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.

[28] We thank M. Sidler-Ansermet and M. Bernard for technical assistance. This work was supported in part by grants from Fonds National Suisse de la Recherche Scientifique and by a grant-in-aid from Hoechst-Pharmaceuticals, Frankfurt-Hoechst, Germany. Preliminary findings (L. ORCI, A. E. LAMBERT, Y. KANAZAWA, W. STAUFFACHER, Ch. ROUILLER and A. E. RENOLD, Diabetes *20*, 326, 1971) and the present work (L. ORCI, R. H. UNGER and A. E. RENOLD, Abstr. 7th ann. meetind Europ. Soc. Clin. Invest., Rotterdam, 1973, p. 157) have been reported in an abstract form.

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.

L. ORCI, R. H. UNGER and A. E. RENOLD[28]

Institutes of Histology and Clinical Biochemistry, University of Geneva, CH-1211 Genève 4 (Switzerland), 9 April 1973.

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.

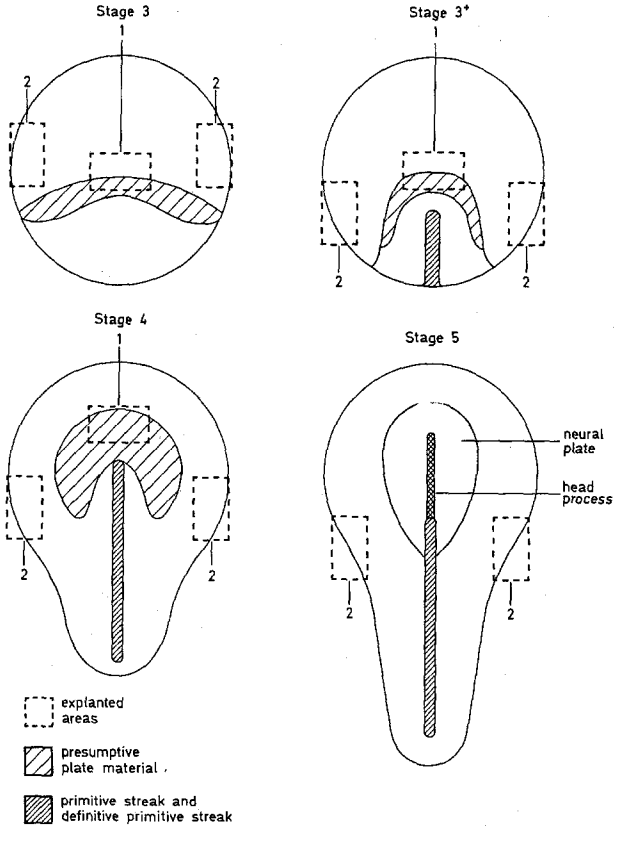

Fig. 1. Stages 3–5. Explanted areas from presumptive neural plate at stages 3–4 (area 1) and from presumptive epidermal ectoderm at stages 3–5 (area 2).

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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Table I. Explants made from presumptive epidermal ectoderm at stage 4. The amount of NGF added to the culture medium and the time that NGF was present in it are indicated. Fraction of cultures producing neural antigens.

Addition to the culture medium	The time that NGF was present in the culture medium	Results (%)
About 100 BU of NGF	Day 1	12/23 (52)
	Days 1–2	12/23 (52)
	Days 1–4	16/23 (70)
	Days 1–8	18/22 (82)
	Days 2–8	7/22 (32)
About 10 BU of NGF	Days 1–8	11/25 (44)
Without NGF	—	9/28 (32)

Table II. Explants made from presumptive neural plate at stages 3–4 (area 1) and from presumptive epidermal ectoderm at stages 3–5 (area 2). About 100 BU of NGF was added to each experimental culture. Controls were grown without NGF. Fraction of cultures producing neural antigens.

Stage of explantation	Explanted area	Control series series (%)	NGF-treated series (%)
Stage 3	Area 1	7/19 (37)	20/24 (83)
	Area 2	6/19 (32)	19/29 (66)
Stage 3+	Area 1	8/21 (38)	22/24 (92)
	Area 2	7/23 (30)	16/23 (70)
Stage 4	Area 1	9/25 (36)	22/24 (92)
	Area 2	9/28 (32)	18/22 (82)
Stage 5	Area 2	3/21 (14)	7/20 (35)

7 S NGF preparation used in the present investigation gave an optimum (4+) fibre response at a protein concentration of about 10 ng/ml. A biological unit (BU) would thus correspond to about 10 ng/ml of this NGF preparation, 1 BU/ml being arbitrarily defined as the NGF concentration in an assay system showing a maximum (4+) fibre response (cf. ref. 8).

Results and discussion. 574 cultures were examined; 119 of these, being necrotic, were discarded. Thus, 79% of the cultures were useful. Those cultures containing neural antigens showed fluorescence mostly in large areas of the culture. As Table I shows, the addition of about 100, but not 10, BU of NGF to the culture medium on the first day of incubation of the explanted ectoderm, taken at stage 4 from the presumptive epidermal region, markedly increased the frequency of neural antigen-producing cultures. The most pronounced effect of NGF was observed when it was present in the medium for the whole culture period, and gradually decreased when it was present for successively shorter periods. These data suggest that the most important mode of action of NGF in the present system is to support the development of cells neuralized by the conditions of the culture procedure and the culture milieu, rather than merely to influence directly the competent ectoderm, even though such a more immediate influence might also contribute to the NGF-induced effect. Thus NGF must be present in the culture medium from the first day of incubation and for more than 4 days onwards to permit the development of a maximum frequency of neural antigen-producing cultures. BJERRE[10] previously suggested a supporting or permissive mode of action of NGF in a similar in vitro system, anti-NGF serum being shown to inhibit in vitro the neural antigen production of chick explants during the neural induction and the early neuralization. Moreover, it was demonstrated that NGF stimulated, and its antiserum inhibited, the development of catecholamine-containing cells from young chick embryos in vitro, suggesting that NGF in this system primarly had a preserving role which permitted a development of catecholamine-containing cells that otherwise would not have survived[11].

The addition of about 100 BU of NGF to the culture medium, after the competent ectoderm taken at stage 4 had grown in vitro for 1 day, did not affect its capacity to produce neural antigen-containing cultures (Table I). Even if NGF in the present system had some effect on the presumptive epidermal ectoderm taken at stage 5, the frequency of neural antigen-producing cultures was low in this series compared with the series of explants taken from the presumptive epidermal ectoderm at younger stages (Table II). These results agree that the presumptive epidermal ectoderm, either developed in ovo or in vitro, rapidly loses its neural competence after stage 4.

The control series in Table II demonstrate a fairly high capacity of autoneuralization for the competent ectoderm (taken at stages 3 to 4) in the present system; i.e. about 1 out of 3 control cultures produce neural antigens. In addition, Table II shows slight differences in the frequency of autoneuralized cultures between the different regions of the ectoderm through stages 3 to 4. Thus the presumptive neural plate ectoderm has a slightly higher tendency for autoneuralization than has the presumptive epidermal ectoderm. With the presence of about 100 BU of NGF during the whole culture period, the frequency of neural antigen-producing cultures is markedly increased in all series of explants taken at stages 3 to 4. The stimulatory effect of NGF seems to be more or less the same in all these series, and the differences in the capacity of autoneuralization between presumptive epidermal and presumptive neural plate ectoderm seem to be reflected also after NGF-treatment. Thus the presumptive neural plate ectoderm has a higher capacity than the presumptive epidermal ectoderm to form neural antigen-producing cultures also when treated with NGF[12].

Zusammenfassung. Mit einer Immunofluoreszent-Technik wird gezeigt, dass der neurale Wachstumsfaktor in vitro sowohl auf kompetentes Ectoderm wie auch auf die eigentliche Region der Neuralplatte einen stimulierenden Einfluss ausübt, indem mehr neurale Zellen entstehen.

B. BJERRE and L. NORD

Department of Anatomy and Tornblad Institute, University of Lund Biskopsgatan 7, S–223 62 Lund (Sweden), 6 March 1973.

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[12] Supported by grants from the Faculty of Medicine, University of Lund and from the Swedish Medical Research Council (grant No. 12X-3926-01).