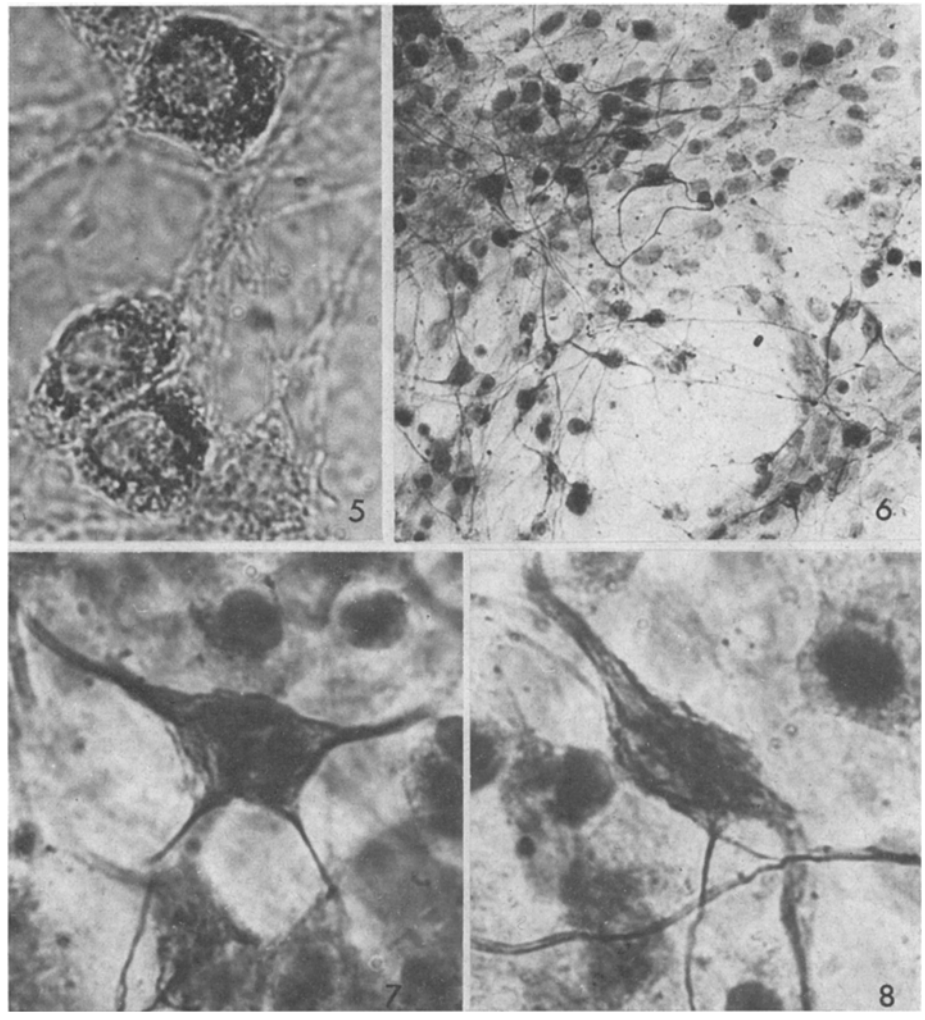

Fig. 5. Neurons showing AChE activity 14 days in culture. ×1000. Fig. 6. A group of neurons and their fibres 14 days in culture in presence of brain extract. Holmes method. × 250. Fig. 7 and 8. Neurons with neurofibrils in their soma; 14 days in culture in presence of brain extract. Holmes method. ×1000.

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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[5] Acknowledgments. This work was supported in part by the 'Actions Thématiques sur Programmes: Différenciation Cellulaire' No. 4112.

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

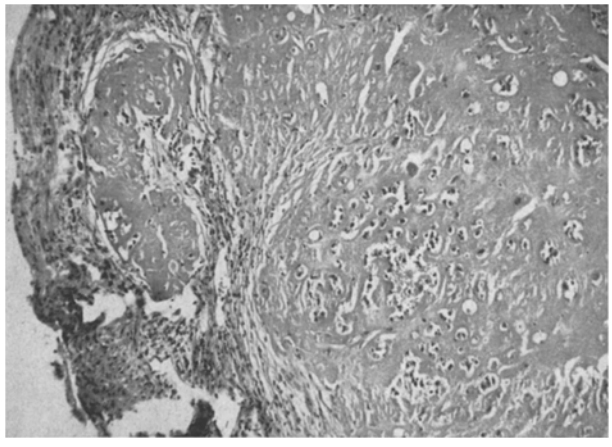
Explantation of embryonic portions of the mouse egg cylinder leads to formation of teratomas and/or teratocarcinomas composed of somatic tissues[1,2]. The developmental potentials of the extraembryonic part (EP) of the mouse egg cylinder were not tested, notwithstanding the unsuccessful attempt to cultivate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

bryonic portion of the 7-day mouse egg cylinder grafted under the kidney capsule.

Seven-day-old egg cylinders were isolated from uterine swellings of pregnant C3H/H mice under the dissecting microscope and bisected into two portions, corresponding to the embryonic and extraembryonic part, and stripped of the parietal entoderm together with the Reichert's membrane. The cutting was done deep into the extraembryonic portion in order to completely remove the embryonic part of the egg cylinder. The cleaned EP was thereafter transplanted under the kidney capsule of isogeneic adult mice with a pipette. The animals with the grafts were sacrificed 1 month after the operation, the grafts were recovered and serially sectioned for histological examination, on slides stained with haematoxylin and eosin.

Twenty grafts were performed and all were recovered except two. Underneath the kidney capsule of recipients a tiny grayish disc, measuring up to 2 mm in diameter was found at the site of transplantation in all the 'positive' animals.

Histologically all the grafts had essentially the same basic appearance. The most conspicuous finding was a hyalin containing tissue almost identical with the so-called murine yolk sac carcinoma[4,5]. The only difference between the yolk sac carcinoma of mice and the tissue found in our grafts was that the cells in the latter were more uniform, smaller and showed no tendency for proliferation. No mitoses could be found, and many cells had pycnotic nuclei. Focally large portions of hyalin and adjecent cells were calcified. Besides these hyalin-forming cells and the extracellular hyalin, some grafts showed cysts containing cellular dentritus or blood and areas composed of granulation tissue. It was not possible to determine whether this vascular connective tissue was part of the graft or the hosts reaction to it. The only graft that did not contain hyalin-forming cells was nevertheless composed of vascular connective tissue. In the connective tissue of 1 graft, there was a nidus of cartilage and 2 grafts contained small pearls of squamous epithelium. No other differentiated tissue was recognized.



Portion of a graft composed predominantly of hyalinforming cells surrounded by it. To the left can be seen connective tissue, which might be also the thickenned renal capsule. H.E. × 160.

This study has shown that explanted cells of EP can survive in favorable extrauterine sites. In addition to this they are able to secrete a hyalin material, similar to the extracellular material found in yolk sac carcinomas of mice. PIERCE et al.[3–5] have given ample proof that this extracellular hyalin in the yolk sac carcinoma corresponds to the epithelial basement membrane of Reichert, which is normally produced by the cells of the parietal entoderm. Morphological similarity between the appearance of our grafts and the yolk sac carcinoma of PIERCE et al.[3–5] indicates that the cells of the extraembryonic portion of the egg cylinder produce the same hyalin as these tumor cells and/or their so-far claimed anatomically normal equivalent, i.e. the cells of the parietal entoderm.

In contradistinction to yolk sac carcinoma cells, the hyalin producing cells obtained from EP grafts showed no tendency to proliferate. The cells of the extraembryonic part of the egg cylinder differ thus from the cells from the embryonic portion, as they never displayed a propensity for proliferation as was observed with explanted embryonic portions of the egg cylinder[1,2]. The comparison between the cells of EP and the explanted trophoblastic tissue[6], since they have evidently a similarly limited life span and capacity for proliferation in extrauterine sites, might be in order.

The finding of connective tissue in the grafts is an indication that the transplanted EP might differentiate in this direction also, but a reaction of the host or inadvertant transplantation of the portions of the embryonic part of the egg cylinder cannot be excluded with certainty. The latter is possibly the only correct explanation for the finding of squamous epithelium in two of the grafts examined.

Zusammenfassung. Von 7 Tage alten Mäuseembryoanlagen wurden extraembryonale Teile der Gewebszylinder entnommen und unter die Nierenkapsel erwachsener isogenetischer Tiere transplantiert, wo sich 1 Monat später kleine Tumorknötchen bildeten, welche jedoch keine Wachstumstendenz zeigten. Es wird auf den Begriff des Dottersackcarcinoms und der Reichertschen Membran eingegangen.

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20 November 1972.

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[7] This work was supported in part by PL-480 grant No. 02–038–1 from the National Institute of Health and by funds obtained from the Council for Scientific Affairs of the Socialistic Republic of Croatia.