ganglia (n=4) and 2.0±0.1 μg/g in the superior cervical ganglia (n=5), and noradrenaline to 38±3.0 ng/g and 7.7±0.3 μg/g, respectively. The adrenaline content was not significant in either ganglion. Examination of sections for formaldehyde-induced fluorescence revealed that nodose ganglia except for SIF-cells and greenish fluorescent axons (possible source of noradrenaline) around the blood vessels and among the ganglion cells, showed no specific fluorescence, whereas superior cervical ganglion cells exhibited a characteristic greenish fluorescence. SIF-cells in both ganglia were often seen in close apposition to blood vessels. The number of SIF-cells in the nodose ganglion was, however, much smaller than in the superior cervical ganglion. It was noted that the SIF-cells in the nodose ganglion were devoid of any prominent processes protruding from their somata, whereas single SIF-cells with a process were often observed in the superior cervical ganglion. Microspectrofluorometrically, the excitation peak of a SIF-cell cluster (7 cells) in the nodose ganglion was 420 nm with a secondary peak at 325 nm, and the peak of emission was 470–490 nm (fig. A). After a short exposure (60 sec) of the section containing the SIF-cells to HCl vapor, the excitation spectrum peak shifted to 370–380 nm. This peak persisted during continued exposure to HCl vapor (10 min) (fig. A). The shift in the excitation spectrum of the SIF-cells agrees with that found for the fluorophore of authentic dopamine[5].

As shown in the figure B, the SIF-cell in the superior cervical ganglion, which contains dopamine[10], showed similar excitation characteristics, whereas the excitation spectrum maximum of the sympathetic ganglion cells in the same section, which contain noradrenaline[10], was changed from the original 420 nm over 370 nm to 320 nm with increasing exposure to HCl vapor.

The observed low concentrations of catecholamines in the nodose ganglion as compared to those in the superior cervical ganglion is in keeping with the histofluorescent finding that the number of catecholamine-containing elements such as SIF-cells and adrenergic terminals was much smaller in the nodose ganglion[4] than in the superior cervical ganglion[10]. In the nodose ganglion, the dopamine content was higher than that of noradrenaline. This suggests that certain dopaminergic elements exist independently from noradrenergic elements in the ganglion. Indeed, the close similarity in excitation spectrum between the SIF-cells in the nodose and the superior cervical ganglion indicates that the SIF-cells of the nodose ganglion store dopamine exclusively.

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Calcification of the deep zone in pig femoral head cartilage[1]

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Summary. X-Ray diffraction shows an almost random arrangement of collagen fibrils in the region of uncalcified pig femoral head cartilage furthest from the articular surface. The characteristic radial orientation of the deep zone of articular cartilage is revealed in the underlying tissue after decalcification.

X-Ray diffraction shows that the deep zone of articular cartilage coating the femoral head may be masked by calcification. It is generally accepted that uncalcified articular cartilage consists of three zones: a) in the surface zone collagen fibrils are oriented roughly parallel to the articular surface, b) in the transition zone there is nearly random fibril orientation and c) in the deep zone fibrils tend to be oriented radially i.e. perpendicular to their orientation near the surface[2]. We have used X-ray diffraction to confirm this pattern of fibril orientations in adult human patellar cartilage; the zones gradually merge into each other without discontinuity[3]. (The advantages of X-ray diffraction for this purpose are discussed elsewhere[3,4].) Radial orientation was observed in the deep zone of human and dog femoral head cartilage in the SEM by McCall[5] and by Speer and Dahners[6]. Using the same technique Clarke[7] observed a random orientation in human tissue. Random orientation was also observed in the deep zone of human and mouse femoral head cartilage in the TEM[8,9].

We have measured the orientation of collagen fibrils as a function of depth from the articular surface in femoral head cartilage of an adult pig (fully grown Göttingen minipig, less than 1-year-old). Split lines were made to indicate the preferred alignment of collagen fibrils in the plane of the articular surface[10].

The femoral head was sliced in two along a line passing mediolaterally through the fovea, a block was cut from the most superior aspect and fixed in formol saline (9.5 g NaCl in 900 cm^3 distilled water and 100 cm^3 of formalin); fixation in formol saline does not appear to affect the

orientations of the collagen fibrils in connective tissue matrices[11]. This block whose cross-section was a square of side 1 mm and whose height contained the uncalcified and some calcified tissue contained a split line on its upper surface; the total height of uncalcified tissue in the block was about 0.6 mm. X-Ray diffraction patterns were recorded at various depths from the articular surface with the beam perpendicular to the split line direction and parallel to the plane of the articular surface. At no time was the cartilage allowed to dehydrate. Each pattern yielded the direction of preferred orientation and the probability of finding a fibril at an angle to this direction for the tissue site from which it was recorded. (Further details of the experimental method and the interpretation of the results are given elsewhere[3,4].) When patterns had been recorded from the uncalcified cartilage the underlying tissue was decalcified with trimethylammonium EDTA (0.2 M in 80% v/v aqueous ethanol)[12]. Further X-ray diffraction patterns were then recorded from this artificially decalcified tissue.

Direction of preferred orientation, ϕ_0, and the spread, $\Delta\phi$, of collagen fibrils about this direction as a function of depth from the articular surface

	Depth (μm)	ϕ_0	$\Delta\phi$
Uncalcified	100	−81°	60°
Uncalcified	300	−90°	74°
Uncalcified	500	−77°	72°
Calcified	600	10°	76°
Calcified	700	10°	56°
Calcified	800	10°	42°

ϕ_0 is measured with respect to the normal to the cartilage surface. X-Ray diffraction yields an orientation distribution function, $g(\phi)$, which represents the probability of finding a fibril at an angle ϕ to ϕ_0[4]. $\Delta\phi$ is the width of $g(\phi)$ at half its maximum height.

The deep zone of the pig femoral head cartilage was calcified so that the characteristic radial orientation of its collagen fibrils was not detected until the tissue was decalcified. In our specimen the surface zone was about 0.3 mm thick and the transition zone occupied the remainder of the depth of uncalcified tissue. After decalcification the radial orientation of the deep zone was revealed below the transition zone. Further details are given in the table. We believe that our results explain the conflicting observations on the structure of the deep zone of femoral head cartilage obtained by electron microscopy. When the deep zone is calcified, sections of cartilage cut for electron microscopy will reveal only the nearly random fibril orientation of the intermediate zone above the underlying calcified tissue.

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Circadian rhythm in the subcommissural organ of the frog, *Rana arvalis* Nilsson, under natural conditions

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Summary. A circadian activity of SCO ependymal cells, judged by changes in the nuclear volume, has been found in juvenile frogs *(Rana arvalis)* under natural summer conditions. The nuclear volume reaches its maximum at 12.00 h and a minimum at 24.00 h. A significant increase in activity occurs between 06.00 and 09.00 h and a gradual decrease is observed from 12.00 to 24.00 h.

The subcommissural organ (SCO) is generally regarded as an independent structural entity with its own function. It is believed that its secretion takes place mainly in the ependymal cells and functions in the control of water-salt balance; it may be involved in the regulation of the composition of the cerebrospinal fluid. It would thus be an auxiliary neurosecretory system more primitive in character than the hypothalamic system[1-7].

In some vertebrates the secretory activity of the SCO seems to show annual variations[8,9]. Also, the influence of light and darkness as well as temperature on the secretion of the SCO was studied[10-12]. The object of the present work was to discover possible changes in the activity of the SCO throughout the 24-h rhythm in free-living frogs.

Material and methods. The work was performed on 56 sexually immature frogs, *Rana arvalis*, measuring approximately 20-25 mm. The animals were caught in their natural habitat (Giby near Suwatki in northern Poland, 21-22 July) every 3 h during a 24-h period and were sacrificed immediately thereafter. Following decapitation after capture, the heads were fixed by immersion in Bouin's fluid and then, after 24 h, transferred to 75% alcohol. In the laboratory they were dehydrated and embedded in paraffin. Serial sagittal sections, 7 μm thick, were prepared and stained with Gomori's chrome-alum-haematoxylin-phloxin method. The longest and shortest nuclear diameters of 50 SCO ependymal cells from each brain were determined with an ocular micrometer and the volume of each nucleus was calculated. Statistical analysis of the differences between the mean values for the consecutive times were determined by Student's t-test. A probability (p) value of 0.05 or less was considered as being significant.

Results and discussion. A change in the nuclear volume of a secretory cell may be considered as a sign of a change in the secretory activity of that cell. It has been confirmed many times[13,14] that an increase in the nuclear volume is associated with increased activity, while a decrease corresponds to diminished activity. In the course of the present investiga-