Histochemie 9, 78—83 (1967)

HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE CELLS OF THE HUMAN FOETAL ADRENAL AND HYPOPHYSIS IN TISSUE CULTURE

A. GYÉVAI, E. STARK and K. SZ. SZALAY

Department of Morphology and Pathophysiology, Research Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Received December 19, 1966

Summary. The histological and histochemical changes taking place in explants and outgrowing cells of the human foetal adrenals and hypophyses have been studied on the 5th, 10th, 20th, and 28th day of the culture period. In the outgrowth from adrenals, both the epithelial-type and the fibroblast-type cells contain 3β-ol steroid dehydrogenase activity. The cells growing out from hypophyseal explants appear to be morphologically undifferentiated. Explants contain differentiated cells in the first 10 to 13 days of the culture period.

In previous tissue culture experiments we have supplied evidence that the adrenal of the human foetus continues to secrete corticosteroids, and the hypophysis to secrete ACTH, for 10 to 13, and in rare cases up to 28, days (STARK, GYÉVAI, SZALAY, ÁCS, 1965). The present work describes histological and histochemical studies of explants of human foetal adrenal and hypophysis cultured for different periods. The published histological studies refer to the adrenals and the hypophysis of animal species other than man (GUILLÉMIN and ROSENBERG, 1955; KAHR, 1966; MARTINOVITCH, 1953; SCHAEBERG and DE GROOT, 1958).

Materials and Methods

The adrenals and the hypophysis of 83 human foetuses of either sex measuring 16 to 36 cm in crown-rump length, derived from spontaneous or surgical abortions, were aseptically removed, washed with Tyrode's solution, and cut into fragments 1 to 2 mm across. In one series of experiments, adrenal fragments and hypophyseal fragments were cultivated separately on top of coagulated chicken plasma and chicken embryo juice on a glass surface, and the glasses were placed into Leighton tubes. To some adrenal fragments ACTH was added (20 mU/2 ml). In another experimental series, adrenal and hypophyseal fragments were explanted together in the manner just described. The Leighton tubes contained in both series 2 ml of a culture medium consisting of a 9:1 mixture of Parker 199 and human serum, with 400 U of penicillin added to it per ml. The cultures were kept at a temperature of 37°C and the medium was exchanged daily. Five, 10, 20, and 28 days after explantation, the adrenal and hypophyseal fragments were explanted together in the manner just described. The Leighton tubes contained in both series 2 ml of a culture medium consisting of a 9:1 mixture of Parker 199 and human serum, with 400 U of penicillin added to it per ml. The cultures were kept at a temperature of 37°C and the medium was exchanged daily. Five, 10, 20, and 28 days after explantation, the adrenal and hypophyseal fragments were fixed with Helly's fluid, embedded in paraffin, cut into sections of 5 to 7 μ thickness, and stained with methyl-green-pyronine. Outgrowth was stained according to May-Grünwald-Giemsa. Adrenal cultures were assayed for 3β-ol steroid dehydrogenase, succinic dehydrogenase, and non-specific esterase (WATTENBERG, 1958; PEARSE, 1960).

Observations

Adrenal Cultures. Growth was of the epithelial type in the first four or five days (Fig. 1). Mitosis was common in the outgrowing cells. After the fifth day, the explants began to die off, and less and less intact cells were seen in them (Fig. 2). As the cultures grew older, the number of cells of the fibroblast type increased, forming several layers anf fixing the explants (Fig. 3).
Enzymic activity was absent in the explants as early as the second or third day of cultivation, but the outgrowing cells exhibited activity to each of the three enzymes studied. In the first five days or so, this was intense in the epithelial-type cells (Fig. 4). The cells of fibroblast type, which appeared later, also gave positive reactions with the three enzymes (Fig. 5). In these, activity seemed to be less intense than in the epithelial-type cells of earlier appearance. 3β-ol steroid dehydrogenase activity was only seen in cells growing out from adrenals cultured in combination with hypophysis and in adrenal cultures to which ACTH had been added. In some cultures the fibroblasts showed 3β-ol steroid dehydrogenase activity for periods up to 28 days.