Summary. Alkylation of RNA from brain, liver and kidney in rats after application of ^{14}C-methylnitrosourea and 1-^{14}C-ethylnitrosourea was investigated. After administration of ^{14}C-methylnitrosourea the amount of 7-methylguanine in the RNA of brain and liver is about the same. Analogic experiments using 1-^{14}C-ethylnitrosourea showed no alkylation of the RNA in the organs investigated. This leads to the conclusion that there is no direct correlation between the alkylation of RNA and organotropic carcinogenic activity of the compounds studied.

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Cytochemical Localization of Monoamine Stores in Sheep Thyroid Gland at the Electron Microscope Level

The determination of the cellular localization of monoamines present in some endocrine organs is of great importance in the assortment of the functional role of these compounds. The thyroid gland of the sheep, in which high amounts of 5-hydroxytryptamine (5-HT) have been demonstrated[1], was found upon examination with the fluorescence histochemical method of Falck and Hillarp for the demonstration of monoamines[2,3], to contain 2 different monoaminergic cell systems. Cells emitting a granular green fluorescence characteristic of primary catecholamines and exhibiting metachromasia were identified as mast cells, which in ruminants are known to store dopamine (DA)[4]. Other cells appearing in clusters between follicles or within follicular epithelium, emitted a yellow light characteristic of tryptamine derivatives and failed to give metachromasia. These cells, probably storing 5-HT, were identified as parafollicular cells named by Nonidez[5], whose existence as a different cell population in mammalian thyroid gland has long been a matter of controversy. However, electron microscopic studies have demonstrated striking morphological differences between follicular and parafollicular cells[6–9]. These findings, as well as the existence of monoaminergic mechanisms operating in those cells in species in which they do not store amines[10–11], support the assumption that the parafollicular cells constitute an independent cell system in the thyroid gland. Recent experiences seem to confirm this hypothesis by demonstrating that the parafollicular cells are involved in the regulation of calcium metabolism mediated by thyrocalcitonin[12].

In order to localize amine stores of sheep thyroid gland at a finer structural level, a cytochemical reaction that was shown to be efficient in differentiating between catechol- and indoleamine containing structures[13–15], was performed in this organ. Morphological findings were correlated with the results obtained with the assay of biogenic amines present in the gland.

Thyroid glands were obtained from sheep at the slaughter house immediately after death. Small pieces were processed for electron microscopy as previously described[13–15]. Fixation was carried on in 3% glutaraldehyde followed by 2.5% potassium dichromate in 0.2 *M* acetate buffer pH 4.1 (glutaraldehyde-dichromate or GD reaction), or in 8% formaldehyde followed by glutaraldehyde and potassium dichromate as above (formaldehyde-glutaraldehyde-dichromate or FGD technique). Some blocks were routinely fixed in glutaraldehyde followed by osmium tetroxide. After dehydration in ethanol, the blocks were embedded in Epon 812 and sections observed without further staining under a Siemens Elmiskop I electron microscope. Fluorometric assays of 5-HT[16], noradrenaline[17], DA[18] and histamine[19] were performed in thyroid tissue.

In material processed with GD reaction revealing catechol- and indole-reactive structures, dense precipitates indicating a positive reaction were observed in 2 different cell populations. Some cells, widely distributed in the perifollicular connective tissue, were characterized by large granules of about 4000–6000 Å displaying a positive reaction and exhibiting varying shapes and internal densities (Figure 1a). Their ultrastructural appearance was characteristic of that of mast cells and corresponded to the numerous metachromatic cells observed in the sheep thyroid under the light microscope. The other cells giving a positive reaction were very sparsely and unevenly distributed within the gland and localized between the follicular epithelium and the perifollicular connective tissue or in clusters between follicular cells. The reaction observed in these cells, less intense than that of mast cells, was localized in cytoplasmic granules of varying size and density, most of them being oval or round-shaped.

After prefixation with formaldehyde, prior to glutaraldehyde-dichromate treatment, a procedure resulting in

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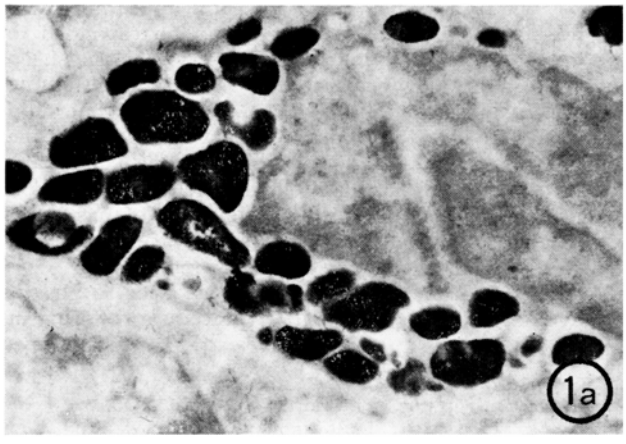
the negativization of catechol-reacting sites, cells similar to those identified as mast cells with GD reaction were present. Only clear empty spaces were observed in the cytoplasm (Figure 1b) while some granules, less dense than those revealed by GD technique, were occasionally seen. The other cells characterized by a positive reaction exhibited reactive granules in their cytoplasm similar to those observed with GD reaction, even after formaldehyde prefixation (Figure 2).

The results of amine determinations are given in the Table and expressed as μg of amine/g wet weight.

The cytochemical reaction used for the differentiation between catecholamines and indoleamines at the electron microscope level is the result of recent experiments made at our laboratory, based on the property of formaldehyde fixation to render negative the chromaffin reaction given by catecholamines, leaving unaffected indolic reactive sites, as was determined by previous light microscopic studies[20,21]. The possibility of differentiating these compounds under the electron microscope was demonstrated in a study of the specificity of the reaction performed in isolated blood platelets[13,14]. The fact that mast cells react with GD technique, giving negative results after the prefixation with formaldehyde, is in accordance with previous findings indicating that mast cells of ruminants are characterized by storing DA[4], and that formaldehyde fixation prior to chromation gives negative results in such cells at the optical level[4]. That DA is stored in mast cell granules is hereby directly demonstrated under the electron microscope since histamine also contained in those cells does not interfere with the reactions[14]. The high amount of DA present in sheep thyroid gland probably corresponds to the abundant mast cell population of the glands examined and is in line with the finding of a large quantity of histamine. The discrepancy between the results of FALCK et al.[2] and those here reported about the concentration of DA may be explained by the fact that these authors observed only few mast cells in sheep thyroid gland, while in those here examined a great number of such cells was apparent. The presence of some scanty granules reacting with dichromate in mast cell cytoplasm after formaldehyde fixation suggest that they may also store a tryptamine derivative, a point that deserves further investigation.

The other cell type having reactive granules bears the ultrastructural characteristics of the granule-containing parafollicular cell[6-9]. The fact that reactive granules are observed after the FGD reaction strongly suggests that they represent the site of storage of 5-HT, as was already suggested in view of pharmacological results[10]. The finding of a less intense reaction in these granules than in those of mast cells may be explained by the lower quantities of 5-HT present in the gland compared with those of DA. The presence of other amine in the granule together with 5-HT cannot be discarded in view of present results.



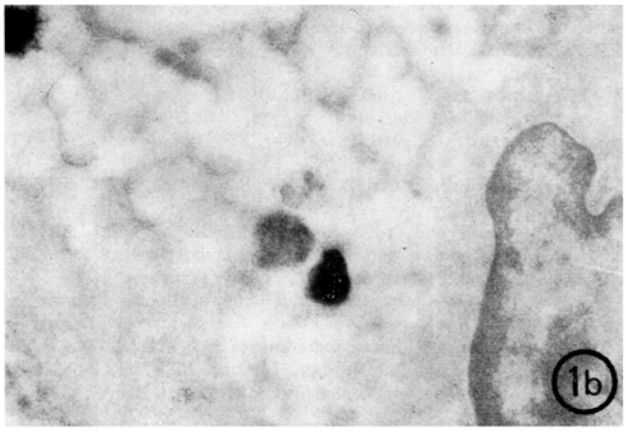


Fig. 1. Electron micrographs showing a portion of mast cells of sheep thyroid gland. (a) The glutaraldehyde fixation and subsequent dichromate treatment depicts dense reactive granules. × 15,000. (b) Following prefixation with formaldehyde a treatment similar to that used in (a) fails to give a positive reaction in the majority of the granules. Some smaller and less dense reactive sites are observed. × 21,000.

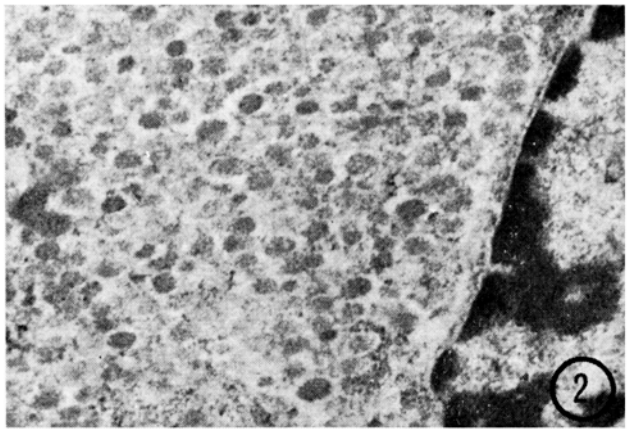


Fig. 2. Portion of the cytoplasm of parafollicular cells of sheep thyroid gland. Dense reactive granules are observed when the tissue is processed with the formaldehyde-glutaraldehyde-dichromate technique. The omission of formaldehyde fixation gives an essentially similar image. × 25,000.

Content of biogenic amines in the thyroid gland of the sheep. Figures represent the mean values of 5 experiments expressed as μg/g wet weight $\pm$ SE

5-Hydroxytryptamine	1.03 $\pm$ 0.09	Histamine	11.71 $\pm$ 1.89
Dopamine	4.51 $\pm$ 0.33	Noradrenaline	0.37 $\pm$ 0.05

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The suggestion that thyrocalcitonin is stored within granules of parafollicular cells[12], 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[10]. 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[22, 23].

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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[23] The kind cooperation of Dr. F. Iturriza and Dr. J. López Aranguren and the technical assistance of Mrs. Cristina Llorente and R. Castelli are gratefully acknowledged. Supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina and U.S. Air Force (AF-AFOSR 963-67).

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[1-4]. 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*[5], *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[6,7] arranged in 1–3 layers around a fin explant[8], 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).

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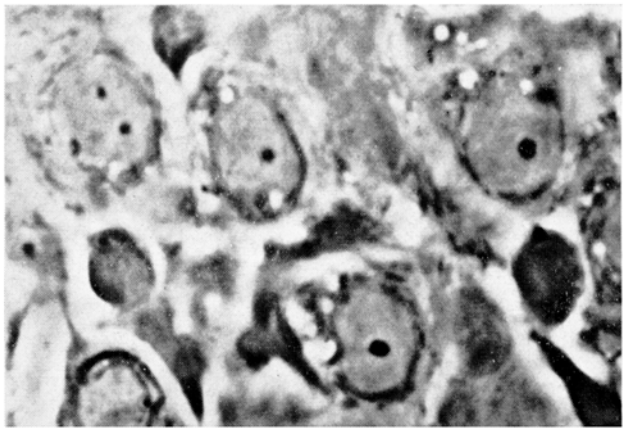

Fig. 1a. Nuclei (with 1 or 2 nucleoli) of living tissue culture cells of *Xiphophorus variatus* in phase contrast. × 2000.

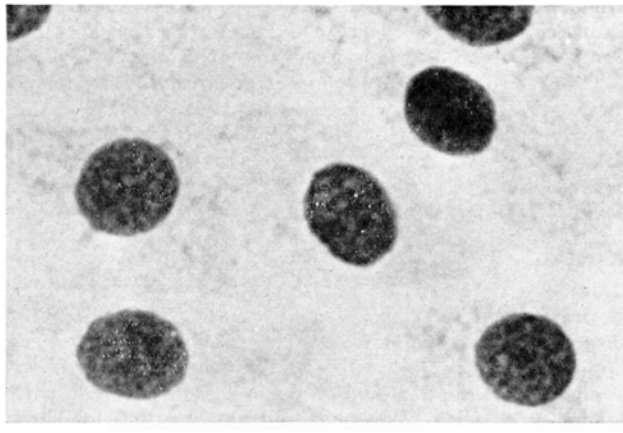

Fig. 1b. Stained nuclei of *X. variatus*. Tissues exposed to colchicine (1:40,000) 3 h, treated with hypotonic solution 45 min, fixed in acetic-alcohol, dried and stained with aceto-orcein. × 2000.