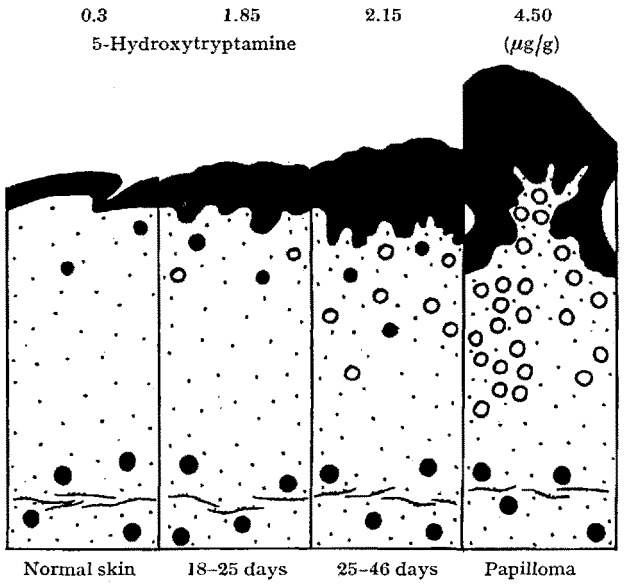
Mast Cell Reaction during Chemical Skin Carcinogenesis of the Lizard *Lacerta agilis*

CRAMER, SIMPSON and RILEY[1] observed, in skin carcinogenesis of the mouse induced by chemical means, a striking accumulation of mast cells in the altered dermis during the precancerous stages of tumour formation. These accumulating mast cells have been described as exhibiting a strong primary golden-yellow fluorescence when the slides are studied in ultraviolet light, after formalin fixation. The mast cells of the normal mouse skin did not show this fluorescence. Since the normal[2] as well as the tumourous[3] mast cells of the mouse are known to contain significant stores of 5-hydroxytryptamine, together with heparin and histamine, it has been suggested that the fluorescence acquired by dermal mast cells in the skin carcinogenesis was due to a remarkable increase of the 5-hydroxytryptamine cellular content[1,4]. The problem regarding the morphology and the functional significance of the mast cell response, associated with hyperplastic and neoplastic changes of the skin, remains obscure. Recently, FIORE-DONATI et al.[5] studied the development of the mast cell reaction during the chemical skin carcinogenesis of the mouse. To establish whether a more general significance should be attached to the results obtained, it seemed desirable to us to perform a corresponding investigation with the chemical skin carcinogenesis of the lizard, *Lacerta agilis*. As in the mouse[5], the morphology of the mast cell reaction occurring in the dermis has been followed closely from the early skin changes induced by the carcinogen to the full development of the papillomas. The 5-hydroxytryptamine content of the treated regions of the lizard skin has also been determined at different subsequent stages of the carcinogenesis.

Adult female lizards received on the skin of the back a single application of 0.05 ml of a 0.5% solution of 9:10-dimethyl-1:2-benzanthracene in acetone, thus containing 250 µg of the substance. The lizards were killed by decapitation in groups every few days from the sixth day after painting to the stage of the development of the papillomas. According to the method of FIORE-DONATI et al.[5], treated portions of skin were excised, slightly stretched on filter paper and fixed in 10% neutral formalin. Frozen sections received an additional fixation in formalin vapour at 37°C for 3 h and were examined for ultraviolet fluorescence using a Leitz Ortholux microscope with a *CS*-150 mercury lamp, and ultraviolet filters with a maximum of transmittance of 365 mµ. Toluidine blue (0.001 aqueous solution) was then applied on the same section. Other fragments from treated skin were pooled and extracted with four parts (w/v) of acetone for 24 h, then re-extracted with 80% acetone for another 24 h. According to the method of ERSPAMER[6], the extracts were assayed on the atropinized oestrus uterus of ovariectomized rats. The standard was 5-hydroxytryptamine creatinine sulphate. The values were expressed as 5-hydroxytryptamine base.

The Figure shows the changes which were found in the morphology, the distribution and the number of the mast cells as well as the level of 5-hydroxytryptamine in the lizard skin observed at different stages of the chemical carcinogenesis. The mast cell reaction appeared to become evident as early as 18–25 days after painting with 9:10-dimethyl-1:2-benzanthracene and increased progressively until the appearance of the papillomas, which occurred after a mean latent period of 50 days. The accumulating mast cells were observed to differ qualitatively from the mast cells of the normal lizard skin in being of very small size, poorly metachromatic and scarcely granulated. Staining from about 18th day after the 9:10-dimethyl-1:2-benzanthracene, these mast cells acquire a peculiar golden-yellow fluorescence. The intensity of this fluorescence gradually increases in the further development of the skin lesions.

The mast cell reaction appeared to be a cellular response, which was strictly localized within the treated region and restricted to the superficial part of the dermis. The mast cells situated in the subcutis and in the deepest part of dermis, heavily granulated and larger in size, did not show any modification in morphology and number, and failed to acquire the golden-yellow fluorescence. As in the mouse[5], also in the lizard a prolonged treatment with acetone, preceding formalin fixation, was observed to abolish completely the fluorescence, leaving the metachromatic appearance of the accumulated mast cells unstained. From these data it would appear that golden-yellow fluorescence and metachromasia are apparently non-related phenomena, depending on the presence within the mast cells of different substances. As to the exact nature of the fluorescing material, no conclusive evidence can be given. The close relationship of the increase of the mast cell number with the rise of the 5-hydroxytryptamine level and the intensity of fluorescence supports the hypothesis that a high content of 5-hydroxytryptamine accounts for



Schematic representation of 5-hydroxytryptamine content and mast cell changes of lizard skin at different stages of the papilloma formation after a single application of 9:10-dimethyl-1:2-benzanthracene. O = fluorescing mast cells. ● = non-fluorescing mast cells.

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the fluorescing properties of the reacting mast cells. According to BARTER and PEARSE[7], a similar golden-yellow fluorescence, after formalin fixation, is exhibited by enterochromaffin cells, the physiological cellular source of 5-hydroxytryptamine as well as by a gelatin model of 5-hydroxytryptamine *in vitro*. BENDITT and WONG[8] found that the lack of fluorescence of normal mast cells can be ascribed to a 5-hydroxytryptamine content lower than the minimum required for the histochemical visibility of 5-hydroxytryptamine.

As the results are in good agreement with those obtained in the mouse[5], they must undoubtedly be regarded as being of more general significance.

Zusammenfassung. Beschreibung der Mastzellenreaktion während der chemisch-induzierten Carcinogenese bei der Eidechse *Lacerta agilis*.

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Localized Areas of High Alkaline Phosphatase Activity in the Endothelium of Arteries in the Axolotl

Activity of phosphatases in the alkaline pH range has been known for a long time in the adventitia of medium-sized arterioles, in the endothelium of blood vessels and in the endothelium of capillaries[1,2], whereas differences in the activity of this enzyme were observed in capillaries of various organs. BARROWS and CHOW[3], LEHNINGER[4], and ZWEIFACH[5] could demonstrate no specific localization of alkaline phosphatase activity in the vascular tree. An intense alkaline phosphatase activity was observed by ROMANUL and BANNISTER[6] in the endothelium of arterioles and small arteries of rat, rabbit and human at their origin from larger vessels; we personally obtained corresponding results with the axolotl *Ambystoma mexicanum*.

The experiments were carried out in skeletal muscles, and also the skin, the spinal cord and the mesentery were surveyed. Fresh-frozen specimens were sectioned in the cryostat and placed in substrate solutions for the demonstration of the alkaline phosphatase activity. As in the rat, rabbit and human[6], two methods were used, *viz.* a modified coupling azo dye method at pH 9.5, using α-naphthyl phosphate and blue *BBN*[7], and that of Gomori with the substrate solution at pH 9.0[1]. The sections were afterwards fixed, counterstained and mounted in 50% polyvinyl pyrrolidone.

The endothelium of the larger arteries showed no alkaline phosphatase activity. The primary branches of such arteries demonstrated moderately intense phosphatase activity, starting abruptly at the point of origin and fading gradually distally. The activity of the secondary branches, which stained identically at the point of origin, continued for a greater length distally, and was more intense. The small arterioles also stained abruptly and intensely at the origin and continued staining with lesser intensity throughout the course, whereas the capillaries appeared to stain with even intensity. The staining at the point of branching of the arterioles started earlier during the incubation of the tissue section and was usually more intense than in the capillaries. In many arterial branchings, the enzymatic activity was restricted to the endothelium, the circular muscular layer being clearly visible around it. In several such branchings the lumen of the arterial branch, as well as the outside diameter of the branch were decreased over the most proximal portion of the vessel near its origin, the endothelial cells bulging inside the lumen of the blood vessel. In the endothelium of arteries at y-shaped bifurcations, no staining was observed, even when their side branches originating proximally showed an intense staining of the endothelium. No staining was observed at the point of branching of veins. The findings in the vascular tree with the azo dye method and with the Gomori technique were identical. The high alkaline phosphatase activity in the endothelium of the arterioles and the arteries at the point of origin from larger blood vessels, if present *in vivo*, may indicate some active transport functions at such points. According to ROMANUL and BANNISTER[6] transport of chemical from the blood stream through the endothelium at these locations in the vascular tree is most unlikely to serve the purpose of supply to tissues surrounding the arteries. It seems more likely that such transport through the endothelium at these sites might serve as a system for sampling continuously the chemical content of the blood for purpose of regulation of the lumen of the artery.

As these results obtained in the axolotl correspond very well with the results obtained in the rat, rabbit and human[6], a general significance may be attached to them.

Zusammenfassung. Es werden lokalisierte Regionen von grosser alkalischer Phosphataseaktivität im Endothelium der Arterien beim Axolotl beschrieben.

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