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

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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.

Although the proposed methods may be effective in reducing maternal cannibalism, they appear to be somewhat cumbersome. Using hypothermia as anaesthetic, we have performed gubernaculotomy in a large series of newborn rats by means of a 4-mm-long transversal incision through the lower abdominal wall[2]. The wound was closed with 6-0 atraumatic silk sutures; 2 were put in the muscle layer and 3 in the skin. After testing various procedures we would like to emphasize the importance of closing the wound very carefully, so that no s.c. tissue is exposed. Moreover, it was necessary to dry away all traces of blood from the skin. Finally, the wound was covered by spraying a thin plastic film (Nobecutan®, Astra). After warming the animals to normal body temperature they were returned to their mothers. There was no special handling of the mothers before or after the operation, and the cages were cleaned within 1 week. When these procedures were used on about 500 newborn rats, less than 5% were lost because of maternal cannibalism.

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The aggregation pheromone of some terrestrial isopod crustaceans

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Summary. Gregariousness has been found in some terrestrial isopod crustaceans. Their faeces were shown to contain the active principle responsible for the initiation and maintenance of aggregation. The active principle was isolated from the mid gut or hind gut and is presumably secreted into the lumen when faecal materials pass and then excreted with the faeces. One of the results of aggregation was shown to be the acceleration of body growth. The active principle was suggested to be an aggregation pheromone.

Gregariousness is common in the animal kingdom. Wilson and Bossert[1] found an active principle which stimulated separate animals to form a group and named it the aggregation pheromone. Since then research on the aggregation pheromone has advanced[2-4]. However, no aggregation pheromone has been reported in invertebrates other than insects, although suggestive results have been found in some species[5-8].

Gregariousness has been found in some terrestrial isopod crustaceans such as the sow bugs, *Porcellionides pruinosus, Porcellio scaber, Alloniscus perconvexus* and *Armadilloniscus tuberculatus,* the pill bugs, *Armadillidium vulgare* and *Tylos granulatus,* and the sea lice, *Ligia exotica* and *Ligidium japonicum.* The present report deals with the behavioural and physiological aspects of aggregation in these isopods.

When a group of these isopods was introduced into a petri dish, after wandering for several min, they aggregated in a certain area of the petri dish and rested. Aggregation was observed in animals kept in the dark or blinded. When the body surface was washed with alcohol, the time necessary for aggregation increased remarkably. Furthermore, when the antennae of these isopods were cut off, no aggregation was observed.

When a piece of filter paper which had been used as a shelter in the stock boxes of these isopods for several days was put into a petri dish in which isopods had been released, the isopods tended to aggregate on this filter paper. This filter paper was contaminated with faeces and presumably odours emitted from the isopods themselves. As the majority of the contamination was faecal, two-choice experiments were adopted for the biological assay. The faeces, about 300 mg in wet weight, were homogenized with acetone in a glass mortar. Then a filter paper was impregnated with the extracted medium. The biological assay was carried out with filter paper impregnated with an acetone extract of the faeces and a clean filter paper. As a result, all the individuals gathered on the filter paper treated with the faecal extract. Thus the active principle responsible for aggregation in these isopods was shown to be contained in the faeces and was presumably communicated by a response to chemical stimuli through their antennae.

A filter paper impregnated with concentrated alcohol washing of the body surface of these isopods was found to elicit a low aggregation response. Although these isopods have many tegmental glands on their cuticular layer, no aggregation activity was found in cuticular extract themselves. Initial aggregation of these isopods appear to be induced by

Bioassay of aggregation pheromone in *Armadillidium vulgare* with Y-maze olfactometer[a]

	Response			
	+	−	Total	χ^2
Control	51	49	100	0.01
Conditioned filter paper[b]	71	29	100	18.91
Faeces[c]	127	73	200	14.05
Faeces in gut*	73	27	100	20.25
Gut**	64	26	90	15.21
Gut (+) and hepatopancreas (−)**	68	12	80	37.81
Fore gut*	39	51	90	1.34
Mid gut*	58	32	90	6.94
Hind gut*	59	31	90	8.10
Ventral part (+) and dorsal part (typhlosole) (−) of mid gut*	41	59	100	2.89

[a] Calculation of χ^2 was made between (+) and (−). Only χ^2 values of 6.64 or greater have significance (p-values of 0.01 or less). [b] This filter paper was contaminated with faeces and odours emitted from the animal themselves. [c] About 1 g in wet weight. * Each sample prepared from 100 individuals. ** Each sample prepared from 20 individuals.