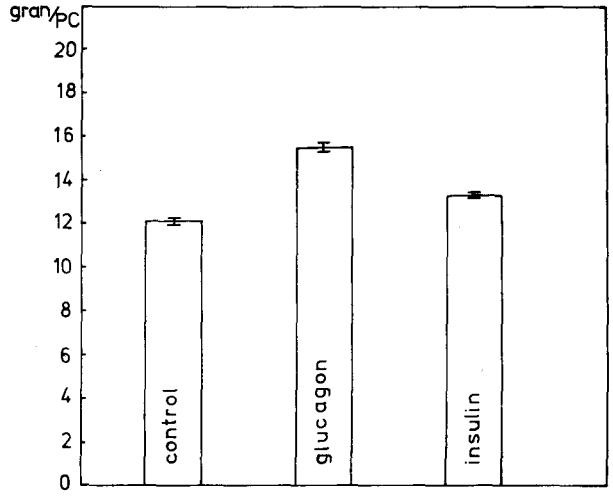

Fig. 1. The effect of glucagon and insulin on the number of secretory granules of PC of the mouse duodenum (means and SEM). The counting was made from 600 paneth cells derived equally from 6 different animals in each group. 100 µg of glucagon or 4 IU of insulin per mouse was administrated 1 h before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

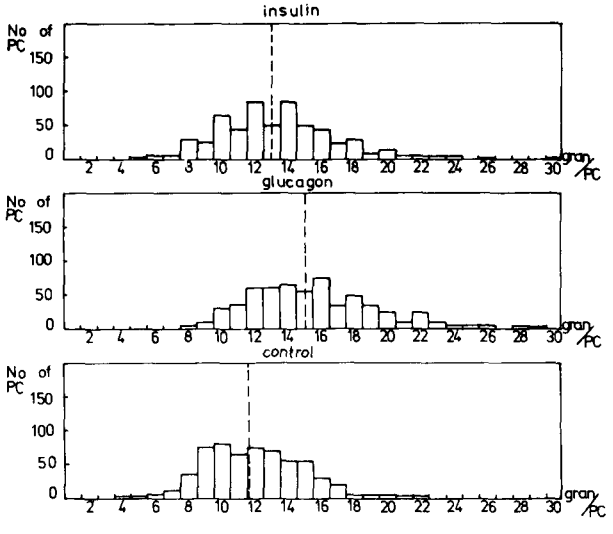

Fig. 2. Distribution of PC as a function of the number of granules. The mean values of the granule counts are marked with dotted lines.

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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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

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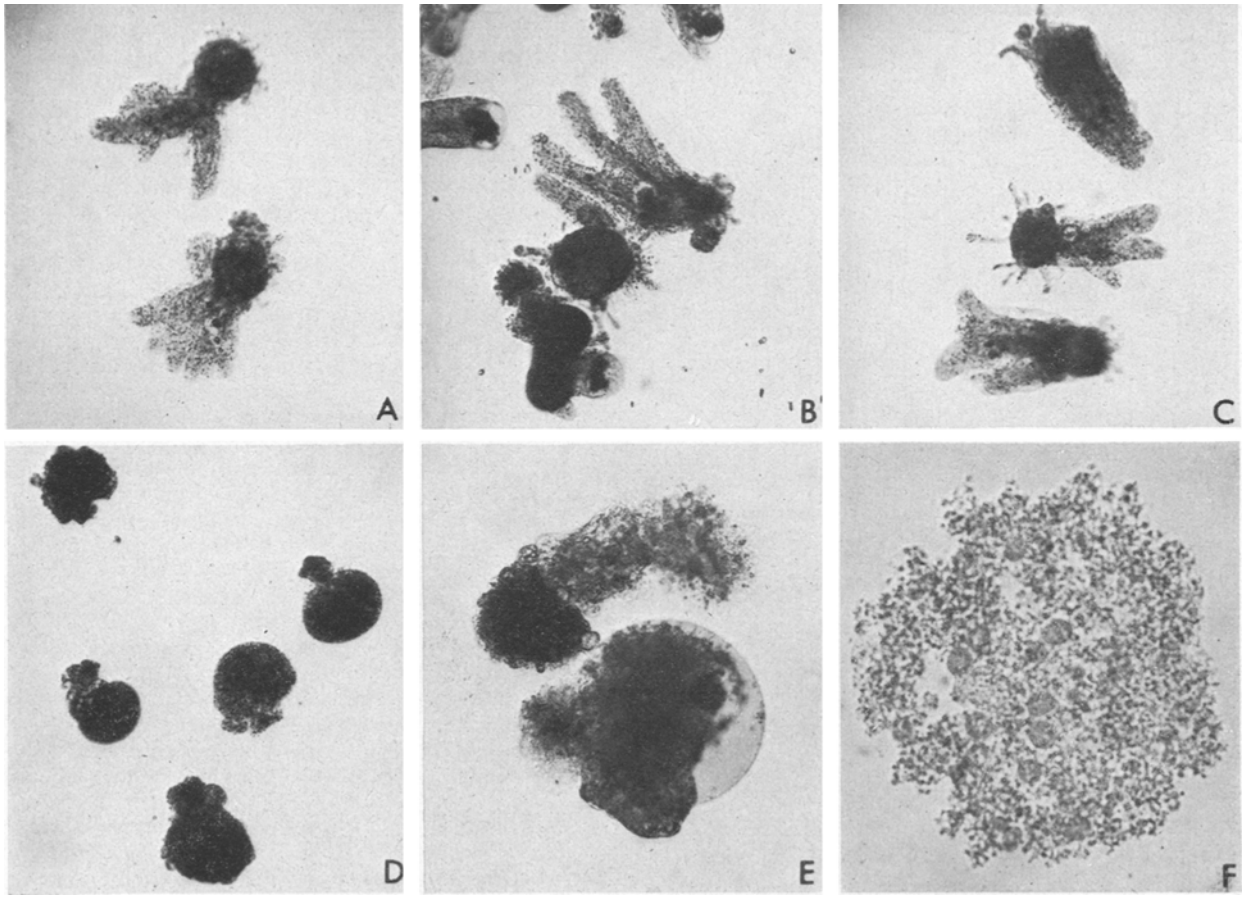

Control amoeba (A); Amoeba treated with 25 units of VCN (B, 15 min; C, 60 min); Amoeba treated with 50 units of VCN (D, 2–3 min; E, 15 min, and F, 60 min). Magnifications: Figures A–D ×75; E–F ×200.

suggested by us[7]. While working in such a system, we have observed that the rigidity of the cell surface structure is lost followed by lysis of the cell body of *Amoeba* when sialic acid residues are removed from the cell membrane with *Vibrio cholerae* neuraminidase (VCN). This enzyme specifically liberated the terminal sialic acid molecules from the cell membrane structure[8] after cleaving the 2–3, 2–6, and 2–8 α-glucosidic linkages between the sialic acid and carbohydrate of the cell membrane structure[9].

VCN was obtained from Behringwerke A.G., Marburg, Lahn, Western Germany. This preparation contains 500 units of enzyme/ml (1 unit of enzyme activity has been described by the manufacturer as being equivalent to the release of 1 μg of N-acetyl neuraminic acid from a glycoprotein substrate at 37 °C in 15 min at pH 5.5). This enzyme preparation is declared to be free of proteinases, aldolases and lecithinase C activity. It liberates sialic acid from the cell surface[10] and its activity can be inhibited in the presence of the specific feed back inhibitor of the enzyme i.e., N-acetyl neuraminic acid[11].

Amoeba proteus cultured[12] at room temperature (23 ± 1 °C), were incubated (37 °C, 20 min) with VCN (25–50 units of VCN/50 amoeba/0.1 ml) in amoeba medium (0.036 m*M* $CaHPO_4$, 0.08 m*M* KCl and 0.033 m*M* $MgSO_4$, pH 6.8). Sham treated cells were also maintained in indentical conditions. At the end of the incubation the cells were washed with amoeba medium and placed in a depression slide. Then both treated and sham treated controls were monitored with a light microscope at various intervals of time.

Cells treated with VCN take a spherical shape immediately after the treatment. Most of the cells treated with 25 units of VCN regain their characteristic normal appearance approximately after 15 min (Figure B). By 60 min they look like the normal amoeba (Figure C). However, cells which were treated with 50 units of VCN cannot maintain the rigidity of the surface structure. Within 2–3 min after the VCN treatment, these cells show localized protrusion of the cytoplasmic mass (Figure D). At a later period a greater mass of cytoplasm is extruded outside, leaving behind the ghost membrane (Figure E). A typical view after 60 min shows absence of the rigid membrane strucutre (Figure F). Cell bursting takes place followed by liberation of the cytoplasmic granules in single and compact masses. It appears that the integrity of the membrane structure is lost, followed by the total dissolution of the cell body of amoeba. The score is more than 98% for the VCN treated cells, while the control amoebae, otherwise maintained in the same conditions, do not show any such effect. These effects

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were quantitatively reproducible. Lower concentrations of VCN are ineffective, perhaps because of their inability to release the optimum amount of sialic acid to bring about cellular deformabiliy. Thus it appears that the observed effect as depicted in the Figure is due to the action of VCN on the cell membrane structure of amoebae.

The exact mechanism as to how VCN leads to the disintegration of the membrane structure followed by the lysis of the cell body of amoeba is not known. Removal of sialic acid might be associated with the loss of the rigidity of the cell membrane structure[5] leading to deformation[6] of the surface structure of the cell. These phenomena might change the modes of normal transport across cell membranes[13–15], thus creating an imbalance in the osmotic potential inside and outside the membrane. In this situation, at some points, any two parts of the membrane structure may fall apart leading to the consequence detailed above.

It might be relevant to point out that this could be an isolated example of the observed effects because of the unique surface configuration of amoeba. In the case of mammalian cells, this kind of situation is not normally observed in vitro, even if incubation is extended for longer periods and higher concentrations of VCN are used[10, 11].

Summary. Removal of cell surface sialic acid with neuraminidase brings about cell deformation in amoeba. The membranes of these deformed cells are eventually ruptured leading to the liberation of the cell mass.

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Two Types of Bipolar Cells in the Chick Retinal Development[1]

Bipolar cells comprise the bulk of the inner nuclear layer of the retina. These cells are important intermediates of the retinal pathways because they form synaptic contacts with both photoreceptor terminals, processes of amacrine cells and ganglion cells[2–5]. Extensive studies by Ramón y Cajal[6–8] indicated that, for example in the chicken, there are 2 types of bipolar cells. However, there appears to be little existing data on when these two types of bipolar cells arise and what their morphological characteristics are. This study is an attempt to shed some insight on this problem.

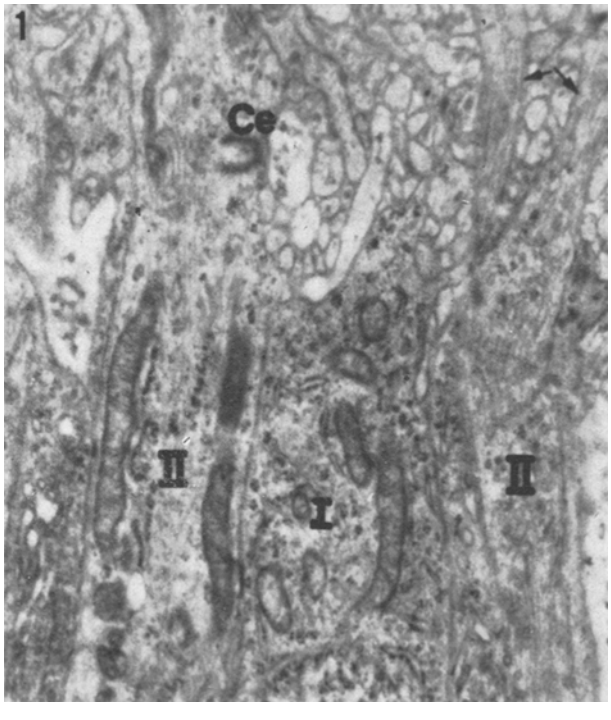

Fig. 1. Type I and II bipolar cells are distinguished at stage 40 of chick embryonic retina. Note a centriole (Ce) in one of the type II cells and external projections (arrows) in the other. ×28,800.

Materials and method. Fertile white leghorn chicken eggs were incubated in standard conditions and staged according to Hamburger and Hamilton[9]. Chick embryonic eyes from stages 36 to hatching were taken out and bisected into halves. Then the posterior halves were prefixed with 6% buffered glutaraldehyde and postfixed with 1% buffered osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide and embedded in Epon. Sections were cut with a Porter-Blum MT 2 microtome and stained with uranyl acetate and lead citrate, and then examined with a Zeiss EM 9S2 electron-microscope.

Results. Bipolar cells cannot be distinguished until stage 36 when both plexiform layers are formed and the presumptive bipolar nuclei become distinguishable by their ellipsoidal shape. The cytoplasm of these cells at stage 36 have a few mitochondria, some rough endoplasmic reticulum and few ribosomes. Some bipolar cells often lie close together resembling a telophase phenomenon, indicating that they have just completed a mitotic division. At stage 40, dendritic projections can be seen arizing from the bipolar cell bodies towards the outer plexiform layer (Figure 1) and at this time 2 types of bipolar cells can be distinguished. The ultrastructural details of these 2 types of bipolars are outlined in the

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