TABLE I. Breakdown of I^{131} Labelled l-Thyroxine (T_4) and l-3:5:3′-Triiodothyronine (T_3) by Rat Brain and Muscle Homogenates (1 g Tissue/15 ml Medium).

Tissue	Hr incubation	l-Thyroxine, 2×10^{-9}M: % total I^{131} distributed as* T_4	I^-	Or.	Others†	l-3:5:3′-Triiodothyronine, 3.5×10^{-9}M: % total I^{131} distributed as* T_3	I^-	Or.	Others†
Muscle	.5	57.1	40.4	1.3	+	76.6	22.7	+	1.4
	1	34.5	63.5		2.0	69.0	29.0	2.0	
	3	17.3	79.1	3.6		56.6	32.4		1.0
	6	12.6	84.2	2.5	+	53.0	35.5	1.5	
Boiled muscle	6	86.1	12.0	+	+	92.4	6.8	+	
Brain	1	68.7	15.9	+	14.8	80.1	9.2	+	10.3
	3	60.4	27.0	3.8	8.8	69.3	23.4	2.7	4.6
	6	51.0	33.2	3.8	2.0	64.8	34.2	1.0	
Boiled brain	6	89.8	10.2			90.6	8.3	+	

* + = Less than 1%; Or. = Fraction of I^{131} immobile in chromatography.
† In case of the brain this group includes acetic acid and/or propionic acid analogues of T_4 and T_3.

Summary. From the present study it can be concluded that brain and muscle have certain similarities as well as differences in the metabolism of thyroxine and triiodothyronine. The deiodinating enzyme is present in both the tissues but the deaminating activity found in brain preparations is virtually absent in skeletal muscle. The nature of the enzymes as well as other factors involved in the metabolism of these hormones require further clarification and are being studied.

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Inhibitory Effects of Cortisone Acetate and Hydrocortisone on Growth of Fibroblasts.* (23223)

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Diverse evidence has been presented by several investigators concerning the direct action of cortisone acetate and 17-hydroxycorticosterone on growth of fibroblasts *in vitro*. The technics have differed as to methods of culture and application of the test agents(1-7).

The purpose of the present work was to study the effect of cortisone and hydrocortisone on a single cell system. The results indicate that addition of these steroids to cultures of actively proliferating fibroblastic cells inhibits their growth and induces development of abnormal cytologic changes.

Materials and methods. The steroids, cortisone acetate and hydrocortisone (free alcohol) were supplied through the courtesy of Dr. Frederick Heath, Merck & Co. Cortisone was diluted in aqueous vehicle to contain 5 mg ml and further diluted to desired strength with Earle's balanced salt solution (8) prior to combining with the culture media. Hydrocortisone was prepared according to the method of Grossfeld and Ragan(3). The original vehicle was removed from this

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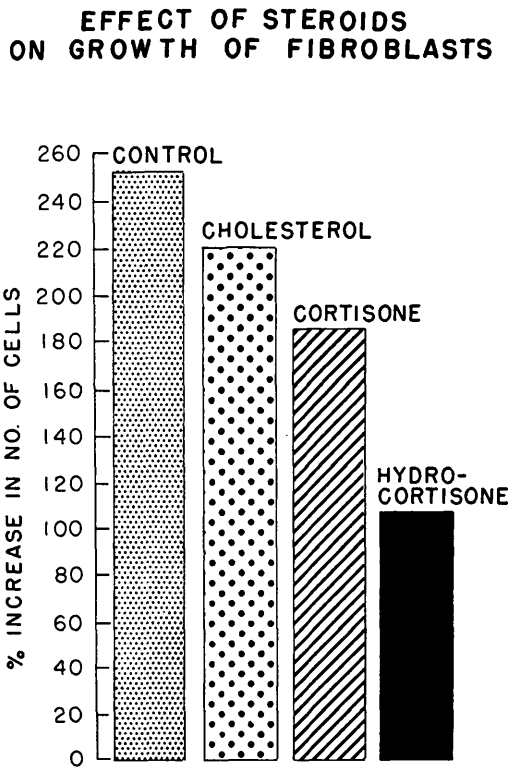
steroid and replaced by saline. To 1 ml of this suspension were added 1.25 ml of 95% ethyl alcohol and 0.75 ml of distilled water. A clear solution containing 8.33 mg/ml of hydrocortisone thus obtained was further diluted with saline to proper concentration for use in the tests. Cholesterol suspended in saline was employed as a control in several experiments. Stock cultures of fibroblasts (L strain, Earle)(9) were grown on the surface of T-60 flasks in a nutrient mixture of horse serum (40%), embryo extract (20%), and Earle's saline (40%). After 7 to 10 days of cultivation the cells were loosened from the glass surface by gentle shaking, suspended in 0.025% trypsin solution at a pH 7.4 to 7.6, incubated at 37°C for 20 to 30 minutes to break cellular clumps and occasionally agitated to obtain an uniform suspension of single cells. Following centrifugation at 1000 rpm for 10 minutes and removal of the supernatant trypsin solution, the cells were resuspended in nutrient medium alone or in medium containing 10 μg/ml of cholesterol, cortisone or hydrocortisone. The cellular concentration was measured by direct count in a hemocytometer. In earlier studies the cells were diluted in balanced salt solution containing neutral red but the vital dye was later discontinued, since healthy-appearing cells were readily discernible without staining. Two and five-tenths milliliter aliquots of the suspensions were dispensed by an automatic syringe into wide mouth, flint glass, screw top bottles. Four bottles were set up for each combination of cells and reagents and incubated at 37°C. During the test period the nutrient medium, with or without steroids, was replaced every 2 or 3 days to maintain the cells. After 11 days the cells from the bottles of each combination were pooled, treated with trypsin, centrifuged, resuspended in saline and enumerated as before. Growth rates were studied in cultures containing 10, 20, and 50 μg/ml of hydrocortisone. In these experiments the treated and untreated cells from 2 bottles of each combination were pooled and counted at 3- or 4-day intervals for 14 days.

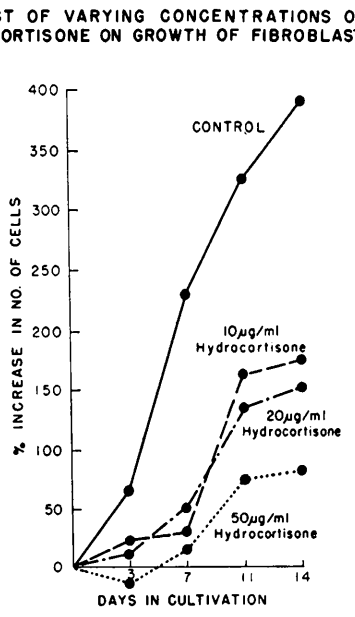
Cultures of fibroblasts derived from subcutaneous areolar tissue of adult rats were used



GRAPH I. Retarding effect of 10 μg/ml of cholesterol, cortisone or hydrocortisone on mouse fibroblasts grown for 11 days. The data from 9 experiments have been pooled.

in a series of experiments designed to test effect of cortisone acetate and hydrocortisone on cell mitosis. The cultures were carried for 15 days or longer in roller tubes and then transferred to Maximow slides(10). After 5 days, when they were about to reach their maximal growth rate, they were exposed to 20 μg/ml of the steroids in the feeding solution. In control cultures saline was substituted for the test agents. In these experiments the feeding solution was composed of human placental serum (20%), beef serum ultrafiltrate (20%), embryo extract (20%), and saline (40%). At 4, 8, 12, 16, 20, and 44 hours, 2 to 4 slides containing 6 to 12 explants were fixed in methyl alcohol and stained with Jenner Giemsa. An average of 2000 cells for each time interval and for each agent were counted, using a Howard ocular grating. The percentage of these cells in the 4 phases of mitosis was determined.

Results. The data in Graph 1, compiled



GRAPH II. Curves illustrate retarding effect of 10, 20 or 50 μg/ml of hydrocortisone on mouse fibroblasts over a 14-day period. The data from 4 experiments have been pooled.

from 9 experiments, show the average percentage increase in number of cells over the original cell count in the control and steroid-treated cultures. The counts were made after 11 days. In control cultures there was a 253% increase in number of cells over the initial inoculum, whereas in cultures containing 10 μg/ml of cholesterol, cortisone or hydrocortisone, the percentage increase in number of cells was 220%, 185%, and 106%, respectively.

Having obtained evidence that hydrocortisone produced more inhibition than cortisone acetate, the next experiments were carried out using only hydrocortisone. Three concentrations of hydrocortisone were studied. The percentage increase of cell count over the original count at intervals of 3 or 4 days is shown in Graph II, which represents the average results of 4 experiments. This graph indicates that all 3 concentrations of hydrocortisone, 10, 20, and 50 μg/ml, produce a definite inhibiting effect on growth of fibroblasts. The inhibitory effect is most noticeable within the first 7 days. Both 10 and 20 μg/ml of hydrocortisone produce approximately the same degree of inhibition of growth. It may be observed that cultures containing 10 or 20 μg/ml of hydrocortisone produce about 50% fewer cells when compared to cell growth in control cultures. Fifty micrograms per ml are roughly twice as inhibitory to growth as the lesser amounts of hydrocortisone.

To determine whether damage to the cells by the steroids was reversible, the treated cultures were transferred into medium without the addition of the test agents; complete recovery of the culture with growth equal to that of control cultures ensued.

Typical changes in morphology were readily seen in unstained as well as in stained fresh preparations. Fig. 1 illustrates a fresh preparation of a normal control 10-day-old culture. The typically fibroblastic cells with the characteristic spindle-shape or, less frequently, with triangular shapes and long terminal processes may be seen. An occasional giant cell is present. In contrast, hydrocortisone-treated cells, Fig. 2, are less spindle-shaped and show definite shortening of their terminal processes; the cells appear swollen, transparent, flattened, and tend to cohere in

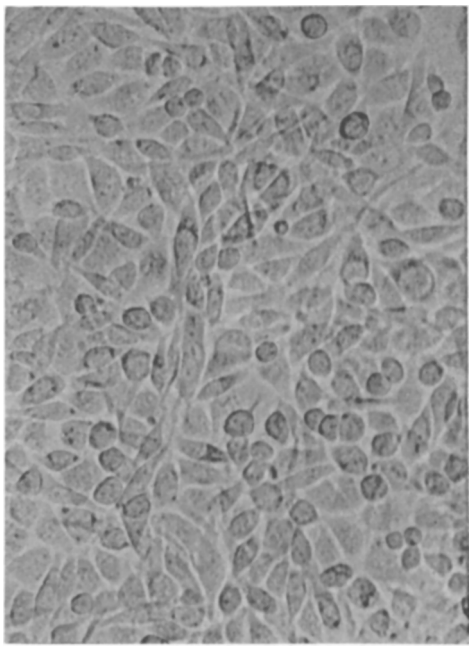


FIG. 1. Fresh preparation of normal control mouse fibroblasts (L strain, Earle) from a 10-day culture. Characteristic spindle-shaped cells and an occasional giant cell can be seen. X 100, approximately.

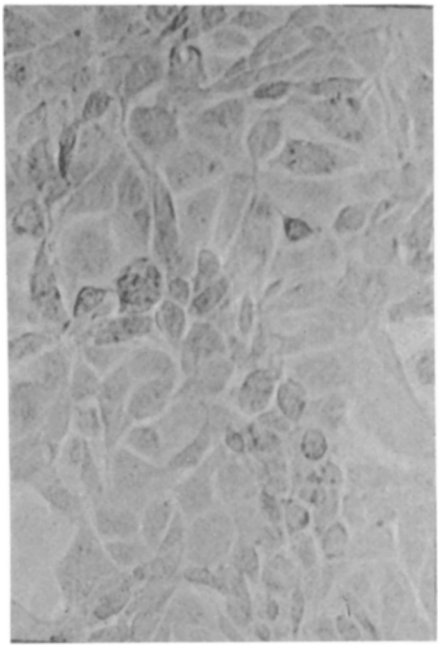


FIG. 2. Fresh preparation of mouse fibroblasts grown for 10 days with 50 μg/ml of hydrocortisone. In comparison to Fig. 1, the cells are swollen and less spindle-shaped. More giant cells are present. X 100, approximately.

sheets; some contain one single enormous nucleus, others 4 to 6 nuclei. Another constant effect of hydrocortisone on fibroblasts is the production of numerous cytoplasmic vacuoles (Fig. 3). These morphologic changes are present in cortisone acetate as well as in hydrocortisone-treated cells and become more pronounced with increasing concentrations of the steroids.

The reaction to the hormone was also noticeable following trypsinization. After 3 or more days' growth in the media containing steroids, detached normal cells, seen in the counting chamber, were round and uniform in size, unlike steroid-treated cells which were large, ameboid and irregular in outline.

Cortisone acetate and hydrocortisone in a concentration of 20 μg/ml did not arrest mitosis in any particular phase and there was no accumulation of one type of mitotic figure at any time during cultivation. In the 4 experiments carried out, there was no appreciable difference in mitotic index between the control and the steroid-treated cells. However, it should be noted that in these experiments the cells were rat fibroblasts and cultivated in a plasma clot.

Discussion. The experimental data indicate that hydrocortisone, and to a lesser degree cortisone acetate, inhibited growth of fibroblasts in tissue culture. Cholesterol caused minimal inhibition. The question arises whether the lesser effect of cortisone, as compared with hydrocortisone, might be due to the fact that cortisone (acetate) was in suspension and was inhomogeneously distributed in the medium, whereas hydrocortisone was in solution. Grossfeld and Ragan (3) tested these steroids in hanging drop chick embryo cultures and found that inhibition by hydrocortisone in suspension was greater than by cortisone acetate in suspension. They believed this indicated that factors other than water solubility make hydrocortisone more effective than cortisone acetate.

The finding that the inhibitory effect on growth of fibroblasts was greater when the concentration of steroids was increased from 10 or 20 to 50 μg/ml conforms with the observation of Kaufman and his associates (2), who, using embryo chick heart tissue embedded in a plasma clot, observed inhibi-

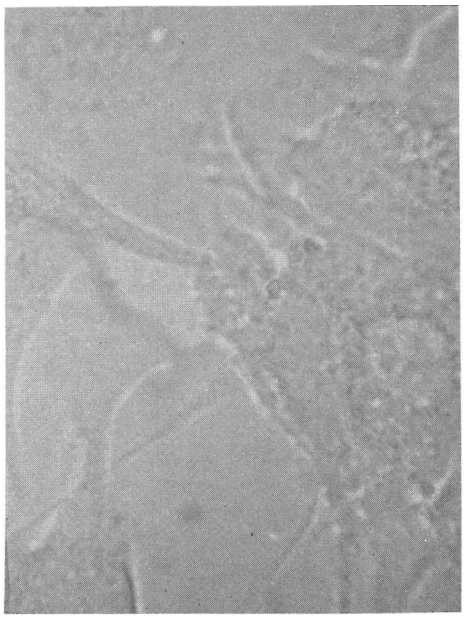


FIG. 3. A higher magnification of Fig. 2, illustrating cytoplasmic vacuoles. X 450.

tion of migration of fibroblasts with 10, 25, 50, and 100 μg/ml quantities of cortisone (free alcohol) and hydrocortisone and concluded that amount of inhibition was directly related to concentration of the steroids.

The formation of cytoplasmic vacuoles in fibroblasts grown together with cortisone or hydrocortisone has been observed by other workers. Paff and Stewart(7), who precipitated crystals of cortisone (free alcohol) by evaporation on coverslips before addition of nutrients and chick embryonic dermal fragments, reported that the first apparent reaction of the fibroblast to the hormone was formation of cytoplasmic vacuoles. Steen(1), using hanging drop cultures containing cortisone acetate, also observed cytoplasmic vacuoles in fibroblastic outgrowth from chick embryo heart. The presence of large fat vacuoles in an unusually early stage of growth was noted by Grossfeld and Ragan(3) in chick embryo cultures submitted to the action of soluble hydrocortisone.

The failure of cortisone or hydrocortisone to influence the mitotic index of rat fibroblasts or alter distribution of the 4 phases of mitosis accords with the work of Grossfeld and Ragan(3), who found no arrest of mitosis in any particular phase.

Summary. 1) The effect of cortisone acetate and hydrocortisone (free alcohol) on growth of L strain mouse fibroblasts was studied. 2) Addition of 10 μg/ml of either steroid to the culture fluid, after 11 days of incubation, resulted in a decrease in total cell count when compared with counts in control cultures. Hydrocortisone was the more inhibitory agent. 3) The effect of 3 concentrations of hydrocortisone (10, 20, and 50 μg/ml) on growth of fibroblasts over a 14-day period was established. The most marked inhibitory effect was produced during the first 7 days of culture. Fifty micrograms per ml were approximately twice as inhibitory as the lesser amounts of hydrocortisone. 4) Deviations from normal morphology of fibroblasts were seen in the steroid-treated cells. The cell processes were shortened, cells were swollen and flattened and tended to cohere in sheets. A great number of giant cells, often multinuclear or with nuclei irregularly shaped, were present. The cytoplasm contained many vacuoles.

The authors are indebted to Dr. C. Andrew L. Bassett for the photomicrographs.

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Phagocytosis of Periodate-Treated and Virus-Coated Red Blood Cells By White Blood Cells *in vitro*. (23224)

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Hemolytic anemia has been reported in virus diseases, such as infectious mononucleosis, virus pneumonia, Coxsackie A infection, influenza A infection and measles (reviewed by Dacie(1)). Several viruses are known to be adsorbed to red blood cells, and cause irre-