of physical activity may at least partly explain the lack of the daily rhythms in the $O_2$ consumption and the RQ of SCN-lesioned rats. Figure 2A shows that in sham-operated rats the RQ value was about 1 (mean values 0.996) during the 12-h dark period, and decreased to about 0.9 in the middle of the light period. These findings suggest that carbohydrate is used as the main energy source during the 12-h dark period, and that more lipid is used as an energy source during the light period than during the dark period. These findings are consistent with the report by Le Magnen[3] that in rats the RQ has a daily rhythm with a higher value in the dark period than in the light period. Le Magnen suggested that the VMH was responsible for generation of the daily rhythm of the RQ, since in his experiments bilateral lesions of the VMH resulted in a constant RQ value of above 1 throughout the 24 h[3]. His finding is consistent with reports that bilateral lesions of the VMH eliminate the circadian rhythm of feeding behavior in rats[6–8].

However, we found that bilateral lesions of the VMH do not cause complete disappearance of the circadian feeding behavior in rats, though they increase food intake in the 12-h light period to 35% of the total daily intake[9]. Rietveld et al.[10] also found that bilateral lesions of the VMH do not eliminate the circadian feeding rhythm in rats. On the other hand, we showed that bilateral lesions of the SCN completely eliminate the circadian rhythm of feeding in rats, as mentioned previously[1,2]. Moreover, we obtained evidence suggesting that in rats the circadian feeding rhythm is due to a time signal from the SCN that might be transmitted to the VMH and LH[9,11]. Recently, we observed that bilateral lesions of the SCN enhance insulin secretion from the pancreas[12–14] and abolish hyperglycemia, hyperphagia and the lipolytic response due to intracranial injection of 2-deoxyglucose[12,15]. These findings suggest that the SCN has important roles in the central regulation of glucose homeostasis. Neural activity in the SCN of rats was reported to be higher in the light period than in the dark period[16,17].

From these findings it can be speculated that in the light period the SCN with higher neural activity suppresses insulin secretion, decreases glucose utilization, and increases lipid utilization, and thus lowers the RQ value, and that in the dark period the SCN with lower neural activity has the opposite effects, resulting in an increase of the RQ value to about 1. This would explain why in rats with SCN lesions, the RQ value remained constant at an intermediate value between the maximum and minimum values in sham-operated rats. The constant RQ suggests that in rats with SCN lesions fairly constant proportions of carbohydrate and lipid are utilized throughout the 24-h period. Further studies are required to test these speculations and to determine the mechanisms involved in the control of energy metabolism.


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## Collagen of the calcified layer of human articular cartilage

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*Summary.* The distribution patterns of collagen types I, II and III were studied using immunofluorescent staining techniques in human articular cartilage, including the calcified layer. Tissue taken from femoral heads was stained with the appropriate antiserum. Adjacent sections were stained with von Kossa or Alizarin red to determine the distribution of calcium salts. Results indicate that endochondral ossification at this site occurs by calcium being deposited initially within a matrix of type II collagen.
*Key words.* Calcified cartilage; collagen types; immunofluorescence.

The predominant collagen type present in articular cartilage is type II, which is comprised of three identical chains with the chain composition $[\propto 1(II)]_3$. In bone type I is predominant with a chain composition $[\propto 1(I)]_2 \propto 2^1$. The major part of skeletal mineralization proceeds through endochondral ossification, with systematic calcification of cartilaginous limb rudiments and their replacement by bone to form, for example, the long bones of the body[2]. The complete sequence of processes that must occur to enable such a change is not clear. Study of the layer of calcified cartilage lying between non calcified hyaline cartilage and bone may elucidate some of the mechanisms involved.

*Materials and methods.* Fourteen femoral heads were collected randomly at autopsy in the age range of 6 to 85 years (mean 49.5 ± 27.3). Samples of full depth cartilage together with approximately 2 mm of underlying bone were taken, rapidly frozen and stored under liquid nitrogen. Antisera were prepared and

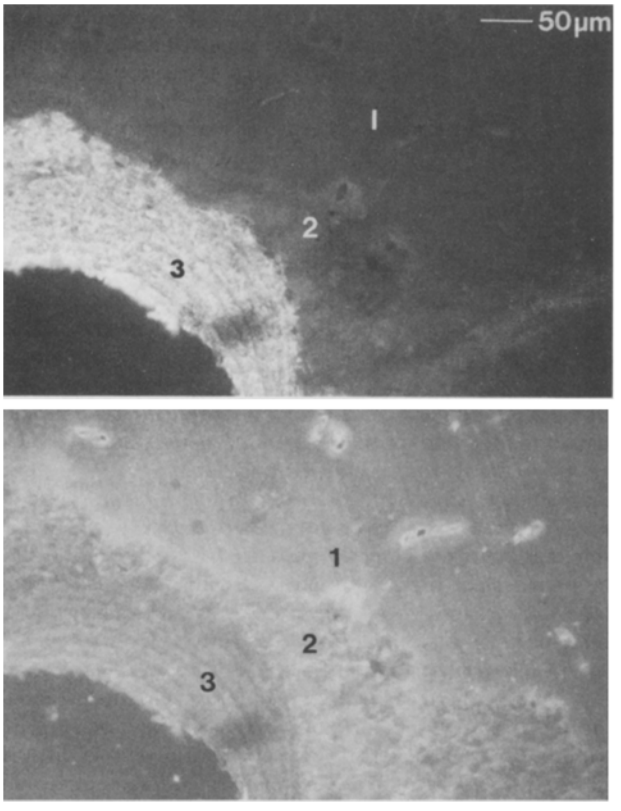


Figure 1. Staining for collagen types I(a) and II(b). Cartilage, particularly the calcified zone, was positive for type II whilst bone was positive for type I. *1* Cartilage, *2* calcified cartilage, *3* bone (29-year-old male).

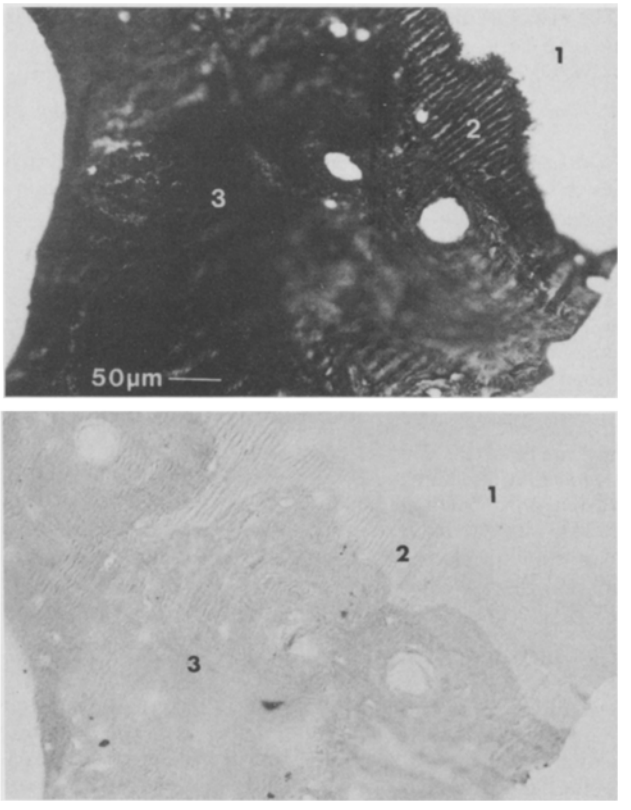


Figure 2. Staining for calcium salts with von Kossa a) without and b) with citrate (negative control). Sections were counterstained with van Gieson. *1* Cartilage, *2* calcified cartilage, *3* bone (29-year-old male).

kindly donated by Dr Helen K. Beard. Antisera to types I and III were raised in guinea pigs to collagens prepared from human placenta, whilst anti-type II was raised in rabbits with collagen prepared from human intervertebral discs[3,4].

The cartilage tissue was sectioned on a Brights rotary cryostat to a thickness of 6 µm and stained by indirect immunofluorescence. The collagen type was located by antiserum labeled with fluorescein in the case of one animal and rhodamine for the other. Hence double labeling of the same section could be achieved. Appropriate negative controls were set up with normal serum and phosphate buffered saline as described previously[3]. Before staining the sections were pretreated with 0.1 mg $cm^{-3}$ trypsin for 15 min at 37°C. This was found to enhance the staining, perhaps by removal of proteins sheathing the collagen fibers. This treatment has been shown previously to release less than 0.2% of the tissue collagen[5]. Adjacent sections were stained with Alizarin red or von Kossa[6] to demonstrate the location of calcium salts.

*Results and discussion.* Staining for type I collagen was positive for the bone in all specimens (see fig. 1a) but negative in the cartilage, including the calcified layer, for all but one specimen. In this specimen, which was from a 6-year-old, a thin strip in the superficial layer stained positively for type I. This may be typical of immature cartilage since a similar pattern has been found in approximately 6-month-old porcine articular cartilage[7].

Antiserum to type III collagen showed a pericellular distribution in the cartilage but was negative elsewhere. This was very similar to that seen previously in human intervertebral discs[5]. Since another collagen, type M, has been shown to exist in articular cartilage, also pericellularly[8], the type III antiserum used in this study was tested for cross reaction with it (kindly carried out by Dr V. Duance). The results showed no such cross reaction.

Antiserum to type II collagen was negative for bone and positive for the cartilage, particularly the calcified layer (see fig. 1b). This is similar to the staining pattern found in embryonic chick tibiae[9]. Von Kossa and Alizarin red staining demonstrated, as expected, the presence of calcium salts in the calcified cartilage and bone, but not in the remaining cartilage (see fig.2). These results indicate that calcification at this location occurs by calcium being deposited within a matrix of type II collagen, rather than of type I, bone collagen.

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