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Some Effects of Desoxycorticosterone Glycoside on Chick Fibroblasts *in vitro.** (20254)

RONALD GILLETTE AND RALPH BUCHSBAUM.

From the Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa.

Although desoxycorticosterone is known to elicit a definite response from connective tissues when injected into the intact body, the effect of this substance upon connective tissues *in vitro* is comparatively little known. Adrenal cortical extracts have been reported by von Haam and Cappel(1), to prolong the survival of fibroblasts grown in Carrel flasks. Using desoxycorticosterone at concentrations of 0.03 mg/ml, Cornman(2) showed that there was a reversible inhibition of the beat of heart fragments taken from chick embryos; he also showed that fibroblasts are selectively damaged in mixed cultures of fibroblasts and endothelium grown in roller tubes. The investigation reported here was undertaken to study the effects of low concentrations of desoxycorticosterone on the rate of growth and the morphology of cells in tissue culture *in vitro.*

Materials and procedures. Stock cultures of connective tissue cells in a medium of chicken plasma and embryo extract were derived from explanted fragments of 7-day-old chick femurs. The cultures were cut into fragments as nearly equal in size as possible. One fragment was explanted into a medium consisting of equal parts of heparinized chicken plasma and chick embryo extract, the latter containing varying amounts of desoxycorticosterone glycoside.† The control fragment was explanted into the same medium without the desoxycorticosterone. The final concentrations of the desoxycorticosterone glycoside in the medium were: 30, 15, 7.5, 3.5, and 2 μg/ml. For each concentration about 20 pairs of cultures were made. Some other cultures were studied with this steroid at concentrations up to 240 μg/ml. The areas of outgrowth of the cultures were measured after 48 hours and the relative increases treated statistically(4). At the same time, photomicrographs were taken of cells in selected pairs of cultures to record morphological changes.

Results. Concentrations of desoxycorticosterone glycoside above 30 μg/ml were found to decrease the growth rate of tissue cultures of chick embryo fibroblasts. Below this concentration the steroid increases the rate of growth of fibroblasts. The stimulation of the growth rate of fibroblasts increases with decreasing concentration until a maximum stimulation is reached at 7.5 μg/ml. Below this concentration, the stimulation decreases until, at 2 μg/ml it is no longer measurable by these methods. The average differences in the relative growth of experimental and con-

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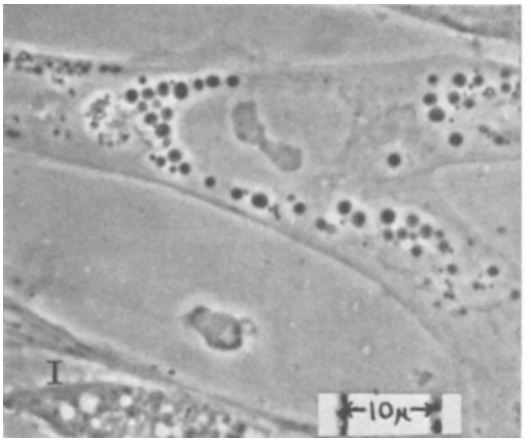

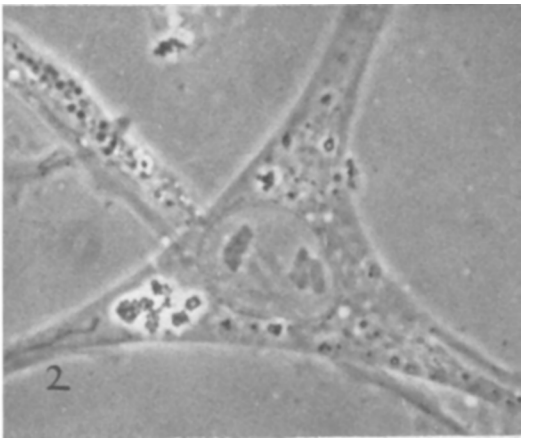

FIG. 1. Normal, untreated chick connective tissue cell in tissue culture *in vitro*. Living; phase microscopy. Distance between two dark bars in rectangular white area is 10 μ.

FIG. 2. Chick connective tissue cell in culture containing 7.5 mg/ml of desoxycorticosterone glycoside. Note reduction of cytoplasmic lipoid material. Living; phase microscopy. Same magnification as in Fig. 1.

trol cultures (based upon about 20 pairs for each concentration) were as follows: 30 μg/ml, –6.2; 15 μg/ml, 2.4; 7.5 μg/ml, 6.3; 3.5 μg/ml, 2.2; 2 μg/ml, 0.0.

Structurally, cells treated with concentrations of desoxycorticosterone glycoside above 30 μg/ml were characterized by enlarged nuclei and an increase in the rate of accumulation of fat droplets in the cytoplasm. At the highest concentrations used, (240 μg/ml) the nuclear membranes appeared to have been ruptured. Below 30 μg/ml photomicrographs show that the fibroblasts accumulated lipids in the cytoplasm at a much reduced rate (Fig. 1 and 2). At these concentrations there were few, if any, enlarged nuclei.

Summary. Concentrations of 15 μg/ml or lower of desoxycorticosterone glycoside stimulate growth of chick embryo fibroblasts and decrease the accumulation of cytoplasmic lipids. Concentrations of 30 μg/ml reduce growth and damage the nuclei of the cells.

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Action of Cortisone on Developing Chick Embryo.* (20255)

HIRAM J. EVANS. (Introduced by V. F. Lindeman.)

From the Department of Zoology, Syracuse University, Syracuse, N. Y.

This study was undertaken to determine whether the growth inhibition produced by cortisone is generalized or is limited to certain organs or regions. Previous studies on chick embryos(3,4) and on newly-hatched chicks (2) have emphasized growth-inhibition following injection of cortisone. In the present experiments, effects of cortisone are evidenced by a proportionate weight reduction of all parts tested except for the liver (smaller), eyes (larger), and gonads (larger), when com-

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