Angiotensin II given alone showed the tendency to suppress the renin levels beneath the control values, phenylephrine had no effect on the plasma renin concentration. The increase in plasma renin concentration induced by isoprenaline was markedly inhibited by simultaneous infusion of angiotensin II or phenylephrine.

Figure 2 shows a similar experiment with vasopressin (10.0 mU/kg min). Given alone, the peptide had no significant effect on plasma renin concentration. Infused in combination with isoprenaline (0.4 µg/kg min), it prevented the renin release caused by the β-adrenergic stimulation.

The effect of phenylephrine (60 µg/kg min) on the PAH-clearance of otherwise untreated animals was studied next. Since isoprenaline causes a strong antidiuresis, it was impossible to study PAH-clearance in isoprenaline-treated rats. PAH-clearance was 6.9 ml/min ($n = 4$) in controls and 7.2 ml/min ($n = 4$) in phenylephrine treated rats (not significant). This indicates that the vasoconstrictors do not suppress renin release by decreasing renal plasma flow and thus the access of isoprenaline to its intrarenal sites of action.

Finally the effects of isoprenaline and the vasoconstrictors on mean arterial pressure (MAP) and heart rate (HR) were analyzed. MAP and HR before and 10 min after the start of the infusions are given. Angiotensin II (1.0 µg/kg min) increased MAP from 104.8 to 149.3 mmHg ($p < 0.01$) and lowered HR from 460 to 315 beats/min ($p < 0.001$). Vasopressin (10 mU/ kg min) increased MAP from 90.0 to 112.0 mmHg ($p < 0.01$); HR was lowered from 478 to 302 beats/min ($p < 0.005$). Isoprenaline given alone increased HR from 470 to 540 ($p < 0.005$) and lowered MAP from 102.4 to 61.8 mmHg ($p < 0.005$). When infused simultaneously angiotensin II partially antagonized the effect of isoprenaline on MAP and HR. During the combined infusion, MAP was lowered from 92,0 to 72.0 mmHg ($p < 0.001$); HR rose slightly from 478.1 to 517.1 beats/min (n.s.). When isoprenaline and vasopressin were infused simultaneously, MAP fell slightly from 95.9 to 88.7 mmHg ($p < 0.01$) and HR rose from 498 to 507 (n.s.). Thus, 3 different vasoconstrictors markedly inhibit the isoprenaline-induced renin release and its vascular and cardiac effects.

Isoprenaline releases renin by an action on intrarenal sites[4]. The suppressive effect on renin release of angiotensin is also due to an intrarenal action[5]. The observations reported may be explained by the assumption that isoprenaline causes an intrarenal vasodilatation, e.g. in the baroreceptor area, and thus stimulates renin release. The vasoconstrictors antagonize the isoprenaline-induced vasodilation in a similar way as they inhibit its effect on systemic blood pressure. Thus they attenuate the stimulus for the baroreceptors and inhibit renin release.

The suppression of the isoprenaline-induced renin release by the vasoconstrictors strongly resembles their inhibitory effect on renin release caused by furosemide[9]. A similar mechanism was proposed to explain this phenomenon.

According to the hypothesis of GANONG[10] isoprenaline causes renin release by a direct secretomotoric stimulation of the renin-releasing cells. NOLLY et al.[11], however, could only observe a weak stimulation of renin release when they added catecholamines to an incubate of rat kidney slices. AOI et al.[12] reported a strong stimulation, but they had to use very high concentrations of epinephrine or norepinephrine. In case of a secretomotoric mechnism of the isoprenaline-induced renin release, which is still unproved, the effect of the vasoconstrictors must be due to a direct inhibitory action on the renin-secreting cells. On the molecular aspects of this action, we can only speculate.

Summary. The vasoconstrictors angiotensin II, vasopressin and the α-sympathomimetic phenylephrine significantly inhibit the renin release caused by the β-sympathomimetic isoprenaline. The mechanism of the inhibition is discussed.

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Scanning Electron Microscope Observations of the Canaliculi in the Rat Pineal Gland

Several authors have described large intercellular and pericapillary spaces in the rat pineal gland[1-8]. In these spaces there are polar terminals of the pineal cells, interstitial cells with their processes, adrenergic nerve endings, capillaries, collagenous fibres and an amorphous, weakly osmiophile substance.

After a parenchymal perfusion of a rat pineal gland, QUAY[9] observed that its canalicular system shows a 24-hour rhythm. According to the same author, this system of channels may be significant for transport activities between pinealocytes and capillaries. The rhythmic changes of the pineal parenchymal channels would be regulated partly by the release of 5-hydroxytryptamine and partly by calcium ions.

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The present study describes the pineal parenchymatous canalicular system under the scanning electron microscope.

Material and method. The pineal glands of adult male Wistar rats weighing 200–250 g are prepared at 09.00 h, prefixed by immersion in a mixture containing 2% glutaraldehyde and 1% formol buffered with cacodylate buffer (pH 7.4). After being briefly rinsed in the same buffer, the glands are postfixed in 1% OsO_4, cut in half with a razor blade, dehydrated and dried by the critical point method. Following gold metallization, the specimens are examined in the Cambridge Stereoscan S4-10 scanning electron microscope at 10 kV[10].

Results. The intercellular spaces in the rat pineal gland can be up to 100 µm long, with a diameter of 1 to 20 µm (Figure 1). The pineal and interstitial cells constitute the pineal channel walls. Within the canalicular spaces extend the pineal and interstitial cell processes, adrenergic nerve endings and a variable quantity of collagenous fibres. The pericapillary spaces communicate largely with the gland's parenchymatous canalicular system.

Under the scanning electron microscope, the dimensions of the intrapineal canaliculi are comparable to the dimensions mentioned above. We notice their large three-dimensional ramifications, creating a veritable intraglandular labyrinth. However, there is always a principal canaliculus that produces a secondary and tertiary branching (Figure 2).

The interior of the pineal channels shows a great quantity of different ramified extensions. They form a

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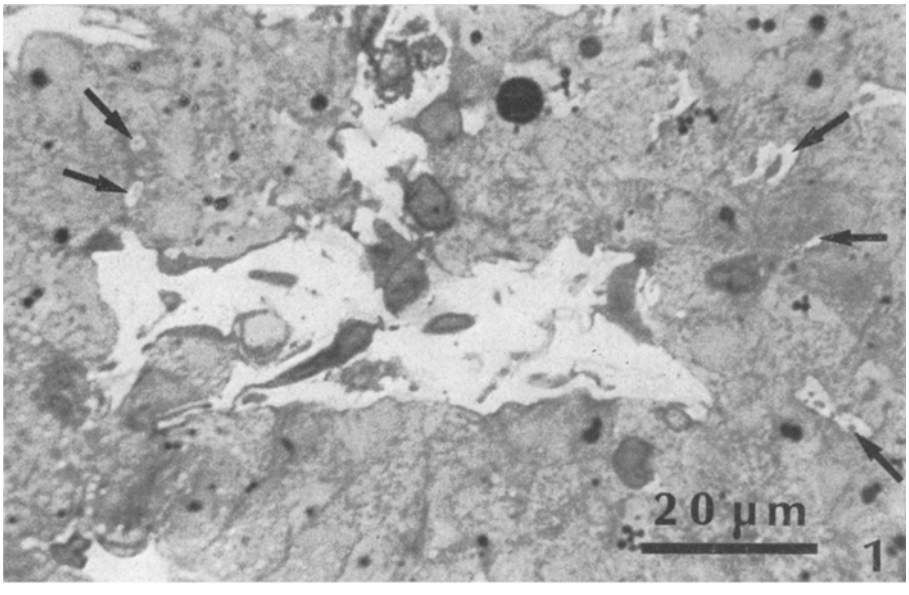

Fig. 1. Pineal canaliculi under light microscope. Some pinealocyte processes, interstitial cells, and collagenous fibre bundles can be seen in their lumina. The arrows indicate small parenchymal channels.

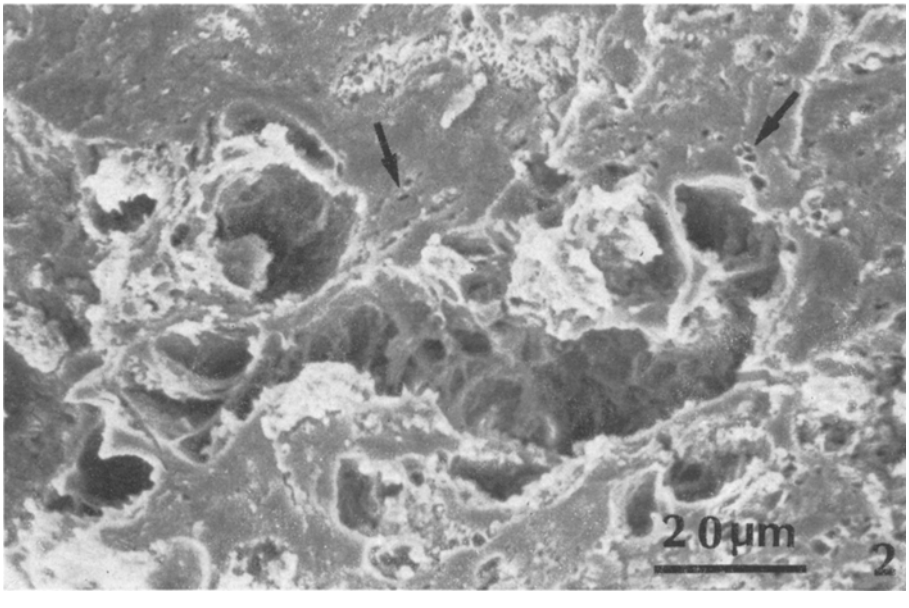

Fig. 2. Scanning electron microscope view of pineal canaliculi. Note their abundant ramifications, the presence of various cellular processes within, and the existence of small channels in the pineal parenchyme (arrows).

very dense framework through which the canaliculi anastomose.

In the pineal parenchyme, one can also observe a considerable number of fine canaliculi measuring 1–3 μm in diameter (Figure 2, arrows).

The nerve endings and collagenous fibre bundles are not easy to distinguish from pineal and interstitial cell processes.

Discussion. The scanning electron microscope shows a surprizing structure of the parenchymal pineal canaliculi. It not only confirms the existence of 'interfacial lakes' and 'cavities of circumluminal arrays'[1,3], but also clearly demonstrates their numerous ramifications. The abundance of fine canaliculi of 1–3 μm forms a network of labyrinthal intercellular spaces which, very probably, come into contact with every pineal cell. The analysis of the pineal gland's parenchymatous channels with the scanning electron microscope suggests a continuity of all canaliculi and casts a new light on their significance. QUAY's experiments, showing the existence of a daily rhythm of pineal canaliculi, suggest their functional dynamism. According to QUAY's observations[9] and our results, it is evident that the pineal gland parenchymatous channels have a much more important histophysiological role than was assumed up to now.

Summary. The scanning electron microscope has shown rich ramifications of the parenchymal canaliculi forming a three-dimensional network of anastomosing intercellular spaces in the rat pineal gland. Every pineal cell seems to be in contact with this channel system. An abundance of cellular processes can be found within the canaliculi which may play an important role in the histophysiology of the pineal body.

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[11] Dedicated to Prof. Dr. med. W. BARGMANN on the occasion of his 70th birthday.

Effects of Glucagon and Insulin on the Paneth Cells of the Mouse Duodenum

The coarse cytoplasmic secretory granules are characteristic of Paneth cells (PC). The function of these cells has remained largely unknown. However, it has been shown that PC secrete digestive enzymes, especially peptidases, into the intestinal lumen[1], and the secretion mechanism seems to be regulated by the sympathetic and parasympathetic nervous systems[2]. It was shown earlier that ordinary food does not have any apparent effect on these cells[3]. However, the recent observations by the present authors indicated that, following fasting, the secretion mechanism is stimulated by food and fasting itself retards the secretory activity of PC[4]. Trasylol®, an inhibitor of trypsin and proteinase activity, also seems to inhibit the secretion mechanism of PC[5]. Therefore, the present preliminary investigation was designed to see whether glucagon and insulin do have any effect on PC.

Material and methods. 18 male and female adult albino mice, descendants of a strain used in the Department of Anatomy, were used. The mice were fasted for 1 day before the experiments but were allowed to drink tap water ad libitum.

The material consisted of 3 groups of mice. The mice of the glucagon group received an i.p. dose of 100 μg of glucagon (Novo). These mice were killed 1 h later and intestinal samples were taken immediately from the mid-duodenum; fixed in a buffered aqueous 4% formaldehyde solution at pH 7.2; embedded in paraffin wax; sectioned at 7 μm and stained with Best's carmine method[6]. The mice of the insulin group received an i.p. dose of 4 IU insulin (Novo). The samples were taken 1 h later and treated as above. The control mice were only fasted for 1 day and the samples were taken as above.

Each group consisted of 6 mice. The number of secretory granules of 100 PC derived from each mouse was counted. Only the relative number of secretory granules was determined and the significance of the differences between the experimental and control groups was estimated, using the Student's *t*-test. The light microscopical morphometric method, together with its reproductibility, have been discussed in detail previously[2].

Results. After glucagon treatment the number of secretory granules of PC increased highly significantly ($p < 0.001$), i.e. from 12.1 ± 0.1 (SEM) to 15.5 ± 0.2 (Figures 1 and 2), in the duodenum. The size of secretory granules of PC, as estimated visually, remained quite unchanged following glucagon treatment.

One dose of insulin also increased significantly ($p < 0.01$) the count of PC granules; i.e. from 12.1 ± 0.2 to 13.0 ± 0.1 (Figures 1 and 2) 1 h after administration, whereas the size of the granules remained unchanged.

Discussion. The present results showed that glucagon and insulin were able to increase the number of secretory granules of PC, which obviously indicates that the secretion of cytoplasmic granules from PC is inhibited. Glucagon plays an important role as a stress hormone and regulates e.g. the concentration of glucose of the serum[7,8]. Glucagon is also able to inhibit the secretion of gastrin[9], hydrochloric acid[10,11], the exocrine secretion of the pancreas[12], and that of various proteins from the salivary glands[13]. The above and the present observations suggest the active role of glucagon in the regulation of digestion.

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