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العدوى الصناعية للكثاكت الفيومي بفيروس مرض الجصورو

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FUNGI OF BROILER

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INFECTIOUS BURSAL DISEASE IN FAYOUMI CHICKENS

(With 1 Table & 7 Figures)

By

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SUMMARY

Precipitating and neutralizing antibodies to infectious bursal disease (IBD) virus were detected in newly hatched Fayoumi chicks, which protected them up to 3 weeks from infection. Clinical signs, pathological lesions, pathogenesis, and serological response after experimental infection with a highly pathogenic strain of IBD virus were described and discussed.

INTRODUCTION

Infectious bursal disease (IBD), an acute viral infection of young chickens, is characterized by damage of lymphoid tissue in the bursa of Fabricius, spleen, thymus and caecal tonsil. The disease is not only immunosuppressive but can also produce clinical signs and mortalities in affected birds (ALLAN *et al.*, 1972; ALMASSY and KAKUK, 1976; ANDERSON, 1977; CHEVILLE, 1967; FADLY *et al.*, 1976; HELMBOLDT and GARNER, 1964).

IBD virus infection results in production of precipitating and neutralizing antibodies at 3 to 15 days postinoculation (SKEELES *et al.*, 1979 and VINDERVOGEL *et al.*, 1975).

It has been recognized that breeding flocks exposed to IBD virus deposit antibodies in the yolk of their eggs which can interfere with the propagation of the virus in the embryonated eggs and protect progenies from infection (BAXENDALE, 1976; HITCHNER, 1970 & 1971 and LUKERT *et al.*, 1975).

In Egypt the disease was reported by EL-SERCANY *et al.*, 1974; AYOUB & MALLICK, 1976 and BASTANI, 1979. The disease picture and the causative agent were reported only in imported foreign breeds.

The present work was planned to study the susceptibility of Fayoumi chicken to IBD; determine the levels, persistence and degree of protection afforded by maternally derived IBD antibodies; and study the clinical and pathological picture of the disease, pathogenesis and antibody response in experimentally infected Fayoumi chickens.

MATERIAL and METHODS

Hyperimmune serum A reference antiserum⁺ was used in virus neutralization and agar-gel precipitin tests.

Virus: The Cu-1 strain⁺ of IBD virus was used in this study. Infected chorioallantoic membranes (CAM) were homogenized, suspended approximately 1:1 in normal saline, centrifuged, and the supernate was stored as stock virus at -20°C. Its 50% infective dose (EID₅₀) was determined by the method of REED and MUENCH (1938) by CAM inoculation of groups of 5 chick-embryos per virus dilution.

Chick embryos: 10 to 11-day-old embryonated eggs were provided by the farm of the Faculty of Agriculture, Assiut. Eggs were inoculated by the dropped CAM method.

Chick: A total of 200 day-old Fayoumi chicks were kindly provided by Beni-Mur governmental farm and reared under strict isolation.

Sera: Experimental chicks were bled by cardiac puncture at different intervals and sera were separated and subjected individually to serological examination.

Agar-gel precipitin (AGP) test: Qualitatively, the test was carried out according to NANSI (1958) to detect precipitins in the sera and precipitinogen in internal organs of experimental chicks. Quantitatively, it

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was employed to measure the levels of serum precipitins after CULLN and WYETH (1975).

Virus neutralization test: All virus neutralization (VN) tests were carried out by chick embryo inoculation using the constant serum-virus dilution method. Sera were considered positive to IBD virus neutralizing antibodies when they reduced the virus titre by one log₁₀ or more (CUNNINGHAM, 1966).

Experimental exposure: Chicks were infected intraocularly with 1000 EID₅₀ of IBD virus/bird. They were isolated and observed daily for 7 days for clinical signs of the disease.

Histopathological examination: Portions from bursa, liver, spleen, kidneys, thymus and caecal tonsil from sacrificed and died chicks as well as controls were fixed in 10% neutral buffered formalin. Samples were further processed for paraffin embedding section, 5 to 7 μ thick, stained with haematoxylin and eosin and examined.

Antigen detection and virus reisolation: Bursae of dead and sacrificed chicks were subjected to ACP test. Pooled samples from each of bursa, kidney, thymus and caecal tonsil of experimentally infected chicks were homogenized, suspended and inoculated on the CAM of five embryonated chicken eggs. Embryos were examined for lesions suggestive of IBD virus (GIAMBRONE *et al.*, 1976).

EXPERIMENTS

Exp. (1):

It was designed to determine the rate of waning of maternally derived IBD antibodies at 1,5,10,20 and 30 days of age by ACP, VN and challenge tests. For this purpose groups of 15 birds each were used at each interval 5 were bled for the serological tests and the remainder were subjected to challenge.

Exp. (2):

A total of 125 day-old chicks were reared under strict isolation. At 30 days of age they were infected intraocularly with 1000 EID₅₀ of the virulent IBD virus, another group served as uninfected control. At 6, 12, 24, 36, 48 and 72 hours; 4, 5,6,7,8, and 9 days postinoculation, birds were examined clinically and 5 birds were necropsied. Organs were subjected to histopathological examination, virus reisolation and ACP test. Sera were subjected to ACP and VN tests.

RESULTS

Exp. (1):

The levels of maternally derived precipitating and neutralizing antibodies at different intervals after hatching are shown in table (1).

Results of challenge revealed the protection of all 1, 5 and 10 day-old chicks, 40% of 20 day-old chicks and none of 30 day-old chicks. Protected chicks showed neither signs of illness nor bursal lesions.

Exp. (2):

Clinical signs were observed 36 hours p.i. in the form of depression, anorexia, whitish diarrhoea, and trembling. Later, birds falled on their sides with eyes partially closed (Fig. 1). Morbidity rate was 100% within 5 days and all infected birds died within 9 days p.i.

Sacrificed or dead birds showed the following lesions: 2 days p.i., bursae were enlarged; oedematous and yellowish white to reddish in colour. At 3 rd. day p.i. bursae returned to their normal size but were covered with gelatinous exudate and bleeding was prominent on their surface. At the 5th. day bursae were markedly atrophied and greyish in colour. Lesions in kidneys could be easily observed at the 5th. day p.i. they were enlarged and the urinary passages were prominent and greatly distended with urates (Fig. 2). Petichae on thigh muscles were prominent at the 3rd. day. Other lesions such as petichae on proventriculus and caecal tonsil, enlargement of spleen, or catarrhal enteritis were sometimes seen between 5th. and 7th. day p.i.

Till 36 hours p.i. all samples showed severe congestion of the vascular beds. 36 hours p.i. proliferative changes manifested in focal round cell infiltration in the liver (Fig. 3) were markedly observed. Kidneys showed increased cellularity of the glomeruli, in which both of the epithelial and endothelial cells showed

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proliferative changes (Fig. 4). Moderate lymphocytic hyperplasia was seen in the spleen (Fig. 5) and the bursa 3 days p.i. the spleen showed severe enlargement of the follicles, while the center of most of the white pulps showed severe necrosis and debilitation of lymphocytes.

Many pulps showed absence of the lymphoid elements and their corpuscles appeared as acellular structures of reticular fibres (Fig. 6).

The bursa of Fabricius suffered from atrophy of lymphoid follicles, necrosis in the centres of the lymphoid elements and relative prominence of the stromal tissue from the 2nd. day p.i. (Fig. 7). In many lymphoid follicles, the centres were completely necrosed and appeared as empty spaces at the 5th. day p.i.

Kidneys showed severe tubular nephrosis which reached to complete epithelial necrosis and resulted in tubular casts formation, cystic dilatation of the urinary passages could also be observed.

IBD virus could be detected 24 hours p.i. in the cecal tonsils, 36, hours p.i. in the bursa of Fabricius and the spleen, 48 hours p.i. in the kidneys, and 72 hours p.i. in the liver and thymus. Precipitating antigen could be detected 4 days p.i. in the bursa of Fabricius and 6 days p.i. in the kidneys, but the antigen could not be detected in other organs.

Precipitating antibodies to IBD virus could be detected 6 days p.i., while neutralizing antibodies appeared 8 days p.i.

DISCUSSION

Estimation of maternally derived antibodies to IBD virus should be useful in designing optimal vaccination programme for young chicks.

Our results showed that newly hatched Fayoumi chicks possessed relatively high titres of precipitating and neutralizing antibodies up to 10 days of age, which protected chicks from infection with the highly pathogenic strain of IBD virus. On the other hand, although 20 day-old chicks possessed no detectable precipitating antibodies, chicks were partially (40%) protected from IBD virus infection. It seems clearly from our results that degree of protection goes hand in hand with presence of neutralizing antibodies, which could be detected in 20 day-old chicks. These results lead to a significant conclusion that the ACP test, while useful for screening purposes because of its ease and economy, may be unreliable and the virus neutralization test is more sensitive for detecting prior exposure to IBD virus, which could be due to rapid disappearance of precipitating antibodies and the persistence of neutralizing antibodies (HITCHNER, 1971; KOESTERS and GEISLER, 1971; VINDERVOGEL, 1975; WYETH and CULLEN, 1976).

A highly pathogenic strain of IBD virus was used for experimental infection in order to give a considerable chance for detecting both virus replication and pathological lesions. Birds were infected intraocularly as the disease is of longer incubation period (KAEUFER and WEISS, 1976) and this route allows better study of the pathogenesis of the disease.

In exp. 2 : 30 day-old chicks used for experimental infection proved to be free from precipitating and neutralizing antibodies, as they were completely susceptible to infection in exp. 1.

Clinical signs, post mortem lesions and histopathological lesions were in great similarity to those described by ALMASSY and KAKUK, 1976; CHEVILLE, 1976; FADLY *et al.*, 1976. In our results the incubation period was shorter, lesions in kidneys were more prominent and haemorrhages were less obvious. Similar morbidity and mortality rate was reported by KAEUFER & WEISS (1976).

Proliferation in lymphoid elements in the spleen, thymus and caecal tonsils as well as activation and proliferation of RES cells in liver and kidneys were clear in early stages of infection which explain why the virus in early stages of infection initiates homologous titre of antibodies.

As regard to our results, 6 days p.i., degenerative; necrobiotic and even necrotic changes in the bursa of Fabricius, thymus, caecal tonsils and spleen clarify the immunosuppressive phenomena of the virus.

KAUEFER and WEISS, 1976 concluded that after oral infection, the virus undergoes primary multiplication in a yet unknown organ or tissue causing a transient viremia during which the bursa as the target organ becomes infected. Our virological and histological findings gave a great evidence that this early multiplication occurs in the caecal tonsils which was probably followed by a state of viremia in which the virus was distributed to liver, spleen, kidneys, thymus and bursa of Fabricius.

In accordance with VENDERVOGEL *et al.*, 1975 and SKEELES *et al.*, 1979 precipitating and neutralizing antibodies were detected on the 6th. and 8th. day p.i. respectively.

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Table (1)
Levels of maternally derived precipitating and neutralizing antibodies to IBD virus at different intervals after hatching.

Serum samples	Age (days)									
	1		5		10		20		30	
	ACP titer	NI	ACP titer	NI	ACP titer	NI	ACP titer	NI	ACP titer	NI
1	2*	3.65+	4	2.65+	0	0.75-	-	0.95-	-	0-
2	3	3.50+	4	3.03+	2	2.66+	-	1.20+	-	0-
3	3	2.50+	2	1.20+	3	2.50+	-	0.66-	-	0.2-
4	2	3.75+	1	2.50+	1	3.33+	-	1.50+	-	0-
5	4	3.33+	1	2.25+	0	1.10+	-	0.75-	-	0-
GM	2.8	3.35+	2.4	2.33+	1.2	2.07+	-	1.01+	-	0.04-
Hyperimmune serum	8	4.1 +								

* = Precipitin titers expressed as \log_2 .

GM = Geometric mean.

NI = Neutralizing index (log. 10).

+ = NI of 1 or higher was interpreted as (+) and $\frac{1}{2}$ as (-).

0 (ACP) = Undiluted.



Fig. (1)



Fig. (2)

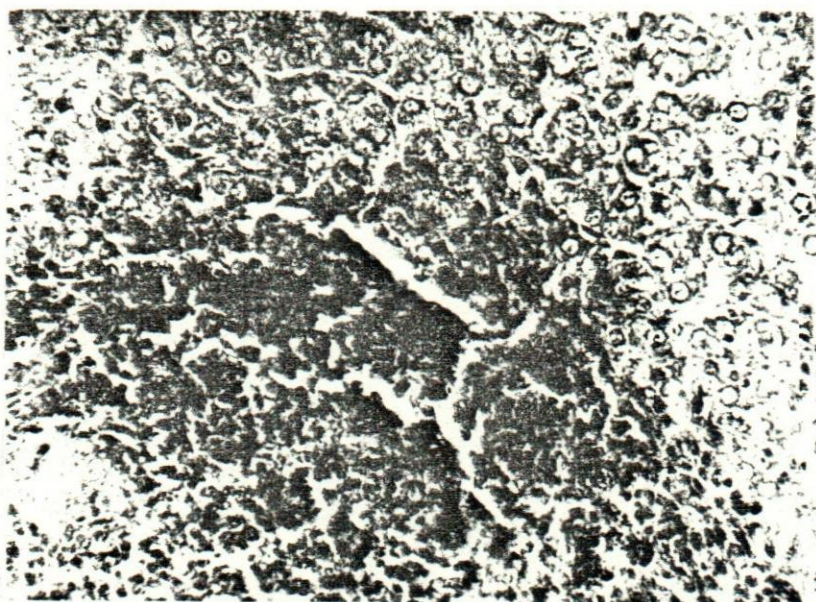


Fig. (3)

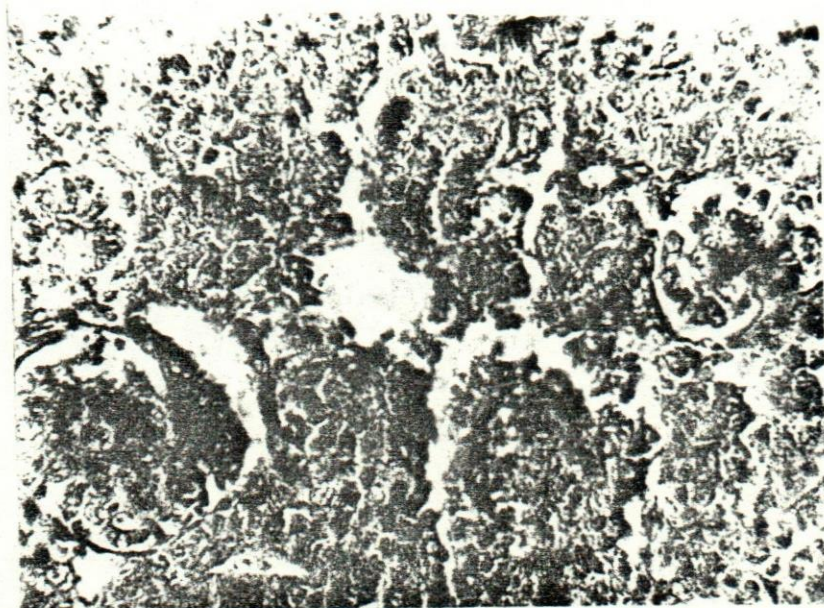


Fig. (4)

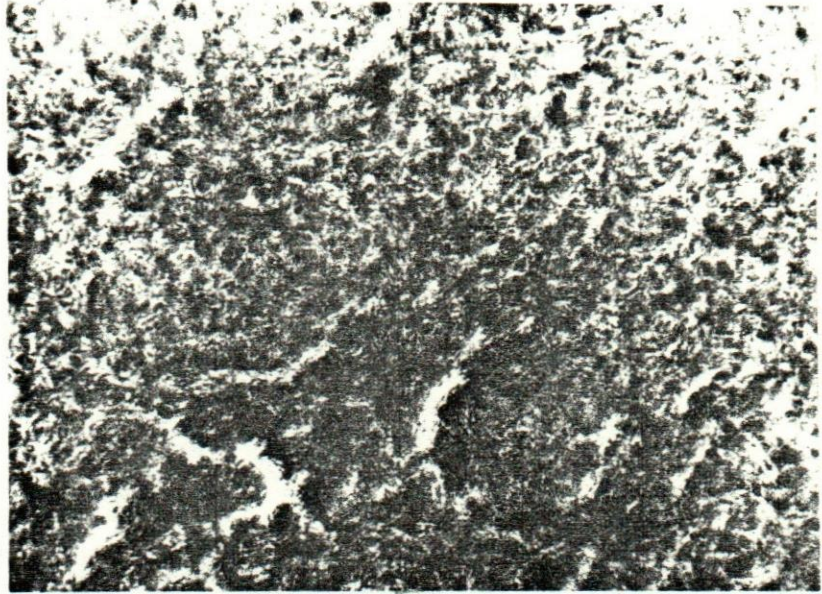


Fig. (5)

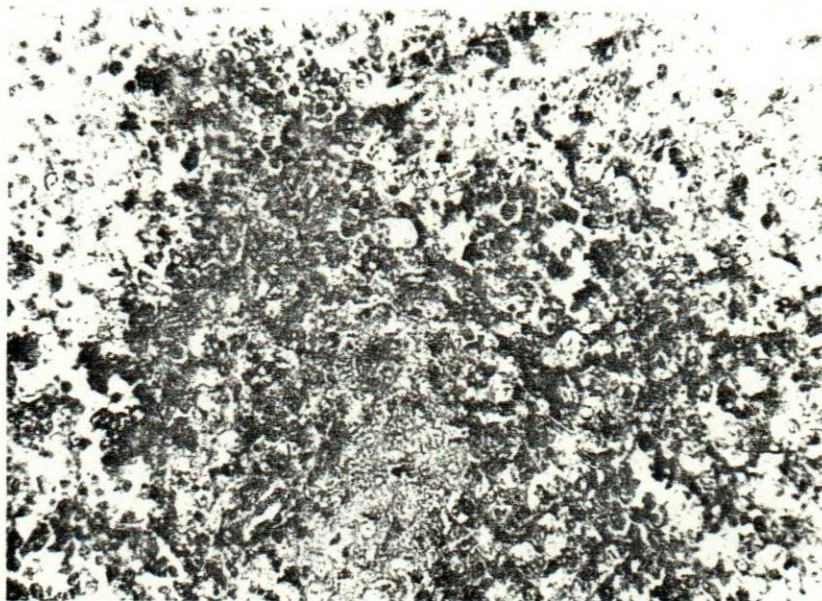
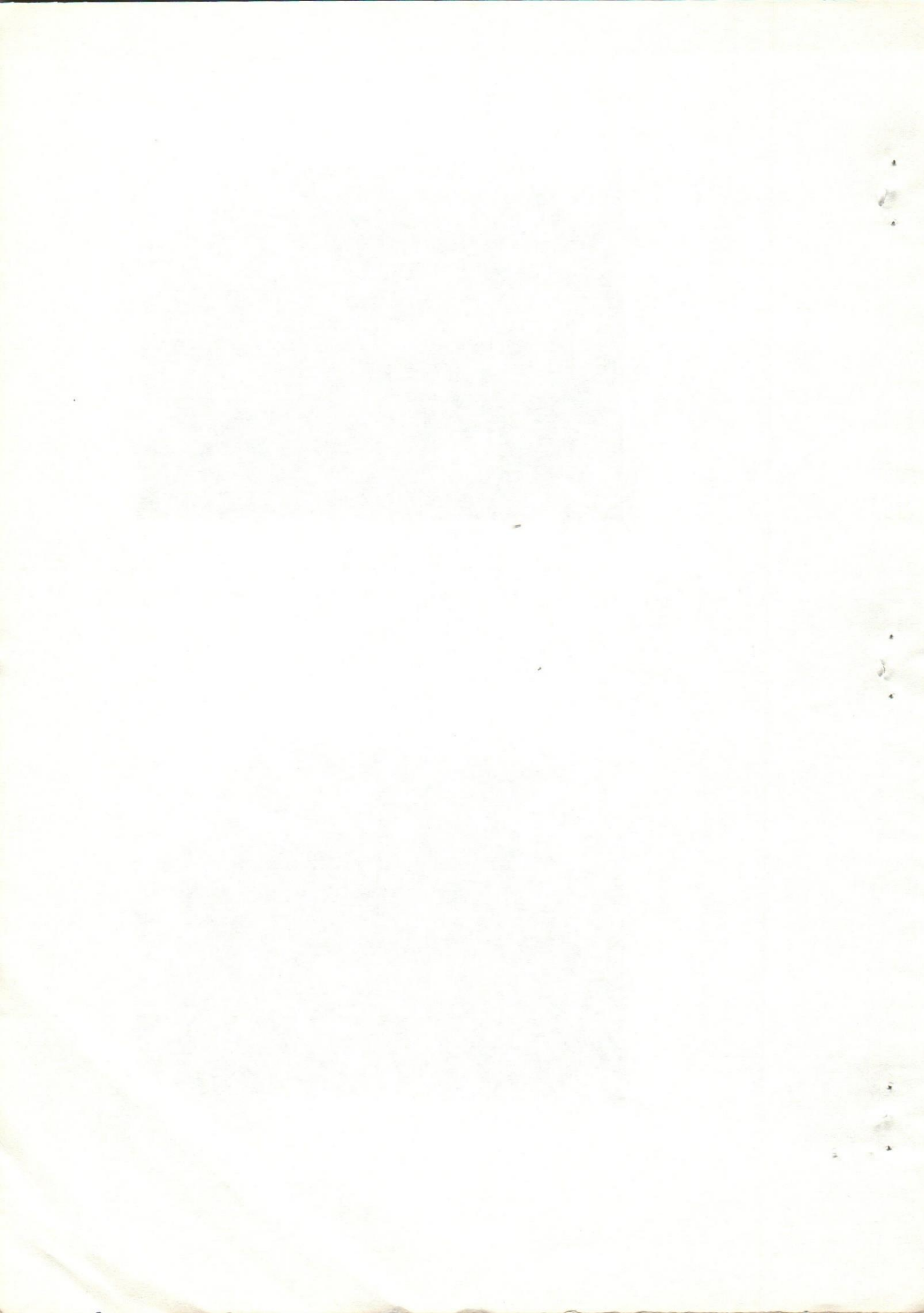


Fig. (6)



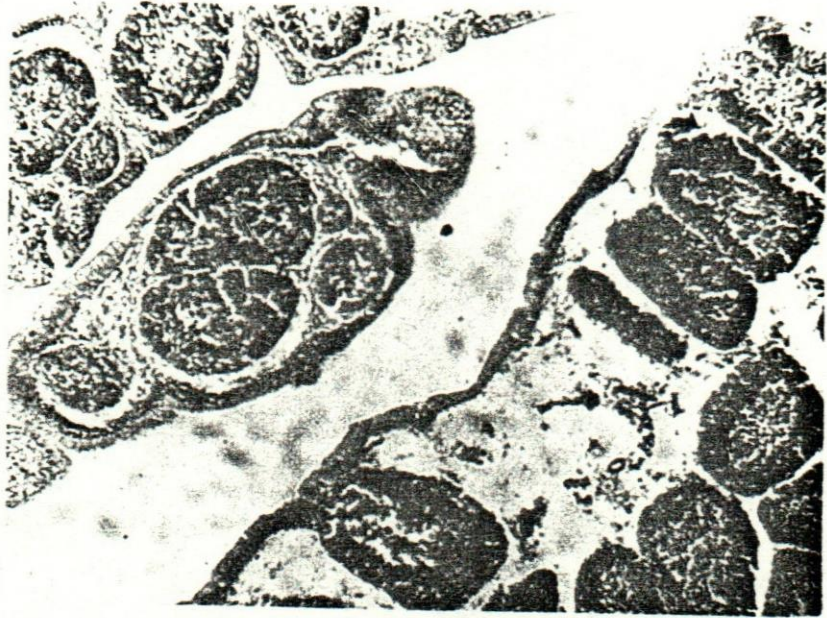


Fig. (7)

LIST OF FIGURES

- Fig. (1): Shows clinical signs of IBD, birds falled on their sides with eyes partially closed.
- Fig. (2): Shows enlargement of kidneys and urinary passages greatly distended with urates.
- Fig. (3): Shows focal round cell infiltration in the liver.
- Fig. (4): Shows endothelial and epithelial cell proliferation of the glomeruli of the kidneys.
- Fig. (5): Shows moderate lymphocytic hyperplasia in the spleen.
- Fig. (6): Shows absence of the lymphoid elements of the white pulps of the spleen.
- Fig. (7): Shows atrophy of the lymphoid follicles of the bursa of Fabricius.

