Summary. Immunological tolerance of AZ F_1 hybrid tissue homograft induced in adult Z mice by virtue of parabiosis of 150 days duration can be transferred to isologous Z strain mice by injecting these animals at birth with spleen cells taken from Z donors tolerant of AZ F_1 tissue. Tolerance resulting from parabiosis of only 30 days duration failed to be transferred by the same procedure.

In the light of these results the mechanism of tolerance induced in adult individuals by the parabiosis procedure is discussed.

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Received May 8, 1961. P.S.E.B.M., 1961, v107.

Effect of Hydrocortisone on Human Cells in Tissue Culture.* (26687)

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The cytotoxic and growth-inhibiting effects of adrenal cortical steroids on various cell strains growing *in vitro* have been observed frequently(1-5). Recently we reported that hydrocortisone inhibited cell growth in proportion to the concentration used(6). The purpose of the present study was to determine the quantitative effects of hydrocortisone on protein synthesis and on nuclear multiplication and to investigate the qualitative effects on enzymes of cells in tissue culture.

Methods. A strain of cells derived from human liver by Chang(7) was grown in stock culture in 95% medium 199 and 5% horse serum. The cells were removed from the flasks by replacing the growth medium with 0.0125% trypsin in Hanks' balanced salt solution(8) for 15 minutes at 35°C and centrifuging the resulting cell suspension for 5 minutes at 1000 rpm. The supernatant was removed, the cells were washed in medium 199, and resuspended in medium 199 with 5% horse serum in a concentration of 100,000 cells/ml. Five ml aliquots were placed in T-flasks for studies on the rates of nuclear multiplication and protein synthesis. All cultures were incubated at 37°C for 24 hours, after which the culture medium was removed from the flasks and the flasks rinsed with 5.0 ml balanced salt solution. Determinations of protein nitrogen content of each of 3 cultures were made with a phenol reagent according to the method of Oyama and Eagle(9). Crystalline bovine albumin was used as a standard. The number of nuclei was determined in 4 different samples from each of 3 flasks by the method of Sanford *et al.*(10) using 6% citric acid to remove the cytoplasm and 0.1% crystal violet to stain the nuclei.

The culture medium to be tested was added to the remaining flasks; 6 flasks were used for each group.

At the end of the experiment 3 flasks from each group were used for determination of protein content and 3 flasks for the number of nuclei.

For microscopic studies coverslips were placed into Leighton tubes containing 100,000 cells suspended in 1 ml of medium 199 with 5% horse serum. After incubation for 24 hours at 37°C, the culture medium was

* Supported in part by grants from Am. Cancer Soc. and USPHS.

TABLE I. Effect of Concentration of Hydrocortisone on Growth of Cells.

Hydrocortisone, μg/ml	Incubation period, hr	Nitrogen, μg/ml ± S.E.		Nuclei, 1000/ml ± S.E.		Nitrogen/cell, mμg
0	0	11.1	.14	81	2.1	.138
0	72	14.2	.34	219	11.8	.065
50	"	14.3	.50	168	5.5	.085
100	"	11.7	.42	114	7.4	.103
200	"	12.8	.59	91	3.4	.141

removed and the tubes were rinsed with balanced salt solution. Culture media corresponding to those used in the flasks were added to the Leighton tubes and the culture incubated at 37°C. At the end of the experiment the coverslips were removed from the Leighton tubes. One coverslip from each group was stained with hematoxylin and eosin; some coverslips were stained by the Feulgen reaction. The remaining coverslips were used for histochemical demonstration of succinic dehydrogenase(11), diphosphopyridine nucleotide diaphorase(12), glucose-6-phosphate dehydrogenase(13), acid and alkaline phosphatase, and esterase(14).

Results. Effect of concentration of hydrocortisone. The experimental groups were cultured in medium 199 containing 50, 100 and 200 μg/ml hydrocortisone and controls were grown in medium 199 alone. At the end of 72 hours of incubation at 37°C the experiment was terminated. In each group protein content was determined in each of 3 flasks, and number of nuclei determined in each of the remaining 3 flasks.

In the controls nitrogen content had increased from an initial value of 11.1 μg/ml to 14.2 μg/ml (Table I). In the cultures grown in the medium with varying concentrations of hydrocortisone nitrogen values varied from 14.3 to 11.7 μg/ml. In the controls the number of nuclei had increased from 81,000 to 219,000/ml, while in the experimental cultures with hydrocortisone concentrations of 50, 100 and 200 μg/ml the nuclei had increased to 168,000, 114,000 and 91,000/ml respectively. Thus degree of inhibition of nuclear multiplication was proportional to concentration of the hormone. However, the nitrogen content of the cultures did not show an equivalent reduction. This difference in effect of hydrocortisone on the nucleus as compared with the cytoplasm was reflected in the nitrogen/cell values which had progressively increased with increasing concentrations of hydrocortisone.

Effect of length of incubation. In these experiments the dosage of hydrocortisone was 150 μg/ml in all experimental groups. Each group was incubated for 24, 48 or 72 hours. At the end of each period protein content and number of nuclei were determined in experimental and corresponding control groups.

Both number of nuclei and nitrogen content of the cells grown in media with hydrocortisone were less than those of the controls. Moreover, the differences between the experimental groups and their respective controls tended to increase with longer periods of incubation.

In the controls there was a progressive increase in nitrogen content and number of nuclei proportional to the length of incubation period (Table II and Fig. 1). The initial value for nitrogen content was 10.8 μg/ml and for number of nuclei, 77,000/ml. These values increased to a maximum nitrogen content of 15.2 μg/ml and to 178,000 nuclei/ml at 72 hours. In the experimental group cultured in 150 μg/ml hydrocortisone nitrogen content was 10.2 μg/ml at 24 hours, 10.8 μg/ml at 48 hours, and 12.2 μg/ml at 72 hours. Numbers of nuclei in all of the experimental groups were less than those in the corresponding controls, *i.e.*, 86,000, 119,-

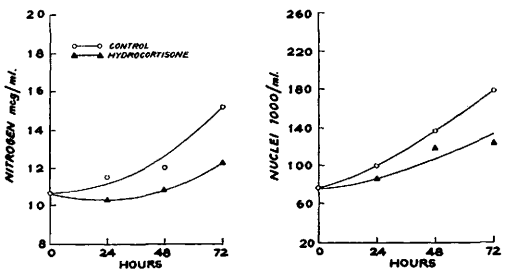

FIG. 1. Cells grown in medium 199 with 150 μg/ml hydrocortisone for 24, 48 and 72 hr.

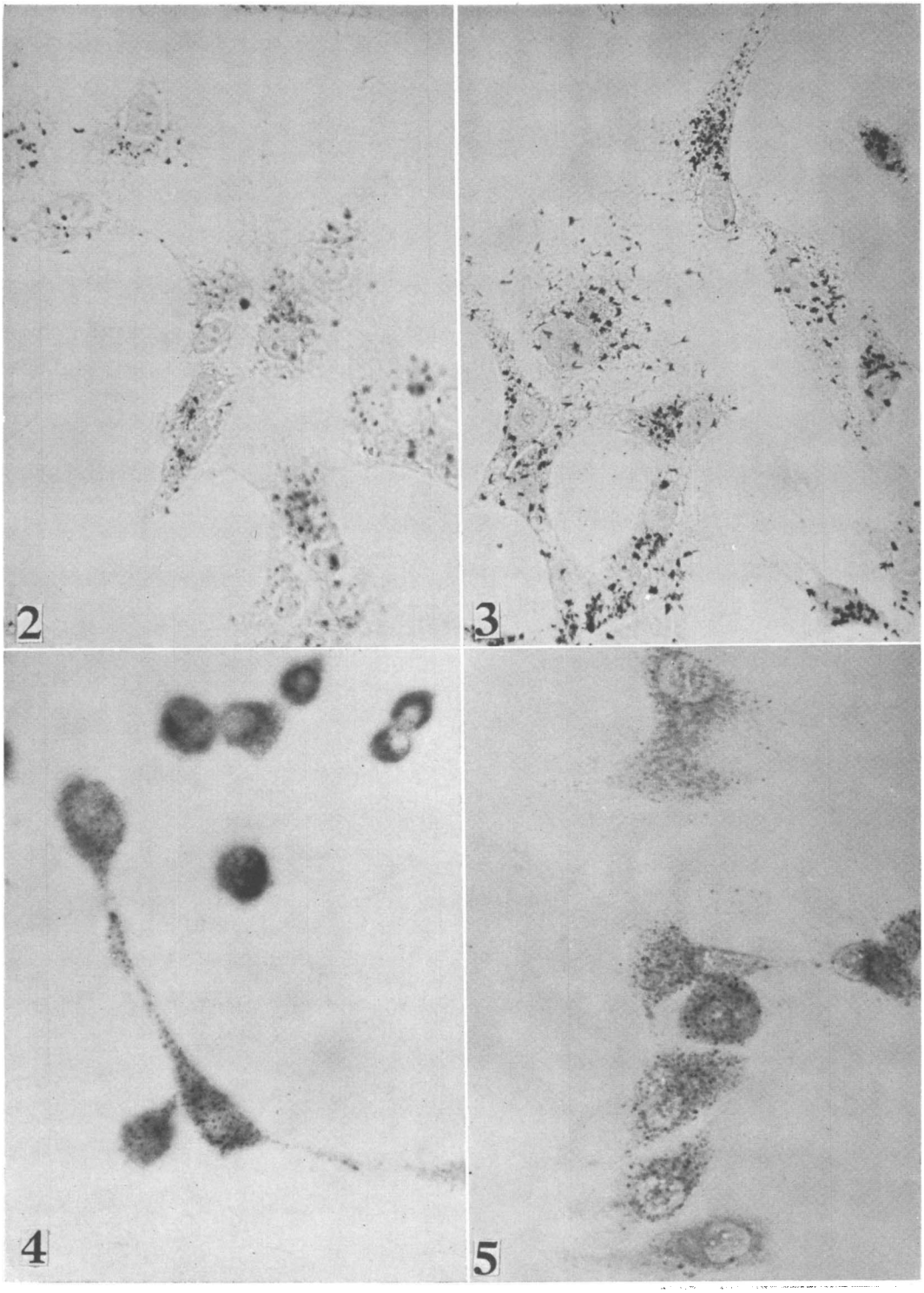

FIG. 2. Control cells cultured in medium 199 with small granules of nonspecific esterase-positive material in the cytoplasm. × 450.

FIG. 3. Experimental cells cultured in medium 199 with 150 μg/ml hydrocortisone. Cytoplasm contains an increased number of esterase-positive granules. × 450.

FIG. 4. Control cells cultured in medium 199. Cytoplasm contains a large amount of succinic dehydrogenase-positive granules. × 450.

FIG. 5. Experimental cells cultured in medium 199 with 150 μg/ml hydrocortisone showing a decreased amount of succinic dehydrogenase-positive material. × 450.

TABLE II. Effect of Length of Incubation on Growth of Cells.

Group	Incubation	Nitrogen, μg/ml ± S.E.		Nuclei, 1000/ml ± S.E.		Nitrogen/cell, mμg
Controls	0	10.8	.43	77	3.3	.140
	24	11.5	.14	100	5.2	.115
	48	12.0	.00	137	5.4	.087
	72	15.2	.46	178	10.4	.085
Hydrocortisone, 150 μg/ml	24	10.2	.38	86	4.0	.120
	48	10.8	.58	119	8.7	.090
	72	12.2	.38	124	3.1	.098

000 and 124,000/ml.

Morphologic and histochemical changes. There were consistent differences between hydrocortisone-treated cells and controls. The nuclei of the cells treated with hydrocortisone were larger, more irregular, and showed greater variation in size than the controls. These nuclei were stained less intensely by hematoxylin; multinucleated cells were more abundant. Mitoses were about two-thirds as numerous in hydrocortisone-treated cells as in controls. The cytoplasm of the hydrocortisone-treated cells was more abundant but less dense than that of the controls.

In the hydrocortisone-treated cells the nuclei stained less intensely with the Feulgen reaction. Acid phosphatase and nonspecific esterase (Fig. 2 and 3) had increased; succinic dehydrogenase (Fig. 4 and 5) and glucose-6-phosphate dehydrogenase were decreased in the cells treated with hydrocortisone. Alkaline phosphatase and diphosphopyridine nucleotide diaphorase reactions showed no difference in experimental and control groups.

Summary. Hydrocortisone inhibited the growth of cells in a synthetic medium. Degree of inhibition was proportional to concentration of the hormone and duration of exposure. The inhibition in nuclear multiplication was relatively greater than the inhibition of protein synthesis. The cells grown in media with hydrocortisone had larger, more variable nuclei, increased amounts of acid phosphatase and nonspecific esterase and decreased amounts of succinic dehydrogenase and glucose-6-phosphate dehydrogenase.

The authors gratefully acknowledge the technical assistance of Miss Virginia Jentoft and Miss Barbara Jennings.

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Received May 8, 1961. P.S.E.B.M., 1961, v107.