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IMMUNOHISTOCHEMICAL LOCALISATION OF STEROID HORMONES IN THE BOVINE PLACENTA AT DIFFERENT STAGE OF PREGNANCY

(With One Table & 6 Figure)

By

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البحث عن الهرمونات التناسليه فى مشيمة الأبقار وتحديد مكان انتاجها فى أطوار الحمل المختلفة بواسطة المناعه لهستوكيميائيه

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مازالت المعلومات المتوفره لدينا عن تكوين الهرمونات التناسليه (السيتروئيدات) وعن وظيفتها فى أطوار الحمل المختلفه عند الأبقار قليلة جدا. فهناك رأى سائد من الدراسات المخبريه على الأنسجه الخلويه المأخوذه من المشيمه (In Vitro) بأن المشيمه عند الأبقار لا تنتج البروجستيرون Proges teron بل مجموعة هرمونات البروجستين Progestin مثل Pregnandiol (البرويجناندول) كما هو الحال عند الماعز. وان انتاج هذه الهرمونات قد يكون هو السبب فى حماية الأبقار من الأجهاض أثناء الحمل فى الفتره ما بين (١٢٠ – ٢٥٠ يوما) فيما اذا تم حقنها بهرمون البروستانملاندين فى هذه الفتره. مجموعه أخرى من الباحثين يعتقدون بأن الغده جارة الكليه هى المصدر الاساسى لانتاج هرمون البروجستيرون فى مراحل الحمل الأخيره. من جهة أخرى فان انتاج الاستروجين Oestrogen فى أنسجة المشيمه له أهميه كبرى وخاصه فى النصف الثانى من مراحل الحمل عند الأبقار. وفى حين ان الاستروجين المرتبط Conjugated Ostogen فى البلاسما الدمويه يكون مرتفعا نسبيا فان الاسترون والاستراديول ١٧ بيتا (Ostradiol 17B, Ostron) يرتفعان بشكل ملحوظ فى حوالى العشرين يوما السابقه للولاده. وإن تحديد مكان إنتاج هذه الهرمونات المذكوره أعلاه على مستوى الخليه فى أنسجة المشيمة عند الابقار ما زال لم يحدد بالضبط.

SUMMARY

The aim of this research was to localize steroid homone synthesis (progesterone and estrogen) in the bovine placenta at different stages of pregnancy, using immunohistocchemical technique (peroxidase anti-peroxidase). Twenty-one gravid bovine uteri at various stages of pregnancy were investigated. Placenta tissues (fetal and maternal) were fixed in paraformaldehyde and glutaraldehyde or snapfrozen without any fixation. The immunohistochemical staining of placenta tissues was

positive with antibody to progesterone for all stages of pregnancy investigated. Whereas no immunoreactive production for estrogen was observed before parturition, it appears clearly on the sample collected during parturition (Cesarean Section) and was localized solely in binucleate giant cells.

Keywords: Immunohistochemical, localization, steroid hormones, bovine placerte, different stages, pregnancy.

INTRODUCTION

Little is known about placental steroid synthesis and function through mid-pregnancy in the cow. It has been suggested, form in vitro studies that the bovine placenta does not produce progesterone but other progestines, such as pregnandiols as in the goat *(SHELDRICK et al, 1980). PIMENTAL et al., (1986)* demonstrated that the placenta on day 250 post coitus is capable of producing progesterone. The production of steroids by the placenta and its localization effect on the uterus may be significant for the mainterance of pregnancty and protect against the abortive effect of exogenous prostaglandines between day 120 to day 250 of gestation *(ERB et al, 1971, THORBURN et al, 1977, FLINT et al, 1979, JOHNSON and JACKSON, 1980). SHREMESH et al, (1983, 1984)* have shown that bovine placental cells produce progesterone in culture, Similar studies in vitro *(REIMERS et al, 1985, WILLIAMS and GROSS, 1986)* have demonstrated the possible role of binucleate cells from bovine placenta as early as 120 days and late as 235 days of gestation. The binucleate cells showed a remarkable ability to produce progesterone and PGI2, PGE.

On the other hand there is a gradual decline in progesterone concentration with a concomitant increase in estrogen value during the last month of preganancy *(SMITH et al, 1973, DOBSON and DEAN, 1974)*. The mean source of estrogens in late pregnancy is the fetal portion of the placenta (villi, cotyledon) *(HOFFMANN et al, 1977, EVANS and WAGNER, 1981)*.

In vitro, the bovine placenta synthesizes free and conjugated estrogens from various androgens *(AINSWORTH and RAJAN, 1966, PIEREPOINT et al., 1969 and MOESTEL et al., 1987)*.

Many prvious studies of placental endocrine funtion in vitro have relied on the use of heterogeneous placental explants *(HOFFMANN et al., 1978, SCHNEIDER, 1988, KIESENHOFER, 1988 and WAGNER, 1988)* or cell supseions *(SHEMESH et al., 1984)*. Such

studies have contributed to elucidate the mecanism controlling placental steroid production but do not allow detailed endocrine examin-ation of the individual cell types within the placenta.

A technique has been described for immunohistochemical localisation of immunoreactive steroids in human chorionic villi *(NAKAMUR et al., 1981)*. In that study progesterone and estrogen were localized and differentiated unconjugated steroids in human term placenta. By applying the above histochemical technique to tissues obtained throughout pregnancy in the cow, as this species has morphologically different placentation from that in the human *(STEVEN, 1975)*, we tried to localize the steroid hormones (progesterone and estrogen) in the bovine placenta cells to provide conciese information on steroid secreting cells.

MATERIAL and WETHODS

Gravid bovine uteri (n=21) at various stages of pregnancy (125 d, 143 d, 157d, 173 d, 204 d, 270 d, post coitus) were obtained from abattoirs and another sample was taken during caesarean section at parturition.

The placentomes (maternal and fetal) were collected in less than 10 min post mortem. Specimens were pecocessed in three different ways (A, B, C).

The sections were stained by a modification of the technique described by STERNBERGER, (1979). All asections were sequentially incubated with the following reagnets:

1. Solution of 1% v/v hydrogen peroxidase in PBS for half to block endgenous peroxidase activity.
2. Normal 0.5% ovalbumin diluted 1:5 in PBS for half an hour to reduce non specific background staining.
3. Rabbit anti progesterone serum diluted with PBS (1:40) for three hours.
4. Swine anti rabbit immunoglobline G (IgG)diluted 1:50 in PBS for an hour at 37 C in a wet chamber. (Dakopatts, lot No. 018)
5. Horse-radish peroxidase rabbit anti horse-radish complex (PAP) 1:50 for one hour at 37 C. (Sigma, lot 117 F-8970).

After each incubation the sections were washed with tris-buffered saline for 10 min. Sections were washed with water, counterstained with Meyer's haemotoxylin, dehydarted with ethanol and mounted in DPX.

The specific reaction were observed with a light microscope.

RESULTS

In this study, attempts were made to fix and localize the steroid homones in bovine placenta using a 0.75% glutaraldehyde and

4% paraformaldehyde solution, which leads to better preservation of tissue structure and enzyme activity *(PEATRSE, 1975)*. However, leakage of steroid hormones from the placental tissues was unavoidable and the immunostaining of steeroids was hampered by diffusion of unbound steroid molecules Fig. (1-3).

Cryostat sectioning of directly snap frozen placenta tissue leads to a damage of the cells and a large irregular shape. The immunospecific reaction is very weak and localized uniformally intra-and extracellular (A).

On the other hand, fixation of the placenta tissue immediately followed snap freezing (B) is the most adaeqate method for histological preparation, steroid hormone preservation and immunospecific staining.

Tissues prepared with method C involving fixation of specimens, embedding in parafin and dehydration of tissues in alcohol lead to a better morphology, but the specific reaction diminished as the steroid hormones eluted from the tissues.

The immunohistochemical staining of placenta tissue reacted positively with variation in immunoreaction intensity to the antibody of progesteron at all stages of pregnancy investigated Fig. (1-3). Furthermore, no immunoreactive staining for estrogen was found at any stage of pregnncy investigted before prturition. It ws observed only in the smple collected t prturition (Fig. 5).

(Fig. 6) Control, no specific immunohistochemical staining for estrogen was observed. The sections were counterstained with Maayer's hematoxylin.

Figs. 1 and 2:

Illustrate the localization of progesterone in the placenta tissues at 125 and 270 days post coitus using peroxidase technique. Very week specific staining for progesterone was noticed at 125 d of pregnancy but a high intensity of staining was observed in the late pregnancy 270 d (Fig. 2), indicating an increased concentration of progesterone. On the other hand, the progesterone was distributed diffusely in the placenta cells and between the cells. Sections were counterstained using Mayer's hematoxylin.

Fig. 3:

Control section for progesterone. No detectable immunoreactive staining was observed.

Figs. 4 and 5:

Immunoperoxidase staining of bovine placenta specimens collected at parturition and stained with antibody directed against estrogen. The PAP immunreaction was localized excusively in the large binucleate cells (arrows). Sections were counterstained using Mayer's hematoxylin.

Fig. 6:
Control for estrogen seial section. The cells do not display any immunoreactive staining.

DISCUSSION

Many studies have utilized placental explants, tissue minces or dispersed cell preparations to examine placental steroidogenesis. Immunohistochemical detection of steroids in endocrine tissues has been attempted serveral investigators.

Immunohisto-chemical localization of estosterone *(BUBENIK et al., 1975)* was reoprted in frozen sections of rat and monkey testis. *KAWAOI et al. (1978)* showed immunofluorescent and immunoperxidase localization of the progesterone in a progesterone-secreting mouse adrenocortical adenoma cell line.

The trophoblastic componet of the ruminat chorion consists of numerous cubioidal uninucleate principal cells while about 20% are binucleate giant cells (BNC). The morphology *(BOSHIER and HOLLOWAY, 1977)*, histochemically and migraytory characteristics of binucleate trophoblastic cells in the ruminant *(WOODING and WATHES , 1980)* suggest a close relationship to the endocrinologically active syncytiotropholblast of the deciduate placenta of humans *(SIMPSON and McDONALD, 1981). WIENER (1976)* demonstrated that the bovine peripartal placental tissue is able to synthesize progesterone. *GROSS and WILLIAMS , 1988, ULLIMANN and REIMERS, 1989*, reported in vitro progesterone synthesis by mid - gestation placental BNC which may be involved in pregnancy maintenance via at least limited progesterone production and in parturition via estrogen synthesis.
In this immunhistochemical study, findings indicate that many cells in the placente synthesize progesterone at aall stages of pregnacy investigatec. Antisera to this hormone produced postive staining. THe intensity increased as pregnancy progressed (Fig.1& 2).

On the other hand, immunoreactive production for estrgene could be observed only on the sample collected parturition (Caesarean Section) and the immunoperoxidase staining for estrogene was localized solely in binucleate giant cells (Fig. 4 and 5). This supports previous results demonstrating that the bincleate cell is the site of steroid hormone synthesis in bovine placenta.

A precise understanding of the role of binucleate cells in the physiological regulation of steroido-gensis requires further investigation. It may be possible that the pogesterone shortly before parturition is trans-formed to estrogen in the binucleate cells. Further research including elec-

tron microsopic studies, is required to elucidate the exact sites of production of steroide hormones in the bovine placenta.

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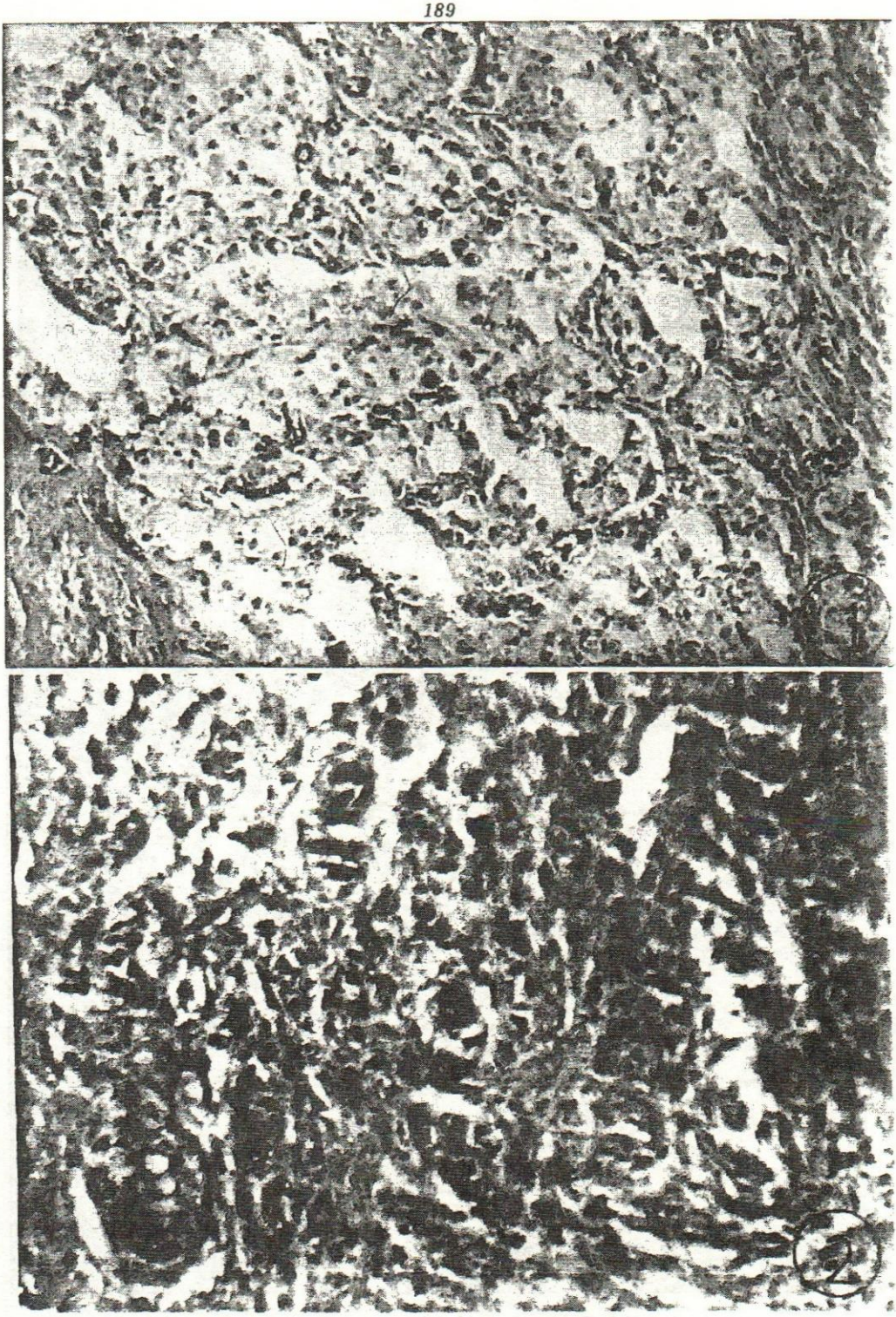

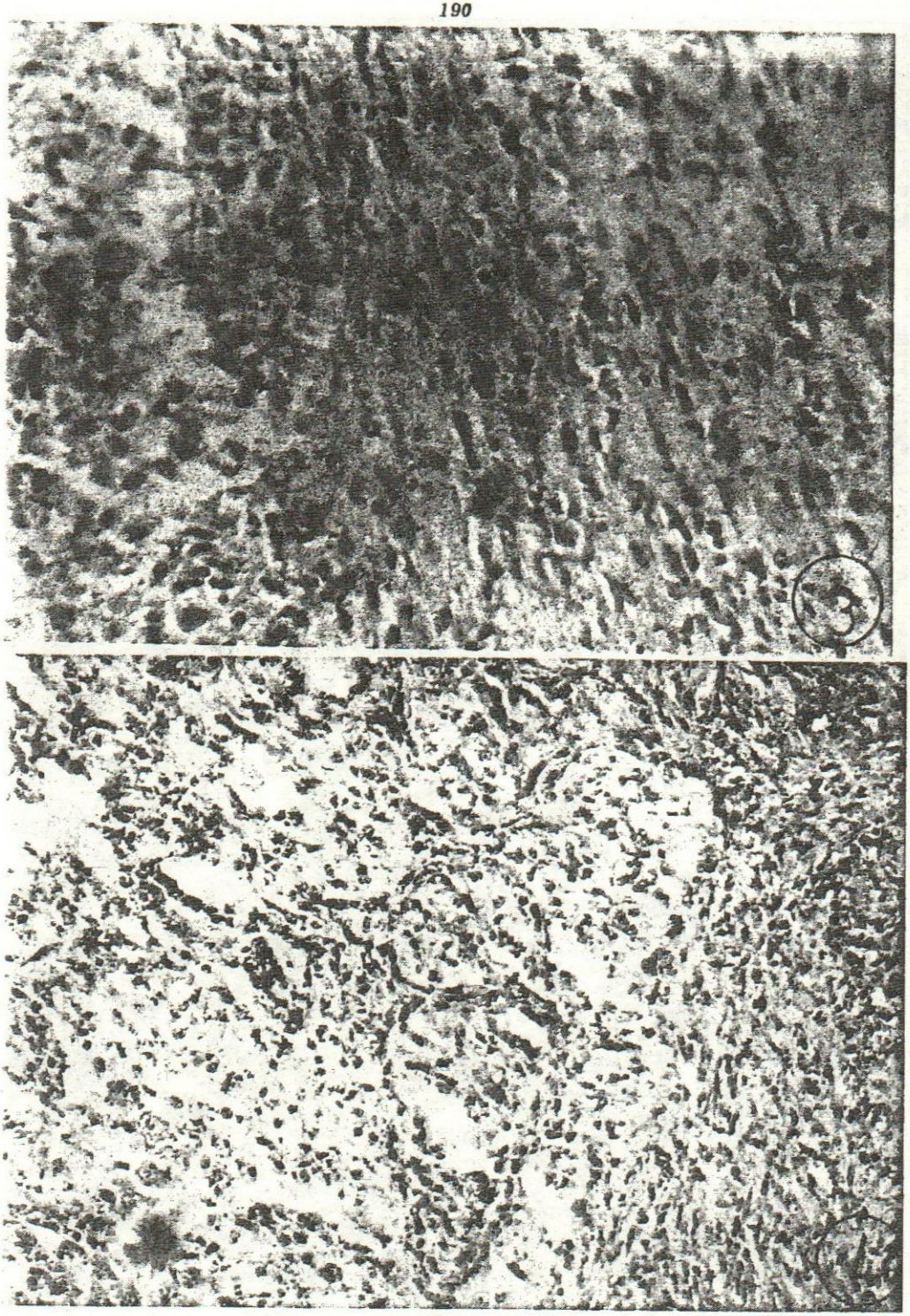

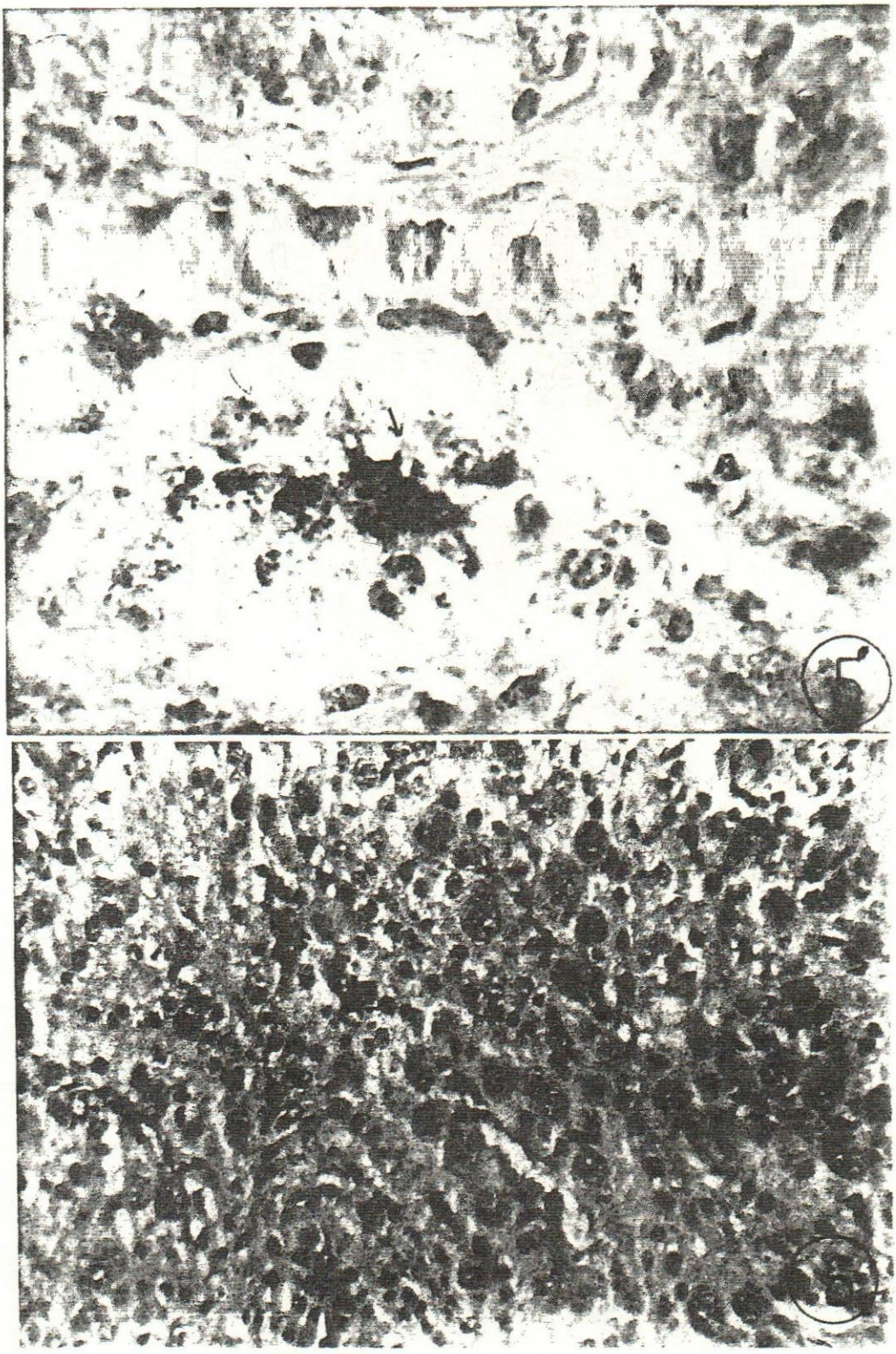

PREPARATION OF HISTOLOGICAL SPECIMEN

A) Direct snap frezing	(B) Fixation of tissues followed by snap freezing	(C) Fixation of the tissues followed by embedding in paraffin
- Dicing the tissues in 1 cm² blocks	- Dicing the tissues in 1 cm² blocks	- Dicing the tissues 1 cm² blocks
- Embedding in O.C.T. compound	- Fixation with 0.75 % glutaraldehyde, 4 % paraformaldhyde in PBS pH 7.4 for 24 hours	- Fixation with 0.75 % glutaraldehyde, 4 % paraformaldehyde in PBS pH 7.4 for 24 hours
- Freezing to -70°C with isopentane cooled by dry ice	- Rinsing in 20 % sucrose solution for 24 hours	- Processing and embedding in paraffin
- Microtoming in 5 µm thickness in a cryostat	- Freezing to -70°C with isopentane cooled by dry ice	- Cutting in 5 mm thick sections
- Rinsing in PBS pH 7.4	- Microtoming in 5 µm thickness in a crystat	- Deparaffinizing in xylen and alcohol
- Immunostaining with PAP-technique	- Rinsing in PBS pH 7.4	- Rinsing in PBS pH 7.4
	- Immunostaining with PAP-technique	- Immunostaining with PAP-technique