6. SUMMARY

Immunhistochemical investigations for identifying the histogenesis of basaloid neoplasias and hyperplasias in the mamma parenchyma of the bitch, for the use of the human nuclear protein marker p63 in canine tissue and on previous as basal cell tumours described and now reclassified neoplasias.

In this present study immunhistochemical investigations were made to three different topics.
The central position in this study are the investigations of the basaloid hyperplasias and neoplasms of the canine mammary gland.
Mammary tumours are the most common neoplasia in the bitch and malignant ones are the frequent cause of death of dogs. Because a multitude of various types of mammary tumours with different biologic behaviour occurs in this species a precise knowledge of all the neoplasias and a solid and reliable diagnostic of these tumours are necessary. The object of academic effort is to determine the histogenesis of the basaloid mammary lesions which are little investigated till now and further to be able to categorize them properly. That enables to make prognostic and therapeutic estimations.

Out of an amount of 220 HE stained sections 50 cases were chosen which showed the typical characteristic basaloid cell morphology and the specific histological architecture. These 50 cases were investigated immunhistochemical by using 5 different antibodies and histochemical by using the PAS- reaction. In the present study 22 cases and their 154 stainings are discussed representatively.

At first an assessment of the histomorphological appearance of each tissue sample in the HE stain was made and the different types of tumours were diagnosed by using the WHO-classification of mammary tumours in the dog and cat. The striking features of the histological structures are the palisaded peripheral cells, with their centrally located cells showing glandular or squamous differentiation or sometimes forming solid structures and the formation of long cords by palisaded basaloid cells in two rows.
After having evaluated the immunhistochemical and PAS stains a renewed diagnostic of the basaloid mammary lesions was made - now knowing the exact histogenesis of the hyperplastic and neoplastic basaloid cells. In the following these diagnoses are compared with those made in the HE stain and possibilities of misdiagnosing are discussed.

The use of the cytoplasmatic antibodies AE1, LP34, CK14, and HHF35 and the nuclear protein marker p63 as well enables a more specific diagnostic of these basaloid mammary lesions. By finding additional knowledge recommendations for modifying or classifying individual tumour types in the WHO classification of the mammary tumours of the dogs could be performed. The immunhistochemical results support to make a more specific classification of the simple adenomas and to reclassify the basaloid adenomas. Three different types of simple adenomas should be described: 1. Glandular Epitheliom, 2. Myoepitheliom and 3.

Basaloid Adenoma. A subdivision of the simple adenomas in glandular epitheliomas and myoepitheliomas is also possible. By classifying the simple adenomas in these two types the basaloid adenomas belong in the first category, because of the determined histogenesis of the neoplastic cells which shows their exclusive origin from glandular epithel cells. But when classifying the basaloid adenomas in this category of the glandular epitheliomas they should be described as a specific variant because of their characteristic histological features.
From the methodology of the investigations no definite statements concerning the dignity of the basaloid adenomas can be made, only observations can be demonstrated. The striking analogies of the histomorphological appearance in 7 of the malignant cases imply the assumption of a malignant transition of basaloid adenomas.

In order to modify the WHO classification the most remarkable finding in the immunhistochemical stainings, particularly in the p63 staining, of a basaloid epithel-like phenotype of the myoepithelial cell is necessary and fundamental. It is shown in more than half of the cases. Without the knowledge of this phenotype respectively without the verifying immunstainings wrong diagnosis in the routine diagnostic are possible, because of falsely assigned neoplastic cells to the glandular epithelium. Incorrect prognostic assessments are the result because the type of tumour could not be recognized. As it is shown in the present study this concerns the myoepithelcarcinomas which are accompanied with a worse prognosis. It also concerns the complex tumours and tumours which are associated with a myoepithelial hyperplasia which have better prognosis because of the involvement of the myoepithel. The possibility that the canine myoepithelial cell can occur in a basaloid epitheloid phenotype should be giving expression in the WHO classification of mammary tumours of the dog (myoepithelioma). So the pathologist can consider the use of more specific diagnostic when examining basaloid lesions.
The occurrence of adenosquamous carcinomas as complex and not only as simple neoplasias should also be included in the description of this tumour type in the WHO classification.
Further more the present study reveals that the complex adenoma is not adequate described in the classification. Only with the immunhistochemical stainings using the antibodies CK14 and p63 the diagnosis of a complex adenoma could be verified because the present histomorphological description in the classification did not allow suspecting this tumour type in the HE stain. Exclusively the simple adenomas and not the complex adenomas may be a precancerous state so that also here the more specific diagnostic has relevance although a benigne tumour is getting diagnosed.

The results of the investigated epithelial and ductal hyperplasias are worth mentioning. In a third of all cases a layer of myoepithelial cells is only in parts developed or the myoepithel cells are missing at all. This observation is a hallmark of invasive mammary lesions and therefore a possible infiltrative quality of the investigated lesions is suspectable. At the moment only ductal hyperplasia showing moderate and marked atypia are considered precancerous. They are thought to have a greater chance of developing into invasiv

carcinomas. But in the present study the missing of myoepithel cell layers is observed in ductal hyperplasias with normotypic cells and in epithelial hyperplasias.

Statements of the occurrence of stem cell tumours can only be speculatively be made. The knowledge that in adult epithelia the p63 expression is restricted to the progenitor cells implies the question about the role of the myoepithelial cells in the genesis of the mammary reserve cells and on the other hand in 4 of the investigated cases the existence of mammary stem cell tumours. The existence of stem cells in the mammary gland has not been proven in the dog yet, but it is likely that they do exist because mammary parenchyma undergoes changes in association within estrus cycle. It is permanently decomposed and rebuild. An analogy of the phenotype of the mammary reserve cells to the epidermal basal cells can be suspected hypothetically, because of the ectodermal origin of the mamma and the fact that it represents a modified sweat gland. It is conceivable that these cells could show by neoplastic degeneration a histological appearance similar to the basal cell tumours of the skin (according to the definition of WEISS and FRESE 1974 and SANDERSLEBEN 1989). Far reaching investigations are necessary for the clarification of the existence of canine mammary stem cells. Further their role in the tumour genesis of mammary neoplasias has to be looked for to give a statement if the canine mammary gland develop stem cell tumours with a specific morphology or if all mammary neoplasias have their origin in stem cells and they develop during the tumour genesis into different phenotypes which simulate the degeneration of the single cell types of the canine mammary gland.

Aptitude tests of the human antibody (clone 4A4) marking the nuclear protein p63 in basal and myoepithelial cells in canine tissue were made before the basaloid lesions of the canine mammary gland were investigated. The immunhistochemical results of the investigations of canine skin and canine mammary gland reveal its high specifity and sensitivity to identify basal and myoepithelial cells in the dog. The p63 protein of both species is recognized by the same antibody (cross reaction) which implies structural similarities between the human and the canine epitops.
The identification of intact, continuous myoepithel cell layers which are characteristic for non invasive mammary lesions is clearly improved by the nuclear staining. This could be shown in the investigations of the basaloid mammary lesions. The cytoplasmatic staining profile of the antibody CK14 cause problems in the evaluation of the carcinoma in situ lesions and epithelial and ductal hyperplasias diagnosed in the HE stain because of the different cut fusiforme cytoplasm ends of the myoepithelial cells. So the continuity of the myoepithel cell layer could only be suspected but not be surely confirmed by the CK14 staining. Owing to the nuclear staining by the p63 antibody a present layer of myoepithel cells is seen by the stained nuclei of the myoepithelial cells, lying on the same level forming a string of pearls. Consequently a localized loss or the absence of the myoepithelial cell layer can be diagnosed with a greater reliability. The nuclear staining pattern causes a better visual evaluation.

In the third part of the present study tumours which previous were described as basal cell tumours but now have been reclassified as trichoblastomas were immunhistochemcal investigated. The primary intention was the inspection of the marking of neoplastic degenerated basal cells by the nuclear protein marker p63. The chosen neoplasias being basal cell tumours as WEISS und FRESE (1974) defined them were first rediagnosed in the HE stain by the amended WHO classification of epithelial and melanotic tumours of the skin of domestic animals by GOLDSCHMIDT et al. (1998). Looking at the diagnostic in the HE stain sections it becomes obvious that reclassifying the basal cell tumours exclusively as trichoblastomas as the authors of the amendment of the classification emphasized do not express the entinity of these neoplasms. The definitions made do not encircle all phenotypes of the neoplasms. For categorizing the previous as basal cell tumours defined neoplasias by the renewed classification the termini of the basal cell carcinoma and basosquamous carcinoma have to be used beside the different subtypes of the trichoblastomas as well. Problems in assigning the neoplasias to the new made categories concern the neoplasias which show medusoid growth patterns combined with infiltrative growth because these histological structures were not get described in the termini of the basal cell carcinoma. To classify these neoplasias as trichoblastomas of the medusoid subtype would not reflect their dignity. On the other hand tumours which are composed of islands of tumour cells or cords of basaloid cells showing infiltrativ growth have to be diagnosed as basal cell carcinomas which do not seem to be in accordance with the intension of the authors who regard these neoplasias as uncommon in the dog.

In the present study 3 basal cell carcinomas, 2 basosquamous carcinomas and 5 trichoblastomas were diagnosed by using the new classification system. 2 of the last named trichoblastomas showed medusoid growth patterns but also infiltrative growth.

All of the immunhistochemically with p63 stained sections revealed a positive immunreactivity of the neoplastic cells forming the different neoplasms. Consequently also by accepting the renewed WHO classification the marking of neoplastic degenerated cells by the antibody p63 is proven by the positive reaction of the basal cell and basosquamous carcinomas.

Looking at the specifity of the p63 marker an origin of the investigated group of neoplasias seems to be possible from the basal cells of the epidermis or from the cells of the outer epithelial root sheath of the hair. A more specific differentiation between the last named structures is not possible. Also the 4 used antibodies by SCHNEIDER (2002) in the immunhistochemical investigations which are the basic for the reclassifying of the basal cell tumours seemed not to be able to differentiate between these two structures. SCHNEIDER (2002) used 3 broad spectrum antibodies (AE1/3, AE1 and AE3) and the CK19 antibody which is marked by its insufficient specifity and its poor sensitivity to show the decent of these tumours from primitive hair germ cells. Schneider's modus of interpretation of the results of the individual antibody stainings is not uniform and seemed to be reprehensible. Qualitative and quantitative criterions for evaluating were used in the assessment and consequently the conclusions are not in accordance with the results of the different immunhistochemical stainings. Furthermore a positive immunreactivity of the basal and

suprabasal cells of the epidermis with the antibody AE1 has to be pointed out which is demonstrated in the present study. In the immunhistochemical investigations for the reclassifying a negative immunreactivity of the epidermis has been observed and is subsequently used for argumentation that the positive reacting trichoblastomas are not traced back to epidermal structures.

From the immunhistochemical investigations made at the moment an exclusive origin from primitive hair germ cells can not be proven for these neoplasias. The results rather show the possibility that these tumours can originate from basal cells of the epidermis or from structures of the hair follicles.