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ACUTE AND CHRONIC TOXICITY OF SOME AROMATIC HYDROCARBONS ON TILAPIA ZILLII (GERV.)

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ABSTRACT

The presence and concentrations of four soluble aromatic hydrocarbons from oil origin (toluene, O-xylene, 1,2,4-dimethyl benzene and naphthalene were determined by gas liquid chromatography in samples of the natural subsurface water at fur stations on the River Nile (Helwan, mid-Cairo, entrance of Ismailia canal and Moustorod) during January and August 1989 and April 1990. The highest concentrations of these pollutants were recorded at Moustorod. The concentration of each pollutant was higher in winter than summer. The percentage frequency of their distribution was in this order: toluene > O-xylene > 1,2,4-trimethyl benzene > naphthalene.

The toxic effects of these aromatic hydrocarbons on the freshwater fish **Tilapia zillii** *(Gerv.) Were investigated. The study included the determination of the 96 hours* LC_{50} *for each toxicant on* **T. zillii** *and the chronic effects induced by sublethal doses (35 % of the 96 hr.* LC_{50}*) of these hydrocarbons on its behaviour, growth and microscopic structure of its gills.*

The hydrocarbons induced behavioural changes in the fish. They also had adverse effects on its growth rate measurements, naphthalene causing maximum reduction in growth rate. The histopathologic changes induced by the toxicants on the gills after 7, 21 and 30 days included drooping and curling of the filaments and lamellae, hyperplasia and lifting of lamellar epithelium, fusion of the lamellae and

filaments and necrosis in varying degrees. The gills showed a progressive damage with prolonged exposure and with the type of hydrocarbon used.

Toluene had the least damaging effect followed by 1.2,4-trimethyl benzene. The effects produced by xylene on the gills were more, deleterious than those produced by 1,2,3-trimethyl benzene inspite of the higher toxicity of the latter, and even ranked with those produced by naphthalene.

INTRODUCTION

Environmental pollution is considered among the most serious problems of universal interest. Pollution of the aquatic environment with hydrcarbons can occur from a variety of sources such as oily leakage during transport, discharge of industrial wastewater and engine exhaust emissions into rivers and streams. There is a general agreement that the aromatic hydrocarbons are responsible for the toxic effects in the aquatic biota. Hence, the relative toxicity of various aromatic hydrocarbons to some aquatic organisms have been explored (Benville and Korn 1977; Moles ***et al.***, 1981; De Graeve ***et al.***, 1982; Devlin ***et al.***, 1982 and Vandermeulen, 1987).

River Nile is the main water supply for drinking purposes, irrigation and industry in Egypt. Despite this fact, Nile water has been polluted significantly by various hydrocarbons (Moursy ***et al.***, 1978; Moursy, 1983). The toxic effects of crude oil on the freshwater fishes of the Nile were studied by some researchers (Saleh ***et al.***, 1983; Mazhar ***et al.***, 1987 a & b; Ghazaly, 1989 and Abdel Wahab, 1990) but none, to the best of the author's knowledge, have investigated the effects of some of its individual aromatic hydrocarbons.

Thus, the present study was designed to identify and measure the concentrations of some soluble aromatic hydrocarbons in River Nile and investigate the toxic effects of some soluble aromatic hydrocarbons namely toluene, O- xylene, 1,2,40 trimethyl benzene and naphthalene on Tilapia zillii (Gerv.).

MATERIAL AND METHODS

Tilapia zillii (Gerv.) Were originally collected from a stream located at Abu-Rawash near Cairo, and kept in dechlorinated aerated tap water under laboratory conditions for 15 days prior to the experiment. The fish were fed daily using dried commercial foodstuff.

CHEMICAL ANALYSIS

The natural subsurface water analyzed in this study was collected from River Nile at four stations : Station 1 Helwan, Station 2 mid-Cairo, station 3 entrance of Ismailia Canal and station 4 Moustorod during the months of January and August 1989 and April 1990. The chloroform-extracted water samples were dehydrated with anhydrous sodium sulphate and concentrated by evaporation under vacuum. Stock standard solutions were prepared as 1 % solutions in chloroform. Residues of soluble aromatic hydrocarbons (toluene, O-xylene, 1,2,4-trimethyl benzene and naphthalene) were identified and determined by a Varian Gas Liquid Chromatography (GLC 3700) apparatus equipped with a flame ionization detector (FID). The concentrations of the aromatic hydrocarbons in the water sample were calculated by means of a Chromatography Data System II (CDS III) attached to the GLC apparatus.

SHORT-TERM STATIC TOXICITY TEST (ACUTE TOXICITY):

This was conducted by exposure of T. Zillii for 96 hours to increasing concentrations of each toxicant. A total number of 230 test fish were used and divided into 4 groups for testing the four toxicants (60 fish for each of toluene, O-xylene and 1,2,4-trimethyl benzene and 50 fish for naphthalene).

Within each group, fish were subdivided into groups of 10 and introduced into large aquaria containing dechlorinated aerated tap water. After 30 minutes, increasing concentrations of each toxicant were added to the aquaria of each group. A control group of fish were kept in non-treated aerated and dechlorinated tapwater under similar conditions. Test and control fish were kept off food during the run of the experiment.

Statistical calculations for the 96 hr. LC_{50}), 95 % confidence limits (C.L.) And slope function (S.F.) For each toxicant was done by probit analysis according to Reish and Oshida (1987).

LONG-TERM RENEWAL TOXICITY TEST (CHRONIC TOXICITY) :

Fish were exposed to sublethal doses (35% of the 96 hr. LC_{50}) of the toxicants for 30 days by renewal type, i.e. test solutions in each aquarium was changed periodically with fresh solutions. The fish were fed daily with dried commercial foodstuff and the temperature was maintained at 25 $\pm$ IC.

The long-term toxicity test was subdivided into 2 main experiments :

a- Behaviour and growth Response Experiment :

A total of 50 fish (10 as control and 10 for each toxicant) were introduced into 5 aquaria. Mean total length and mean total weight were calculated at the beginning and end of the experiment. Swimming and feeding behaviour of the fish was observed at frequent time intervals following the addition of fresh hydrocarbon solutions to the test aquaria.

b- Gill Histopathology Experiment :

A similar number of fish as in the previous experiment were subjected to similar test conditions. The selected test organisms for this experiment were 9 + 1 cm in total length. Three fish from each test group and one control were sampled at 7,21 and 30 days after exposure to the individual toxicants. Gills from the sampled fish were isolated, fixed in Bouin's fluid and processed. Paraffin section (4-6 microns) were cut, stained with Harris's haematoxylin and eosin and examined.

RESULTS

Chemical Analysis :

The concentrations of the four aromatic hydrocarbons (toluene, 0-xylene, 1,2,4-trimethyl benzene and naphthalene) chosen for this investigation in the water samples from River Nile and Ismailia Canal are given in table 1. The highest concentrations were found in the water sample of station 4. The concentration of each component was always highest in winter and lowest in summer. In general, the percentage frequency of distribution given in table 2 shows that toluene, 0-benzene and 1,2,4-trimethyl benzene were more frequently distributed than naphthalene in River Nile and Ismailia Canal during the period of sampling

Acute Toxicity :

The results are given in table 3. The 96 hr. LC50 values for toluene, 0-xylene, 1,2,4-trimethyl benzene and naphthalene were calculated graphically using probit graph papers.

Inspection of table 3 shows that naphthalene was the most toxic while toluene was the least toxic. There were no differences between the observed and expected LC_{50} values foall four compounds.

Chronic Toxicity :
a- Behaviour and growth

Within 2-3 minutes after exposure to toluene, the fish became hyperactive and disoriented and remained out of their normal schooling pattern for about half an hour before regrouping. In the case of the other three hydrocarbons, the fish were less violent within 2-3 minutes of exposure but some started to swim erratically. Many attached to the side of the aquarium and few, surfaced, but all of them appeared in state of stupor. After about one hour, the fish returned to normal behaviour.

Table (1) : Concentration of Some Aromatic Hydrocarbon in River Nile and Ismailia Canal Water (mg/L).

Station of sampling	Date of sampling	Toluene	Compound 0-xylene	1,2,4-Trimethyl-benzene	Naphthalene
Helwan (1)	January 1989	1.25	1.25	1.15	--
	August 1989	1.7	--	--	--
	April 1990	2.4	--	--	--
Mid-Cairo (2)	January 1989	6.75	2.65	1.5	--
	Ausust 1989	2.7	--	--	--
	April 1990	3.8	1.3	--	--
Entrance of Ismailia canal (3)	January 1989	5.2	2.15	1.4	--
	August 1989	2.2	--	--	--
	April 1990	4.05	1.8	--	--
Moustorod (4)	January 1989	8.65	3.4	2.1	--
	August 1989	1.15	1.15	0.55	--
	April 1990	5.72	2.1	1.22	0.25

Table (2) : Percentage Frequency of Distribution of Recovered Aromatic Hydrocarbons.

Compounds	% Frequency
Toluene	100
0-xylene	66.7
1,2,4-Trimethylbenzene	50
Naphthalene	16.7

The opercular movement of the fishes in all cases of exposure decreased markedly as compared to control fish. All fish refrained from feeding under the abnormal behaviour, then two hours later began to feed activity: These behaviour, responses were observed following each renewal of the aromatic hydrocarbons. After about 15 days, the fish feeding ability was greatly reduced.

The solutions of all aromatic hydrocarbons had an adverse effect of ***T. zillii*** in growth rate as expressed by length and weight measurements (Table 4). Fish held in naphthalene solution were reduced in growth than in other aromatic hydrocarbons.

Histopathologic Changes in the Gills :

The histomorphology of gills of control T. Zillii is shown in figures 1 & 2.

T. Zillii exposed to sublethal dose of the aromatic hydrocarbons under investigation showed varying degrees of histopathologic changes along the period of the experiment the changes were generally more severe at the distal ends of the filaments that at the base.

After 7 days of exposure to toluene, drooping of the filaments as well as curling of the lamellae were observed (Fig. 3) Moderate lamellar epithelium lifting and mild hyperplasia of interlamellar epithelium were evident in some parts (Fig. 4). After 21 days, extensive lifting of lamellar epithelium and hyperplasia spread downwards along the filament (Fig. 5). This was accompanied by dilation and congestion of the filament vessels and lamellar capillaries. Sometimes rupture of the lamellar epitheluim was observed and hemorrhagic exudes could be seen in the branchial cavity. The severe hyperplasia which dominated after 30 days exposure to toluene resulted in fusion between the lamellae as well as neighboring filaments. Sometimes Detachment of some filaments from the branchial arch occurred (Fig. 6 & 7). Exposure to O-xylene resulted in swelling of the distal ends of the filaments and severe hyperplasia within 7 days (Fig. 8). As a result of the hyperplasia, fusion of the lamellae occurred. In the more proximal areas of the filaments, hyperplasia and lamellar fusion were less severe but curling of the lamellae was also observed. After 21 days of exposure, the distal ends of the filaments were necrotic. At the proximal parts, the lamellar epithelium was swollen and hyperplasia and severe congestion of the lamellae was exhibited. The lamellar

Table (3) : 96hr. LC 50, 95% Confidence Intervals (C.I) in mg/l and slope function (S.F.) of the aromatic hydrocarbons.

Hydrocarbon solutions	*96-hr. Lc50*		*95% C.I*	*S.F.*
	observed	*Expected*		
Toluene	133.4	132.9	122.7-143.9	3.0
O-xylene	39.8	39.5	36.5 - 42.7	3.4
1,2,4-Trimethyl-benzene	22.4	22.0	20.2 - 24.1	2.5
Naphthalene	5.9	5.6	4 5 - 6.9	1.4

Table (4) : Effect of soluble aromatic hydrocarbone on Growth rate of *Tilapia zillii* (Gerv.)

Compounds	*At the beging*		*30 days offer xposure*		*Increment*	
	mean length	*mean wet weight*	*mean length*	*mean wet weight*	*length*	*weight*
		weight		*weight*		
	± S.D.	*± S.D.*	*± S.D.*	*± S.D.*		
Control	9.03+1.4	11.4+4.3	10.14+1.3	15.44+4.3	1.11	4.04
Toluene	9.02+1.2	11.28+3.9	9.88+1.2	14.84+3.9	0.86	3.56
O-xylene	9.05+1.2	11.38+3.9	9.89+102	14.87+3.8	0.84	3.49
1,2,4- Trimethyl benzene	9.06+1.3	11.52+3.9	9.89+1.2	14.94+3.9	0.83	3.42
Naphthalene	9.0+1.3	11.25+4.0	9.61+1.3	14.23+4.0	0.61	2.98

. mean total length expressed in cm
. mean wet weight expressed in gm

epithelium was even ruptured at many points leaking haemorrhagic exudates into the branchial cavity (Fig. 9). By 30 days, the damage was most severe particularly at the distal ends of the filaments which became necrotic (Fig. 10).

The rest of the filaments showed very severe hyperplasia, complete lamellar fusion and vascular changes. Necrotic changes were also observed in many cells.

Seven days exposure to 1,2,4-trimethyl benzene caused moderate hyperplasia and building of the tips of the lamellae. In wards, the most common feature, beside hyperplasia, was the severe lifting of lamellar epithelium in many parts. (Fig. 11).

After 21 days, severe swelling of the lamellae and congestion of lamellar capillaries were observed with some rupture of the lamellar epithelium (Fig. 12). Within 30 days, the lamellae were completely fused as a result of the hyperplasia. Blood vessel walls were ruptured and necrotic changes were evident in many cells. (Fig. 13).

On the other hand, naphthalene caused severe hyperplasia and rupture of the lamellar epithelium as early as 7 days from exposure. The blood vessels were congested (Fig. 14). Twenty one days exposure caused progression of these symptoms along the entire length of the filaments, with necrotic changes in many cells (Fig. 15). After 30 days, the branchial cells were mainly necrotic. Complete fusion of lameller epitheluim as well as of neighboring filaments were observed. The blood vessels were collapsed or ruptured in many parts. In some areas, advanced necrotic changes were seen as well as lamellar fragmentation and dissociation (Fig. 16 & 17).

DISCUSSION

The four aromatic hydrocarbons under investigation were recovered from River Nile, their concentrations showing some variation along the four stations according to the industrial activities and other different sources which discharge their waste into the aquatic environment. The highest concentrations of these

compounds were recorded at Moustorod due to the increasing industrial activities in this area, especially oil refinery industries, whereas the lowest concentrations were detected at Helwan where the width of the Nile and speed of water flow led to the dilution of the pollutant material. The concentrations of the pollutants also varied according to the time of sampling being higher in winter than spring or summer, mainly due to the evaporation process. In general, the percentage frequency of distribution revealed that toluene has the maximum ratio while naphthalene the lowest in accordance with their solubility's. These findings are in agreement with those of Moursy et al. (1978) who found these aromatic hydrocarbons at the same sites of the present study distributed in the same order but in lower percentages.

The long term static bioassay test performed in the present study indicated that there were toxicity differences between the four aromatic hydrocarbons under investigation of the fresh water fish ***T. Zillii*** and can be ranked in this order: toluene < O-xylene < 1,2,4-trimethyl benzene < naphthalene. In this respect, we may recall the results of previous investigators (Neff ***et al.***, 1976, Benville and Kornm 1977., Rice ***et al.***, 1977 and Caldwell ***et al.***, 1977). That the toxicity of aromatic hydrocarbons increases with the number of rings and the degree of alkyl substitution.

The long-term fish bioassay conducted in this study using a sublethal concentration (35 % of the 96 hr. LC50) of each compound revealed that they have several different sublethal effects ranging from behavioural (erratic swimming or narcotized activity) to transitory physiological effects (increase in respiration) and long term physiological effects (reduced growth). This is in agreement with the previous reports in this context (e.g. Thomas and Rice 1979, Solongi and Overstreet, 1982 and Moles and Rice, 1983).

Also the increase in energy demand during hydrocarbon exposure to sublethal doses may cause energy reserves to be shunted from growth to hydrocarbon metabolism and excretion. Similar results and conclusion were reported by Moles ***et al.*** (1981), Woodward ***et al.*** (1981) and Moles & Rrice (1983). In freshwater intoxication, branchial epithelium lifting was reported to be due in part to an influx of the hyposomatic external medium through physiologically impaired epithelial cells (Rombough and Garside, 1977). Lifting of lamellar epithelium could also serve as a defense mechanism to

increase the distance through which the toxicant has to travel to reach the blood stream (Morgen & Tovell, 1973 and Mallatt, 1985).

Hyperplasia & lamellar Fusion were the most evident histopathologic alterations induced by the four compounds but in varying degrees. These changes were more severe in case of orthoxylene & naphthalene and were accompanied by filament fusion after 30 days. Like epithelial, lifting, hyperplasia and lamellar fusion may serve as a protective function by decreasing the amount of vulnerable gill surface area (Mallatt, 1985).

Rupture of the branchial epithelium and necrosis were induced earlier in the case of xylene and naphthalene but were also observed with prolonged exposure to toluene & 1,2,4-trimethyl benzene, and rupture of branchial epithelium are believed, Necrosis and rupture of branchial epithelium are believed to reflect the direct deleterious effects of the irritants (Temmink ***et al.***, 1983). Furthermore, it has been reported that under highly toxic condition, necrosis and rupture are the only gill lesions that occur (Abel, 1976).

In conclusion, the results revealed that although 1,2,4-Trimethyl benzene was more toxic to ***T. Zillii*** than xylene, the effects of the latter on the gills were more deleterious and even ranked with those produced by naphthalene.

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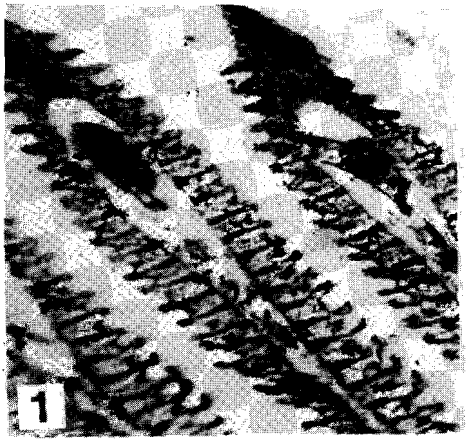
FIGURES

Figure 1: Section through gills of control ***T. Zillii***. HE. X120.
Figure 2: Section through gills of control ***T. Zillii***. HE. X240.
Figure 3: Section through gills of ***T. Zillii*** exposed to a sublethal dose of toluene for 7 days. HE. X120.
Figure 4: Section through gills of ***T. Zillii*** exposed to a sublethal dose of toluene for 7 days. HE. X240.
Figure 5: Section through gills of ***T. Zillii*** exposed to a sublethal dose of toluene for 21 days. HE. X240.
Figure 6: Section through gills of ***T. Zillii*** exposed to a sublethal dose of toluene for 30 days. HE. X120.
Figure 7: Section through gills of ***T. Zillii*** exposed to a sublethal dose of toluene for 30 days. HE. X240. Opposite arrows point to fusion site between two.
Figure 8: Section through gills of ***T. Zillii*** exposed to a sublethal dose of o-xylene for 7 days. HE. X240.
Figure 9: Section through gills of ***T. Zillii*** exposed to a sublethal dose of o-xylene for 21 days. HE. X480.
Figure 10: Section through gills of ***T. Zillii*** exposed to a sublethal dose of o-xylene for 30 days. HE. X120.
Figure 11: Section through gills of ***T. Zillii*** exposed to a sublethal dose of 1,2,4-trimethyl benzene for 7 days. HE. X240.
Figure 12: Section through gills of ***T. Zillii*** exposed to a sublethal dose of 1,2,4-trimethyl benzene for 21 days. HE. X480.
Figure 13: Section through gills of ***T. Zillii*** exposed to a sublethal dose of 1,2,4-trimethyl benzene for 30 days. HE. X480.
Figure 14: Section through gills of ***T. Zillii*** exposed to a sublethal dose of naphthalene for 7 days. HE. X240.
Figure 15: Section through gills of ***T. Zillii*** exposed to a sublethal dose of naphthalene for 21 days. HE. X240.
Figure 16: Section through gills of ***T. Zillii*** exposed to a sublethal dose of naphthalene for 30 days. HE. Z240.
Figure 17: Section through gills of ***T. Zillii*** exposed to a sublethal dose of naphthalene for 30 days. HE. Z240.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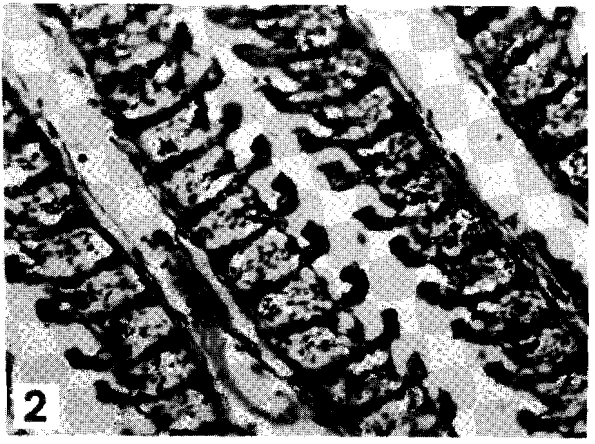
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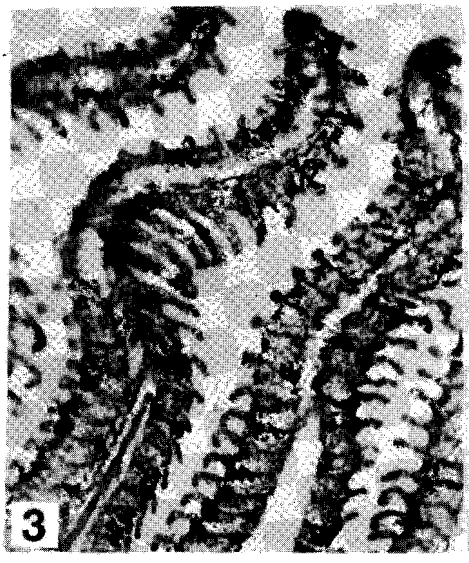
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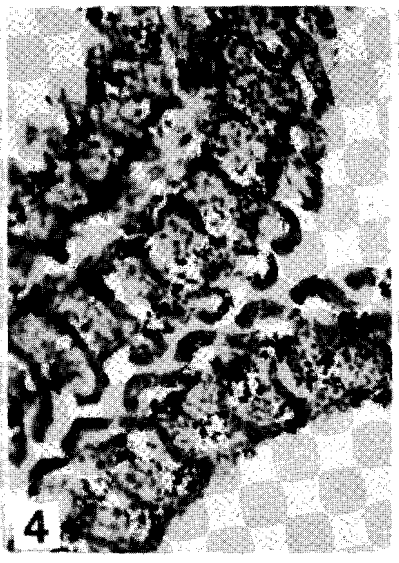
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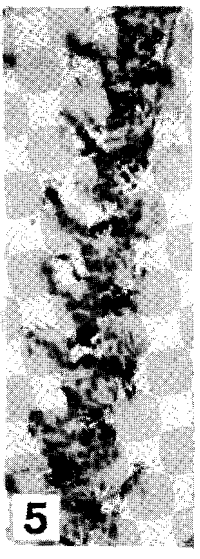
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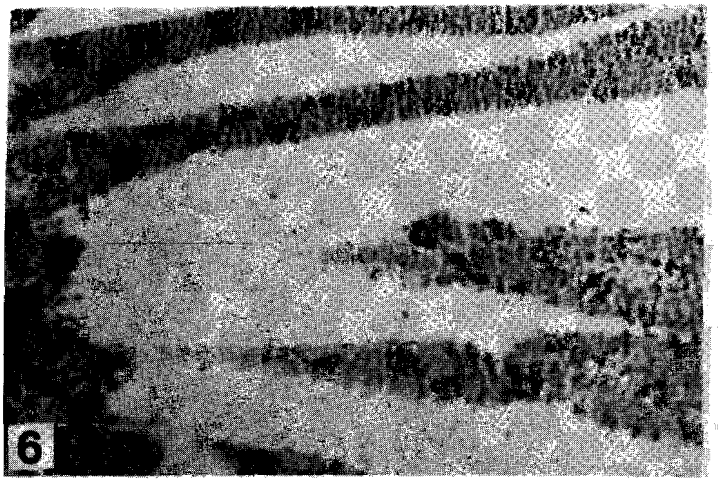
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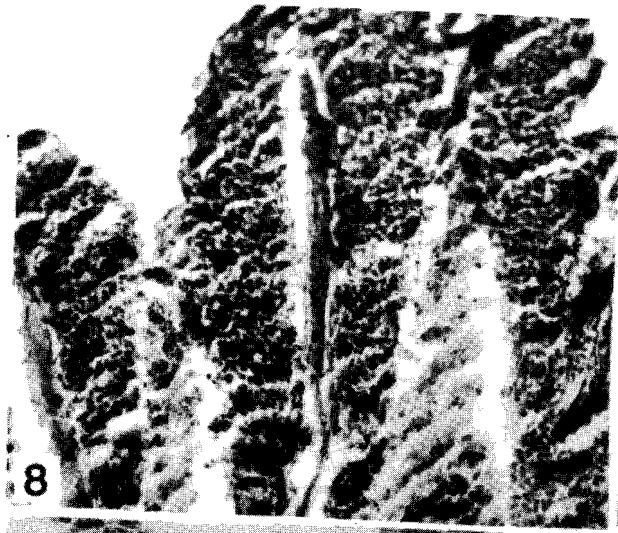
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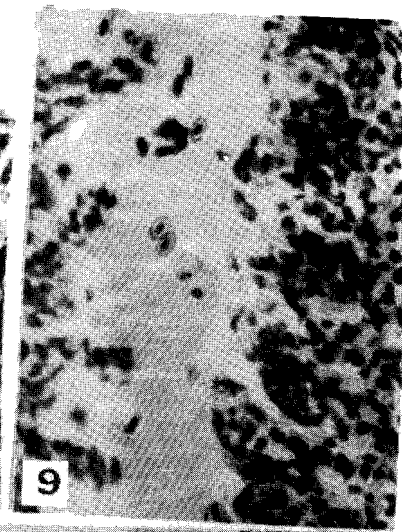
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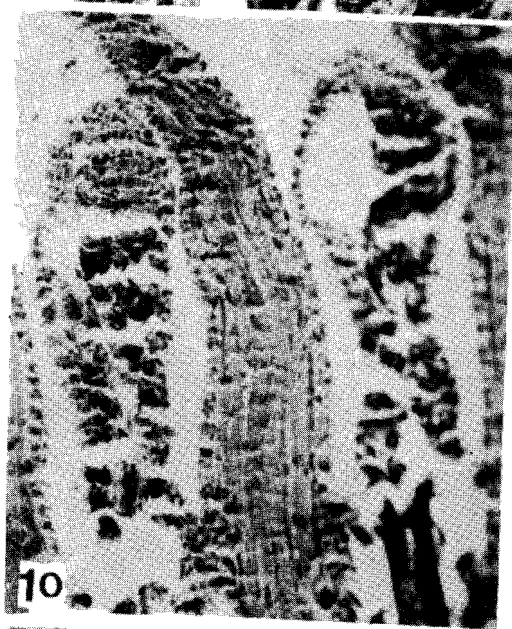
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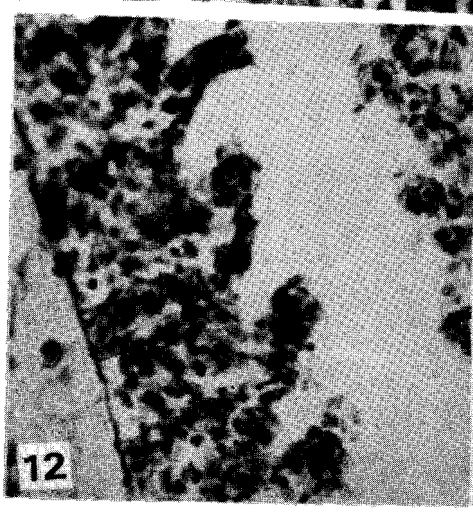
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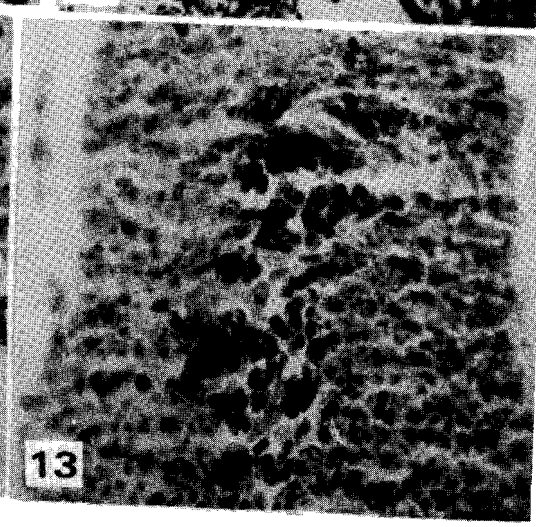
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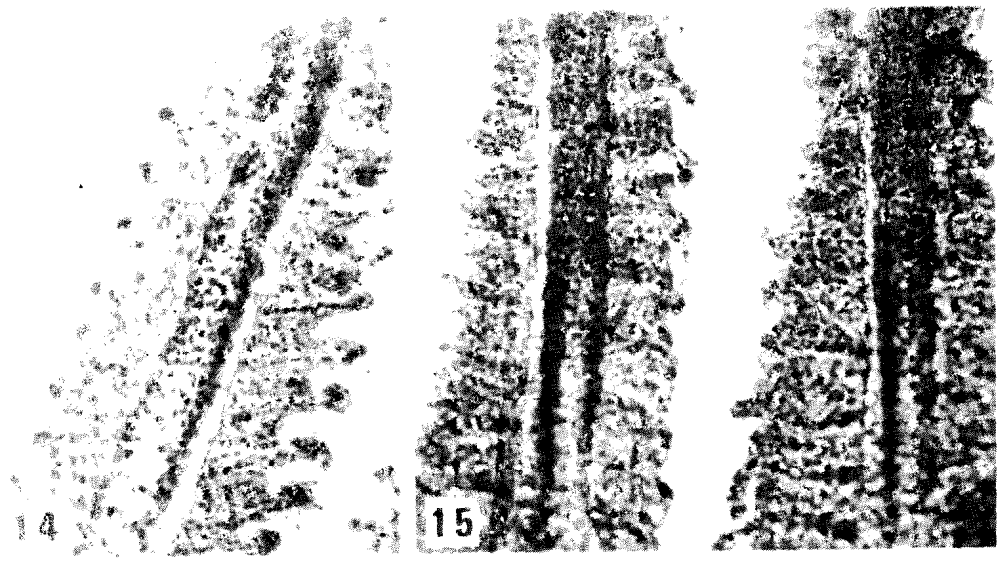
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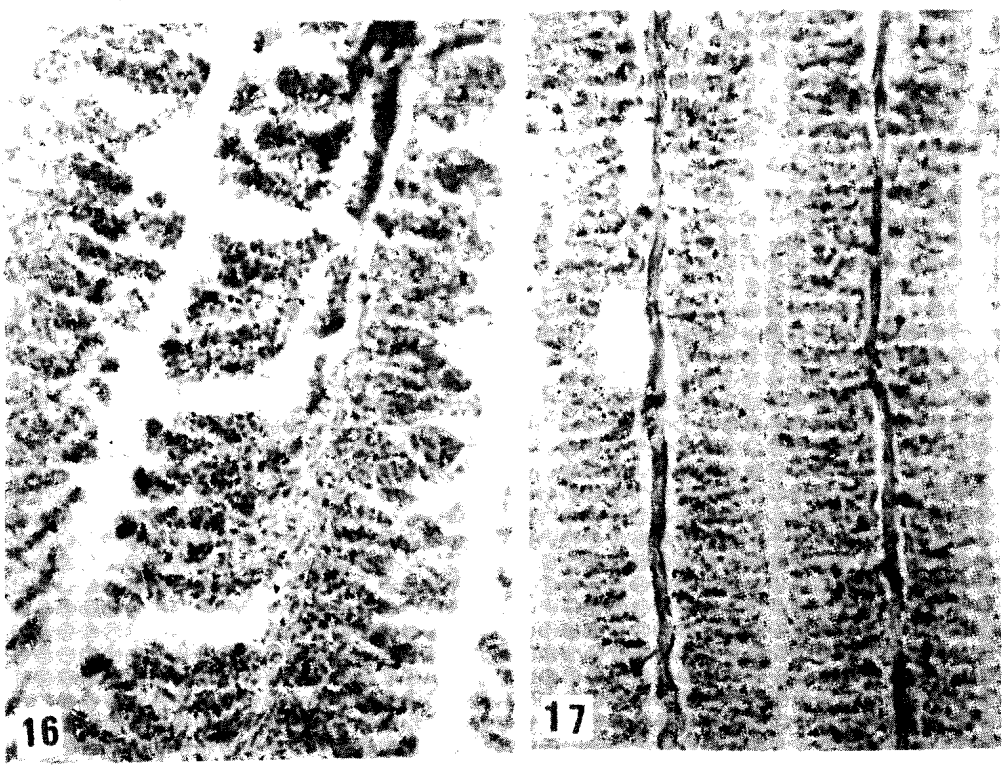
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