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-

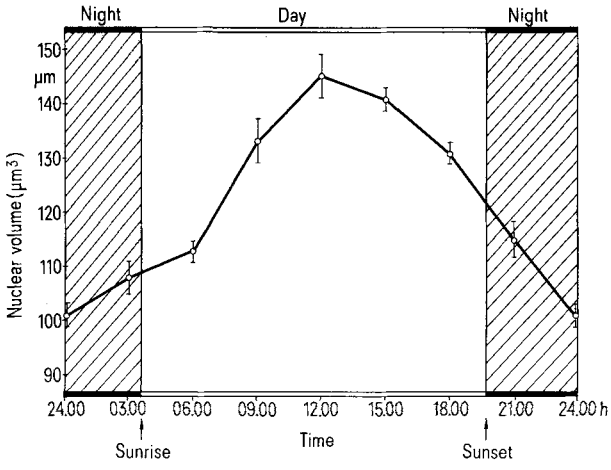

Figure 1. Circadian variations in nuclear volume of summer SCO ependymal cells of juvenile frogs *Rana arvalis*. 7 animals were sampled every 3 h. Each point corresponds to the mean for a given time; vertical bar is standard error (SE). Changes in nuclear volume of cells between 06.00 and 09.00, 09.00 and 12.00, 15.00 and 18.00, 18.00 and 21.00, 21.00 and 24.00 h were significant of the difference at $p < 0.05$.

tion it was shown that the minimum activity of SCO cells occurred at 24.00 h and the maximum at 12.00 h (figs. 1 and 2). The increase of nuclear volume starts from midnight, but up to 06.00 h is of minor importance. The greatest change in the activity of SCO appears between 06.00 and 09.00 h, reaching a peak at 12.00 h (145.25 µm³). Afterwards, there is a gradual reduction in nuclear size to 100.40 µm³ at 24.00 h. These results are in harmony with the biological rhythm of *Rana arvalis*. This frog is believed to have a typical diurnal activity. It is well known that the metabolic activity in poikilothermic animals depends on environmental temperature and is reflected in the central nervous system. Increase in ambient temperature stimulates the secretory activity of the SCO, at least as far as the production of Reissner's fiber (RF) is concerned[12]. These data agree with my finding of a rapid rise in the nuclear volume with temperature increase between 06.00 and 09.00 h. On the other hand, the exposure of frogs *(Rana temporaria)* to room temperature during 3–4 weeks in February did not influence the SCO[15]. The secretory substance released by the SCO cells develops into a thick fiber (RF) slowly moving through the 3rd ventricle and the central canal of the spinal cord in a caudal direction[5]. Changes in metabolic activity may cause changes in the composition of the cerebrospinal fluid.

There is a possible functional connection between the SCO and the preoptic nucleus. The activity of SCO stimulated by the stress of cold develops parallel with depression in the activity of the supraoptic nucleus of rat hypothalamus, which is engaged in the regulation of thermogenesis. This lower activity suggests a correlative blockade of secretion of the antidiuretic hormone under the influence of cold[16]. Some investigations were performed on the cyclic changes in the nucleus preopticus in adult *Rana esculenta* in the course of the year. The greatest volume of cell nuclei in females was recorded during the first 10 days of April, which is probably due to a significant increase in metabolism after the animals have left their winter lairs. In males, however, the maximum increase in nuclear volume was found in the last 10 days of January[17], and this fact is enigmatic. These considerations suggest the influence of not only environmental factors but also the parallel presence of endogenous rhythms connected with the seasonal and 24-h life cycle. Investigations which may contribute to

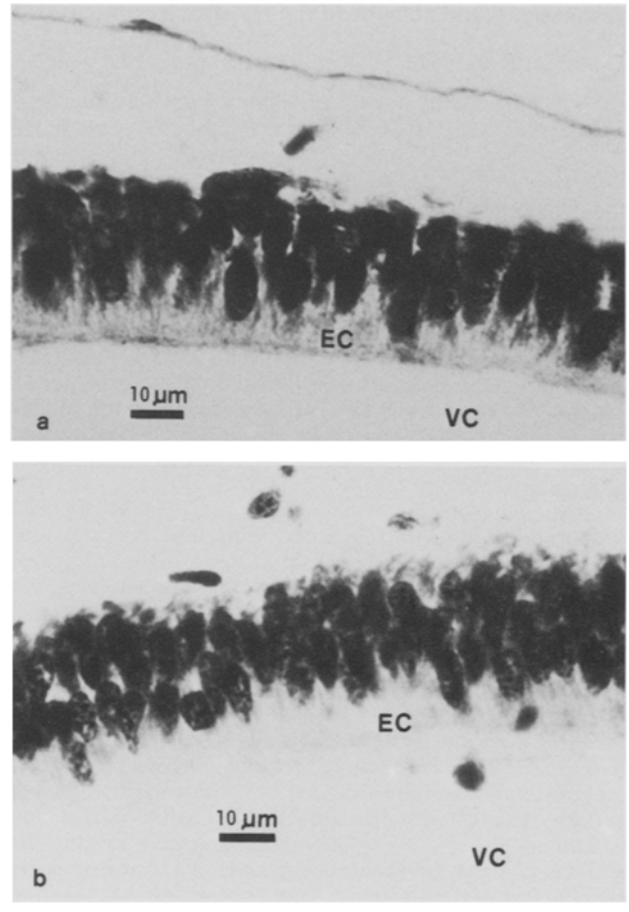

Figure 2. Sagittal section of the SCO of *Rana arvalis* stain with chrome-alum-haematoxylin-phloxin. *a* 12.00 h, *b* 24.00 h; EC, ependymal cells of SCO; VC, ventricular cavity.

an understanding of the circadian activity in the nucleus preopticus in relation to the SCO are in progress.

It has been found in *Rana temporaria* and *R. esculenta* that light/darkness conditions only slightly influence the secretory activity of the SCO cells, but the possible biological significance of this minor effect of light and darkness on the SCO remains totally unknown[10].

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