

From the Department of Pathology, College of Veterinary Medicine,
Helsinki, Finland.

STUDIES ON THE PATHOLOGY
OF DICROCOELIASIS AND FASCIOLIASIS
IN THE GOAT

III. THE HISTOCHEMISTRY OF MAST CELLS AND GLOBULE
LEUCOCYTES *

By
Timo Rahko

RAHKO, TIMO: *Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. III. The histochemistry of mast cells and globule leucocytes.* Acta vet. scand. 1972, 13, 575—584. — Comparative studies were made on the structure and histochemistry of mast cells and globule leucocytes in dicrocoeliasis and fascioliasis of the goat. Investigations showed that *D. dendriticum* and *F. hepatica* infections in the goat produce an increase in the number of hepatic mast cells and frequent occurrence of globule leucocytes in the epithelium of the bile ducts. The only difference between these infections was that globule leucocytes were more frequent in small bile ducts in dicrocoeliasis than in fascioliasis.

Histochemical methods showed the presence of sulphomucins in the granules of mast cells, while both neutral mucins and carboxymucins were revealed in the globules of globule leucocytes. The acidity of the mucosubstances could not be shown to result from hyaluronic acid, chondroitin-4 and -6-sulphates, or sialic acid. The globules of globule leucocytes also contained histochemically identifiable highly basic proteins.

Dicrocoelium dendriticum; *Fasciola hepatica*;
liver; bile ducts; mast cell; globule leucocyte.

The mast cell and globule leucocyte are both types of mammalian cells which possess intracytoplasmic granules. Mast cells are normal elements of connective tissue, while the globule leuco-

* Supported by a grant from the Finnish Veterinary Medical Foundation.

cyte is absent or infrequent in the tissues of parasite-free animals. In parasitic infections globule leucocytes occur frequently within the epithelium of different mucous membranes and several studies have shown that the mast cell proliferates, too (e.g. Wells 1962, Fernex 1968, Rahko 1971).

The appearance of mast cells and globule leucocytes in the walls of bile ducts has previously been studied in fascioliasis of cattle (Rahko 1970a, 1971), sheep (Murray *et al.* 1968, Rahko 1970b) and mice (Rahko 1971) and in dicrocoeliasis of cattle (Rahko 1970a) and sheep (Rahko 1970b). So far no studies have been published on these cells in the goat. This report describes some histochemical properties of carbohydrate-rich compounds in the granules of mast cells and globule leucocytes in the bile-duct walls of goats infected either with *Dicrocoelium dendriticum* or with *Fasciola hepatica*.

MATERIAL AND METHODS

The material and some histochemical methods are described in previous papers (Rahko 1972a, b). The terminology and symbols presented earlier for various stainings (Rahko 1972 b) are used in the present work, too. Additional staining methods employed are described below.

The globule leucocyte was identified by staining the sections with amidoblack (Puchtler & Leblond 1958). Toluidine blue was used according to Enerbäck 1966 a, b) as a 1.0 % aqueous solution in McIlvaine's citric acid disodium phosphate buffer pH 4.0 (TB 4.0) or in 0.5 N-HCL pH 0.5 (TB 0.5). Alcian blue was employed as an 0.1 % aqueous solution at pH 0.3 for 10 min. followed by staining with safranin at pH 1.0 (AB/S) for 30 min. (Enerbäck 1966 b). Basic proteins at the sites of carbohydrate-rich compounds were studied by staining with 0.04 % biebriich scarlet (BS) in Laskey's glycine buffer adjusted to pH 8.0—10.5 with NaOH, as described by Spicer *et al.* (1967). The sequence of alcian blue at pH 2.5 followed by biebriich scarlet at pH 9.5 (AB/BS) was also used, according to Spicer *et al.*

RESULTS

Mast cells were normally present in the connective tissues of interlobular areas and in the lamina propria and peripheral fibrous layer of the walls of bile ducts (Fig. 1). In dicrocoeliasis

and fascioliasis the distribution of mast cells remained normal, but the cells also proliferated in the connective tissues produced by these diseases. Consequently, there was an increase in the total number of hepatic mast cells in the infected livers. The density of the cells, however, seemed to be normal, their quantity

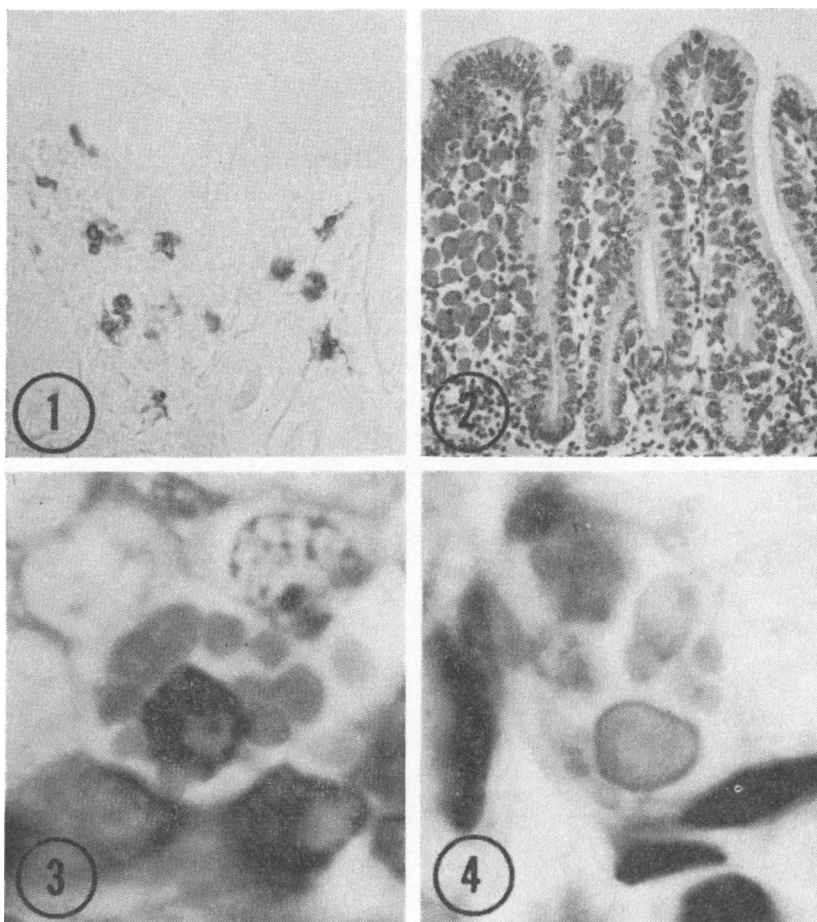


Figure 1. Section through wall of uninfected bile duct, showing mast cells in subepithelial tissues. AB/S \times 320.

Figure 2. High frequency of globule leucocytes in the epithelium and lamina propria of a main bile duct infected with liver flukes. HE \times 180.

Figure 3. Globule leucocyte showing regular eosinophilic globules in the cytoplasm. HE \times 2500.

Figure 4. Globule leucocyte showing two large irregularly formed globules. HE \times 2500.

being apparently correlated with the amount of connective tissues.

Mast cells could not be visualized by ordinary histological stainings, being revealed distinctly only by staining methods for acid mucosubstances. The cells were more or less spindle-shaped, and presented numerous small intracytoplasmic granules, which showed a tendency to become dissolved in the cytoplasm. The nucleus of the cells varied in size, form and chromatin density.

Globule leucocytes were extremely rare in normal bile ducts, whereas in dicrocoeliasis and fascioliasis the cells were frequent in varying degrees within the epithelium of the larger intrahepatic and the main bile ducts. In livers with particularly numerous globule leucocytes the cells occurred in the tissues of the lamina propria, too (Fig. 2). Intrahepatic bile ducts which were too small to harbour liver flukes seemed to display more frequent globule leucocytes in dicrocoeliasis than in fascioliasis.

Globule leucocytes showed striking differences in the structure of the intracytoplasmic globules (Figs. 3 and 4). The globules were usually liable to coalesce, forming more or less large aggregations in the cytoplasm, and only a few cells presented regular spherical globules (Fig. 5). An experiment using other fixation than formalin showed that Bouin's fluid preserved the

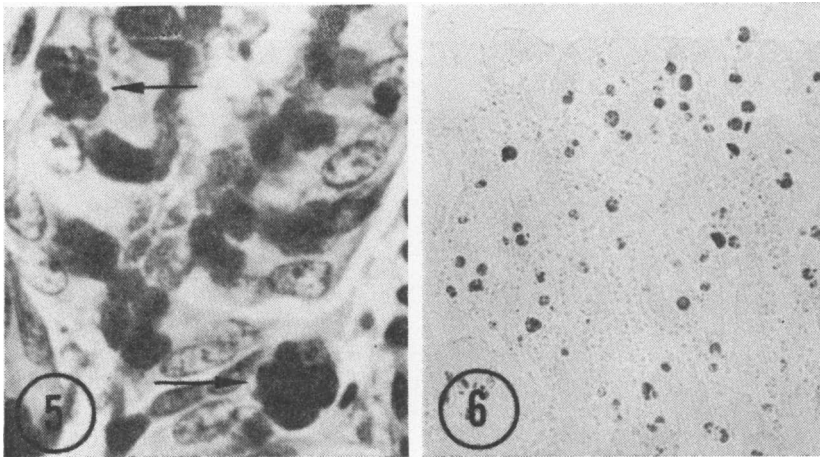


Figure 5. Globule leucocytes (arrows), showing periodate-reactive mucosubstances in the globules. PAS \times 630.

Figure 6. Basic proteins are present in the globules of globule leucocytes. BS \times 160.

structure of the globules better. The nucleus of the cells appeared pycnotic or was elongated with marginal chromatin. Globule leucocytes were readily differentiated from eosinophilic granulocytes, which were sometimes present in the epithelium but displayed only small granules in the cytoplasm.

The globules of globule leucocytes were strongly acidophilic, stained with eosin after HE, but turned either bright-red or orange after Ladewig's modification of Mallory's method. With amidoblack staining the globules became rimmed with a deep-staining cortex while the cores remained weakly stained. The above stainings did not reveal mast cells. The acidophilia of the globules in globule leucocytes evidently originated from strongly

Table 1. Histochemical characteristics of mucosubstances in the granules of mast cells and the globules of globule leucocytes (0: no reaction; 1+: weak positive reaction; 2+: moderate or strong positive reaction).

Staining method	Mast cells	Globule leucocytes
PAS	0	red 2+
diastase-PAS	0	red 2+
acetylation-PAS	0	0
deacetylation-PAS	0	red 2+
methylation-PAS	0	red 1+
methylation-saponification-PAS	0	red 1+
MC	0	0
TB 4.0	blue or purple 2+	0
TB 0.5	blue 1+	0
AB 2.5	blue 2+	blue 1+*
methylation-AB 2.5	0	0
methylation-saponification-AB 2.5	0	0
hyaluronidase-AB	blue 2+	blue 1+*
sialidase-AB	blue 2+	blue 1+*
deacetylation-sialidase-AB	blue 2+	blue 1+*
AF	purple 2+	0
AB 1.0	blue 2+	0
methylation-AB 1.0	0	0
methylation-saponification-AB 1.0	0	0
AB 2.5/PAS	blue 2+	blue 1+*, red 2+
AB 1.0/PAS	blue 2+	red 2+
AF/AB	purple 2+	blue 1+*
AB/S	blue 1+	0

* blue-staining substances usually formed precipitates in the cytoplasm between the globules.

basic proteins, which were stainable with BS even at pH 10.5 (Fig. 6). Mast cells, on the other hand, remained colourless after this staining, and in the lamina propria only eosinophilic granulocytes displayed red-staining substances in the granules.

A summary of the histochemical characteristics of carbohydrate-rich compounds in mast cells and globule leucocytes is given in Table 1, and brief comments on the results are made below.

The mucosubstances in the granules of mast cells were not periodate-reactive after ordinary PAS or after the removal of acetyl and acidic radicals. In contrast, the mucosubstances in the globules of the globule leucocytes were characterized by diastase-resistant, moderate or strong periodate-reactivity, which was not increased after the removal of acetyl radicals and became weakened after the removal of the acidic or the sulphate radicals (Fig. 5). The carbohydrate-rich compound of the mast-cell granules, judged by its staining characteristics in single and sequential stainings, consisted of acid mucosubstances, the acidity of which was produced by sulphate esters. Staining failed to reveal the presence of carboxyl groups and the staining with AB/S indicated only a low or moderate degree of sulphation in these mucosubstances. The globule leucocytes displayed carboxymucins, too, but no evidence for the presence of sulphate groups was obtained.

DISCUSSION

On the basis of the present and previous studies by the author it is well established that the frequent occurrence of globule leucocytes in bile ducts of cattle, sheep, goats and mice is associated with an infection with liver flukes (*Rahko* 1970 a, b, 1971). The studies have indicated that globule leucocytes are most frequently present in sections of bile ducts harbouring parasites. This agrees well with the occurrence of intestinal globule leucocytes (e.g. *Whur* 1966). In studies still in progress, the author has also observed that globule leucocytes may occur in the epithelium of interlobular bile ducts when parasites other than the liver fluke have burrowed near such interlobular areas during their migration through the liver.

Globule leucocytes are large cells and owing to their striking morphology and frequent occurrence readily distinguishable from the other cells in the bile-duct epithelium. Therefore it is

surprising that the literature available to the author on spontaneous dicrocoeliasis and fascioliasis contains only one publication (*Asdrubali & Mughetti* 1966) describing the presence of globule leucocytes in the epithelium of infected bile ducts. It is possible that other authors have also detected globule leucocytes but mistaken this type of cell for the eosinophilic granulocyte, which is occasionally found in the epithelium of bile ducts. For instance, *Joest* (1970) reported that during distomiasis eosinophilic granulocytes are transformed into a peculiar cell, the descriptions of which incline the present author to identify it as a globule leucocyte.

Previous studies have shown differences between different species of animals in the structure of the globule leucocyte in the intestinal wall (e.g. *Carr & Whur* 1968). The globule leucocytes in the bile-duct walls of cattle, sheep, goats and mice present not only morphological differences but also differences in the histochemistry of the globules. As with the mast cell (*Selye* 1965), the behaviour of the globule leucocyte towards formalin varied between the animal species studied. Thus the globules of globule leucocytes seemed to be most sensitive in the goat, and those in cattle and sheep were less sensitive, while those in mice were quite resistant to the dissolving action of formalin. In the present formalin-fixed material the alcianophilic substances of the globule leucocytes were mostly located in the cytoplasm, between the globules. *Ahlqvist & Kohonen* (1959) also reported that the metachromatic material in globule leucocytes in the urinary bladder was located in the cytoplasmic matrix of the cells.

The present histochemical studies indicated that the mucosubstances of mast-cell granules in the goat were sulphomucins, whereas those in the globules of globule leucocytes consisted of both neutral mucins and carboxymucins. The alcianophilic substances of these cells were resistant to hyaluronidase and sialidase, with and without previous deacetylation. According to *Pearse* (1961), *Gibbons* (1963) and *Ravetto* (1968), these phenomena indicate that the observed alcianophilia is not attributable to hyaluronic acid, chondroitin-4 or -6-sulphates, or sialic acid and acetylated forms of sialic acid. The globules also contained highly basic proteins, showing affinity to bieberich scarlet at high alkalinity. This method did not produce red-staining in the mast-cell granules. According to *Selye* basic proteins are

present in the granules, too, and the absence of staining reaction in the present material is probably due to the fact that they were blocked by formalin (*Spicer et al.* 1967). Staining of formalin-fixed material has also failed to reveal their presence in cattle, sheep and mice (*Rahko* 1970 a, b, 1971).

The staining for sulphate radicals did not reveal these strongly acidic groups in the mucosubstances of globule leucocytes. This does not necessarily indicate the absence of sulphate groups in the globules, because at high acidity basic proteins may mask the binding of copper phthalocyanine dyes (*Scott & Dorling* 1965). On the other hand, the globule leucocytes of cattle and mice showed a staining reaction for sulphate groups in the mucosubstances of the globules, although basic proteins were also present in these globules. Thus, it is possible that in the goat formalin fixation was unsuitable for the demonstration of sulphate esters, and further investigations employing other fixatives seem indicated.

REFERENCES

- Ahlqvist, J. & J. Kohonen*: On the granulated cells of the urinary tract in rats infected with *Trichosomoides crassicauda*. *Acta path. microbiol. scand.* 1959, *46*, 313—319.
- Asdrubali, G. & L. Mughetti*: Sul comportamento della cictifella nella distomatosi epatica del bovino. (The behaviour of the gall-bladder in hepatic distomiasis of cattle). *Atti Soc. Ital. Sci. vet.* 1966, *20*, 553—557.
- Carr, K. E. & P. Whur*: Ultrastructure of globule leucocyte inclusions in the rat and mouse. *Z. Zellforsch.* 1968, *86*, 153—162.
- Enerbäck, L.*: Mast cells in rat gastrointestinal mucosa. 1. Effects of fixation. *Acta path. microbiol. scand.* 1966 a, *66*, 289—302.
- Enerbäck, L.*: Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. *Acta path. microbiol. scand.* 1966 b, *66*, 303—312.
- Fernex, M.*: The Mast-Cell System: Its Relationship to Atherosclerosis, Fibrosis and Eosinophils. S. Karger, Basel 1968.
- Gibbons, R. A.*: The sensitivity of the neuraminocidic linkage in mucosubstances towards acid and towards neuraminidase. *Biochem. J.* 1963, *89*, 380—391.
- Joest, E.*: Handbuch der Speziellen Pathologischen Anatomie der Haustiere. Band VI. Digestionsapparat. (Handbook of the special pathological anatomy of domestic animals. Vol. VI. Alimentary system). Paul Parey, Berlin und Hamburg 1970.
- Murray, M., H. R. P. Miller & W. F. H. Jarrett*: The globule leucocyte and its derivation from the subepithelial mast cell. *Lab. Invest.* 1968, *19*, 222—234.

- Pearse, A. G. E.*: Histochemistry: Theoretical and Applied. J. & A. Churchill, Ltd., London 1961.
- Puchtler, H. & C. P. Leblond*: Histochemical analysis of cell membrane and associated structures as seen in the intestinal epithelium. Amer. J. Anat. 1958, 102, 1—22.
- Rahko, T.*: Globule leucocyte and mast cell in bile ducts of cattle naturally infected with liver flukes. Acta vet. scand. 1970 a, 11, 219—227.
- Rahko, T.*: Mast cells and globule leucocytes in bile ducts of cattle and sheep with fascioliasis and dicrocoeliasis. Nytt Mag. Zool. 1970 b, 18, 111—112.
- Rahko, T.*: 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. A 5 1971, 148, 1—62.
- Rahko, T.*: Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. I. The histopathology of the liver and bile ducts. Acta vet. scand. 1972 a, 13, 554—562.
- Rahko, T.*: Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. II. The histochemistry of bile-duct mucosubstances. Acta vet. scand. 1972 b, 13, 563—574.
- Ravetto, C.*: Histochemical identification of N-acetyl-O-diacetyl-neuraminic acid resistant to neuraminidase. J. Histochem. Cytochem. 1968, 16, 663.
- Scott, J. E. & J. Dorling*: Differential staining of acid glycosaminoglycans (mucopolysaccharides) by alcian blue in salt solutions. Histochemie 1965, 5, 221—233.
- Selye, H.*: The Mast Cells. Butterworth, Inc., Washington 1965.
- Spicer, S. S., M. W. Staley, M. G. Wetzel & B. K. Wetzel*: Acid mucosubstances and basic protein in mouse Paneth cells. J. Histochem. Cytochem. 1967, 15, 225—242.
- Wells, P. D.*: Mast cell, eosinophil and histamine levels in *Nippostrongylus brasiliensis* infected rats. Exp. Parasit. 1962, 12, 82—101.
- Whur, P.*: Relationship of globule leucocytes to gastrointestinal nematodes in the sheep and *Nippostrongylus brasiliensis* and *Hymenolepis nana* infections in rats. J. comp. Path. 1966, 76, 57—65.

SAMMANFATTNING

Undersökningar med avseende på dicrocoeliasis- och fascioliasis-infektionens patologi hos get. III. Mastcellernas och globule leukocyternas histokemi.

Jämförande undersökningar med avseende på mastcellernas och globule leukocyternas histokemi vid dicrocoeliasis och fascioliasis hos get har utförts. Resultatet visade att infektioner med *D. dendriticum* och *F. hepatica* orsakar en ökning av antalet mastceller i levern och en frekvent förekomst av globule leukocyter i gallgångsepitelet.

Enda observerade differens mellan dessa infektioner var att dicrocoeliasis visade rikligare förekomst av globule leukocyter i de små gallgångarna än fascioliasis.

Histokemiska metoder visade förekomst av sulfomuciner i granula hos mastceller medan neutrala- och karboxymuciner demonstrerades i globuler hos globule leukocyter. Hyaluronsyra, chondroitin-4 och -6-sulfater och sialinsyra kunde inte visas producera aciditet hos mukosubstanser. Globuler hos globule leukocyter innehöll starkt basiska proteiner, som kunde påvisas med histokemiska metoder.

(Received November 19, 1971).

Reprints may be requested from: Timo Rahko, Department of Pathology, College of Veterinary Medicine, Hämeentie 57, Helsinki 55, Finland.