cytes in vivo and in isolated liver nuclei in vitro is evidently effected by the same enzyme system, although it responds differently in different cells to glucocorticoids.

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CRITERIA OF SENSITIVITY OF ZAJDELA AND MORRIS HEPATOMA CELLS TO GLUCOCORTICOIDS

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Liver and hepatoma cells, in which glucocorticoids induce tyrosine aminotransferase (TAT) synthesis, are the classical model with which to study the molecular mechanism of action of steroid hormones on a target cell.

Morris hepatoma 7777 and Zajdela hepatoma cells were studied in the investigation described below. Glucocorticoids induce TAT synthesis in the former, whereas the latter are resistant to the hormone in this respect. The writers showed previously that cells of both hepatomas contain glucocorticoid receptors and form hormone-receptor complexes which can interact with nuclear structures [1, 2]. Analysis of interaction of glucocorticoids with isolated plasma membranes, and also with whole cells revealed, however, differences between the cells of the above hepatomas [3]. It was therefore decided to compare the response of the two hepatomas to this hormone with respect to several criteria.

EXPERIMENTAL METHOD

Cultures of exponentially growing cells of Morris and Zajdela hepatomas were used. The former were grown in Leibovitz L-15 medium (Flow Laboratories, England) with 10% embryonic serum, the latter in Eagle's medium with 10% bovine serum. The cells were subcultured twice a week, removed mechanically, washed 3 times with Hanks' solution (1000 rpm, 5 min, 4°C), and used in a concentration of $(0.5\text{-}1.0)\cdot 10^6$/ml.

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TABLE 1. Interaction of Hepatoma Cells With Hydrocortisone Immobilized on Gauze (in cpm/mg gauze; M ± m, n = 3)

Conditions of incubation	Morris hepatoma	Zajdela hepatoma
20 °C:		
control	10 530	16 290
+ hormone	14 410	19 370
37 °C:		
control	27 820	62 010±5 800
+ hormone	12 470	17 360±4 900

TABLE 2. Action of Glucocorticoids on TAT and Alkaline PDE I Activity (in conventional optical units, OU)

Hepatoma	TAT, OU/μg protein			PDE I, mOU/min/10^6 cells		
	hormone	control	hormone / control	hormone	control	hormone / control
Morris	44	7,2	6,1	1,5	0,66	2,25
	39	4,1	9,5	7,0	4,8	1,47
Zajdela	Not determined			42	19	2,15
				55	23	2,34
				36	20	1,8

Triamcinolone acetonide (TA) in a concentration of 10^{-7} M was used as inducer, and in this case serum was excluded from the medium. TAT activity was determined by the method in [8]. The protein concentration was measured by Lowry's method.

Activity of alkaline phosphodiesterase I (PDE I, 5'-nucleotide hydrolase) (oligonucleate 5'-nucleotidohydrolase) was determined by the method described previously [7]. Whole cells were incubated for 5-20 min at 25°C in 0.3-0.5 ml of buffer containing 0.11 M Tris-HCl (pH 9.0), 0.11 M NaCl, and 15 mM $MgCl_2$. The substrate for alkaline PDE I was Na-bis-(4-nitrophenyl) phosphate, the optimal concentration of which (2 mM) was determined in a preliminary series of experiments.

Adhesion of the cells to glass after culture for 24 h with or without TA was estimated by the method in [6].

To estimate interaction of cells with the hormone immobilized on an inert carrier, hydrocortisone hemisuccinate was bound to a cotton gauze matrix through an albumin insert [11]. One gram of the matrix contained 200 mg of albumin and 15 mg of hydrocortisone. The quantity of tissue used in the experiment was 4-5 mg. The quantity of hormone on the matrix was estimated spectrophotometrically (E_{245}) after hydrolysis by 1% KOH solution in methanol for 15 min. The cells were agitated on a shaker together with the matrix carrying the hormone, or in control experiments with the matrix and without the hormone for 30-60 min at 4, 20, and 37°C, in a volume of 2-5 ml. The matrix was then removed, washed 3 times in 5-10 ml of Hanks' solution, after which either the cells remaining in the solution or cells bound with the matrix were counted (the cells were labeled beforehand with ^{3}H-thymidine for 24-48 h). After incubation of the cells with the matrix at different temperature, hydrolysis of the hormone did not exceed 10%.

The cells were prepared for scanning electron microscopy by the method in [5].

The specimens were examined on a Backman (USA) spectrophotometer. Radioactivity was determined in a homogeneous counting system in ZhS-8 scintillation fluid or a Triton-toluene scintillator on a Mark II counter (USA).

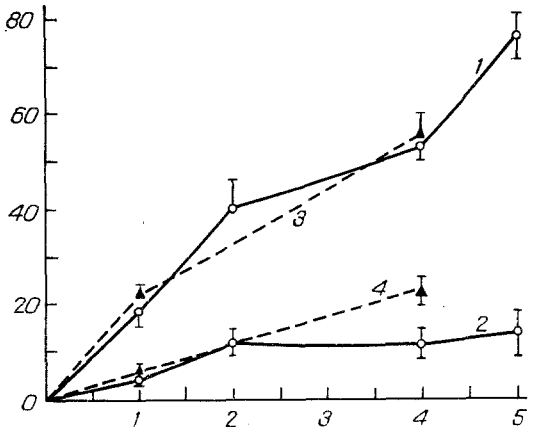

Fig. 1. Changes in adhesion of cells to glass under the influence of glucocorticoids. Abscissa, time (in h); ordinate, number of cells adherent to glass, determined from difference in spectrophotometer readings at 450 nm, taking the initial density of the cell suspension ($2 \cdot 10^5$ cells/ml) as 100%. 1, 2) Zajdela hepatoma cells; 3, 4) Morris hepatoma cells, 1, 3) Cells incubated with TA; 2, 4) control.

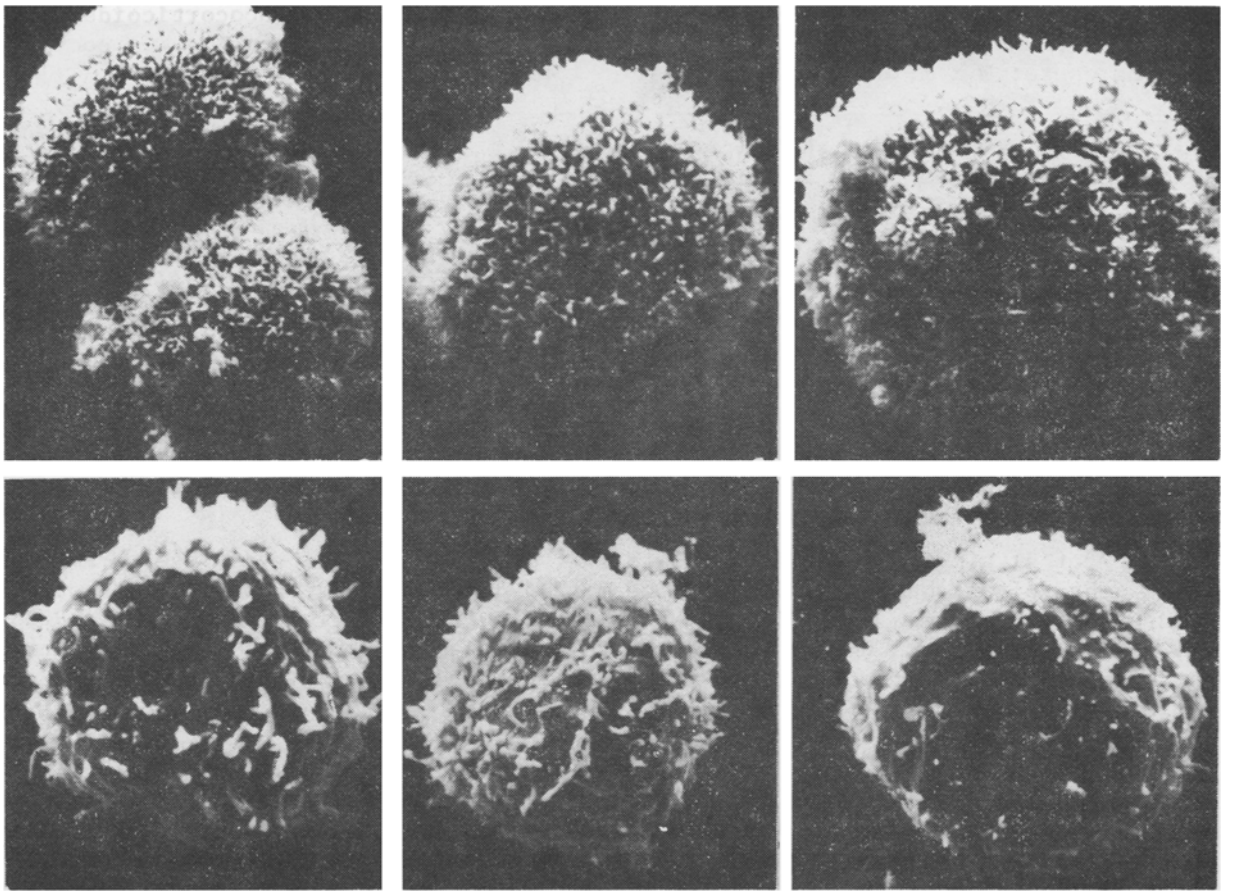

Fig. 2. Zajdela hepatoma cells. Top row — control; bottom row — cells cultured with hormone. Electron microscopy. 3500 ×.

EXPERIMENTAL RESULTS

Interaction of cells of both hepatomas with the matrix carrying immobilized-hydrocortisone was similar in character. Preincubation of the cells at 37°C with excess of hormone in the presence of TA in the medium led to a two-threefold decrease in the number of cells immobilized on the gauze (Table 1). An active binding process was observed only at a physiological temperature (37°C), and at 20°C interaction between hepatoma cells and matrix was evidently nonspecific, and no decrease in binding was observed. Increasing the duration of preincubation of cells of both hepatomas with the hormone to 15-24 h also reduced the number of cells remaining on the matrix with immobilized hydrocortisone by 50-67%. In the control experiments, when matrix with or without albumin and subjected to identical treatment was used, no decrease in the number of cells interacting with the matrix was observed (results not given).

In a series of experiments with induction of adhesion of cells of both hepatomas to a glass surface by the hormone TA, we found that TA increased by several times adhesion of the cells to glass (Fig. 1): among cells preincubated with the hormone for 4 h 55-75% adhered to the glass, compared with only 15-20% among control cells. Similar potentiation of adhesion to glass under the influence of glucocorticoids also was observed in cells of another Morris hepatoma (HTC [6]). The authors cited showed that RNA and protein synthesis is essential for the induction of adhesion, and it develops in hepatoma cells parallel with binding of the hormone with cytoplasmic receptors and induction of TAT synthesis in the cells.

A change in the adhesive properties of cells is known to be accompanied by a change in the microarchitecture of the cell surface and, in particular of the microvili, and it is coupled with the state of transformation of the cells [10]. It will be clear from the example of Zajdela hepatoma cells (Fig. 2) that glucocorticoids reduce the number of microvili on the surface and also induce a change in their shape. Similar changes in the microvili of

the cell surface under the influence of glucocorticoids have been described in cells of other strains of hepatomas sensitive to the hormone: one in suspension, and two growing in a monolayer [9]. It is considered that glucocorticoids induce a program of membrane changes in the cells, apparently imitating reversion of the transformed phenotype of these cells toward the normal phenotype.

To sum up the results, it can be concluded that the response of Zajdela hepatoma cells to the hormonal signal is manifested phenotypically in just the same way as in Morris 7777 hepatoma cells or in other cells sensitive to the hormone.

The biochemical test used to assess hormonal sensitivity of Zajdela hepatoma cells was alkaline PDE I determination. As Table 2 shows, glucocorticoids induced PDE I in cells of both hepatomas and increased its activity by 50-100%. Moreover, induction of PDE I depends on the hormone concentration in the medium just like induction of TAT in Morris hepatoma 7777 cells (results not given). The increase in PDE I activity in Zajdela hepatoma cells took place on account of an increase in the maximal rate (V_{max}) by 1.5-2.5 times, whereas the Michaelis constant (K_M) remained unchanged at 0.8 mM. Similar results were obtained on cells of the other Morris hepatoma (HTC), and the authors cited proved that alkaline PDE I is located in the plasma membranes of the cells [12]. It also follows from Table 2 that, when TAT synthesis was used as the criterion, Zajdela hepatome cells were hormone resistant. However, it was shown previously by hybridization of total RNA with a TAT DNA-copy, and also by hybridization of ^{125}I-mRNA$_{TAT}$ with DNA of Zajdela hepatoma cells, that both the TAT gene and nuclear pre-mRNA$_{TAT}$ are preserved in the nucleus of hepatoma cells [1]. It can be tentatively suggested that disturbance of the cell response to the hormone evidently takes place at the stage of mRNA$_{TAT}$ processing. The present writers showed previously by two-dimensional electrophoresis of proteins in polyacrylamide gel that glucocorticoids regulate the synthesis of at least four proteins in Zajdela hepatoma cells: the rate of synthesis of three of them was increased and of one it was reduced [4].

It can accordingly be concluded that Zajdela hepatoma cells, arising from hepatocytes as a result of chemical hepatocarcinogenesis and resistant to the hormonal stimulus with respect to the classical criterion of synthesis and induction of TAT, were inducible with respect to certain other glucocorticoid-reactive functions, namely: adhesion to glass, induction of alkaline PDE I synthesis, and also interaction with hydrocortisone immobilized on an inert carrier. It can be postulated that as a result of carcinogenic action on hepatocytes a whole group of responses is lost, whereas other responses to the hormone are preserved.

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