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EFFECT OF *CLOSTRIDIUM PERFRINGENS* INFECTION ON SOME BIOCHEMICAL, PHYSIOLOGICAL AND PATHOLOGICAL PARAMETERS IN *OREOCHROMIS NILOTICUS*

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) was injected intraperitoneally with types A, D and A+D of *Clostridium perfringens* under controlled laboratory conditions. The biochemical and haematological analyses were carried out on the 3rd, 7th and 14th days post injection. The obtained results showed that:

1. Clinically, the infected fish showed loss of appetite, sluggish and erratic swimming, loss of equilibrium and listless. At the site of injection, there was brownish caseous material which easily separated from the muscle. Gills were congested. Internally, the organs were friable and had a generalized hyperemic appearance.
2. Histopathologically, the striated muscle, at the site of injection showed edema and necrosis with leucocytic infiltration. The gills showed sloughing of secondary lamellae and desquamation of the epithelial covering of secondary lamellae.
3. Significantly decreased numbers of erythrocytes were detected after injection with *C. perfringens* type A or D at all periods. Sporadically significant decreases in haemoglobin and haematocrit were found. Other haematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration

(MCHC) revealed inconsistent fluctuations.

4. The leucocytes were decreased significantly in fish infected with *C. perfringens* types D and A+D on the 7th and 14th days respectively.
5. Plasma and muscle total proteins showed a significant decrease whereas liver total proteins recorded a significant increase in comparison with the corresponding control values.
6. Plasma glucose was significantly increased in fish injected with *C. perfringens* type D at all periods, but significantly decreased with the combination of A+D.
7. Plasma total lipids were significantly decreased in fishes injected with *C. perfringens* type D as well as with the combination of A+D.
8. Plasma alkaline phosphatase activity significantly decreased in all groups at all periods studied.
9. A general tendency of a significant decrease was observed in plasma aspartate aminotransferase (AST) activity paralleled with an increase in alanine aminotransferase (ALT) activity after injection with the two strains of *C. perfringens* or their combination. There were some changes following this trend with respect to liver AST and muscle ALT.

In conclusion. *Clostridium perfringens* bacteria caused a high mortality rate among the cultured fish, while the drastic effect of the mild bacteria infection was inflected more on the fish physiological status and as a consequence decreases the farm production. Either mild or severe bacterial infection should receive an immediate eye of inspection.

INTRODUCTION

In the past, diagnostic laboratories did not normally use anaerobic methods. Therefore, it was unlikely that isolation of an offending anaerobic pathogen would ever be achieved. Consequently, the cause of disease might have not been recognized or it could have been attributed to aerobic secondary invaders (Austin and Austin, 1993).

Clostridia occur commonly where oxygen levels are restricted in soil, sewage, marine sediment, decaying vegeTable and animal products. They are numerous in the intestinal tract of man and animals. Some are opportunist pathogens of man and animals, causing wound and soft tissue infection and food poisoning and their pathogenicity are associated with potent exotoxins (Inglis *et al.*,

1993). *Clostridium perfringens* continues to be a common cause of food-borne disease. It produces an enterotoxin which is released upon lysis of the vegetative cell during sporulation in the intestinal tract (Schalch *et al.*, 1999). The health hazards produced as a result of consumption of contaminated fish with *C. perfringens* were reviewed by Panebianco *et al.* (1993); Niewolak and Tucholski (1995, $2000_{a,b}$) and El-Deeb (1998). *C. perfringens* causes clinical signs of gastroenteritis, which often resolve within 24 hours with minimal or no treatment (Stoskopf, 1993).

In Egypt, El-Ged *et al.* (1985) recorded the presence of *C. perfringens* in diseased Nile catfish *Clarias gariepinus* in a higher incidence (45 %) than apparently healthy ones (16 %). Abd El-Rahman *et al.* (1989) found that the incidence of *C. perfringens* in examined *Oreochromis niloticus* was 68, 76 and 20 % in gills, surface and muscles respectively. Enany *et al.* (1989) subjected *O. niloticus*, *Clarias gariepinus* and *Mugil cephalus* to anaerobic bacteriological examinations. They reported that *C. perfringens* type D was the most common isolates in the examined fish with incidence of 2.7, 5.7 and 2.0 % respectively. Rizkalla *et al.* (1999) dealt with the isolation of *C. perfringens* from apparent healthy *Clarias gariepinus* from Mariut Lake. All fish revealed the presence of non toxogenic strains of *C. perfringens* in both intestinal and muscle samples.

Tilapia is possibly the most important freshwater aquaculture species worldwide. The increasing scale of production demands new accurate and efficient tools to screen and monitor the health status of these fish. Histology-based monitoring is time-consuming, and cannot detect disease until moderate tissue damage is apparent. The evaluation of blood chemistry parameters in animals is a routine and important tool in clinical practices. This simple approach can provide essential information on the physiological status of the animal; therefore help the biologists to make proper decisions. So, the aims of the present study are : (1) isolation, identification and typing of *C. perfringens* among *O. niloticus*. (2) assessment of the temporal changes in selected haematological and biochemical parameters in blood, plasma, liver and muscle after administration of *C. perfringens* to *O. niloticus* (i.e. how to identify the chemistry profile pattern associated with a diseased state) and (3) assessment of changes in the histopathology of the muscle, liver, spleen, kidney and gills after 3, 7 and 14 days of injection of *C. perfringens*.

MATERIAL AND METHODS

Clostridium perfringens isolation and preparation:

Sixteen apparently healthy Nile tilapia (*Oreochromis niloticus*) were brought from Giza market, Cairo and subjected to bacteriological examination immediately. Samples from the intestine were collected from each fish and inoculated in one tube of sterile freshly prepared thioglycolate broth and incubated anaerobically for 24-48 hours at 37 °C using GS pack anaerobic Jar. A loopfull from the tube was streaked on to the surface of 10 % sheep blood agar with neomycin sulphate (200 µg/ml) for isolation of *Clostridium perfringens*. All plates were incubated anaerobically for 24 hours (Willis, 1977). All suspected colonies were isolated and identified microscopically using Gram stain (Quinn *et al.*, 1994). Identification of toxin was carried out according to Sterns and Batty (1975). The positive toxogenic strain for *C. perfringens* types A has been purified by reinoculation onto one tube of sterile freshly prepared cooked meat broth, which has been boiled and cooled before, and incubated anaerobically for 24-48 hours at 37 °C. Gram stain was made for assurance of the culture purification. The whole culture was then centrifuged at 3000 r.p.m. for 15 minutes. The supernatant was discarded and the sediment was streaked on to sheep blood agar (10 %) with neomycin sulphate 200 µg/ml and incubated anaerobically for 24 hours at 37 °C. The pure and well isolated colonies were picked up, resuspended in 0.5 % formal saline, adjusted and compared with the turbidity of Mc farland tube No. 1 "approx. 300 million of *C. perfringes* cell/ml" (Quinn *et al.*, 1994). The same procedures were used with *C. perfringes* type D.

Experimental design:

Male Nile tilapia "*O. niloticus*" with an average weight 50-60 g were obtained from the Central Laboratory for Aquaculture Research, Abbassa, Sharkia. Fish were acclimated in indoor tanks for 2 weeks and then randomly distributed in glass aquaria of 80 liter capacity at a rate of 10 fish/aquarium. The aquaria were supplied with aerated water and temperature was kept at 25±1 °C. Fish were fed on fish pellet diet (32 % CP) at a rate of 3 % of their live body weight twice daily. A semi-dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of fresh water.

After the acclimatization period, fish were classified into four groups (each group contains 6 aquaria). The first group was injected

intraperitoneally with 0.3 ml sterile saline as a control. The second group was injected intraperitoneally with 0.3 ml of the culture of *C. perfringens* type A (3X10^{7} cell/ml). The third group was injected intraperitoneally with 0.3 ml of the culture of *C. perfringens* type D (3X10^{7} cell/ml). The fourth group was injected intraperitoneally with a mixture of the two cultures. 0.15 ml of type A and 0.15 ml of type D. Fish were sampled 3 times (after 3, 7 and 14 days of injection). Each time consisted of 2 replicate aquaria.

Clinical and post-mortem examinations:

The fish were observed for the signs of disease over the period of experiment. The clinical and post-mortem examinations of fish were carried out according to Lucky (1977).

Physiological parameters:

Blood samples were taken from the caudal vein of non anaesthetized fish by a sterile syringe containing EDTA as an anticoagulant. A portion of each blood sample was used for erythrocyte count and leucocyte count (Dacie and Lewis 1984), haemoglobin content (Vankampen, 1961) and haematocrit value (Britton, 1963). MCV, MCH and MCHC were calculated using the formulae mentioned by Dacie and Lewis (1984). The other blood portion was centrifuged and the non haemolyzed plasma was stored in a deep freezer at-20 °C pending biochemical analyses.

Tissue samples from the white dorsal muscle and liver were excised for the determination of protein content and enzyme activities. Plasma and tissue protein content was determined by Biuret method (Wootton, 1964). Enzyme activities were measured colorimetrically using kits supplied by Diamond Diagnostics. AST and ALT were determined according to Reitman and Frankel (1957) and alkaline phosphatase (ALP) according to Rec (1972). Plasma glucose concentration was measured according to Trinder (1969) using Boehring Mannheium kits and total lipids were determined colorimetrically using kits supplied by El Nasr Pharmaceutical Chemical Co. (Joseph *et al.*, 1972).

Histopathological examination:

For histopathological examination, tissue specimens from muscle, liver, spleen, kidney and gills were fixed in 10 % phosphate buffered formalin and 5 micron-thick paraffin sections were prepared and stained with hematoxylin and eosin (H & E) and examined microscopically (Roberts, 1989).

Statistical analysis:

The results were statistically analyzed (mean, standard

deviation, t-test and F-test) using the different equations recommended by Hill (1977).

RESULTS

Pathological findings:

Clinically, the injected *Oreochromis niloticus* with toxogenic Clostridia showed loss of appetite, sluggish and erratic swimming, loss of equilibrium, listless and the fish activity was reduced as manifested by loss of any reflex action. At the site of injection, there was brownish caseous material which easily separated from the muscle, whereas gills were congested. Internally, the organs were friable and had generalized hyperemic appearance. The liver was often mottled with hemorrhage and had over distended gall bladder with bile. The kidneys and spleen were swollen and congested. The abdominal cavity usually contained yellowish turbid fluid.

Figures from 1 to 10 illustrate the pathological changes of different tissues and organs in the experiments. The striated muscles, at the site of injection (Fig. 1) showed edema and necrosis (n) with leucocytic infiltration (y). The gills (Fig. 2) showed sloughing of secondary lamella (sl) and desquamation of epithelial covering of secondary lamella. Edema, congestion (c), hemorrhage (h) and leucocytic infiltration were detected in the gill arch (Fig. 3). Fatty changes were evident in the liver (v) (Fig. 4). The blood sinusoids and the central vein were congested (c) with thickening of the wall of blood vessels (Fig. 6). Leucocytic infiltration was noticed. The kidneys showed hyaline droplet degeneration (hy) and degenerative changes in the renal tubules (Fig. 7). Hyaline casts inside the lumina of the renal tubules were observed with hemorrhage of peritubular blood vessels. Thickening of renal capsule (t) (Fig. 8) and proliferation of fibrous tissue (f) (Fig. 9) were also noticed. The spleen revealed proliferation of melanomacrophage center (m) (Fig. 10).

Haematological findings:

These findings are summarized in Table 1. After 3 days of infection of *O. niloticus* with *Clostridium perfringens* types A, D or a mixture of the 2 types, the erythrocytic counts (RBCs) and haemoglobin concentrations (Hb) recorded significantly lower values than those of the control fish. Haematocrit value (Hct) showed a significant reduction with type D only. In the same trend, significantly lower values of RBCs were detected in fishes infected

with types A and D after 7 and 14 days of injection as compared with the control value. Also Hb contents and Hct values of fishes infected with type A showed significantly lower values after 7 days. The significant reduction of Hb (type D) and of Hct (type A+D) was recorded in fishes after 14 days of injection. There were significant ($P<0.05$) effects due to infection with different types of *C. perfringens* on the Hb contents of fishes at the three periods studied, whereas RBCs were significantly ($P<0.01$) affected by *C. perfringens* on the 3rd and 14th days and Hct values were reduced on the 3rd day ($P<0.05$). No Effect of time was not significant with regard to RBCs and Hct except for the Hb content of fishes infected with type D ($P<0.01$).

Significantly higher values of MCV were detected in fishes infected with *C. perfringens* type D at the three periods studied and type A after both 3 and 14 days of injection as compared with the control values, whereas such a reduction was found only after 14 days in fishes injected with type A+D (Table 1). Also, the MCH values increased significantly in fishes injected with type D after 3 and 7 days of injection and with type A after 14 days as compared with the corresponding control values. On the other hand, MCHC values decreased significantly in fishes injected with types A and D after 3 and 14 days of injection respectively. The effect of types of *C. perfringens* infection on haematological indices were significantly ($P<0.05$) demonstrated in MCV after 3 and 14 days of infection and in MCH after 7 days. Significant ($P<0.05$) time effect was found in the MCV of fishes infected with type A.

The total leucocytic count (WBCs) decreased significantly in fishes injected with *C. perfringens* type D after 7 and 14 days and with a mixture of types A+D after 14 days only. The F test shows that there were no differences among means of groups treated with different strains. Asignificant ($P<0.05$) time effect was recorded in the control, type D and A+D groups (Table 1).

Biochemical findings:

Table 2 shows that plasma total protein and total lipid concentrations decreased significantly in fishes infected with *C. perfringens* type D at the three periods studied and type A+D after 3 and 7 days (for total proteins) and after 7 and 14 days (for total lipids). Plasma glucose concentrations showed significantly higher values than the control groups in fishes injected with *C. perfringens* type D at the three periods studied, whereas glucose decreased significantly after 3 and 14 days post injection with a mixture of *C.*

perfringens types A+D.

Both plasma AST and alkaline phosphatase (ALP) activities showed significantly lower values in fishes infected with type A, D or their combination at the three periods studied than the control ones. The exception of that was the AST activity in fishes after 14 days of injection with type A where no significant effect was found (Table 2). On the contrary, ALT activity showed significantly higher values after 3 days in fishes injected with *C. perfringens* type A+D; after 7 days with types A and D and after 14 days with the three types of *C. perfringens* than the control groups.

It is obvious that significant changes were detected in response to infections with different types of *C. perfringens* on the studied plasma parameters except on the plasma total proteins after 7 and 14 days. Effect of time was significant in plasma total proteins of fishes infected with *C. perfringens* type D; glucose and AST with type A+D; glucose of the control group and ALT and ALP of the control and fish groups infected with *C. perfringens* types A and D (Table 2).

The biochemical changes occurred in liver are presented in Table 3. Total proteins significantly increased in fishes injected with *C. perfringens* type A (on day 3); type D (on days 7 and 14) and types A+D (after 14 days). Differences among means of groups treated with different strains were significant ($P<0.05$) on day 7 only. Significant ($P<0.05$) time effect was detected in fishes injected with *C. perfringens* types D and A+D. Liver AST activities were significantly decreased in fishes injected with *C. perfringens* types A (after 7 and 14 days); D (after 7 days) and A+D (after 3 days), whereas AST significantly increased in fishes injected with *C. perfringens* types A+D after 7 and 14 days of injection. A significant increase in liver ALT activities was observed in fishes injected with the three types of treatments after 3 days and with types A+D after 7 and 14 days. A significant ($P<0.01$) decrease was detected in the fish group injected with *C. perfringens* type A after 14 days. Both liver AST and ALT activities were significantly influenced by the type of *C. perfringens* infection in the three periods studied except AST after 3 days. Time effect was significant in AST activity of control group and fishes injected with *C. perfringens* types A+D and in ALT in fishes injected with the three types of treatments.

The biochemical changes occurred in muscles are presented in Table 4. Total proteins significantly decreased in fishes infected with *C. perfringens* type A at the three periods studied; type D after 7 and

14 days and types A–D after 3 and 7 days post injection. Influence of *C. perfringens* strains on muscle total protein was significantly detected at the three periods. Time effect was significant ($P<0.05$) in groups infected with *C. perfringens* types D and A+D. Both muscle AST and ALT activities were significantly decreased in fishes injected with *C. perfringens* type A after 3 and 7 days and type D after 14 days. A similar significant decrease was observed in fishes infected with *C. perfringens* type A+D after 7 and 14 days in AST and after 3 days in ALT. On the other hand, higher ALT values than those of the control were recorded in fishes injected with *C. perfringens* types D and A+D after 3 and 14 days respectively. A significant effect of *C. perfringens* strains was detected for both muscle AST and ALT activities at the three periods except for ALT activity after 7 days. The effect of time was significant for the AST activity ($P<0.05$) in the fish groups infected with *C. perfringens* types A and D and in ALT activity ($P<0.01$) in the fish group infected with *C. perfringens* type D as well as in the control group.

DISCUSSION

McKeon *et al.* (2000) mentioned that *C. perfringens* is present in all tilapia commercial systems indicating fecal contamination. Moreover, it was considered likely that Clostridia become established in the mud and bottom living invertebrates in trout ponds (Huss *et al.*, 1974). Clostridial microorganisms are considered among the most important species that cause a large scale spoilage of fish and fishery products as they are present in the skin, gills, and intestine of nearly caught fish and may be picked up adventitiously during handling (Hibbs *et al.*, 1991). In the present work, the signs and postmortem lesions in the injected fish with toxogenic clostridia showed loss of equilibrium, sluggish swimming and generalized hyperemic appearance of the internal organs. These alterations were in harmony with those found by Can and Taylor (1982); El-Ged *et al.* (1985); Panebianco *et al.* (1993); Austin and Austin (1993) and Rizkalla *et al.* (1999). In the natural environment, the fish need to feed repeatedly on toxic material before eventually succumbing to the disease (Can and Taylor, 1982).

Concerning the Histopathological studies, our observation showed that degeneration, congestion, edema, leucocytic infiltration and necrosis were observed in the tissue specimens collected from different organs. Such findings are in concurrence with those

reported by El-Ged *et al.* (1985); Roberts (1989) and Rizkalla *et al.* (1999).

Rizkalla and Tanios (1994) recorded that haemograms and blood indices of tilapia showed a pronounced variation under different rearing conditions. Snieszko (1958) mentioned that the presence of anaerobic microorganisms in fish may invade the tissues causing pathogenic conditions when fish were injured or their resistances were lowered by adverse environmental conditions. Our haematological results tabulated in Table (1) declared significantly lower values of RBCs, Hb and Hct in *O. niloticus* injected with different types of *C. perfringens* compared to the control group. El-Deeb (1998) mentioned that, regardless of type of *C. perfringens* infection, RBCs, Hb and Hct of catfish *Clarias gariepinus* insignificantly decreased from the first week to the third week. RBCs, Hb and Hct appeared significantly higher after infection with *C. perfringens* type A and type D when compared to those of the control. Also, Rizkalla *et al.* (1999), working on wild *Clarias gariepinus* collected from Mariut lake, detected significantly lower values of Hct in fishes their intestine contained positive non-toxogenic *C. perfringens* compared to the negative ones. The significantly higher values of MCV observed in this study (Table 1) are contradictory to the results of El-Deeb (1998) and Rizkalla *et al.* (1999) on *Clarias gariepinus*. El-Deeb (1998) did not find significant differences in MCV between fish infected with types A or D and the control group. This author found that infection with type A *C. perfringens* produced significantly higher MCH and MCHC values than those of the control or the group infected with type D, whereas the latter two groups were not statistically different from each other. The present results on *O. niloticus* align with those which revealed a significant increase in MCH, however for *O. niloticus* injected with *C. perfringens* type D rather than type A (Table 1), where type A was insignificantly different from the control group, except after 14 days of infection. MCHC in our study recorded significantly lower values in two cases (type A after 3 days and type D after 14 days), but the other cases were not significant (Table 1). The common agreement between this study and that of El-Deeb (1998) is reflected by the fact that there were no significant differences in MCV, MCH and MCHC in correlation to the time of infection. Coles (1986) reported that MCV, MCH and MCHC are corpuscular indices that have particular importance in most animals in differentiating between types of anaemia and can be used in diagnosis and therapy. The present study

indicated that the fish were suffering from haemolytic anaemia after infection with *C. perfringens*.

The decline in RBCs, Hb and Hct of *O. niloticus* observed in the present study after injection with different types of *C. perfringens* aligns with the effects of other pathogens that infect different fish species. For example: infection of *O. niloticus* with *Flexibacter columnaris*, *Pseudomonas fluoreescence*, *Flavobacterium* and *Yersinia ruckeri* (Marzouk, 1991; Husien and Elias, 2000 ; Husien and Younis, 2000); *Aeromonas* bacteria infecting sand gobies "*Oxyeleotris marmoratus*, *Channa punctatus* and rainbow trout "*Oncorhynchus mykiss*" (Charnchit, 1985; Pradhan and Pal, 1995 ; Ehulka *et al.*, 1998); cyanobacterial water blooms infecting juvenile carp "*Cyprinus carpio*" (Kopp and Hetesa, 2000); infection of rainbow trout "*O. mykiss*" with *Campylobacter cryaerophila* and *Arcobacter cryaerophilus* (Aydin *et al.*, 2000, 2002); hybrid tilapia infected with *Streptococcus* spp. (Bunch and Bejerano, 1997); juvenile spring Chinook salmon "*Oncorhynchus tshawytscha*" infected with *Renibacterium salmoninarum* (Mesa *et al.*, 2000) and sheatfish "*Silurus glanis*" infected with *Edwardsiella tarda* (Caruso *et al.*, 2002). Also Pearson *et al.* (1994) and Rauthan *et al.* (1995), working on hybrid *Clarias* and hill stream fish respectively, obtained results that support our results, where MCV increased and MCHC decreased with jaundice disease and trypanosomal infection respectively. Haematological examination of chronic aflatoxicated *O. niloticus* (Essa, 1993) and *O. aureus* (Rizkalla *et al.*, 1997) revealed significantly decreased values of RBCs, Hb and Hct.

The influence of *C. perfringens* infection on total leucocytic count of *O. niloticus* was clear in fish injected with type D where it decreased after 7 and 14 days of injection (Table 1). This result is in contrast to that of El-Deeb (1998). This author reported that WBCs count increased significantly after infection with *C. perfringens* type A when compared to infection with type D, but the difference was not significant from the control. He also mentioned that regardless of the type of infection, WBCs appeared to decrease insignificantly from the 1st week to the 3rd week. The work of Charnchit (1985) supports our result where injection of sand gobies (*Oxyeleotris marmoratus*) intraperitoneally with a moderate or large numbers of *Aeromonas hydrophila* lowered total leucocytes counts in comparison with the controls at 48 hours while WBCs, one week after challenge Javenese carp (*Puntius gonionotus*) with *A. hydrophila*, were not significantly different from the control (Shariff *et al.*, 2001). Husien and Younis

(2000) reported lower WBCs count in Nile tilapia "*O. niloticus*" intramuscularly injected with *Yersinia ruckeri*. Also, Caruso *et al.* (2002) reported an increase in leucocrit in the unstressed juvenile sheatfish after intraperitoneal injection of *Edwardsiella tarda*. whereas stressed fish showed significantly decreased values. Essa (1993) on *O. niloticus* and Rizkalla *et al.* (1997) on *O. aureus* recorded a significant decrease in WBCs when fed crude aflatoxin-contaminated diet. Roberts (1989) suggested that the underlying reason for the previous findings is that bacterial infection causes destruction of haematopoietic tissue in the kidney and spleen and subsequently decreases blood cell production. It is likely that the hazardous impact of bacterial infection substantial stress which is reflected passively on the haematological parameters.

Normal plasma total protein concentrations of *O. niloticus* in our study (Table 2) recorded lower values than those reported in the same species by different authors: 5.3 and 4.1 g/dl for male and female respectively (Terao and Ogawa, 1984); 3.40 g/dl (Hussein *et al.*, 1996); 4.60 g/dl (Yavuzcan Yildiz *et al.*, 1997) and 3.68 g/dl (Chen *et al.*, 2003). Upon injection with *C. perfringens*, plasma total proteins were significantly reduced with types D and A+D (Table 2). Similarly, a statistically significant fall occurred in plasma total proteins of sand gobies, rainbow trout and *O. niloticus* infected with *Aeromonas hydrophila* (Charnchit, 1985; Ehulka *et al.*, 1998 ; El-Ashram, 2002); rainbow trout naturally infected with *Campylobacter cryaerophila* (Aydin *et al.*, 2000); juvenile carp exposed to the cyanobacterial water blooms (Kopp and Hetesa, 2000) and nephrocalcinosis-affected Nile tilapia obtained from Lake Nasser (Chen *et al.*, 2003). The common cause of decreased plasma proteins is a decline in hepatic protein synthesis either a result of liver function impairment or because the amino acid building blocks of proteins are not available because of malnutrition and poor amino acid intestinal absorption. However, in this study, there was an increase in hepatic proteins content with a decrease in muscle proteins, so it seems that excessive muscle protein breakdown provided an amino acids flow to the liver to synthesize proteins. The decrease in plasma proteins, which are synthesized in the liver, is likely due to an increase in the permeability of glomerular capillaries leading to loss of plasma proteins in urine (proteinurea). The amount of protein excreted in urine exceeds the rate of hepatic synthesis in case of endotoxin-induced nephrosis.

The significant fall of plasma total proteins due to *C. perfringens* infection was also detected in muscle total proteins (Table 4). The metabolic pathways of fish can be severely altered by a variety of biological, chemical and physiological factor, which could be assessed throughout several biochemical procedures. Many workers studied the influence of toxicant on total protein content of fish. Verma and Tonk (1983) observed a decrease in muscle protein content of mercury-exposed fish "*Notopterus notopterus*"; common carp "*Cyprinus carpio*" and in Nile catfish "*Clarias lazera*" exposed to iron (Haggag *et al.*, 1993) and *C. carpio* exposed to cadmium (Shalaby, 1997). The reduction in muscle protein may be attributed to the great energy demands imposed on fish during the periods of infection-induced stress. Under those circumstances, stress hormones, particularly cortisol, stimulate the breakdown of protein molecules and their amino acid constituents are used for gluconeogenesis or deamination and their hydrocarbon skeletons are oxidized in exergonic reactions. A sustained portion of the released energy is used for tissue repair and to affset the cellular damage that occurred in the tissue of toxicated fish.

The obtained results (Table 3) indicated that hepatic total protein increased following infection with *C. perfringens*. Similar results were obtained by (Abd El-Tawwab *et al.*, 2001) who recorded a significant increase in liver total protein in Nile tilapia (*O. niloticus*) exposed to sublethal concentration of inorganic mercury. Also Shalaby (1997) recorded a significant increase in liver total protein in common carp (*Cyprinus carpio*) exposed to Cu, Cd and Zn. It could be suggested that fish exposed to toxicant might compensate for possible protein loss by increasing hepatic protein synthesis which depends mainly on the influx of amino acids resulting from excessive muscle protein catabolism.

In the present study, infection of *O. niloticus* with *C. perfringens* showed a significant decrease in plasma total lipids (Table 2). This reduction is in agreement with the studies of Gluth and Hanke (1983) on *Oreochromis aureus* and in *Clarias gariepinus* exposed to copper and lead and with Haggag *et al.* (1993) on *C. gariepinus* and *Cyprinus carpio* exposed to a sublethal concentration of iron. Abu El-Ella (1996) reported a decrease in serum and muscle total lipids in grass carp exposed to cadmium. The author attributed this decrease to the great demand of energy for confronting the stress. This assumption is highly supported by Abbas (1998). Also El-Sayed *et al.* (1996) illustrated that the decrease in the body proteins and

lipids in appropriate habitats was a direct indicator of utilization of body protein and/or fat in energy production to meet the increase in physiological demands.

Normal plasma glucose level of *O. niloticus* in the present study (Table 2) showed lower values than that recorded on the same species by Terao and Ogawa (1984) "408 and 197 mg/dl for male and female respectively" and Chen *et al.* (2003) "85.4 mg/dl" and in the same level "52.33 mg/dl" as reported by Hussein *et al.* (1996). Following infection with *C. perfringens*, plasma glucose showed significant elevation and depression in fish injected with types D and A+D respectively, whereas plasma total lipid levels decreased significantly in fish injected with both types D and A+D (Table 2). Depressed levels of plasma glucose in fish have been reported by others assessing the physiological effects of various pathogens (Mesa *et al.*, 1999, 2000 ; Aydin *et al.*, 2000) indicating that the disease is stressful particularly during the later stages (Mesa *et al.*, 2000), and are probably due to excessive use of this energy substrate to help combat the infection (Mesa *et al.*, 1998). On the other hand, Charnchit (1985) and Bury *et al.* (1996) found that exposure of sand gobies and brown trout to *Aeromonas hydrophila* and cyanobacteria *Microcystis aeruginosa* respectively raised plasma glucose levels. So, abnormal variation in plasma glucose levels, at both high and low extremes, may reflect severe disturbance of carbohydrate metabolism in the fish (Brown *et al.*, 1987). Also Palti *et al.* (1999) revealed that glucose concentration of *Oreochromis aureus* and *O. mossambicus* showed significant fluctuations in response to stress.

The fall of plasma and muscle total proteins, plasma glucose and total lipids and the rise of liver total protein in fish injected with different types of *C. perfringens* may be interpreted histopathologically by the presence of fatty livers in these fish.

Control values of plasma AST obtained in the present study (Table 2) are in the middle between that obtained by Hrubec *et al.* (2000) from hybrid tilapia "102 U/L" and Chen *et al.* (2003) from *O. niloticus* "278 U/L". It is reported that plasma and hepatic AST and ALT activities of the continuous-fed hybrid tilapia were lower than those of the meal-fed fish (Yen and Shyan, 1999) and the activities of these enzymes in liver and muscle *O. mossambica* were significantly altered throughout the stressful situations (Rani *et al.*, 2001). In the present study, infection of *O. niloticus* with *C. perfringens* caused a significantly decreased activity of plasma, liver and muscle AST concomitant with a significant increase activity of plasma and liver

ALT, while muscle ALT showed inconsistent fluctuations (Tables 2 - 4). Kotak *et al.* (1994); Bury *et al.* (1997) and Kopp and Hetesa (2000) reported that exposure of rainbow trout, brown trout and juvenile carp to the liver toxin cyanobacterial caused a dramatic increase in the enzymes ALT and AST released into the bloodstream (an indication of severe liver trauma). Rizkalla *et al.* (2003) observed an increase in plasma ALT and AST of *Clarias gariepinus* resulting from liver injury caused by antibiotics and ALT was more sensitive to antibiotic administration. Siau and Ip (1987) mentioned that AST was at least eight times more active than ALT in the liver of different fish species. ALT activity declined significantly with a change in diet from a carnivorous (high protein) diet in juveniles to an omnivorous (lower protein) diet in adult fish as in tilapia fish (Gallagher *et al.*, 2001). Changes in AST and ALT due to injection of *C. perfringens*; in addition to the significant decrease in plasma ALP in all injected types of *C. perfringens* in the three periods of the present investigation (Table 2) revealed that the dose used in the present study was too low and not sufficient to cause substantial liver damage. Young *et al.* (1994) reported that elevated ALP level is a consistent feature of the moribund condition in striped bass and high ALP levels may reflect the degree of tissue damage seen in liver, kidney and intestine of moribund striped bass. Also Rizkalla *et al.* (2003) mentioned that the elevated plasma ALP activity in *Clarias gariepinus* treated with three antibiotics corresponded to an inflammatory reaction of the bile ducts.

From the present study, it is concluded that the virulent bacteria cause a high mortality rate among the cultured fish while the drastic effect of the mild bacteria is inflected more on the fish physiological status and as a consequence decreases the farm production. Either mild or severe bacterial infection should receive an immediate eye of inspection.

REFERENCES

Abbas. H. H. (1998). "Toxicological Effects of Copper and Lead on Some Physiological Aspects in Two Fish Species. Blue Tilapia (*Oreochromis aureus*) and African Catfish (*Clarias gariepinus*)." Ph.D. Thesis. Fac. of Science. Cario Univ., Egypt.

Abd El-Rahman, H. ; Eisa, L. A. M. and Yassien, M. A. (1989). Occurrence of *C. perfringens* in fish and mollusks. Assuit Vet. Med., *42* (21):80-83.

Abd El-Tawwab, M. ; Shalaby, A. M. E. ; Ahamed, M. H. and Khattab, Y. A. E. (2001). Effect of supplemental dietary L-Ascorbic acid (Vitamin C) on mercury intoxication and growth performance of Nile tilapia (*Oreochromis niloticus* L). Annals Agric. Sci. Moshtohor., *39* (2): 961-973.

Abu El-Ella, S. M. (1996). "Studies on the Toxicity and Bioconcentration of Cadmium on Grass Carp. *Ctenopharyngodon idella.*" M.Sc. Thesis, Fac. of Science, Helwan Univ., Egypt.

Austin, B. and Austin, D. A. (1993). "Bacterial Fish Pathogens Disease in Farmed and Wild Fish." 2nd Ed., Ellis Horwood Limited, U.K.

Aydin, S. ; Gueltepe, N. and Yildiz, H. (2000). Natural and experimental infections of *Campylobacter cryaerophila* in rainbow trout: Gross pathology, bacteriology, clinical pathology and chemotherapy. Fish Pathol., *35* (3): 117-123.

Aydin, S. ; Engin, M. and Bircan, R. (2002). A Comparative investigation of *Arcobacter cryaerophilus* infection among albino crosses and high- and low-body weight rainbow trout. J. Aquat. Anim. Health, *14* (1): 39-44.

Britton, C. J. (1963). "Disorders of the Blood" 9th ed. I.A. Churchill Ld., London.

Brown, C. L. ; Young, G. ; Nishioka, R. S. and Bern, H. A. (1987). Preliminary report on the physiological status of striped bass in the Carquinez Strait die-off. Fish. Res., *6* (1): 5-16.

Bunch, E. C. and Bejerano, I. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* to Streptococcosis. Isr. J. Aquacult., Bamidgeh. *49* (2) 67-76.

Bury, N. R. ; Eddy, F. B. and Codd, G. A. (1996). Stress responses of brown trout, *Salmo trutta* L., to the cyanobacterium, *Microcystis aeruginosa.* Environ. Toxicol. and Water Quality, *11* (3): 187-193.

Bury, N. R. ; McGeer, J. C. ; Eddy, F. B. and Codd, G. A. (1997). Liver damage in brown trout, *Salmo trutta* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), following administration of the cyanobacterial hepatotoxin microcystin-LR via the dorsal aorta. J. Fish Dis., *20* (3): 209-215.

Can, D. C. and Taylor, L. Y. (1982). An outbreak of botulism in rainbow trout, *Salmo gairdneri* Richardson, farmed in Britain. J. Fish Dis., *5*: 393-399.

Caruso, D. ; Schlumberger, O. ; Dahm, C. and Proteau, J. (2002). Plasma lysozyme levels in sheatfish *Silurus glanis* (L.) subjected to stress and experimental infection with *Edwardsiella tarda.* Aquacult. Res., *33* (12): 999-1008.

Charnchit, K. (1985): Pathogenicity and therapeutic studies of *Aeromonas hydrophila* infection in Sand Goby (*Oxyeleotris marmoratus* Bleeker). Abstracts of Master of Science Theses Fisheries Science 2000 p. 2.

Chen, C. ; Wooster, G. A. ; Getchell, R. G. ; Bowser, P. R. and Timmons, M. B. (2003). Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. Aquacult., *218* (1-4): 89-102.

Coles, E. H. (1986). "Veterinary Clinical Pathology". W.B. Saunders. Philadelphia.

Congleton, J. and La Voie, W. J. (2001). Comparison of blood chemistry values for samples collected from juvenile Chinook salmon by three methods. J. Aquat. Anim. Health, *13*: 168-172.

Crisley, F. D. (1960). Routine methods for gold fish assay of toxin in crude culture centrifugates of *Clostridium botulinum* type A. Applied Microbiol., *8*: 282-285.

Dacie. J. V. and Lewis. S. M. (1984). "Practical Haematology." 6th ed Churchill Livingstone. Edinburgh. London. Mellbourne and New York. 22 .pp.

Ehulka. J. ; Baranyiova. E. ; Vykusova. B. ; Svobodova. Z. (1998). The blood indices of the rainbow trout. *Oncorhynchus mykiss* (Walbaum) in *Aeromonas*-induced ulcerous dermatitis. Acta Vet. Brno. *67* (4): 317-322.

El-Ashram. A. M. M. (2002). On *Aeromonas hydrophila* infection among cultured tilapia: A biological. histopathological and management study. Egypt. J. Aquat. Biol. & Fish.. *6* (3):181-202.

El-Deeb. M. I. M. (1998). "Studies on Some Clostridial Microorganisms Isolated From Some Fresh Water Fishes." Ph.D. Thesis. (Microbiology). Fac. Vet. Med.. Zagazig Univ.

El-Ged. A. ; Hafez. M. A. M. ; Eisa. L. A. M. and Hassan. N. R. (1985). Prevalence of *C. perfringens* in catfishes (*Clarias lazera*) in Egypt with special references to physiological and histopathological changes. Ann. Agric. Sci. Moshtohor, *23* (2): 947-959.

El-Sayed, A. F. ; El-Goboshy. A. and Al-Amoundi. M. (1996). Effect of pond depth and water temperature on growth. mortality and body. composition of Nile tilapia (*Oreochromis niloticus*). Aquaculture Res.. *27*: 681–687.

Enany. M. ; El-Sayed. Z. M. ; El-Bouhy. G. S. and El-Kenawy. A. (1989). Preliminary studies on clostridial infection in some fresh water fish. Bull. Fac. Sci. Zagazig Univ., *11*: 9-25.

Essa. M. A. A. (1993). "Studies on the Role of Mycosis and Mycotoxicosis in Freshwater Fishes." Ph.D. Thesis, (Infectious Diseases), Fac. of Vet. Med., Cairo Univ.

Gallagher.. M. L. ; Luczkovich, J. J. and Stellwag, E. J. (2001). Characterization of the ultrastructure of the gastrointestinal tract mucosa, stomach contents and liver enzyme activity of the pinfish during development. J. Fish Biol., *58* (6): 1704-1713.

Gluth, G. and Hanke, W. (1983). The effect of temperature on physiological changes in carp "*Cyprinus carpio*" induced by phenol. Ecotoxicol. Environ. Safety. 7: 373- 389.

Haggag, A. M. ; Marie, M. A. S. ; Zaghloul, K. H. and Eissa, S. M. (1993). Treatment of underground water for fish culture in Abbassa farm, Sharika. II. Biochemical and histological studies. Bull. Fac. Sci. Cairo Univ., *61*: 43–69.

Hibbs, R. G. ; Mishu, B. ; Darwish, A. ; Weber, J. T. ; Hatheway, C. L. ; El-Sharkawy, S. and Corwin, A. (1991). A food borne outbreak of type E botulism in Cairo, Egypt. Am. J. Trop. Med. Hyg., *45* (3): 109-110.

Hill, A. B. (1977). "A Short Textbook of Medical Statistics." 10 ed., The English Language Book Soc. Hodder and Stoughton, London.

Hrubec, T. C. ; Cardinate, J. L. and Smith, S. A. (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Vet. Clin. Pathol., *29*: 7-12.

Husien, M. M. and Elias, N. S. (2000). The role of comparative analysis for enzymatic efficiency with blood picture in early diagnosis of some bacterial freshwater fish diseases. SCVMJ, *III* (2) :625-634.

Husien, M. M. and Younis, A. A. (2000). Efficacy and residues of oxolinic acid in *Oreochromis niloticus* fish infected with enteric red mouth disease (ERM). Egypt. J. Agric. Res., *78* (1): 399-409.

Huss, H. H. : Pedersen, A. and Can, D. C. (1974). The incidence of *C. botulinum* in Danish trout farms. J. Food Technol., *9*: 443-450.

Hussein, S. Y. : El-Nasser, M. A. and Ahmed, S. M. (1996). Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut. Egypt. Bull. Environ. Contam. Toxicol., *57*: 503-510.

Inglis, V. ; Roberts, R. J. and Bromage, N. R. (1993). "Bacterial Diseases of Fish." Institute of Aquaculture. Halsted Press: and Imprint of John Wiley & Sons. INC. N.Y. and Toronto.

Joseph, A. ; Kinght, T. ; Anderson, S. ; James, M. and Rawie, H. (1972). Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipid. Chin. Chem., *18* (3): 198-201.

Kopp, R. and Hetesa, J. (2000). Changes of haematological indices of juvenile carp (*Cyprinus carpio* L.) under the influence of natural populations of cyanobacterial water blooms. Acta Vet. Brno, *69*: 131-137.

Kotak, B. ; Serediak, M. ; Prepas, E. ; Hrudey, S. ; Semalulu, S. and Coppock, R. (1994). Cyanobacterial liver toxins in the aquatic environment: Implications for fish health. Lake Reserv. Manage., *9* (2): 90.

Lucky, Z. (1977). "Methods of Diagnosis of Fish Diseases." Amerind Publishing Co., PVT Ltd, New Delhi, Bombay, Calcutta and N.Y.

Marzouk, M. S. M. (1991). Some haematological studies on Nile tilapia experimentally infected with *flexbacter columinaris* bacteria. J. Egypt. Vet. Med. Ass., *51*, (1-2): 129-136.

Mesa, M. G. ; Maule, A. G. ; Poe, T. P. and Schreck, C. B. (1999). Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? Aquaculture, *174* (1-2): 25-41.

Mesa, M. G. ; Maule, A. G. and Schreck, C. B. (2000). Interaction of infection with *Renibacterium salmoninarum* and physical stress in juvenile Chinook salmon: Physiological responses, disease progression, and mortality. Trans. Am. Fish. Soc., *129* (1): 158-173.

McKeon, C. ; Glenn, E. ; Gerba, C. P. and Fitzsimmons, K. (2000). Microbiological hazards of Tilapia culture system. 5th International Symposium on Tilapia Aquaculture, Brazil.

Niewolak, S. and Tucholski, S. (1995). Sanitary and bacteriological study of common carp reared in ponds supplied with biologically pretreated sewage. Arch. Pol. Fish., *3* (2): 203-215.

Niewolak, S. and Tucholski, S. (2000_a). Ability of bacteriological self-purification of common carp, tench and crucian carp fingerlings reared in pond receiving the discharge from sewage treatment plant and then kept in running river water of different quality. Arch. Pol. Fish., *8* (1): 49-61.

Niewolak, S. and Tucholski, S. (2000_b). Reduction of bacterial contamination of common carp under various rearing conditions. Arch. Pol. Fish., *8* (1): 63-74.

Palti, Y. ; Tinman, S. ; Cnaani, A. ; Avidar, Y. ; Ron, M. and Hulata, G. (1999). Comparative study of biochemical and nonspecific immunological parameters in two tilapia species (*Oreochromis aureus* and *O. mossambicus*). Isr. J. Aquacult.; Bamidgeh, *51* (4): 148-156.

Panebianco, A. ; Minnit, A. ; Conte, F. and Iannuzyi, L. (1993). Study on *C. perfringens* in marine fishes. Arch. Vet. Ital., *44* (2): 64-70.

Pearson, M. D. ; Chinabut, S. ; Karnchanakharn, S. and Somsiri, T. (1994). Jaundice disease in the farmed catfish hybrid, *Clarias macrocephalus* (Gunther) x *C. gariepinus* (Burchell), in Thailand. J. Fish Dis., *17* (4): 325-336.

Pradhan, K. and Pal, J. (1995). Effect of cultures of bacteria isolated from ulcers of air-breathing fishes on RBC count in *Channa punctatus*. Environ. Ecol., *13* (1): 227-232.

Quinn, P. J. ; Carter, M. E. ; Markey, B. K. and Carter, G. R. (1994). "Clinical Veterinary Microbiology." An imprint of Mosby-Year Book Europe Limited. Wolfe Publishing. London. England.

Rani, A..S. ; Sudharsan, R. ; Reddy, T. N. ; Reddy, P. U. M. and Raju, T. N. (2001). Effect of arsenite on certain aspects of protein metabolism in fresh water teleost, *Tilapia mossambica* (Peters). J. Environ. Biol., *22* (2): 101-104.

Rauthan, J. V. S. : Grover, S. P. and Jaiwal, P. (1995). Studies on some haematological changes in a hill stream fish *Barilius bendelisis* (Hamilton) infected with trypanosomes. Flora Fauna Jhansi. *1* (2): 165-166.

Rec. G. S. (1972). Determination of alkaline phosphatase. J. Clin. Chem. Clin. Biochem.. *10*: 182.

Reitman, S. and Frankel, S. (1957). Colorimetric determination of glutamic oxaloacetic and glutamic pyruvite transaminase. .J. Clin. Pathol., *28*: 56.

Rizkalla, E. H. and Tanios, N. I. (1994). Physiological responses of intermittent feeding in fish farms reared under cold natural conditions. Bull. Zool. Soc. Egypt. *42*: 25-44.

Rizkalla, E. H. ; Zahra, M. H. ; Deab, A. A. and Farag, M. E. (1997). Haematological responsiveness of *Oreochromis aureus* to dietary aflatoxins contamination and the role of selenium and vitamin E. Alex. J. Vet. Science. *13* (4): 423-438.

Rizkalla, E. H. ; Yanni, A. A. and Ali, A. A. (1999). Incidence of *Clostridium perfringens* infection in catfish *Clarias lazera* and its effect on some biochemical and histopathological parameters. Alex. J. Vet. Sci., *15* (5): 1077- 1096.

Rizkalla, E. H. ; Fahmy, B. G. ; Zaghloul, H. H. ; El-Sadek, S. and Feisal, R. A. (2003). Comparative study of some antibiotics on biochemical changes in plasma of the sharptooth catfish *Clarias gariepinus*. Egypt. J. Aquatic Biol. & Fish., *7* (4): 381-402.

Roberts, R. J. (1989). "Fish Pathology." Bailliere Tindall, London.

Schalch, B. ; Spemer, B. ; Eisgruber, H. and Stolle, A. (1999). Molecular methods for the analysis of Clostridium perfringens relevant to food hygiene. Immunol. Med. Microbiol., *24* (3): 281-286.

Shalaby, A. M. E. (1997). "Biochemical and Physiological Studies on Metal Contamination in the Common Carp (*Cyprinus carpio* L)." Ph.D. Thesis, Zoology Dept., Fac. of Science, Zagazig Univ. (Benha).

Shariff, M. ; Jayawardena, P. A. ; Yusoff, F. M. and Subasinghe, R. (2001): Immunological parameters of Javanese carp *Puntius gonionotus* (Bleeker) exposed to copper and challenged with *Aeromonas hydrophila*. Fish Shellfish Immunol., *11* (4): 281-291.

Siau, H. and Ip, Y. K. (1987). Activities of enzymes associated with phosphoenolpyruvate metabolism in the mudskippers. *Boleophthalmus boddaerti* and *Periophthalmodon schlosseri*. Comp. Biochem. Physiol., *88* B (1): 119-125.

Snieszko, S. F. (1958). Natural resistance and susceptibility to infection progressive. Fish Culture, *20*: 133-138.

Stern, M. and batty, I. (1975). "Pathogenic Clostridia." Butter Worth, London, Boston.

Stoskopf, M. K. (1993). "Fish Medicine." W.B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.

Terao, T. and Ogawa, T. (1984). On the biochemical components in blood of the cultured cichlid fish, *Tilapia nilotica*. Sci. Pap. Hokkaido Fish Hatch., *39*: 83-88.

Trinder, P. (1969). Determination of glucose concentration in the blood. Aann. Clin. Biochem., *6*: 24.

Vankampen, E. J. (1961). Determination of haemoglobin. Clin. Chem. Acta., *6*: 538- 544.

Verma, S. R. and Tonk, I. P. (1983). Effect of sublethal concentration of mercury on the composition of liver, muscle and ovary of *Notopterus notopterus*. Water, air & Soil Pollution, *20*: 287-292.

Willis, A. T. (1977). "Anaerobic Bacteriology, Clinical and Laboratory Practical." 3 rd. Ed. Butter Worth, London, Boston, pp. 131-133.

Wootton, L. D. P. (1964). "Micro-analysis in Medical Biochemistry in Micrometer." 4th ed., p: 264, Churchill, London, Basel, Kager.

Yavuzcan Yildiz, H. : Pulatsu, S. and Kurtoglu, F. (1997). Baseline haematological and serological parameters of healthy Nile tilapia (*Oreochromis niloticus* L.). Anim. Sci. Pap. Rep., *15*: 213-217.

Yen, S. S. and Shyan, L. M. (1999). Feeding strategy does affect carbohydrate utilization by hybrid tilapia *Oreochromis niloticus* x *O. aureus*. Fish Sci., *65* (4): 553-557.

Young, G. ; Brown, C. L. ; Nishioka, R. S. ; Folmar, LC. ; Andrews, M. ; Cashman, J. R. and Berm, H. A. (1994). Histopathology, blood chemistry, and physiological status of normal and moribund striped bass (*Morone saxatilis*) involved in summer mortality "die-off" in the Sacrament San Joaquin Doaquin Delta of California. J. Fish Biol., *44* (3): 491-512.

Table (1): Mean ± Standard deviation of erythrocytic count (RBCs), haemoglobin content (Hb), haematocrit value (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total leucocytic count (WBCs) in blood of control and *Clostridium perfringens* (types A, D and A+D) infected *Oreochromis niloticus* after 3, 7 and 14 days of intraperitoneal injection.

Parameter	treatment	Exposure period (days) 3 Mean	± S.D.	7 Mean	± S.D.	14 Mean	± S.D.	$_{18}F^{3}$-test
RBCs ($X10^6/mm^3$)	Control	1.330	0.134	1.684	0.192	1.822	0.298	N.S.
	C. perf. A	0.870	0.329**	1.105	0.280***	1.328	0.191**	N.S.
	C. perf. D	0.630	0.114***	1.020	0.329***	0.962	0.070***	N.S.
	C. perf. A+D	0.977	0.068***	1.513	0.458	1.610 *	0.302	N.S.
	$_{24}F^{4}$-test	0.01		N.S.		0.01		
Hb (g/dl)	Control	7.397	0.943	8.213	0.734	9.312	1.268	N.S.
	C. perf. A	4.920	1.966*	5.060	1.313***	8.023	1.629	N.S.
	C. perf. D	4.330	0.274***	10.01	2.267	5.143	0.771***	0.01
	C. perf. A+D	4.367	1.583**	7.432	1.824	7.840	1.734	N.S.
	$_{24}F^{4}$-test	0.05		0.05		0.05		
Hct (%)	Control	12.67	2.582	12.34	2.491	12.23	1.323	N.S.
	C. perf. A	13.67	2.733	8.600	1.855**	12.20	2.227	N.S.
	C. perf. D	8.50	2.665*	11.67	2.805	11.33	4.274	N.S.
	C. perf. A+D	10.42	0.492	10.00	3.347	9.333	1.633**	N.S.
	$_{24}F^{4}$-test	0.05		N.S.		N.S.		
MCV (μm^3)	Control	9.500	1.648	7.345	1.453	6.779	0.562	N.S.
	C. perf. A	16.64	3.202***	7.939	1.393	9.405	2.344*	0.05
	C. perf. D	13.29	2.582**	11.95	3.490**	11.84	4.612*	N.S.
	C. perf. A+D	10.71	0.956	7.228	3.420	5.85	0.837*	N.S.
	$_{24}F^{4}$-test	0.05		N.S.		0.05		
MCH (pg)	Control	54.73	2.107	49.18	6.090	51.48	4.797	N.S.
	C. perf. A	57.77	14.65	46.73	9.482	60.20	6.519*	N.S.
	C. perf. D	71.13	16.40*	104.9	37.26**	53.62	8.330	N.S.
	C. perf. A+D	44.52	14.89	51.73	23.81	49.83	13.28	N.S.
	$_{24}F^{4}$-test	N.S.		0.05		N.S.		
MCHC (%)	Control	59.14	10.64	68.50	12.00	76.24	7.961	N.S.
	C. perf. A	35.22	8.532***	60.22	15.36	68.73	24.12	N.S.
	C. perf. D	56.31	22.56	88.29	24.81	49.37	15.07**	N.S
	C. perf. A+D	42.39	16.86	77.75	20.28	83.74	21.88	N.S.
	$_{24}F^{4}$-test	N.S.		N.S.		N.S.		
WBCs ($X10^3/mm^3$)	Control	4.267	0.677	8.581	0.663	14.50	4.570	0.05
	C. perf. A	5.400	1.239	7.800	2.635	10.47	5.061	N.S.
	C. perf. D	3.667	0.882	5.183	1.099***	8.300	1.657**	0.05
	C. perf. A+D	5.333	1.294	7.017	1.701	8.911	0.863**	0.05
	$_{24}F^{4}$-test	N.S.		N.S.		N.S.		

Number of fish in each time and group = 6 N.S.: Not significant

Significant in comparison with the control: * $P < 0.05$. ** $P < 0.01$ and *** $P < 0.001$

Table (2): Mean ± Standard deviation of plasma total proteins (TP), glucose, total lipids (TL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in control and *Clostridium perfringens* (types A, D and A+D) infected *Oreochromis niloticus* after 3, 7 and 14 days of intraperitoneal injection.

Parameter	treatment	Exposure period (days)						$_{ts}F^{1}$-test
		3		7		14		
		Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	
TP (g/dl)	Control	1.647	0.201	2.449	0.578	1.743	0.645	N.S.
	C. perf. A	1.557	0.369	2.273	0.882	1.497	0.336	N.S.
	C. perf. D	0.953	0.179***	1.723	0.509*	0.930	0.105**	0.05
	C. perf. A+D	0.867	0.411***	1.187*	0.561**	1.367	0.306	N.S.
	$_{24}F^{1}$-test	0.05		N.S.		N.S.		
glucose (mg/dl)	Control	52.40	4.774	59.79	1.223	51.42	4.112	0.05
	C. perf. A	64.16	15.06	54.10	10.19	58.52	9.416	N.S.
	C. perf. D	70.23	8.295***	88.41	24.55*	76.55	8.555***	N.S.
	C. perf. A+D	37.78	4.212***	59.00	10.54	42.69	6.343*	0.05
	$_{24}F^{1}$-test	0.01		0.05		0.01		
TL (g/dl)	Control	9.685	1.694	9.712	0.600	10.67	1.666	N.S.
	C. perf. A	12.21	3.602	10.51	1.810	11.91	1.684	N.S.
	C. perf. D	6.509	1.306**	6.552	1.823***	4.735	0.951***	N.S.
	C. perf. A+D	8.822	1.675	5.427	1.307***	7.180	1.993**	N.S.
	$_{24}F^{1}$-test	0.05		0.01		0.01		
AST (IU/L)	Control	145.7	18.68	160.0	4.063	128.7	15.46	N.S.
	C. perf. A	103.3	18.13**	126.5	32.57*	112.9	22.82	N.S.
	C. perf. D	66.41	19.12***	91.13	14.30***	89.18	10.86***	N.S.
	C. perf. A+D	71.45	15.43***	117.22	20.13***	41.15	7.036***	0.01
	$_{24}F^{1}$-test	0.01		0.05		0.01		
ALT (IU/L)	Control	16.67	2.251	18.69	2.017	10.23	0.896	0.01
	C. perf. A	15.27	3.048	30.17	2.507***	15.27	1.369***	0.01
	C. perf. D	15.33	0.186	38.20	6.537***	14.17	0.723***	0.01
	C. perf. A+D	26.33	6.088**	17.00	2.869	21.70	2.250***	N.S.
	$_{24}F^{1}$-test	0.05		0.01		0.01		
ALP (IU/L)	Control	87.26	12.62	83.48	9.848	43.63	4.875	0.01
	C. perf. A	55.30	12.92***	72.10	4.601*	32.70	8.597*	0.01
	C. perf. D	49.08	0.809***	31.51	6.994***	27.27	3.725***	0.01
	C. perf. A+D	21.19	4.974***	27.87	1.242***	20.61	3.749***	N.S.
	$_{24}F^{1}$-test	0.01		0.01		0.01		

Number of fish in each time and group = 6 N.S.: Not significant

Significant in comparison with the control: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

Table (3): Mean ± Standard deviation of liver total proteins (TP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in control and *Clostridium perfringens* (types A, D and A+D) infected *Oreochromis niloticus* after 3, 7 and 14 days of intraperitoneal injection.

Parameter	treatment	Exposure period (days) 3 Mean	3 ± S.D.	7 Mean	7 ± S.D.	14 Mean	14 ± S.D.	$_{18}F^{3}$-test
TP (mg/g)	Control	9.467	2.686	11.19	0.786	13.28	1.912	N.S.
	C. perf. A	12.29	1.556*	12.52	2.030	12.23	2.701	N.S.
	C. perf. D	10.42	1.845	16.14	1.967***	15.57	1.563*	0.05
	C. perf. A+D	8.733	1.878	10.97	3.120	21.30	8.240*	0.05
	$_{24}F^{3}$-test	N.S.		0.05		N.S.		
AST (IU/g)	Control	122.6	14.47	186.1	3.119	141.1	16.07	0.01
	C. perf. A	141.8	22.48	120.4	13.19***	120.6	14.31*	N.S.
	C. perf. D	107.1	21.77	108.0	21.73***	124.5	11.63	N.S.
	C. perf. A+D	101.5	17.10*	309.3	45.40***	166.5	18.26*	0.01
	$_{24}F^{3}$-test	N.S.		0.01		0.05		
ALT (IU/g)	Control	23.00	4.419	21.55	4.400	21.47	1.529	N.S.
	C. perf. A	31.26	4.305**	17.79	4.499	17.83	1.819**	0.05
	C. perf. D	34.13	3.780***	24.93	4.419	22.53	4.741	0.05
	C. perf. A+D	35.10	4.929***	51.03	12.54***	28.53	5.532**	0.05
	$_{24}F^{3}$-test	0.05		0.01		0.05		

Number of fish in each time and group = 6 N.S.: Not significant
Significant in comparison with the control: * P < 0.05, ** P < 0.01 and *** P < 0.001

Table (4): Mean ± Standard deviation of muscle total proteins (TP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in control and *Clostridium perfringens* (types A, D and A+D) infected *Oreochromis niloticus* after 3, 7 and 14 days of intraperitoneal injection.

Parameter	treatment	Exposure period (days) 3 Mean	3 ± S.D.	7 Mean	7 ± S.D.	14 Mean	14 ± S.D.	$_{18}F^{3}$-test
TP (mg/g)	Control	8.913	1.178	6.963	0.948	7.437	1.931	N.S.
	C. perf. A	4.513	1.164***	4.993	0.631***	4.553	0.530**	N.S.
	C. perf. D	7.993	1.763	4.430	0.521***	4.357	0.581**	0.05
	C. perf. A+D	4.463	0.614***	4.050	0.220***	5.760	0.769	0.05
	$_{24}F^{3}$-test	0.01		0.01		0.05		
AST (IU/g)	Control	220.8	28.37	228.0	17.28	231.4	21.31	N.S.
	C. perf. A	160.4	24.11**	189.3	17.33**	233.8	23.25	0.05
	C. perf. D	216.0	18.36	220.0	15.86	164.6	22.42***	0.05
	C. perf. A+D	200.7	24.34	183.4	8.640***	200.0	6.059**	N.S.
	$_{24}F^{3}$-test	0.05		0.05		0.01		
ALT (IU/g)	Control	12.35	1.004	9.231	0.283	7.853	0.564	0.01
	C. perf. A	7.807	1.390***	7.620	1.498*	9.333	2.357	N.S.
	C. perf. D	25.47	6.549***	12.64	4.733	4.533	0.696***	0.01
	C. perf. A+D	7.813	2.174***	10.35	2.218	12.02	2.842**	N.S.
	$_{24}F^{3}$-test	0.01		N.S.		0.01		

Number of fish in each time and group = 6 N.S.: Not significant
Significant in comparison with the control: * P < 0.05, ** P < 0.01 and *** P < 0.001

EXPLANATION OF FIGURES

Fig. (1): Muscles showing edema, necrosis and leucocytic infiltration. H & E stain (x 150).

Fig. (2): Gills showing sloughing of secondary lamella (Sl) and desquamation of epithelial covering. H & E stain (x 150).

Fig. (3): Gill arch showing congestion (C), hemorrhage (h) and edema. H & E stain (x 150).

Fig. (4): High power of Fig. (3) showing intact erythrocytes (er). H & E stain (x 300).

Fig. (5): Liver showing fatty changes (V). H & E stain (x 600).

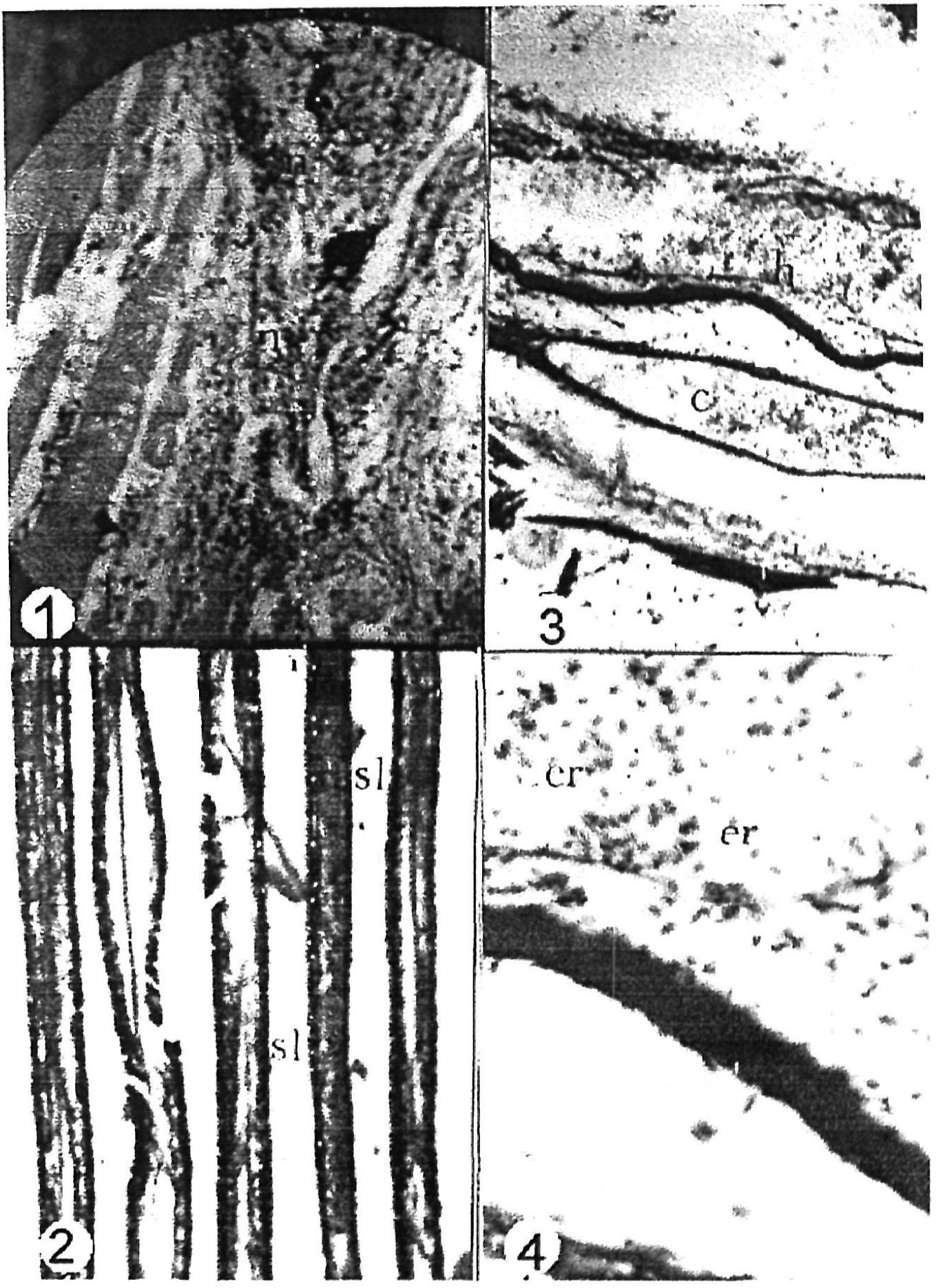
Fig. (6): Liver showing congestion (C) and thickening of blood vessel wall and leucocytic infiltration. H & E stain (x 600).

Fig. (7): Kidney showing hyaline casts (hy) and degenerative changes in the renal tubules. H & E stain (x 150).

Fig. (8): Kidney showing thickening of renal capsule (t), hemorrhage and hyaline droplets degeneration. H & E stain (x 150).

Fig. (9): High power of Fig. (8) showing fibrous tissue proliferation of renal capsules (f). H & E stain (x 600).

Fig. (10): Spleen showing proliferation of melanomacrophage center. H & E stain (x 150).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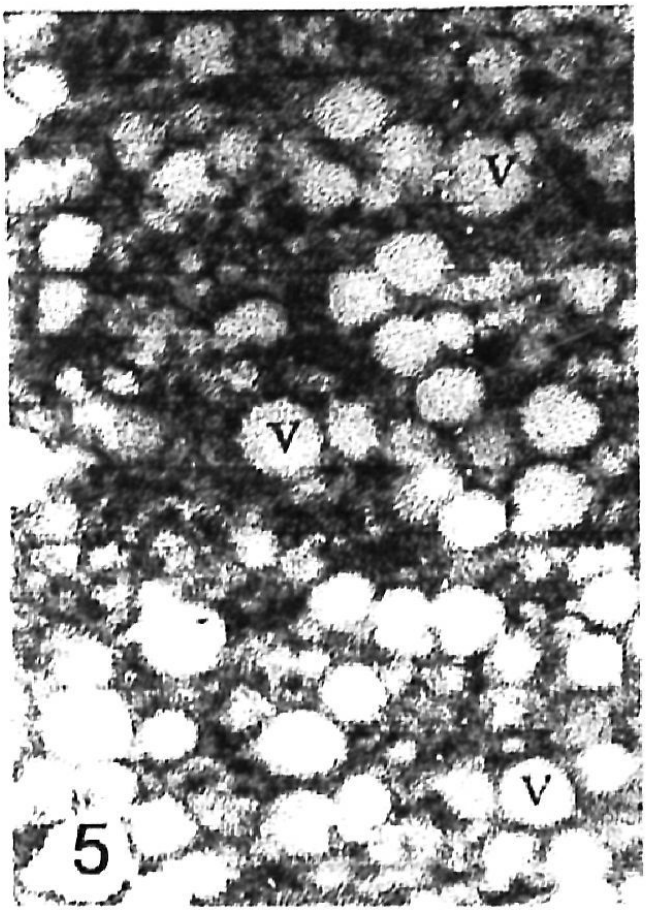

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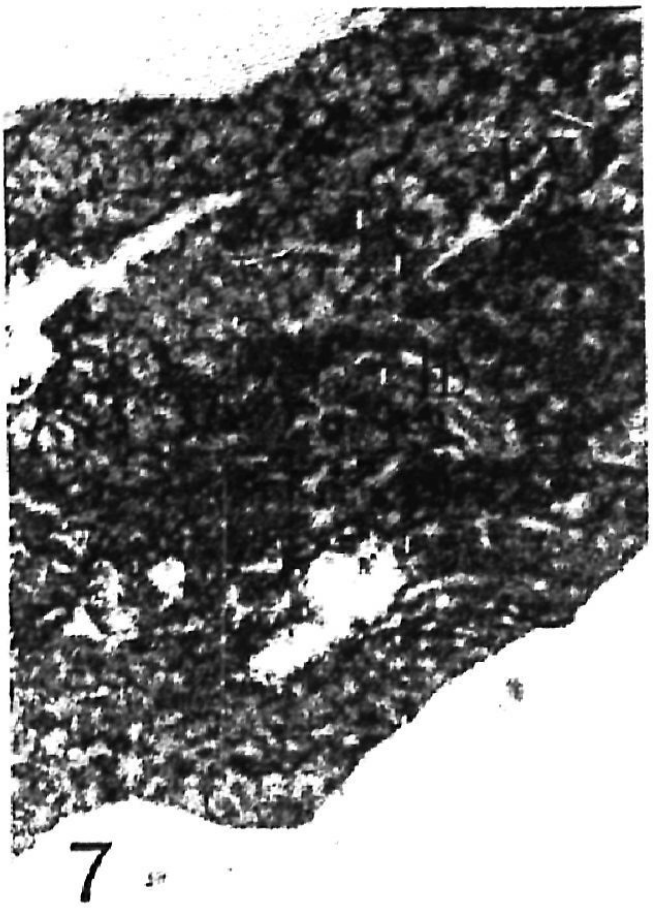

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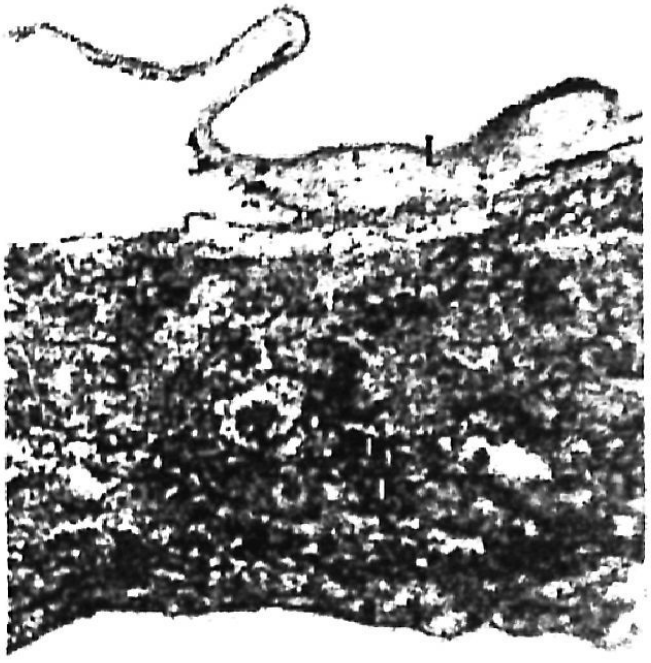

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