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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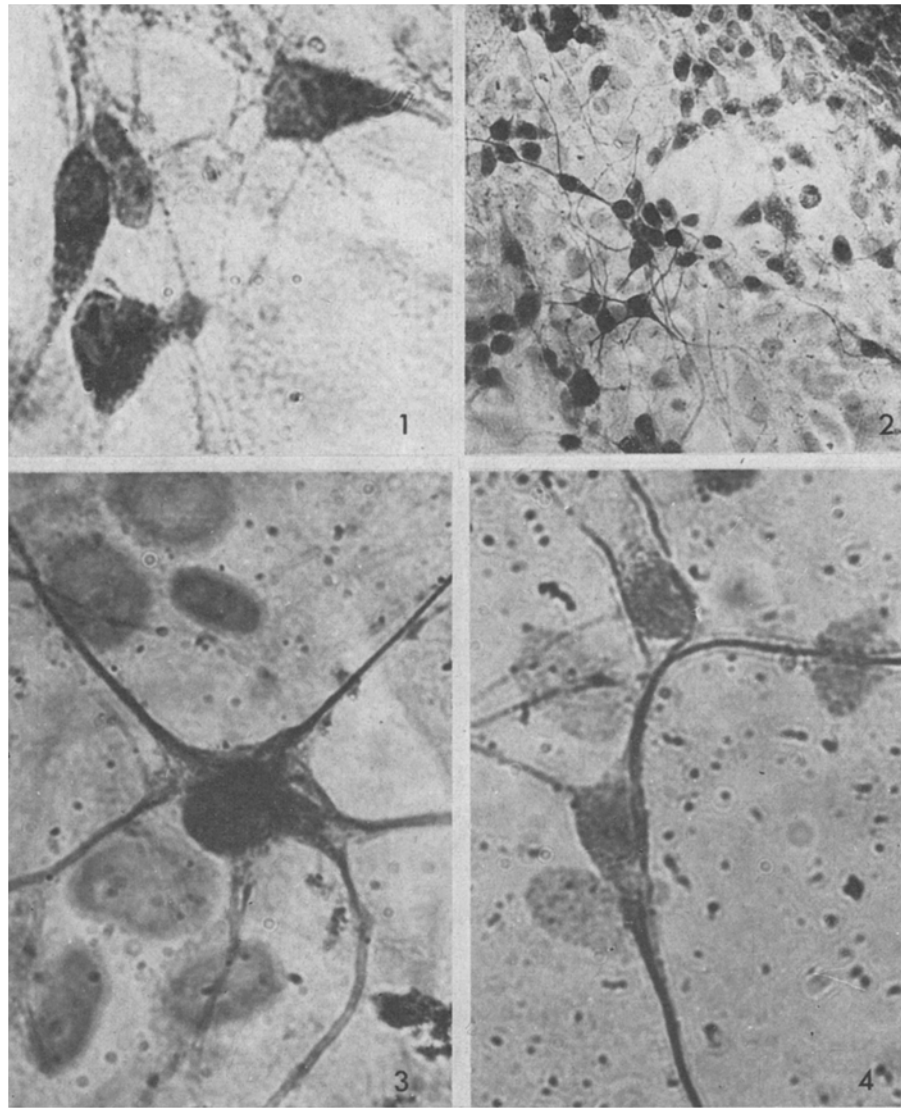

Fig. 1. Nissl bodies in multipolar and bipolar neurons 14 days in culture. Thionine stain. ×1000. Fig. 2. A group of neurons and their fibres 14 days in culture. Holmes method. × 250. Fig. 3. A multipolar neuron with neurofibrils in the soma 14 days in culture. Holmes method. ×1000. Fig. 4. A neuron without neurofibrils in the soma 14 days in culture. Holmes method. ×1000.

fixed for 1 week at 4 °C and impregnated with silver by the modified Holmes method for neurofibrils[3].

From a phase contrast study[1], we had previously shown that dissociated brain cells from 8-day-old chick embryos, which were still in an undifferentiated morphological stage, reaggregated in clumps after 24 h cultivation in plastic flasks. The cells eventually began to migrate and to proliferate. Multipolar and bipolar neuronal-like cells differentiated after 7 days in culture and were dispersed upon a monolayer of polygonal-shaped undifferentiated cells.

After 2 weeks cultivation in plastic flasks, most multipolar and bipolar cells contained Nissl bodies in their perikaryon (Figure 1), indicating that these cells are neurons. In older cultures, increased amounts of Nissl substance could be demonstrated. This shows a further differentiation of these nerve cells.

Silver impregnation revealed a network of nerve fibres throughout the cultures after 2 weeks in vitro (Figure 2). Large multipolar neurons containing densely stained neurofibrils within the soma and fibres were observed (Figure 3). Also bipolar neurons, which contained neurofibrils, were seen in these cultures. However, many neurons had slightly stained somas but long, well-developed, extensively branched processes (Figure 4).

Many AChE-containing neurons were observed after 7 days in culture. Later, the activity increased and, after 2 weeks cultivation, a high AChE content was present in the cytoplasm of the soma of most neurons (Figure 5). However, very little AChE activity could be demonstrated in the fibres.

In a previous paper we had shown that embryonic brain extract stimulates the differentiation of CNS neurons cultivated in Rose chambers[4]. Therefore, in some of the flask cultures we added brain extract prepared from 8-day-old chick embryos. The suspension homogenate was centrifuged at 105,000 × g for 1 h. The supernatant

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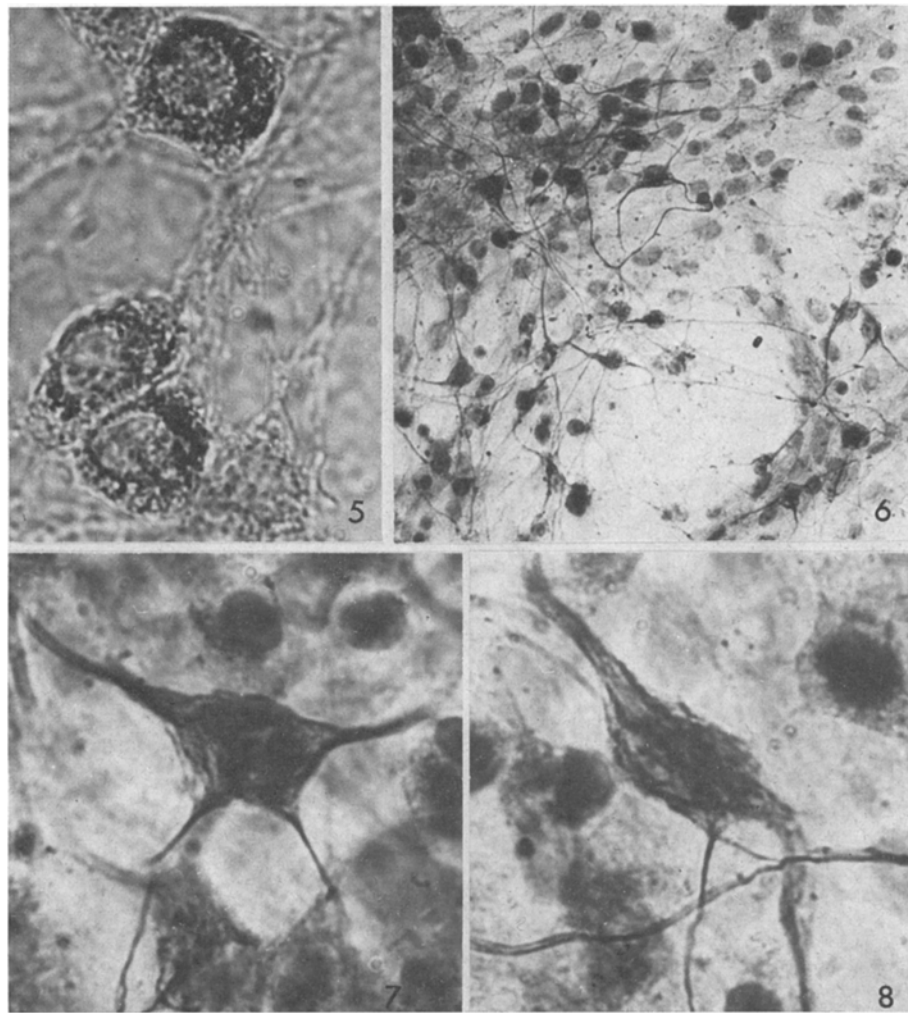

Fig. 5. Neurons showing AChE activity 14 days in culture. ×1000. Fig. 6. A group of neurons and their fibres 14 days in culture in presence of brain extract. Holmes method. × 250. Fig. 7 and 8. Neurons with neurofibrils in their soma; 14 days in culture in presence of brain extract. Holmes method. ×1000.

was then added after the 4th day of cultivation to the nutrient medium as previously described[4]. Differentiation of the nerve cells cultivated in flasks seemed to be influenced in a similar manner to cultures grown in Rose chambers by the brain extract. We could observe after 14 days cultivation a very dense network of thick nerve fibres (Figure 6). Almost all the differentiated neurons possessed a dense concentration of neurofibrils within the soma as well as in their processes (Figures 7 and 8).

Histochemical demonstration of Nissl substance, neurofibrils and AChE activity provides evidence for good differentiation of neurons cultivated in plastic flasks. Addition of brain extract to the nutrient medium stimulates this differentiation[5].

Zusammenfassung. Die Differenzierung von dissoziierten Grosshirnneuronen in Plastikflaschenkulturen wurde mit histochemischen Methoden, Nisslfärbung, Silberimprägnation der Nervenfasern und Acetylcholinesteraseaktivität untersucht.

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Explantation of Extraembryonic Parts of 7-day-old Mouse Egg Cylinders

Explantation of embryonic portions of the mouse egg cylinder leads to formation of teratomas and/or teratocarcinomas composed of somatic tissues[1,2]. The developmental potentials of the extraembryonic part (EP) of the mouse egg cylinder were not tested, notwithstanding the unsuccessful attempt to cultivate the parietal yolk sac from somewhat older embryos[3]. The purpose of this study was to determine what happens to the extraem-