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A STUDY ON THE EFFECT OF AMMONIA ON THE MATURATION OF GONADS IN COMMON CARP (CYPRINUS CARPIO) L., 1758 .

(With One Table & 29 Figures)

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دراسة تأثير الأمونيا على نضج المناسل في المبروك العادي

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أجريت هذه الدراسة على مائة سمكة من أسماك المبروك العادي بمتوسط وزن 54.64 ± 5.96 جم ومتوسط طول 15.18 ± 0.69 سم حيث تم تربية هذه الأسماك في أحواض مزودة بماء جاري بمعدل 8 لتر/ساعة. عرضت هذه الأسماك الى معدل مستمر من التركيزات 1، 5، 10، 20 مجم أمونيا لكل لتر ماء بالإضافة الى المجموعة الضابطة لمدة 9 أسابيع وفي نهاية الدراسة أخذت مناسل هذه الأسماك ووزنت في الحال وكذلك تم عمل قطاعات هستولوجية منها وفحصت بالميكروسكوب الضوئي. أظهرت الدراسة أن معامل الفدة التناسلية (GSI) يتناقص نقصاً معنوياً ($P < 0.05$) بزيادة تركيز الأمونيا وكانت نسبة نقص معامل الفدة 28.94 ، 28.73 ، 49.18 ، 55.28 % للتركيزات 1، 5، 10، 20، مجم أمونيا / لتر ماء على التوالي مقارنة بمجموعة الأسماك الضابطة وكان النقص في معامل الفدة (GSI) يرتبط ارتباطاً سالباً مع الزيادة في مستويات الأمونيا ($r = -0.9193$). أوضحت الدراسة وجود تغيرات هستولوجية في حالة المبيض والخصية إلا أنه لا توجد اختلافات ملحوظة عند المستوى المنخفض من الأمونيا (1 مجم / لتر) في عملية تكوين البويضات والحيوانات المنوية، أما في التركيزات الأعلى من الأمونيا (5، 10، 20 مجم / لتر) فإنه قد وجد نقص معنوي ($P < 0.01$) في أعداد الأطوار المختلفة لمراحل تكوين البويضات في المبيض وكذلك تثبيط في عملية تكوين الحيوانات المنوية.

SUMMARY

This study was carried out on 100 of healthy live common carp, (Cyprinus carpio) with an average body weight of 54.64 ± 5.96 g and average total length 15.18 ± 0.69 cm. The fish were reared in aerated metallic tanks with flow rate of 8 L/h water. Fish were exposed to 1, 5, 10, and 20 mg/L ammonia-N for 9 weeks. Gonad specimens were taken from the fish and immediately weighed and fixed for histological sections and examined with light microscopy. The gonado-somatic index (GSI) decreased significantly ($P < 0.05$) with the increase of ammonia concentration. The percentage decrease of GSI was 28.94, 28.73, 49.18 and 55.28 % for 1, 5, 10 and 20 mg/L ammonia-N, respectively. The decrease of GSI was correlated with the increase of ammonia-N levels ($r = -0.9193$). The histological examination showed no remarkable changes in the oogenesis and spermatogenesis, at 1mg/L ammonia, but at higher concentrations of ammonia-N (5, 10 and 20 mg/L) there was a significant reduction ($P < 0.01$) in the number of yolk vesicles and mature stages of the oocytes in the ovary and there was an inhibition in the spermatogenesis.

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INTRODUCTION

Fish is a good source of animal protein that attracts attention of many researchers to increase its productivity by farming (SHEPHERD and BROMAGE, 1988). Common carp (*Cyprinus carpio*) is one of the most important cultured fish in the world nowadays and its production is more than any other freshwater species (HEPHER and PRUGININ, 1981 AND BILLARD and PERCHEC, 1993).

In recent years, public alarm raised about pollution of the river Nile and other native bodies. Ammonia enters inland waters from several sources (THURSTON and RUSSO, 1983), where it is the normal catabolism of protein by fish and also a natural degradation product of nitrogenous organic matter. The effect of ammonia on fishes has been studied, however most information within this field is derived from mortality studies (DABROWSKA and WLASOW, 1986), and little is known about ammonia effects on internal fish organs (SMITH and PIPER, 1975 and SODERBERG *et al.*, 1983). Also, although the evidence for environmental regulation of sexual maturation is extensive (de VALMING, 1974; BILLARD and BRETON, 1978 and BAGGERMAN, 1980), the study of ammonia effects on gonad maturation is poorly represented. The most crucial factor affecting the success or failure of a fish farming venture is the water supply where removing of ammonia or organic compounds is very necessary in fish reproduction (Lowe-McConnell, 1975; SCHWASSMANN, 1978, SUNDARARAJ, 1981 and SHEPHERD and BROMAGE, 1988).

Not only the temperature and photoperiod interact with the endogenous productive cycle are responsible for maturation of carp gonads but also require other factors (STACEY, 1984). The future success of common carp culture and mass propagation of juveniles requires a better understanding of the reproductive biology and the effect of water quality on its life cycle. So, the economic importance of cyprinids has made the subjects of extensive research on reproduction and its control (POTTS and WOOTTON, 1984).

The objective of the present study is to correlate between gonads maturation of common carp (*Cyprinus carpio*) as necessary organs and exposure to different levels of ammonia - N. Also, a trial of recovering from ammonia - N effect by exposure to freshwater free ammonia.

MATERIAL AND METHODS

A total of 100 healthy live common carp (*Cyprinus carpio*) were obtained from ponds of Faculty of Agriculture, Assiut University and were transported immediately to the laboratory. The fish were reared in aquaria 180 x 60 x 70 cm. with water flow 8L/h. Each aquarium was aerated by electric air pump (RENA 301). The average water temperature was thermostatically adjusted at 26.37 ± 0.21 °C, dissolved oxygen was 6.17 ± 0.09 mg/L and pH was 6.88 ± 0.18 which were measured three times daily. Fish were acclimated to laboratory conditions for two weeks.

Fish were individually weighed, measured the total and standard length and were divided randomly into five

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equal groups of 20 fish each and exposed to 1, 5, 10 and 20 mg/L NH₄-N and the 5th group was left as control. The mean weight of fish was 54.64 ± 5.96g and the mean total length was 15.18 ± 0.69 cm. The required level of ammonia was attained by continuous addition of ammonium chloride. Ammonia stock solution was prepared by dissolving requisite amount of ammonium chloride (Merck reagent grade, 19.09 g/5L distilled water) according to CHEN and KOU (1992). Concentrations of ammonia-N in tanks were verified daily to determine the actual ammonia in each treatment by using analysis Kits (Sera Aquaristik, GmbH, D-5138, Heinsberg., Germany). The equivalents in terms of un-ionized ammonia were calculated from total ammonia-N (TAN) concentration using the tables of Solorzano (1969). The fish were fed commercial pellets containing the optimum protein level (Mohamed, 1993) twice daily at a rate 2% of wet body weight. At the end of study (9 weeks) ten fish from each treatment were sacrificed and quickly dissected, the gonads were removed and weighed at once. The gonadosomatic index (GSI) was calculated as following: $GSI = \frac{\text{gonad weight (g)}}{\text{body weight (g)}} \times 100$.

The gonads were immediately fixed in Bouin's fluid, the samples were embedded in paraffin and 5-7 µm thick sections were made. The slides were stained with hematoxylin-eosin and examined with light microscopy.

After 9 weeks the treated fish were transferred to other aquaria containing

ammonia free water and were examined after two weeks. Fish were killed, GSI was calculated and the histological sections were also made. In examination of ovary sections it could be classified the developing oocytes into three stages: Yolk nuclear stage, yolk vesicle stage and mature stage. The number of oocytes in various stages were calculated in different treatments.

Statistical analysis

Data were subjected to analysis of variance using general linear model (GLM) producers of SAS (1987) for personal computer, by one-way analysis of variance. Least Significant Differences (LSD) were used to identify significant differences between treatments according to Steel and Torrie (1980).

RESULTS

1- Changes in gonadosomatic index (GSI)

The gonadosomatic index decreased significantly ($P < 0.05$) with the increase of ammonia-N concentrations (Table 1 & Fig.1). There was no significant differences ($P > 0.05$) between the GSI of control fish and the fish which were exposed 1 and 5 mg/L NH₄-N. While, in case of exposure to 10 and 20 mg/L NH₄-N the GSI decreased significantly ($p < 0.05$, Table 1). On the other hand, the GSI of recovering fish was not different from control ones.

2. Histological changes:

a-Ovary

*S. Y. HUSSEIN & A. M. KELANY**(1) Control*

In the present study the cytoplasmic and nuclear changes in the developing oocytes are classified into three stages : yolk nuclear stage , yolk vesicle stage and mature stage (Fig.2,3& 4).The percentage of yolk nuclear stage was 36.35%, yolk vesicle stage was 40.05%, while the mature follicle percentage was 23.60% of the total calculated stages (Table 1 & Fig. 5) .

(2) 1mg /L NH₄-N (0.0625mg/L NH₃-N)

There was no remarkable histological changes in the ovaries of the fish which exposed to 1 mg / L NH₄ - N as compared with the control group (Fig 6) while there was an increase in the number of yolk nuclear stage (43.01%) and in mature stage (26.88%), while a decrease in yolk vesicle stage (30.11)was found.

(3)5 mg /L NH₄-N (0.3125mg/L NH₃-N)

The histological findings of the ovary in this group revealed that, there was a slight changes such as an increase in the affinity of the cytoplasm of the developing follicles to the basophilic stain, and appeared more darker than the previous treatment especially in the primary and secondary follicles. Also, appearance of atretic follicles and connective tissue fibers inbetween the follicles towards the lumen of the ovary was noticed (Fig. 7).

In the larger follicles , atresia was characterized by dissolution of the thecal and follicular cells collapse and degeneration of zona pellucida of follicular

and thecal cells, coagulation and lysis of yolk material and leucocytic infiltration (Fig. 8). The percentage of yolk nuclear stage, yolk vesicle stage and mature stage was nearly similar to fish group exposed to 1 mg / L (Table 1 & Fig5).

(4)10mg/LNH₄-N (0.6087 mg/L NH₃-N)

In ovaries of the exposed fish to 10 mg/L , there were variable changes among the individuals in the same group. In some specimens the ovigerous lamellae appeared intact , radiated and contain no mature follicles (Fig. 9), but in other specimens there were remarkable decrease in the number of mature follicles. An appearance of connective tissue fibers , connective tissue cells inbetween the developing follicles (Fig. 10) and some atretic follicles were observed. There was a significant ($P<0.01$) reduction in yolk vesicle stage and mature stage (12.20 % and 3.63 %, Table 1 & Fig. 5).

(5)20mg/L NH₄-N(1.2095 mg/NH₃ -N)

In exposed fish to 20 mg/L, the microscopical examination of the ovaries revealed that, the ovigerous lamellae in some specimens appeared disrupted and showed no radiating pattern with different stages of the developing follicles and also contained atretic follicles (Fig. 11). The cytoplasm of the developing oocytes appeared more darker and basophilic than the yolk vesicle stage which appeared lightly stained (Fig. 12). There was a significant ($P<0.01$) reduction in yolk vesicle stage and mature

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stage (20.52 % and 10.72%, Table 1 & Fig 5).

(6) *Recovered from 1mg/LNH4-N*

There was no histological changes comparing with the exposed fish to 1mg/LNH4-N.

(7) *Recovered from 5mg/LNH4-N*

The histomorphological findings in the ovaries of this group revealed that there was an increase in the atretic follicles; which appeared inbetween the developing follicles (Fig. 13) and in the yolk nuclear stage, where it increased from 39.37 % in exposed fish to 59.71 % in recovered animals, while the yolk vesicle stage and mature stage percentage were decreased (Table 1).

(8) *Recovered from 10 mg /LNH4-N*

The picture of the ovary in this group showed that the developing follicles especially the primary and secondary follicles appeared with darkly stained cytoplasm and the germ cells were slightly increased in number and present in groups as nests with slightly stained acidophilic cytoplasm. The nuclei were large rounded and contained a few peripherally located chromatin clumps (Fig. 14). The atretic follicles were present in various stages of atresia (Figs. 15 and 16). There was a decrease in the yolk nuclear stage percent (from 84.17 to 54.21 %), while there was an increase in yolk vesicle stage and mature stage, where it became 24.30 and 28.85 % versus 12.20 and 3.63 % (Table 1).

(9) *Recovered from 20 mg /L NH4-N*

The uniform radiating pattern of the ovigerous lamellae appeared and each lamella was surrounded by a delicate membrane with different stages of the follicles (Fig 17). Atretic follicles were also observed. The percentage of the yolk nuclear stage was 41.63 %, yolk vesicle stage was 28.85 and the mature stage was 29.52 %.

b- Testis

(1) *Control*

The normal histological picture of the testis was represented. The testicular sheath was thick and the connective tissue septa inbetween the seminiferous tubules were noticeable, thin and accompanied with small blood vessels (Fig 18).

The seminiferous tubules in the peripheral region of the testis have no lumina and were lined with spermatogenic cells which appeared rounded or polyhedral. Their nuclei were rounded with various amount of chromatin network. The nucleoli were prominent as deeply stained spherules while the primary and secondary spermatocytes were relatively deeply stained (Fig 19).

(2) *1 mg/L NH4-N*

The microscopical examination of the testis of this exposed group revealed that the spermatogenesis increased in activity (Fig. 20). The seminiferous tubules appeared widely large due to the decrease in the height of the early stages of the spermatogenic cells therefore represented as one to two layers of rounded

faintly stained cells with large rounded vesicular nuclei (Fig. 21).

(3) 5 mg/L NH₄-N

The testicular sheath in these specimens was somewhat thicker than the sheath of the previous treatment. The early stages of the spermatogenic cells (primary and secondary spermatocytes) were less represented (Fig. 22).

(4) 10 mg/L NH₄-N.

The testicular sheath in these specimens was more thickened than the preceding treatments (Fig. 23) and contain blood vessels and pigment cells, primary and secondary spermatocytes were mostly represented, but the spermatogonia became less remarkable and represented as large rounded cells with rounded nuclei.

(5) 20 mg/L NH₄-N.

The histological examination revealed that the testicular wall was comparatively thick and contain pigment cells and blood vessels. Spermatogenic cells were less represented. There was a deterioration of the seminiferous tubules which leads to presence of spaces especially in the central region of the testis (Fig. 24). The connective tissue septa in-between the tubules were increased in thickness and contained blood vessels, the tubules were increased in thickness (Fig. 25). Sertoli cells were prominent in some specimens of this group.

(6) Recovered from 1 mg/L NH₄-N

There were no remarkable changes in the recovered fish and treated with 1 mg/L NH₄-N.

(7) Recovered from 5 mg/L NH₄-N

The testicular sheath became more thin. The different stages of spermatogenic cells were represented. The connective tissue and the blood vessels in-between the tubules were detected (Fig. 26).

(8) Recovered from 10 mg/L NH₄-N

In this group of fish, there was an improvement in the histological picture of the testis, which represented in the appearance of the primary and secondary spermatocytes which lined the seminiferous tubules (Fig. 27). The spermatogonia appeared in some tubules underwent mitotic divisions (Fig. 28). The testicular sheath was thin.

(9) Recovered from 20 mg/L NH₄-N

The improvement in this group of fish was less recorded where the deterioration of the seminiferous tubules was still noticed and the testicular sheath became thinner (Fig. 29). The early stages of spermatogenic cells were less represented and appeared as few cells on the periphery of the tubules.

DISCUSSION

The results of the present study show that the exposure of common carp (*Cyprinus carpio*) to ammonia concentrations had inhibitor effect on ovarian and testicular activity as shown by significant reduction ($P < 0.05$) in GSI and histological changes of the ovary

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and testis. HAYWOOD (1983) showed that the maximal permissible levels of total ammonia was 2.5 mg L / L for freshwater fishes.

The gonadosomatic index (GSI) is accurate indicator in predicting the gonad growth (MAGDA and MESSAEDA, 1991), ripening (TSENG and CHAN, 1982) and reproduction (CALOW, 1978). In this study the increase of ammonia-N levels decreased significantly ($P < 0.05$) both of GSI and percentage of mature follicles (Table 1). The GSI differs according to the number of eggs in the ovary (CALOW, 1978 and TSENG and CHAN, 1982). The presence of mature follicles and other stages in the ovary and different stages of spermatogenesis in the testis of control and treated fish are in agreement with BILLARD *et al.* (1978); HEPHER and PRUGININ (1981) and LAM (1982) who showed that carp at 20-22°C begin the oogenesis and spermatogenesis at the same age of our studied carp.

The lack of relevant literature leads to the conclusion that higher levels of ammonia-N produce irreversible changes in gonads of common carp. At low level (1 mg / L NH₄-N) there were no remarkable changes in the ovaries of treated fish. While, in increasing the level to 5, 10 and 20 mg / L NH₄-N, was strong enough to reduce significantly ($P < 0.01$) the mature follicles, thickening of the tunica albuginea, appearance of atretic follicles and connective tissue inbetween

the developing follicles. Therefore, the fecundity of studied fish decreased with the increase of NH₄-N concentration. Since, the fecundity of the fishes is defined as the number of mature eggs found in the ovary (BAGENAL, 1967 and GUNDERSON, 1980).

Concerning the testes of treated fish, the histological changes were evident in exposed fish to 5, 10 and 20 mg / L NH₄-N. The thickening of testicular wall containing blood vessels. Also, less of spermatogenic cells, deterioration of seminiferous tubules, presence of sertoli cells and scattered areas of degeneration. These histological observations indicate that spermatogenesis was impaired. The interstitial leydig cells which are steroidogenic in the testes of several teleosts showed histological signs of progressive degenerative changes such as hyperactivity and pyknosis. These cellular alterations reflect adversely on the steroidogenic potential of the cells.

The obtained results suggests that ammonia has harmful effects on gonad. This is in harmony with STOTT *et al.* (1981) who remarked that the gonads are sensitive indicator of physiological disturbances. Thus, the signs of damage seen in the ovary and testis even with the minute sublethal concentration are a strong proof of its deleterious effects. These findings run in agreement with those of BILLARD *et al.* (1981) who mentioned that physical and chemical properties of the water have been shown

to exert facilitate or inhibit effect on carp reproduction. Also, mixture of organic compounds play an important role in fish reproduction (LOWE-McCONNELL, 1975; SCHWASSMANN, 1978 and SUNDARARAJ, 1981). It is noteworthy to mention that STACEY (1984) declared that not only temperature and photoperiod interact with the endogenous reproductive cycle are responsible for maturation of carp gonads but also ovulation, spermiation and gamete release require other factors. MUNKITTRICK and DIXON (1988) showed that fish from contaminated lakes suffered from decrease in egg size and fecundity. However, SCHWASSMANN, (1971,1978) and POTTS and WOOTTON (1984) mentioned that the environmental conditions play an important role in maintenance of the final stage of maturity. Also, the reproduction cycle is controlled by a combination of environmental factors which initiate gonadal development.

Yet, no specific reports have been published regarding the effect of ammonia-N to gonad maturation, its dysfunction appears to have few deleterious effects of the body as whole (WHEATER *et al.*, 1987). Since the physiological processes which control the allocation of energy reserves to the gonads and the rest of the body would be most instructive. There are many theories to explain the effect of ammonia-N on gonad maturation. The inhibition of oogenesis and

spermatogenesis according to the increase of $\text{NH}_4\text{-N}$ may be due to the important role played in regulation of protein and carbohydrate metabolism (SHADIA, 1989). Also, the increase of ammonia - N levels led to significant changes in serum cholesterol, glucose, creatinine, sodium, potassium and especially significant decrease ($P < 0.01$) of haemoglobin for the same fish (HUSSEIN *et al.*, 1994). Otherwise, the impairment of spermatogenesis and oogenesis might be due to a direct action of ammonia on steroid biosynthesis or indirectly via hormonal feedback. Ammonia induce stress effects in fishes (SWIFT, 1981 and HOLT and ARNOLD, 1983). This stress has primary effects enter on neuroendocrine changes that appear at the systemic level; secondary effects often follow neuroendocrine stimulation (SPOTTE and ANDERSON, 1989).

On the other hand SCHLÜTER and GROENEWEG (1985) showed that 3 mg/ L $\text{NH}_3\text{-N}$ inhibited the reproduction of *Brachionus rubens*. Also, GUCHTE and MASS-DIEPEVEEN (1988) mentioned that the level of > 2 mg/L $\text{NH}_4\text{-N}$ had significant deleterious effects where it inhibited the reproduction of *Daphnia*. Taking into account the difference of the substance employed, these findings are in fairly good agreement with KIRUBAGARAN and JOY (1992) using MERCURY and

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STOTT *et al.* (1981) and MAZHAR *et al.* (1987) using petroleum water pollutant.

The relatively improvement of the ovary and testis from ammonia-N effect after recovering in freshwater (free from ammonia) for two weeks are in coincidence with findings of HUEY *et al.* (1980) who suggested that replacing contaminated water with fresh water can be one of the practices proper for the management in fish culture. Also, WEDEMEYER and YASUTAKE (1978) found that fresh water was an effective treatment. Moreover, SCARANO and SAROGLIA (1984) showed that the mechanism of recovery might help to minimize losses at fish-farming facilities. There was generally complete recovery in one to three years (NELSON-SMITH, 1970; STRAUGHAN, 1971 and CHAN, 1975). Also, stimulation of ovulation by

water from males and inhibition of ovulation by water from crowded aquaria indicates that the mechanism regulation spontaneous ovulation (LOWE-McCONNELL, 1975; SCHWASSMANN, 1978 and SUNDARARAJ, 1981).

CONCLUSION

Studies conducted by the present investigation has clearly evidence that inhibition of common carp (*Cyprinus carpio*) gonad maturation is proportional to the concentration of ammonia-N. The results point out to the potential danger of the ammonia-N to fish reproduction. The damage of the gonads affected both oogenesis and spermatogenesis processes which is of course a form of partial sterility and reduced fecundity causing inhibition of reproduction and a decrease of the fishery wealth.

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(All the sections were stained with Harris Haematoxylin and Eosin)

Fig. 2: Control ovary showing oocytes in yolk nuclear stage (arrows) ..(X25).

Fig. 3: Control ovary showing oocytes in yolk vesicle stage with yolk vesicles (Y)... (X25).

Fig. 4: Control ovary showing the mature follicles(X 25).

Fig.6: Ovary from exposed fish to 1 mg /L NH₄-N showing the different stages of oocytes in radiating ovigerous lamellae (R), ovarian wall (W).....(X4).

Fig. 7: Ovary of exposed fish to 5 mg /L NH₄-N showing the general view, connective tissue (C), atretic follicles (a)(X4).

- Fig. 8: Ovary from exposed fish to 5 mg /L NH₄-N showing some atretic follicles, one in advanced atresia (a)(X10).
- Fig. 9: Ovary from exposed fish to 10 mg /L NH₄- N showing developing and no mature follicles(X4).
- Fig. 10: Ovary from exposed fish to 10 mg /L NH₄-N showing connective tissue fibers and cells inbetween the developing follicles (c).....(X25).
- Fig. 11 : Ovary from exposed fish to 20 mg /L NH₄- N showing disrupted radiating pattern of lamella and atretic follicles (a).....(X4).
- Fig. 12: Ovary from exposed fish to 20 mg /L NH₄-N showing some atretic follicles (arrow) and developing follicles appeared more darker than yolk vesicle stage(X10).
- Fig. 13: Ovary of recovered fish from 5 mg /L NH₄-N showing atretic follicles (a) connective tissue (c)(X4).
- Fig. 14: Ovary of recovered fish from 10 mg /L NH₄- N showing an increase of the germ cells , which appeared slightly stained (arrows), while the developing follicles appeared dark(X25).
- Fig. 15: and 16: Ovary of recovered fish from 10 mg /L NH₄-N showing some mature follicles (m) and early stages of atretic follicles (e) and advanced stage of atretic follicle (E).....(15 X4 & 16 x10).
- Fig. 17: Ovary of recovered fish from 20 mg /L NH₄-N showing the radiating pattern of the ovigerous lamella (R), each lamellae surrounded by delicate membrane (arrow).....(15 X4& 16 x10).
- Fig. 18: A control testis showing the testicular capsule (T) and the spermatozoa (Z) localized in the center of the testis(X10).
- Fig. 19: A Control testis showing the seminiferous tubules which lined with spermatogenic cells tubule, containing both primary and secondary spermatocytes (arrow) spermatogonia (g) spermatid (d)(x 40).
- Fig. 20: Testis from exposed fish to 1 mg/L NH₄-N, showing increased activity of spermatogenesis(x 10).
- Fig. 21: Higher magnification of figure 20, showing the seminiferous tubules which filled with spermatid, and lined with one to two layers of spermatogenic cells (g) (x 40).
- Fig. 22: Testis from exposed fish to 5 mg/ L NH₄-N, showing the thick testicular capsule and the tremendous amount of spermatids and spermatozoa(x 25).
- Fig. 23: Testis from exposed fish to 10 mg/L NH₄-N, showing the thickened testicular capsule and the large spermatogonia with large rounded nuclei (arrows)(x 25)
- Fig. 24: Testis from exposed fish to 20 mg/L NH₄-N, showing the deterioration of the seminiferous tubules and presence of spaces (x 10).

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- Fig. 25: Testis from exposed fish to 20 mg/L NH₄-N showing the thick interstitial connective tissue contained blood vessel (v) with valves (.....x 25).
- Fig. 26: Recovered testis from 5 mg/L NH₄-N showing thin testicular wall and different stages of spermatogenic cells(x 25).
- Fig. 27: Recovered testis from 10 mg /L NH₄-N, showing the seminiferous tubules lined with spermatogonia (g) , primary spermatocytes (p) and secondary spermatocytes (s), spermatids (d)(x 40).
- Fig. 28 a , b : Recovered testis from 10 mg / L NH₄-N showing the mitotic divisions in the spermatogonia (arrows).....(x 40).
- Fig. 29: Recovered testis from 20 mg /L NH₄-N showing less improvement in the testis, the deterioration of the seminiferous tubules was still noticed(x10).

Table (1) The gonadosomatic index (GSI), average number and percentage of oocytes in different stages of common carp exposed to different ammonia-N concentrations.

| | | Treatment (mg/L NH ₄ -N) | | | | | Recovered from | | | | level of significant |
|-------------------|-----|-------------------------------------|--------|--------|--------|--------|----------------|--------|--------|--------|----------------------|
| | | control | 1.0 | 5.0 | 10.0 | 20.0 | 1.0 | 5.0 | 10.0 | 20.0 | |
| GSI | Me. | 1.69 a | 1.20 a | 1.20 a | 0.85 d | 0.75 d | 1.74 a | 1.47 a | 1.13ad | 1.16ad | 0.05 |
| | ± | 0.44 | 0.63 | 0.71 | 0.51 | 0.55 | 0.25 | 0.47 | 0.44 | 0.94 | |
| Yolk. nucl. stage | Me. | 4.42 | 4.40 | 4.60 | 7.56 | 5.90 | 4.52 | 6.58 | 5.17 | 4.33 | 0.68 (N.S) |
| | ± | 1.71 | 1.64 | 1.24 | 2.37 | 2.46 | 1.30 | 1.67 | 1.31 | 1.49 | |
| Yolk. stage | Me. | 36.35 | 43.01 | 39.37 | 24.17 | 28.76 | 49.66 | 59.71 | 54.21 | 41.63 | 0.01 |
| | ± | 4.87 | 3.8 | 3.25 | 1.11 | 1.75 | 2.39 | 2.30 | 2.75 | 3.20 | |
| Mature stage | Me. | 1.24 | 1.31 | 0.62 | 0.92 | 1.30 | 0.61 | 0.36 | 2.12 | 1.22 | 0.01 |
| | ± | 40.05 | 30.11 | 31.99 | 12.30 | 30.52 | 30.21 | 25.41 | 24.50 | 28.85 | |
| Mature stage | Me. | 2.78 | 2.75 | 2.91 | 0.33 | 0.92 | 2.01 | 1.64 | 2.39 | 3.07 | 0.01 |
| | ± | 1.24 | 1.60 | 1.08 | 0.51 | 0.99 | 1.02 | 0.92 | 2.48 | 1.11 | |
| Mature stage | Me. | 23.60 | 26.88 | 23.64 | 03.53 | 10.72 | 20.15 | 14.88 | 21.49 | 29.52 | |
| | ± | | | | | | | | | | |

Means with the same letter are not significantly different.

N.S : not significant .

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Fig. 1. Gonadosomatic index of common carp exposed to different levels of ammonia.

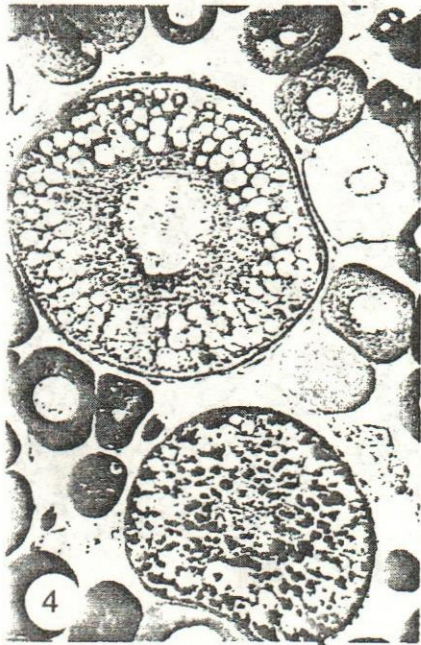
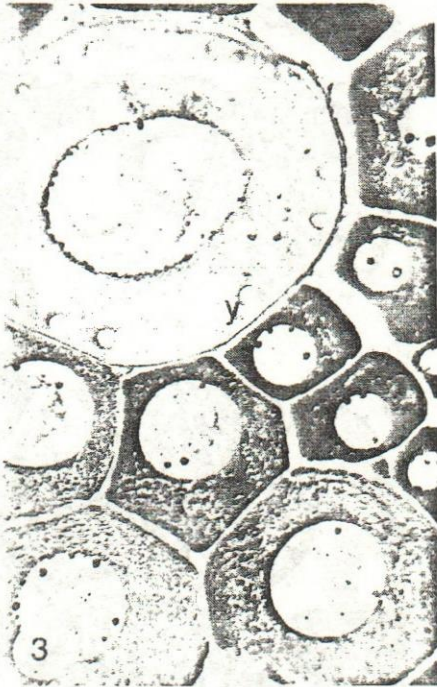
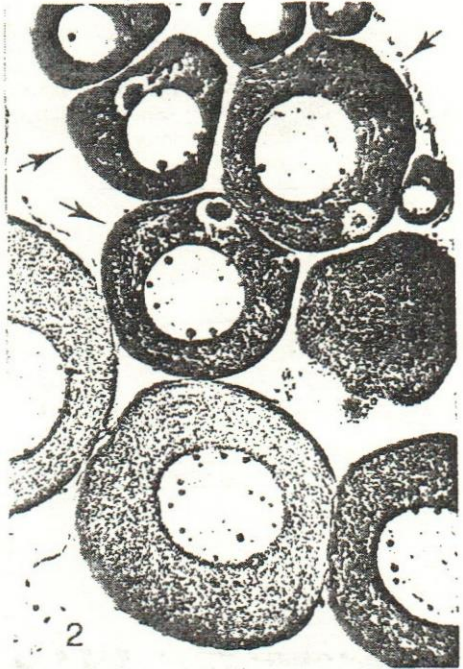
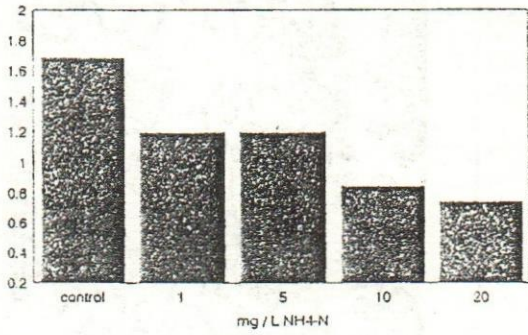
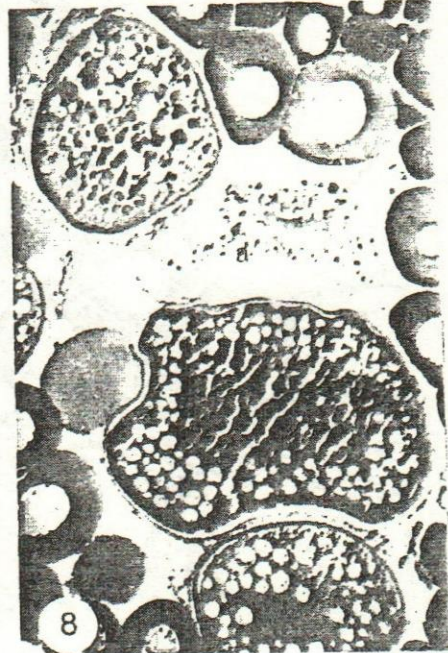
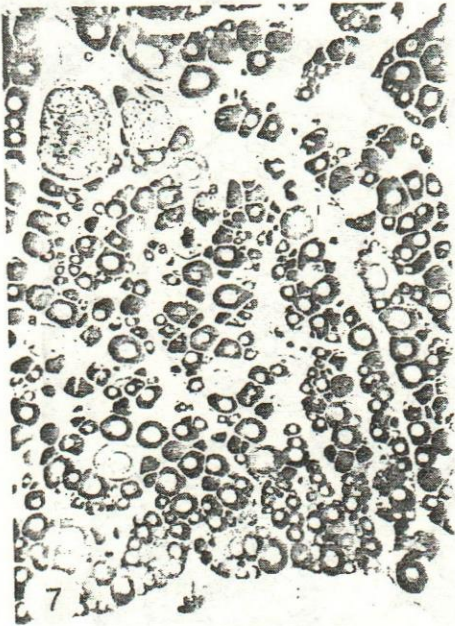
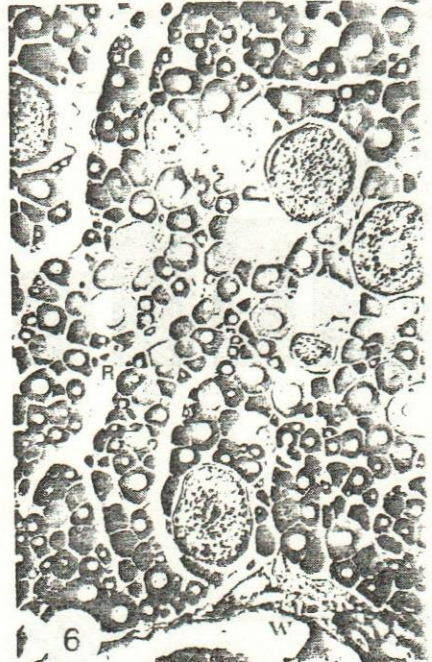
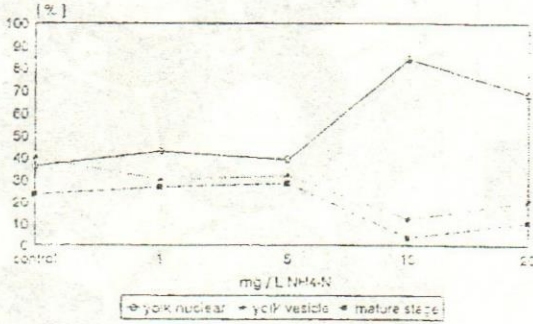
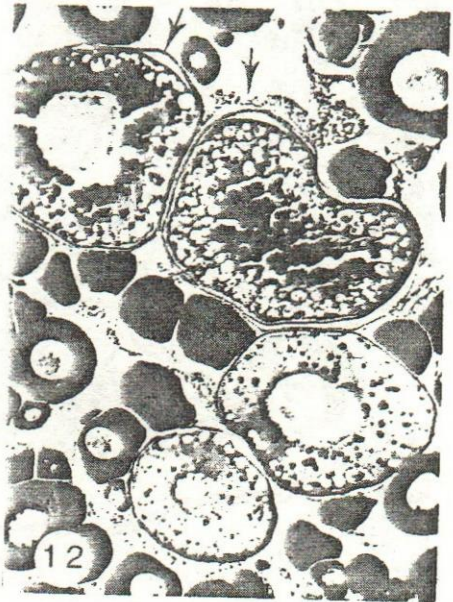
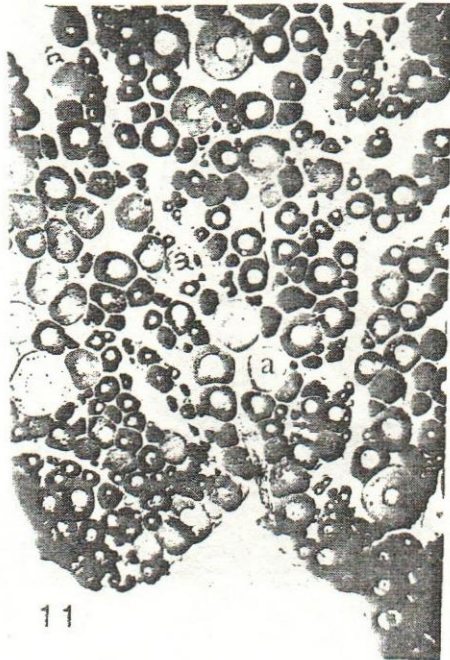
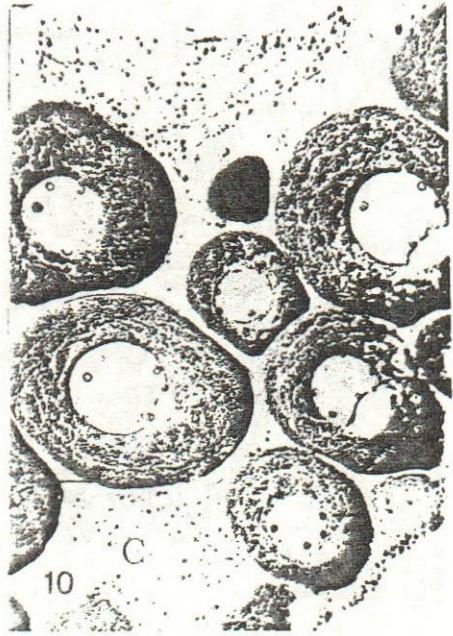
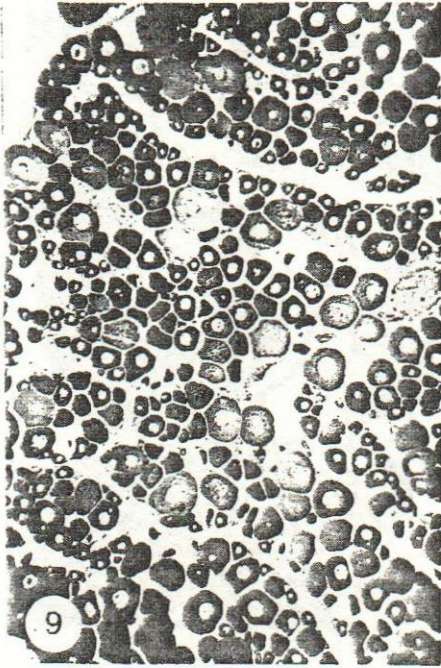
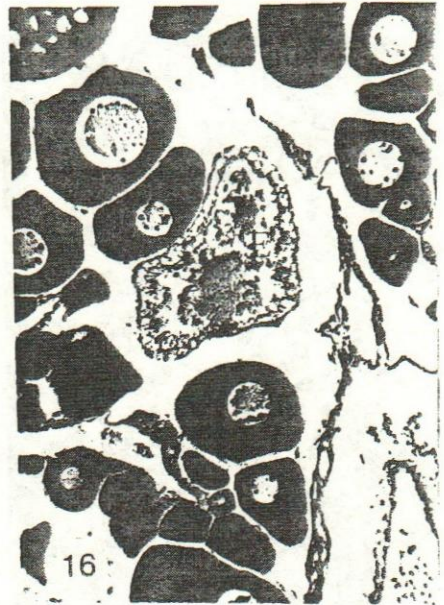
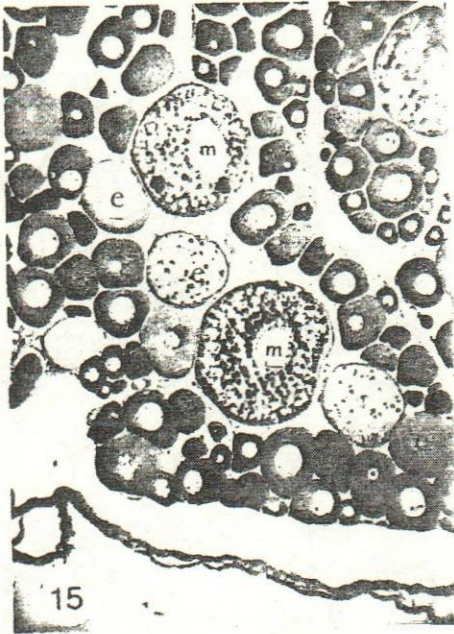
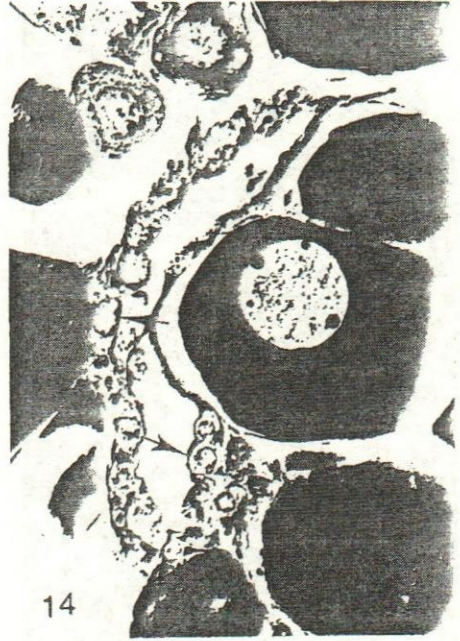
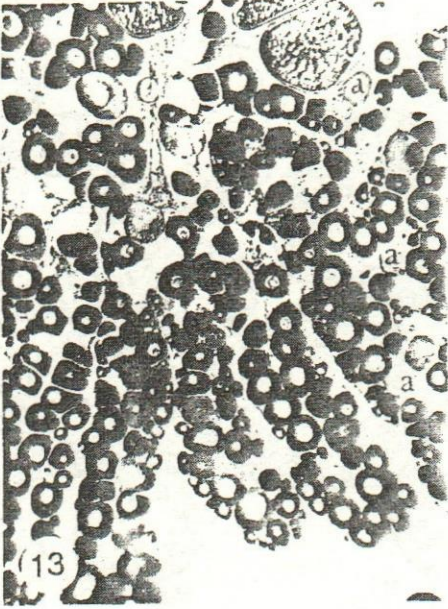


Fig. (5): The yolk nuclear, yolk vesicle and mature stages percentage in ovary of common carp exposed to various levels of ammonia.

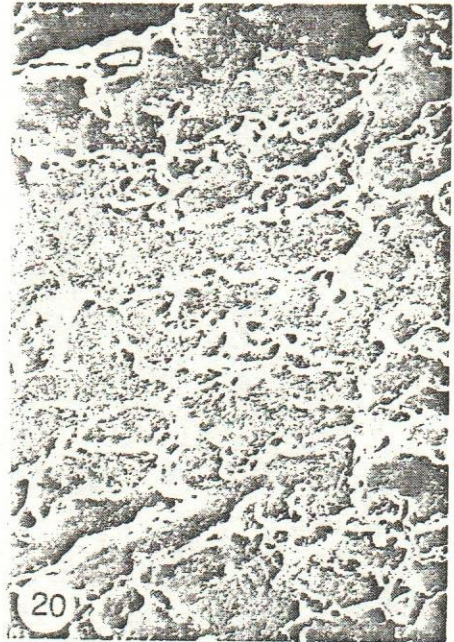
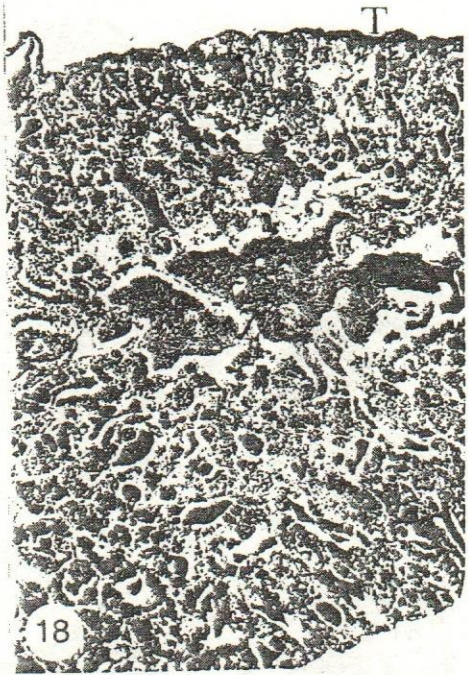
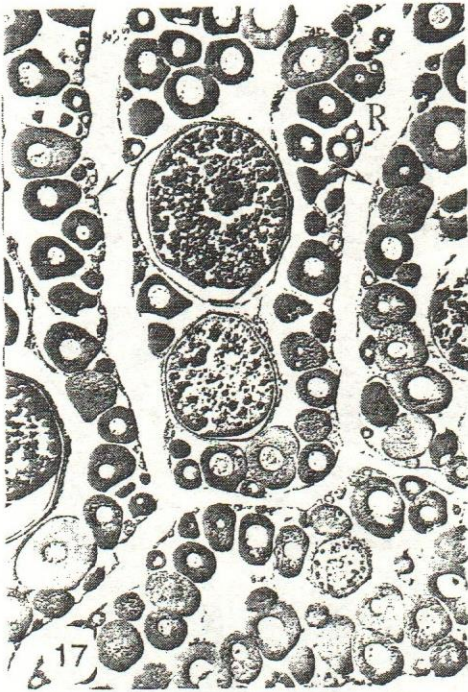


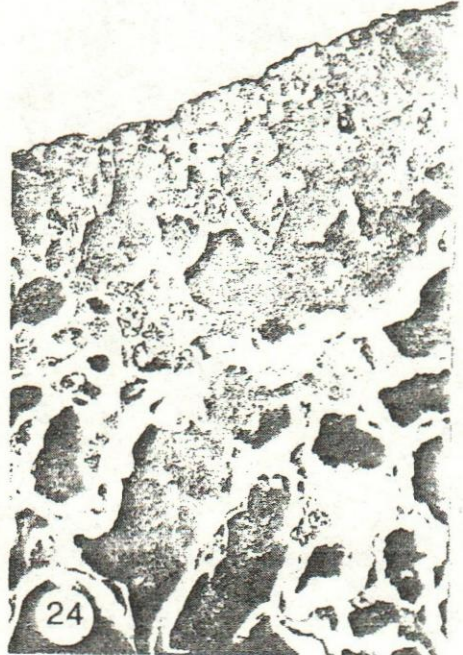
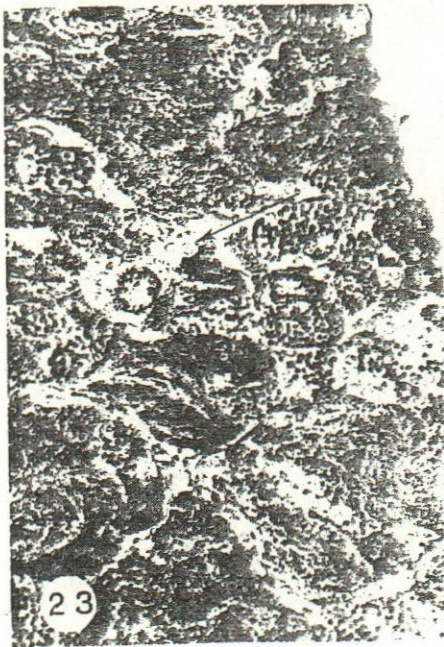
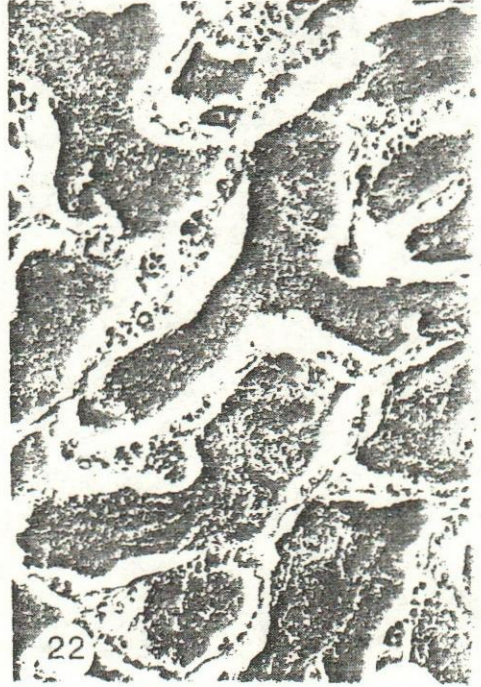
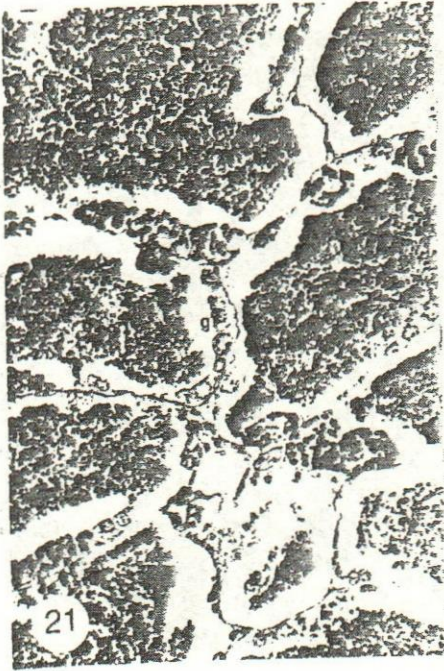
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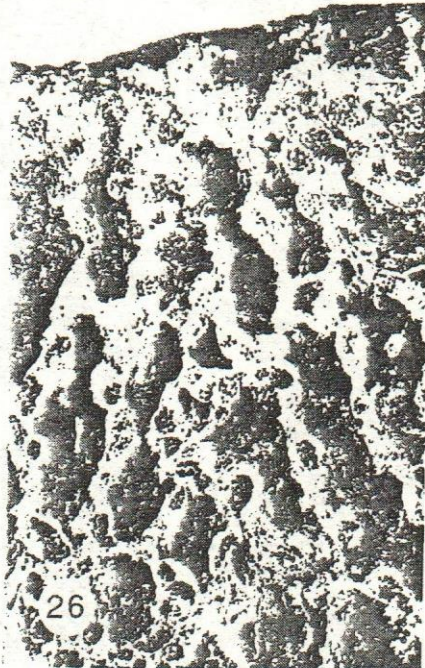




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