Results and discussion. The results obtained with the group of oxytocin analogues are given in Table I and can be summarized as follows: (1) The kidney homogenate inactivated all three peptides (rate differences were not great between groups) and the inactivation capacity was somewhat increased after dialysis. (2) DCOT was not split by cytoplasm, inactivation of DOT decreased markedly after dialysis of the cytoplasmatic fraction. Since both analogues differ only in the type of bridge which can undergo reduction in DOT but not in DCOT, we may assume the existence of an inactivation via reduction of the S-S-bond. In this cell fraction oxytocin is inactivated apparently by enzymatic systems other than DOT as can be concluded with regard to the effect of dialysis on the inactivation of both peptides. (3) The inactivation of oxytocin by microsomal fraction is decreased after the dialysis in contrast to the inactivation of DOT and DCOT.

The results obtained with the vasopressin group are shown in Table II. Both, the homogenate and microsomes and the cytoplasm split DAVP and DLVP at the same rate as their corresponding L-forms. There were also insignificant differences between dialysed and nondialysed preparations.

As shown by pharmacokinetic evaluation, elimination of analogues in the target organ does not markedly differ from that of the maternal hormone; using the antidiuretic test[13] we found an index of persistence[14,15] for all of these substances equal approximately to unity when doses producing a 10- to 35-min anuria were assayed. None of the above analogues of oxytocin had a protracted uterotonic activity.

These results suggest the following: (1) A two-stage mechanism of inactivation (reduction and aminopeptidase splitting) can be localized in the cytoplasm of kidney cells. (2) In the microsomal fraction there is apparently a combined action of undefined endo- and aminopeptidases (the dialysis removes a low molecular weight cofactor of one system, as well as a dialyzable inhibitor of the other one). (3) A decisive action of trypsin-like enzymes in inactivation of vasopressin can probably be excluded. The physiological significance of these conclusions is uncertain, since it is not known whether neurohypophysial hormones penetrate through the cell membrane into the cytoplasm or into the endoplasmatic reticulum.

Table II. Inactivation of vasopressin analogues by kidney cell particles

Substance	Hydrolytic rate constant[a] ($sec^{-1} \times mg\ protein^{-1} \times 10^{-3}$)					
	Homogenate		Microsomes		Cytoplasm	
	N	D	N	D	N	D
[L-Lys^8]-Vasopressin	3.4	11.0	25.0	25.4	9.7	16.5
[D-Lys^8]-Vasopressin	10.4	9.4	19.9	9.9	19.6	21.7
[L-Arg^8]-Vasopressin	10.4	11.1	28.2	25.4	19.6	12.5
[D-Arg^8]-Vasopressin	7.7	11.5	45.1	40.7	20.3	15.0

[a] Explanation see Table I.

Zusammenfassung. Die Inaktivierung von Carba-, Deamino- und 8-D-substituierten Analogen der neurohypophysäuren Hormone durch Schweinenierenhomogenate verläuft mit der gleichen Geschwindigkeit wie die des Oxytocins und Vasopressins.

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Behaviour of Glycogen and Related Enzymes in the Sertoli Cell Syndrome

The presence and the behaviour of glycogen and of 1–4 amylophosphorylase (1–4 AP) in human testis under normal and pathological conditions has already been described in an earlier report[1,2]. There is evidence that intratubular glycogen represents an important source of energy for spermatogenesis[3–9] whereas the presence of this substance in the cytoplasm of some peritubular cells (muscle cells, Ross[10]) seems to demonstrate that glycogen is also involved in tubular motility[11].

Among the pathological conditions of the human testis, the 'germinal aplasia' or 'Sertoli cell Syndrome'[12] seems to be characterized from a histochemical point of view, by the scarcity or absence of intratubular glycogen and 1–4 AP, and by the large amounts of both substances in the peritubular zone[1]. The present paper reports the results of further investigations on histochemical behaviour of glycogen and the 1–4 AP in this particular condition.

Material and methods. Bioptic specimens were obtained from testes of 14 subjects who were found to be affected by complete absence of seminiferous epithelium (Sertoli cell syndrome). Eight of them gave no history of exogenous

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or endogenous factors of testicular morbility and therefore were considered as being affected by 'true germinal aplasia'; on the other hand, prolonged X-ray treatment on the scrotal region for eczema was recorded in the past history of the other 6 patients; these were defined as suffering from 'acquired germinal aplasia'. A part of each fragment was fixed in Bouin, submitted to the standard histological procedure and stained with PAS-Haematoxylin, whereas control slices were incubated with α-amylase prior to staining. The remaining part of each specimen was frozen at − 20 °C; cryostat sections of 16 μ were treated according to the technique described by TAKEUCHI et al.[13,14] and reported in a previous paper[1].

Results. 'True germinal aplasia'. The histochemical examination showed no interstitial or vascular changes in these cases. In 2 cases a Leydig cell hyperplasia was evident. The germinal epithelium was completely absent; in only one case were glycogen and 1–4 AP histochemically detectable. On the contrary, strong positivity for both substances was observed in the area corresponding to the peritubular zone in which the muscle cells are situated (Figure 1).

'Acquired germinal aplasia'. Histological patterns in this group of patients did not differ from the picture found in the true germinal aplasia. In 3 cases vascular changes (arteriosclerosis) were evident. Glycogen was present in variable quantity within the cytoplasm of the Sertoli cells, some of which appeared full of this substance. The method for 1–4 AP gave medium to high intratubular positivity. The content of this enzyme of the peritubular elements appeared to be normal (Figure 2).

Discussion. No morphological differences have so far been reported between idiopathic and acquired Sertoli cell syndrome[15,16]. In the opinion of some authors[17], the existence of 'true germinal aplasia' has not yet been established and the past history is still considered the only valid element for the differentiation between the 2 forms. The results of the present study, however, would seem to demonstrate that the histochemical behaviour of Sertoli cell syndrome is not constant in all cases. In the above patients, with the exception of one case, a relationship was found between the clinical and histochemical data. The scarce amount or the absence of intratubular glycogen and 1–4 AP in the cases who can clini-

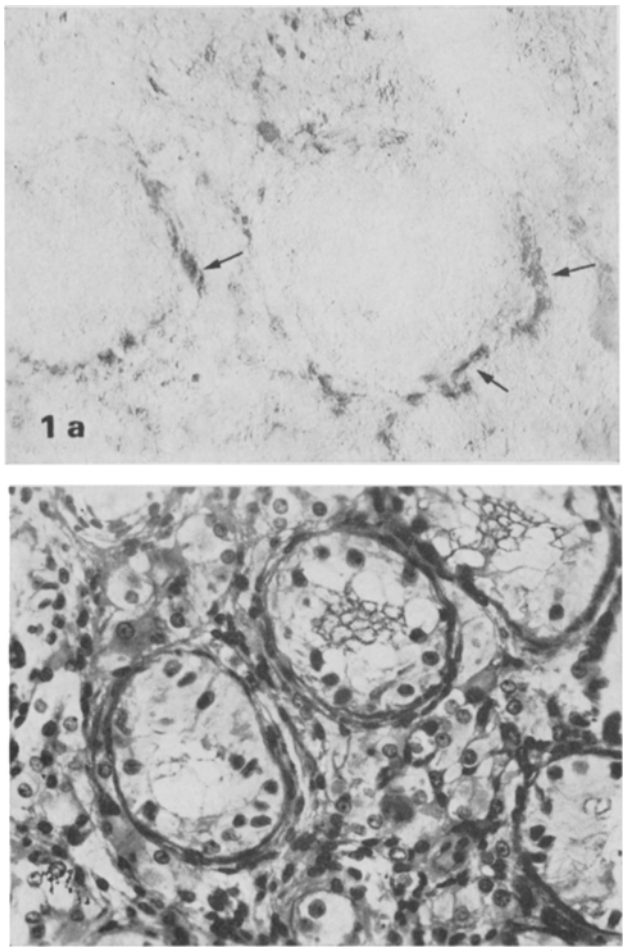

Fig. 1. Testis of a patient affected by 'true germinal aplasia'. (a) Negativity of 1–4 AP in the tubular epithelium, whereas positivity of peritubular cells can be observed (arrows); (b) histological pattern of the same case. Note the scarcity of sertolian glycogen. PAS staining. × 140.

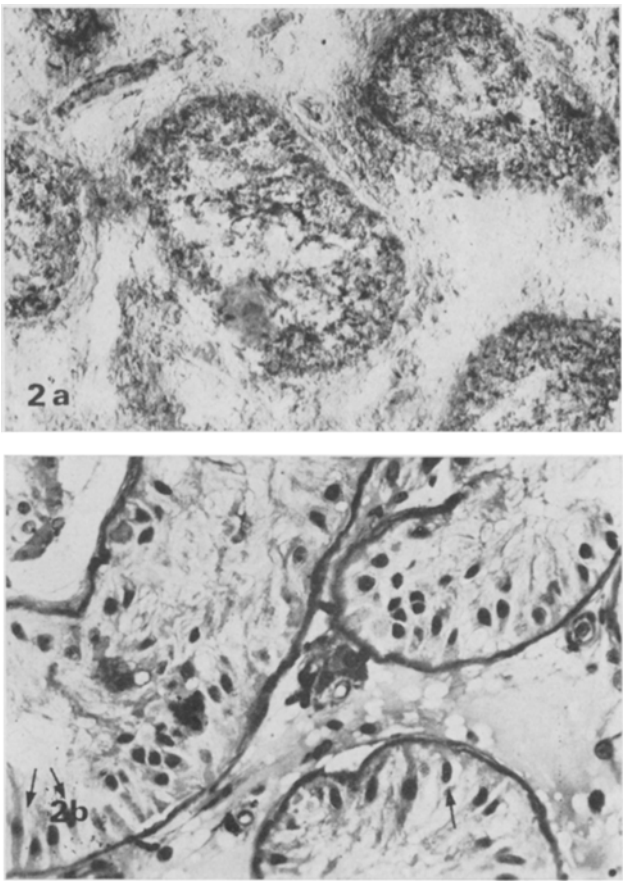

Fig. 2. Testis of a patient affected by 'acquired germinal aplasia'. (a) Strong intratubular positivity of 1–4 AP; (b) histological pattern of the same case. Note the variable amount of sertolian glycogen (arrows). PAS staining. × 140.

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cally be classified as 'idiopathic' may indicate that Sertoli cells are congenitally lacking in normal content of energetic substances and related enzymes; on the other hand, the presence and distribution of glycogen and 1–4 AP in the 'acquired' case may represent a condition similar to the normal Sertoli cell. The large amount of 1–4 AP, in correspondence to the peritubular muscle cells found in the 'true aplasia', may be related to lack of utilization of glycogen, caused by the absence of the tubular motility, which probably plays a role in the spermatogenesis[18].

Riassunto. Nella «sindrome da sole cellule di Sertoli congenita o idiopatica» (aplasia germinale vera) la 1–4 AP, ricercata con metodi istochimici, risulta assente in corrispondenza dell'epitelio del tubulo seminifero e si presenta in quantità apprezzabile in sede peritubulare (cellule muscolari). Nella «sindrome da sole cellule di Sertoli acquisita», conseguente a trattamento radiante, il comportamento della 1–4 AP è del tutto simile a quello del testicolo normale. Il metodo appare pertanto utile per la diagnosi differenziale fra le due forme, su base istochimica.

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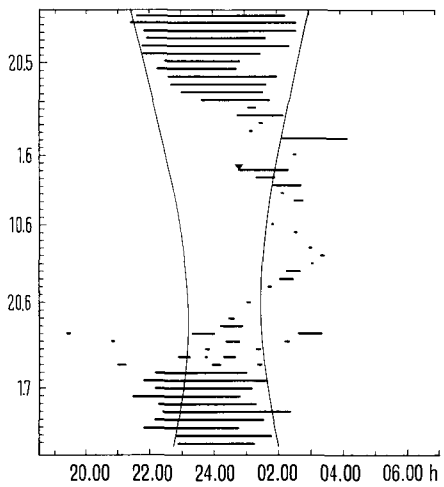
Free-Running Circadian Rhythm in Wood Mouse (*Apodemus flavicollis* Melch.) under Natural Light-Dark-Cycle

Under natural conditions, circadian rhythms are always influenced by periodically changing circumstances and therefore not free-running. For mammals and birds at least, light is the most important 'Zeitgeber', which develops the spontaneous rhythm to exactly 24 h. The locomotory activity of the wood mouse (*Apodemus flavicollis* Melch.) was registered continuously in the running wheel in Oulu (Finland) (65°1′ N, 25°28′ E). The mouse was in an isolated room with naturally fluctuating temperature and with a window looking NW.

From 20.5 to 1.6 the mouse ran with the period $\tau = 24.4$ h with reduced activity. In the last 2 of these days the beginning of activity took place after sunrise. On 3.6. the mouse was disturbed by feeding, and after a phase-shift it ran again with the same length of period, $\tau = 24.4$ h, as before, until 7.6. The last 3 beginnings of activity again took place after sunrise. After 2 days, with completely reduced activity and after a new phase-shift it ran 5 days again with $\tau = 24.4$. The beginning of activity took place during these 5 days always after sunrise: on 10.6. 7 min and on 14.6. 1 h 46 min after sunrise. Thereafter, although the nights were growing still shorter and average light-intensity of the day was higher, the circadian rhythm became shorter ($\tau = 23.6$) in the time of 14..6 to 30.6., after which it was again synchronized to 24 h by means of natural light-dark-cycle. One special feature was that the resynchronization took a long time, more than a fortnight.

The natural light-dark-cycle has a rather constant range of oscillation, but towards the north and south pole it changes greatly according to the season. In the polar regions the range of oscillation is much smaller in winter and summer than in spring and autumn. Although Oulu is situated 172 km south of the Arctic circle, the light intensity, on the bright nights in the beginning of June, is at midnight 50 lux and in midsummer 100 lux according to my measurements. Because the minimum value of the light intensity in the night is so great, the range of oscillation becomes smaller and also the 'Zeitgeber' weaker. On the other hand, it is known that if the 2 light intensities have the same duration (in nature LD 12:12), the weak 'Zeitgeber' is strongest, but the more the LD-ratio deviates from this, the weaker becomes the ‚Zeitgeber.' And lastly if (as in this case) dark-time becomes too short, the range of development is exceeded and the biological oscillation is no longer synchronized, in spite of the 24 h 'Zeitgeber' periodicity[1]. Because the range of forcing oscillation became small owing to the great light intensity in the night, and, at the same time, the LD-ratio grew to its greatest value 21.7:2.3, the 'Zeitgeber' was too weak to synchronize the locomotory activity of the wood mouse.



The activity of a wood mouse in the summer 1967. Horizontal bars, time when the mouse was active; black triangle, feeding on 3.6. The vertical curves show sunset and sunrise.

Zusammenfassung. Bei Versuchen mit Waldmäusen (*Apodemus flavicollis* Melch.) wurde im Sommer 1967 172 km südlich des Polarkreises (kürzeste Zeit zwischen Sonnenuntergang und -aufgang 2,3 h, Beleuchtungsstärke um Mitternacht bis 100 Lux) freilaufende zirkadiane Periodik gemessen.

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