results have been so consistent over many weeks and months that they can well serve as a basis for a working hypothesis that rat pineal function exhibits a 7-day rhythmic activity.

We are aware that the postulate of a 7-day functional cycle is an unusual one. Admittedly, there is no known prominent geophysical counterpart for such a rhythm[8]. On the other hand, the results reported here do not represent the first hint for such a rhythm. Circaseptan rhythms in thermovariance[9] and 17-ketosteroid excretion[8] have been described in humans. The fact that the latter rhythm was not always synchronized with the weekly societal rhythm led to the speculation that a weekly component in physiologic function might have preceded the societal 7-day week and that the sizeable gap between circadian rhythms on the one hand and monthly and annual rhythms on the other might be reduced by about weekly rhythms and that this could facilitate the temporal integration of organisms[8].

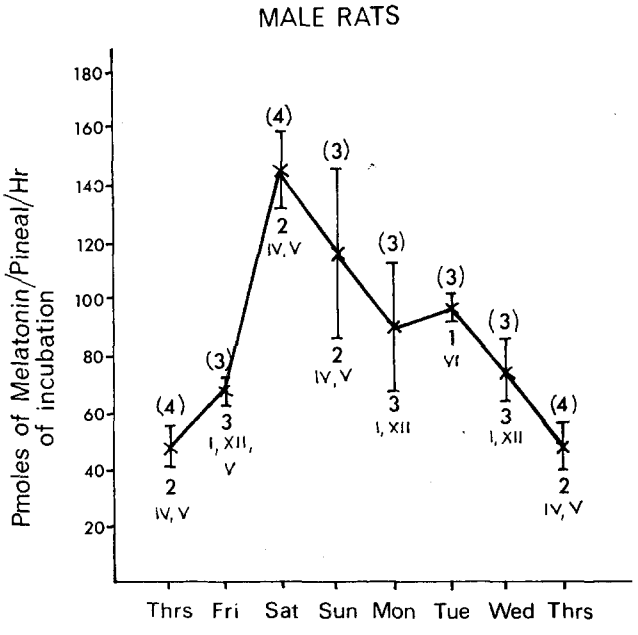

Fig. 3. HIOMT activity in pineal glands of male Wistar rats. For details see Figure 1, for the explanation of the Roman numerals Figure 2. Statistical analysis: Sat + Sun vs Wed + Thurs + Fri $p < 0.001$.

From our results we are inclined to conclude that, if the 7-day cycle in the rat pineal gland is not endogenous, it is probably the change in the environmental conditions over the weekend which has a profound and long-lasting effect on the organ. Such an interpretation is all the more likely because most pronounced changes occurred in relation to the weekend. Furthermore it is becoming increasingly apparent that factors other than environmental lighting affect pineal function as well[10,11]. Seasonal rhythms[11], the presence of β-receptors whose sensitivity undergoes characteristic changes[12] and the close association with the sympathetic nervous system[1] make the pineal an ideal target for external and internal stimuli which could easily lead to a superimposition of a 7-day functional cycle on 24 h cycles. In this context it is interesting to note that recently the HIOMT activity pattern during the estrous cycle in the rat has been described as the sum of the oscillations of at least 2 rhythms whose frequencies differ slightly[13]. Further studies are in progress to elucidate the factor(s) responsible for the 7-day cycle and to unravel its physiological significance.

Zusammenfassung. In der Zirbeldrüse männlicher und weiblicher Ratten deutet das Verhalten des an der Melatoninbildung beteiligten Enzyms Hydroxyindol-*O*-methyltransferase auf das Vorhandensein eines 7tägigen Funktionszyklus hin.

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Uncoupling of Heart Cells Produced by Intracellular Sodium Injection

It is known that the low intracellular calcium concentration in muscle cells is maintained by an active uptake by mitochondria and sarcoplasmic reticulum, and also by active extrusion of the ion through the surface cell membrane.

In other cells, like red blood cells, ATP is used directly to extrude calcium from the cell interior[1]. In excitable tissues, however, evidence has been obtained that the inward movement of Na^+ and probably the outward movement of K^+ provides energy for calcium extrusion[2,3]. In this case, the breakdown of ATP is indirectly involved, since it is essential for the maintenance of sodium gradient.

In cardiac muscle the stochiometric relationship seems to be the exchange of two sodium ions for one calcium ion[4]. Studies of BAKER and BLAUSTEIN[5], and BAKER et al.[3] showed, indeed, that a small raise in the intracellular sodium concentration of the squid axon, results in a large increase in the intracellular calcium concentration.

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According to these authors, the increment of the intracellular calcium concentration follows at least the square of the internal sodium concentration.

Calcium ions play an important role in the control of junctional permeability in epithelia[6] and in cardiac muscle[7]. Intracellular calcium injection produces electrical uncoupling in salivary glands of Chironomus[8] and in cardiac Purkinje fibres[9,10].

Considering the fact that the increase of intracellular sodium concentration causes a large increment in internal calcium content[3], we thought it is important to investigate the influence of intracellular sodium injection on electrical coupling of heart cells.

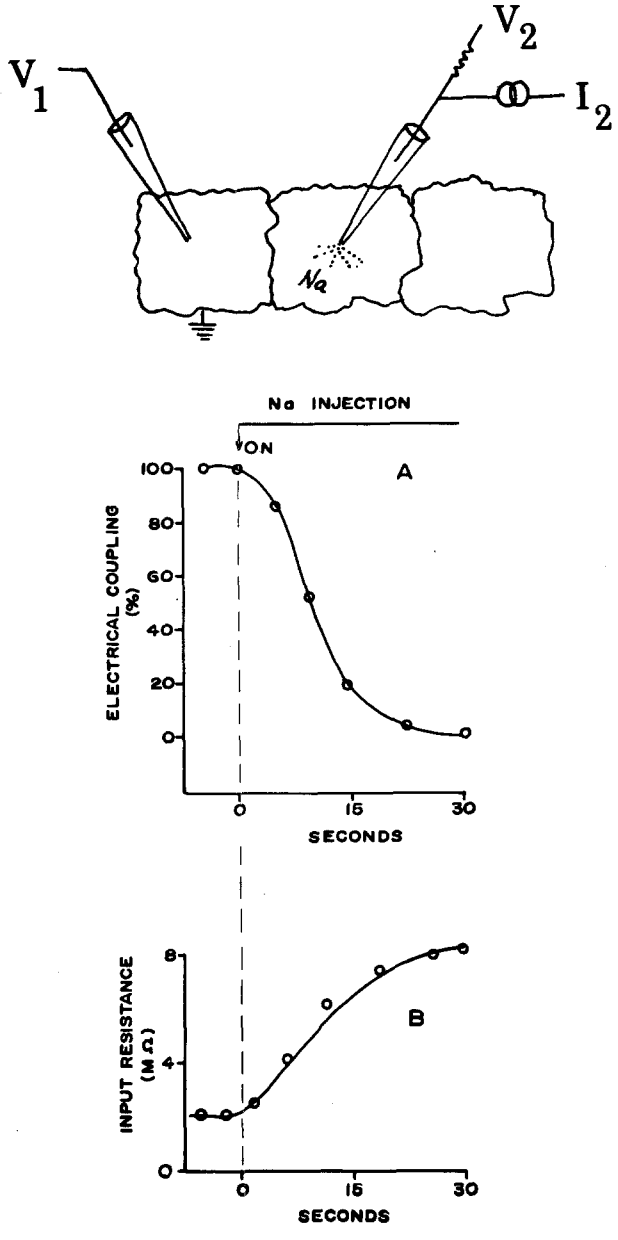

Fig. 1. Top: diagram showing arrangement for measuring input resistance and electrical coupling. In cell 2, the NaCl microelectrode was connected to a bridge circuit which was used to record voltage and pass current. A) Suppression of electrical coupling of myocardial cells produced by intracellular sodium injection. B) Increase of input resistance of the injected cell recorded simultaneously with change in intercellular communication. Temperature —36 °C.

Experiments were performed on isolated strips of rabbit atrium. The animals were killed with a blow on the head and the heart was immediately removed and transferred to a transparent chamber through which Tyrode's solution with the following composition flowed continuously: (m*M*) Na^+ — 137; K^+ — 2.7; Ca^{2+} — 2.7; Cl^- — 137; HCO_3^- — 12; $H_2PO^2_4$ — 3.6; Mg^{2+} — 0.5; dextrose — 5.5. This solution was saturated with a gas mixture of 95% O_2 and 5% CO_2 and kept at 36 °C. Sodium was injected into the cell using the techniques of Nastuk[11] and del Castillo and Katz[12]. Glass microelectrodes (10 *MΩ* resistance) were filled with NaCl 1 *M* and used to inject sodium electrophoretically into the cytoplasm. The injection was made by applying outward rectangular current pulses (40 msec duration, 4 cps) to the interior of the microelectrode. Measurements of input resistance of the injected cell were made by connecting the sodium microelectrode to a balance bridge circuit serving to pass current and record voltage changes. In these experiments, a precision electrometer (Model M-4) from W-P Instruments, was used. An adjacent cell was also impaled with a conventional KCl microelectrode (15 to 20 *MΩ* resistance) which was connected to a standard cathode follower and D.C. amplifier, and the changes in membrane potentials were displayed simultaneously on a second beam of a Tektronix oscilloscope. The electrical coupling between the two cells was measured before, during, and after sodium injection.

The results obtained indicated that the injection of sodium produced a gradual decline of cell communication that culminated in complete failure of electrical coupling. The time required for total uncoupling varied from 30 to 45 sec, but in some experiments only after 70 sec complete uncoupling was achieved.

Figures 1 and 2 show the effect of sodium injection on electrical coupling and input resistance from a typical experiment. The increment of input resistance which occurred concomitantly with sodium injection (see Figure 1B) means that the fall in size of the electrotonic potentials recorded from a cell adjacent to the site of injection was not due to an increase in membrane conductance of the injected cell.

The electrical uncoupling caused by raising the intracellular sodium concentration seems to be unrelated to any harmful effect of sodium injection, since no drastic depolarization or apparent change in transparence of the injected cells was found. On the contrary, in some experiments a slight hyperpolarization was seen even in the cell adjacent to site of injection. This increase in membrane polarization was completely reversed on interrupting the release of sodium. On the other hand, the failure of cell communication caused by sodium injection was completely reversible, but the time required for total re-establishment of electrical coupling varied. In many experiments, however, 400 sec or more were necessary for total recovery of cell communication.

The effect of intracellular sodium injection on electrical coupling of myocardial cells is probably explained by the increment of the internal calcium concentration produced by the increase on intracellular sodium content[3]. It is also

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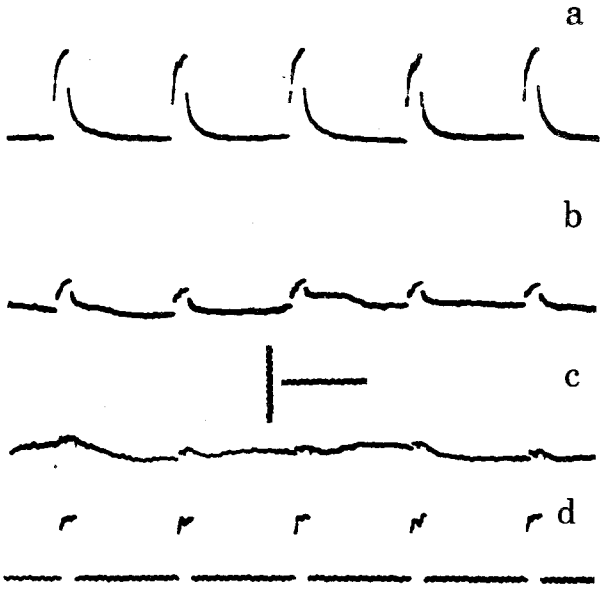

Fig. 2. Electrical uncoupling of myocardial cells produced by intracellular sodium injection. a) control values of electrotonic potentials recorded from a myocardial cell adjacent to the site of sodium injection immediately after the beginning of sodium release; b) and c) after 15 and 30 sec of injection respectively. Resting potential in a) −80 mV and in c) −83 mV. Vertical calibration, 5 mV; horizontal calibration, 200 msec. Lower trace: outward current pulses (2.5×10^{-7} A). Temperature 36 °C.

known that a rise in internal sodium concentration releases calcium from mitochondria, and this can also contribute to the development of the electrical uncoupling.

The present results rise the possibility that the Na-K pump in heart and in other cells has an indirect role on the control of cellular communication. It is reasonable to think that the failure of the sodium in a cell or group of cells can interfere markedly with intercellular communication through a marked change on the intracellular calcium concentration. It is conceivable, indeed, that the block of impulse conduction produced by cardiac glycosides in heart tissues can be related, in part, to changes on junctional conductance, secondary to its blocking effect on sodium extrusion. Experiments are in progress in our laboratory in order to investigate this hypothesis[13].

Résumé. L'influence de l'augmentation de la teneur en Na^+ intracellulaire sur le processus de communication entre les cellules cardiaques du lapin est étudiée. Une suppression de la communication électrique est provoquée par l'injection de Na^+ dans la cellule. Ce résultat est probablement expliqué par une augmentation de la concentration en Ca^{2+} intracellulaire, subséquente à l'injection de Na^+ et par une diminution de la perméabilité des jonctions intercellulaires.

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Quantification of Secretin Release by Acid, Using Immunocytochemistry and Radioimmunoassay

When secretin was successfully purified by JORPES and MUTT[1], sequenced by MUTT and JORPES[2], and its synthesis undertaken by BODANSZKY, ONDETTI, LEVINE, NARAYANAN, VON SALTZA, SHEEHAN, WILLIAMS and SABO[3], and by BODANSZKY and WILLIAMS[4], availability of the hormone in pure form gave rise to numerous studies designed to elucidate its physiological role in digestion. The production of suitable antisera permitted identification of scattered secretin-producing endocrine cells in the upper intestinal mucosa[5–7], and the development of a radioimmunoassay for the hormone in blood and tissue extracts[8]. Doubts remained, however, as to the precise response of the endocrine cells of the upper intestine to instillation of acid into the duodenum, and therefore the primacy and degree of the secretin response to acid remained unknown.

For this reason we proposed to investigate the effect of such acid instillation on the secretin and other endocrine cells by 1. Measurement of secretin release into the blood stream, 2. Assay of the secretin content of the mucosa, 3. Assessment of immunocytochemical changes in the endocrine cells and, 4. Quantification of intracellular secretin levels.

Material and methods. Healthy English White pigs were obtained from the same breeder. Their weight was between 24 and 30 kg. For 1 week before each experiment they were kept in an animal house, washed, dewormed and then starved for 18 h before experimentation. The pigs were anaesthetized with halothane induction, and received no barbiturates. They were then intubated with an endotracheal tube and given continuous nitrous oxide and oxygen. The mean arterial blood pressure was continually monitored and maintained at normal levels by i.v. saline infusion. The bile duct, pancreatic duct and stomach were canulated and catheters placed in the portal vein and femoral artery. All blood and juice samples were collected at 5 min intervals.

The duodenum and proximal 20 cm of jejunum were isolated and canulated for acid perfusion. After a 15 min basal period, biopsies (approximately 4×2 cm) of the duodenal mucosa, 5 cm distal to the pylorus, were taken for histology, immunocytochemistry and tissue extract assays. 0.1 *N* HCl was then perfused at a constant rate of 11 ml/min for 30 min, and a second mucosal biopsy taken.

Secretin levels in blood and tissue extracts were carried out by radioimmunoassay[9]. The biopsy samples, taken before and after acid instillation, were subjected to the

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