mainly in the terminal position occur in morphologically identical cell types. In the case of intracellular staining of galactose residues we do not know whether the latter represent terminal saccharides or incompletely glycosylated glycoconjugates. The distribution of binding sites for DBA (specific for N-acetyl-galactosamine[20]) and BSA I (specific for D-galactopyranosyl[21]) in gastrointestinal cells showed a lack of correlation between PAS reactivity and lectin binding. While surface epithelium and Brunner's gland cells were mainly positive for DBA, PAS positive neck cells usually failed to stain with DBA-HRP (fig.). Antral gland cells also gave an irregular staining pattern; nonreacting and reacting cells were seen in the same histological preparation. PAS negative parietal cells were positive with DBA; chief cells were almost negative. In the case of BSA I binding, a similar pattern was found. Goblet cells and columnar striated cells exhibited an irregular staining pattern with the 4 lectins used (see table). Generally, the lectins did not bind only to cells with secretory activity but also to glycosylated nonmucus cell products. The heterogeneous lectin binding in histological preparations of normal mucosa suggests qualitative and quantitative differences in the composition of glycoconjugates. This might be due to different functional states and maturation stages; studies including diseased mucosae are needed.

1 This study was supported by the Deutsche Forschungsgemeinschaft (Ku 257/5-2 and Ku 257/6-3) Bonn (FRG).

2 Lev, R., Lab. Invest. *14* (1966) 2080.
3 Goldman, H., and Ming, S.C., Lab. Invest. *18* (1968) 203.
4 Leeson, T.S., and Leeson, C.R., J. Anat. *103* (1968) 263.
5 Rubin, W., Ross, L.L., Sleisenger, M.H., and Jeffries, G.H., Lab. Invest. *19* (1968) 598.
6 Filipe, M.I., Invest. Cell Path. *2* (1979) 195.
7 Morson, B.C., and Dawson, J.M.P., Gastrointestinal Pathology. Blackwell Sci. Publ., Oxford 1979.
8 Lis, H., and Sharon, N., A. Rev. Biochem. *42* (1973) 541.
9 Etzler, M.E., and Branstrator, M.L., J. Cell Biol. *62* (1974) 329.
10 Boland, C.R., Montgomery, C.K., and Kim, Y.S., Proc. natl Acad. Sci. USA *79* (1982) 2051.
11 Sato, A., and Spicer, S.S., Histochem. J. *14* (1982) 125.
12 Kuhlmann, W.D., Histochemistry *44* (1975) 155.
13 Wurster, K., Kuhlmann, W.D., and Rapp, W., Virchows Arch. Path. Anat. *378* (1978) 213.
14 Graham, R.C., and Karnovsky, M.J., J. Histochem. Cytochem. *14* (1966) 291.
15 Slocombe, G.W., Berry, C.L., and Swettenham, K.V., Virchows Arch. Path. Anat. *387* (1980) 289.
16 Matsumoto, J., and Osawa, T., Biochim. biophys. Acta *194* (1969) 180.
17 Stemmerman, G.N., J. natl Cancer Inst. *39* (1967) 375.
18 Gottschalk, A., Ann. N.Y. Acad. Sci. *106* (1963) 168.
19 Lotan, R., Skutelsky, E., Danon, D., and Sharon, N., J. biol. Chem. *250* (1975) 8518.
20 Etzler, M.E., and Kabat, E.A., Biochemistry *9* (1970) 869.
21 Murphy, L.A., and Goldstein, I.J., J. biol. Chem. *252* (1977) 4739.

0014-4754/83/030286-02$1.50 + 0.20/0

Mast cells in the pars flaccida of the tympanic membrane. A quantitative morphological and biochemical study in the rat[1]

P.E. Alm, G.D. Bloom, S. Hellström, L-E. Stenfors and L. Widemar[2]

Division of Experimental Medicine, National Defence Research Institute, Department 4, Umeå (Sweden), Departments of Anatomy, Histology and Otolaryngology, University of Umeå (Sweden), and Department of Otolaryngology, Huddinge University Hospital, S-141 86 Huddinge (Sweden), July 12, 1982

Summary. The present study has shown that the pars flaccida of the tympanic membrane of the rat is extremely rich in mast cells. The findings were compared with those from earlier investigations in the rat; the pars flaccida is one of the tissues in this animal in wich mast cells are most abundant.

Since its discovery by Ehrlich in 1877, the mast cell has attracted great interest and has been shown to be widely distributed in the connective tissue throughout the body in humans as well as in other species. The mast cell stores pharmacologically potent mediators, which after appropriate stimulation are released, and which participate in various inflammatory and hypersensitivity reactions (see eg. Selye[3], Bloom[4]).

Until lately, mast cells of the middle ear have been exclusively demonstrated in the hypotympanon, mainly in the connective tissue of the mucosal lining close to the tympanal orifice of the Eustachian tube[5]. However, in a recent study on the morphology of the pars flaccida of the tympanic membrane, this membrane portion was found to be extremely rich in mast cells[6]. In the present paper quantitative data will be presented on these pars flaccida mast cells and their possible role in the pathogenesis of a common pathological entity - otitis media with effusion - will be discussed.

Material and methods. 30 male Sprague-Dawley rats, average weight 200 g, were used for the study. They were anesthetized by i.v. injection of sodium pentobarbital. For light microscope studies, a mixture of ethanol-formalin (9:1) was used as fixative and instilled in the external auditory canals of 20 rats. After decapitation their tympanic membranes and, in 6 rats, parts of their mesenteries were carefully dissected out and immediately fixed. All specimens were stained with toluidine blue. Directly after staining, mast cell numbers in individual pars flaccidas were calculated and related to the total area of pars flaccida using a point counting method[7]. Thickness measurements of pars flaccida were performed on semithin sections (1 µm) of glutaraldehyde-fixed and Epon-embedded tissue. For determination of histamine content, 10 anesthetized animals were decapitated and the pars flaccida was dissected out. Its wet weight was determined, and the specimens were then rapidly frozen. Histamine was assayed according to the method of Shore et al.[8].

Results. Mast cells were randomly distributed throughout the pars flaccida (fig. 1) but were confined to a rather narrow layer of the propria immediately below the keratinized squamous epithelium (figs 2 and 3). It should be noted that pars tensa was prepared in the same manner but was totally lacking in mast cells. For comparison the

Table 1. Quantitative data for pars flaccida, its mast cells, some reference tissues and histamine values

Tissue/tissue component		Units	Mean ± SEM	
Pars flaccida (pf)	Thickness	mm	0.0295 ± 0.013	(10)
	Area	mm^2	1.10 ± 0.05	(16)
	Volume	mm^3	0.0325	
	Wet weight	µg	Range 100–200	
Pars tensa	Thickness	mm	0.0059 ± 0.0002	(5)
Mast cells				
Pars flaccida	Total number		596 ± 40	(11)
	Number per mm^2		561 ± 45	(11)
	Number per mm^3		18338	
	Density per mm^2-%		4.8 ± 0.37	(11)
Pars tensa	Total number		0 ± 0	(5)
Mesenterium	Density per mm^2-%		1.2 ± 0.21	(6)
Histamine	Concentration ng/pf		Range 8.0–8.5	(20)
	Concentration pg/ mast cell		Range 13.4–14.3	

Number of samples within parenthesis.

thickness of pars tensa was measured and found to be 1/5 of that of pars flaccida, 6 µm vs 30 µm. Pars flaccida was remarkably rich in mast cells. Our quantitative data are listed in table 1. In table 2 the results from earlier studies on mast cell distribution in 10 different tissues of the rat are listed[9].

Histamine content was determined in 20 flaccidas and the mean value was found to be 8.25 µg per flaccida (table 1).

Discussion. Comparing our results with those reported by Coleman and De Salva[9] it is evident that the number of mast cells per unit volume of pars flaccida is only exceeded by that found in the reticular layer of the lip and cheek of the rat. However, considering that the mast cells of the pars flaccida are confined to a very narrow plane beneath the epithelium their actual density is far higher than that reported for any other rat tissue.

Rat peritoneal fluid is a common source of mast cells for experimental purposes due to its known richness in these cells. According to Padawer and Gordon[10] peritoneal mast cell numbers vary somewhat between the values 3340/mm^3 in male rats and 3150/mm^3 in females. These figures should be compared with the present finding of 18,000 mast cells/mm^3 in the pars flaccida. In the present study we also compared, on an area basis, rat mesenteries, known to be rich in mast cells, with our flaccidas. We found mast cell numbers in the latter to be 4 times greater than those found in the mesenteries.

With respect to the histamine content determined it was observed that the values correlated well with the mast cell numbers in individual flaccidas. Total numbers of mast cells in the flaccidas we studied varied somewhat between 450 and 750. Histamine values ranged from 8–8.5 ng per flaccida. A rough estimate of amount of histamine per mast cell lies within the range 13.4–14.3 pg per cell. This corresponds well with values reported in the literature 8.6–40 pg/cell[11].

The presence of unusually large numbers of mast cells within this specific area of the tympanic membrane raises

Table 2. Mast cell distribution in different tissues of the rat. (Coleman and De Salva[9])

	Number/mm^3
Lip-papillary	7.450 (8)
Lip-reticular	27.137 (8)
Cheek	18.500 (8)
Distal ear	14.512 (8)
Dorsum-papillary	4.537 (8)
Dorsum-reticular	6.200 (8)
Ventrum	3.300 (8)
Tail	2.537 (8)
Footpad-papillary	5.312 (8)
Footpad-reticular	14.627 (8)

Number of samples within parenthesis.

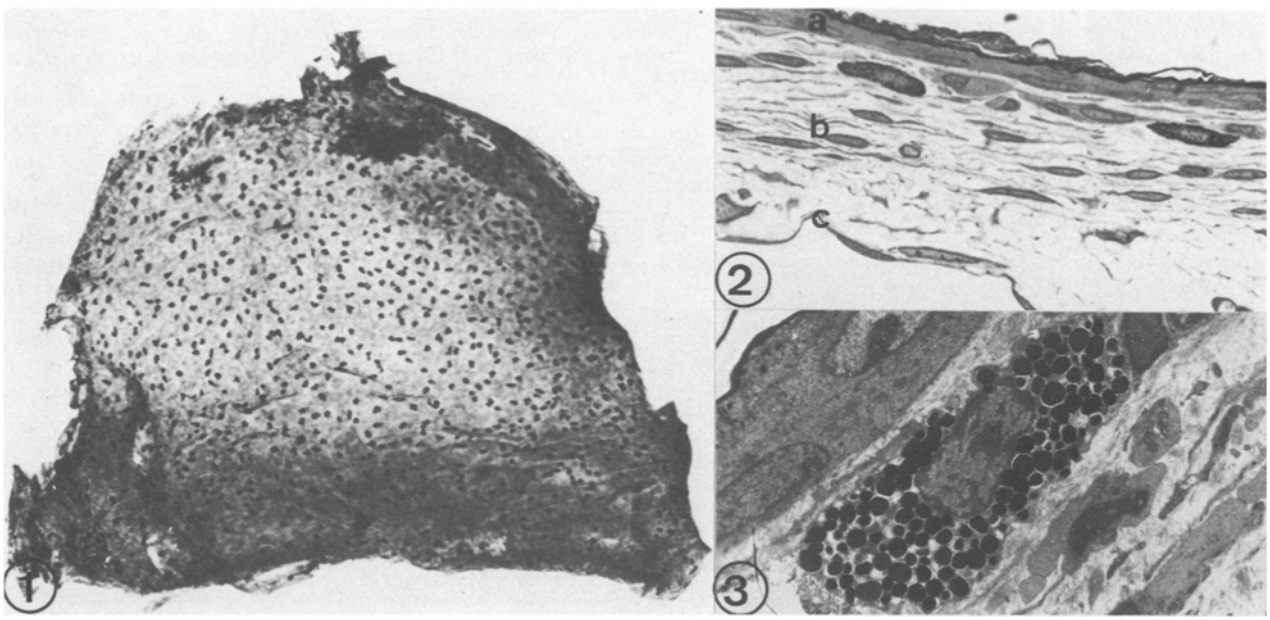


Figure 1. Light micrograph of a whole mounted pars flaccida fixed in ethanol-formalin and stained with toluidine blue. The numerous mast cells are easily distinguished. × 60.

Figure 2. Light micrograph of a semithin (1 µm) section of an Epon embedded pars flaccida. *a* Stratified squamous epithelium. *b* Lamina propria. *c* Inner epithelial lining. Note the mast cells immediately below the stratified squamous epithelium. × 600.

Figure 3. Electron micrograph of a mast cell from pars flaccida. × 5000.

the important question of their possible participation in pathophysiological conditions which affect the middle ear compartment. In recent experimental studies it has been shown that the initial effusion material in otitis media with effusion (OME) originates from the attic space - the compartment which is laterally bordered by pars flaccida[12]. Moreover the induction of effusion e.g. by a blocked tympanic isthmus or mechanical stimulation of the external auditory canal is accompanied by a degranulation of the pars flaccida mast cells and a subsequent release of histamine into the middle ear cavity[6,13]. It has also been found that onset of effusion may be brought about by instilling the potent mast cell degranulating drug, compound 48/80, into the external auditory canal[4]. The mechanisms involved in this pathological entity are at present being further explored.

1 Acknowledgments. The study was supported by grants from the Swedish Medical Research Council (B83-17X-06578-01), the Magnus Bergvall Foundation and the Medical Faculty, University of Umeå.

2 Reprint requests to L.W., Department of Otolaryngology, Huddinge University Hospital, S-14186 Huddinge (Sweden).
3 Selye, H., The Mast Cell. Butterworth, Washington 1965.
4 Bloom, G.D., The inflammatory process. Eds B.W. Zweifach, L. Grant and R.T. McCluskey. Academic Press, New York 1974.
5 Hentzer, E., Ann. Otol. Rhinol. Lar. *79* (1970) 1143.
6 Alm, P.E., Bloom, G.D., Hellström, S., Salén, B., and Stenfors, L-E., Acta oto-lar. *94* (1982) 517.
7 Weibel, E.R., Stäubli, W., Gnägi, H.R., and Hess, F.A., J. Cell Biol. *42* (1969) 68.
8 Shore, P.A., Burkhalter, A., and Cohn, Jr, V.H., J. Pharmac. exp. Ther. *127* (1959) 182.
9 Coleman, E.J., and De Salva, S.J., Proc. Soc. exp. Biol. Med. *122* (1966) 945.
10 Padawer, J., and Gordon, A.S., J. Geront. *11N*(1956) 268.
11 Helander, H.F., and Bloom, G.D., J. Microsc. *100* (1974) 315.
12 Hellström, S., Salén, B., and Stenfors, L-E., Acta oto-lar. *93* (1982) 435.
13 Alm, P.E., Bloom, G.D., Hellström, S., Stenfors, L-E., and Widemar, L., Acta oto-lar. (1982) in press.

0014-4754/83/030287-03$1.50+0.20/0

Diethylpropion decreases food intake in fish[1]

W. Mrowka

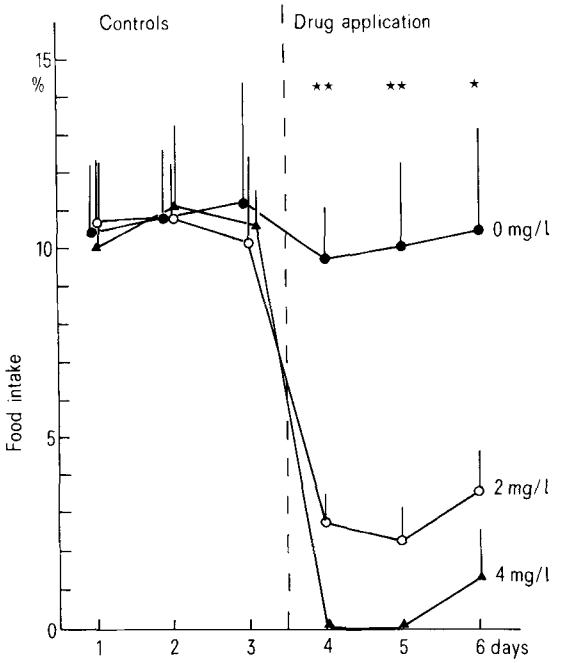
Zoologisches Institut, Technische Universität Braunschweig, Pockelsstrasse 10a, D-3300 Braunschweig (Federal Republic of Germany), June 18, 1982

Summary. Daily food intake of 5 specimens of *Haplochromis burtoni,* measured in a 30-min feeding session, was reduced by the application of water solutions of 2 and 4 mg/l diethylpropion (2-diethylamino-propiophenon).

Diethylpropion (2-diethylamino-propiophenon) is known for its anorectic effects in rats and dogs[2-7]and is used therapeutically in man[8,9]. The purpose of this study was to investigate how this anorectic drug influences feeding in fish.

Material and methods. 5 nonbreeding female *Haplochromis burtoni (Cichlidae)* served as test animals. Their weight ranged from 4.8 to 7.7 g. They were housed individually at 27 °C in optically isolated tanks containing 5-6 l water and fine gravel. A daily 30-min feeding session was held between 14.00 and 15.00 h. In preparation for this feeding session, 1-1.5 g Tubifex worms per fish were washed, dried for 5 min on blotting paper, weighed (with an accuracy of 10 mg) and then placed in special feeding containers, each consisting of a small pot (2 cm diameter) with a perforated plastic cover. The Tubifex worms could not leave the container on their own, but the fish could pull them out. At the end of the feeding session the remaining Tubifex worms were washed, dried and weighed again as described above. The differences between the initial weights and the end weights were expressed as a percentage of the body weight of the individual fish. The same 5 fish were used in 3 consecutive experimental series with an interval of at least 1 week between each series. One week proved ample time for complete recovery from previous experimental effects on daily food intake. Each series began with 3 days of normal feeding according to the schedule described above. On day 4 at 9.00 h diethylpropion was added to the tanks so that the concentrations were 0 mg/l (controls), 2 mg/l and 4 mg/l respectively. Water conditions remained unchanged for the next 3 days. Then the solutions were replaced by fresh water.

Results. As can be seen from the figure, diethylpropion decreases dose-dependently the daily food intake of the fish. Application of 4 mg/l results in a complete inhibition of feeding. Intermittent observation of the feeding behavior of the fish during the feeding session did not reveal any



Effects of different concentrations of diethylpropion on daily food intake (in percent b.wt) of 5 *Haplochromis burtoni.* Means and SD. Differences were tested using Friedman's 1-way analysis of variance; * $p < 0.05$; ** $p < 0.001$.