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HYDROCORTISONE MODULATES THE PRODUCTION OF EXTRACELLULAR MATERIAL AND ALBUMIN IN LONG-TERM COCULTURES OF ADULT RAT HEPATOCYTES WITH OTHER LIVER EPITHELIAL CELLS.

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SUMMARY : Adult rat hepatocytes were cocultured with other liver epithelial cells in serum-free medium with or without hydrocortisone. In the presence of hormone hepatocytes survived for several weeks and an abundant extracellular material was produced. The amounts of secreted extracellular material were hormone dose-dependent. In the absence of hydrocortisone a number of hepatocytes were altered or dead as soon as day 8, and only low amounts of extracellular material were secreted. The amounts of secreted albumin paralleled those of extracellular material. Hydrocortisone could act by exerting a permissive effect at the plasma membrane level in cocultured cells.

A number of works support the concept that hepatocytes adapt to in vitro environmental conditions by losing partly or totally many differentiated functions within a few days of culture. In a recent study we have shown that rat parenchymal cells remained capable of producing high albumin levels for several weeks when cocultured with another liver cell type. In this coculture system an abundant extracellular material was rapidly secreted (1).

Corticosteroid hormones exert various effects on several hepatic functions (2-6), including the synthesis of components of the extracellular matrix (7). Since these hormones have been reported to stimulate fibronectin synthesis in cultured hepatocytes and to decrease collagen production in the liver (8), the question arose whether hydrocortisone modulated the synthesis of extracellular material in our coculture system. We show that hydrocortisone has a dose-dependent effect on the accumulation of extracellular material in cocultures of adult rat hepatocytes with liver epithelial cells. The hepatocyte survival rate and the levels of secreted albumin parallel the amounts of accumulated material.

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MATERIAL AND METHODS

Cell cultures : Cocultures of adult rat hepatocytes with epithelial cells were performed as previously described (1). Briefly after their isolation by collagenase perfusion 1.5 x 10^6 hepatocytes suspended in 3 ml of Ham F_{12} medium containing 20 µg/ml of bovine albumin, 10 µg/ml of insulin and 10 % fetal calf serum were plated in 25 cm^2 culture flasks. Within 3 h, about 70 % of the seeded hepatocytes attached to the plastic. At this time, the medium was removed and 2 x 10^6 epithelial cells suspended in 3ml of serum-free medium were added. The epithelial cell line was derived from a 10-day old Fischer rat and was not yet transformed. Indeed the combination of hepatocytes with transformed liver epithelial cells resulted in only a minor improvment of hepatocyte survival rate and functions (manuscript in preparation). After 24 h, the time necessary to obtain cell confluency and every day thereafter, the medium with or without hydrocortisone hemisuccinate (Roussel France) was renewed. Various hormone concentrations ranging between 7 x 10^{-7} and 5 x 10^{-5}M were tested.

Albumin assay : Culture media were collected daily and stored at - 20°C. Albumin content was quantified by laser immunonephelometry (1).

Reticulin staining was performed according to the silver impregnation method described by Gordon and Sweets (9) after cell fixation with a mixture of 4 % paraformaldehyde - 0.2 % glutaraldehyde buffered with sodium cacodylate 0.1 M for 15 min at + 4°C.

RESULTS

Cell culture characteristics

In the absence of hydrocortisone, most of the cells did not survive for more than about 1-2 weeks (fig. 1a). After a few days hepatocytes appeared poorly spread and formed compact cell cords in which cellular boundaries and refringent structures corresponding to bile canaliculi were difficult to visualize. By day 6 a number of parenchymal cells exhibited cytoplasmic alterations consisting mainly in accumulation of dense granules and some cells began to detach.

In the presence of hormone cell survival rate was prolonged for several weeks (4 to 8 weeks). A dose-related response was observed. With 7 x 10^{-7}M hormone cell spreading was moderate and in places spaces corresponding to bile canaliculi were visible (fig. 1b). With hormone concentrations close to 1.4 x 10^{-6}M, hepatocytes were well-spread and maintained the typical aspect of granular epithelial cells with bile canaliculus-like structures for several weeks (fig. 1c). In the presence of hormone concentrations equal to or higher than 7 x 10^{-6}M, the cells were less spread, exhibited hypertrophied bile canaliculi (fig. 1d) and after day 8, some cells became necrotic. With 5 x 10^{-5}M hydrocortisone numerous parenchymal cells rapidly died.

Albumin secretion

In the absence of hydrocortisone the amounts of secreted albumin remained low and gradually declined after day 6. Differences were found

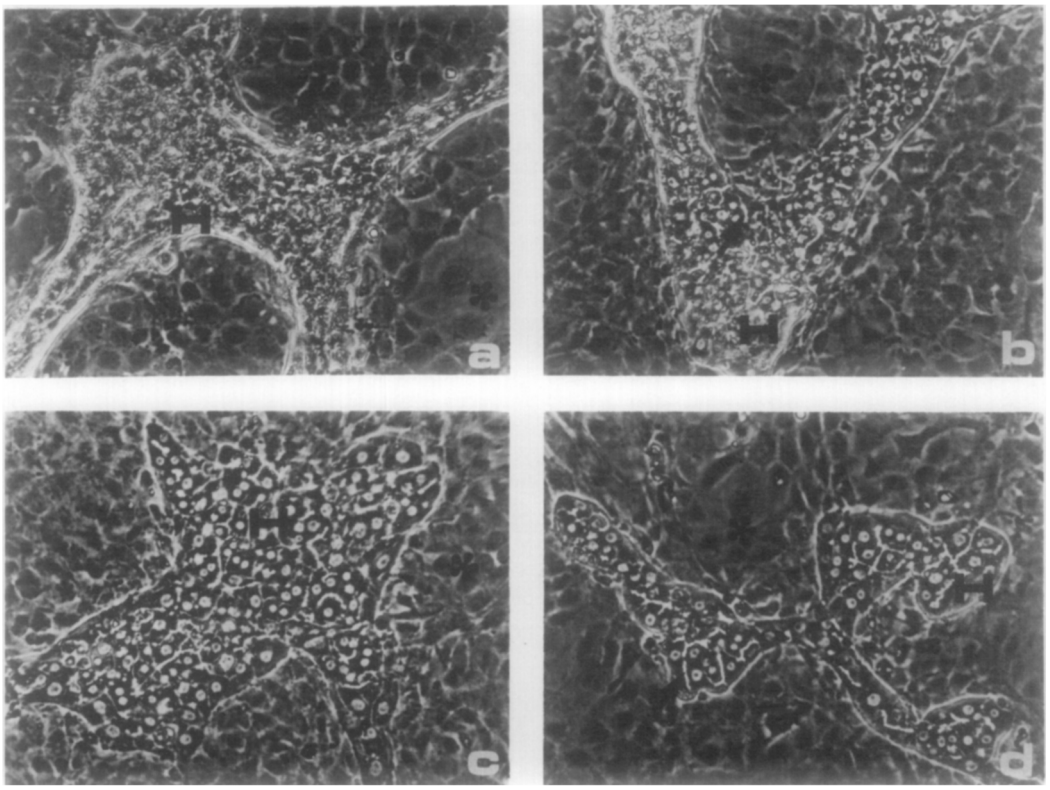

Fig. 1 Phase-contrast micrographs of adult rat hepatocytes (H) cocultured for 10 days with liver epithelial cells (*) in the absence (a) or presence of 7 x10^{-7} (b), 1.4 x 10^{-6} (c) or 7 x 10^{-6}M (d) hydrocortisone. In places, refringent intercellular spaces corresponding to bile canaliculi are visible (arrow) x 140.

according to the hormone concentration. The highest levels of secreted albumin were obtained with a concentration of 1.4 x 10^{-6}M hydrocortisone (fig. 2).

Extracellular material accumulation

Strong differences were found in the number and the distribution of reticulin fibers according to the presence or absence of hormone and to hormone concentration. In the absence of hormone, only a few fibers were secreted between the two cell populations and over hepatocyte cords. Most of the fibers were thick ; they could form bridges between hepatocyte groups. Rare fibers infiltrated epithelial cell areas (fig. 3a). In the presence of 7 x 10^{-7}M hydrocortisone both the number and the length of the fibers strongly increased. Mainly localized over and around hepatocyte cords, the fibers were also present in epithelial cell areas (fig. 3b). With 1.4 to 7 x 10^{-6}M hormone the number of argyrophilic fibers still increased and particularly

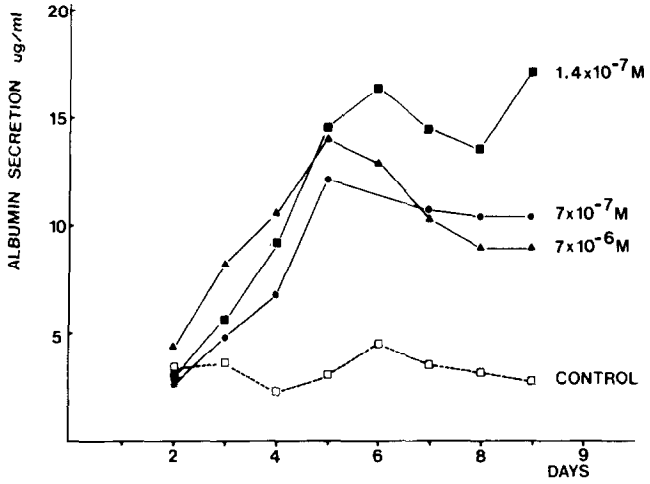

Fig. 2 Secretion of albumin in the medium of cocultures of rat hepatocytes with liver epithelial cells. The cells were cultured in the absence or presence of various hydrocortisone concentrations. The medium with or without hormone was renewed every day. Albumin release is expressed in µg/ml of medium/ 24 h of culture and the values are the average of triplicate experiments.

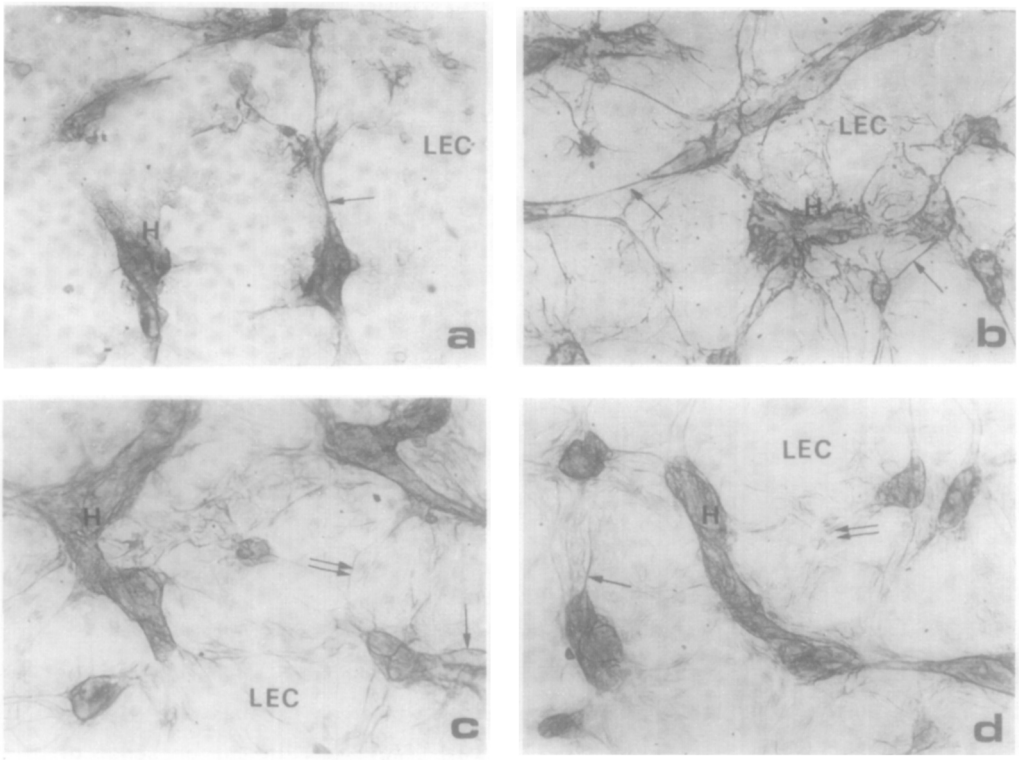

Fig. 3 Silver impregnation of primary cultures of adult rat hepatocytes (H) mixed with liver epithelial cells (LEC) on day 10. The cells were incubated in the absence (a) or presence of 7×10^{-7} (b), 1.4×10^{-6} (c) or 7×10^{-6} M (d) hydrocortisone. Thick (arrow) and thin (double arrow) reticulin fibers are observed. Some of them form bridges between different hepatocyte cords. x 80.

numerous thin fibers were seen over both hepatocyte cords and epithelial cells surrounding hepatocyte groups (figs 3c and 3d).

DISCUSSION

A number of reports have documented extension of cell viability and short-term modulation of several functions by corticosteroids in cultured hepatocytes (2-5). However although significant, these beneficial hormone effects were limited to a few days especially when parenchymal cells were incubated in serum-free medium. The findings described here demonstrate that actively albumin secreting hepatocytes might survive for several weeks when they were cultured in the presence of both hydrocortisone and another liver cell type. Glucocorticoïd hormones do not seem to regulate albumin synthesis (10) but are known to exhibit a permissive or synergic action toward various factors on several functions (4, 5). As cell contacts between the two liver cell populations were as a prerequisite for obtaining an active albumin secretion by adult rat hepatocytes, it could be postulated that hydrocortisone performed a permissive effect with other factors resulting in changes in plasma membrane properties and in production of an abundant extracellular material allowing long-term stability of hepatic functions.

Large amounts of reticulin fibers were secreted in coculture maintained in the presence of hydrocortisone. Preliminary analysis of the extracellular material secreted in these cocultures by immunofluorescence using specific antibodies has revealed that this material was composed of various collagen types, particularly type III collagen as well as of fibronectin (11). Fibronectin has already been shown to be increased in primary cultures of rat hepatocytes in the presence of dexamethasone (7). As reticulin fibers are mainly made up of various collagen types (12, 13) it is likely that hydrocortisone strongly increased collagen production in our coculture system. To our knowledge corticosteroids have not been reported to stimulate collagen synthesis in either primary hepatocyte cultures or liver epithelial cell lines and numerous works have shown that these hormones decreased collagen production in various tissues, including the liver and cultured non hepatic cell types (8). However in that case the mechanism of action of these hormones remains unclear ; they could be effective in a non specific fashion, e.g. in the liver by suppressing fibroblastic activity.

It is well established that both cultured hepatocytes (14-16) and liver epithelial cell lines (17, 18) may secrete collagen. The location of most of reticulin fibers close to hepatocyte cords strongly suggests that an important part of the extracellular material was secreted by parenchymal cells. Moreover hormonal dose-related difference in both the number and the distribution of the fibers could represent qualitative and quantitative

changes in the extracellular material and favor the idea that certain components could be as a prerequisite for obtaining in vitro stability of specific hepatic functions.

Finally, these observations emphasize the need of corticosteroids for culturing hepatocytes as well as the interest of the original coculture system associating two liver epithelial cell types for studying hormonal regulation of hepatic functions in relation to the presence of an extracellular matrix and the effects of various exogenous factors on the synthesis of the different components of this matrix.

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