

EFFECTS OF 17- β -ESTRADIOL AND OF GROWTH
HORMONE ON THE PRODUCTION OF ACID
MUCOPOLYSACCHARIDES BY HUMAN EMBRYONAL
FIBROBLASTS *IN VITRO*

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In recent years several reports have appeared in the literature regarding the ability of various cell types to produce acid mucopolysaccharides (AMPS) *in vitro*. At the same time, numerous observations have been made on humans and on experimental animals pointing out that the metabolism of AMPS is influenced by various hormones. Interesting information could, therefore, be gained by studying the production of AMPS by cells grown *in vitro* and fed with media supplemented by hormones. This note refers to observations made on replicate cultures of human embryonal fibroblasts exposed to 17- β -estradiol and to human growth hormone.

MATERIALS AND METHODS

Subcutaneous tissue isolated from a 9-week-old human embryo was minced with scissors and explanted on chicken plasma in T-30 flasks. Cells grew out rapidly exhibiting the morphology of fibroblasts. Subculturing was started 2 weeks after primary explantation, and replicate cultures were prepared every 10 days thereafter. Cell suspensions were prepared by trypsinization (trypsin 0.05 per cent, Versene 0.002 per cent in Moscona's saline solution). In subculturing, 1.25×10^6 cells suspended in 2.5 ml of medium were seeded in each of several T-30 flasks previously coated with an extremely thin layer of chicken plasma. The feeding solution consisted of: Eagle's HeLa medium (supplemented with glutamine) 50 per cent; whole egg ultrafiltrate 20 per cent; human placental serum 20 per cent; medium from the previous feeding period 10 per cent. Between transfers, the cultures were washed and fed twice. The control cultures were fed with the above medium, while for

the experimental cultures the following hormones were added to the medium: 17- β -estradiol, 0.1 $\mu\text{g}/\text{ml}$ and 0.4 $\mu\text{g}/\text{ml}$; human growth hormone, 20 $\mu\text{g}/\text{ml}$ and 100 $\mu\text{g}/\text{ml}$. All experimental and control groups of cultures were carried on through 3 transfers. Cells were counted with a hemocytometer. The quotient of the number of cells at the beginning and at the end of each culture period represented the growth increment (GI). The medium of all the flasks from each group of cultures were pooled for AMPS determination. The AMPS were extracted, using the method described by Prince and Castor (1), and their amount estimated as uronic acid (UA-AMPS) with the carbazole method of Dische (2). Glucuronolactone was used as standard. Cell-bound AMPS were not determined. The standard deviation calculated on 22 samples of glucuronolactone solution (10 $\mu\text{g}/\text{ml}$) was ± 0.891 . The amount of UA-AMPS present in fresh medium averaged 0.866 $\mu\text{g}/\text{ml}$ (± 0.179).

OBSERVATIONS AND COMMENTS

The cells exhibited constant morphological and behavioral features throughout this study. Seen with the light microscope they appeared as fibroblast-like cells (Fig. 1). Electron micrographs prepared by Dr. George D. Pappas of the Anatomy Department, College of Physicians and Surgeons, Columbia University, indicated that these cells were acceptable as fibroblasts. Degenerative changes were occasionally seen especially during the first 10 to 20 days after exposure to a new hormone or to a different hormonal concentration. Experiments in which the cultures exhibited degenerative changes were excluded.

The control cultures produced AMPS at a

reasonably constant rate throughout this study. Although the capacity for AMPS production by synovial cells may be decreased or lost after prolonged cultivation (3-6), fibroblasts from different sources have been reported to produce AMPS *in vitro* at a constant rate for long periods of time (7-9). Table I illustrates the relationship between GI and amount of UA-AMPS produced by four groups of control cultures. It is apparent that with these cells an increase in cell population is attended by an increase in UA-AMPS production. This is in agreement with the observation that in cultures of

with 17- β -estradiol with the GI of the control cultures, it appears that the hormone has caused a depression of the growth rate. Several reports in the past have indicated that estradiol exerts *in vitro* an inhibitory effect on the growth of various cell types (12-16). With the exception of an inhibitory effect exerted by estradiol on the incorporation of S³⁵ in cartilage and aorta of rats (17, 18), by and large the estrogens seem to induce an increase in AMPS of the ground substance of various organs (10, 19-25). It is important to note that in quantitative studies of AMPS produced

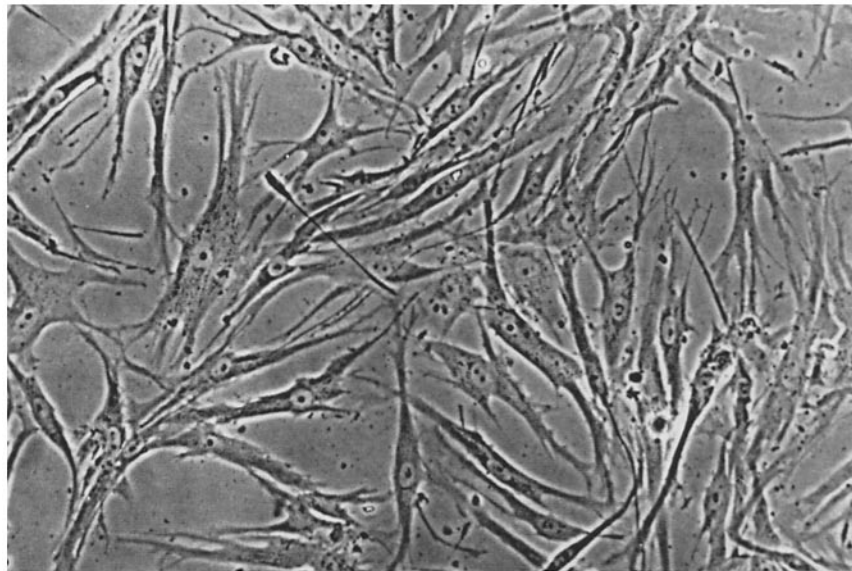


FIGURE 1 Living culture of human embryonal fibroblasts 20 hours after the 12th transfer. Phase contrast. $\times 140$.

rat fibroblasts there seems to be a direct over-all relation between rate of cell population increase and synthesis of AMPS (8, 9). It has also been observed that in the normal human breast the periodical accumulation of AMPS in the intralobular and periductal stroma coincides with an increase in number of fibroblasts (10).

The effects of 17- β -estradiol on the growth and on the AMPS production of these cells are summarized in Table II. According to Diczfaluzi and Magnusson (11), the mean value for *free* 17- β -estradiol in human cord blood is 3.6 $\mu\text{g}/\text{kg}$. Therefore, the addition of 0.1 γ of 17- β -estradiol per ml of medium represents approximately a 50-fold increase. Comparing the GI of the cultures treated

in vitro one is mainly concerned with the non-sulfated AMPS since it has been observed that cultures of human and rat fibroblasts and of human synovial cells produced predominantly non-sulfated AMPS (3, 8, 26, 27), and that the small amount of chondroitin sulfate produced by some of these cells is not fully sulfated (26). In the present experiments the cultures treated with 0.1 $\mu\text{g}/\text{ml}$ and 0.4 $\mu\text{g}/\text{ml}$ of 17- β -estradiol produced significantly greater amounts of UA-AMPS than the control cultures in spite of a much slower growth rate. It has been reported that in deteriorating cultures of synovial cells with decreasing cell populations the apparent rate of hyaluronate production is higher than that of healthy cultures (5).

In the experiments reported here the estradiol-treated cultures did not show any deterioration of the cells but only a depression of the mitotic activity. Furthermore, somewhat greater amounts of UA-AMPS were produced by those cultures with a less depressed GI.

The effects of the human growth hormone on

TABLE I
UA-AMPS Production by Control Cultures*

GI	UA-AMPS	UA-AMPS per unit of GI
	$\mu\text{g/ml}$	$\mu\text{g/ml}$
1.506	2.259	1.5
1.636	2.408	1.41
1.711	2.839	1.659
1.908	4.077	2.136

* Average values of pooled material from each group of control cultures.

TABLE II
Effects of 17- β -Estradiol and of Growth Hormone on Rate of Growth and UA-AMPS Production*

Hormone	GI	UA-AMPS per unit of GI	
		$\mu\text{g/ml}$	$\mu\text{g/ml}$
17- β -estradiol			
0.1	0.763	3.664	4.802
0.1	1.049	4.544	4.331
0.4	0.95	3.114	3.277
0.4	1.00	4.043	4.043
Growth hormone			
20	1.071	2.971	2.774
20	1.566	3.467	2.213
100	1.564	2.322	1.484
100	2.067	1.939	0.938

* Average values of pooled material from each group of experimental cultures.

these cells (Table II) are difficult to evaluate because of the very small differences between the behavior of the experimental cultures and that of the controls. The apparent lack of a stimulatory action on the GI is unexpected and is at variance with the increase in growth rate of human liver cells caused by growth hormone *in vitro* (28). The amounts of UA-AMPS produced by these fibroblasts when ex-

posed to growth hormone are small and very close to those of the control cultures. The values for UA-AMPS produced per unit of GI indicated that the AMPS production was just above the upper limits of the normal when the growth hormone was used at the concentration of 20 $\mu\text{g/ml}$ and just below the lower limits of the normal when it was used at the concentration of 100 $\mu\text{g/ml}$. This result is not entirely unexpected since growth hormone is known to produce an increase in uptake of S^{35} in cartilage (29-34), but its effects seem to be confined to sulfated AMPS (33, 35). As mentioned earlier, the AMPS produced by cell cultures are largely non-sulfated. Nevertheless, in spite of their smallness, the differences in AMPS production by the cultures fed with these two concentrations of growth hormone might indicate a trend to be revealed better by the use of smaller and larger doses of the hormone.

SUMMARY AND CONCLUSIONS

17- β -estradiol added to the medium (0.1 $\mu\text{g/ml}$ and 0.4 $\mu\text{g/ml}$) of replicate cultures of human embryonal fibroblasts elicited an increase in production of AMPS while depressing the growth rate of the cells. No noticeable effect on growth rate of these cells nor on their AMPS production was exerted by human growth hormone (20 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$).

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