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SPERMATOGENIC PROCESS IN CARBENDAZIMTREATED RATS

(With 1 Table & 12 Figures)

by
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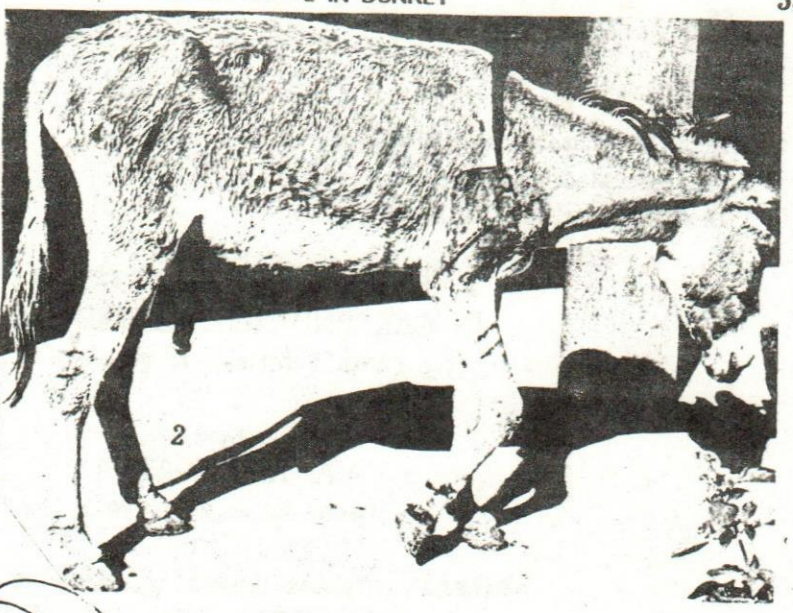
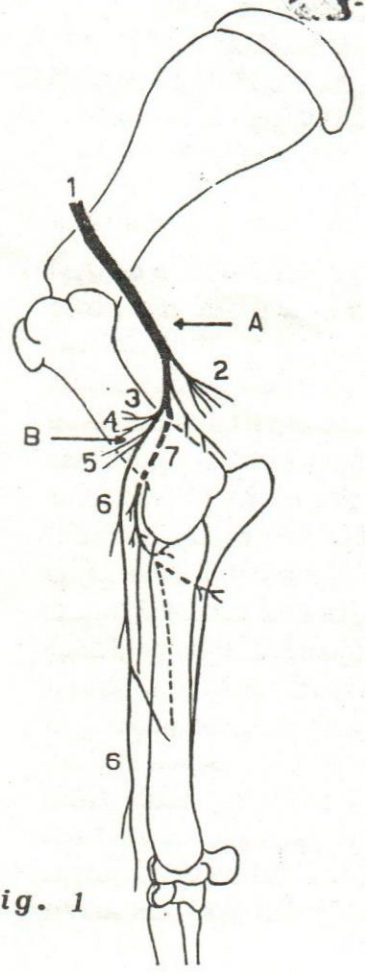
النسيج الطلائي المنوى للفئران المعاملة بالمبيد الفطرى / كاربندازيم / بالاشارة الى تأثيره على تكرارية كل من المراحل الحساسة من النسيج الطلائي المنوى

أحمد أبو العطا

أجرى هذا البحث على ذكور فئران بالغة وسليمة ظاهريا ، ظلت بالمعمل لمدة أسبوع للأقلمة تحت نظام ١٢ ساعة ضوء و١٢ ساعة ظلام . تم تقسيم ذكور الفئران فى ٦ مجموعات كل منها يحتوى على ثلاث ذكور. المجموعة الأولى وهى مجموعة ضابطة تم تجريعها زيت أذرة معقم ٢ سم باستخدام ابرة تجريع مباشرة الى المعدة . وبالنسبة للمجموعات الثانية الى السادسة تم تجريع حيوانات كل مجموعة بجرعة واحدة من مادة الكاربندازيم ، كمستحلب مع زيت الاذرة . والجرعات بالنسبة للمجموعات الثانية الى السادسة ٢٠٠ ، ١٠٠ ، ٥٠ ، ٢٥ ، ١٠ مج /كجم وزن بالترتيب . وتم تجريع جرعات المستحلب والتى تحتوى على تلك التركيزات من الكاربندازيم بالنسبة لكل حيوان وقبل التجريع ، تم رفع العلف من أمام الحيوانات أثناء الليل لتسهيل انسياب المستحلب فى المعدة التعريض أو المعاملة كانت ساعتين بعد التجريع . وقد تبين من نتائج البحث أن نتائج البحث أن جرعة واحدة من الكاربندازيم تحدث تغيرات فى النسيج الطلائى المنوى تتمثل فى ظهور خلايا عملاقة متعددة الانوية وهى من نوع خلايا مدورة مولدة حيا من وأيضا ظهور انفصال الحيوانات المنوية فى دور الاستطالة وذلك قبل انطلاقها الطبيعى خصوصا فى مراحل VII, XII-XII & XIV. كما أن الاستجابة بتأثير هذه المادة السامة يختلف من مرحلة لأخرى فيظهر انفصال الحيوانات المنوية فى حوالى ٥٠ ٪ من مرحلة VII و١٠٠ ٪ من مراحل XII-XIII & IV بعد جرعة واحدة ٢٠٠مج و١٠٠ مج /كجم . كاربندازيم . وكانت أقل جرعة مؤثرة ٢٥ مجم/كجم وزن ويتمثل تأثيرها فى مرحلة VIIفقط التى يظهر انفصال الحيوانات المنوية فيها ، كما ان الاختلاف فى تكرارية





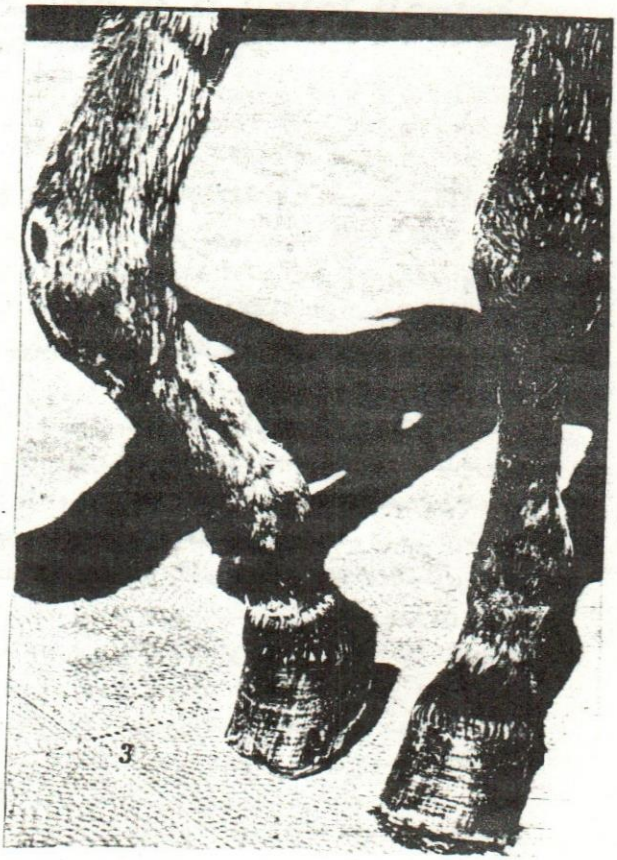


Fig. 1

مراحل VII,XII–XIII & XIV تتغير تغير معنوى بعد الجرعات العالية ٢٠٠، ١٠٠ مجم ماعدا مراحل XII–XIII & XIV (عند جرعة ٢٥ مجم) ومراحل الغشاء المنوى بعد جرعة ١٠مجم التى تظهر غير متأثرة وطبيعية . ومثل هذه التغيرات الناتجة فى تكرارية المراحل المدروسة تعتبر مؤشرا سلبيا فى عملية تكون الحيوانات المنوية والتى تؤثر تماما على الصحة التناسلية للفئران وللعاملين الذين قد يتعرضوا للكاربندازيم اثناء عملهم . ويستنتج من البحث أن المبيد الفطرى كاربندازين يعتبر من المواد السامة التى لها علاقة بالتناسل فى الحيوانات بصفة عامة والقوارض بصفة خاصة ، الأمر الذى يمكن أن يؤخذ فى الاعتبار من العلماء والباحثين فى مجال علم السموم وعلم مقاومة القوارض عند تحضير مبيدات حديثة لمكافحة الفئران ، كما أن النتائج التالية للتعرض بمادة الكربندازيم تؤثر تأثيرا مباشرا على النسيج الطلائى المنوى ، الأمر الذى يحث العاملين فى مجال تصنيع الكاربندازيم والمجال الحقلى ، على أن يلتزمو بكافة مقاييس الأمان وعدم التعرض له أثناء التعامل به وذلك لتجنب تأثير الكاربندازيم السريع على خصية الانسان .

SUMMARY

Six groups of adult male albino rats (3 animals each) were employed in this study. The 1st griyo (control) was given 2 ml corn oil/rat. From the fungicide Carbendazim; 200, 100, 50, 25 and 10 mg/Kg, emulsified in corn oil. A single dose of Carbendazim produced both dose- and stage- related changes, especially at certain stages (VII, XII–XIII & XIV) of the seminiferous epithelium. These changes were represented as multinucleated giant round spermatids, and premature release (sloughing) of the elongated spermatids. The least effective dose (25 mg/Kg), caused sloughing of elongated spermatids only at stage VII. Meanwhile, at dose 200 and 100 mg, the sloughing appeared at about 50% of stage VII and extended to be 100% of stages XII–XIII & XIV.

INTRODUCTION

The systemic fungicide Carbendazim (methyl 2–benzimidazole carbamate) is used to control a wide range of plant pathogens in vegetable fields. The Carbendazim effects were severe in adult male rats that received 400 mg/Kg body weight for 10 days, causing retardation of spermatogenesis in 7 of 12 adult male rats (CARTER et al., 1987). HESS et al. (1985) reported that a premature release of spermatids from the seminferous tubules occured as early as 2 hours after a single dose 400 mg Carbendazim/Kg body weight.

Both carbendazim and Benomyl compounds are capable of disrupting spermatogenesis in adult rat (CARTER et al., 1987). However, the effect of Carbendazim on frequency of the stages of spermatogenic epithelium could not be traced in the availabe literature.

The objective of this investigation is to study the short-term (2 hours post-treatment) sequellae of Carbendazim-exposed spermatogenic epithelium in adult albino rats. This short exposure time was designed to recognize the changes of the spermatogenic cycle, as well as, to figure out the early sensitve stages and their frequency following the exposure to Carbendazim.

MATERIAL and METHODS

Adult male albino rats, 3 months old, were utilized for conduting this work. The animals spent a week for acclamatization in the laboratory (12 hours light/12 hours dark, fed barely and milk, and tap water ad libitum). The rats were divided into 6 groups (3 animals each). The 1st group received 2 ml steralized corn oil only as a control group; the 2nd to the 6th griyo received 200, 100, 50, 25 and 10 mg Carbendazim (Methyl 2-Benzimidazole Carbamate, MBC, provided by E.I. Du Pont de Nemours and Co., Wilmington, DE USA) per Kg body weight respectively. The MBC was emulsified in corn oil and given by oral gavage in volumes containing the designed MBC dose per rat. The rats were kept unfed for overnight. The post-treatment period was 2 hours. The rats were then aneasthetized using sodium pentabarbital, the testes were fixed by vascular perfusion (FORSSMANN et al., 1977), using Bouin's fixative. Paraffin sections were stained with Hematoxylin and Eosin (DRURY and WALLINGTON, 1980), and also with Periodic Acid Schiff technique, counterstained with Hematoxylin to help in recognizing the stages of spermatogenesis (HESS, 1990). The number of each stage was counted per one hundred seminfierous tubules from each treated testis and the same stages were counted parallely in testes of the conrol rats. The frequency (%) of each of the sensitive stages in MBC-treated rats was studied in comparison with the control testes. Statistical Analysis System "SAS" (1987) was adopted to interpretate the data.

RESULTS

Comparing the seminiferous tubules of the control with those of the Carbendazim-treated rats, the sensitive and the early affected cell associations

(stages), which showed a detectable changes, were stages VII, XII–XIII and XIV, except in the 10 mg MBC–treated testes which showed normal spermatogenic epithelial lining similar to those of the control (corn oil–treated) testes (Fig. 1 and 2).

Corn oil (control) testes: Stage VII: It was characterized by the presence of two spermatids types: the elongated step 19 spermatids, aligned along the luminal border of the spermatogenic epithelium, followed by the rounded step 7 spermatids with centrally located rounded nuclei and an acrosome covering about 1/3 of their basal surfaces. Stage VIII, showing residual bodies, which were present beneath step 19 spermatids (Fig. 2).

Stage XII–XIII: They were characterized by absence of the rounded spermatids. The elongated spermatids had blunt acrosomes, with long and narrow slightly bent heads (Fig. 3 and 4). Spermatogonial mitotic figures were seen at stage XII only on the basement membrane (Fig. 3).

Stage XIV: It was defined by the presence of numerous divisions, together with step 14 spermatids. Transition between stage XIII and XIV was present (Fig. 5 and 6).

MBC–treated testes:

Carbendazim (MBC) induced short–term sequellae amoung the spermatogenic epithelium at doses of 200, 100, 50 & 25 mg MBC/Kg body weight. The 25 MBC dose was the least effective dose, while the 10 mg MBC dose didn't show any detectable change and the spermatogenic epithelium was unaffected and appeared normal.

The MBC–induced changes were manifested as multinucleated giant round spermatids, premature release (sloughing) of elongated spermatids, and exfoliation of some round spermatids. These changes were mainly detectable at certain cell associations (stages); VII, XII–XIII & XIV of the spermatogenic epithelium (Fig. 7 to 12).

Of particular interest, the MBC–induced changes appeared variable among the affected stages. The frequencies (%) of the sensitive (affected) stages VII, XII–XIII & XIV (showing sloughing or not) in testes of control and MBC–exposed rats were caliculated, the least square means and standard errors of these frequencies were shown in table (1). At 200 & 100 mg MBC doses, about 50% or less of stage VII and 100% of stages XII–XIII & XIV exhibited sloughed elongated spermatids. At 50 mg MBC dose, more than 50% of stage VII, less than 50% of XII–XIII, and about 50% of stage XIV possessed

sloughed spermatids. Later on, at 25 mg MBC dose, despite less than 50% of stage VII showed sloughed spermatids, stages XII–XIII and XIV didn't show any detectable sloughing. The frequency of stages XII–XIII (showing sloughed spermatids in rat subjected to 200 or 100 mg MBC dose), was significantly increased (Table 1).

DISCUSSION

The present study revealed that a single dose of Carbendazim produced dose– and stage– related effects (200, 100, 50 & 25 mg MBC/Kg body weight) on the spermatogenic epithelium especially at certain stages; VII, XII–XIII & XIV. The MBC–induced changes of these stages were disorganization of their epithelia, appearance of multinucleated giant round spermatids, and premature release (sloughing) of the elongated spermatids. The least effective MBC–dose was 25 mg/Kg body weight. Of particular interest, such damage resulted in a dramatic alterations in the frequency of the sensitive stages of the spermatogenic cycle.

Similar sloughing was reported as early as 2 hours after a single dose of 400 mg MBC/Kg body weight (HESS et al., 1985). Meanwhile, 400 mg Benomyl or Carbendazim/Kg body weight for 10 successive days, followed by 2 weeks post–treatment period, induced disruption of all stages of spermatogenesis (CARTER and LASKEY, 1982), since MBC is identical to the metabolite of Benomyl (GARDINER et al., 1974). The least effective MBC dose of the current study, was 25 mg/Kg body weight. Benomyl has the same least effective dose, as announced by HESS et al. (1991), who added that sloughing was found primarily in stages VII at lower dosages, and extended to be in all stages at higher Benomyl dosages except for stages VIII–XI.

The sloughing of elongated spermatids in our work is similar to that induced after a single dose of 400 mg MBC/Kg (HESS et al., 1985), that could be attributed to the removal of barely overnight before gavage of MBC. Thus, the amount of food inside the stomach could possess a dramatic effect on the testicular response.

The current study revealed a premature release (sloughing) of elongated spermatids resulting 2 hours after MBC exposure, which seems to be dose– or/and stage–dependant response. FAWCETT et al. (1971), mentioned that acrosome formation, nuclear condensation and elongation of spermatids take place during spermatogenesis. Moreover, spermatids elongation is dependant upon normal microtubulin formation (CARTER and LASKEY, 1982),

which has been inhibited by Carbendazim (DAVIDSE and FLACH, 1977). Concurrently, sloughing of elongated spermatids after MBC exposure, could be due to disruption of Sertoli cell apices, which may be a result to impaired structure and function of the microtubules, especially at the sensitive stages; VII, XII–XIII & XIV of the seminiferous epithelium.

The microtubule inhibitor such as MBC may interfere during the early stages of spermatogenesis (DAVIDSE and FACH, 1977). In addition, development and maintenance of the shape of spermatozoa during the final stages of spermiogenesis, are dependant upon microtubules (FAWCETT et al., 1971). Similar findings were concluded too, since the MBC–sensitive stages were VII, XII–XIII and XIV (which present early in the cycle of the spermatogenic epithelium), and stage VII (late of the cycle), because the cycle of spermatogenesis begins with a spermatogonial mitosis at stage IX and ends with a release of spermatozoa at the end of stage VII (ROOSEN–RUNGE and GIESEL, 1950).

The frequency of a particular stage in a cross section of testis is proportional to the percent of time occupied by that stage (CLERMONT and HARVEY, 1965). The amount of time per cells of each stage is constant (COUROT et al., 1970). From the present results, the frequency in table 1 per each studied stage, was significantly changed, particularly at dose 200, 100 mg MBC, an indication of a disturbance in the kinetics of spermatogenesis.

In conclusion, the MBC–induced changes could be considered from the toxicologists during the preparation and formulation of a new effective rodenticide, containing Carbendazim, which had the potential to affect the reproductive health of rodents. Moreover, the handling of such toxic chemical in factories or fields must be faced with the highest safety precutions, in order to avoid the MBC–induced testicular response in human may exposed to such reproductive toxin by hazards.

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LEGENDS

Fig. 1: Seminiferous tubules of control rats. PAS–Haematoylin stain, Obj.: X20, Oc.: X10.

Fig. 2: Seminiferous tubules of control rats, showing stages VII and VIII. PAS–Haematoxylin stain, Obj.: X40, Oc.:x10.

Fig. 3: Seminiferous tubules of control rats, showing mitotic activity (arrow) at the spermatogonial cells. PAS–Haematoxylin stain, Obj.: X40, Oc. X10.

Fig. 4: Seminiferous tubules of control rats, showing stages XII and XIII. PAS–Haematoxylin stain, Obj.: X40, Oc.: x10.

Fig. 5: Seminiferous tubules of control rats, showing transition between stage XIII and stage XIV. PAS–Haematoxylin stain, Obj.: X40, Oc.: x10.

Fig. 6: Seminiferous tubules of control rats, showing stage XIV. PAS–Haematoxylin stain. Obj.: X40, Oc.: x10.

Fig. 7: Seminiferous tubules of 25 mg MBC–treated rat, showing multinucleated giant rounded spermatids. Haematoxylin and Eosin stain. Obj.: X40, Oc.: x10.

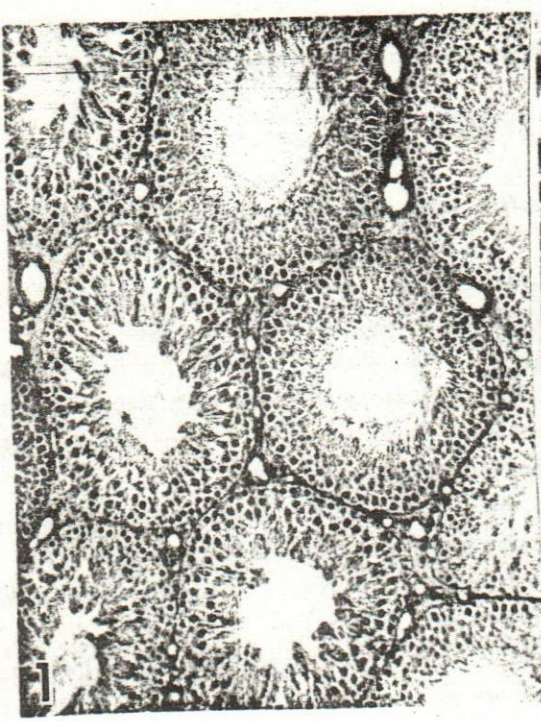
Fig. 8: Seminiferous tubules of 25 mg MBC–treated rat, showing stage VII with sloughed elongated spermatids (S) and stage VII which appears normal (N). PAS–Haematoxylin stain. Obj.: X40, Oc.: X10.

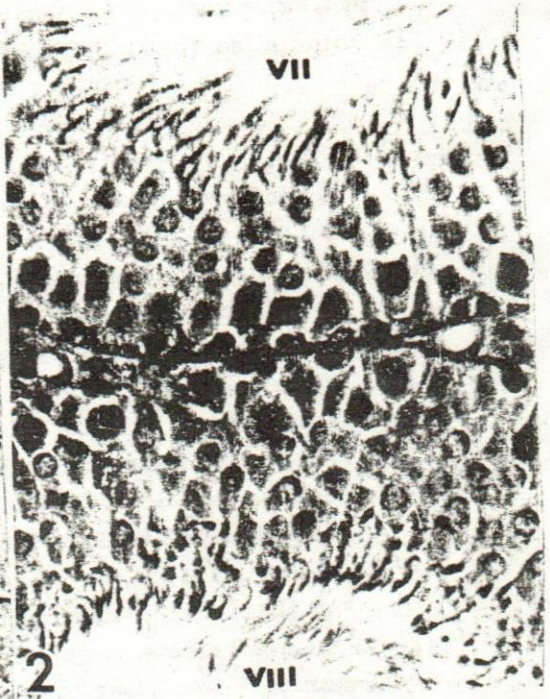
Fig. 9: Seminiferous tubules of 50 mg MBC–treated rat, showing sloughing of elongated spermatids within the lumina of the tubules. PAS–Haematoxylin stain. Obj.: X20, Oc.: x10.

Fig. 10: Seminiferous tubules of 100 mg MBC–treated rat, showing sloughed elongated spermatids at stage XII and stage XIV. Obj.: X40, Oc.: x10.

Fig. 11: Seminiferous tubules of 100 mg MBC–treated rat, showing sloughed elongated spermatids at a transition between stage XIII and stage XIV. PAS–Haematoxylin stain. Obj.: X40, Oc.: x10.

Fig. 12: Seminiferous tubules of 200 mg MBC–treated rat, showing sloughed spermatids within the lumina of the tubules. PAS–Haematoxylin stain, Obj.: X20, Oc.: x10.

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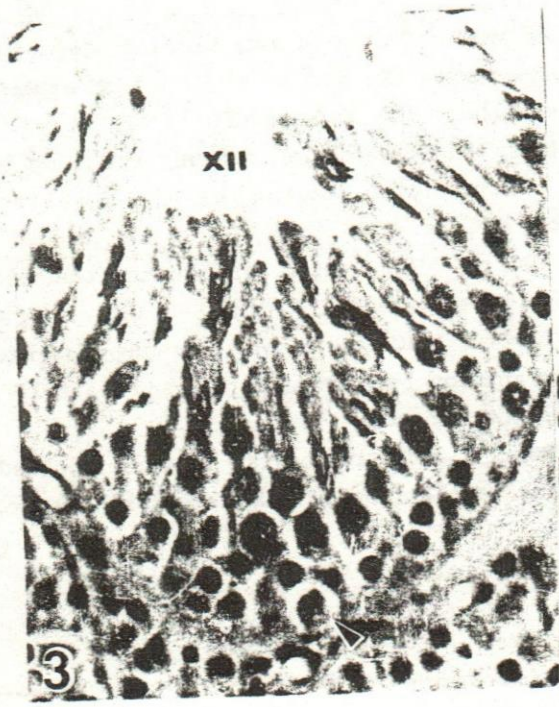
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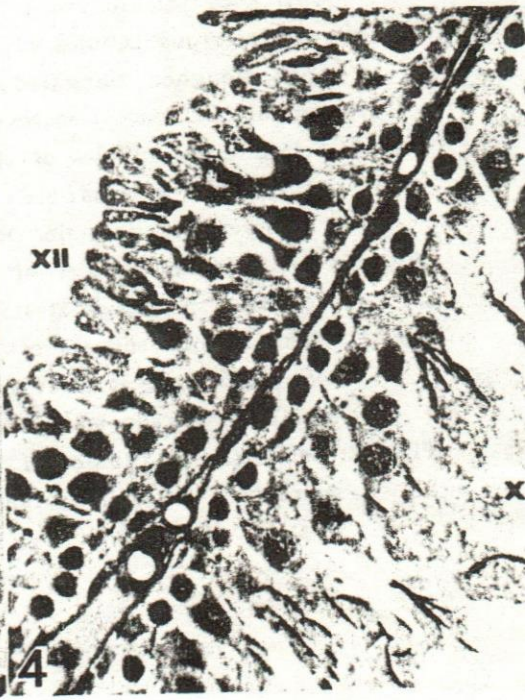
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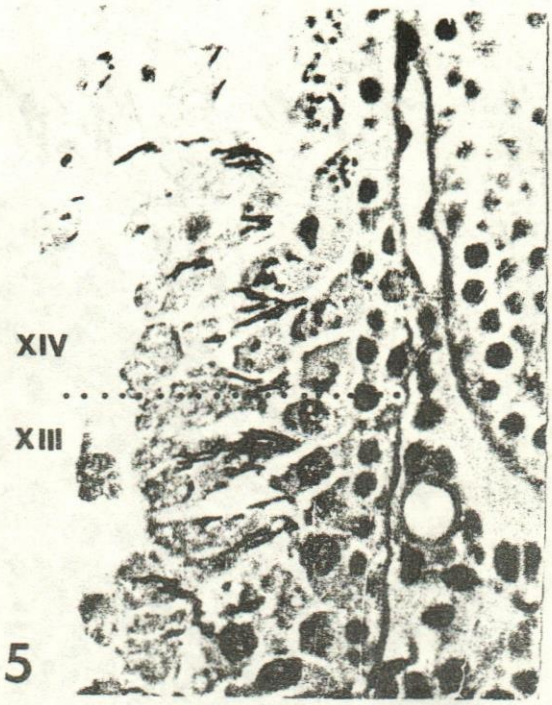

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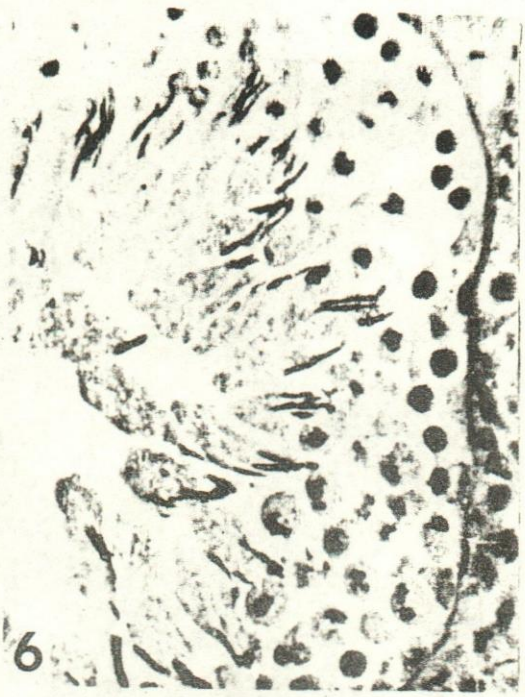

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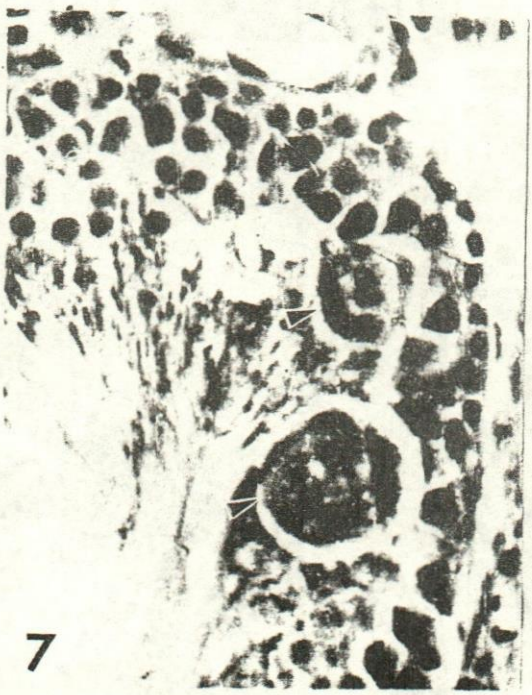

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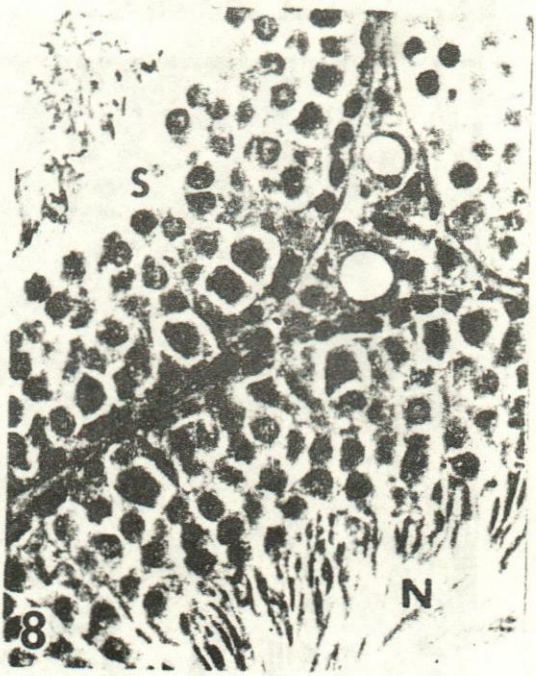

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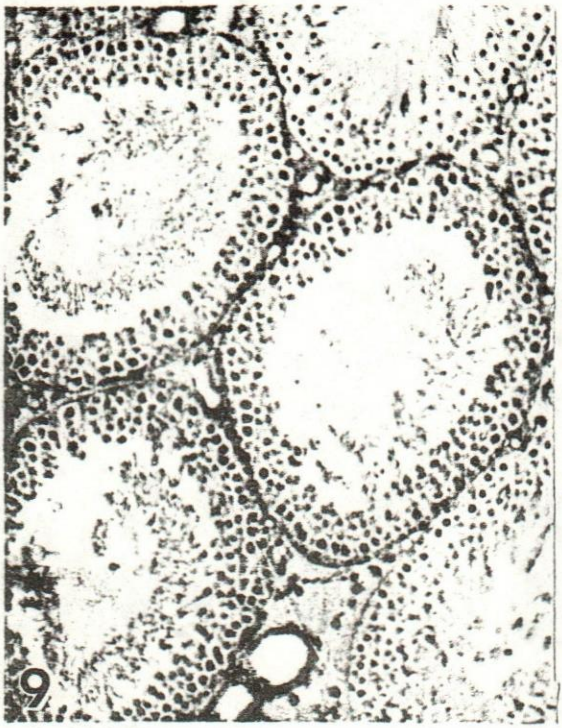

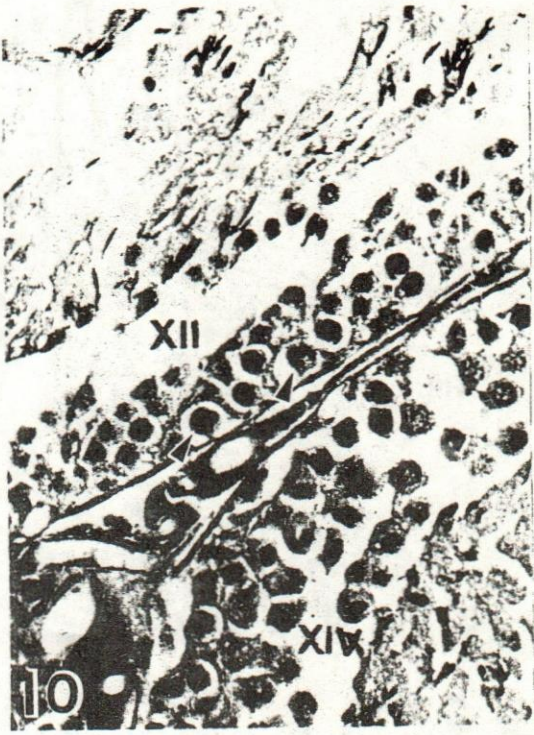
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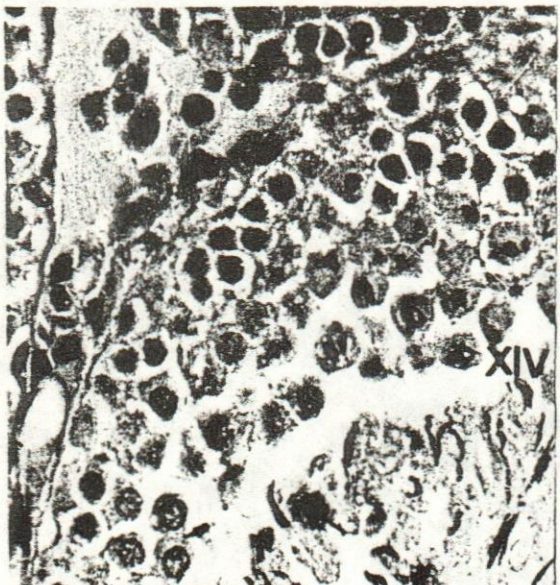
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