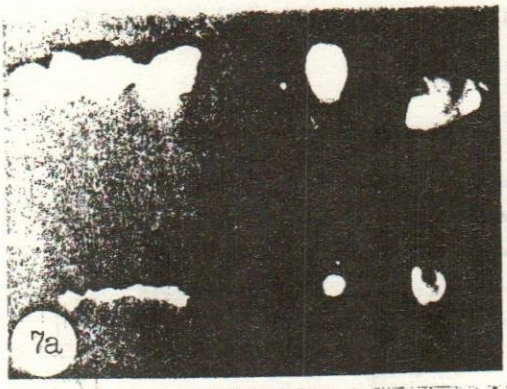
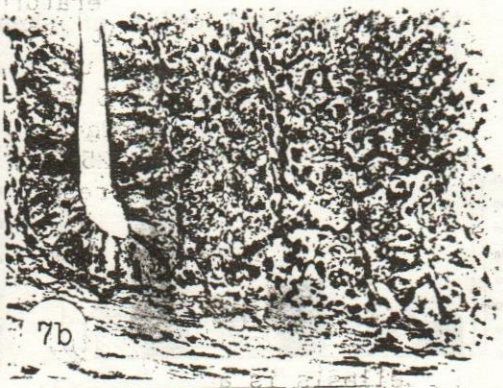
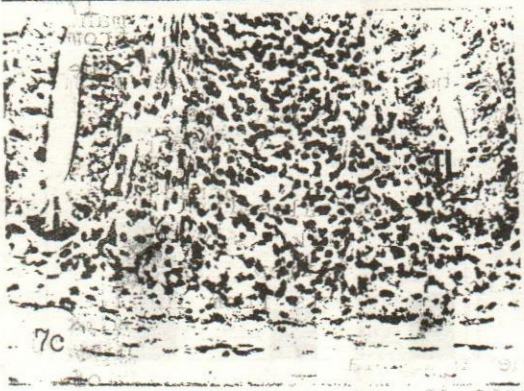


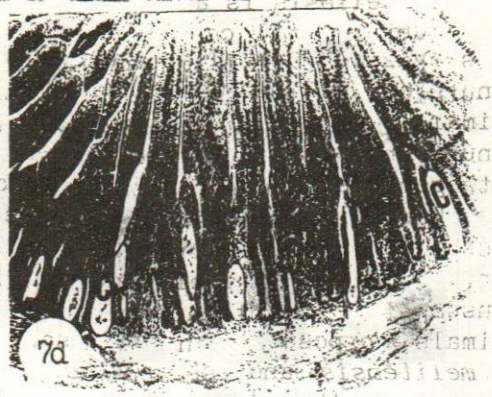
Fig.(6) showing:
Changes in the spleen weight



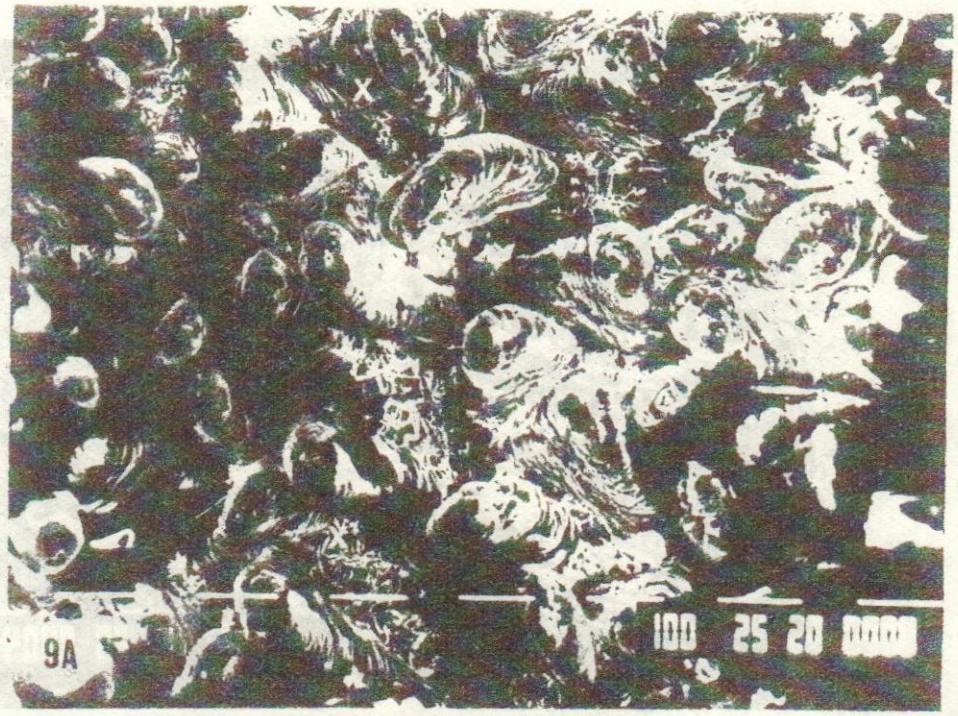

7a

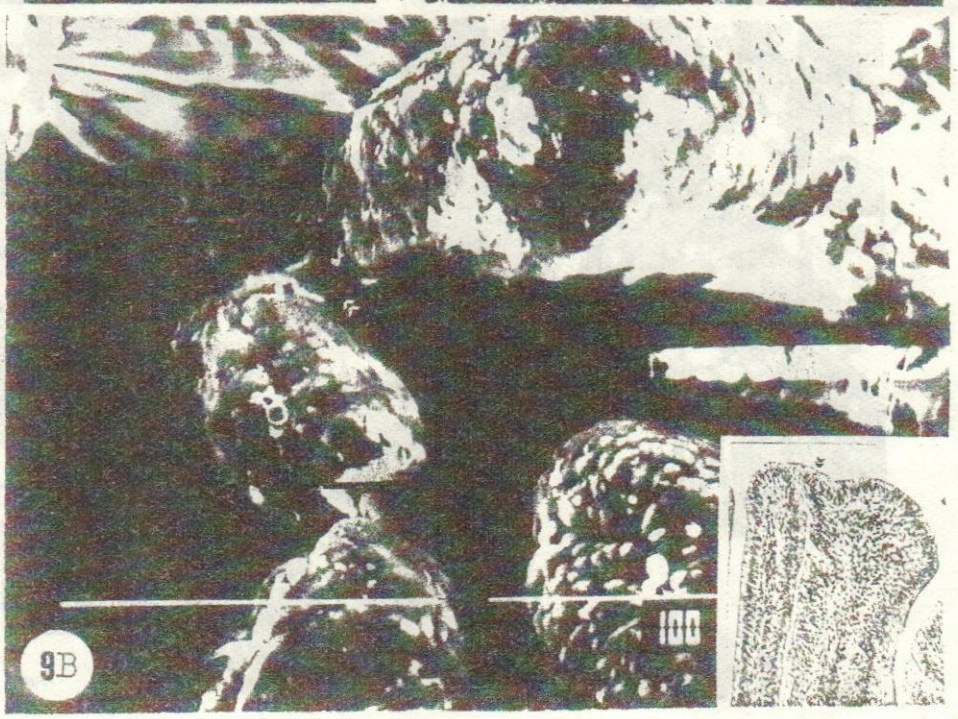

7b

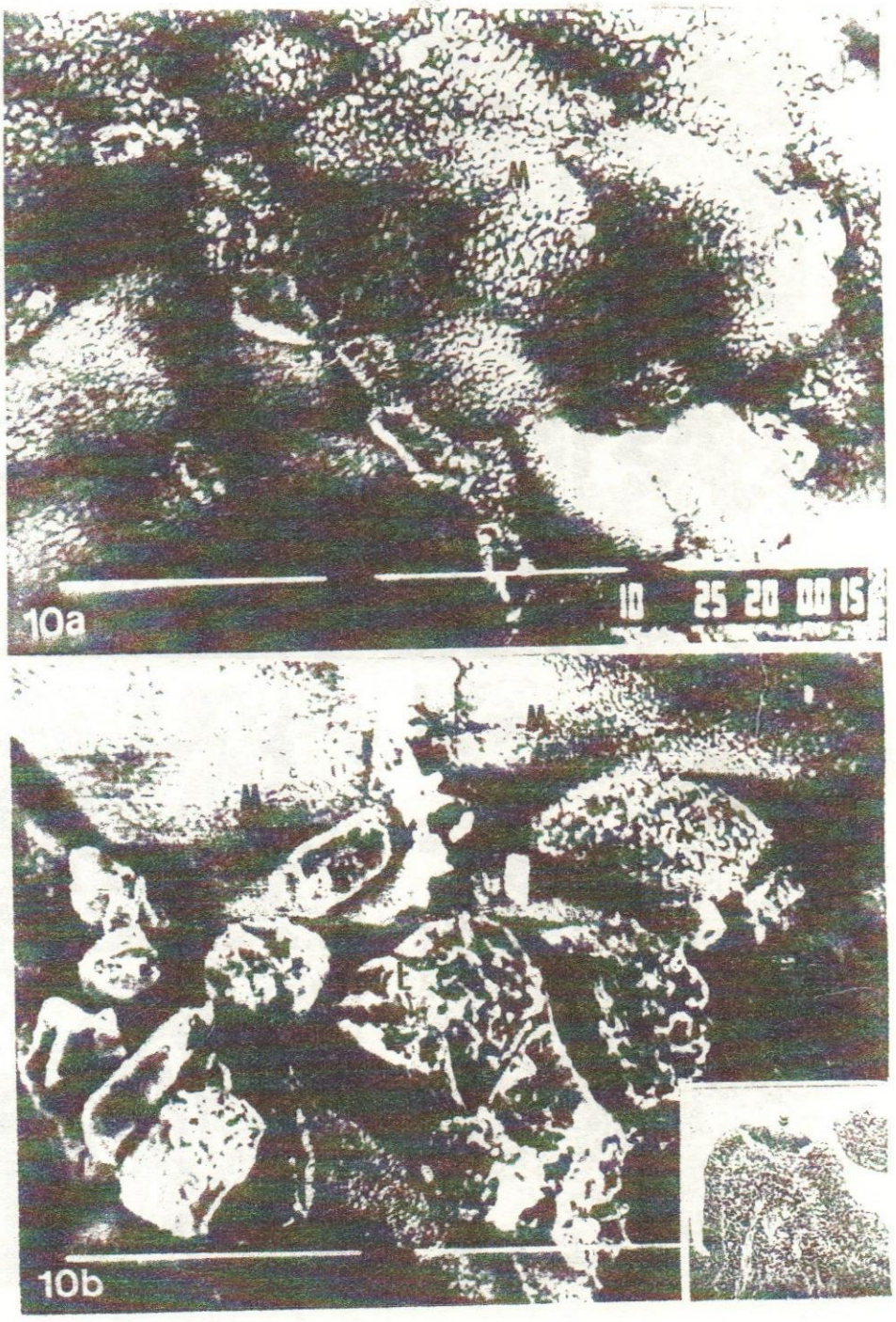

7c

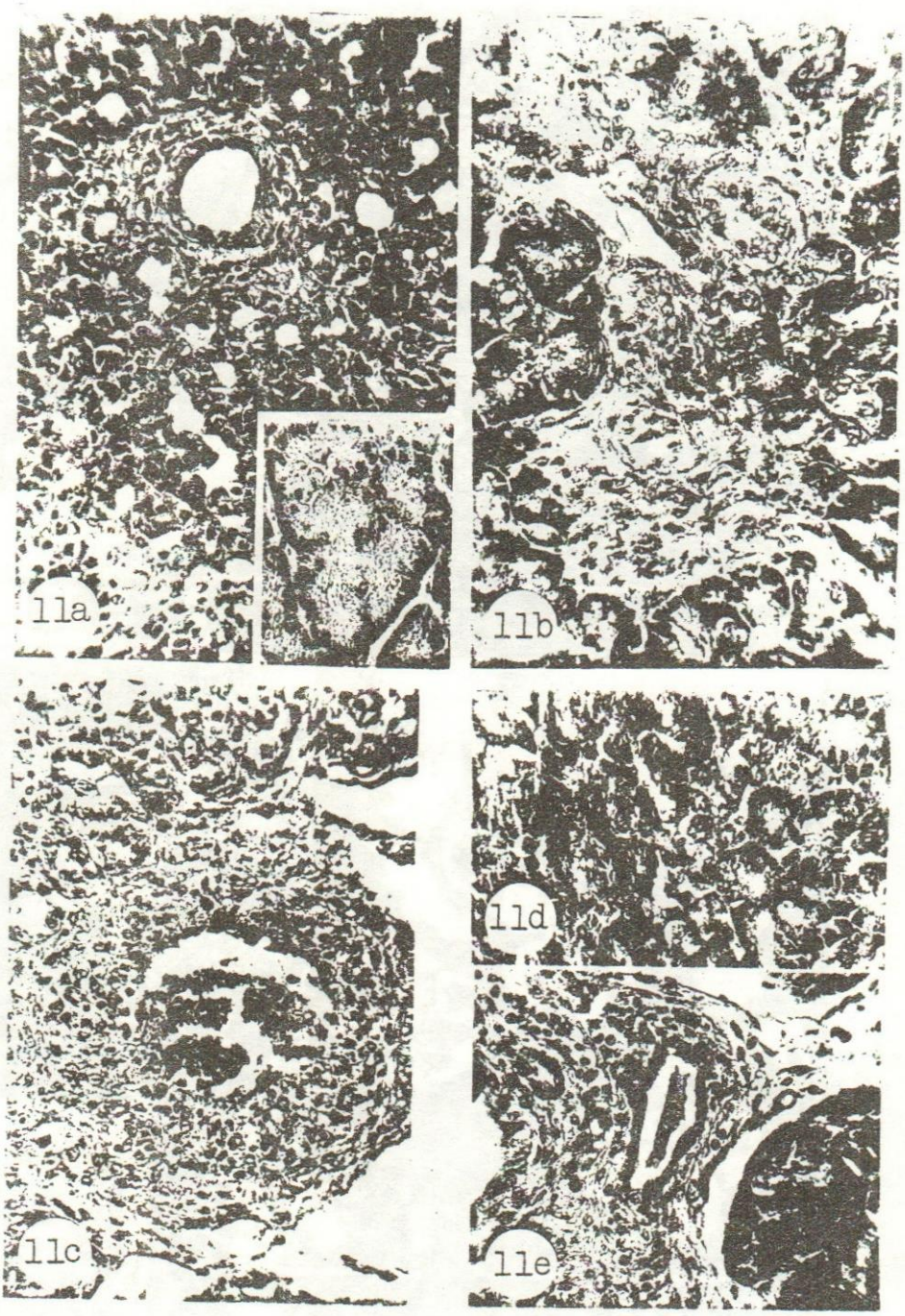

7d

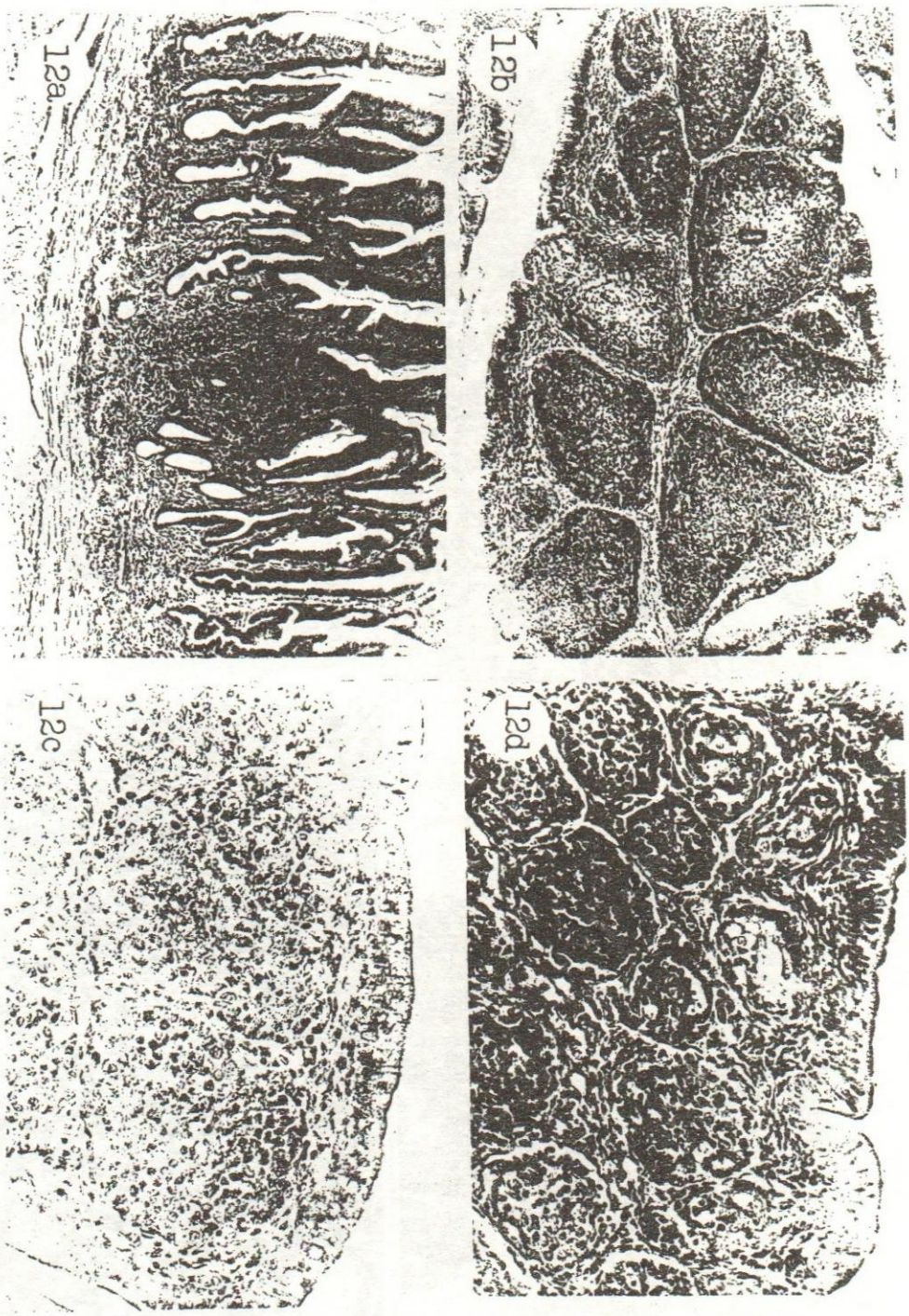

C
100 25 20 0004
8A
E
C
100 25 20 0011
8B

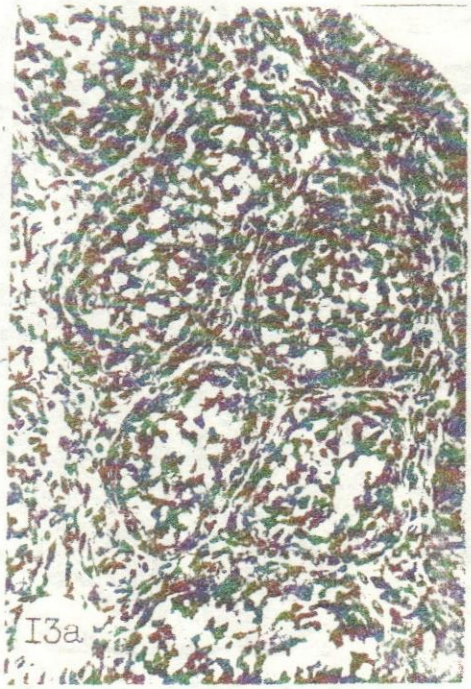

X
9A
100 25 20 0000


9B
100

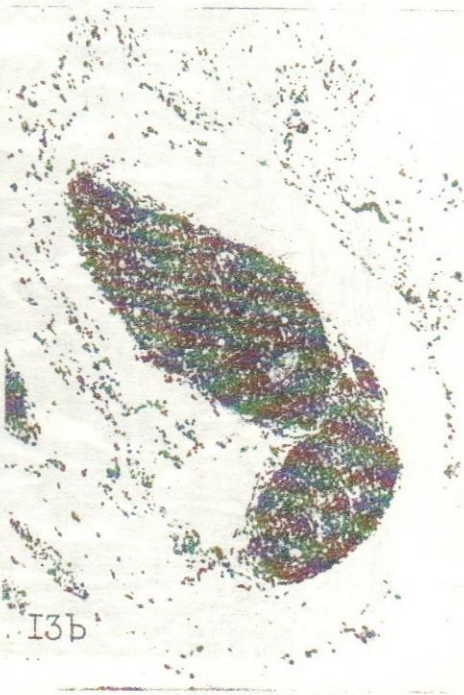

10a
10b


11a
11b
11c
11d
11e


12a
12b
12c
12d

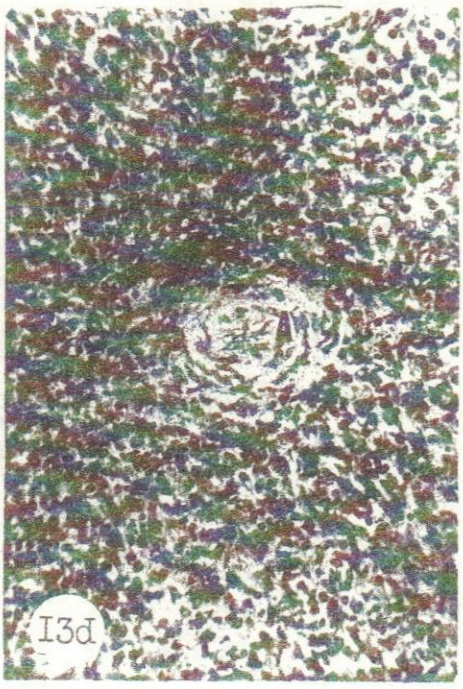


I3a



I3b

I3c



I3d