differences between the olfactory mucosa of the two strains with respect to mucus composition, mucus thickness, receptor density and receptor type. These differences may influence the ability of the odorants to stimulate the epithelium (there is no evidence to support this) and the effectiveness of the concanavalin A superfusion. Consistent with this notion is the observation that 10 times as much concanavalin A is required to selectively reduce EOG's in the frog[18]. This may be explained by the presence of a thicker layer of mucus overlying the frog olfactory epithelium than is seen in the rat[25].

Alpha-methyl-D-mannoside protection of EOGs. The results of the mannoside protection experiment seen in figure 4 show that concanavalin A modification of the olfactory response to all three odorants could be prevented by competing for the sugar residue binding site on the concanavalin A molecule with mannoside.

One-way analysis of variance showed for each odour that there was a difference in the R values for the three treatments on the Wistar rat (cineole and i-pentanoic acid $p < 0.001$, nicotine $0.01 > p > 0.001$). To identify which of the treatments contributed most to this difference, the control R values were compared with R values for concanavalin A and R values for concanavalin A and mannoside, using the Dunnett test for multiple comparisons to a control group. The results of this test showed that for each odour, only the concanavalin A treatment R values were significantly different from the control R values (cineole, nicotine and i-pentanoic acid, $p < 0.01$).

Treatment of the epithelium with mannoside after a concanavalin A superfusion does not reverse the concanavalin effect[15], but the EOGs which were protected in the concanavalin A and mannoside treatment in this study, could be reduced by subsequent treatment with concanavalin A alone ($n = 3$). These observations suggest that alpha-methyl-D-mannoside is binding to the concanavalin A molecule's sugar residue binding-site, preventing modification of the EOGs. This is evidence that the olfactory receptors which respond to cineole, nicotine and i-pentanoic acid are glycosylated and/or are close to a portion of sensory membrane which is glycosylated. Other workers have shown that there are glycoproteins unique to sensory cilia and have suggested that these proteins play a role in olfactory reception[26].

Nicotine is the key chemical found in the smoke from cigarettes and other tobacco products. We have shown that nicotine acts as an odorant in addition to its well-known role as a pharmacological agent. It is of interest that in human experiments (unpublished) subjects could smell the pure nicotine used in this work. We can speculate that other pharmacologically active odorants exist.

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Short Communications

Identification of a pre-hibernating state in myocardium from nonhibernating chipmunks

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Summary. During the hibernating season, the amplitude of the cardiac action potential plateau of nonhibernating chipmunks was reduced. Replacing external Ca by Sr inhibited the electromechanical responses of these preparations. Similar properties were observed in hibernating animal preparations, suggesting that changes in cardiac function are already triggered before hibernation begins.

Key words. Pre-hibernating state; hibernating and nonhibernating seasons; chipmunks myocardium; strontium; plateau potential.

Recently, it has been reported that the amplitude of the action potential plateau of cardiac muscle from chipmunks is reduced in the hibernating state[1]. This reduction has been shown to be due to a reduced contribution of the slow inward

current to the electrical response of caridac muscle[2,3]. This difference between nonhibernating and hibernating animals was observed simply by replacing external Ca by Sr[3]. Sr, which can permeate the slow Ca channels as a charge carrier of the slow inward current[4,5], will still sustain an action potential plateau in cardiac muscle from nonhibernating animals, but not from hibernating animals. It is suggested that this procedure might be an useful tool for identifying the cardiac responsiveness seen in the hibernating state. By using this feature, the seasonal variations in the electromechanical responses in nonhibernating animals were investigated. The present findings suggest that during the hibernating season, pre-hibernation changes in cardiac function have already been induced even in nonhibernating animals. This finding may play an important role in the study of the induction and the regulation of the hibernating state.

Materials and methods. Asian chipmunks *(Tamias sibiricus)* of either sex were trapped in April or in October, 1984, and kept in two separate groups throughout. Most of the animals in both groups were maintained at 25 °C and used for experiments on nonhibernating preparations. The rest were kept in the dark at 4 °C from the end of November with a standard laboratory rat chow diet and water ad libitum. Within 3 weeks most of these animals had exhibited preliminary bouts of hibernation and by the following March had exhibited several consecutive periods of hibernation longer than 1 week in duration.

Deeply-hibernating animals were used for experiments on hibernating preparations. Animals were killed by cervical dislocation. The heart was quickly excised, and a papillary muscle, 2–3 mm in length and less than 1 mm in diameter, was isolated form the right ventricle. The preparation was suspended and then equilibrated for 2 h in a tissue bath of Krebs-Ringer solution aerated with 95% O_2 and 5% CO_2. The composition of the Krebs-Ringer solution in mmoles l^{-1} was: NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7\ H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 24.2; and glucose, 5.5 (pH 7.4). In the strontium-substitution experiments, 2 mM $SrCl_2$ was used in place of the 1.2 mM $CaCl_2$. The temperature of the superfusate was maintained at 30 °C. The preparations were stimulated at 0.2 Hz with pulses 1 ms in duration and twice the diastolic threshold. The amplitude of the action potential plateau was measured by using a stimulating frequency of 1 Hz. Membrane action potentials were recorded through a glass microelectrode filled with 3 M KCl. The action potentials were displayed on a storage oscilloscope (Tektronix 7613) and the mechanical tension was recorded on a polygraph (Nihon Kohden TB612T).

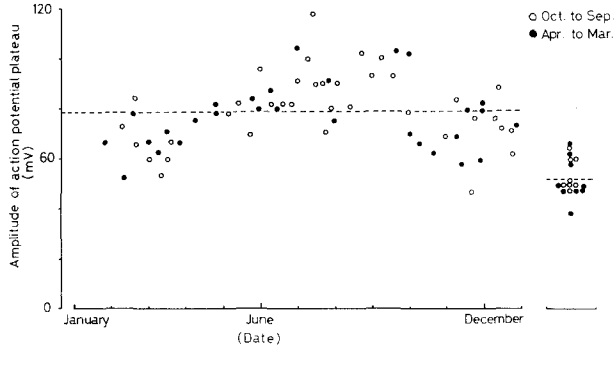

Figure 1. The seasonal variations in the amplitude of the membrane action potential plateau (mV) of myocardium from nonhibernating animals (left column) and the amplitude of the membrane action potential plateau of myocardium from hibernating animals (right column). The horizontal line indicates the date at which animals were killed. The animals in the first group (○) were killed for experiments between October in 1984 and the next September, and those in the second group (●) were killed between April in 1985 and the next March. Hibernating animals were killed for experiments between February and March. The broken line indicates the mean value. All preparations were driven at 1 Hz.

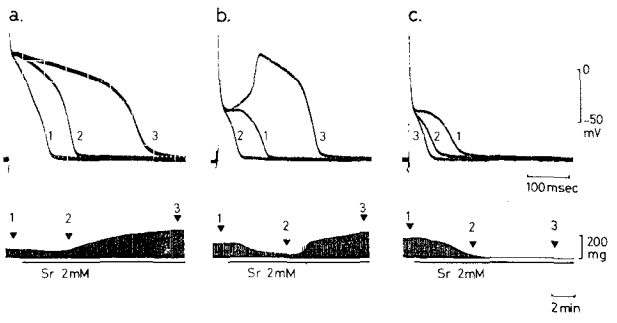

Figure 2. Electromechanical effects on myocardium from nonhibernating and hibernating animals as a result of the substitution of Sr for external Ca. *a* Typical preparation from nonhibernating animals in summer; *b* typical preparation from nonhibernating animals in winter ; *c* typical preparation from hibernating animals. Superimposed tracing in upper panels shows the membrane action potential and lower trace shows a continuous record of isometric tension. Numbered arrowheads (1,2,3) indicate recording points of the respective membrane action potential shown in upper panel.

Results and discussion. Figure 1 shows the amplitude of the action potential plateau (APp) of cardiac muscles from nonhibernating and hibernating chipmunks. These data were obtained from two different groups, one (trapped in April) in which animals were killed between October and the following September and the other (trapped in October) in which animals were killed between the April of the next year and the following March. As can be seen from the figure, the amplitude of APp was smaller in those animals killed during the hibernating season (autumn, winter and early spring) than in those killed during the nonhibernating season (summer). It has been previously reported that the reduced amplitude of the APp is a characteristic feature of myocardium of hibernating animals[1-3]. The amplitude of APp in nonhibernating animals during all seasons was 78.4 ± 1.6 mV (n = 66). Most preparations obtained during the hibernating season (31 out of the 36 preparations) exhibited a reduced plateau amplitude. Furthermore, the amplitude of APp in 20 out of these 36 preparations were not significantly different from that (54.1 ± 1.7 mV, n = 17) in hibernating animals[6]. These observations suggest that during the hibernating season, the electrical properties of the myocardium of chipmunks is already altered before hibernation begins.

Substitution of Sr for the external Ca caused a marked positive inotropic effect accompanied by a prolongation of APp in nonhibernating animals during the nonhibernating season, while in hibernating animals, Sr substitution abolished both the contraction and the APp (fig. 2a, c). It may be proposed to attribute this difference to a reduction of the slow Ca inward current during the APp of hibernating animals as has been suggested earlier[2,3]. These same studies also suggested that the APp in hibernating animal preparations is mediated by some mechanism linked to internal Ca release rather than influx of external Ca.

In preparations from nonhibernating animals obtained during the hibernating season (fig. 2b), Sr substitution produced a negative inotropic effect accompanied by marked inhibition of the APp. Similar effects were observed on preparations from hibernating animals. However, prolonging the exposure to Sr increased contraction, which was accompanied by a marked augmentation of APp. An increase in either APp or contraction was blocked by nifedipine (10^{-6} M), a Ca channel blocker (data not shown), suggesting that

the delayed effects of Sr were mediated by an increase in the slow inward current carried by Sr. These results were quite consistent with the properties of nonhibernating animals during the nonhibernating season. Thus, the myocardium of nonhibernating animals obtained during the hibernating season has a dual nature, one observed in hibernating animals and another observed in nonhibernating animals obtained during the nonhibernating season. This suggests that the cardiac function is changed during the hibernating season whether the animals hibernate or not. Paradoxically, some of the preparations obtained from nonhibernating animals during the hibernating season exhibited APps with relatively high amplitudes. The electromechanical characteristics of these preparations were similar to those seen in nonhibernating animals during the nonhibernating season (fig. 2a).

Nifedipine-sensitive electromechanical responses were induced by prolonged exposure to Sr in nonhibernating animals during the hibernating season, but not in the hibernating animals. A previous study[3] indicates that the lack of effect of Sr in hibernating animals is due to the inhibition of the slow inward current by a large transient outward current which has been shown to be independent on intracellular Ca. In nonhibernating animals during the hibernating season, however, blockade of Ca-activated potassium outward current[7,8] by Sr cannot be eliminated as a possible mechanism. Although the explanation for this difference between these two preparations is not clear, it is interesting that the electrophysiological changes in the myocardium during the hibernating season may be closely correlated to an outward current which is less sensitive to Sr.

In conclusion, the electrical and mechanical characteristics of cardiac muscle seen in hibernating animals occurs, at least in part, before the animals begin hibernating; the changes in cardiac function are not simply the result of hibernation. This suggests the possibility that hibernation is induced or regulated by as yet unknown factors[9–13]. If this is so, some responsible substance(s) may be present in animals during the hibernating season. Cardiac muscle may be one of the target organs affected by such substance(s). The present findings could provide a useful model for studying the mechanism of hibernation and the existence of some hibernation trigger substance(s).

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Renal handling of bilirubin photoderivatives[1]

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Summary. The renal handling of unconjugated bilirubin in the dark and during light exposure was analyzed using an isolated rat kidney preparation. The parameters tested were pigment disappearance from the perfusion medium, pigment uptake by tissue, and its renal clearance. The results indicated that despite the fact that pigment disappearance from the medium was similar for both forms of pigment, the extraction ratio was higher for irradiated pigment than for pigment in the dark. When renal clearance of pigment was plotted vs pigment uptake of tissue, the results indicated that irradiated pigment may be more efficiently removed by the kidney. In addition, data on the rate of secretion of p-aminohippurate suggested that both pigment forms shared a common site for secretion.

Key words. Bilirubin; phototherapy; organic anion transport; renal clearance.

Since 1958, phototherapy treatment of jaundiced infants has been widely employed both to prevent and to control neonatal hyperbilirubinemia[2]. Despite its widespread use, debate continues about several important points such as the most effective light source[3,4], sites of light action[5,6], intermediates, and final products of the photochemical reaction in vivo[7–10] and excretion fate of these products[7,8,10–15].

Experiments on Gunn rats provided the first key to the chemistry of phototherapy. First, wavelength-dependence studies indicated that the photoreceptor is unconjugated bilirubin (UB) itself[10,16]. Second, excretion studies showed that the slow decline in serum UB during phototherapy is preceded by a much faster, almost instantaneous excretion of yellow pigment in bile[8,11,12]. Moreover, despite some uncertainties, most data indicate that the photoisomerization pathway is far more important quantitatively than photooxidation in human infants and rats[15,17–19].

The role of the kidney in the excretion of UB is not clear enough[20–25], and data on the participation of that organ during phototherapy are scarce[12,14,15,26–29]. The main point analyzed was the chemical structure of the yellow pigment which appeared in the urine shortly after the phototherapy[14,15,26]. However, the mechanisms involved in the renal excretion of UB photoderivatives are poorly known. Therefore, in this study, the urinary excretion rate of UB photoderivatives was analyzed using an isolated rat kidney preparation, in comparison with the excretory rate of UB not exposed to light.

Materials and methods. Animals. Male Wistar rats weighing 300–350 g were used as kidney donors for all studies. Animals were allowed free access to a standard diet and tap water until used.

Perfusion procedure and apparatus. The animals were anesthetized with sodium pentobarbital (40 mg/kg b.wt, i.p.). The right kidney was prepared as previously described[24,25]. Arterial samples were collected from a catheter inserted in the mesenteric artery, and urine samples from an ureteral catheter. Venous effluent drained into a reservoir and recirculated. The perfusion medium (pH 7.5) consisted of Krebs-