denature by mitomycin C, were hardly seen in the above sites, and hyperplasia or excessive cell proliferation were present. It is speculated that the inherited multi-systemic diseases may be related to the cells derived from the same origin, especially the neural crest[6,11]. On the other hand, it is said that the cells from patients with the hereditary disease, xeroderma pigmentosum, bring about mutation such that the repair replication of DNA is either absent or much reduced, and patients with xeroderma pigmentosum develop fetal skin cancers when exposed to sunlight, and so the failure of the DNA to repair in the skin must be related to carcinogenesis[12]. It is speculated that the hyperplasia or excessive cell proliferation and the heterotopic deposition might occur by the dysdifferentiation[13] of the neural crest cells.

Zusammenfassung. Nachweis, dass bei Entwicklungsstadien von Mäusesäuglingen, die mit Mitomycin C injiziert wurden, Hyperplasien mit Melanin-Ablagerungen auftraten, was mit «Dysdifferenzierungen» von Zellen der Neuralleisten zusammenhängen dürfte.

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Histophotometric Measurements of the DNA Content in the Ovarian Follicle Cells of *Lacerta sicula* Raf.[1]

In *Lacerta sicula*[2], in the ovarian follicles ranging from about 100 to 1500 µm in diameter, the follicular epithelium consists of 3 different types of cells (Figure 1). Outstanding are the 'pyriform cells', with enlarged body containing a large nucleus and the apex toward the oocyte. These cells represent the only clear example in vertebrates so far studied by electron microscopy, of cells in direct connection with the oocyte through intercellular bridges[2-6].

By electron microscopy Taddei[6] and Taddei and Barsacchi-Pilone[7], with cytological, cytochemical and autoradiographic methods, have observed that, in contrast to the first phase of oocyte growth characterized by a monolayered follicular epithelium, typical nucleoli are lacking in the germinal vesicle after differentiation of the pyriform cells and incorporation of ^{3}H uridine, very high in these cells, is absent in the oocyte nucleus[6,7]. Taddei[6] has suggested that, at this stage, together with other materials, ribosomes are synthesized by the pyriform cells and transferred to the oocyte through intercellular bridges, so that these cells, by constituting a highly integrated system with the oocyte, seem to function as 'nurse cells'.

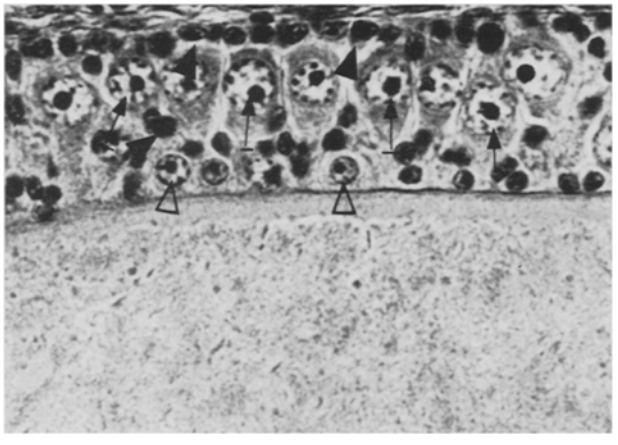

Fig. 1. Light micrograph of the polymorphic follicular epithelium of *Lacerta sicula*. Note the nuclear characteristics of the three types of follicular cells: small follicular cells (black arrow), pyriform cells (arrow with bar), intermediate cells (white arrow) (450×).

Nurse cells are described in the oogenesis of some invertebrates; especially in the meroistic insect ovary, the function of these cells of synthesizing and transferring to the growing oocyte RNA through intercellular bridges is well-documented; it has further been observed that during differentiation of these cells, the nucleus undergoes endomitotic polyploidization[8-10].

The present work investigates whether the differentiation of the lizard's pyriform cells is accompanied by a process of polyploidization, with a view to better understanding of the function of these cells in the oogenesis of *Lacerta sicula*.

Materials and methods. For the histophotometric determination, oocytes of the lizard *Lacerta s. sicula* Raf., taken in November, were manually removed from their connectival theca under the dissecting microscope using watchmaker's forceps. The oocytes surrounded by the follicular epithelium were fixed in ethanol-acetic acid (3:1) and transferred into 45% acetic acid on a slide previously coated with gelatin (0.1%) chrome alum (0.001%) solution, covered with a siliconized cover slip and then squashed. The preparations were frozen on dry ice and the cover slip removed with a razor blade[11].

After 5 min in 95% alcohol, the Feulgen reaction was carried out on the air-dried slides. After hydrolysis in 1 *N* HCl (12 min at a constant temperature of 60 °C.), the slides were stained for 90 min with Schiff's reagent and rinsed in 3 baths of freshly-prepared SO_2 solution.

[1] This research was supported by a C.N.R. grant.
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Absorption measurements were taken by a Leitz MPV microphotofluorimeter, with monochromatic light at 546 nm, according to the 'multiple plug'[12] and the 'two area'[13] methods. The Feulgen DNA content was expressed in arbitrary units (AU) as the product of optical extinction and nuclear area, and the values obtained for each follicular cell group were compared with the Feulgen DNA content of the erythrocytes, which were used in the experiment as typical diploid cells. The number of nuclei investigated and the results obtained for each cell group are reported in the Table.

Results and discussion. Either in the sections or in the squashes, the nuclei of the 3 types of follicular cells may be distinguished by their size, by the nucleolar size and by the chromatin distribution pattern.

Nuclear DNA amounts of the 3 types of follicular cells and erythrocytes

	No.	Mean	S.D.	S.E.
Erythrocytes	119	7.23	± 2.36	± 0.22
Small follicular cells	254	8.32	± 3.32	± 0.21
Intermediate follicular cells	83	7.28	± 2.11	± 0.23
Large follicular cells (pyriform cells)	55	7.84	± 3.10	± 0.42

Average values in arbitrary units (mean), standard deviation (S.D.), standard error (S.E.) and number of nuclei studied (No.) for each cell group.

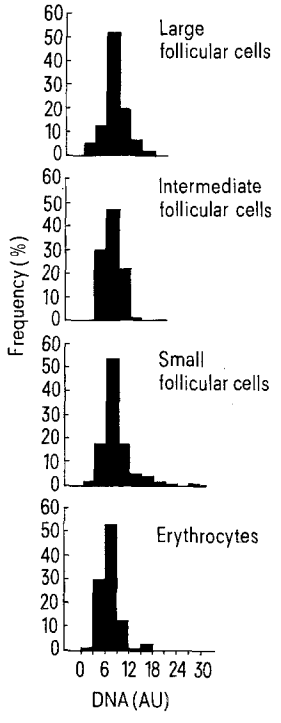

Fig. 2. Frequency diagrams showing the distribution of the DNA content in the 3 types of follicular cells and erythrocytes. The DNA values, in arbitrary units (AU), sub-divided into frequency classes of 3 AU (in abscissa), are plotted against the number of nuclei (in %) for each frequency class.

Nuclei of small follicular cells generally show an almost regular distribution of small heterochromatic clumps and a relatively small nucleolus. Those of the pyriform cells possess typically a larger nucleolus and more scattered but larger heterochromatic clumps, sometimes linked together with fine chromatin threads. Those of the cells in an intermediate stage show an aspect that seems intermediate between both the small and pyriform cells, and may be distinguished especially by their size and the size of their nucleolus. In the sections, the diameter of the nucleus of the small follicular cells is about 7 μm that of the pyriform cells is 15–16 μm, and that of the intermediate cells 10 μm; in the squashes, the diameters are about 8, 16–19 and 12 μm, respectively. The larger diameters found in the squashes in comparison with those found in the sections are probably due to the pressure exerted during the preparation.

The results of the Feulgen DNA measurements on the nuclei of the 3 cell types are summarized in Figure 2, where also the measurements of the erythrocyte nuclei are reported as a control. There are no differences between the main peaks of the 3 types of follicular cells examined and those of the erythrocytes, ranging from 6 to 9 AU of DNA, suggesting that most of the cells have the same DNA content, which is in the range of a diploid value.

Only in the small follicular cells there is a small percentage of nuclei with a significantly higher Feulgen DNA content that can reach 30 AU. Also considering the mean of all the results (Table), the small follicular cells show a higher value with respect to the erythrocytes and other follicular cells; the significance of this difference was evaluated by statistical analysis. The data on the Feulgen DNA content, therefore, show that the pyriform cells bear a diploid genome and that their differentiation is not accompanied by a process of endopolyploidization.

The reported data on the significantly higher amount of DNA in a small percentage only of small folicular cells confirm the morphological and autoradiographic results that only these cells in the lizard's follicular epithelium can divide; they also agree with the hypothesis that the pyriform cells differentiate from the small follicular cells[14–16].

Riassunto. Dallo studio comparato sul contenuto di DNA dei vari tipi di cellule follicolari della *Lacerta sicula*, è emerso che il nucleo delle cellule piriformi e di quelle intermedie presenta un contenuto diploide di DNA, e che solo una parte delle cellule follicolari piccole raggiunge dei valori superiori. L'attività delle cellule piriformi, in diretta connessione con l'ovocita attraverso ponti citoplasmatici, è sotto il controllo di un genoma diploide.

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