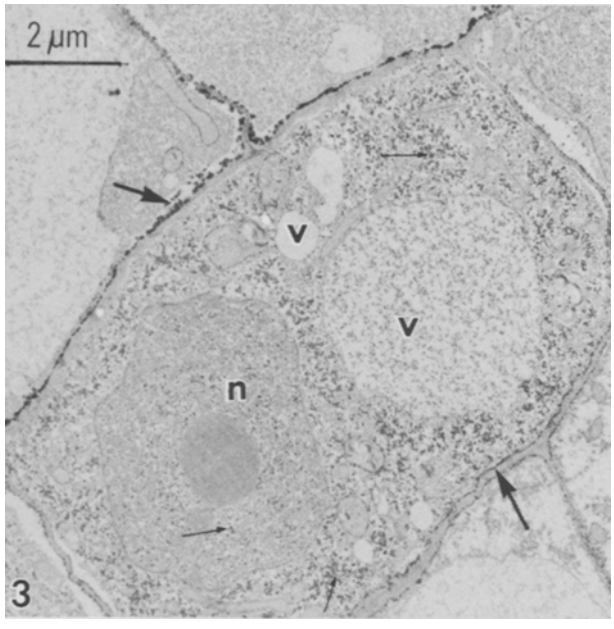


Fig. 3. Electron micrograph showing localization of polyphenols by $FeCl_3$. Note the Fe-polyphenolic complexes in the cytoplasmic and nuclear matrix (small arrows). The preparation was stained with uranyl acetate and lead citrate. The vacuoles (v) do not show reaction product; the fine granulation in the vacuole is not due to Fe-polyphenol complex and does not appear in unstained sections. Reaction product can also be seen at the interface of the cell wall and the plasma membrane (large arrows).

be localized in the vacuole and therefore may not be in the storage form. Their localization within the cell matrix suggests an active physiological participation within the cytosol.

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## In situ aging of auricular chondrocytes is not due to the exhaustion of their replicative potential

S. Moskalewski, A. Gołaszewska and T. Książek

*Department of Histology and Embryology, School of Medicine, Chałubińskiego 5, 02-004 Warsaw (Poland), 28 January 1980*

*Summary.* Multiplication of chondrocytes during growth of rabbit auricular cartilage was estimated on the basis of total DNA determination and compared with the population doubling level reached by these chondrocytes in vitro. The results indicate that the in situ aging of auricular chondrocytes is caused by factors other than the intrinsic depletion of their growth potential.

It has been shown that diploid cells have a finite capacity for replication in vitro[1]. Growth potential of cells seems to be related to aging since it is decreased in older donors[2,3]. Nevertheless, recent studies indicate that the cessation of growth in cultures of diploid cells may be due to terminal differentiation of cells rather than to aging[4,5]. It also appears that the finite limit of replication occurring in vitro is rarely, if ever, reached by cells in vivo and therefore is not responsible for the aging of the organism[6,7].

While the aging of auricular cartilage is hardly critical for the individual, this organ offers a good opportunity for studying the latter point. Auricular chondrocytes taken from 7-day-old rabbits pass in culture 10–14 population doublings (PD) before the onset of phase III[8] and may be easily matched with chondrocytes aging in situ, since auricular cartilage forms a closed system in which migration of cells is prevented by an intercellular matrix. It is also noteworthy that nearly all chondrocytes in auricular cartilage from 7-day-old rabbits are mononuclear, while in 4-week and older rabbits centrally located chondrocytes contain 2 nuclei and are much larger[9]. These chondrocytes, in adult rabbits, display pycnotic condensation of the chromatin and accumulate a large amount of fat[10]. Binuclear chondrocytes seem to represent terminally differentiated cell types; mononuclear chondrocytes from older rabbits are able to grow in culture for a limited period[11]. It appears, therefore, that in mature auricular cartilage there exist both cells which are capable of replication and those that are not, they show a characteristic spatial distribution, and in the latter cells changes which may be interpreted as aging occur relatively early. In this work actual multiplication of auricular chondrocytes during growth of cartilage was estimated on the basis of total DNA determination in cartilage of varied ages to see how it corresponds with the replicative potential displayed by these cells in culture.

*Materials and methods.* Auricular cartilages were carefully cleared of surrounding tissues, including, as far as possible, perichondrium, then lyophilized and pulverized in an agate mortar. The pulverized material was dried to constant weight, weighed, and divided into suitable samples. The samples were digested overnight in preactivated 0.5% papain in 0.1 M phosphate buffer a t 37 °C[12]. The partially digested material was cooled and precipitated with trichloroacetic acid (TCA) added to the final concentration of 5%.

Changes in DNA content of rabbit auricular cartilage in relation to age

| Age of rabbits | Dry mass of cartilage (mg)* Mean | SD | Amount of DNA per mg of dry cartilage (µg) Mean | SD | Total amount of DNA per cartilage (µg) Mean | SD | Mean number cells per cartilage** ($\times 10^7$) |
|---|---|---|---|---|---|---|---|
| 7 days | 13.8 | 1.5 | 30.2 | 2.1 | 415.3 | 32.1 | 7.8 |
| 4 weeks | 265.8 | 31.6 | 4.5 | 0.7 | 1193.8 | 183.5 | 22.5 |
| Adult (3–3.5 kg) | 1225.5 | 404.0 | 1.0 | 0.2 | 1309.4 | 616.0 | 24.7 |

* In each group, n=6; ** Calculated on the basis of DNA value in rabbit cells given by Rees and Jones[15]. The cell number in cartilage from 4-week and adult rabbits is overestimated since these cartilage contain a considerable number of binuclear cells, reaching 30% in enzymatically prepared cell suspensions[9].

The precipitate was defatted, DNA extracted with hot TCA[13] and determined by the diphenylamine method[14]. Highly polymerized DNA from calf thymus (Sigma) was used as a standard.

*Results and discussion.* As the table shows, the amount of DNA per cartilage increases about 2.5 times between week 1 and 4 of postnatal life and only insignificantly afterwards. This rise corresponds to approximately 1.5 PD. The results of DNA determination accord well with observations indicating that in cartilage of 4-week-old rabbits mitotic figures are absent[16]. The results could be influenced by differentiation of chondrocytes from the perichondrium and disintegration of cells within the cartilage, but morphological observations[9,10] do not suggest that these factors are significant. Dilution of DNA content per mg of cartilage powder occurring in older cartilage is undoubtedly due to the increase in the amount of intercellular substance. Comparison of PD level reached by chondrocytes from 7-day-old rabbits in vitro and in vivo, amounting to 10–14 and 1.5 (table) respectively[9], indicates that factors limiting and finally preventing replication of cells operate during development of auricular cartilage. In the case of peripheral, mononuclear chondrocytes from mature cartilage, cessation of growth could be simply due to the accumulation of intercellular substance, since after liberation from matrix these cells resume growth in culture[11]. Terminal differentiation of centrally located chondrocytes as manifested by their enlargement and binuclearity could be, as discussed previously[9], either determined genetically or caused by local factors, such as hypoxia or inadequate supply of nutrients and growth factors.

Since PD levels achieved by chondrocytes in vivo and in vitro represent average values for the whole population it could still be argued that the in vivo terminal differentiation of centrally located chondrocytes is caused by the loss of their ability to divide. If so, then it would be necessary to assume that in cultures of auricular chondrocytes from 7-day-old rabbits, mainly chondrocytes from the periphery of the cartilage multiply, since the centrally located cells would be at the verge of terminal differentiation. Such an assumption is, however, not plausible. Enlarged and sometimes binuclear cells are continually produced in cultures of chondrocytes from immature cartilage and are particularly pronounced at the onset of phase III[9]. If precursors of these cells were unable to multiply, or divided only once, they would become too diluted in whole population to be noticed at the 10–14 PD level. It appears therefore, that the in situ terminal differentiation and subsequent aging of centrally located auricular chondrocytes is caused by factors other than the intrinsic depletion of their growth potential. These factors may operate less effectively in culture resulting in the prolongation of the period of growth before the onset of terminal differentiation.


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## How to avoid maternal cannibalism after neonatal surgery in rats


H.F. Helander and A. Bergh

*Department of Anatomy, University of Umeå, S-901 87 Umeå (Sweden), 29 February 1980*



*Summary.* Following surgery newborn rats are often eaten by their mothers. This can be avoided if the wound is closed carefully, the blood removed from the skin, and the wound area covered by a plastic film.


In a recent article Libbin and Person[1] describe methods of avoiding maternal cannibalism after surgery in newborn rats. Their methods include preparation of the pregnant rats from the 13th day of gestation by certain breeding and handling procedures. The cages were not cleaned between confinement and weaning, a period of some 30 days.