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# BINDING OF DEXAMETHASONE AND INHIBITION OF TRANSPORT OF 3-*O*-METHYLGLUCOSE IN RAT THYMOCYTES

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## Summary

The effect of temperature (21—37°C) on binding of [$^3$H]dexamethasone in intact rat thymocytes was investigated. Receptor binding was correlated with inhibition by dexamethasone of in vitro transport of 3-*O*-[$^{14}$C]methyl-D-glucose at 37°C.

Receptor binding of dexamethasone was dependent on temperature. The number of receptors per cell was about 3000 at all temperatures studied. However, an increase of temperature from 21 to 37°C changed the rate constant of dissociation from $6.0 \cdot 10^{-3}$ min$^{-1}$ to $55.0 \cdot 10^{-3}$ min$^{-1}$, and the dissociation constant from 1.1 to 5.9 nM.

The effect of dexamethasone (0.8—790 nM) on transport of 3-*O*-[$^{14}$C]-methyl-D-glucose (10—50 $\mu$M) was investigated during the initial 30 s of uptake at 37°C. A dose-dependent inhibition of transport was found at 60 and 120 min after the addition of hormone. Correlation between effect and receptor occupancy was linear. Maximal inhibition was achieved at concentrations of dexamethasone resulting in 95—97% receptor occupancy, and 50% of the maximal effect was obtained at a concentration of dexamethasone corresponding to 50% receptor occupancy.

Inhibition of transport differed in latency between cell preparations. However, this could not be ascribed to change of either amount or affinity of dexamethasone receptors.

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

## Introduction

Kinetic analyses of inhibition by glucocorticoids of methylglucose transport into rat thymocytes have been described recently [1,2]. Glucocorticoids did not change the final volume of distribution of methylglucose, but $V$ of the carrier-mediated transport was reduced without concomitant change in affinity of the carrier to methylglucose [2]. The inhibition appeared to be receptor-mediated, as judged by the potency of equimolar concentrations of various steroids to produce this effect [1]. However, no dose dependence has been presented so far.

Affinity of glucocorticoids to the hormone receptor changed with temperature in rat thymocytes [3,4], rat splenic lymphocytes [5] and hepatoma tissue culture cells [6], but no such change was found in mouse thymocytes [7]. Unless receptor binding is independent of temperature, meaningful correlations between binding affinity and biological affinity can be made only on the basis of isothermal experiments.

The purpose of the present study was to clarify the effect of temperature on the binding of dexamethasone in a whole cell assay and to correlate occupancy of receptors with inhibition by dexamethasone of transport of methylglucose.

## Materials and Methods

Radiochemicals were from the Radiochemical Centre, Amersham: [$^{14}$C]-methylglucose (spec. act. 50—60 Ci/mol), L-[1-$^{14}$C]glucose (spec. act. 2.99 Ci/mol), $^{3}H_2O$ and [1,2-$^{3}$H]dexamethasone (spec. act. 25—40 Ci/mmol). Tritiated dexamethasone was purified by thin-layer chromatography. Dexamethasone and Hepes were supplied by Sigma, St. Louis, MO; silicone oil AR 200 ($d$ = 1.04) from Serva Feinbiochemica, Heidelberg; Lumagel from Lumac, Basel, and phloretin from K and K Labs. Division, Plain View, NY.

The source and preparation of cells were as described previously [2]. However, all experiments were done in buffered media without bovine serum albumin added. The media were Hanks' balanced salt solution buffered with 10 mM Hepes [2], with or without 5 mM glucose added, or phosphate buffered salt solution [3] with or without 8.1 mM glucose added.

For binding studies, oil centrifugation [2,8] was used to separate cell-bound hormone from free hormone. This technique has the advantage of being rapid, and dilution procedures or sudden changes in temperature are avoided. Aliquots of 900 $\mu$l cell suspension were incubated in 0.9 × 10 cm glass vials containing ethanolic solutions of [$^{3}$H]dexamethasone with or without appropriate amounts of unlabelled dexamethasone added. 14—15 concentrations within the range of $5 \cdot 10^{-11}$—$5 \cdot 10^{-6}$ M were included. The concentrations of ethanol did not exceed 0.5% (v/v). The stoppered tubes were incubated at the appropriate temperatures for periods of time that were adequate to obtain steady-state binding at low hormone concentrations ($10^{-10}$ M), i.e. 8.5 h at 21°C, 5 h at 30°C, and 60—90 min at 37°C. The cells were resuspended every 15 min. Incubations were terminated by withdrawal of duplicate aliquots of 300 $\mu$l for oil centrifugation for 30 s [2]. 150 $\mu$l supernatant and the cut tip of the tube containing the cell pellet were passed into individual scintillation vials containing 5

ml Lumagel. After vigorous shaking, pellets were extracted for 12 h at 37°C and subsequently counted. No detectable radioactivity was found in 50—75 μl aliquots from the separating layers of silicone oil. Thus, partitioning of dexamethasone to the oil did not occur during the separation procedure. The efficiency of the extraction producedure was assured by the identity of counting results from extracted and combusted cell pellets.

Results were expressed as the ratio of bound $^3H$ activity per cell pellet from 300 μl cell suspension ($B$) and free $^3H$ activity per 150 μl supernatant ($F$). This ratio was plotted vs. log of free concentration of dexamethasone [3]. Assuming a reversible reaction between dexamethasone and one class of homogeneous, noninteracting receptors, the equilibrium values of $B/F$ can be calculated as [3] $B/F = R_0/K_d + [D]) + Q_1$. $R_0$ is concentration of receptors (pmol/2 ml cell suspension). $K_d$ is the dissociation constant (nM) and [D] the concentration of free dexamethasone (nM). $Q_1$ is the value of $B/F$ in the presence of excess unlabelled dexamethasone, i.e. the contribution of nonspecific binding. Consequently, the best fit of the above mentioned equation to the experimental data displays the concentration of receptors, the dissociation constant and the amount of nonspecific binding.

For dissociation experiments, a 300-μl aliquot from a dense cell suspension ($1—15 \cdot 10^8$ cells/ml) preloaded with [$^3H$]dexamethasone was transferred to 15 ml buffer containing $5 \cdot 10^{-7}$ M unlabelled dexamethasone. The diluted suspension was stirred, and duplicate aliquots of 300 μl were withdrawn at appropriate times. From semi-logarithmic plots of receptor-bound dexamethasone vs. time, $t_{1/2}$ for dissociation was estimated, and by extrapolation to time zero the number of receptors occupied per cell could be calculated.

Assay of transport was as follows. Aliquots of cell suspensions were transferred to glass vials that were kept at 37°C in a water bath placed on a magnetic stirring board. The cell suspensions were stirred synchronously by teflon-coated magnets. After 15-min temperature equilibration, ethanolic (0.4%, v/v) solutions of dexamethasone (0—790 nM) were added. Two methods were applied for the measurement of transport: (a) the oil centrifugation technique described in Ref. 2 was used for the measurement of uptake of [$^{14}C$]methylglucose (10—20 μM) during periods of 30 s; (b) the phloretin technique of Whitesell and Gliemann [9] with modification.

40 μl cell suspension ($4—6 \cdot 10^8$ cells/ml) in a prewarmed constriction pipette were splashed into 1.5 ml polypropylene tubes containing 10 μl [$^{14}C$]-methylglucose giving a final concentration of 25—50 μM. At indicated times, uptake was terminated by the addition of 1.0 ml 0.4 mM phloretin in buffer (21°C). 150 μl silicone oil were immediately layered beneath the cell suspension using a needle-mounted syringe, and tubes were centrifuged for 45 s in a Beckman microfuge. Zero time was determined by adding first the phloretin and then the cells. Liquid scintillation counting and determination of DNA were done as described previously [2].

## Results

Dissociation of [$^3H$]dexamethasone from preloaded thymocytes was followed using a combination of dilution and displacement (Fig. 1). After an ini-

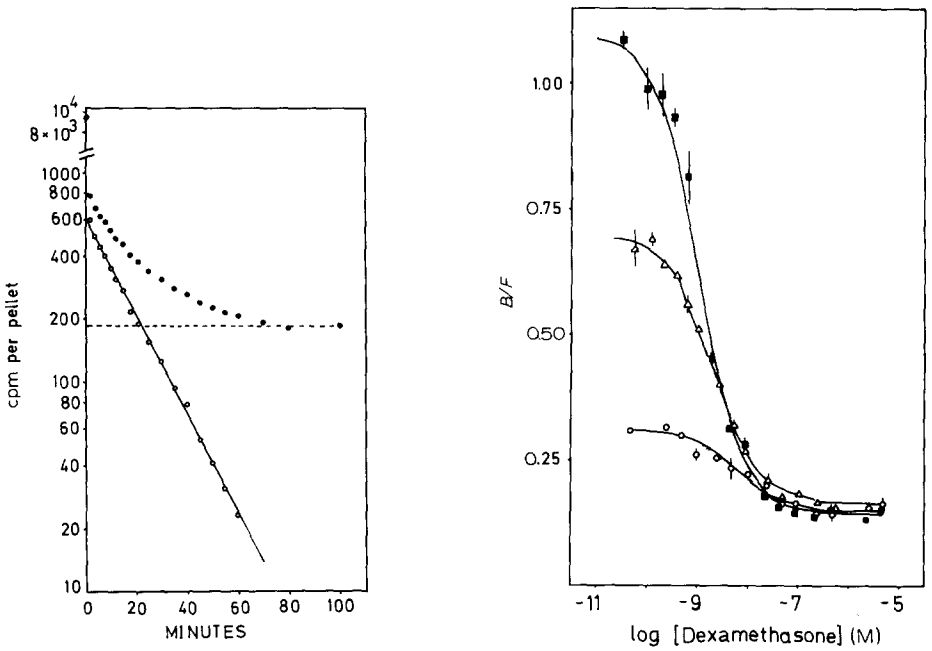



Fig. 1. Dissociation of [$^3$H]dexamethasone from rat thymocytes at 37°C. The procedures were as described in Materials and Methods. The dense cell suspension ($9.4 \cdot 10^8$ cells/ml) was preloaded with $10^{-7}$ M [$^3$H]dexamethasone for 15 min at 37°C. Just before dilution, duplicate 300-µl aliquots were withdrawn for oil centrifugation. Dilution took place at time zero and dissociation was followed for 100 min. Results are presented in a semilogarithmic plot of radioactivity (cpm) per cell pellet from 300 µl cell suspension vs. time (min). The zero time value was computed as $B_0/F_0 \times F$. $B_0$ was radioactivity in cell pellet from 300 µl undiluted cell suspension. $F_0$ was radioactivity of 150 µl supernatant from undiluted cell suspension. $F$ was radioactivity in 150 µl supernatant from diluted cell suspension. (•) Means of duplicates, (○) values after subtraction of new steady state (- - - - -). Lines were fitted by eye. $t_{1/2}$ of dissociation was 13 min corresponding to rate constant of dissociation of $53.3 \cdot 10^{-3}$ min$^{-1}$. The number of receptors occupied at time zero was 3830 per cell.

Fig. 2. Steady-state binding of [$^3$H]dexamethasone to rat thymocytes ($10^8$ cells/ml) at 21°C (■), 30°C (△) and 37°C (○). Ordinate represents ratio between cpm in cell pellets from 300 µl cell suspension ($B$) and cpm in 150 µl supernatant ($F$). The abscissa is log of the free concentration of dexamethasone [D]. Ranges not covered by the symbols are indicated by bars. The fitted curves have the equations:

$$B/F = \frac{1.056 \text{ pmol/2} \cdot 10^8 \text{ cells}}{1.1 \text{ nM} + [\text{D}]\,(\text{nM})} + 0.140 \quad \text{at } 21°\text{C}$$

$$B/F = \frac{1.214 \text{ pmol/2} \cdot 10^8 \text{ cells}}{2.3 \text{ nM} + [\text{D}]\,(\text{nM})} + 0.165 \quad \text{at } 30°\text{C}$$

$$B/F = \frac{1.01 \text{ pmol/2} \cdot 10^8 \text{ cells}}{6.3 \text{ nM} + [\text{D}]\,(\text{nM})} + 0.150 \quad \text{at } 37°\text{C}$$

These equations correspond to 3160, 3640 and 3020 receptors per cell at 21, 30 and 37°C, respectively.

tial rapid phase of 60 s (i.e. nonspecific binding), the dissociation curve followed an exponential course approaching a new steady state. $t_{1/2}$ for dissociation was estimated in semilogarithmic plots of log bound hormone vs. time. The mean values of $t_{1/2}$ for dissociation were 115 min at 21°C, 35 min at 30°C, and 12.6 min at 37°C. The temperature dependence of the rate constant of dissociation is given in Table I. Six independent experiments of dissociation were

TABLE I

DEPENDENCE OF TEMPERATURE ON THE INTERACTION BETWEEN DEXAMETHASONE AND GLUCOCORTICOID RECEPTORS OF INTACT RAT THYMOCYTES

$k_{-1}$ = rate constant of dissociation, $K_d$ = dissociation constant. Numbers in parentheses indicate the number of experiments. Values are given ± S.E.

| Temperature (°C) | $k_{-1}$ ($min^{-1}$) | $K_d$ (nM) | Receptor amount (sites per cell) |
|---|---|---|---|
| 21 | $6.0 \cdot 10^{-3}$ (1) | 1.1 ± 0.1 (3) | 2535 ± 118 (3) |
| 30 | $(19.8 \pm 1.4) \cdot 10^{-3}$ (3) | 2.3 ± 0.1 (12) | 3140 ± 240 (12) |
| 37 | $(55.0 \pm 5.4) \cdot 10^{-3}$ (15) | 5.9 ± 0.2 (4) | 3077 ± 175 (4) |

done at 37°C using cells preloaded for 15 min with $5 \cdot 10^{-7}$ M [$^3$H]dexamethasone. On the basis of extrapolation to time zero, the number of receptors per cell was estimated to be 3252 ± 400. Net uptake of receptor-bound [$^3$H]dexamethasone was followed. A steady state of binding was achieved within 4—5-times $t_{1/2}$ for dissociation at low concentrations of dexamethasone (1/10—1/100 of $K_d$).

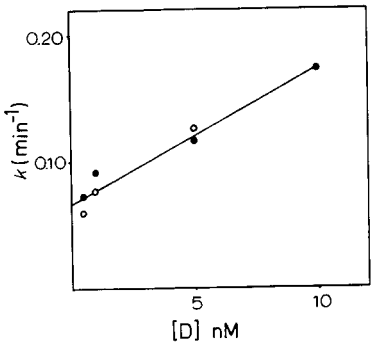
Fig. 2 illustrates the dependence of temperature on steady-state binding of dexamethasone to thymocytes from three representative experiments. On the basis of results from 19 experiments $K_d$ and number of receptors per cell were estimated (Table I). In conclusion, (a) nonspecific binding at identical cell concentrations was not dependent on temperature. Fig. 1 visualizes that $B/F$ values at three temperatures approach the same value at concentrations of dexamethasone above $5 \cdot 10^{-7}$ M. The nonspecific $B/F$ values were 0.140 at 21°C, 0.165 at 30°C and 0.150 at 37°C; (b) the number of receptors per cell was not dependent on temperature, but (c) receptor affinity increased with decreasing tem-

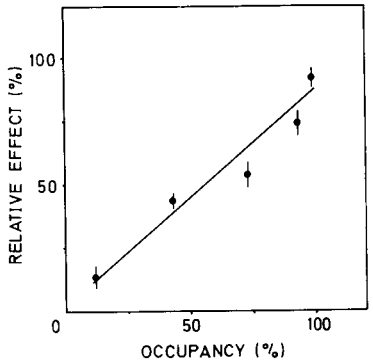
TABLE II

DOSE-DEPENDENT INHIBITION BY DEXAMETHASONE OF INITIAL 30-s UPTAKE OF [$^{14}$C]METHYLGLUCOSE AT 25—50 μM

Methods were as described in Materials and Methods. In experiments 1—4, the uptake of methylglucose was stopped by the addition of phloretin to cells. Cells were preincubated at 37° C for 15 min in six vessels. The 30-s uptakes of [$^{14}$C]methylglucose were tested and found identical after the preincubation. Subsequently, dexamethasone was added to give final concentrations of 0—790 nM in the six vessels. 60 min later, control cells were tested for uptake of [$^{14}$C]methylglucose at 0, 10, 20 and 30 s, and afterwards cells incubated with dexamethasone were tested in duplicate at 0 and 30 s of uptake. Finally, control cells were tested again at 0, 10, 20 and 30 s of uptake. After the transport assays, cell counts and cell viability were controlled in all vessels, and finally intracellular $^3H_2O$ spaces were measured in pellets from all vessels using L-[$^{14}$C]glucose as an extracellular marker [2]. In experiments 5—8, termination of uptake was defined as starting time of the microfuge for oil centrifugation, and transport assay was carried out as described in Ref. 2. Results are given as percentage of initial velocity of transport of control cells. Figures are means ± S.E. ($n$ = 4).

| Expt. No. | Dexamethasone concentration (nM) | | | | |
|---|---|---|---|---|---|
| | 790 | 79 | 15.8 | 7.9 | 0.8 |
| + phloretin 1—4 | 76.8 ± 1.8 | 75.3 ± 4.3 | 83.5 ± 2.5 | 86.3 ± 2.6 | 97 ± 0.9 |
| — phloretin 5—8 | 73.5 ± 4.4 | 79.0 ± 2.8 | 88.3 ± 0.8 | 87.8 ± 1.1 | 96.3 ± 1.9 |



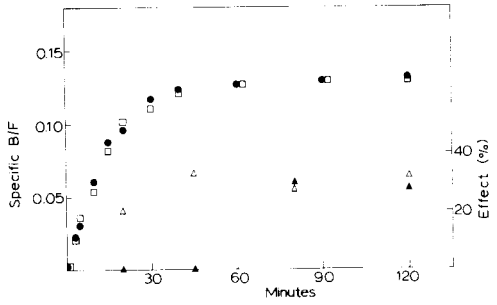




Fig. 3. (Left). The dependence of concentration on the rate constant of net uptake ($k$) of [$^3$H]dexamethasone at 37° C. Thymocytes ($7 \cdot 10^7$ cells/ml) were incubated under stirring at 37° C. After 15 min of temperature equilibration, [$^3$H]dexamethasone was added to give final concentrations of 0.5, 1.0, 5.0 or 10.0 nM. Uptake was monitored by withdrawal of duplicate aliquots of 300 µl for oil centrifugation at appropriate times from 0 to 60 min after the addition of hormone. Non-specific binding at these concentrations was determined in parallel incubations containing the same concentrations of [$^3$H]dexamethasone plus $10^{-6}$ M unlabelled dexamethasone. Net uptake of receptor-bound dexamethasone was exponential. From semilogarithmic plots of 1 — (fractional steady-state values) vs. time, $t_{1/2}$, for net uptake was determined, and the rate constants of net uptake were calculated as ln $2/t_{1/2}$ of net uptake. The results from two experiments (●, ○) are shown. The equation of the regression line was:

$k = 0.011\ \text{nM}^{-1} \cdot \text{min}^{-1} \times [\text{D}] + 0.067\ \text{min}^{-1}$ .

[D] represents concentration of dexamethasone (nM).

Fig. 4. (Centre). Comparison of receptor occupancy with inhibition of [$^{14}$C]methylglucose transport at 37° C. Data came from eight experiments presented in Table II. Each experiment was normalized on a relative scale from 0—100 according to its maximal inhibition by dexamethasone of methylglucose uptake, i.e. at the $j$'th concentration of dexamethasone $[\text{D}]_j$, the relative effect (%) equaled: 100 × (inhibition at $[\text{D}]_j$/maximal inhibition). Ordinate = relative effect (%); abscissa = receptor occupancy (%) computed assuming $K_d$ = 5.9 nM at 37° C. Results are means, with bars indicating S.E. ($n$ = 8). The equation of the regression line was:

$y = 0.85\ x + 2.90$ .

Fig. 5. (Right). Simultaneous measurement of [$^3$H]dexamethasone receptor binding and latency for inhibitory effect of dexamethasone on [$^{14}$C]methylglucose transport. Two separate experiments are shown (filled and open symbols). They were done at identical conditions and identical cell concentrations ($10^8$ cells/ml). Duplicate vials with 15 ml cell suspension were incubated under stirring at 37° C. After 15 min, 15 µl of an ethanolic solution of dexamethasone and 5 µl [$^3$H]dexamethasone were added to one vial giving concentration of $5 \cdot 10^{-7}$ M dexamethasone and $10^{-10}$ M [$^3$H]dexamethasone. The second vial, to which 15 µl ethanol and also 5 µl [$^3$H]dexamethasone were added, served as a control. The additions were done at zero time. Uptake of [$^3$H]dexamethasone was followed in both vessels, withdrawing duplicate aliquots of 300 µl at indicated times for oil centrifugation. $B$ was tritum activity (cpm) in cell pellets from 300 µl cell suspension. $F$ was tritium activity (cpm) in 150 µl supernatant. Specific $B/F$ (●, □) were computed as the differences between time-matched $B/F$ values from control vessel (uncompeted) and dexamethasone vessel (competed). The 30-s uptake of 11 µM [$^{14}$C]methylglucose and of 10 µM L-[$^{14}$C]glucose were tested on 1 ml aliquots from each vessel at times indicated [2]. The effect (▲, △) was expressed as per cent inhibition of methylglucose uptake into control cells. At the end of the experiment, numbers of viable cells and content of DNA were found to be identical in both vessels. Values are means of duplicates.

perature. An Arrhenius plot (log $K_d$ vs. $1/T$) of the data gives a straight line with a delta $H^0$ of 18.3 kcal · $mol^{-1}$. No difference in binding characteristics was found between experiments done with or without glucose added.

Fig. 3 illustrates the linear relationship between the rate constant of net uptake of receptor-bound [$^3$H]dexamethasone and the concentration of free hormone. The total amount of hormone taken up by the cells from the medium was less than 8%. Therefore, the hormone concentration was considered constant throughout the period of uptake. From the kinetic values of rate constants of association (0.011 $nM^{-1} \cdot min^{-1}$) and dissociation (0.067 $min^{-1}$), a dissociation constant of 6.1 nM was calculated. On the basis of the initial specific uptake of [$^3$H]dexamethasone, of the rate constant of association (0.011 $nM^{-1} \cdot min^{-1}$), and of the hormone concentration, the number of receptor sites was calculated to be 2650 per cell.

Phloretin at a concentration of 0.4 mM inhibits efflux of [$^{14}$C]methylglucose at 37°C [2]. In the present study, the effect of 40 nM—0.4 mM phloretin on uptake of 11 μM [$^{14}$C]methylglucose was investigated. At 37°C, 20 μM phloretin caused half maximal inhibition of uptake of methylglucose during 30 and 150 s. Inhibition was complete at 0.4 mM phloretin. At 21°C, inhibition of uptake lasted for at least 4 min.

Table II shows the dose-dependent inhibition of the initial, linear 30-s uptake of methylglucose after 60 min preincubation with dexamethasone. For the lowest concentrations of dexamethasone, this period of incubation would suffice for binding to reach a steady state. Results are from eight experiments of which four used oil centrifugation alone and four used phloretin combined with oil centrifugation. The maximal inhibition of 30-s uptake of methylglucose ranged in these eight experiments from 19 to 38% of uptake into control cells.

In order to compare dose dependency of these eight experiments exhibiting different maximal responses, each experiment was normalized (control cells = 0% relative effect; maximal inhibition = 100% relative effect). Given a $K_d$ of 5.9 nM at 37°C, the correlation between receptor occupancy and relative inhibitory effect is shown in Fig. 4. Maximal relative effect was obtained at concentrations of dexamethasone resulting in 95—97% receptor occupancy, and 50% of the maximal effect was obtained at a concentration not different from $K_d$ at 37°C (5.9 nM). The computed regression line of the means did not differ significantly from the line of identity. More accurate graduation of the effect of 20—40% was not possible using the present methods. Two experiments were extended to 120 min of incubation with dexamethasone without changes in the dose dependency.

Various cell preparations showed different latencies for dexamethasone to exhibit this effect [2]. This might be explained by change of either amount or affinity of receptors. These possibilities were tested. Fig. 5 shows results of two experiments done at identical conditions and cell concentrations. In one experiment, inhibition of transport was 15% 20 min after addition of $5 \cdot 10^{-7}$ M dexamethasone. The inhibition amounted to 33% after 45 min, and thereafter it was constant 28—32% up to 120 min. In the other experiment, no inhibition was measurable at 20 or 45 min after hormone addition but at 80 and 120 min, the inhibition reached 30 and 28%, respectively. In spite of this difference in

latency of 60 min, the association curves of specific bound [$^3$H]dexamethasone at $10^{-10}$ M were identical in respect to $t_{1/2}$ (10.5 min) and to maximal ratio of bound to free hormone. Thus, isolated changes in amount or affinity of receptors cannot explain the observed variation of latency. In the experiments of Fig. 5, control cells were in fact treated with $10^{-10}$ M [$^3$H]dexamethasone. However, no significant effect on transport would be expected at this receptor occupancy of 1.6%.

## Discussion

Only free glucocorticoids are biologically active [10]. In the present experiments, all dexamethasone, except for the cell-bound hormone, was free because albumin and serum were omitted from the media and because no detectable metabolism of dexamethasone occurred in rat thymocytes during the short incubation times (Ref. 4, and own unpublished results). Glucocorticoid receptors in rat thymocytes were first described by Munck and Brinck-Johnsen [11], and Schaumburg and Bojesen [3]. Temperature-dependent binding of [$^3$H]corticosterone was reported by Schaumburg and Bojesen [3]. Enthalpy was constant in the range of temperature from 4 to 37°C. However, the measurements at 37°C were inaccurate, because of relatively low affinity of the receptor to corticosterone at physiological temperatures [3]. Taking advantage of the higher affinity of dexamethasone to the receptor, the present study extends the dependence of dexamethasone-binding on temperature in the range of 21—37°C. The results were consistent with those of Schaumburg and Bojesen [3]. The failure of Dausse et al. [7] to demonstrate an effect of temperature on the binding of dexamethasone to mouse thymocytes can probably be explained by the fact that they used 2-h periods of incubation at 0—4°C. This might be insufficient to obtain equilibrium between the receptor and the lowest concentrations of dexamethasone. The effect of temperature on binding of dexamethasone has previously been observed in nuclei and cytosol of rat thymocytes [4,12] and in rat splenic lymphocytes [5]. Wolff et al. [6] have characterized the cytosolar binding of corticosterone in hepatoma tissue culture cells. In a temperature range from −2 to +16°C, the best fit of the plot of ln $K$ vs. $1/T$ was a second degree polynomium with maximum at 5°C. Our results were linear. This discrepancy might reflect organ-specific differences, but it appears more likely that the impact of temperature on the different binding assays (whole cells vs. cytosol) complicates the thermodynamic considerations, which have to be taken with some reservations.

The number of receptors per cell was calculated to be 3000—3500. This is in accordance with the range of 2500—5000 sites per cell previously found in rat thymocytes [3,11], in human lymphocytes [13], and in mouse thymocytes [7], but a little lower than that in macrophages from mouse, man and rabbit [14]. The dissociation constant of 5.9 nM at 37°C agrees with that found in macrophages (2—8 nM) [14]. In rat thymocytes at 37°C, $K_d$ was about 10 nM [11,15] but $t_{1/2}$ for dissociation of dexamethasone from receptors was 5 min as compared to 12 min in the present study. The reason for this discrepancy is obscure. The fact that we found $t_{1/2}$ of net uptake of receptor-bound dexamethasone at low concentrations to be more than 10 min supports our value of 12 min for $t_{1/2}$ of dissociation.

Assuming that $t_{1/2}$ of dissociation is 10—12 min at 37°C, an incubation time of 30 min as used by Sloman and Bell [15] would be insufficient to obtain equilibrium of binding at low hormone concentrations. This would tend towards an increase of the apparent dissociation constant and a slight increase of number of receptors. However, these arguments do not suffice to explain the very high dissociation constant of 40 nM found in mouse thymocytes [7].

Given the temperature dependence of the receptor affinity to individual steroids and the possibility of changes in specificity of the receptor for different steroids with temperature [4,5], it follows that receptor binding and biological activity ought to be correlated only when measurements of both are made at identical temperatures. Many studies do not fulfill this condition. In the present study, a linear correlation was established between inhibition of transport of methylglucose and receptor occupancy at 37°C. No indications of spare receptors were recognized.

The reason for the previously described difference in latency of dexamethasone to inhibit transport of methylglucose into different cell preparations cannot be due to isolated changes in either dissociation constant or numbers of receptors. A remote possibility is balanced changes in the rate constant of association and in amount of receptors that will give identical association curves at $10^{-10}$ M dexamethasone. However, it seems more likely that events distant to involvement of receptors are responsible for this phenomenon.

Approx. 6 nM dexamethasone causes half maximal inhibition of transport of methylglucose. This corresponds with $LD_{50}$ of 5.6 nM dexamethasone previously found for rat thymocytes [16], and with 8 nM dexamethasone which induces 50% of maximal activity of tyrosine aminotransferase in hepatoma tissue culture cells [17]. In this order of magnitude were also the half maximal inhibitory concentrations of dexamethasone for the concanavalin A-induced incorporation of thymidine into mouse thymocytes [18], human lymphocytes [19] and enzyme secretion from human and murine macrophages [20].

In conclusion, specific binding of dexamethasone in rat thymocytes was dependent on temperature. Correlation between inhibition of transport of methylglucose and receptor binding of dexamethasone at 37°C was linear.

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