Dispersed Cell Culture of Bovine Pars Intermedia[1]

Because of the complexity of the controlling elements and their interrelated effects in the whole animal, we have attempted to establish an in vitro system to study the mechanism by which differentiated functions within a single type of cells are controlled. Functional clonal strains of several endocrine cell types have been established in culture. These investigations include steroid-secreting Leydig cells and steroid-secreting adrenal cells[2,3]. In culture the pituitary tissue displays the ability to secrete hormones. BRAUMAN et al.[4] have presented evidence that growth hormone can be detected in medium from organ cultures of fetal human pituitary gland. YAMARA[5] established a clonal strain of pituitary cells that synthesize and secrete ACTH. Clonal strain of cells derived from a rat pituitary tumor secretes growth hormone into the culture medium[6]. The goal of the present experiment is to conduct such a study utilizing dispersed cell culture from bovine pars intermedia and to evaluate the degree of specialization by its ability to synthesize the melanocyte-stimulating hormone (MSH).

Pituitary glands were taken from up to 4-month-old calves. The pars intermedias were placed immediately in an Erlenmeyer flask containing cold nutrient medium. The medium consisted of 75% Evans NCTC 109. Single primary culture in rubber-stoppered plastic flasks was prepared involving cutting and mincing of the tissue, digestion with 0.25% trypsin and suspension of the digested and washed cells in the nutrient medium. Synthetic culture medium was suplemented with fetal and calf serum. The cells were incubated at 37 °C in humidified atmosphere of 5% CO_2 and 95% air. Subcultures were prepared by trypsinization of half of the cells in the primary dispersed culture after 30 days in vitro in a rubber-stoppered plastic flask culture. Media from subcultures were changed every 3-4 days. With progress of culture age the explant showed extensive propagation from the central region toward the periphery. Small cells containing oval nuclei were present in the central area. In the periphery there were large cells with irregular borders (Figure 1). The cytoplasm of the cells is homogeneous and poor in density. It contains granular elements and small vacuoles. The most peripheral cells showed large accumulation of lipid dropplets, indicators of cellular 'senescence'. In summary, the trypsin-dispersed cells observed up to 32 days after subcultures maintain the epithelioid lineage and their cytological characteristics resemble those of the glandular cells in vivo (Figure 2). The preservation of these cells in culture may be explained by the fact that pars

No. of days after subcultures	Medium MSH (Units/ml) Exp. A	Exp. B	Exp. C
9	63	79	78
14	127	128	105
25	78	148	142
32	99	90	138

Data represents the mean values of total hormones in the media collected each 3 or 4 day period. Experiments A, B and C are different subcultures.

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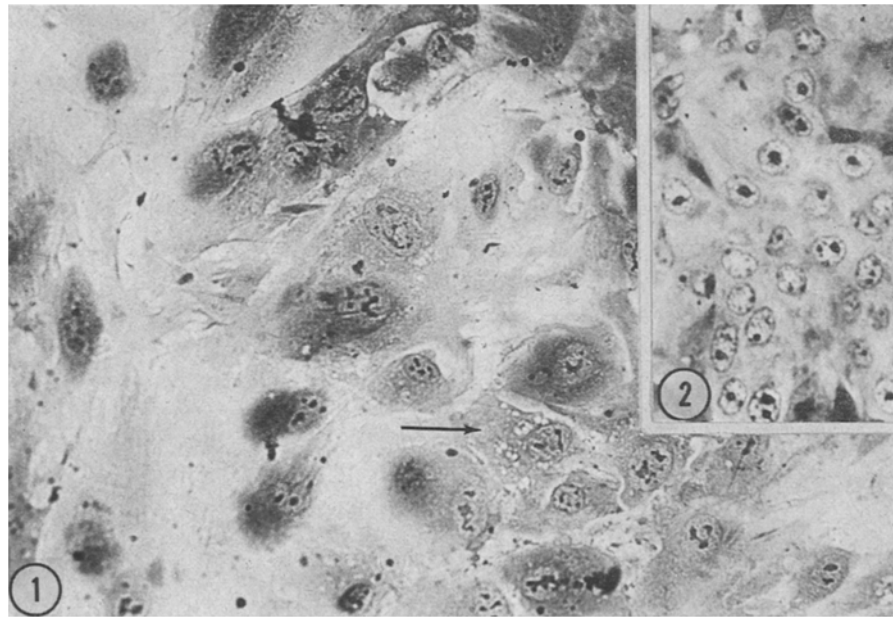

Fig. 1. Explant from bovine pars intermedia. Appearance of cells 32 days after subculture. The nuclei are ovoid. Arrow indicates a cell showing small vacuoles in the cytoplasm. Fixed in Bouin and stained with 1% toluidine-blue. ×700. Fig. 2. Thick section (lum) fixed in Karnovsky and stained with toluidine blue of a portion of in vivo bovine pars intermedia. The glandular cells show a round and light nucleus with evident nucleolus. The other type is elongated and dense. Compare the cells in culture with the glandular cells in vivo. ×400.

intermedia is an epithelioid tissue with a little blood supply normally inhibited by the central nervous system. This inhibition is absent in vitro.

Organ specific function of cells in culture was determined by examination of the media for a characteristic product of the tissue from wich the cells derived. With this purpose the media from subcultures were tested on the skin of *Rana pipiens* frog using the reflectometric method of LERNER and WRIGHT[7] in order to assay the MSH activity. Since the hormone was accumulating in the culture medium during 3 or 4 days between changes, the values given for the concentration of hormones in the medium represent the average of different collection period (Table). The results presented seem to be consistent with the small amount of MSH synthesized even in multiplying cells in vitro, and suggest that they retain the specific ability to elaborate the hormone. Using this pattern, different aspects of the regulation of pars intermedia cells by humoral factors can be studied.

Resumen. Células de pars intermedia bovina, dispersas por tripsina y cultivadas por 60 dias mantienen las caracteristicas morfológicas del tipo glandular descripto in vivo. Los medios muestran actividad melanocito-dispersante sobre la piel de *Rana pipiens*. Estos resultados sugieren que las células cultivadas retienen la específica capacidad de sintetizar MSH.

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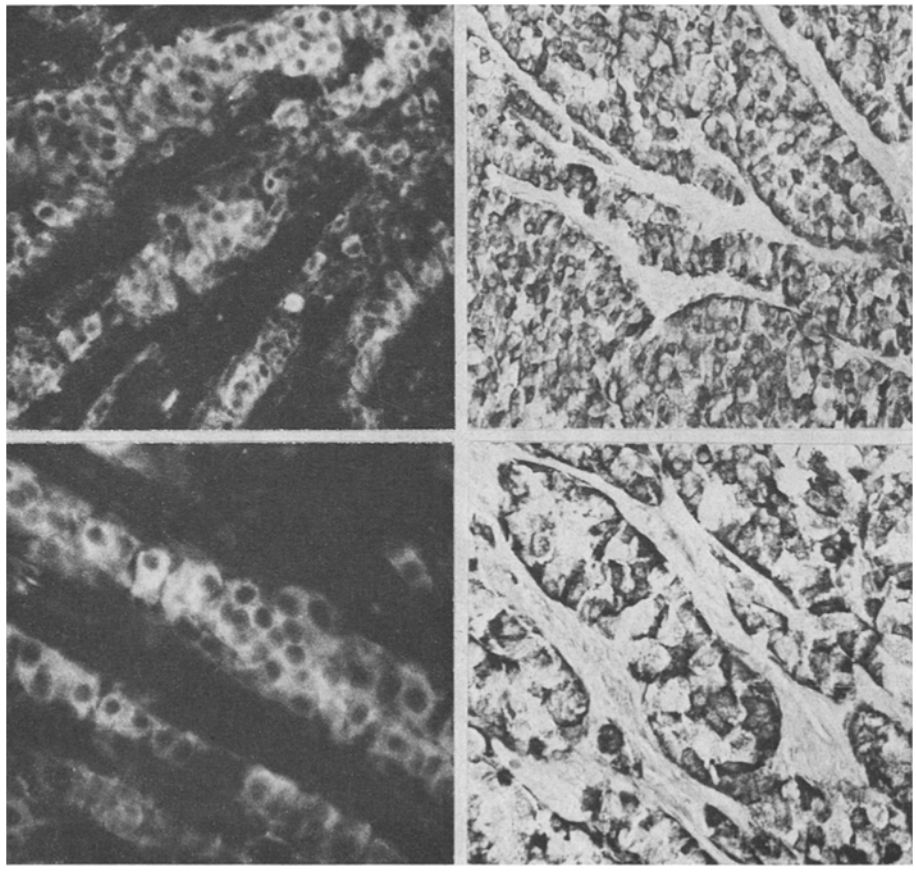
Immunohistochemical Demonstration of Glucagon in an A_2-Cell Carcinoma

Glucagonoma, i.e. tumours arising from pancreatic A_2-cells constitute a rare type of endocrine adenoma, usually associated with diabetes mellitus[1–5]. Such tumours sometimes occur as part of the polyglandular adenoma syndrome[6].

In 1969 GRIMELIUS et al.[7] described an islet cell carcinoma in a 62-year-old woman, who suffered from episodes of hypoglycemic coma and had extremely low fasting blood glucose values. A small tumour was observed in the tail portion of the pancreas with several metastases in the regional lymph nodes and in the liver parenchyma. Most tumour cells were stained with the Grimelius and Bodian silver staining methods but did not stain with that of Davenport. The cells did not stain with aldehyde fuchsin nor did they show metachromasia with the pseudoisocyanin method. Further, the cells gave no argentaffin reaction with the technique of Masson-Hamperl. These results indicated that the tumour cells were of the A_2-type.



Left: Immunofluorescence of glucagon in most cells of a glucagon-producing tumour in human pancreas. Right: Silver staining showing most tumour cells to be argyrophil (Grimelius technique). Top: ×270, bottom: ×440.