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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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.

[1] A. D. HALLY, J. Anat. *92*, 268 (1958).
[2] A. AHONEN, Acta physiol. scand. Suppl. *398*, 1 (1973).
[3] J. S. TRIER, V. LORENZON and K. GROEHLER, Gastroenterology *53*, 240 (1967).
[4] A. AHONEN and A. PENTTILÄ, Scand. J. Gastroenterol., *10*, 347 (1975).
[5] A. AHONEN and A. PENTTILÄ, Experientia, *31*, 577 (1975).
[6] P. LAUREN and T. E. SORVARI, Stain Technol. *42*, 311 (1967).
[7] S. R. BLOOM, Postgrad. med. J. *49*, 607 (1973).
[8] A. LINDSEY, F. SANTEUSANIO, J. BRAATEN, G. R. FALOONA and R. H. UNGER, J. Am. med. Ass. *227*, 757 (1974).
[9] H. D. BECKER, D. D. REEDER and J. C. THOMPSON, Gastroenterology *65*, 28 (1973).
[10] T. M. LIN, D. C. EVANS and G. F. SPRAY, Archs. int. Pharmacodyn. Thér. *202*, 304 (1973).
[11] T. M. LIN, D. C. EVANS and G. F. SPRAY, Archs. int. Pharmacodyn. Thér. *202*, 314 (1973).
[12] M. MERCADIER, J. P. CHIGOT and J. P. CLOT, Méd. Chir. Dig. *3*, 101 (1974).
[13] S. KOJIMA, M. IKEDA and A. TSUJIMOTO, Jap. J. Pharmac. *23*, 588 (1973).

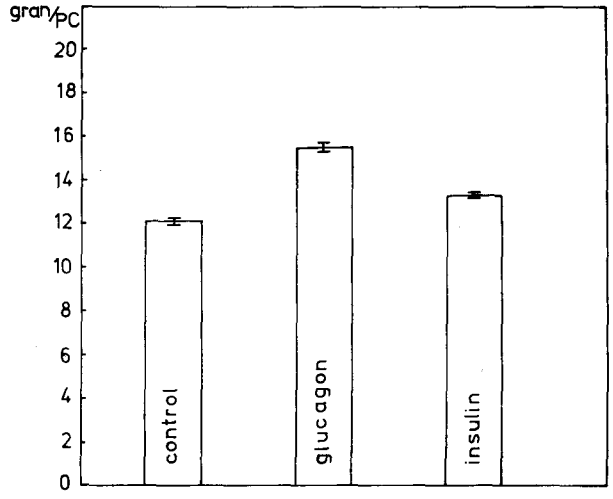

Fig. 1. The effect of glucagon and insulin on the number of secretory granules of PC of the mouse duodenum (means and SEM). The counting was made from 600 paneth cells derived equally from 6 different animals in each group. 100 µg of glucagon or 4 IU of insulin per mouse was administrated 1 h before the samples were taken.

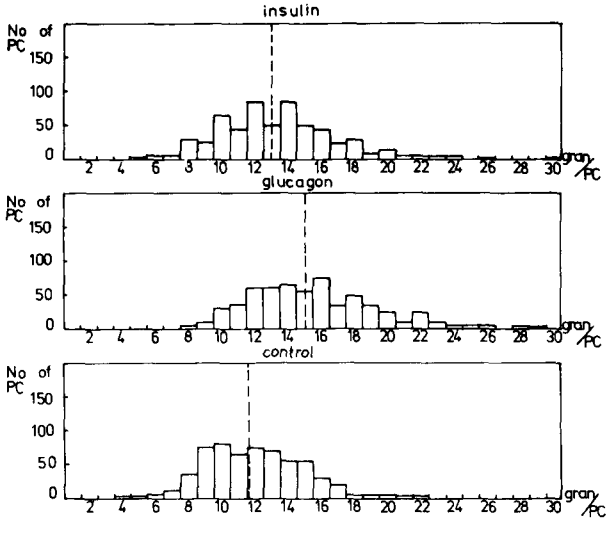

Fig. 2. Distribution of PC as a function of the number of granules. The mean values of the granule counts are marked with dotted lines.

The present results showed that the effect of glucagon on PC of the mouse was quite similar to that of fasting[4]. Prolonged fasting induces hypoglycaemia, which is followed by an increase of serum glucagon[14,15]. The inhibitory effect of fasting on the secretion of the PC granules may therefore be mediated through the action of glucagon. The same would also be valid for the mechanism of the effect of insulin on PC. Insulin increases serum glucagon[16] and the present observations showed that insulin slightly inhibited the secretion of PC; however, this effect was not so strong as the effect of glucagon.

Previous studies have shown that the size of PC granules has increased following vagotomy, sympathectomy, or the treatment of the mice with Trasylol®[2,5]. The size of PC granules was also shown to increase with the age of mice[17,18]. The present observations suggested that glucagon or insuling did not have any apparent effect on the size of PC granules. Glucagon is an important catabolic hormone and markedly inhibits the protein synthesis of hepatocytes[19]. It also exerts a marked effect on the carbohydrate, lipid and protein metabolism of liver cells[19], and may therefore exert its action on PC through metabolic effects.

Summary. The effect of glucagon and insulin on the paneth cells (PC) of the duodenum of the mouse was investigated using light microscopy. Both glucagon and insulin were able to increase significantly the number of the secretory granules of PC. This possibly means that these hormones are capable of inhibiting the secretion of PC.

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[14] J. M. Basset, Aust. J. biol. Sci. *25*, 1277 (1972).
[15] A. Cherrington, M. Vranic, P. Fono and N. Kovacevic, Can. J. Physiol. Pharmac. *50*, 946 (1972).
[16] J. E. Gerich, J. H. Karama and P. H. Forsham, J. clin. Endocr. Metab. *37*, 479 (1973).
[17] K. Kurosumi, Int. Rev. Cytol. *11*, 1 (1961).
[18] H. M. Selzman and R. A. Liebelt, J. cell Biol. *15*, 136 (1962).
[19] I. Weinstein, H. A. Klausner and M. Heimberg, Biochim. biophys. Acta *296*, 300 (1973).
[20] This work was supported by grants from the Emil Aaltonen Foundation, Tampere, and
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Role of Sialic Acid in the Maintenance of Cell Surface Rigidity

Recently, abundant information has been accumulated with respect to the function of the sialic acids at cell surface[1]. It is now well known that sialic acids form an integral component of the carbohydrate prosthetic groups of glycoproteins[2–4] and of acid mucins[2] of the cell surface. It has been postulated that the primary function of the sialic acid molecule is to confer structural rigidity on glycoproteins[5] of cell membranes. Weiss[6] has shown removal of sialic acid residues from the surfaces of sarcoma 37 cells increases the overall cellular deformability. The increase in the deformability may very well be explained as the loss of rigidity of the cell membrane in absence of sialic acid. However, no attempt has been made so far to demonstrate the loss of the rigidity of the cell membrane in absence of sialic acid and the subsequent fate of these cells.

The presence of sialic acid in the cell membrane of a large free living protozoa, *Amoeba*, has recently been

[1] L. Weiss, J. natn. Cancer Inst. *50*, 3 (1973).
[2] A. Gottschalk, *The Chemistry and Biology of Sialic Acids and Related Substances* (Cambridge University Press, Cambridge 1960).
[3] A. Gottschalk, Perspect. Biol. Med. *5*, 327 (1962).
[4] A. Gottschalk, Rev. pure appl. Chem. *12*, 46 (1962).
[5] A. Gottschalk, Nature *186*, 949 (1960).
[6] L. Weiss, J. Cell Biol. *26*, 735 (1965).