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ACUTE TOXICITY OF ALKYLBENZENE SULPHONATE (ABS) DETERGENT TO TILAPIA, OREOCHROMIS SPILURUS SPILURUS (GUNTER) UNDER HYPERSALINE WATER CONDITIONS

(With 1 Table and 13 Figurs)

By

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التأثير السمي لمركب الالكيل بنزين (الامو الأزرق) علي أسماك البلطي تحت ظروف الملوحة العالية

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تم دراسة التأثير السمي لمركب الالكيل بنزين (الامو الأزرق) علي أسماك البلطي المرباة تحت ظروف الملوحة العالية ود لك باستخدام مياه ساكنه وهواء لمده ٩٦ ساعة. تم تحديد الجرعة النصف مميته LC50 وكد لك وصفت التغيرات الباثولوجية بالعين المجردة والميكروسكوب الضوئيي في الخياشيم والجلد والمخ للاسماك المعرضة لمركب الالكليل بنزين مقارنه بضوابط التجربة وكانت الجرعة النصف مميتة LC50 تحت ظروف الملوحة العالية (٤٣جم /لتر) تساوي٧ ٨و٢١ مملجم /لتر بحدود ثقه Confidence Limits ١٥و٣ و٥٥ و. مملجم .أظهرت التغيرات بالعين المجردة احتقان شديد بالخياشيم وتبيض القوس الخيشومي ووجود مساحات بيضاء علي الجلد مع فقدان للمخاط والقشور وكدلك وجود نقط نزيفية علي الزعنفة الصدرية والفك السفلي . أما التغيرات الهستوباثولوجية في الأسماك المعرضة للجرعات٧ ٨و ٢١ و ٢٥ مملجم/ لتر تمثلت بالتحام الرقائق الثانوية وتتكرز الخلايا الغضروفية المدعمة للقوس الخيشومي و تضخم خلايا الكلوريد و تكسير في الخلايا المبطنة للأوعية الدموية مع خروج كرات الدم الحمراء. كانت هناك تغيرات في جلد الاسماك المعرضة للمركب متمثله بتتكرز خلايا الأدمة وانتشار الميلانين في الادمة مقارنه بضوابط التجربة. لوحظ وجود مناطق متعددة ومحدده من نزيف بالنسيج المخي مع انتفاخ للخلايا العصبية.

SUMMARY

Acute toxicity of alkylbenzene sulphonate (ABS, Omo blue) detergent to tilapia, Oreochromis spilurus spilurus (Gunter) raised in hypersaline water conditions was determined using static bioassay with aeration over

a period of 96 h. The 96-h LC_{50}, gross and histopathological changes of the gills, skin, and brain were evaluated. The 96-h LC_{50} was determined to be 21.87 mg l^{-1} with lower and upper confidence limits being 3.15 and 0.55 mg l^{-1} respectively. The gills of dead fish showed severe congestion and whitish discoloration of the gill arch, especially those which died at higher doses (25 mg l^{-1}). Focal whitish area of the skin in the trunk region with loss of mucous and scales were observed. Moreover, petichael haemorrhage at the base of pectoral fins and the curvature of the lower jaws was detected. The brain had no obvious gross lesions. Microscopically, the gills of dead fish exposed to 21.87 mg l^{-1} and higher dose of 25mgl^{-1} had lamellar fusion, necrosis of the secondary lamellar epithelium, necrosis of the chondrocytes supporting the gill arch, hypertrophy of chloride cells and damaged endothelial lining of the blood vessels with extravasated red blood cells were observed. The skin epidermis showed necrotic changes with hypertrophy of mucous cells and dispersion of melanin pigment throughout the epidermis and hypodermis. The brain tissue had multifocal areas of haemorrhage and neuronal swelling. The significance of these changes to behavioral and physiological responses are discussed.

Key words: *Acute toxicity, alkylbenzene sulphonate, tilapia.*

INTRODUCTION

Oreochromis spilurus spilurus, (Gunter) had been introduced and successfully raised under hypersaline water conditions at the Fish Farming Center, Jeddah, and Kingdom of Saudi Arabia since 1982 (Osborne 1979). The ability of tilapias to thrive in saline environment has been studied and tested by several authors (Stickney 1986; Al-Amoudi 1987 a and Jonassen; Pittman & Imslans 1997).

Synthetic detergents are known to be toxic to fish at concentrations between 0.4-40 mg l^{-1} (Abel 1974). Moreover, the lower levels of these detergents will increase the uptake of other pollutants by the fish (Abel & Skidmore, 1977 and Dauvram, 1987). Detergents are commonly used in houses and industrial products and its "after wash" is eventually drained into the aquatic environment, where it constitutes a major pollutant to the aquatic organisms (Okwuosa and Omoregie, 1995). The 96-h LC_{50} of alkylbenzene sulphonate (Omo blue) to the toothed carp, Aphyosemion gairdneri (L) of freshwater was 25.11 $\pm$ 8.4 mg l^{-1} . The exposed fish had erratic swimming, loss of balance and

respiratory distress prior to death. Likewise skin lesions and haemorrhaes of the gill filaments were observed on dead fish (Okwuosa and Omoregie, 1995).

Several acute toxicity studies of different chemicals in normal saline water (36 g L^{-1} had been reported (Wise & Tomasso, 1989, Tudor, Katavic & MarsicLucic, 1994; and Gaikowski, Rach & Ramsay, 1999). However, reports on acute toxicity in hypersaline water are scarce. The objective of this study was to determine the 96-h LC_{50} of alkylbenzene sulphonate to the Oreochromis spilurus spilurus, (Gunter) raised in hypersaline conditions along the Red Sea coast of Saudi Arabia (43 g L^{-1} and temperature of 30 $\pm$ 5 °C) and to describe gross and microscopic changes in acutely exposed fish.

MATERIALS and METHODS

Tilapia fingerlings, Oreochromis spilurus spilurus, (Gunter) were collected from the nursery tanks of the Fish Farming Center facilities. These fingerlings were raised at temperatures of 30 $\pm$ 5 °C and hypersaline water of 43 g L^{-1}. Twenty fish were stocked in four circular tanks of 200 l provided with seawater and aeration. These fish were of 3.95 $\pm$ 0.36 g body weight and acclimated to the indoor temperature of 29 $\pm$ 3 °C for two weeks. The fish were fed twice daily on 38% protein commercial crumbles of 4mm diameter up to satiation at 8 AM and 14 PM. Dissolved oxygen, temperature and salinity were monitored during acclimation and throughout the duration of the experiment..

A stock solution was prepared by dissolving 800 mg of the powder in 1 l of freshwater. From this stock solution, Four concentrations of 25, 22.65, 21.87, and 18.75mg l^{-1} were made. These concentrations were chosen based on several preliminary experimentations. The above concentrations were prepared first in the tanks. Twenty fish were randomly assigned for each treatment with another twenty non-exposed fish used as control. The 96h-LC_{50} was investigated using static bioassay with aeration according to UNEP/FAQ/IAEA, (1989). Fish were observed regularly with mortality and gross lesions recorded every 24h.

Six dead fish exposed to 25 and 21.87 mg l^{-1} and six non-exposed fish were dissected with their gills, skin, and brain were taken. These samples were fixed immediately in 10% formalin, dehydrated, embedded in paraffin, sectioned at 4-6 $_{u,}$ stained by Haematoxylin and Eosin and examined by light microscopy.

RESULTS

The dissolved oxygen concentration, temperature, and salinity were 5.7 mg l, 29 °C; 43 g L^{-1} at the start and during exposure. The mortality rates of Oreochromis spilurus spilurus, (Gunter) exposed to various concentrations of ABS are shown in Table 1. The mean mortality expressed as percent increased with increase in the detergent concentrations.. The linear relationship between percentage mortality and detergent concentrations is shown in Fig.1. The 96h-LC_{50} was observed to be 21.87 mg l^{-1} with lower and upper confidence limits being 3.15 and 0.55 mg l^{-1} respectively. The ($r = 0.861$) value is significant at ($P<0.05$) using the simple linear correlation coefficients. No mortality was observed in non-exposed fish

The color of exposed fish prior to death was darker. The major gross lesions were severe congestion of the gills of fish exposed to higher doses (25; 22.65 mg l^{-1}). Moreover, whitish discolouration of the gill arches and operculum were also observed. Petechael haemorrhage was noticed at the curvature of the lower jaws and the base of pectoral fins. The skin had whitish discolouration . Loss of scales and mucous were also detected in focal areas of the trunk region. No gross lesions were observed in the brains of exposed fish.

Microscopically, the gills of the dead fish using doses of 21.87 and 25 mg l^{-1} have severe hyperaemia of the blood vessels, hypertrophy of the chloride cells, lamellar fusion and necrosis of the secondary lamellar epithelia in comparison with that of the non-exposed fish (Figs.2,3,4;5). Damaged endothelial cell lining the blood vessels in the gill arch with extravasated red blood cells were also noticed (Fig.6). Moreover, necrosis of the chondrocytes supporting the gill arches was evident in exposed fish (Fig.7). Figs. 8; 9 showed necrosis of the epidermal cells of the skin and dispersion of melanin pigment throughout the epidermis and hypodermis were observed compared to the control. The cereberal tissue had multifocal areas of haemorrhages in the brain of the exposed fish compared the non-exposed fish (Figs 10;11). Neuronal swelling with marginated nuclei were also observed (Fig.12; 13).

DISCUSSION

Water quality parameters measured in this study are within the suggested tolerance ranges for O. spilurus spilurus raised at the FFC environmental conditions of 43 g L^{-1} salinity and temperature of 30 ±

5°C (Osborne 1979). Toxicity of ABS to the tested species in this study increased with increasing detergent concentrations. This observation is in agreement with previous acute toxicity studies (Omoregie, Ofojekwu, Anosike & Adeleye, 1998; Gaikowski, Rach & Ramsay, 1999). The 96-h LC_{50} of 21.87 mg l^{-1} with lower and upper confidence limits being 3.15; 0.55 mgl^{-1} determined in this study is within the range of 0.4 to 40.00 mg l^{-1} previously reported by Abel (1974), for several fish species. Okwuosa& Omoregie (1995) determined the 96h-LC_{50} to the toothed carp, Aphyosemion gairdneri L using ABS being 25.11 $\pm$ 8.4 mg l^{-1}. Cairns & Scheier (1964) reported a 96h-LC_{50} of 22 mgl^{-1} to the pumpkin seed fish, Lepomis gibbosus (L) exposed to ABS. The 96h-LC_{50} obtained in this study could be attributed to differences in fish species, age and experimental conditions such as salinity. The present study indicates that ABS is toxic to the O. spiulurus in hypersaline water conditions and high temperature. Although salinity is known to reduce the toxicity of chemicals, increase in temperature is known to increase toxicity (Peppard, Wolters, Avault & Perry 1991).

The black colouration of the tilapia exposed to ABS in this study prior to death had also been reported by De Silva & Ranasinghe 1989, Okwuosa & Omoregie 1995; Omoregie & Okpanachi 1997. The present study showed dispersion of melanin pigment in the skin epidermis and hypodermis of exposed fish resulting in the black colouration of fish prior to death.

Gill lesions caused by toxins are predicted to have several physiological effects on the fish, which include gas exchange, ammonia excretion and ion regulation (Randall & Daxboeck 1984; Heisler1984; Evans 1993). Common lamellar changes include oedema, adhesion, epithelial necrosis, hyperplasia of mucous and chloride cells in response to diverse environmental toxins (Mitchell & Cech 1983; Ewing, Black, Blazer & Kocan 1994). The present study showed hyperaemia of the blood vessels of the gills, and endothelial damage of the blood vessels which explain the congestion and petechial haemorrhage observed grossly and suggesting sign of acute toxicity. Meanwhile, the necrosis of the chondrocytes supporting the gill arches explains the whitish discolouration of the operculum and gill arches observed grossly. The necrosis of lamellar epithelium observed in this study suggests respiratory failure due to the detergent. Previous report on toxicity of ABS to the toothed carp, Aphyosemion gairdeneri (L) indicated that respiratory distress was responsible for the mortalities observed (Okwouosa & Omerogio 1995).

The chloride cells are cells located in the lamellar trough with brightly eosinophilic cytoplasm and are responsible for acclimation of fish in different salinity and has the ability to osmoregulate (Grizzle & Rogers 1976; Hwang 1987 and Hwang, Sun & Wu, 1989). These cells have been shown to increase in number and size in response to toxins such as elevated ammonia levels and chloramine-T exposure in rainbow trout, Onchorhynchus mykiss (Powell, Wright and Speare 1995). The chloride cell hypertrophy observed from the exposed fish in the present study indicate a response due to the toxic effect of the detergent.

The necrotic changes in the epidermis of the skin of fish exposed to ABS suggest osmoregulatory disorder had occurred, which lead to loss of balance. Loss of balance has been reported as a reponse to detergents and agrochemicals exposure in fishes (DeSilva & Ranasinghe 1989 and Okwouosa & Omerogio, 1995). Nervous disorders has been attributed to the mortalities observed in fish exposed to detergents (De Silva & Ranasinghe, 1989 and Ufodike & Omerogio, 1990). The present multifocal areas of haemorrhage and neuronal swelling indicate nervous disturbances in fish which died to ABS exposure.

In conclusion, the 96h-LC50 of ABS to the tilapia, Oreochromis spilurus spilurus (Gunter) raised in hypersaline water conditions of Saudi Arabia (21.87mg l^{-1}) which is lower than that reported in freshwater environment. But still ABS is toxic in hypersaline water. The histopathological changes reported in the present study indicate the acute toxicity signs and had affect the diverse functions and morphology of the gills, skin, and brain of tilapia exposed to ABS detergent in hypersaline water conditions.

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FIGURE LEGENDS

Fig. 1: Linear relationship between percentage mortality and log concentration of O. spilurus spilurus exposed to various concentrations of alkylbenzene sulfonate (ABS) detergent for 96 hours.

Fig. 2: Gills of O. spilurus exposed to 21.87 mg l^{-1} of ABS detergent which died at 96h showing hyperaemia (H) of blood vessels. Haematoxylin and Eosin. X 13.2.

Fig. 3: Gills of O. spilurus exposed to 21.87 mg l^{-1} of ABS detergent which died at 96h showing hypertrophy of chloride cells (C). Haematoxylin and Eosin. X 132.

Fig. 4: Gills of O. spilurus exposed to 25 mg l^{-1} of ABS detergent which died at 24h showing severe lamellar necrosis (n) and lamellar fusion (F). Haematoxylin and Eosin. X 132.

Fig. 5: Gills of non- exposed O. spilurus showing the normal architecture of gill tissue and normal appearance of the chondrocytes supporting the gill arches. Haematoxylin and Eosin. X 33.

Fig. 6: Gills of O. spilurus exposed to 21.87 mg l^{-1} of ABS detergent which died at 96h showing damage of the endothelial lining blood vessels (D) with extravasated red blood cells (R). Haematoxylin and Eosin. X 132.

Fig. 7: Gills of O. spilurus exposed to 25 mg l^{-1} of ABS detergent which died at 24h showing severe necrosis of the chondrocytes supporting the gill arches (N). Haematoxylin and Eosin. X 132.

Fig. 8: Skin of O. spilurus exposed to 21.87 mg l^{-1} of ABS detergent which died at 96h showing necrotic changes of the epidermis (N) and dispersion of melanin pigment (P). Haematoxylin and Eosin. X 132.

Fig. 9: Skin of non-exposed O. spilurus showing the normal appearance of the epidermis (E), mucous cells (C), dermis (D), and muscular layer (M). Haematoxylin and Eosin. X 132.

Fig. 10: Brain tissue of O. spilurus exposed to 21.87 mg l^{-1} of ABS detergent which died at 96h showing multifocal areas of haemorrhages (G). Haematoxylin and Eosin. X33.

Fig. 11: Brain tissue of non-exposed O. spilurus showing the normal architecture . Haematoxylin and Eosin. X 33.

Fig. 12: Brain tissue of exposed O. spilurus to 21.87 mg l^{-1} of ABS detergent which died at 96h showing neuronal swelling with marginated nuclei (Arrow). Haematoxylin and Eosin. X 132.

Fig. 13: Brain tissue of non-exposed O. spilurus showing the normal appearance of the neurons . Haematoxylin and Eosin. X 132.

Table 1: Mortality rate of *Oreochromis spilurus*, spilurus (Günter) exposed to Alkylbenzene sulphonate detergent for 96 h. in hypersaline water conditions.

Toxicant Conc. (mg l^{-1})	Log Conc. (mg l^{-1})	24	48	72	96	Mean Mort. (%)
25	1.39	18	0	0	0	90
22.65	1.35	10	5	1	0	80
21.87	1.33	2	1	5	2	50
18.75	1.27	0	0	0	3	15
0.00	0.00	0	0	0	0	0.0

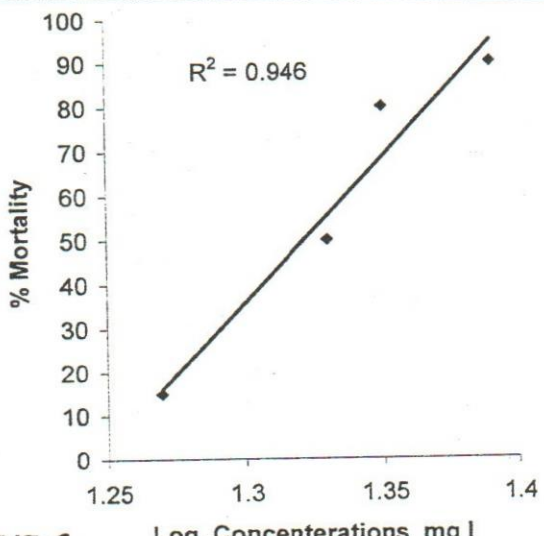
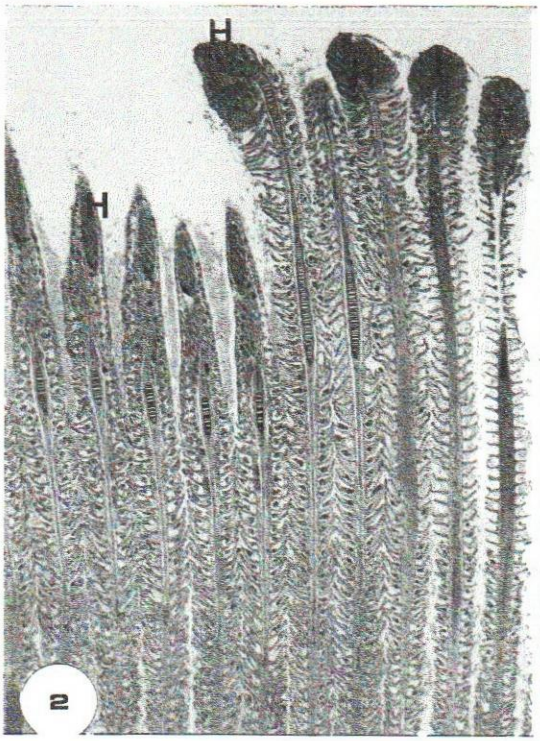


Fig. 1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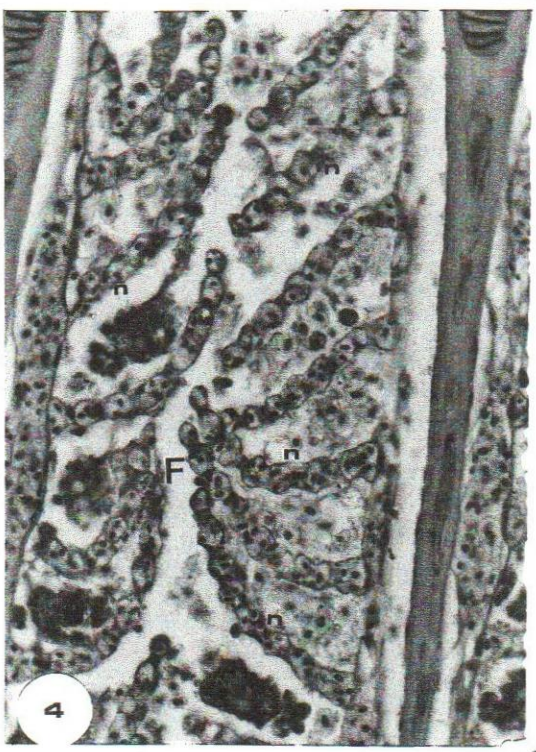
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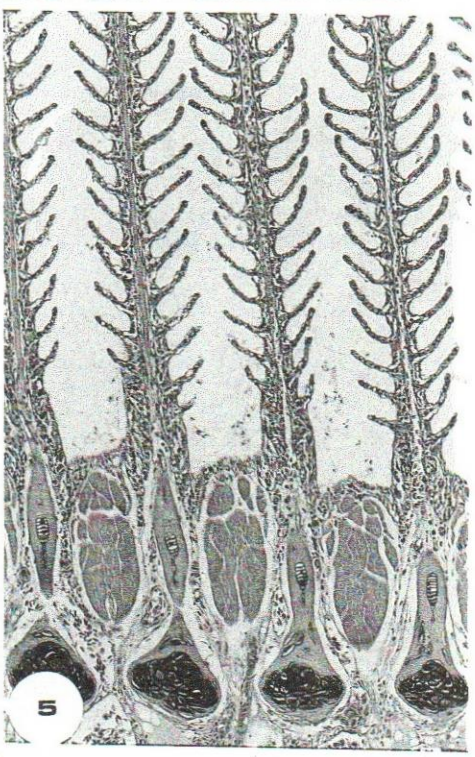
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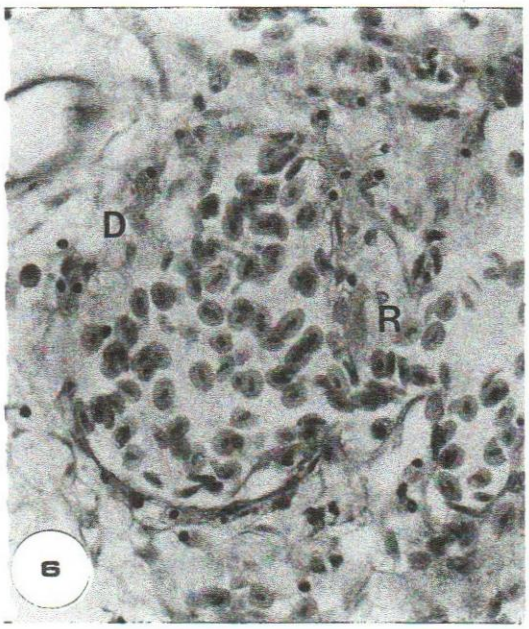
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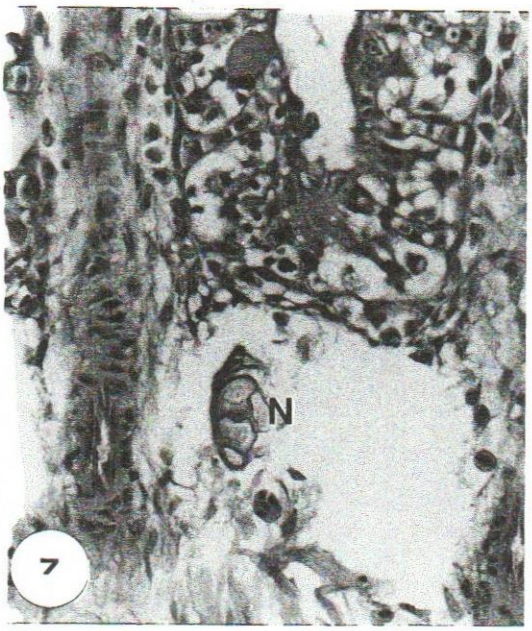
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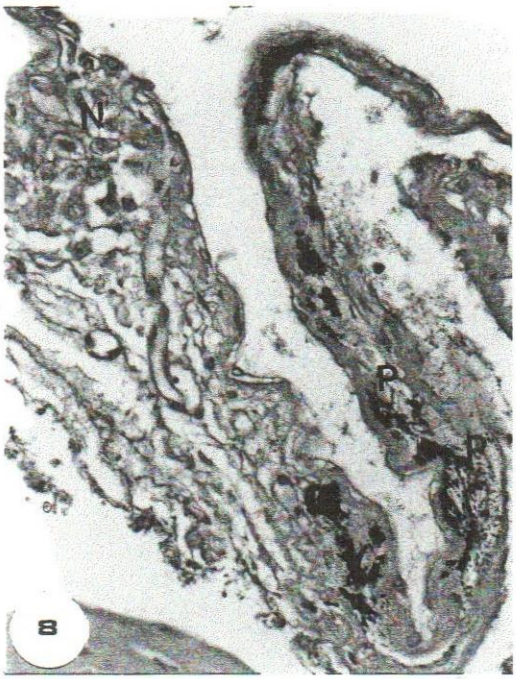
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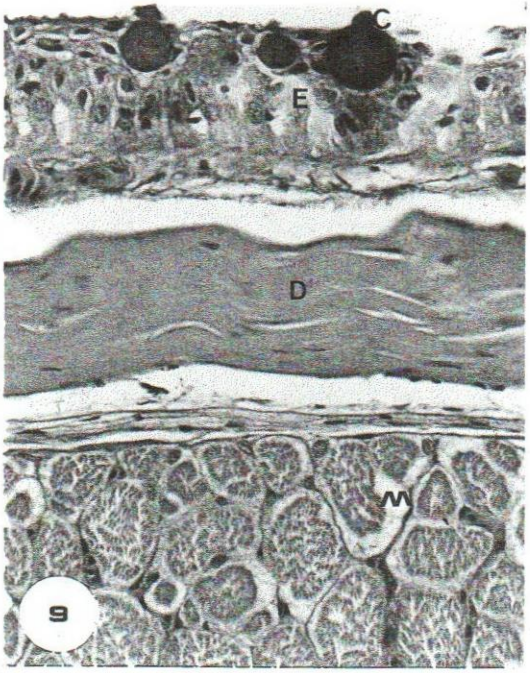
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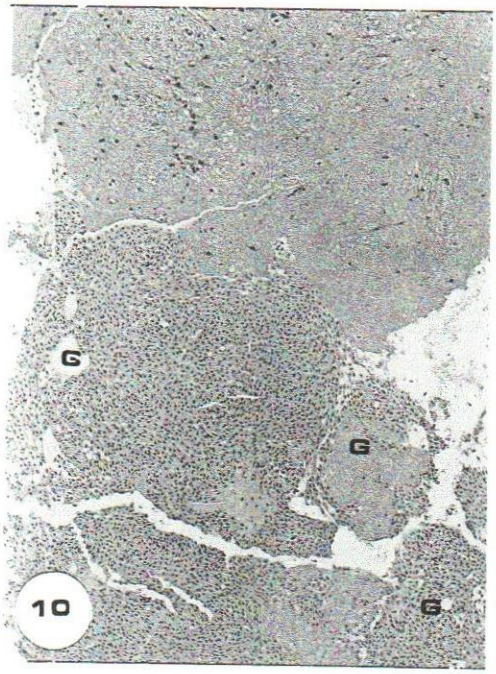
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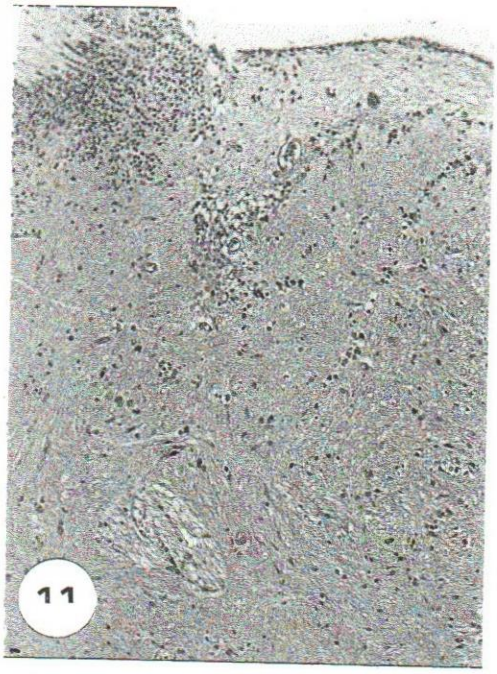
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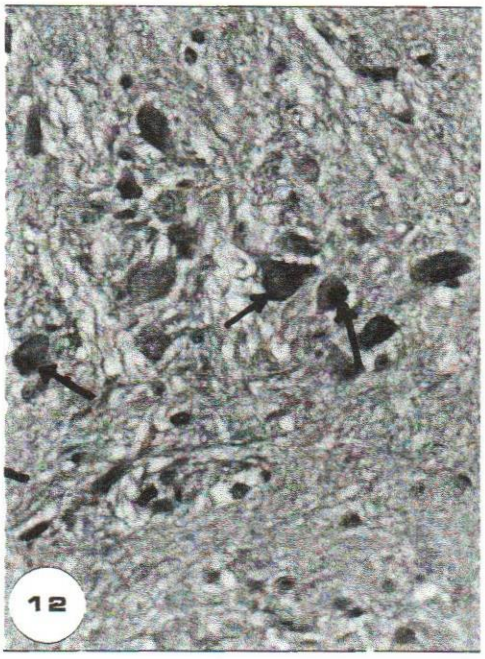
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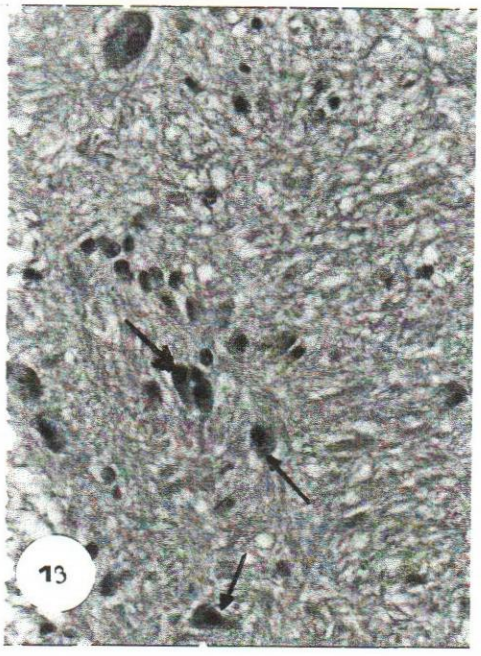
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