been noted earlier[6,8] but the differences between left and right axillae were not significant for men or women in this study. Previously[14] in men we recorded a 'superior' axilla, but the intra- and inter-individual variation found in the present work may have obscured this.

Axillary 5α-androstenone levels were found to be un-related to age, handedness or degree of hirsutism (in women) and to anosmia to 5α-androstenone. A significant linear relationship exists, in men, between this steroid and cholesterol, but not with squalene. As squalene is not found in apocrine secretions[17] but in sebum[28], this suggests that 5α-androstenone does not correlate with sebum secretion, a finding that compares favorably with our earlier studies[14]. Olfactory thresholds to 5α-androstenone varied widely but the lowest recorded in several subjects (0.2 parts per billion) was as found earlier[18,20]. There was no difference in thresholds between men and women (as noted earlier[20]). Women largely (70%) found the smell repellant or unpleasant (20%). Anosmia to the smell of 5α-androstenone did not differ greatly between men and women but the incidence (9–20%) was much lower than the values reported before[18,20]. In this study, a very high proportion (90%) of women were anosmic to the smell of an-α, and this is difficult to explain in relation to data obtained from larger studies[18].

5α-Androstenone is now known to be a product of microbial action in the axilla[14,16,17]. Coryneform bacteria are present especially in the axillae of men and this could explain the higher levels of 5α-androstenone found compared with those in women. Results are also consistent with the more pronounced 'musky' or 'strong' smells of male axillary extracts compared with the 'sweet' smell of those from the female subjects[4] (except for one, recorded as 'strong', with 550 pmol/24 h). Sex discrimination of adult humans by odor is well-documented (see Doty[4] and Schleidt[28] and references therein), and axillary 5α-androstenone may well be involved.

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Bilateral lesions of suprachiasmatic nucleus eliminate circadian rhythms of oxygen consumption and the respiratory quotient in rats

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Summary. Bilateral lesions of the suprachiasmatic nucleus of the hypothalamus of rats abolished circadian rhythms of oxygen consumption and of the respiratory quotient (RQ). The RQ remained constant at a level intermediate between the maximum (about 1.0) and minimum (about 0.9) values in control animals.
Key words. Circadian rhythm; oxygen consumption; respiratory quotient; suprachiasmatic nucleus; rat.

Previously we found that the circadian rhythm of feeding behavior of rats disappeared after bilateral lesions of the suprachiasmatic nucleus (SCN) of the hypothalamus[1,2]. Since oxygen (O_2) consumption and the respiratory quotient (RQ) are closely related to food intake, and both are reported to show a daily rhythm[3], we next examined the effects of bilateral lesions of the SCN on these rhythms.

Materials and methods. Male Wistar strain rats, initially weighing 150–200 g, were used. Animals were housed in stainless steel cages with free access to water and powdered laboratory diet (type M, Oriental Yeast Co., Osaka), which contained 25 g of protein, 5.5 g fat and 58.6 g of carbohydrate per 100 g (359.9 Cal) of diet. The animal room was maintained at 24 ± 1 °C and 60 ± 10% relative humidity, and illuminated by fluorescent

lights for 12 h from 08.00 h every day. One group of rats was subjected to bilateral electrolytic lesions of the SCN, as described previously[1]. The other (control) group was subjected to the same operation but with an anodal current as a sham operation.

6 weeks after the operation, the oxygen consumption and the RQ of these animals were determined using an open respiratory chamber. This chamber was a plastic cylinder of 16 cm diameter and 35 cm length floored with a stainless wire mesh (5 cm in height). Cups of food and water were placed on the wire mesh. Air was drawn through the respiratory chamber at a flow rate of 60 l/h by a pump and the concentrations of oxygen and carbon dioxide were measured with a magnetic oxygen analyzer (MAG-2, Shimadzu Seisakusho Ltd, Kyoto) and an IR gas analyzer (URA-2S, Shimadzu Seisakusho, Ltd, Kyoto), respectively. These analyzers were calibrated with a gas mixture containing O_2 (20%) and CO_2 (1.8%) in N_2 and N_2 (100%). The concentrations of O_2 and CO_2 in gas entering and leaving the respiratory chamber were detremined at intervals of 30 min and the RQ was calculated from the differences between the values. Statistical analyses were done by analysis of variance (ANOVA) and Student's t-test. At the end of the experiment, the animals were given an overdose of sodium pentobarbital, and then perfused with 10% formalin. After fixation, the brain was examined histologically to determine the sites of lesions by the method of Wolf[4], and data on rats with incomplete bilateral lesions of the SCN were discarded.

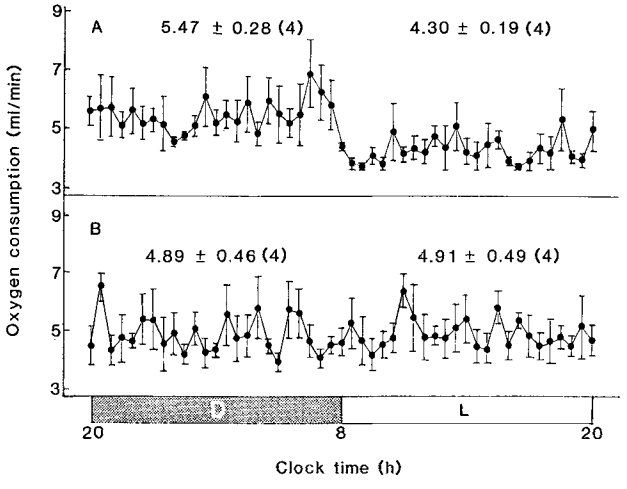

Figure 1. Changes in oxygen consumption of sham-operated (A) and SCN-lesioned rats (B). Data are means ± SEM for O_2 consumption (ml/min STPD) in four sham-operated and four SCN-lesioned rats recorded at 30-min intervals. Numbers are means ± SEM for O_2 consumptions during the 12-hour dark and light periods. The daily change in O_2 consumption in sham-operated rats was statistically significant ($F = 1.468$, $p < 0.05$ by ANOVA). D and L, dark and light period, respectively.

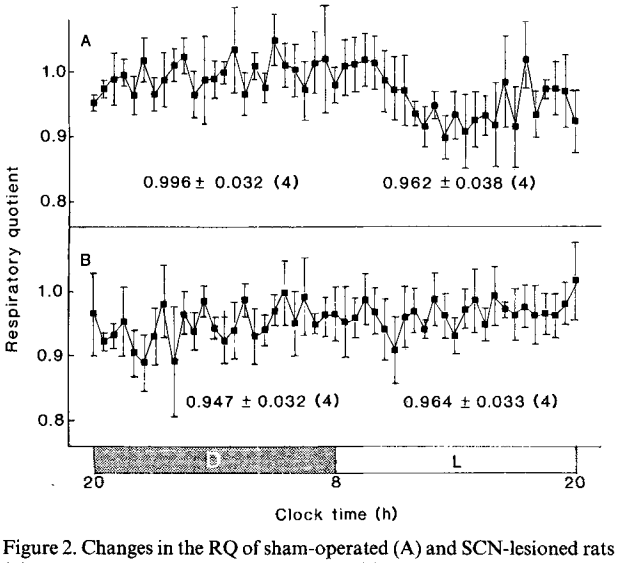

Figure 2. Changes in the RQ of sham-operated (A) and SCN-lesioned rats (B). Data are means ± SEM for the RQs of four sham-operated and four SCN-lesioned rats. Numbers are means ± SEM for the RQs during 12-h dark and light periods. In sham-operated rats, the RQ value at 12.00 h was significantly ($p < 0.05$) different from that at 02.30 h or 04.00 h by the t-test. Other explanations are as for figure 1.

Results and discussion. Figures 1 and 2 show the circadian patterns of O_2 consumption (ml/min STPD) and the RQ, respectively. Data are shown as means SEMs for four sham-operated rats (figs 1A and 2A) and four rats with SCN lesions (figs 1B and 2B). In sham-operated rats, O_2 consumption was higher in the 12-h dark period (5.47 ± 0.28) than in the 12-h light period (4.30 ± 0.19) (fig. 1A). The daily change in O_2 consumption was statistically significant ($p < 0.05$) by ANOVA, and the difference between the mean values in the dark and light periods was also statistically significant ($p < 0.05$) by the t-test. The RQ of sham-operated rats was also higher in the dark period (0.996 ± 0.032) than in the light period (0.962 ± 0.038), though its decrease at the beginning of the light period and increase before the dark period were not so steep as those of O_2 consumption (fig. 2A). The daily change in RQ was not significant by ANOVA, but the differences between the RQ values at 12.00 h and 02.30 h and at 12.00 h and 04.00 h were significant ($p < 0.05$) by the t-test.

In rats with SCN lesions, the level of O_2 consumption and RQ remained at intermediate values between the maxima and minima of those of sham-operated rats throughout 24 h (figs 1B and 2B). The regions of SCN lesions common to all four rats and the surrounding areas with lesions in some rats are shown in figure 3. The lesions common to all four rats included the bilateral SCN, and parts of the periventricular nucleus and anterior hypothalamus, but not the ventromedial hypothalamus (VMH) or lateral hypothalamus (LH). These findings suggest that the SCN is essential for the generation of daily rhythms of O_2 consumption and RQ.

As already reported, the locomotive activity of rats shows a circadian rhythm and the rhythm is abolished by bilateral lesions of the SCN[2,5]. Thus the disappearance of the circadian rhythms

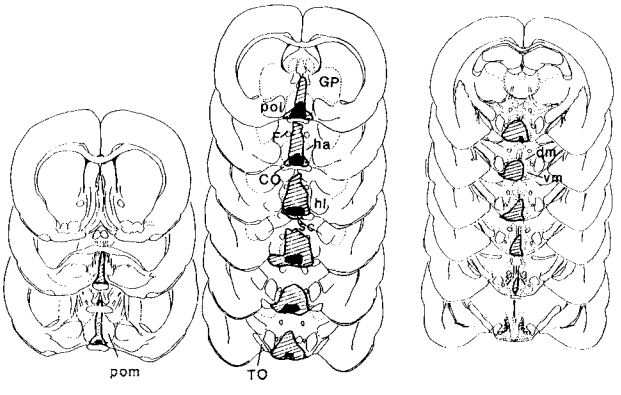

Figure 3. Brain lesions in the four rats. The lesions common to all four rats and those in some rats are shown by black and shaded areas, respectively. Abbreviations: GP, globus pallidus; pol, lateral preoptic nucleus; pom, medial preoptic nucleus; F, fornix; ha, anterior hypothalamic nucleus; CO, optic chiasma; hl, lateral hypothalamus; sc, suprachiasmatic nucleus; TO, optic tract; dm, dorsomedial nucleus; vm, ventromedial nucleus. The map of the brain is a modification of that of König and Klippel[18].

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