From the Dept. of Anat., Okayama Univ. Med. School
(Director: Prof. M. SEKI).

On the Peculiar Clumps Which are Found in the Loose Connective Tissue of the Skin of *Bufo vulgaris* *formosus*.

ヒキガヘルの皮膚の疎性結合組織に見出される
奇異の小塊に就いて.

Gako ZIMBO 神保雅幸.

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On microscopical examination of sections of the skin of *Bufo vulgaris formosus*, prepared by fixation in formalin, alcohol or ZENKER's fluid and embedding in paraffin or celloidin or by freezing method, a very interesting observation may be made. Namely, in the loose connective tissue of the stratum spongiosum of the dorsal skin, there may be found small clumps that are very well stainable with hematoxylin. These clumps are not surrounded by glandular cells nor may there be observed any pathological changes in the neighbouring connective tissue, and it seems as if these clumps were inserted into the connective tissue postmortally (Fig. 1). These clumps are insoluble in alcohol and display a metachromatic staining with toluidin blue.

In the skin of *Rana nigromaculata*, *Rana japonica*, *Rana rugosa*, *Rana limnocharis*, *Rana catesbiana*, *Hynobius neberosus* and *Triturus pyrrhogaster*, such clumps could not be found. Even in the *Bufo* these clumps are not to be seen in the skin of the ventral and lateral abdomen regions, and are found only in the dorsal skin.

Frozen sections of the dorsal skin of *Bufo vulgaris* were examined under the fluorescence microscope. A yellow coloured fluorescence was seen in the horny layer of the epidermis and a light blue coloured fluorescence in the germinal layer and in the connective tissue of the dermis. The clumps in question gave an intense blue fluorescence. The content of mucous and granular glands was bluish. The so-called toad-poison emits a bluish light, but is not so well stainable with hematoxylin and not metachromatically stainable with toluidin blue. But the bufotoxin, abstracted out of the skin of *Bufo vulgaris* by WIELAND (1937), emits a strong blue colour and similar to that of the small clumps display a metachromatic staining with toluidin blue. The FEULGEN's nucleal reaction, iodine reaction, and calcium reaction of the clumps in question were all negative.

Concerning fluorescence and stainability these clumps bear a resemblance

to the sieve-layer on the surface of the stratum compactum of the dermis. But the clumps are found in the stratum spongiosum directly under the epidermis, while the sieve-layer is located far deeper in the dermis, and furthermore, the clumps are to be found only in the skin of the back, while the sieve-layer is to be seen in the skin of all body regions.

The skin of *Rana nigromaculata* is 240μ in thickness and its sieve-layer is 11μ , while the skin of *Bufo vulgaris* is 610μ , and its sieve-layer is $1,3\mu$. The sieve-layer of *Rana nigromaculata* appears at the end of metamorphosis, but that of *Bufo vulgaris* at the seventh month after birth, when its length gains 4 cm. It was found that, when *Bufo vulgaris* grow up to 6.5 cm in length, a part of the sieve-layer seems to emigrate up into the loose connective tissue, then turns into small clumps (Fig. 2). It is only specifically seen in the skin of *Bufo vulgaris* that the sieve-layer is scattered about to the stratum spongiosum.

Summary.

Small clumps were found in the stratum spongiosum of the dorsal skin of *Bufo vulgaris*. The clumps stain themselves metachromatic with toluidin blue, and show a remarkable blue fluorescence. They are located merely in lacunae of connective tissue and are not surrounded by specially organized cells. There are no blood-vessels in the adjacent connective tissue. These small clumps seem to come from the sieve-layer. But their biological significance is not yet known.

内 容 自 抄.

ヒキガヘルの背皮の海綿層で表皮に近いところの疎性結合組織の中にヘマトキシリンに甚だよく染る小塊が認められる。この小塊は腺細胞に囲まれて居らず、また塊の周りの結合組織に少しの病変も見られず、恰も動物の死後結合組織に送入せられた小塊の如くである。これは側腹と腹の皮膚には認められない。トルイデン青で染めれば、この物質は異調に赤紫に染る。また蛍光鏡検すれば強く青い蛍光を放つ。小塊と Bufothionin とは異調染色性と蛍光性がよく似ている。小塊は発生学的には節層より分離したやうであるが、動物体に如何に役立つてゐるかはまだ知られない。

Reference.

Wieland, H. u. T.: Zur Kenntnis der Krötengiftstoffe. X. Ann. Chem. 528 (1937).

Explanation of Plate.

Cl clump, *E* epidermis, *L* loose connective tissue, *Gl* gland, *Me* melanin, *Si* sieve-layer.

Fig. 1. Small clumps in the skin of adult *Bufo vulgaris formosus*. 90×.

Fig. 2. Scattering sieve-layer. *Bufo vulgaris formosus*, 6.5 cm in length. 60×.

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