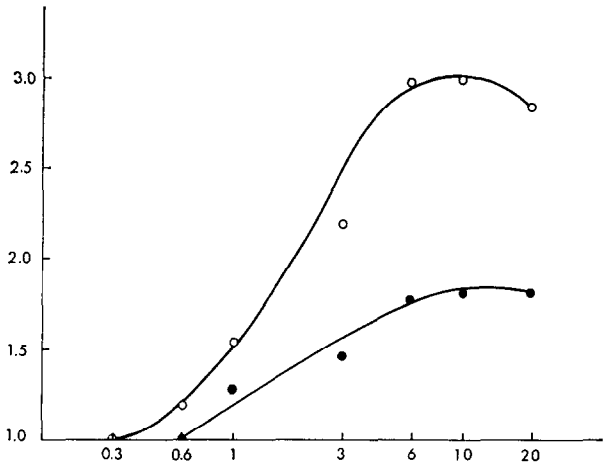

Fig. 3. Abscissa: inoculated cells/Petri dish ($\times 10^{-5}$); *ordinate:* growth rate (N_3/N_0).
Growth rate of adult mouse (AMK) and mouse embryo (MEK) kidney cultured cells in secondary culture. The growth during the 3 day interval (N_3/N_0) after attachment on the glass surface was plotted against various inoculum sizes. ●, AMK cells; ○, MEK cells.

dish. With higher, or lower, sizes of inoculum the growth rate decreased gradually. As seen in fig. 3 the growth rate of MEK cells is higher than that of AMK cells in a secondary culture. Therefore, loss of the degree of the saturation density or the sensitivity to contact inhibition of growth seems to be closely correlated with growth in vivo (fig. 2) and in vitro (fig. 3). Mora et al. reported the decrease of gangliosides in mouse cell line transformed by SV40 virus and this was correlated with increased saturation density [9]. Although the difference in saturation density between adult and embryonic kidney cultured cells remains to be elucidated, it is suggested that cell populations growing rapidly in vivo differ as to their component of the surface membrane from those not growing rapidly in vivo.

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Significance of pyriform cells in ovarian follicle of *Lacerta sicula*

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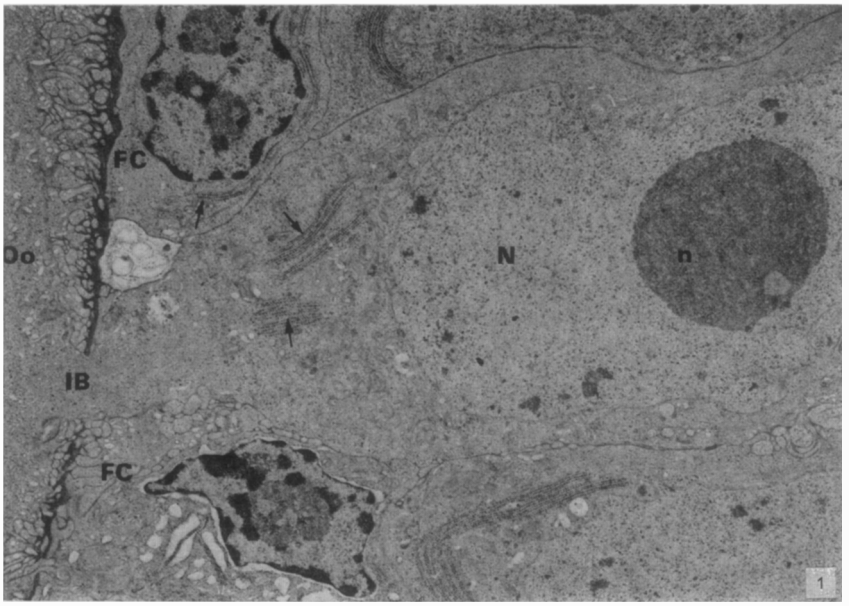
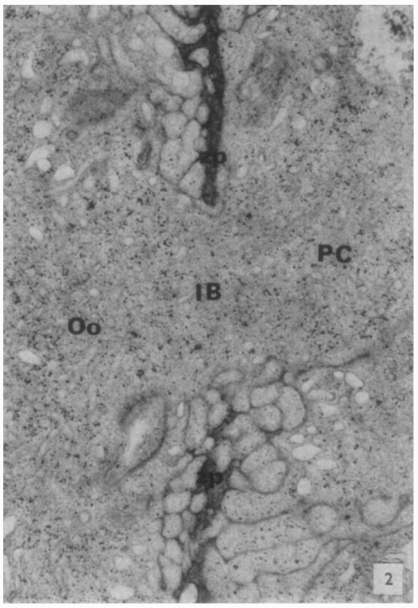
The present paper describes some ultrastructural aspects of the ovarian follicle in *Lacerta sicula* which may help towards an understanding of the function of the pyriform cells [10]. Since these cells regress and degenerate at the beginning of the vitellogenic phase, they have been ascribed a 'nourishing' function, and hence called nurse cells [3]. Their role during oocyte growth is, however, essentially unknown.

The pyriform cells stand out among the cells of the follicular epithelium because of their larger size and characteristic pyriform shape. Their elongated apex points toward the oocyte and the enlarged body contains a





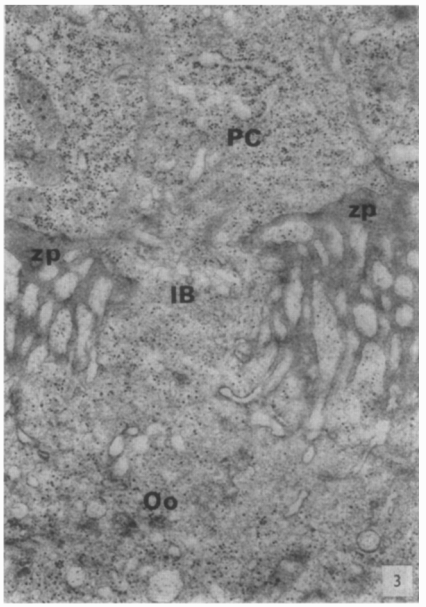
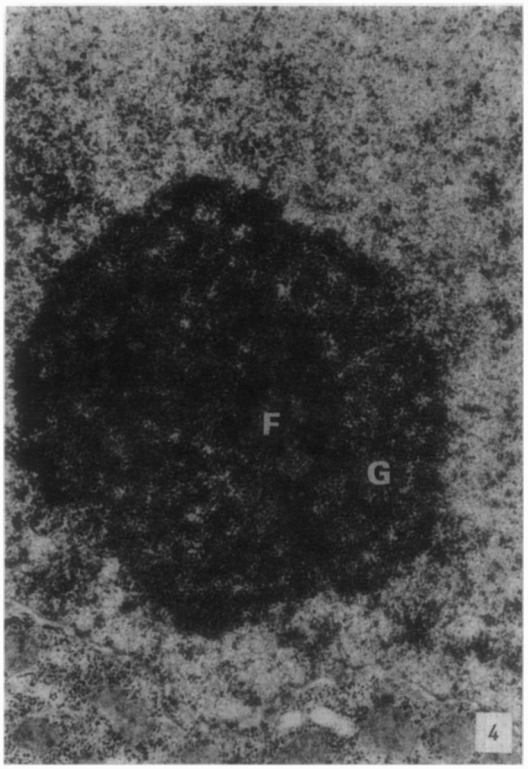
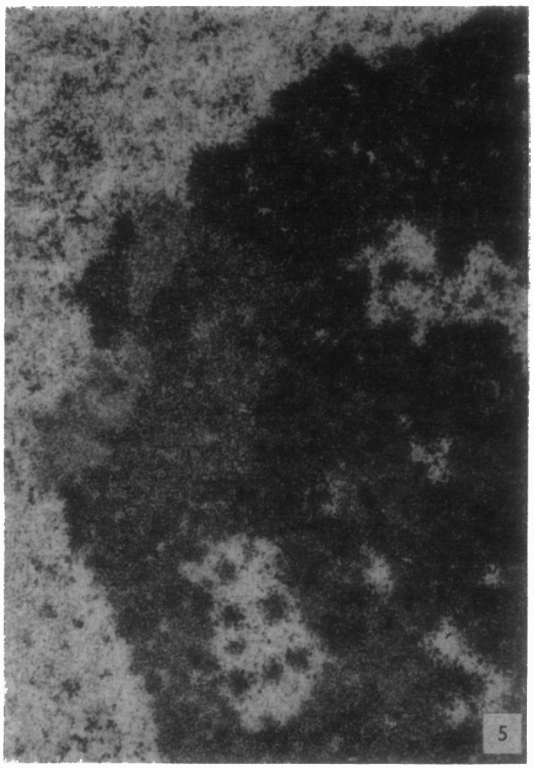


Fig. 1. A pyriform cell of a follicle about 400 μm in diameter in longitudinal section. In its enlarged body, a large vesiculous nucleus (*N*) is evident with thin clusters of scattered chromatin and a well-developed nucleolus (*n*). In the cytoplasm mitochondria and two ribosomal bodies (*arrows*) are evident. The cell apex is connected with the oocyte (*Oo*) through an intercellular bridge (*IB*) crossing the zona pellucida. Around the apex of the pyriform cell there are two small follicular cells (*FC*) with a comparatively small nucleus, a nucleolus and dense clusters of chromatin; also the cytoplasm of these cells during the winter rest contains small ribosomal bodies (*arrow*). ×4 000.

Fig. 2. A higher magnification of the intercellular bridge (*IB*) of fig. 1, connecting through the zona pellucida (*ZP*), the pyriform cell (PC) with the oocyte (Oo); in the cytoplasm of the bridge ribosomes are evident. ×10 000.

Fig. 3. A cytoplasmic bridge crossing the zona pellucida (*ZP*) of a 1 mm in diameter follicle and connecting the elongated apex of a pyriform cell (*PC*) with the oocyte (*Oo*). In the bridge, besides other materials, ribosomes are evident. ×16 000.





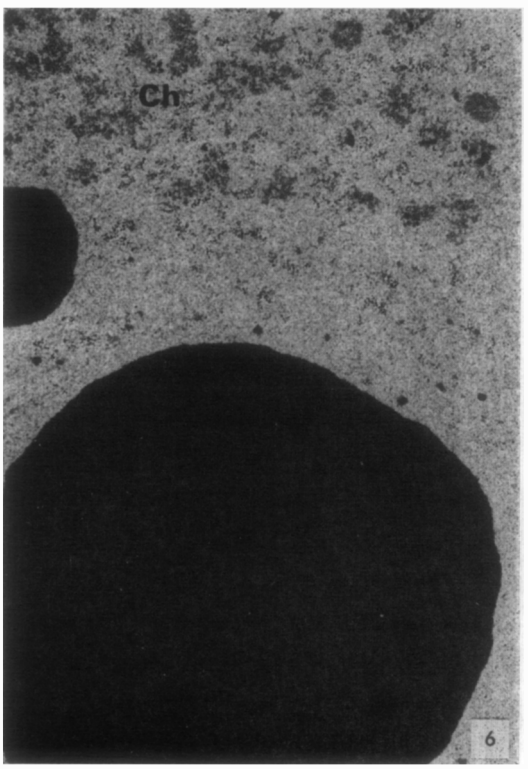


Fig. 4. A pyriform cell nucleolus. The fibrillar core (*F*) and the granular cortex (*G*) are evident. × 13 000.
Fig. 5. A portion of the oocyte nucleolus in a follicle about 100 μm in diameter without pyriform cells. The fibrillar and granular constituents can be noted. × 17 000.
Fig. 6. A section of two dense nucleolus-like bodies, different in size, consisting of electron-dense material, adjacent to chromatin threads (*Ch*). These bodies are located in the central region of the oocyte nucleus in a follicle about 1 mm in diameter, characterized by a completely differentiated follicular epithelium with pyriform cells. × 6 000.

vesicular nucleus which can reach 15 μm in diameter (fig. 1); the nuclear membrane is crossed by many pores, and thin clusters of chromatin lie scattered in the nucleoplasm. The nucleolus is much developed (6–8 μm) without a perinucleolar chromatin ring; it contains typical granular and fibrillar components which may vary in distribution: some nucleoli are rather dense, with a fibrillar core and a granular cortex (fig. 3), others are either ring-shaped, or show a reticular matrix, either loose or vacuolated. This morphological diversity of the nucleolus is similar to that described in amphibian oocytes [1, 5] and may be related to active ribosome synthesis. The ribosomes in the cytoplasm are abundant, both free and attached to the cytomembranes. Moreover, during the winter rest a portion of the ribosomes in the follicular cells aggregates into typical crystalline bodies (ribosomal bodies) (fig. 1), which are, however, smaller than those already described in the subcortical region of the oocyte [2, 8, 9].

The most remarkable feature of these cells is their connection with the oocyte through cytoplasmic bridges. This situation has already been indicated by Ghiara et al. [3] in *Lacerta sicula*. While this paper was in preparation, Hubert [4] and Neaves [7] described similar findings in *Lacerta vivipara* and *Anolis carolinensis*.

In *Lacerta sicula*, these bridges are found both in rather small follicles (300–400 μm in diameter) (figs 1, 2), in which the follicular epithelium is not yet completely differentiated and in follicles in later phases of growth (1 000–1 500 μm) (fig. 3). Furthermore, we can observe regions where both the oocyte and the pyriform cells are found in close contact with each other, due to the contiguity of their plasma membranes. It remains to be ascertained whether these regions have a specific function or represent a phase of the cytoplasmic bridge formation.

The intercellular bridges develop during the formation of the zona pellucida and the early organization of the polymorphic follicular epithelium, owing to the fusion of the plasma membranes both of the oocyte and pyriform cells.

During the growth of the oocyte, as a result of the differentiation of the follicular epithelium, the pyriform cells elongate. In oocytes about 1 mm in diameter, the accumulation of electron-dense amorphous material increases the thickness of the zona pellucida and forms a ring around the cytoplasmic bridge (fig. 2).

The cytoplasm of the bridges has no characteristics of its own and, in fact, can be distinguished neither from that of the pyriform cells nor of the cortical region of the oocyte. It seems reasonable to suggest that the pyriform cells and oocyte may function as a highly integrated system in relation to the physiological needs of the oocyte growth.

Concurrently with the formation of the cytoplasmic bridges, the morphology of the oocyte nucleoli changes drastically. Indeed, in the small follicles (about 100 μm in diameter) with a monolayered follicular epithelium and without pyriform cells, the oocyte contains a well-developed nucleolus with granular and fibrillar components (fig. 4). In the larger follicles (ranging from 100 μm to 2 000 μm), in which pyriform cells connected with the oocyte are present, several nucleolus-like bodies can be seen in the germinal vesicle (fig. 5); they appear to be made of homogeneous electron-dense material. These dense bodies are usually found in the central region of the nucleoplasm, sometimes surrounded by chromatin threads; their appearance is quite different from that of the multiple nucleoli described in amphibian oogenesis [6].

On the basis of these observations, the author suggests that in *Lacerta sicula*, during the early phase of growth (monolayered follicular epithelium), ribosome synthesis is car-

ried out in the germinal vesicle; later on, during the subsequent slow and long phase of growth, when the follicular epithelium becomes polymorphic, and pyriform cells differentiate and make connection with the oocyte through the cytoplasmic bridges, the ribosomes are synthesized by the pyriform cells and then transferred through the cytoplasmic bridges to the oocyte. Experiments are in progress to verify this hypothesis. It is an attractive hypothesis that in the lizard the very large number of pyriform cells in each follicle (10 000 according to Neaves) may in fact provide a mechanism for the increase in the amount of ribosomes present in the oocyte

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Human diploid cell response to variations in relative amino acid concentrations in Eagle medium

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Summary

The growth and longevity of human diploid fibroblasts was studied in Eagle medium modified by adding 1 mM excess of one of the amino acids already present. Excess tyrosine increased the longevity of these cells by at least 20 cell divisions. Excess histidine was also stimulatory; excess isoleucine and cystine were growth-inhibitory. The other amino acids showed little or no effect.

Recently it has been shown that increasing the concentration of the amino acids in Eagle minimum essential medium (MEM) [2] as much as 4-fold had no beneficial effect on the growth rate and longevity of human embryonic diploid lung fibroblasts (HEDLF) [6]. In the present report, the growth response of HEDLF cells was studied in Eagle medium in which the concentration of each amino acid was increased individually by 1 mM.

Material and Methods

The cell strains were grown from lung tissue of human embryos aborted in about the third month of gestation. The cells had the growth characteristics typical of diploid fibroblasts [3, 4] and had been used in other experiments [7, 8]. The growth medium for all experiments was Eagle MEM [2] supplemented with 10% calf serum, 1 mM Na pyruvate, 4 mM L-glutamine, 100 IE/ml penicillin and 100 μg/ml streptomycin.

The general culture and passage techniques have been described elsewhere [6–8]. 1×10^5 cells were inoculated into culture bottles with a growth surface of 46 cm^2. The number of cells attaching to the glass after overnight incubation was measured after each passage according to a procedure described earlier [7, 8]. This value was used in the calculations of cell divisions to correct for the loss of cells in the inoculum, which did not attach to the glass. When the culture became confluent the cells were suspended with 0.25% trypsin and passed.

Results

To independent aliquots of MEM was added 1 mM excess of one of the 12 amino acids already present. HEDLF cells were grown in one particular medium until they were senescent and no longer divided. The growth responses obtained with excess cystine, histidine, isoleucine, leucine and tyrosine are shown in fig. 1. A 1 mM excess of the other amino acids (arginine, lysine, tryptophane, valine, threonine, phenylalanine and methionine) were not significantly different from the control. However, excess arginine ap-