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Original Research Paper

MAST CELLS IN THE SUBEPIDERMAL LAYER OF THE BUDGERIGAR (*MELOPSITTACUS UNDULATUS*) DURING MOULTING

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Summary: We used histological methods to examine skin from the head region of budgerigars (*Melopsittacus undulatus*) during their feather regeneration (moulting period). Sixteen birds were included in the study; 8 were treated with thyroxine to accelerate moulting and another 8 represented the control group. Paraffin-embedded tissue samples were stained with haematoxylin and eosin, trichrome stain (after Goldner) and toluidine blue. Light microscopy was used to locate mast cells and their numbers were assessed by a Lucia-M computer-assisted image analysis system (Optoteam, Vienna).

Mast cells, which were found to be a regular component of budgerigar skin, were predominantly located in subepidermal connective tissue; however, they were also found near the dermal blood vessels. Thyroid hormones are elevated during moulting and lymphocytic infiltrations can be found in the skin, however, we found that the number of mast cells was significantly higher ($P < 0.01$) in thyroxine-treated animals. Although clinically healthy, 37 % of the animals investigated were infected with ectoparasites of the *Cnemidocoptes* genus. In those animals, which were found in both the thyroxine-treated and control groups, the number of mast cells was also significantly higher, probably as a consequence of their immune responses.

Key words: mast cells; budgerigar; skin; feather regeneration; parasitic infection

Introduction

Mast cells are a regular component of loose connective tissue, especially along blood vessels. They are migratory and are of various forms and sizes (1). They are relatively large cells with a centrally-located spherical nucleus and numerous cytoplasmic granules. Immature granules are small and orthochromatic with basic dyes, while the larger, mature granules are metachromatic (2, 3). Mast cells produce, store and excrete various biogenic amines and play an important role in an organism's immediate, delayed, local and systemic hypersensitivity. In mammals, two populations of mast cells are known: mucosal mast cells – associated with gut and lung mucosae, and the ubiquitous connective-tissue mast cells. Besides tissue distribution, they differ in their staining characteristics, receptor numbers and protease content (4). Increased numbers of connective-tissue mast cells are regularly found in the granulated tissue of healing wounds and in various pathological changes such as chronic dermatoses and many tumours (5).

Ehrlich drew attention to the specific metachromatic staining characteristics of these cells as early as 1877 and gave them the name "Mastzellen". Among many species, he described them in pigeons. Danchakoff (6) later described them in connective tissue along the blood vessels of the omentum in domestic fowl and Arvy (7) also described them in the connective tissue of *Gallus domesticus*. They are sparse in fowl compared to other domestic animals according to Boseila (8).

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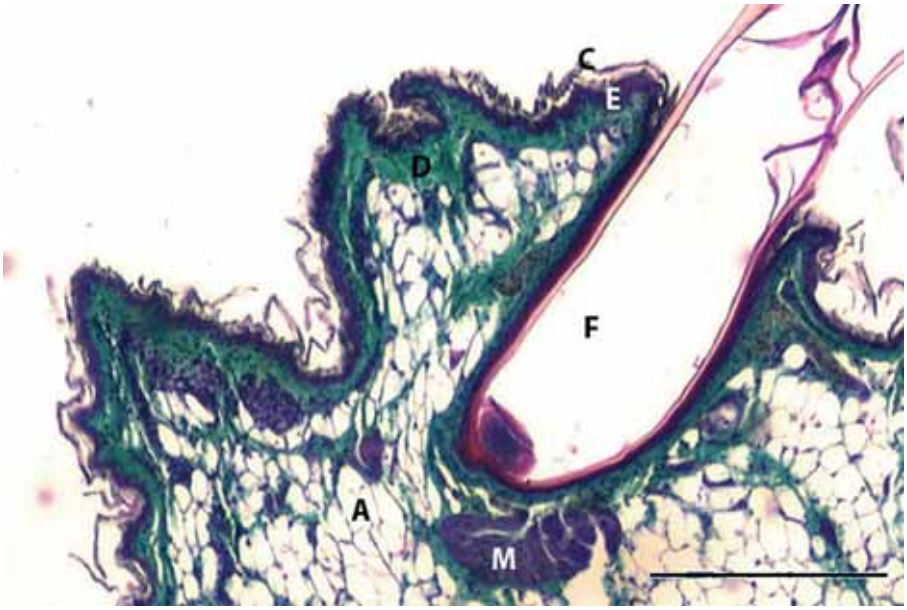

Figure 1: Skin from the head region of a budgerigar. Epidermis (E) with *stratum corneum* (C), dermis (D), feather muscles (*musculi pennarum*, M), feather follicle (F) and fat tissue in the hypodermis (*corpora adiposa*, A). Trichrome staining after Goldner, scale bar = 200 μm

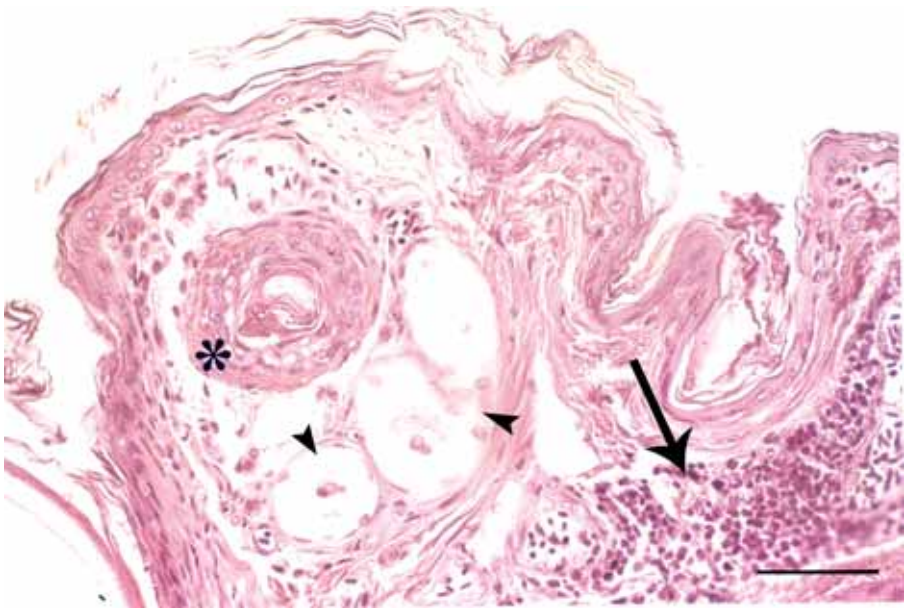

Figure 2: Skin from the head region of a budgerigar during moulting. Lymphocyte infiltrations during feather regeneration can be seen in the dermis (arrow) as can lamellar bodies (Herbst bodies, arrowheads) around a thread feather (filoplume, asterisk). H&E stain, scale bar = 50 μm

Wight (9) extensively researched *Gallus domesticus* and reported that mast cells are mainly concentrated in the infundibulum of the oviduct, the ovary, the periphery nerves and the alimentary tract, particularly the proventriculus and floor of the mouth. He observed that, in contrast to mammals, they are relatively infrequent in loose subcutaneous tissue. He also described the distribution and ultrastructure of the mast cells; their morphological characteristics indicated that there was only one type of mast cell in fowl.

Although physiological, moulting is stressful for birds. Since thyroid hormones are typically elevated, synthetic thyroxine is often used to induce or accelerate moulting for clinical purposes. During feather regeneration there is extensive activity in the skin and it becomes more exposed to environmental influences. While an activation of the immune system during moulting is to be expected there is no systematic description of mast cells in avian skin during moulting to be found in the available literature. Therefore, we added the study of mast cells, in particular the concentration and location of mast cells in the dermis of the head region, to the extensive research already conducted on the characteristics

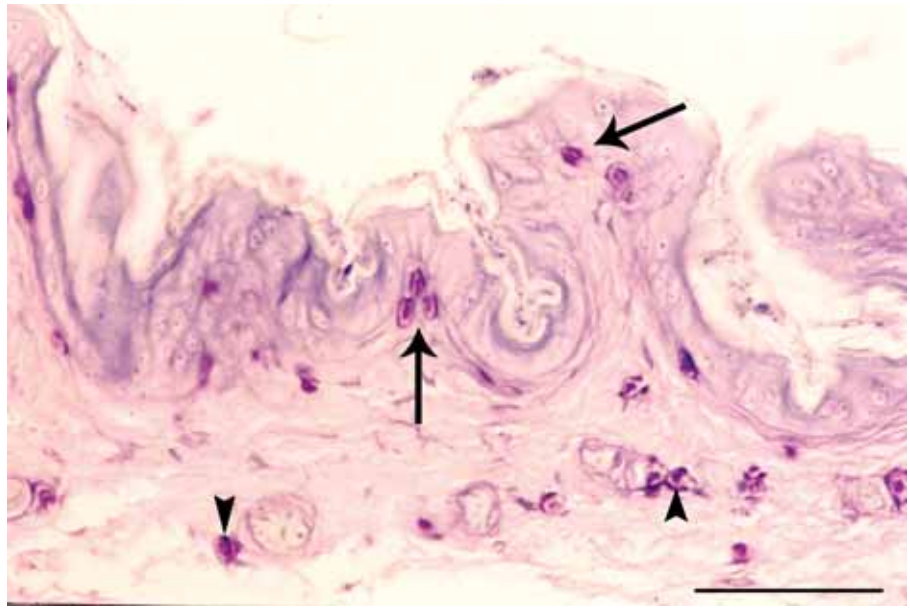

Figure 3: Skin of an L-thyroxine-treated animal. Numerous mast cells can be seen just beneath the epithelium - along the dermo-epidermal juncture (arrows) and in the connective tissue of the dermis near blood vessels (arrowheads). Toluidine blue staining at pH 5.2, scale bar = 50 µm

of budgerigar (*Melopsittacus undulatus*) moulting (10, 11).

Materials and methods

The research was conducted between the 10th and 27th of September on 16 budgerigars during their physiological moulting period. The animals were between 6 and 8 months old and clinically healthy. They were fed the same food and kept in identical climatic conditions with an average temperature of 23.6 °C.

We accelerated the moulting of 8 animals by giving them thyroxine and the other 8 were given a placebo (saline). At 7 p.m. for seventeen consecutive days, 500 µg/kg of body weight of levothyroxin L-T4 (Lek, Ljubljana, Slovenia), a synthetic, thyroid-gland hormone, was applied by cannula into the crop of the treatment group.

Four treated and 4 control animals were sacrificed on September 21st, and the remaining animals on September 27th – at the peak of the moult. Skin samples from the sacrificed animals were then taken from the head region (*pteryla capitalis*), fixed in Bouin's solution and embedded in paraffin. A Leica SM 2000R microtome was used to cut 5 µm tissue sections that were then stained with haematoxylin & eosin as well as with trichrome stain – after Goldner. Metachromasy of mast cells was confirmed by Toluidine blue, in a pH range from of 4.0 to 5.2.

A Nikon Microphot FXA microscope was used to perform the histological analyses and to take microphotographs. The histometry was performed using a Lucia M image analysis system (Optoteam Vienna). Mast cells were enumerated in the subepithelial layer of the dermis, which was 30 µm thick, and a total of 210,000 µm² of skin from each animal was analysed. The statistical differences between the mast cell numbers of each group were analysed with a Student 't'-test.

Results

A general histological analysis of the skin on the head region revealed a typical, thin epidermis with a keratinous layer and a dermis with feather follicles (Fig. 1). The animals whose moulting was accelerated (treatment group) had numerous lymphocyte infiltrations (Fig. 2). There were two locations where mast cells were primarily encountered: near the dermo-epidermal juncture and around the blood vessels (Fig. 3).

The histometric analyses revealed little difference between the animals in the treatment group but varying results within the control group. Some of the control animals had significantly lower numbers of mast cells than others. No differences were observed between the first and second groups of sacrificed animals within either the treatment or control group. An additional histological analysis of histological slides revealed a parasite infection in many of the animals used in our study. Developmental stages of the *Cnemi-*

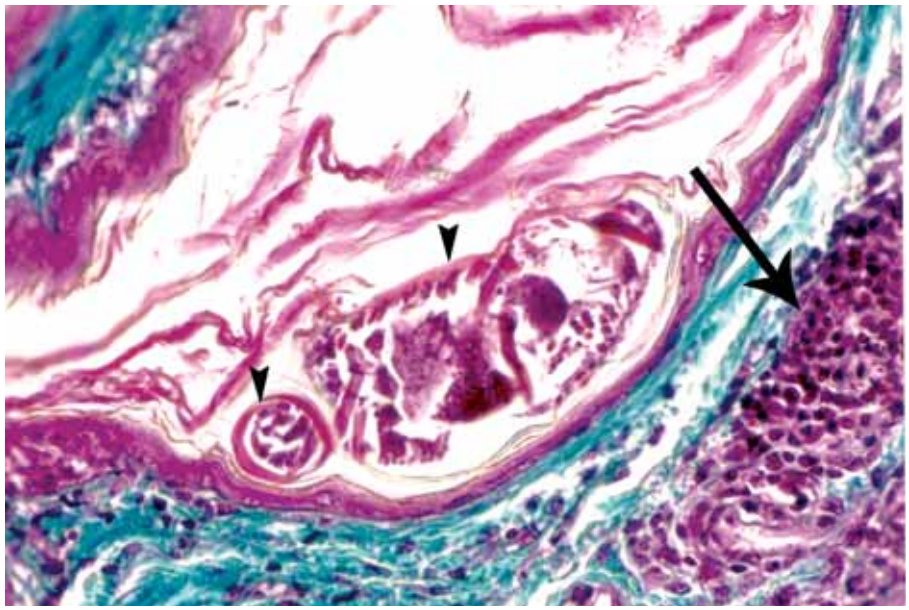

Figure 4: Skin from the head of a budgerigar. Many of the otherwise healthy animals were invaded by parasites of the *Cnemidocoptes* genus, which were found in the epithelial layer (arrowheads). A lymphocyte infiltration can be seen in the nearby dermis (arrow). Trichrome staining after Goldner, magnification x 200

Table 1: Number of mast cells in a 210000 μm² section of dermis taken from budgerigars in a treatment group, a control group and a group of animals invaded by ectoparasites
1 = first sacrifice, 2 = second sacrifice, C = control, T = treated

Treated		Control		Invaded by ectoparasites	
group	No. mast cells	group	No. mast cells	group	No. mast cells
1	86	1	30	1C	111
1	80	1	35	1C	73
1	112	2	29	2C	75
2	91	2	31	2C	91
2	113			1T	120
2	73			2T	178
average	92.5*		31.25		108*
SD	15.2		2.3		35.7

* The number of mast cells in the treated animals and those invaded by parasites (treated or control) were significantly higher than those in the uninfected control group ($P < 0.01$, Students t-test).

docoptes spp. ectoparasite were found in the epidermis of many of the animals (Fig. 4).

Following the diagnosis of ectoparasites and on the basis of experimental procedures, the animals were reclassified into 3 groups for further analysis: animals from the treatment group without parasites, control animals without parasites and animals with parasites. The animals in the control group without parasites had significantly lower numbers of mast cells in the dermis than those in the other two groups (Table 1).

Discussion

Differences in the numbers of mast cells found in the different groups of birds were found in this study. Although clinically healthy, many of the animals were found to be infected with the *Cnemidocoptes* spp. ectoparasite. These animals had a higher number of mast cells in the subepidermal layer of their skin than the uninfected control group. A more complete interpretation of the results was hindered by the relatively small number of animals in the study. It can be concluded, however, that the number of mast cells in

the untreated animals (control group) remained approximately the same from the onset of the moult (first sacrifice) until the time when the moult was at its most intensive (second sacrifice). Also in the L-thyroxine treated group, no significant differences in the number of mast cells could be noticed between the first sacrifice and the second.

However, an important difference between the two groups (treated and control) was found. The group of budgerigars that received treatment had a statistically-significant ($P < 0.01$) higher number of mast cells in the subepidermal layer of the head skin. It could be concluded that the L-thyroxine application induced a proliferation of mast cells in the subepidermal layer of the skin or their migration into this layer. The most likely cause of this was the accelerated moulting (induced by the L-thyroxine application) that compromised the intensified metabolism needed for skin and feather regeneration. This process includes an intensified engagement of the immune system – as was noted in a previous experiment where there were significantly higher numbers of lymphocytes in the skin of treated animals compared to control (untreated) animals (12).

Mast cells are known to be engaged in local immune responses, which includes a response to parasites (4). There are reports of increased numbers of mucosal mast cells in the intestines of fowl following worm or Eimeria invasions (13, 14, 15) but to the best of our knowledge this is one of the first studies to report an in increase in the mast-cell population following parasitosis in avian skin.

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TKIVNI BAZOFILCI V PODEPITELIJSKEM PASU PRI PAPIGI SKOBČEVKI (*Melopsittacus undulatus*) MED GOLJENJEM

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Povzetek: S histološkimi tehnikami smo proučili kožo na glavi med regeneracijo peres (goljenjem) pri papigi skobčevki (*Melopsittacus undulatus*). V poskusu je bilo 16 živali, od tega smo 8 dajali tiroksin, ki pospeši goljenje, 8 živali pa je predstavljalo kontrolno skupino. Vzorce kože na glavi smo fiksirali v raztopini Bouin, zalili v parafin, tkivne rezine pa nato barvali s hematoksilinom in eozinom, touloidinskim modrilom in s trikromnim barvanjem po Goldnerju. S svetlobno mikroskopijo smo ugotavljali položaj tkivnih bazofilcev v koži in z računalniško podprtim sistemom za analizo slike analizirali njihovo številčnost. Ugotovili smo, da so tkivni bazofilci redna sestavina kože pri papigi skobčevki. V naši študiji so bili predvsem na epidermalno-dermalnem stiku, pa tudi v vezivu usnjice v bližini krvnih žil. Čeprav je znano, da se med golitvijo poveča izločanje ščitničnih hormonov, v prejšnji raziskavi pa smo ugotovili tudi pogoste limfocitne inflitracije v koži, je bilo pri s tiroksinom tretiranih živalih število tkivnih bazofilcev statistično značilno ($P < 0,01$) večje. Vse živali v poskusu so bile klinično zdrave, vendar smo z mikroskopsko preiskavo pri 37 % živali ugotovili invadiranost z zajedavci iz rodu *Cnemidocoptes*. Tudi te živali (tako iz tretirane kot kontrolne skupine) so imele povečano število tkivnih bazofilcev, kar lahko povezujemo z imunskim odzivom.

Ključne besede: tkivni bazofilci; papiga skobčevka; koža; golitev; parazitoza