dermis beneath the basal layer of epidermis. Thickened epithelium (Figure 2b) and inflammatory infiltrates were present in skin exposed to GLU.

Pathological changes of internal organs were fibrosis of liver and intestines after i.p. exposure to TYR for 10 days. Perivascular aggregation of eosinophiles were observed in organs which contained high fibrogenic activity after exposure to TYR. Hemorrhage and engorgement with red blood cells in lungs were observed after exposure of B-ALA. Subcapsular hemorrhage of kidneys occurred after i.p. exposure of mice and rats to 0.5 mmole of B-ALA. Morphological changes such as giant cell formation were present in liver (Figure 3a) after exposure to HIS. The liver was normal (Figure 3b) after similar injections with isotonic saline. The bones of these animals injected with HIS were soft and friable and were less calcified than isotonic saline injected controls.

Discussion. Pathological changes which resulted after the series of i.p. injections appeared to be specific for the amino acid in excess. Different pathological changes were seen for each of the amino acids in excess.

The mechanism(s) of action of these essential and non-essential amino acids in producing lesions is unknown. Acidosis per se[4] is not a controlling mechanism since ASP caused the greatest rabbit skin irritation, GLU less, and D-ASP and D-GLU caused the least irritation. Structure and steric configuration of amino acid appears important. Amino acids may become decarboxylated producing very active biogenic amines. Activation of proteolytic enzymes[5] may occur due to amino acids or their products. It is possible that amino acids produced by bacteria[6] or amino acid metabolites activate mycoplasma and viruses present in tissues to produce cytopathology. No precedent appears to exist in literature for viral activation by an amino acid. Viral activation remains a possibility.

Zusammenfassung. Es wird der Einfluss einer Aminosäure auf verschiedene Gewebsarten und auch auf die Haut kontrolliert. Unterschiedlich starke, z.T. schwere Degenerationen, Entzündungen und auch Zellauflösungen waren festzustellen. Teilweise kam es zu Riesenzellbildungen oder zu Fibrosen in der Leber. Knochennekrosen und Blutungen wurden festgestellt.

R. W. LONGTON[7-11]

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Structural Coupling Between Pancreatic Islet Cells

There is circumstantial evidence that ionic[1-3] and metabolic[4] coupling observed between cells in a variety of tissues may be due to intercellular specializations of the membranes known as 'gap junctions'[5], which seem to represent low resistance pathways through which ions and low molecular weight substances can diffuse from one cell to the other[4, 6-13]. Thus far, no such junctions have been identified between pancreatic islet cells. The recent introduction of freeze-etching technique[14, 15] has greatly facilitated the identification of cell junctions[16-19]. This technique has now allowed us clearly to demonstrate for the first time in islet cells the presence of junctional complexes, in the form of small gap junctions and focal tight junctions[20].

Isolated islets were obtained by collagenase digestion[21] from pancreases of albino rats. Rats were chosen because of the characteristic topological relationship between the two main types of islet cells: indeed, glucagon-producing α-cells are disposed peripherally as a mantle around centrally located insulin-producing beta cells[22]. Pelleted islets were briefly fixed with 2% glutaraldehyde in phosphate buffer and soaked in 20% phosphate-buffered glycerol solution before freezing. Freeze-cleaving was performed according to the method of MOOR and MÜHLETHALER[23] in a Balzers Freeze-Etch Unit. Fracturing and etching temperature were –100 °C. Etching time was 1 min. The cleaned replicas were observed in a Philips EM 300

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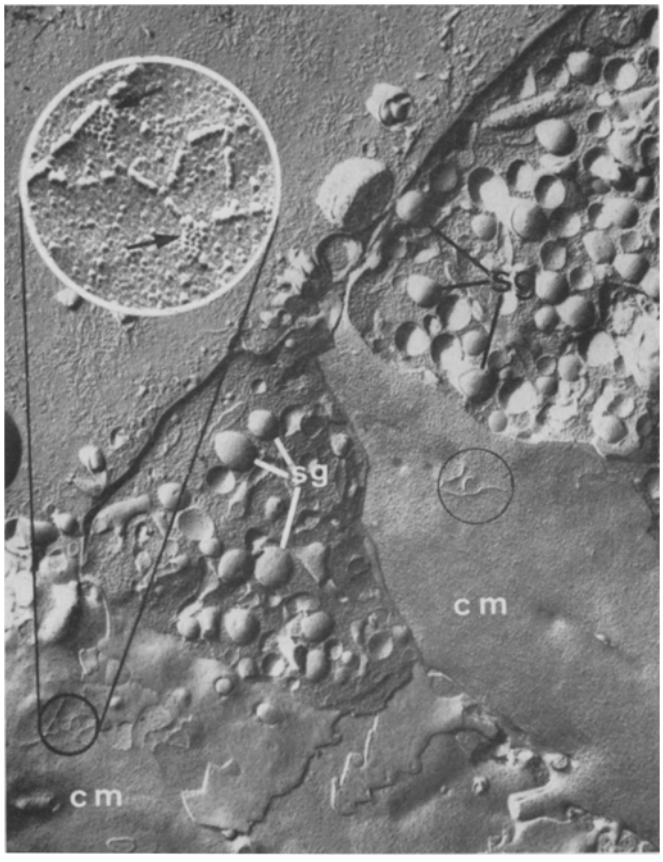

Fig. 1. Low magnification view of the periphery of a freeze-etched islet. The fracture reveals large areas of cell membranes (cm) and cytoplasm of α-cells containing the secretory granules (sg). The encircled areas show the branching linear ridges of focal tight junctions (× 13.000). The insert is a blow-up of one of the encircled areas. Two small groups of aggregated particles (arrows), characteristic of gap junctions, are seen in proximity of the tight junctional elements. (× 75.000).

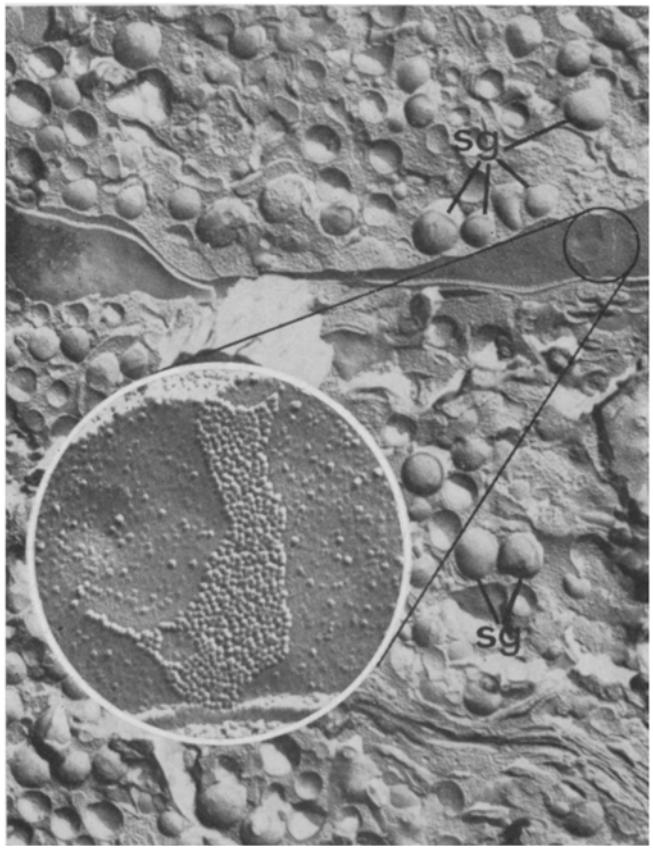

Fig. 2. Low magnification view of the central part of a freeze-etched islet, showing an unusually large gap junction, blown up in the insert, between 2 β-cells (× 14.000; insert × 83.000).

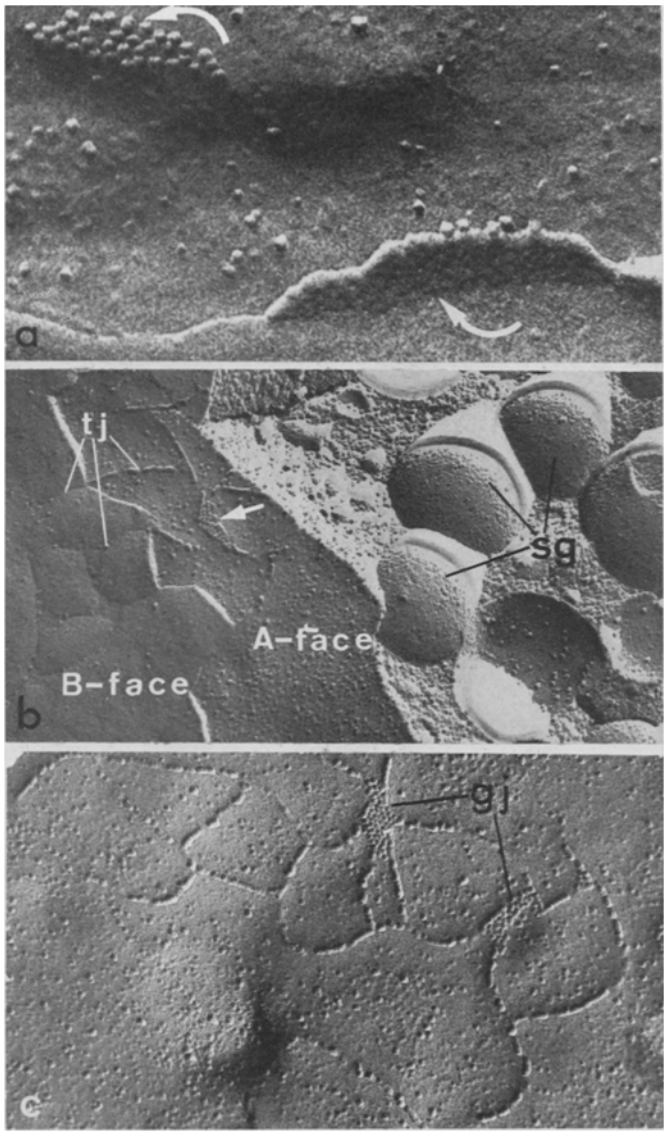

Fig. 3. Replicas of freeze-etched islet cells. a) Gap junctions as seen on A- (upper arrow) and B- (lower arrow) fracture faces (× 172.000). b) Focal tight junction (tj) characterized by linear ridges on the A-face and complementary linear furrows on the B-face. The arrow indicates a gap junction. sg: secretory granules (× 50.000). c) Two gap junctions (gj) associated with branching and anastomosing ridges of focal tight junction (× 63.500).

electron microscope and the micrographs were processed to reveal shadows in white.

A typical replica of most peripherally located islet cells (α-cells) is presented in Figure 1. Figure 2 shows 2 centrally located β-cells. It can be seen that the plane of fracture traversed the cytoplasm, revealing the secretory granules, and followed the cell surface, exposing large areas of membrane faces. According to BRANTON[24], the fracture plane splits the unit membrane in its hydrophobic region, revealing 2 complementary faces, the A-face, oriented towards the extracellular space, and the B-face, oriented towards the cytoplasm[25]. On the A-faces of exposed membranes, the gap junctions (Figures 1, 2, 3a and 3c) appear as closely packed arrays of 80–90 Å particles[17, 25–27], which have a center-to-center spacing of 90–100 Å when arranged in an hexagonal lattice. On B-faces, the gap junctions appear as 'negative' images, i.e., plaques of aggregated pits (Figure 3a)[17, 25–27]. The size of gap junctions was usually small (Figures 1 and 3c), in some cases restricted to a few particular components. Gap junctions were often associated with focal tight junctions (Figures 1, 3b and 3c). The latter appear on A-faces as linear and anastomosing ridges (Figure 3b) and, on B-faces, as linear and anastomosing grooves (Figure 3b)[17, 25–27]. The number and distribution of both gap and tight junctions varied to some extent from one cell to another. These findings on freeze-etched replicas have been confirmed by painstaking examination of standard thin sections containing lanthanum tracer. In these preparations, both gap and tight junctions were found to occur not only between β- and β-cells and between α- and α-cells, but also between α- and β-cells (data not shown).

The asynchronous but coordinated activity of insulin-producing cells and glucagon-producing cells in releasing precisely the appropriate amounts of hormones is poorly explained by present concepts of islet regulation. Previous studies in other tissues have demonstrated that inorganic ions and small molecules (up to M.W. 500) can diffuse through the gap junction channels of adjoining cells[10, 11].

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Thus, in islet cells, gap junctions could theoretically be used for intercellular passage of ions, metabolites and other possible types of signal molecules. In that way, gap junctions may play a role in the control of islet cells' secretory activity. It is not known if tight junctions also participate in intercellular communication.

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Résumé. Ce travail décrit la présence de spécialisations des membranes plasmatiques entre les cellules endocrines des îlots de Langerhans chez le rat. Ces spécialisations sont révélées par le cryodécapage («freeze-etching») et apparaissent sous la forme de nexus («gap-junctions») ou de jonctions serrées («tight-junctions»). Elles représentent probablement la base structurale d'un couplage ionique et métabolique entre les cellules endocrines.

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Effects of Nerve Growth Factor on Competent Chick Ectoderm

Several authors have shown that the whole ectoderm area of the chick embryo at stages 3 to 4 (stages according to HAMBURGER and HAMILTON[1]) is competent to react to neural inductive stimuli[2–5].

BJERRE and NORD[6] could demonstrate that the male mouse submaxillary gland, but not the female, and different nerve growth factor (NGF) preparations purified from the gland of the male mouse, acted strongly neuralizing on competent chick ectoderm in vitro. An immunofluorescence method was used for identification of neural differentiation, as neural antigen production in cultures made from chick ectodermal explants could be used as an indication of such a differentiation[7]. In the investigation by BJERRE and NORD[6], NGF was present in the culture medium of the explanted ectoderm, taken from the presumptive epidermal region at stages 3+ to 4, throughout the culture period (8 days); therefore it could not be concluded whether NGF had a direct action on the ectoderm, or whether NGF rather supported the autoneuralized cells that normally would have died during the culture period. The present study used the immunofluorescence method to rule this out and also to investigate the possibility of a varied effect of NGF on different regions and ages of the ectoderm.

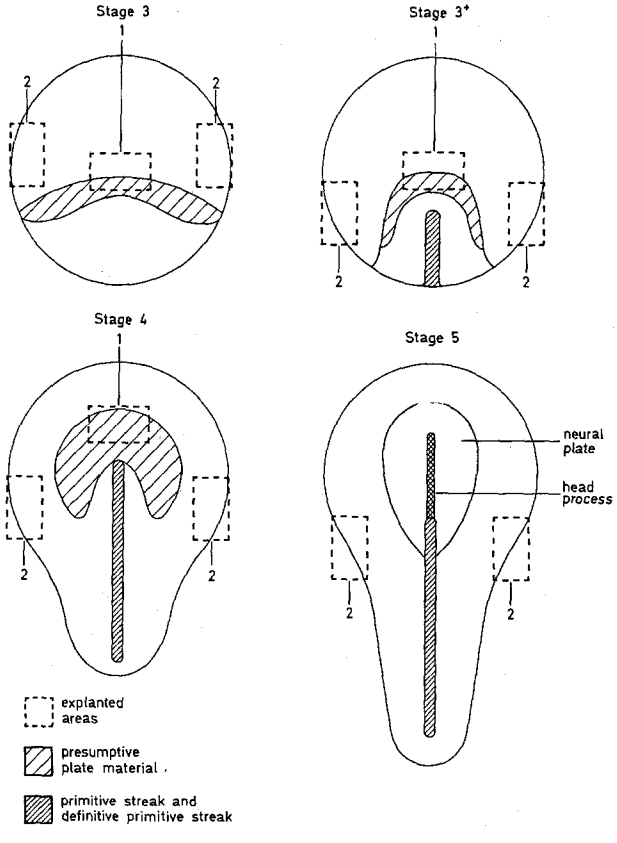

Fig. 1. Stages 3–5. Explanted areas from presumptive neural plate at stages 3–4 (area 1) and from presumptive epidermal ectoderm at stages 3–5 (area 2).

Method. Explants were taken from the presumptive epidermal region of the chick ectoderm at stages 3 to 5 and from the presumptive neural plate at stages 3 to 4 as shown in the Figure. The explanted region, wrapped in a piece of vitelline membrane prepared from an unincubated egg, was put on a millipore filter strip and placed on a piece of gel-foam inside a Leighton tube containing 1 ml culture medium, consisting of 3 ml human serum, 3 ml 50% embryo extract, and 7 ml Tyrode. The Leighton tubes were incubated at 37 °C for 8–10 days; thereafter the cultures were freed, crushed, and processed for the immunofluorescence investigation. Antisera specific to antigens present in the chick central nervous system were used for the first of the four steps in the immunofluorescence process (for further details of the immunofluorescence method and its specificity, see ref.[6]).

7 S NGF prepared according to VARON et al. [8] and tested for the specific activity by the tissue culture method according to LEVI-MONTALCINI et al.[9] was administered at various times during the culture period. The

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