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GLUCOCORTICOID SENSITIVE AND RESISTANT CELL POPULATIONS IN THE MOUSE THYMUS

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SUMMARY

We have studied in parallel the effects of dexamethasone on *in vitro* incorporation of [^{3}H]-uridine and [^{3}H]-thymidine and on cell viability in thymocyte subpopulations isolated by centrifugation on a discontinuous bovine serum albumin gradient from intact, adrenalectomized and hydrocortisone-treated mice. In cell populations isolated from intact and adrenalectomized animals, the steroid induces both inhibition of precursor incorporation and a decrease in cell viability, whereas cells isolated from hydrocortisone-treated mice show marked inhibition of precursor incorporation but no cell death after 24 h. Our present results suggest that differences in steroid sensitivity are not directly related to variations in cell receptor content but may be associated with cell differentiation.

INTRODUCTION

Lymphoid cells are known to be sensitive to glucocorticoid hormones [1, 2]. These compounds induce several events such as inhibition of transport and synthesis of macromolecules [3]. They may also produce nuclear damage and cell lysis [4–7]. However, between species, and within a given species between different cell types, the hormonal sensitivity may vary considerably [8–11]. In addition, even in a single cell lineage, changes in steroid sensitivity occur during maturation and differentiation [8, 11–14]. The mouse thymus, which contains both corticosensitive and corticoresistant subpopulations [15–20], has been widely used to study the molecular bases of steroid sensitivity or resistance. In previous experiments [19, 20], we have used centrifugation on a bovine serum albumin gradient or *in vivo* injection of hydrocortisone to isolate mouse thymus cell subpopulations "resistant" to steroids. Steroid receptor measurements showed that these differences in thymocyte steroid sensitivity could not be explained by differences in the number of steroid binding sites. Moreover, the small "cortico-resistant" cell fraction recovered from the thymus of steroid-treated animals, which is still heterogeneous [18, 20–23] but markedly enriched with immuno-competent cells [15, 24, 25], was found to be partly sensitive *in vitro* [20, 26].

In order to investigate further the mechanism of steroid sensitivity or resistance, we have measured simultaneously in mouse thymocyte subpopulations the effects of glucocorticoids on cell metabolism and steroid-induced cell lysis. As one of the factors responsible for thymus cell proliferation is the level of endogenous steroids [1, 2], we used cells isolated from animals in three different hormonal states: intact, adrenalectomized and hydrocortisone-treated.

MATERIALS AND METHODS

Biological material

C_{57} BL/6 female mice, 6–8 weeks old (15–25 g body wt.), were purchased from CSEAL (Orleans, France).

Intact (control) animals were maintained on a standard diet with free access to drinking water.

Adrenalectomized (ADR) animals were operated under pentobarbitone anesthesia 5–7 days before each experiment and given 0.9% saline to drink.

Hydrocortisone-treated (OH) animals received two intraperitoneal injections of 10 mg of hydrocortisone hemisuccinate 24 h apart 3–4 days after adrenalectomy. OH animals were routinely sacrified 48 h after the second injection.

Reagents

[^{3}H]-dexamethasone ([^{3}H]-DM), 23–29 Ci/mmol was obtained from the Radiochemical Center (Amersham, U.K.). [^{3}H]-uridine, 25 Ci/mmol, and [^{3}H]-thymidine, 30 Ci/mmol, were purchased from CEA (France). Unlabelled dexamethasone was obtained from Sigma. Bovine serum albumin was a sterile 35% solution (Miles Path. O. cyte 5). Conventional reagents were Baker A grade.

Thymocyte isolation

At the time of the experiment, the animals were anesthetized with ether and the thymus was removed and homogenized in ice-cold Hanks balanced salt solution (HBSS, Institut Pasteur, Paris) using a Teflon–glass Potter homogenizer. The homogenate was filtered through nylon gauze and centrifuged at 800 *g* for 10 min. The cells were washed twice, resuspended in HBSS and counted using a Malassez chamber. The entire preparation was carried out at 4°C.

Determination of cell viability

The number of viable cells in the populations under investigation was determined using the trypan blue exclusion procedure. A 50 μl aliquot of the cell suspension was mixed with 200 μl of buffer and 50 μl of a 0.4% solution of trypan blue stain. After a 2–3 min incubation the percentage of cells containing inspissated dye (assumed to represent dead cells) was determined by counting 300 cells under a light microscope [27]. At the end of the cell isolation procedure the viability was always greater than 95%.

Isolation of thymocyte subpopulations

The separation of mouse thymocyte subpopulations by centrifugation on a discontinuous bovine serum albumin (BSA) gradient was performed according to the method of Komuro and Boyse [28]. Thymocytes ($2–4 \times 10^8$ cells), prepared as described above, were resuspended in 1 ml of 35% BSA solution and deposited at the bottom of 1 × 10 cm tubes. One milliliter of the following BSA solutions were then layered successively on this suspension: 29, 26, 23 and 10%. After a 30-min centrifugation at 13,000 *g* (4°C), the cells located at the four interfaces were collected and washed twice with 5 ml HBSS. The cells were then resuspended in minimal essential medium (MEM Gibco) supplemented with 1 mM glutamine, 2 mM sodium pyruvate, 100 U/ml of penicillin and 100 μg/ml streptomycin and 1% v/v solution of non-essential amino acids (L-alanine, 890 mg/l, L-asparagine, 1500 mg/l, L-aspartic acid, 1330 mg/l, L-glutamic acid, 1470 mg/l, L-proline, 1150 mg/l, L-serine, 1050 mg/l, and glycine, 750 mg/l, Gibco ref. 114), counted and assayed for [^{3}H]-uridine and [^{3}H]-thymidine incorporation. "A" cells are those located at the top of the gradient between the 23 and 10% BSA layers, "B" cells are located between 26 and 23% BSA layers, "C" cells are located between 29 and 26% BSA layers and "D" cells are located between 35 and 29% BSA layers.

Binding assays

Point three of a milliliter of cell suspension (10^7 cells/ml) were distributed in 1.5 ml conical plastic vials containing 0.3 ml of [^{3}H]-DM solution (the final concentration of radioactive steroid was in the range of 3×10^{-9} to 10^{-7} M). These samples were incubated for 20 min at 37°C. At the end of the incubation period the samples were centrifuged for 15 s in an Eppendorf 3200 centrifuge, the supernatants discarded and the cells resuspended in 0.3 ml of ice cold medium containing $Na^+ = 133$, $K^+ = 6$, $Ca^{2+} = 1$, $Mg^{2+} = 1$, $Cl^- = 134$, $H_2PO_4 = 6$, Tris–HCl = 5, and glucose = 5, all in mM, pH 7.4 0.2 ml of cell suspension were then filtered through Whatmann GF/A filters and washed with 3 × 10 ml of ice-cold Tris buffer. The radioactivity collected on the filters was counted by liquid scintillation spectrometry. In each experiment the cells were incubated with [^{3}H]-DM $\pm 5 \times 10^{-5}$ M non-radioactive dexamethasone. The residual binding in the presence of non-radioactive dexamethasone represents non-specific binding. The specific binding of the steroid is the difference between the total binding of [^{3}H]-DM alone and this non-specific binding [29].

Inhibition of nucleic acid precursor incorporation in the presence of dexamethasone

To appreciate the steroid sensitivity of the lymphocytes *in vitro* we measured the incorporation of tritiated nucleosides in the absence (control) or presence of dexamethasone using several experimental procedures.

Evolution of steroid sensitivity over a 12-h period

Point six of a milliliter of lymphocyte suspensions ($5–10 \times 10^6$ cells/ml) were preincubated at 37°C in a 5% CO_2–air atmosphere, with or without 10^{-6} M dexamethasone, for various periods of time (2, 4, 6, 8, and 12 h) and then received 1 μCi of either [^{3}H]-uridine or [^{3}H]-thymidine. After a 60-min additional incubation at 37°C, the reactions were terminated by the addition of 1 ml of 5% trichloroacetic acid at 0°C. The precipitates were filtered on Whatman GF/A filters and washed three times with 10 ml of cold 5% trichloroacetic acid. The radioactivity collected on the filters was counted by liquid scintillation spectrometry. To take into account the steroid-induced cell lysis, the viability of the cells was determined at the onset of the incorporation periods, and therefore the results are expressed as c.p.m./10^6 viable cells.

Determination of steroid effect in a 24-hour experiment

In some of the experiments performed with cell subpopulations isolated from OH animals, we used a different protocol with 3-h preincubation in the absence or presence of the steroid followed by a 21-h incubation with 1 μCi of either [^{3}H]-uridine or [^{3}H]-thymidine. Precipitation of the samples by 5% trichloroacetic acid and filtration on glass wool filters were performed as described above.

Determination of whole cell nucleoside uptake

The cells were preincubated for 60 min in the presence of either [^{3}H]-uridine or [^{3}H]-thymidine, washed, and then applied to a BSA gradient. The whole-cell uptake was then determined in each of the sub-populations recovered from the gradient: aliquots of the cell suspension were centrifuged at 11,000 *g* for 15 s, resuspended in ice-cold 0.9% saline and washed twice. The cell pellets were digested overnight in 1 ml of soluene 350 (Packard) and counted by liquid scintillation spectrometry.

DNA measurements by flow system fluorometry

The cells (0.5 ml, 10^7 cells/ml) were fixed in methanol (final concentration 50%), washed in phosphate saline buffer (PSB) pH 7.4 and incubated for 20 min at 37°C in PSB containing 50 μg/ml RNAse (Worthington 3000 U/mg). At the end of the incubation the cells

were washed and resuspended in 1 ml of propidium iodide solution (50 mg propidium iodide and 1 g sodium citrate per liter).

Pulse cytophotometric measurements were made within 1 h of staining using a cytofluorograf (4802 A, Biophysic system Inc., Mahopac, New York). DNA histograms were evaluated by planimetry [30].

RESULTS

Binding studies

The specific binding of [^{3}H]-dexamethasone in the entire population of control and adrenalectomized mice is shown in Fig. 1 (48 h after adrenalectomy). Adrenal ablation, which increases the size of the thymus [1] also produces an elevation in the number of binding sites (from 3,700 to 6,500 per cell) without significant changes in their affinity (K_{D37C} are 4.6×10^{-8} M and 3.9×10^{-8} M respectively in four experiments). Conversely, hydrocortisone treatment induces a marked decrease in the cell number per thymus which, 48 h after the second hydrocortisone injection, represents only 4.9 ± 1.25% of that in the adrenalectomized animals. At the same time, the actual amount of the tritiated tracer specifically bound, using a concentration of 5×10^{-8} M [^{3}H]-dexamethasone, is very low as previously described [20]. However, this decrease appears to be the result of a diminution in the apparent affinity of the receptors for the steroid rather than a real decrease in the number of binding sites. Indeed, the number of steroid receptors calculated from Scatchard plot analysis in the populations isolated from hydrocortisone-treated animals (48 h after the second injection) averaged 4900 binding sites per cell and was not significantly different from that in adrenalectomized mice (see above), whereas the K_D value was significantly higher: 1.06×10^{-7} M in nine experiments.

This drop in affinity after *in vivo* steroid injection has also been demonstrated in the liver cytosol [20]. One explanation for this effect put forth by Beato *et al* [31] points toward hydrocortisone occupation or nuclear sequestration of the receptors.

Time course studies of cell viability

The evolution of thymocyte viability in the absence or presence of 10^{-6} M dexamethasone was followed in the three groups of mice. In the absence of steroid, this pattern was markedly different in each. After 6 h incubation *in vitro* 59.2 ± 12.1% ($n = 4$) of the cells isolated from control animals appeared viable compared with 88 ± 1.4% ($n = 5$) of the cells from the adrenalectomized animals. However, after 24 h of incubation the percentages of viable cells were similar in the two groups: 52.3 ± 5.5% and 55.9 ± 6.9% respectively. In thymocytes isolated from hydrocortisone-treated mice the percentages of viable cells remain high during *in vitro* incubation: 91.2 ± 5.5% and 81.3 ± 3.8% after 6 and 24 h of incubation respectively. The lower panel of Fig. 2 demonstrates the patterns of cell viability in the presence of dexamethasone. In samples isolated both from control and adrenalectomized animals the percentages of viable cells decreased with prolonged incubation in the presence of dexamethasone: the percentages of viable cells were 45.6 ± 16.2% and 38.9 ± 11.8% (6 h) and 4.7 ± 8.1% and 8.8 ± 10.1% respectively (24 h). In contrast, the thymocytes from the hydrocortisone-treated mice appeared resistant to steroid *in vitro*: the percentage of viable cells in the presence of steroid was not significantly different from that determined in the absence of the drug.

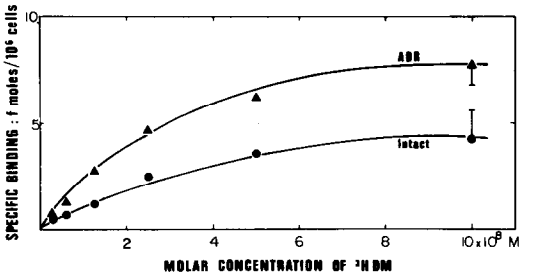

Fig. 1. Specific binding of [^{3}H]-dexamethasone ([^{3}H]DM, 3×10^{-9} to 10^{-7} M, 20 min, 37°C) in thymocytes isolated from intact (●) or adrenalectomized (▲) aminals. Mean values ± standard deviations (SD) of four experiments.

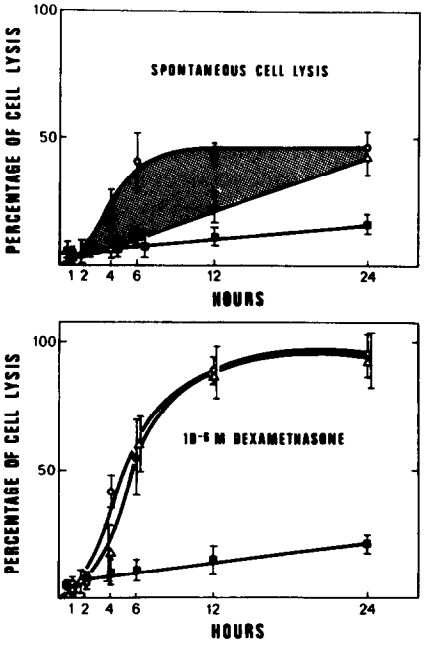

Fig. 2. Top: Time-course pattern of cell viability *in vitro*, in thymocytes isolated from control (○), adrenalectomized (△), or hydrocortisone-treated (■) animals. Cell viability was measured by trypan blue exclusion. Each value represents the mean ± SD of four experiments. Bottom: Evolution of cell viability in the presence of 10^{-6} M dexamethasone. Thymocytes were isolated from control (○), adrenalectomized (△), or hydrocortisone-treated (■) animals. The steroid was added at the start of the incubation.

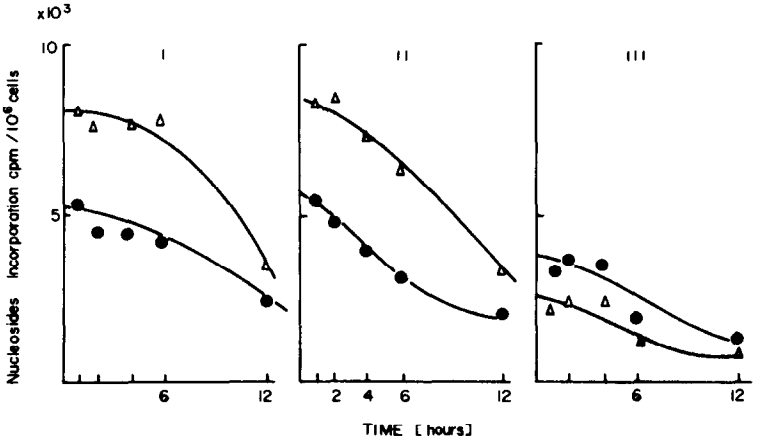

Fig. 3. Incorporation of [^{3}H]-uridine (●) and [3H]-thymidine (△) in the absence of steroid, as a function of the time incubation *in vitro*. Incorporation was measured after a 60-min pulse at 37°C using 1 μCi of the tritiated precursor; cell concentration was 10^7/ml. Thymocytes were isolated from control (I) adrenalectomized (II) or hydrocortisone-treated animals (III).

Effect of dexamethasone on nucleoside incorporation

The incorporation of [^{3}H]-uridine and [^{3}H]-thymidine was studied at various intervals between 0–12 h incubations in the absence (spontaneous incorporations) or presence of 10^{-6} M dexamethasone using a 60-min pulse. Incorporation values were expressed as c.p.m./10^6 viable cells. As shown in Fig. 3, in each group, the level of nucleoside incorporation decreases with prolonged incubation *in vitro* as previously described by Donofrio *et al.* [32]. This phenomenon appears related to the fact that the conditioned medium used for cell suspensions does not contain calcium ions. Similarly, Kaiser and Edelman showed that uridine incorporation by rat thymocytes was lower in the absence than in the presence of calcium [33]. In fact, calcium appears to be necessary to sustain cell proliferation [34]. In thymocytes isolated from hydrocortisone-treated mice the levels of incorporation were very low, particularly for [^{3}H]-thymidine as previously demonstrated [20].

The percentages of steroid-induced inhibition of nucleoside incorporations are shown in Fig. 4. In samples isolated from intact and adrenalectomized mice the inhibitory effect of steroid became perceptible after 2 h and then increased progressively until 7 h, when the percentages of inhibition were 77.7 ± 3.3% and 69.4 ± 9.1% for [^{3}H]-uridine, and 82.6 ± 3.1% and 77.6 ± 5.8% for [^{3}H]-thymidine respectively. After 7 h the extent of inhibition declined slightly, perhaps related to the concomitant decrease of spontaneous incorporation.

In thymocytes isolated from hydrocortisone-treated mice, the inhibitory effect of steroid on nucleoside incorporation increased continuously to reach a percentage of 68.5 ± 12% ([^{3}H]-uridine) and 70.3 ± 13.2% ([^{3}H]-thymidine) at 12 h (n = 3). Thus, the effects of steroid in these cells appeared comparable, although delayed, to those measured in other samples.

Distribution of thymocytes after gradient centrifugation

The circulating level of steroid has been shown to influence the size and cellularity of the thymus: there is a 50–100% increase in thymic cell content of adrenalectomized versus intact animals whereas *in vivo* hydrocortisone treatment decreases the thymic medullary "corticoresistant" cell population to only 3–5% of the initial thymocyte content [1, 20, 22, 35].

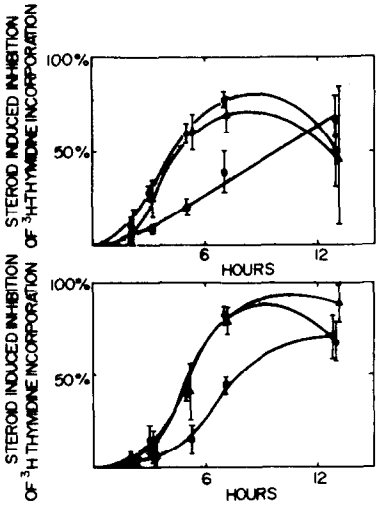

Fig. 4. Top: Steroid induced inhibition of [^{3}H]-uridine incorporation. The cells were preincubated in the absence or presence of 10^{-6} M dexamethasone and then received [^{3}H]-uridine for a 60-min pulse. The incorporation in the absence of steroid is taken as 100% and the values measured in the presence of steroid (corrected for cell viability), expressed as a percentage thereof. (○) = thymocytes isolated from intact animals (n = 3), (△) = thymocytes isolated from adrenalectomized animals (n = 3), (■) = thymocytes isolated from hydrocortisone-treated mice, (n = 3). Bottom: Steroid induced inhibition of [^{3}H]-thymidine incorporation.

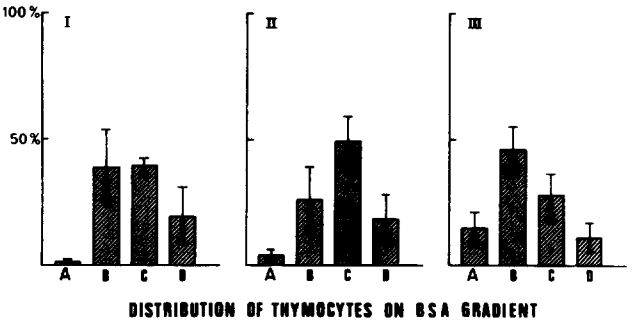

Fig. 5. Distribution profiles of thymocytes after centrifugation on a discontinuous bovine serum albumin gradient (30 min at 10,000 *g*, 4°C). The magnitude of each subpopulation A, B, C, D (from the top of the gradient to the bottom) is expressed as a percentage of the total number of cells harvested after the centrifugation. Usually, 2–4 × 10^8 cells were applied to the gradient and the recovery was about 75%. I = thymocytes isolated from control animals, $n = 3$. II = thymocytes isolated from adrenalectomized animals, $n = 11$. III = thymocytes isolated from hydrocortisone-treated animals, mean value ± SD, $n = 4$.

To investigate the effect of steroid on the various thymocyte subpopulations, we have compared the profile of cell distribution on a density gradient in the three groups of mice. In each sample, about 75% of the cells layered on the gradient (usually 2–4 × 10^8 cells) were collected at the interface after the centrifugation; the results were then expressed as a percentage of the cells recovered. As shown in Fig. 5, these distribution profiles are, on an average, different: the thymocytes isolated from intact animals are equally distributed in B and C fractions (39.4 ± 14.2% and 39.8 ± 2.7% respectively, $n = 3$) with a very low proportion of cells in fraction A (1.2 ± 0.7%); the thymocytes from adrenalectomized animals are located preferentialy in fraction C (51.6 ± 10.4%), whereas the size of fraction B decreases (27.7 ± 10.1%, $n = 11$). The distribution profile of the cells isolated from hydrocortisone-treated animals is characterized by an increase in the population recovered in fraction A (12.3 ± 5.8%) a decrease in the population in fraction D (12.5 ± 4.2%) and a high proportion of the cells in fraction B (44.8 ± 8%, $n = 6$).

Due to individual variations, none of these differences appears statistically significant except for the increase of the A subpopulation in hydrocortisone-treated animals ($P < 0.01$ vs control and $P < 0.05$ vs adrenalectomized animals).

Effects of dexamethasone on nucleoside incorporation in thymocyte subpopulations

We have studied in 24-h assays the effects of 10^{-6} M dexamethasone on [^{3}H]-nucleoside incorporation in thymocyte subpopulations isolated from hydrocortisone-treated animals (OH).

As shown in Fig. 6 (lower panel) the percentage of inhibition of [^{3}H]-uridine incorporation reaches 80% in the entire population, with the extent of inhibition slightly different in the various subpopulations (58.5% for fraction A, 82% for fraction B and 69% in fraction C). In addition, the level of spontaneous incorporation (i.e. in the absence of steroid) decreased from the light fractions (A and B) to the dense fractions (C and D).

Similar results were obtained when measuring the effect of 10^{-6} M dexamethasone on [^{3}H]-thymidine incorporation Fig. 6 (upper panel). The percentage of steroid-induced inhibition of precursor incorporation approaches 50% in the unfractionated population and appears more pronounced in the B fraction than in the A and C fractions. The level of spontaneous incorporation similarly declines from fraction A to fraction D.

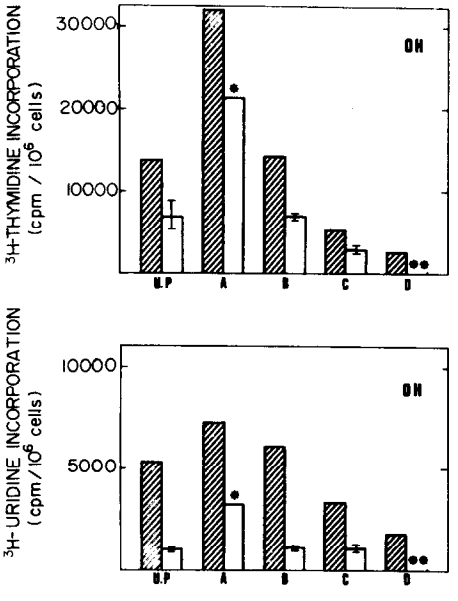

Fig. 6. Top: [^{3}H]-thymidine incorporations in the unfractionated thymocyte population (UP) and in the subpopulations isolated by gradient centrifugation from hydrocortisone-treated animals (24-h experiments). Bottom: [^{3}H]-uridine incorporations in the unfractionated thymocyte population (UP) and in the subpopulations isolated by gradient centrifugation from hydrocortisone-treated animals (24-h experiments). ■ Incorporation in the absence of steroid ($n = 3$). □ Incorporation in the presence of 10^{-6} M dexamethasone ($n = 3$). * $n = 1$. ** Experiment not done.

Table 1. Percentage of cells in G1, S and G2 + M phases in thymocytes isolated from adrenalectomized (ADR) and hydrocortisone-treated (OH) animals; percentage of S cells in subpopulations isolated by gradient centrifugation

	ADR					OH				
	UP	A	B	C	D	UP	A	B	C	D
G1	80.8 ± 4.4					83.7 ± 3.1				
S	9.1 ± 2.1	12.2 ± 3.9	15 ± 1.4	6.6 ± 0.85	5.3 ± 1.7	8.0 ± 1.8	12.6 ± 3.5	6.5 ± 3	5.2 ± 1	7.4 ± 2.5
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
G2 + M	10.1 ± 2.6					8.3 ± 2				

(2) vs (1) ns; (3) vs (1) $P < 0.02$; (3) vs (4) $P < 0.01$; (3) vs (5) $P < 0.01$; (3) vs (2) n.s; (7) vs (6) n.s.; (7) vs (8) $P < 0.1$; (7) vs (9) $P < 0.05$; (7) vs (10) $P < 0.1$.

Evaluation of these percentages was made by planimetric analysis of the DNA histograms. Each value is the mean ± SD of three different experiments. U.P.: unseparated population.

These long-term incubations in the presence of 10^{-6} M dexamethasone could only be performed using cells isolated from hydrocortisone-treated animals. The viability of these thymocytes after *in vitro* incubation in the presence of 10^{-6} M dexamethasone remained over 80%, whereas long term incubation in the presence of steroid of cells isolated from both control and adrenalectomized animals led to pronounced cell lysis (~90%).

DNA measurements by pulse cytofluorometry

The potency of steroid action has been shown to vary throughout the cell cycle [36–38], therefore we determined by pulse cytofluorometry the percentages of cells in G1, S and G2 + M phases in the various populations studied. As shown in Table 1 the thymocyte populations isolated from ADR and OH animals contained similar proportions of cells in the different phases of the cell cycle. These proportions, however, varied among the subpopulations. In thymocytes isolated from ADR animals, the subpopulation B appears significantly enriched in cells in the S phase.

In subpopulations isolated from OH animals, the subpopulation A also contains a high proportion of cells in the S phase.

Uptake of [^{3}H]-nucleosides in ADR thymocytes

The results presented in Fig. 6 show that the levels of spontaneous incorporation differed from one subpopulation to another. To verify that the decrease in incorporation with increasing cell density did not correspond to an experimental artifact occurring during the gradient separation, the following experiment was performed: thymocytes (ADR) were preincubated for 60 min with [^{3}H]-uridine or [^{3}H]-thymidine, washed and then applied to a discontinuous BSA gradient. We then measured both the uptake (whole cell uptake) and incorporation (trichloroacetic precipitable fraction) of the precursors in each cell subpopulation.

The results of typical experiments are presented in Fig. 7. The levels of [^{3}H]-uridine incorporation similarly decreased with increasing cell density. In addition, the uptake values followed a similar pattern: the ratio between [^{3}H]-uridine uptake and incorporation in a given subpopulation, was constant (4.86 ± 0.31). [^{3}H]-Thymidine incorporation levels were higher in fractions A and B than in fraction C and D. This result was easily explained by the significant proportion of S-phase cells in subpopulation B. In each of these subpopulations the ratio between [^{3}H]-thy-

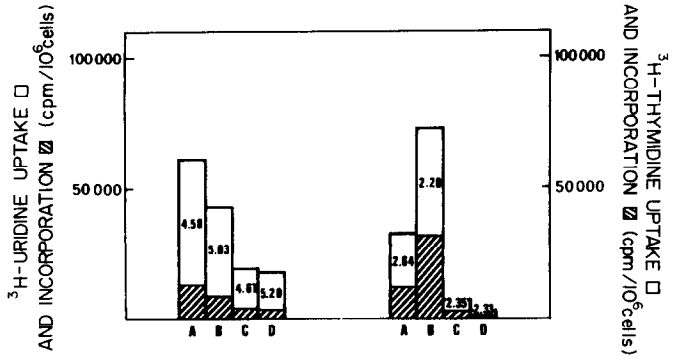

Fig. 7. Left: [^{3}H]-uridine uptake and incorporation in thymocyte subpopulations isolated from ADR animals by gradient centrifugation. The cells were preincubated for 60 min in the presence of [^{3}H]-uridine, washed, and then applied to a BSA gradient. In each subpopulation both the whole-cell uptake (□) and the trichloroacetic acid precipitable fraction (■) (incorporation) were measured. The values in the bars denote the ratio between uptake and incorporation in a given subpopulation. Results of a typical experiment. Right: [^{3}H]-thymidine uptake (□) and incorporation (■) in thymocyte subpopulations isolated from control animals. Results of a typical experiment.

midine uptake and incorporation also appeared constant (2.4 ± 0.16).

DISCUSSION

It has been postulated for several years that a correlation could exist between the sensitivity of lymphoid cells to glucocorticoids and their number of steroid receptors [12–14]. In a previous report however, we showed that the level of dexamethasone binding sites was similar in thymocyte subpopulations with differing sensitivity to glucosteroids [19]. Our present results provide additional evidence suggesting that receptor level does not obligatorily reflect steroid susceptibility. The studies performed with cell populations isolated from hydrocortisone-treated mice showed that these cells, which undergo a pronounced metabolic inhibition in the presence of dexamethasone, are still viable after a 24-h incubation with 10^{-6} M dexamethasone. The receptor content of this "corticoresistant" population was not significantly different from that measured in thymocytes isolated from adrenalectomized mice (4900 ± 1700 sites per cell vs 6500 ± 1200) although 48 h after hydrocortisone injection the apparent affinity of the receptors for the ligand was markedly lowered. In contrast, adrenal ablation was followed by an increase in cellular receptor content. This elevation, which has also been demonstrated in other glucocorticoid target tissues [31, 39, 40], is assumed to represent an increase in the number of sites and not a liberation of sites previously occupied by the endogenous hormone. The effect of dexamethasone on [^{3}H]-nucleoside incorporation and the kinetics of the steroid induced decrease in cell viability were however similar in thymocytes isolated from both control and adrenalectomized animals. These results lend credence to the recent suggestion of Nordeen and Young [41] that the metabolic and lytic effects of glucocorticoids may involve distinct pathways.

Glucocorticoids appear to exert *in vivo* a profound regulatory effect on the thymic cell population. In the absence of endogenous corticosterone (i.e. after adrenalectomy) both the size of the thymus and its cell content increase, whereas conversely, hydrocortisone treatment produces a marked decrease in the thymocyte population [1, 42]. Moreover, the cells present in the thymus after hormonal changes are different from those found in control animals. After adrenal ablation, the proportion of cells which rapidly die *in vitro*, even in the absence of steroid ("Labile cells"), decreases, whereas the thymocytes isolated from hydrocorticosterone-treated animals do not contain an appreciable percentage of labile cells as demonstrated previously by Shortman *et al.*[43]. The distribution profile of thymocytes after centrifugation on a discontinuous BSA gradient is also variable according to the group of animals studied. Thymocytes isolated from hydrocortisone-treated animals are relatively enriched in less dense cells (A and B), in agreement with other reports [18, 20, 23, 44], whereas adrenalectomy is associated with a relative increase in the dense subpopulation (C).

Several authors have studied the immunological properties of the thymocyte subpopulations isolated by density gradient centrifugation. Konda *et al.*[17] and Sabolovic and Dumont[16] showed that in control animals these properties differed markedly from one population to another. The B subpopulation in particular exhibits antigenic and functional properties similar to those of mature immunocompetent cells. Similarly, Mosier and Cantor have shown that the various subpopulations isolated from the thymus of hydrocortisone-treated animals may well correspond to different steps of intrathymic maturation and differentiation [21]. The final product of this process is now located in subpopulation D which contains cells with high buoyant density, high electrophoretic mobility and PHA reactivity [22].

In addition, experiments performed with thymocytes from adrenalectomized animals, using an experimental protocol in which gradient separation followed precursor incorporation, demonstrated that the level of incorporation is an intrinsic property of a given subpopulation and that both uridine and thymidine incorporation differ markedly from one subpopulation to another, although the ratios between precursor uptake and incorporation are constant. These results are comparable to those obtained by Fridlender *et al.* in human lymphocytes, who suggested that a relationship could exist between an increase in precursor transport and DNA synthesis [6, 45].

The discrepancy between the high rate of cell proliferation in the thymus and the low extent of T-cell seeding in peripheral tissues has led to the hypothesis that most of the cells produced in the thymus will die there [46]. Our results are in agreement with the hypothesis of a physiological suppressive regulation exerted by glucocorticoids on thymocyte maturation proposed by Shortman *et al.* Stress, injection of hydrocorticosterone *in vivo*, or the presence of dexamethasone *in vitro* will only accelerate the programmed death of some cells [47] particularly those with high θ, low H_2 and TL positivity, leaving unaffected the immunocompetent cells [42, 44]. Therefore, steroid resistance (i.e. resistance to steroid-induced cell death) does not appear to be directly associated with a decrease in the number of steroid receptors or with an impared function of these receptors, but may well be linked to cell differentiation.

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