

The suggestion that thyrocalcitonin is stored within granules of parafollicular cells<sup>12</sup>, together with the present findings indicating that 5-HT is also present inside those granules, implies that the amine may act in some stage of the processes of metabolism, storage or liberation of the hormone<sup>10</sup>. Preliminary results of experiments now being made with the administration of precursor aminoacids of amine synthesis in thyroid glands of species in which 5-HT and catecholamines cannot be normally demonstrated, suggest that the fate of amines formed is the granule of parafollicular cells. The demonstration of the coexistence of a hormone and 5-HT in a similar organelle was also recently made in the insulin-producing cells of guinea pig endocrine pancreas<sup>22, 23</sup>.

**Resumen.** Se han estudiado los depósitos de catecol- e indolaminas en la tiroides de la oveja mediante una técnica citoquímica que permite la diferenciación entre ambos compuestos a nivel ultraestructural. Los abundantes mastocitos hallados en la glándula presentan granulaciones que contienen una catecolamina, que en base a estudios anteriores se identificó como dopamina. Las células

parafoliculares en cambio, contienen serotonina en los gránulos citoplasmáticos. Dichos gránulos, cuya presencia ha sido vinculada con la de la tirocalcitonina, son por lo tanto capaces de almacenar la hormona y la 5-hidroxitriptamina. Los resultados citoquímicos se analizan conjuntamente con los obtenidos mediante la determinación química del contenido en aminas de la glándula.

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<sup>22</sup> G. JAIM ETCHEVERRY and L.M. ZIEHER, for publication (1968).

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### Difference in Interphase Nucleus Organization Within the Genus *Xiphophorus* (Pisces, Poeciliidae)

The genic background of melanotic tumour formation in Poeciliid fishes has been thoroughly investigated, and biochemical methods were applied to understand the mode of gene regulation involved<sup>1-4</sup>. As the results are thought to be a hopeful basis for studying the problems on the single cell level, now tissue culture is employed. In these experiments an unexpected difference between species of the genus *Xiphophorus* was found, which concerns the structure of interphase nuclei.

Four of the species investigated, i.e., *X. (= Platypoecilus) maculatus*<sup>5</sup>, *X. montezumae* ssp. *cortezi*, *X. variatus*, and *X. xiphidium*, show equal behaviour which is demonstrated first. In living cells of the epithelial and macrophage type<sup>6,7</sup> arranged in 1-3 layers around a fin explant<sup>8</sup>, the nuclei appear homogenous in phase contrast (Figure 1a) as well as in interference contrast, except for 1-3 nucleoli and, sometimes, a very fine, hardly visible granulation. In stained preparations this feature is principally maintained (Figure 1b, methods see legend): the

nuclei are rather dark, slightly scattered, and if some irregular spots can be seen, these are little distinct and few in number.

A completely different situation was found in *X. helleri*. In living nuclei a certain number of rather extended, clearly visible bodies appear (Figure 2a). After staining, there are numerous chromocentres situated on a background which itself is almost uncoloured (Figure 2b).

<sup>1</sup> J. W. ATZ, Zoologica, N.Y. 47, 153 (1962).

<sup>2</sup> F. ANDERS, Experientia 23, 1 (1967).

<sup>3</sup> F. ANDERS und K. KLINKE, Z. VererbLehre 96, 49 (1965).

<sup>4</sup> F. ANDERS, Zool. Anz. 179, 2 (1967).

<sup>5</sup> Nomenclature see C. D. ZANDER, Mitt. zool. Mus. Ham. 64, 87 (1967).

<sup>6</sup> G. v. BARGEN und A. WESSING, Z. wiss. Mikrosk. 64, 356 (1960).

<sup>7</sup> E. N. WILLMER (Ed.), *Cells and Tissues in Culture* (Academic Press, London and New York 1965), vol. 2.

<sup>8</sup> W. LUEKEN and W. FOERSTER, in preparation.

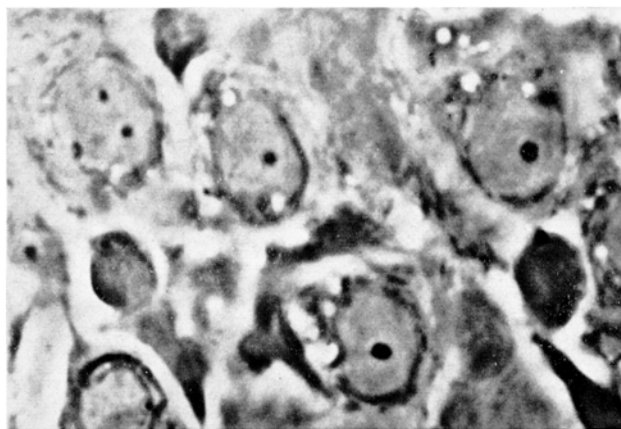


Fig. 1a. Nuclei (with 1 or 2 nucleoli) of living tissue culture cells of *Xiphophorus variatus* in phase contrast.  $\times 2000$ .

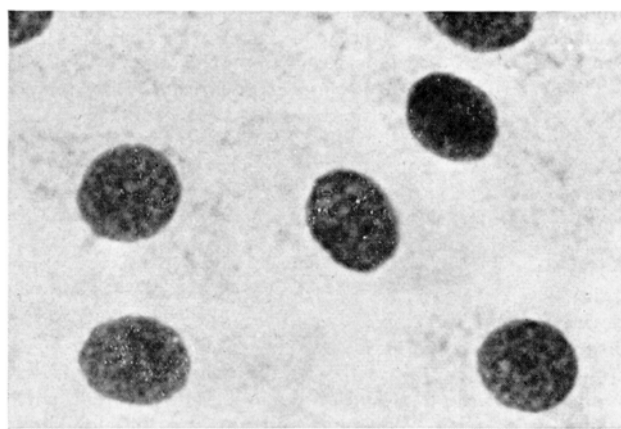


Fig. 1b. Stained nuclei of *X. variatus*. Tissues exposed to colchicine (1:40,000) 3 h, treated with hypotonic solution 45 min, fixed in acetic-alcohol, dried and stained with aceto-orcein.  $\times 2000$ .

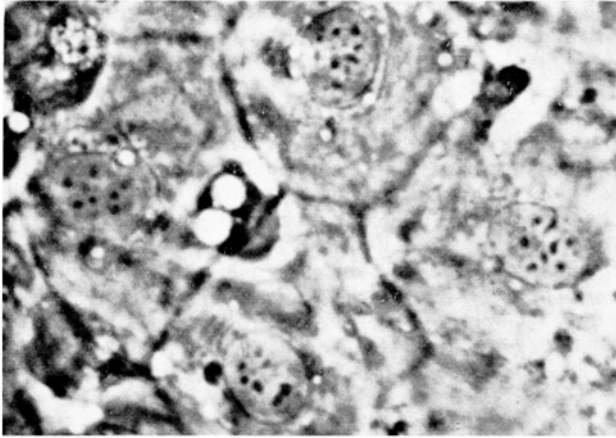


Fig. 2a. Live tissue culture cells of *X. helleri* in phase contrast.  $\times 2000$ .

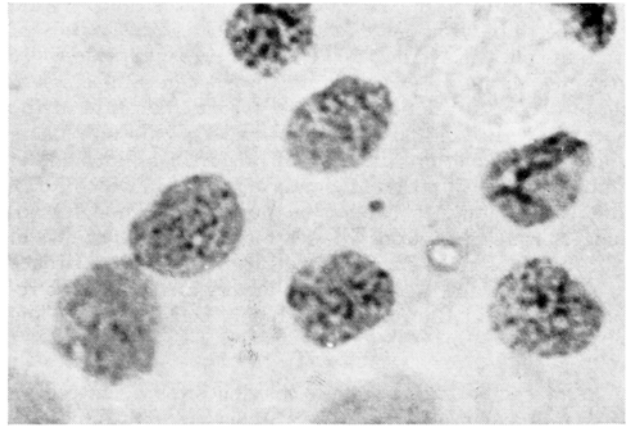


Fig. 3. Nuclei of a *X. helleri*  $\times$  *X. maculatus* hybrid. Same procedure as Figure 1b.  $\times 2000$ .

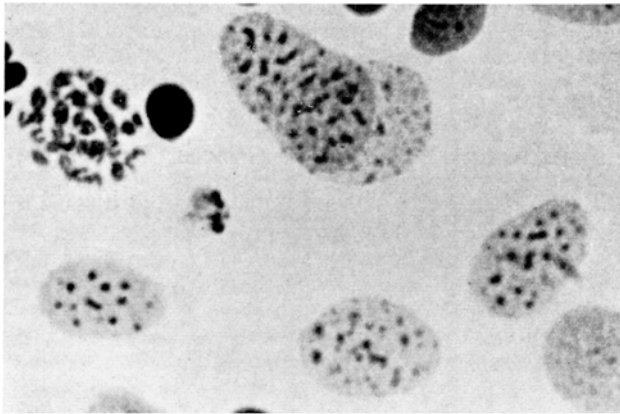


Fig. 2b. Nuclei of *X. helleri*. Same procedure as Figure 1b.  $\times 2000$ .

Very often, the number of chromocentres comes nearly to 48, which is the ordinary  $2n$ -chromosome number of *Xiphophorus* species<sup>9-11</sup>. In no case were more than 48 elements counted. It is still to be mentioned that the pattern of interphase nuclei differs within *X. helleri* according to populations of this wide-spread species. Thus the peculiarity of nucleus structure is most pronounced in a stock coming from Rio Papaloapan in Mexico<sup>12</sup>. In other stocks bred in our laboratory, from Mexico (Rio Jamapa), Honduras (Rio Lancetilla), and British Honduras (Belize River), it is much less distinct.

The inheritability of nucleus structure has been tested by crossing *X. helleri* with the species cited above, and it may be stated that in interspecific hybrids the nuclei show a somewhat intermediate picture (Figure 3). Comparable pictures can even be obtained in  $F_2R$  and later backcross generations. Thus the hereditary character of interphase nucleus organization is revealed.

It must be stated that the views obtained in fixed and stained preparations do not significantly depend on the methods used, as far as number and distinctness of chromocentres or homogeneity of nuclei are concerned. For example, fixation with formalin and staining after Feulgen led to quite similar results. Pretreatment with colchicine or with hypotonic solution also did not show any important effect. In this connection it is to be noted that the aspect of nuclei is not caused by in vitro culture because squash or section preparations of fins gave pic-

tures of stained nuclei which strikingly resemble those described here. As for the live state, nothing can be said, since it can only be observed in tissue cultures.

Some hints, as to the meaning of these phenomena, can be obtained from the following statements: in all cases described above, where animals from pure species were used, the state of nuclear organization in living cells was confirmed by the picture in stained preparations. In contrast to this, in live tissue culture cells of tumour-forming hybrids, by phase contrast as well as by interference contrast microscopy, special nuclear structures could be observed, which did not reappear in the preparations made according to the methods used before. This observation may be interpreted as supporting the view that there are some relationships between visible nuclear organization and the problems dealt with in tumour developing *Xiphophorus* hybrids<sup>13</sup>.

**Zusammenfassung.** Bei mehreren *Xiphophorus*-Spezies wurden Artunterschiede in der Organisation von Interphase-Kernen festgestellt. Die Kerne lebender Zellen in der Gewebekultur erscheinen im Phasen- bzw. Interferenzkontrast homogen bei *X. variatus*, *X. xiphidium*, *X. maculatus* sowie *X. montezumae* ssp. *cortezii*. Dagegen weisen sie bei *X. helleri* eine grössere Zahl stark kontrastierender Körper auf. Nach Anfärbung sind die Nuclei der erstgenannten Spezies wiederum weitgehend homogen oder fein granuliert, während die von *X. helleri* Chromozentren in hoher Zahl (manchmal bis nahe  $48 = 2n$ ) zeigen. Hinsichtlich der Deutlichkeit dieser Struktur bestehen innerhalb der Art *X. helleri* Unterschiede zwischen Populationen.

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63 Giessen (Germany), 8 September 1967.

<sup>9</sup> T. WICKBOM, *Hereditas* 29, 1 (1943).

<sup>10</sup> A. POST, *Z. zool. Syst. und EvolutForsch.* 3, 47 (1965).

<sup>11</sup> W. FOERSTER and W. LUEKEN, in preparation.

<sup>12</sup> For provenience of *Xiphophorus* stocks see K. D. KALLMAN and J. W. ATZ, *Zoologica*, N.Y. 51, 107 (1966).

<sup>13</sup> We are greatly indebted to Prof. Dr. F. ANDERS for kind and constant interest in our work and helpful criticism, and to Miss KÄTE KLINKE for providing us with fish specimens. We thank Mr. R. BENDER very much for going through the manuscript. The work is supported by Deutsche Forschungsgemeinschaft and by Stiftung Volkswagenwerk.