Histochemical studies of the common bile duct in reindeer

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Summary: Histochemical characteristics of bile duct mucosubstances, mast cells and globule leucocytes have not previously been described in the reindeer. Therefore various staining methods were applied on 1 to 6 specimens cut from formaline or Bouin-fixed histological blocks of the *ductus hepaticus communis* from 20 reindeer. The present study showed that bile duct mucins include neutral, carboxy- and sulphomucins located chiefly in goblet cells and in the deep glands and as a thin superficial layer covering the surface epithelium. PAS-reactivity was diastase resistant, indicating that glycogen was not demonstrable in the epithelial layer of reindeer, contrary to previous studies e.g., on carnivores. Furthermore, carboxymucins were sialidase-resistant, as sialic acid could not be identified in the present material. Certain differences were noted in the appearance and composition of intracytoplasmic granules and globules of mast cells and globule leucocytes, respectively. The mucosubstances of the mast cell contained sulphate groups indicative of sulphomucins while both neutral, carboxy- and sulphomucins were identifiable in globule leucocytes. However, due to the sensitivity of mast cells and globule leucocytes to postmortal changes the above interpretations need to be confirmed by further studies.

Key words: anatomy, histology, histochemistry, mucosubstances, globule leucocyte, mast cell.

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Rahko, T. ja **Nikander, S.** 1990. Histokemiallinen tutkimus yhteisen sappikäytävän rakenteesta porolla.

Yhteenveto: Yhteisen sappikäytävän (*ductus hepaticus communis*) seinämän epiteelisolukon ja syöttö- ja kerässolujen lima-aineiden koostumusta porolla ei ole aikaisemmin kuvattu. Sen vuoksi katsottiin aiheelliseksi suorittaa histokemiallinen tutkimus formaliini- ja Bouin-kovetetusta histologisesta aineistosta, jonka kirjoittajat ovat julkaisseet aikaisemmin. Aineisto on peräisin 20 porosta. Erilaisia histokemiallisia värjäyksiä sovellettiin 1–6:een valikoituun leikkeeseen tarkoituksella analysoida nimenomaan lima-aineiden koostumusta.

Tutkimuksissa ilmeni, että sappikäytävien erittämä lima koostuu neutraaleista, karboksy- ja sulfomusiinesta. Eritys tapahtuu pääasiassa pikarisoluista ja seinämien syvistä rauhasista. Myös pintasolukkoa peittää ohut limakerros. Glykogeeniä ja sialiinihappoa ei todettu. Syöttö- ja kerässolujen soluliman jyvästen koostumuksessa todettiin tiettyjä eroavaisuuksia. Syöttösolujen lima-aineet ovat sulfomusiineja, mutta kerässolujen jyväsissä on sulfomusiinien lisäksi myös neutraaleja- ja karboksymusiineja. Syöttö- ja kerässolujen todettiin olevan herkkiä kuolemanjälkeisille muutoksille, mikä vaikeutti varmojen johtopäätösten tekemistä.

Rangifer, 10 (1): 9—15

Rahko, T. och **Nikander, S.** 1990. Histokemiska studier av gallgången hos ren.

Sammandrag: Mucinernas sammasättning i globulära leukocyter, mast- och epitelceller i gallgångarna på ren har inte beskrivits tidigare. Det ansågs motiverat att histokemiskt undersöka det formalin- och Bouin fixerade material som tidigare publiserats av undertecknade. Materialet härstammar från 20 renar. Olika histokemiska färgningar utfördes på 1–6 utvalda preparat i avsikt att analysera muchinernas sammansättningen. Det framgick att slemmet i gallgången innehåller neutrala-, karboxyl- och sulfomuciner. Exkretionen sker i huvudsak från bägarceller och körtlar i gallgångväggen. Ytepitelet täckes också av et tunnt slemskit. Glykogen och sialinsyra påvisades icke. Det konstaterades skillnader i mucinsammansättningen hos mastceller och globulära leukocyter. I mastcellernas granulae påvisades sulfomuciner och de globulära leukocyterna innehöll förutom sulfomuciner, neutrala- och karboxymuciner. Konklusionerna bör verifieras medan mastcellerna och de globulära leukocyterna föreföll att var känsliga för postmortala förändringer.

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Introduction

In the available literature there are several histochemical studies on extrahepatic bile ducts of e.g., small laboratory rodents (McMinn and Kugler, 1961, Rahko, 1971), dogs (Seeliger, 1937, McMinn and Kugler, 1961), goats (Rahko, 1972a, b, Cheema and Hooshmand-Rad, 1985) and cattle (Rahko, 1971) while corresponding information is lacking in reindeer.

In a previous report, the authors have described the histologic structure of the common bile duct the reindeer (Rahko and Nikander, 1990). The present paper describes some histochemical characteristics of bile duct mucosubstances, mast cells and globule leucocytes.

Materials and methods

Staining methods were applied on 1 to 6 specimens cut from formalin- or Bouin-fixed material of 20 reindeer included in the histological material of our previous report (Rahko and Nikander, 1990). Stainings were performed according to the instructions described in the manual of the AFIP (1968) and Pearse (1968) and by Rahko (1971).

Mesenchymal tissues were studied by staining the sections with Herovic's staining and Ladewig's modification of Masson's trichromstaining.

Carbohydrate rich and mucinous compounds were analyzed by the following procedures: Periodic acid-Schiff with and without diastase (d-PAS, PAS) for glycogen and neutral glycoproteins. Acid mucosubstances were investigated by variously staining with alcian blue at pH 2.5 and pH 1.0 (AB 2.5, AB 1.0), nuclear fast red counterstain and with aldehyde fuchsin at pH 1.7.

The presence of sialomucins was studied with a sialidase digestion of the sections prior to AB 2.5 staining (sialidase-AB). The sialidase digestion was carried out in 0.1 M acetate buffer pH 8.9, containing 0.04 M calcium chloride and 0.1–4.5 U/ml sialidase (Neuraminidase, Sigma). The sections were digested at 40°C for 1 hour. Control sections were treated with buffer without the enzyme.

In order to differentiate acid (blue or blue purple) from neutral (red) mucosubstances, the sequence AB 2.5 followed by PAS (AB 2.5-PAS) was employed.

Sulphated mucosubstances were studied with alcian blue staining at pH 1.0 followed by PAS (AB 1.0 -PAS) to differentiate sulphomucins (blue or purple) from neutral mucins (red). Aldehyde fuchsin staining at pH. 1.7 followed by alcian blue at pH 2.5 (AF-AB 2.5) was further used to differentiate sulphomucins (purple) from nonsulphated (blue) acidic mucins.

Mast cells and globule leucocytes were studied by staining selected sections with amidoblack for identification of globule leucocytes, with v. Kossa's staining for calcium, with oil red O for neutral fats, with Fontana-Masson staining for melanin and iron-containing pigments, but no positive staining was observed. Furthermore, the cells were stained with alcian blue at pH 0.3 followed by safranin at pH 1.0 (AB-S) to differentiate heparine (red) from other highly sulphated mucosubstances (blue), with toluidine blue at pH 4.0 and pH 0.5 (TB 4.0, TB 0.5) to study the metachromatic properties of the granules in mast cells and the globules in globule leucocytes.

Results and discussion

Bile duct epithelium

Neutral, carboxy- and sulphomucins were iden-

Table 1. Identification of neutral, carboxy- and sulphomucins.

Staining method	Neutral mucins	Carboxymucins	Sulphomucins
PAS	red	-*	-
d-PAS	red	-	-
AB 2.5	-	blue	blue
sialidase-AB	-	blue**	blue
AB 2.5-PAS	red	blue/purple	blue/purple
AB 1.0	-	-	blue
AF	-	-	purple
AB 1.0-PAS	red	-	blue/purple
AB-S	-	-	purple/red***

* no staining reaction
** sialomucins -
*** heparin

Table 2. Histochemical characteristics of mucosubstances in the granules of mast cells and the globules of globule leucocytes.

	Mast cells	Globule leucocytes
PAS	0	0
d-PAS	0	0
TB 4.0	purple or blue 1+	blue 1+ or 0
TB 0.5	purple or blue 1+	0
AB 2.5	blue 2+	*blue 1+**
AB 1.0	blue 2+	*blue 1+**
AB 2.5-PAS	blue 2+	*blue or red 1+
AB 1.0-PAS	blue 1+	*blue or red 1+ or 0
AF-AB 2.5	blue 2+	*blue 1+ or 0
AB-S	blue 1+	*blue 1+ or 0

0: no reaction;
1+: weak positive reaction;
2+: moderate or strong positive reaction.

* blue staining appeared as intracytoplasmic precipitates between the globules or
** as cortical zones in the globules (in Bouin-fixed sections).

tified on the basis of different histochemical reactions presented in Table I.

The reactions for carbohydrate rich compounds were the most intense in the goblet cells and in the deep glands (Figures 1 to 5). Furthermore, a thin layer of mucins covered the surface epithelium.

Sulphomucins were demonstrable only in the goblet cells and in the deep glands. Carboxymucins, on the other hand, were present also in superficial mucins where the reaction of neutral mucins was also the most intense. Mucins were thus blue or purple with AB 1.0-PAS staining of the deep glands and goblet cells but red in superficial mucins, while AB 2.5-PAS stained superficial mucosubstances blue and mucins of the other areas purple. The carboxymucins did not show digestibility by sialidase, indicating

that sialomucins were not demonstrable in the present material. Otherwise, it appears that the bile duct mucins of reindeer are largely similar to those in cattle, goats and mice (McMinn and Kugler, 1961, Rahko 1971, 1972a and b). Glycogen was not identified in the bile duct epithelial cell as described by Seeliger (1937) in dogs.

Mast cells and globule leucocytes

The histochemical characteristics of carbohydrate rich compounds detected in mast cells and globule leucocytes are presented in Table II and Figures 5 to 8. It is, however, noteworthy to comment that the conclusions made on the staining properties of the intracytoplasmic granules and globules are based on observations of only a restricted number of cells detectable by light microscopy. Difficulties were also encountered due to the diffusion of stainable substances both in Bouin- and formalin-fixed material. Furthermore, when some, but not all, of the granules and globules were stained, the reaction was nevertheless considered positive.

In the AB 2.5 staining of Bouin-fixed material the cores of globules in the globule leucocytes stained red with nuclear fast red counterstain being surrounded by a blue cortical zone. In the Bouin-fixed material globule leucocytes did not show AF 1.7 stainable material.

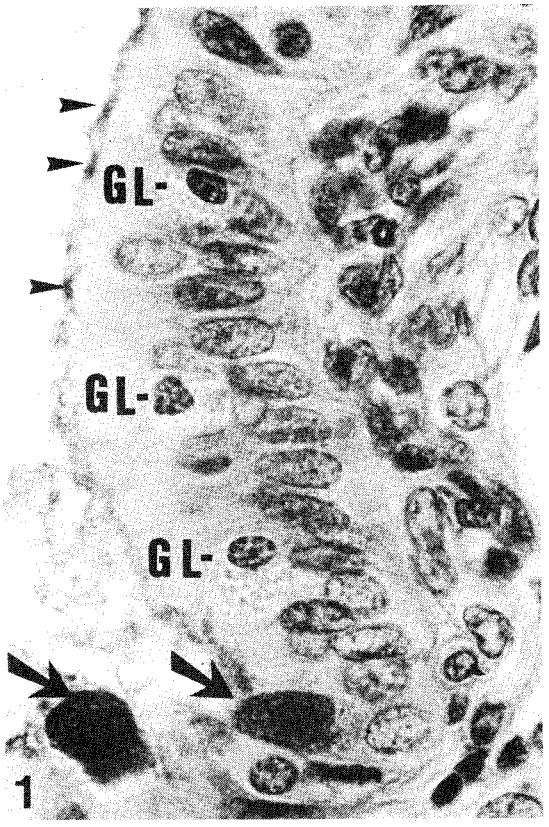
Mast cell granula and globule leucocyte globules displayed a somewhat different pattern in the staining reactions applied. The carbohydrate rich compounds of mast cell granules were more acidic than those in the globules of globule leucocytes. It is obvious that mast cell granula contain sulphomucins while globules of globule leucocytes show the staining reactions of neutral, carboxy- and sulphomucins. However, difficulties were produced by postmortal changes in interpretations of locations and shades of stainable material in mast cells and globule leucocytes. This phenomenon is analogous to that previously described in globule leucocytes in the bile ducts of goats (Rahko, 1972 b) and in the urinary bladder of rats (Ahlqvist and Kohonen, 1959). Also the mast cells of reindeer appeared to be sensitive to artificial changes so that the presence of heparin was not demonstrable by the present methods in the cells.

Fig. 1. Periodate-reactive neutral mucins are present in abundance in goblet cells (arrows) and cover the surface epithelium (arrowheads, GL = globule leucocyte) PAS, x 1040.

Fig. 2. Pancreatic acini are seen as dark-stained islets within mucosa. Arrowheads point to mast cells. TB 4.0, x 256.

Fig. 3. Dark-stained areas of surface and glandular epithelium contain vicinal groups of neutral and carboxymucins. Compare with Figure 4. AB 2.5-PAS, x 400.

Fig. 4. Reaction for hydroxyl and sulphate groups gives a weaker contrast than that for neutral and carboxymucins shown in Figure 3. Compare the figures and note that the stain in Figure 4 is weaker especially in the deep glands. AB 1.0-PAS, x 400.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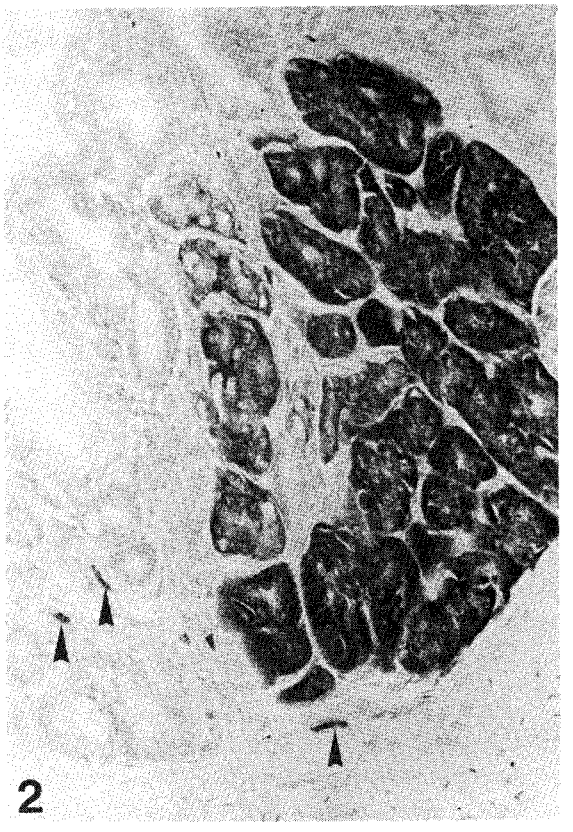
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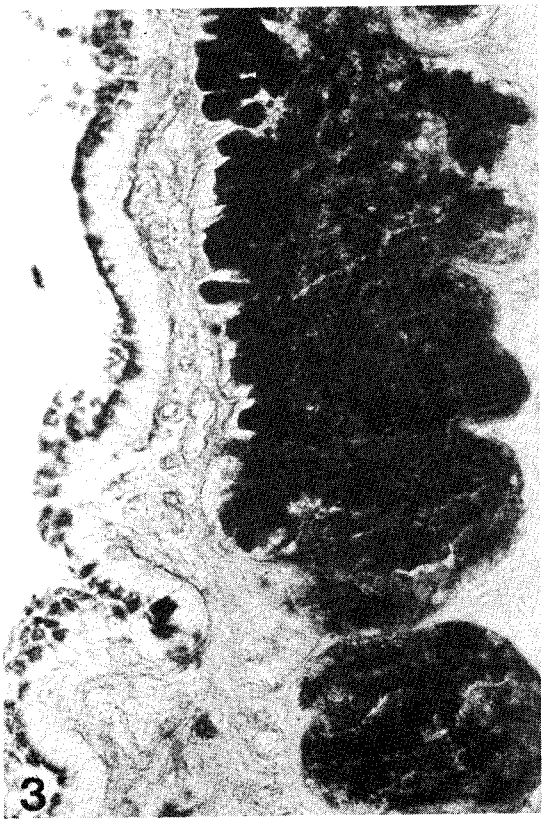
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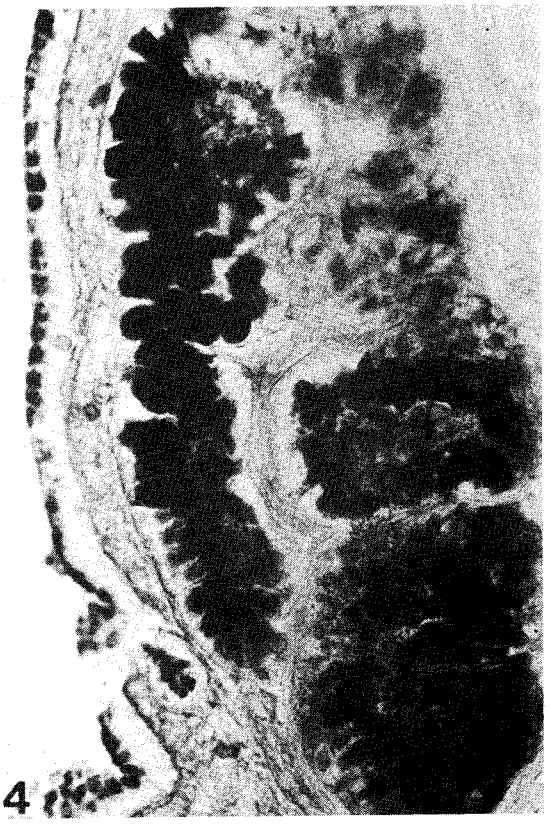
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References

Ahlqvist, J. & **Kohonen, J.** 1959. On the granulated cells of the urinary tract in rats infected with *Trichosomoides crassicauda*. - *Acta. Path. Microbiol. Scand.* 46: 313–319.

Cheema, A. H. & **Hooshmand-Rad, P.** 1985. Experimental cholecystitis in goats caused by mature *Fasciola gigantica*. - *Res. in Vet. Sci.* 38: 292–295.

Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology (AFIP). 1968. 3rd ed. - McGraw-Hill, New York.

McMinn, R. M. H. & **Kugler, J. H.** 1961. The glands of the bile and pancreatic ducts: autoradiographic and histochemical studies. - *J. Anat.* 95: 1–11.

Pearse, A. G. E. 1968. *Histochemistry: Theoretical and Applied. 3rd ed.* - J. & A. Churchill, Ltd. London.

Rahko, T. 1971. Studies on the Pathology of Bovine and Murine Liver infected with *Fasciola Hepatica* with Reference to the Mast Cell and Globule Leucocyte. Thesis. - *Ann. Acad. Sci. Fenn. Ser. A.V. Medica N:o 148.*

Rahko, T. 1972a. Studies on the pathology of dicrocoeliasis and fascioliasis in the goat II. The histochemistry of bile duct mucosubstances. - *Acta Vet. Scand.* 13: 563–574.

Rahko, T. 1972b. Studies on the pathology of dicrocoeliasis and fascioliasis in the goat III. The histochemistry of mast cells and globule leucocytes. - *Acta Vet. Scand.* 13: 575–584.

Rahko, T. & **Nikander, S.** 1990. Macroscopical and microscopical studies of the common bile duct in the reindeer. *Rangifer* 10(1): 3–8 (this issue).

Seeliger, M. 1937. Über den Bau des Gallengangensystems bei den Carnivoren (Hund und Katze) mit besonderer Berücksichtigung der Schleimbildung und des Glykogengehaltes. - *Z. Zellforsch. Mikr. Anat.* 26: 578–602.

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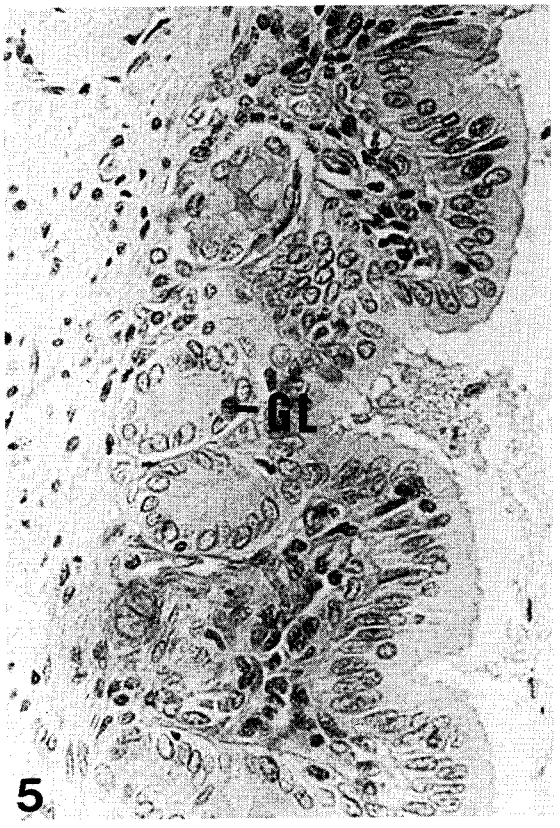
Fig. 5. A weak blue (dark) staining reaction indicative of sulphomucins is present in connective tissue matrix and in epithelial surfactant and in globules of globule leucocyte (GL). AB 1.0, X 400.

Fig. 6. Blue (dark) staining in globules of globule leucocytes (GL) and weakly purple metachromasy in granules of mast cells (MC) differentiate the sulphomucins of the globules and granules. Arrows indicate to pancreatic tissues within the mucosal layer. TB 4.0, x 256.

Fig. 7. An abundance of blue (dark)-stained mast cells and globule leucocytes (e.g. arrowheads) in the wall of the common bile duct. AB-S, x 100.

Fig. 8. Globule leucocytes (arrows) show a blue (dark) stain indicative of sulphomucins concentrated in the cortex of the globules. AB 1.0, x 1040.



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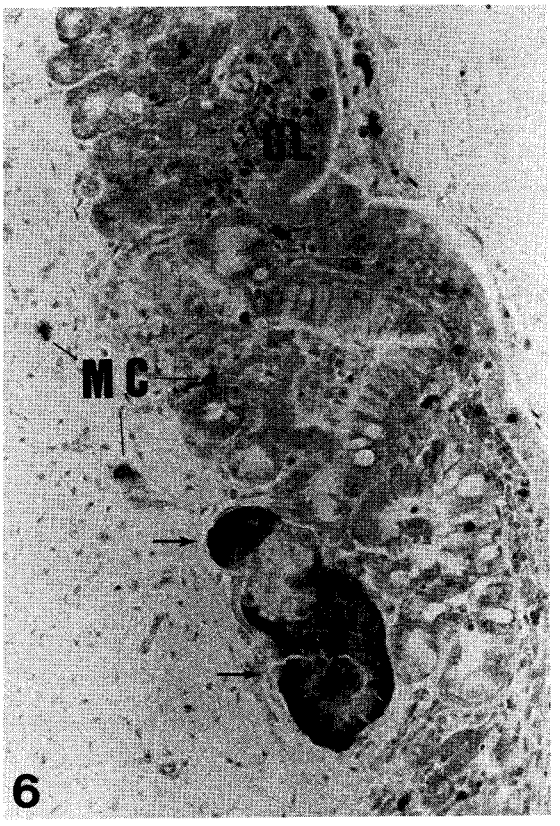

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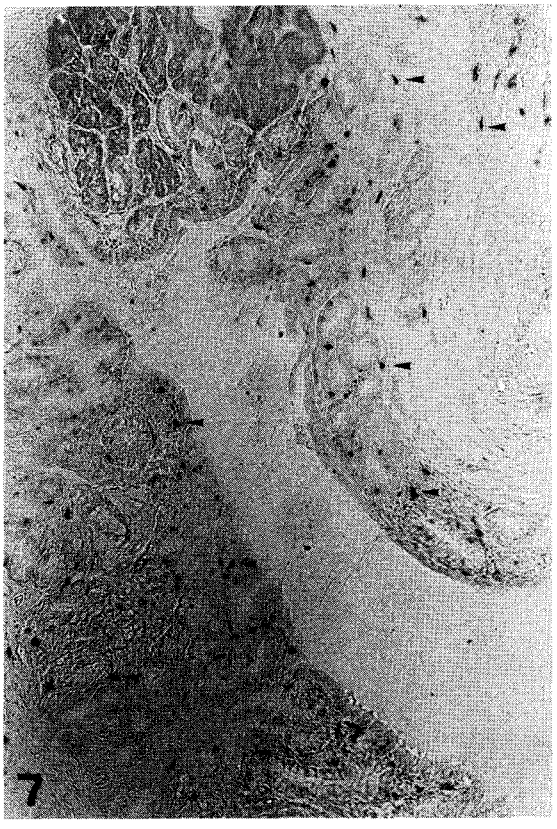

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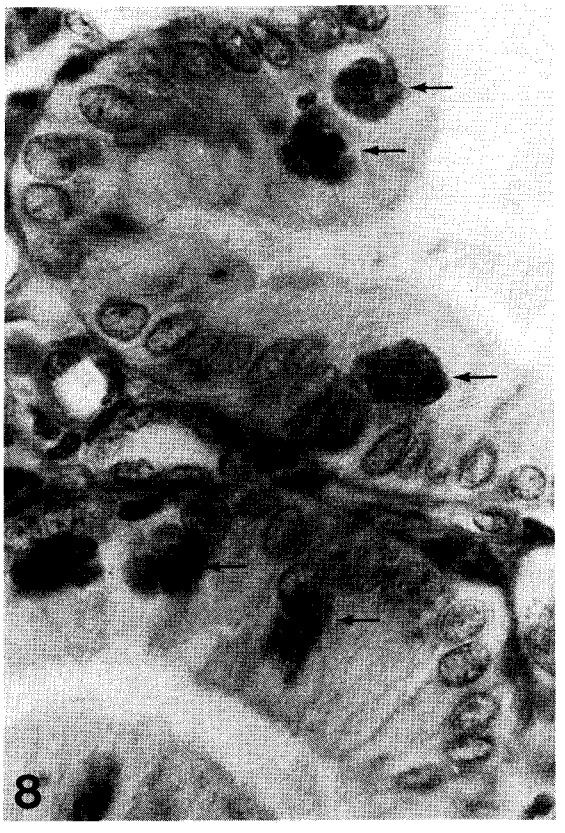

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