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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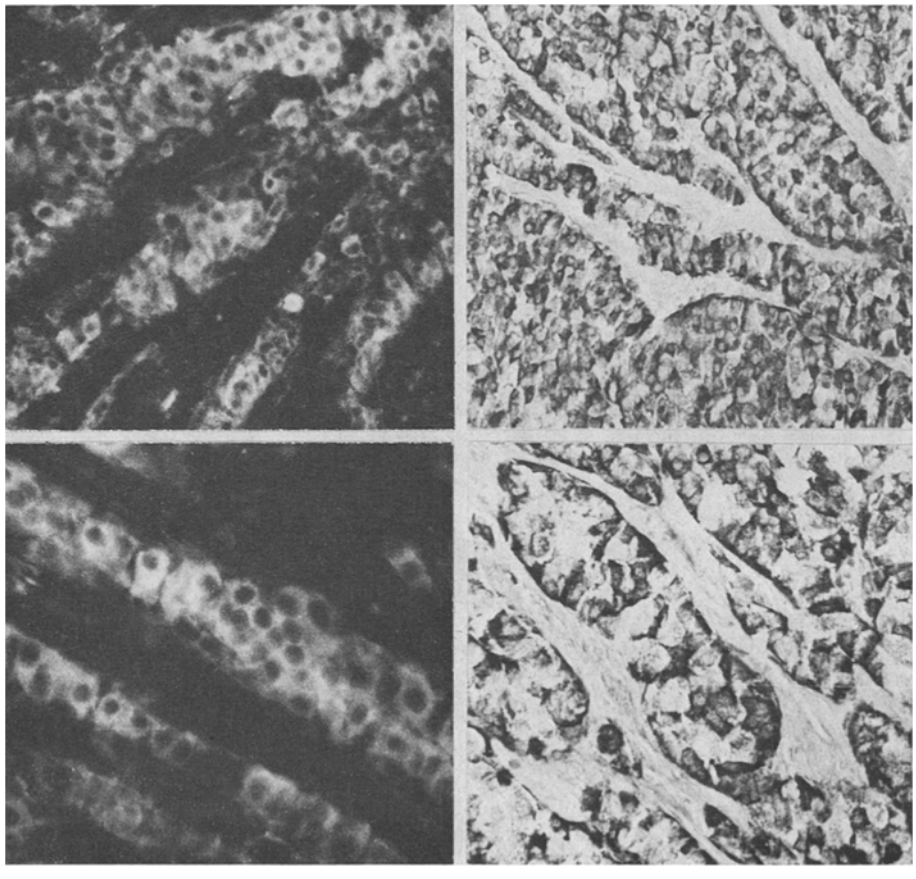
Immunohistochemical Demonstration of Glucagon in an A_2-Cell Carcinoma

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.

Radioimmunoassay of glucagon showed high concentrations of this polypeptide hormone in the metastases. The tumour itself was not assayed for glucagon.

The present report describes the immunohistochemical demonstration of glucagon in the cells of the tumour as well as in the metastases. Paraffin sections were cut at 4–6 μm thickness from specimens fixed in 10% formalin and paraffin-embedded several years earlier. The sections were deparaffinized in xylene and hydrated through graded ethanol solutions. They were rinsed in 0.9% saline (buffered to pH 7.2 with 0.01 *M* phosphate buffer) and subjected to an indirect immunofluorescence method[8] for the demonstration of glucagon. Antisera to pancreatic glucagon were raised in rabbits employing purified porcine glucagon covalently coupled to bovine serum albumin. One of these antisera, used in routine radioimmunoassay of serum glucagon in dilution 1:500, was used undiluted for the first layer. After 30 min incubation at room temperature, the sections were rinsed in several changes of buffered saline. The sections were then exposed for 30 min to a second layer consisting of anti-rabbit-globulin from goat conjugated with fluorescein isothiocyanate (Miles) and diluted 1:10. The sections were again rinsed in buffered saline and mounted in glycerine, buffered to pH 7.2. Some of the sections were counterstained with Evans blue (0.1% in buffered saline, pH 7.2, for 2–3 minutes) in order to improve contrast. Control sections were treated identically except for: a) omitting both layers; b) omitting the second layer; c) letting the antiserum react with glucagon (10 μg/ml) before applying it as the first layer; d) replacing the first layer with normal rabbit serum. The specimens were examined in a fluorescence microscope, equipped with a Schott BG12 as primary (lamp) filter and a Schott OG4 as secondary (barrier) filter.

Most epithelial cells of the primary tumour showed specific reaction with the glucagon antiserum (Figure). Controls were negative. The tumour cells, often showing a trabecular, carcinoid-like growth pattern, had a varying intensity of immunofluorescent staining. Some cells, especially those peripherally located in the trabeculae, were intensely fluorescent, whereas the centrally located cells often showed lower fluorescence intensities. When the sections were restained according to GRIMELIUS[7], all the immunofluorescent cells were found to be argyrophilic. In the primary pancreatic tumour the degree of argyrophilia correlated well with the content of immunohistochemically demonstrable glucagon. A certain discrepancy was noted, however, in a hepatic metastasis. Although most cells were argyrophilic, only a few of them, situated mainly at the periphery, showed immunofluorescent staining for glucagon.

In 1969 LOMSKY et al.[9] described glucagon immunofluorescence in an islet cell adenoma but to our knowledge this report is the first to describe the immunohistochemical demonstration of glucagon in a pancreatic A_2-cell carcinoma and in its metastases[10].

Zusammenfassung. Glucagon ist durch Immunohistofluoreszenz in einem A_2-Zell-Carcinom der menschlichen Bauchspeicheldrüse und in Metastasen in der Leber entdeckt worden.

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Histochemical Study of Dissociated Nerve Cells from Embryonic Chick Cerebral Hemispheres in Flask Cultures

In a previous study we have demonstrated that dissociated nerve cells from young embryos can grow and differentiate when they are cultivated in plastic flasks without a semi-solid substrate as support[1]. Flask cultures offer advantages for biochemical analysis. In the present investigation we have extended our observations of neuronal maturation, using some tests of histochemical reactions.

Cerebral hemispheres from 8-day-old chick embryos were dissociated and cultivated in Falcon plastic 3012 flasks (30 ml) without collagen, as previously described[1]. The nutrient medium consisted of Eagle's basal medium supplemented with 20% fetal calf serum and contained the antibiotics penicillin (50 units/ml) and streptomycin (50 μg/ml). The medium was changed twice a week. The cell cultures were incubated at 37 °C in an atmosphere of 95% air and 5% CO_2. After 2 weeks incubation the cultures were fixed for 20 min at room temperature in neutral buffered formol solution for histochemical analysis. Some were stained with 0.1% thionine to test for Nissl substance. Others were tested for AChE activity by the method of KARNOVSKY and ROOTS[2]. Another series was

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