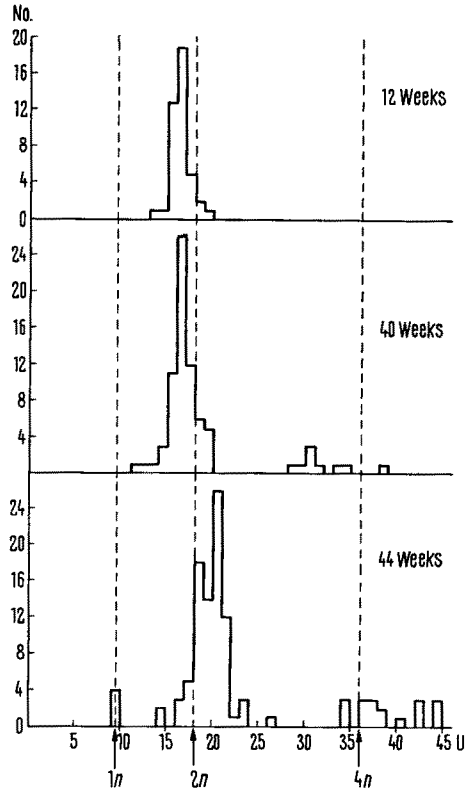

Fig. 2. Nuclear DNA-content of adipose tissue cells of rats aged 12, 40 and 44 weeks. Abscissa: No. of measured nuclei; ordinate: DNA-content in arbitary units (U). The first column represents DNA-content of rat-spermatosomes (reference value). $1n$, $2n$, $4n$ = haploid, diploid and tetraploid DNA-value.

Since an absolute separation of adipose tissue cells from stroma and blood cells proved to be impossible, only those cells were measured that could be clearly identified as adipose tissue cells by means of the nuclear shape (large, mostly oval nuclei, poor in chromatin) and the position of the nucleus in a cytoplasmic structure.

The nuclear DNA-content was found to be close around the diploid DNA-value in animals aged 5–12 weeks (Figures 1 and 2). In older animals (40 weeks and more) tetraploid nuclei appeared besides the diploid ones (Figure 2). The number of tetraploid nuclei amounts to 12–14%. In addition, binucleated adipose tissue cells were noticed in about 5% (Figure 3). The DNA-content of each of these nuclei corresponded with the diploid value.

These findings indicate that biochemical DNA-estimations in the adipose tissue[2–4] do not accurately represent a basis of estimation of the number of cells. Tetraploid adipose tissue cells appear in 12–14% in older animals. In another 5% the appearance of binucleated adipose tissue cells must be expected. These results are in accordance with the findings of LIEBELT[8] but contrast with the observations of HIRSCH and GOLDRICK[2]. Our suggestion is that the cytokinesis is no longer possible in differentiated adipose tissue cells of older animals, while the ability to synthesize DNA may persist[9].

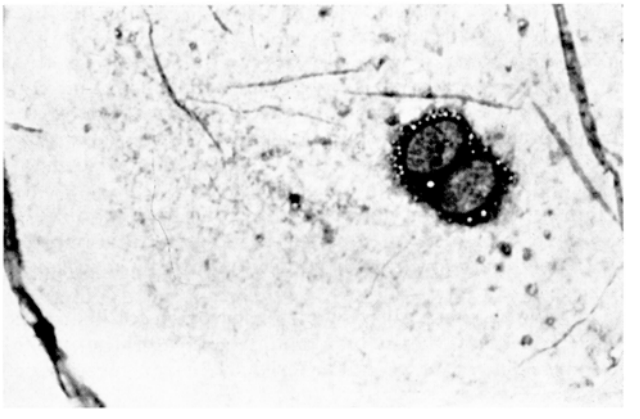

Fig. 3. Binucleated adipose tissue cell, α-Naphthylacetat-esterase reaction. × 1250.

Zusammenfassung. Der DNS-Gehalt isolierter Fettzellen wurde cytophotometrisch bestimmt. Bei älteren Tieren fanden sich 12–14% tetraploide Fettzellen neben 5% zweikernigen Fettzellen. Es wird auf die Folgen für die Bestimmung der Zellzahl aus dem Gesamt-DNS-Gehalt des Fettgewebes hingewiesen.

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[9] This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

Mast Cells and Histamine in a Newt, *Triturus pyrrhogaster* Boié

Although mast cells in the lower vertebrates have been studied by many histologists, it has generally been believed a priori that they contain histamine as mammalian mast cells do. ARVY[1], in his histochemical study, noticed that he failed to demonstrate histamine in mast cells of the frog, but he could not escape the supposition that mast cells are the cells of histamine.

In a previous report[2], we showed the absence of histamine in mast cells of the frog, *Rana catesbiana*, by the combination of histological survey of mast cell population in several organs and bioassay (Code's method) of histamine content in these organs.

We also reported that there is no histamine in mast cells of the vertebrates below the level of reptilia. This agreed with the results of REITE[3], which were obtained by a more indirect method.

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In the present report, the tissue mast cells and the blood basophils of the newt, *Triturus pyrrhogaster* Boié, were studied by the same bioassay method of histamine as used in our previous study[2], by fluorescence microscopy with *o*-phthalaldehyde[4] and by histochemical methods for sulphated mucopolysaccharides. Mast cell distribution in various tissues of the newt was studied with paraffin sections and spread preparations.

Histamine content and the relative mast cell population of the newt tissues are shown in the Table. The heart and tongue contained a large number of dendritic type mast cells among their muscle bundles and under the mucous membrane respectively, thus representing the organs of the newt rich in mast cells. The histamine content, however, as in the frog organs, was at most $^1/_{100}$ as much as that in the rat mesentery.

Higher values of histamine in the digestive tract than in the other organs probably indicate that histamine contained in the newt tissues comes from the flora in the lumen of the digestive tracts, although histamine absorption through the intestinal epithelium is denied in mammals. REITE[3] also postulated the same possibility for the origin of the small amount of histamine he recognized in the newt, although he gave the histamine value only for the whole body and not for individual organs such as the intestine.

The fluorescence microscopic observation for histamine was carried out with *o*-phthalaldehyde[4] on the spread preparations of newt mesenteries. The rat mesenteries were used as controls.

Microscopic observations of the mesenteric spreads virtually agree with the previous descriptions[5] as regards the presence of 2 types of tissue mast cells, dendritic and ovoid, besides the intravascular basophil leucocytes. The networks of the processes of the dendritic type mast cells almost cover the mesentery.

With the *o*-phthalaldehyde treatment of the spreads, no yellow fluorescence corresponding to either of 3 types of cells was recognized, whereas mast cells of the rat mesentery distinctly fluoresced with this method.

Different tissues of the newt were fixed in Bouin's fluid, 10% neutralized formalin and Carnoy's fluid, treated in a conventional manner and embedded in paraffin. The spread preparations of the mesentery, peritoneum and pericardium were also fixed in the above fluids. They were stained in 0.1% toluidine blue O (pH 3), Astra blue[6], Gomori's aldehyde fuchsin, periodic acid Schiff reaction (McManus) and aluminium-toluidine blue which is known to be highly specific to sulphated acidic mucopolysaccharides[7]. They showed distinct metachromasia in toluidine blue O, were stained positively in aldehyde fuchsin, reacted weakly positive in PAS method and were demonstrated selectively in aluminium-toluidine blue. These results indicate that newt mast cells contain sulphated acidic mucopolysaccharides as mammalian mast cells do. It was reported that the presence of sulphated compounds in mast cell granules of a newt, *T. cristatus carnifex* (Laur.) was confirmed by their S 35 uptake in autoradiography[8].

Besides tissues, blood was examined by routine hematological techniques and the basophils were counted. Histamine value of the blood was examined by Code's method. The blood of turtles, *Clemmys japonica* and *Amyda japonica*, was treated in the same way for comparison. The blood of the newt contained, in agreement with the previous authors[9], about 50% basophils (900/mm³ blood) among its leucocytes. The histamine value of the blood turned out to be as low as 0.04 µg/ml. In contrast to this, 60 and 35% basophils among leucocytes and 1.20 and 0.48 µg/ml histamine were found in blood of *C. japonica* and *A. japonica* respectively.

In order to test whether newt mast cells have the ability to concentrate histamine from the surroundings, 500 µg/animal histamine was injected i.p. and the mesenteries of 7 newts were examined under a fluorescence microscope with *o*-phthalaldehyde at 10 and 30 min, 2 and 6 h after injection. The newt showed no change in general appearance with the administration of histamine. None of the 3 types of mast cells came to fluoresce with the *o*-phthalaldehyde method.

From the results of the present study, it is obvious that newt tissues contain only a trace of histamine and that mast cells are, at least, virtually free from histamine and have no ability to concentrate histamine from the surroundings. In spite of this, mast cells of the newt, as those of the frog, seem fully competent as such[10] because they contain sulphated mucopolysaccharides.

It would be almost safe to say from the present and previous studies that there is no histamine in mast cells of the amphibians, both urodele and anuran. Under study are now mast cells and basophils of reptiles and birds which may probably give the key to the appearance of histamine in mast cells in the course of the phylogenetic development of the animal kingdom[11].

Histamine content and mast cell population in newt tissues

Organs of newt	No. of materials	Histamine µg/g wet tissue	Mast cell population
Heart	6	0.13	++
Tongue	6	0.20	++
Digestive tract	5	0.59	+
Body except digestive tract	4	0.05	
Blood	5	0.04 µg/ml	Basophils ca. 50% of leucocytes

Zusammenfassung. Es wird nachgewiesen, dass die Mastzellen von *Triturus pyrrhogaster* Boié kein Histamin enthalten, sie also im Gegensatz zur allgemeinen Annahme keine Histamozyten sind. Dieser Befund wurde sowohl biologisch am Meerschweinchendarm durch Extraktion des Histamins mit Hilfe der Codeschen Methode als auch durch den histochemischen Histaminnachweis mit *o*-Phthalaldehyd an verschiedenen Geweben bestätigt.

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