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The permeability of the human red cell membrane to steroid sex hormones

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Abstract

The release rates of the ^{3}H-labeled steroid sex hormones estrone, estradiol, estriol, progesterone, and testosterone from the human red blood cell and resealed red cell ghosts were studied at 38°C and pH 7.2 by means of the rapid continuous flow tube method which has a time resolution of a few milliseconds. Further, the equilibria between unbound hormone and hormone bound to red cells, resealed red cell ghosts and albumin were studied by partitioning analysis of trace amounts of labeled hormones. The half-times for release from erythrocytes under physiological conditions ranged from 4 ms (testosterone) to 150 ms (estriol). The release from ghosts was significantly faster than from cells preincubated with hormones at unphysiological high concentrations. Affinities of hormone binding to cells and hormone indicate that as much as 15–35% of the total hormone content in whole blood is confined to red cells. The ratio between bound and free hormone in the cell ranged from 5 to 10, and the ratio between cytoplasma-bound and membrane-bound hormone ranged between 3 and 9. The results are compatible with a model of fast transition of hormone through the red cell membrane and intracellular binding of hormone. We suggest that red cells function as carriers of sex hormones in the bloodstream in a manner similar to that of albumin, and that red cells may be responsible for 5–15% of sex hormone delivery to target tissues.

Keywords: Steroid sex hormone; Membrane permeability; Erythrocyte; Red blood cell

1. Introduction

The chemical structure of the steroid sex hormones makes them rather hydrophobic, and the carrying capacity of plasma water would, therefore, be low were hormones not bound to the circulating proteins. Because of the hydrophobic structure of the hormones, it may be assumed that the membranes have no hormone receptor binding sites, as the hormones are supposed to permeate the cell membrane easily and bind to the nucleus and subsequently initiating their signal to the cell. Some earlier studies reported a specific transport of steroids across the cell membrane [1–3]. However, Moll and Brahm [4] found no evidence for the presence of a specific transport system in a study of red blood cells. In the present study we further investigate how fast are the hormones transported across the cell membrane in intact red blood cells and resealed red blood cell ghosts. The erythrocyte membrane was selected because these cells are well-suited for studying rapid transport processes by means of a continuous flow tube system, which has been further developed by Brahm [5]. A subsequent question was, that because of our finding that the hormones are transported extremely rapid across the red cell membrane, to what extent the red cells contribute to the carrying capacity of whole blood to hormones. It is generally accepted that hormones bind to plasma proteins, and that the free plasma concentration is very low [6]. Studies [7,8] suggest that almost all hormone is extracted during the capillary transit time. It follows from such studies that either is the intracellular binding of hormones in the red cells insignificant, or that the dissociation of hormones from the intracellular binding sites and the subsequent passage through the cell membrane are not

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rate limiting processes for hormone uptake in tissues. In the present study we conclude that the latter assumption is the most likely explanation.

2. Materials and methods

2.1. Reagents

Estrone (estra-1,3,5(10)-trien-17-on), estradiol (estra-1,3,5(10)-trien-3,17β-diol), estriol (estra-1,3,5(10)-trien-3,16α,17β-diol), progesterone (pregn-4-en-3,20-dion), and testosterone (17β-hydroxyandrost-4-en-3-on) were purchased from Merck Denmark A/S. [2,4,6,7,(n)-^{3}H]Estrone, [2,4,6,7,(n)-^{3}H]estradiol, [1,2,6,7,(n)-^{3}H]progesterone and [1α,2α,(n)-^{3}H]progesterone were purchased from Amersham International. [2,4,6,7,(n)-^{3}H]Estriol was purchased from Du Pont Industries. Freeze-dried human albumin was obtained from Novo-Nordisk Industries, Denmark. All other chemicals and solvents used were of PA-grade. The hormones were kept for maximum 5 days in cold store in 96% ethanol as 1 mM stock solutions. No change of efflux data was related to the storage time.

The following media were used: A: 150 mM NaCl, 2 mM NaH_2PO_4, pH 7.2 at 38°C. A1: 3 nM estradiol in A. A2: 100 nM estriol in A. A3: 10 nM estrone in A. A4: 20 nM progesterone in A. A5: 1 nM testosterone in A. A6 1 μM estradiol in A. A7: 10 μM estriol in A. A8: 1 μM estrone in A. A9: 1 μM progesterone in A. A10: 1 μM testosterone in A. B: 165 mM NaCl, 2 mM NaH_2PO_4, pH 7.2 at 38°C. B1: 3 nM estradiol in B. B2: 100 nM estriol in B. B3: 10 nM estrone in B. B4: 20 nM progesterone in B. B5: 1 nM testosterone in B. C: 150 mM NaCl, 2 mM NaH_2PO_4, 3 nM estradiol, 100 nM estriol, 10 nM estrone, 20 nM progesterone and 1 nM testosterone, pH 7.2 at 38°C. D: 150 mM NaCl, 2 mM NaH_2PO_4, 1% human albumin, pH 7.2 at 38°C. E: 4 mM $MgSO_4$, 3.8 mM CH_3COOH. F: 1.99 M KCl, 25 mM Tris.

2.2. Labeling and packing of erythrocytes

20 ml freshly drawn, heparinized human blood was centrifuged at room temperature and the plasma and buffy coat were removed. The cells were washed three times at low hematocrit in one of the hormone-containing media listed above to ensure that intracellular concentrations of hormone equalled that of the medium. The cells were gently titrated with CO_2 at 38°C to pH 7.2, next washed two more times in the medium concerned, and resuspended in the medium to a hct $\approx$ 50%. 35–40 kBq ^{3}H-labeled hormone was added, and the suspension was, after 5 min of incubation, centrifuged at 4900 $\times g$ at 0°C for 20 min. The supernatant was removed and the packed cells transferred to syringes which were stored at 38°C for at most 1 h before use in the flow-tube.

2.3. Preparation of resealed ghosts

A suspension of resealed ghosts was prepared from 25 ml of freshly drawn, heparinized human blood, as recently described by Gasbjerg and Brahm [9]. The ghosts were treated as the red cells described above using one of the B-media.

2.4. Determination of hormone release

All experiments were performed under steady-state conditions, that ensures no net flux of solvent and solutes across the membranes. In the present study the efflux of radioactively labeled hormone from the cells was monitored using the continuous flow-tube method [5]. Packed, radioactively labeled cells and non-radioactive medium were simultaneously and continuously injected separately into a chamber where they mixed. The shape of the chamber ensured an optimal mixing of cells and medium. The suspension (hct $< 0.5\%$) was driven out of the chamber through a tube with inserted filtration sites placed at well-defined distances from the mixing chamber. At each filtration site a cell-free filtrate was collected. The linear flow velocities were between 13 m s^{-1} and 43 m s^{-1}, and varied $< 2\%$ during an experiment. Thus, the flow velocity by far exceeded the minimum velocity that maintains both turbulent flow in the pipe and the complete mixing of the medium and the packed cells before the first filtration-site was reached by the suspension [5]. During an experiment the time and volume of suspension were measured; from these values and the radius of the tube (1 mm) a linear velocity (m s^{-1}) was computed. Since the distances of the collecting ports from the mixing chamber are known, the time for the suspension to pass from the chamber to each port can be calculated. These times also represent the reaction times for the tracer efflux process.

The radioactivity of the filtrated samples were measured by liquid scintillation (in a Tri-Carb 2200CA Liquid Scintillation Counter, Packard Instruments, using OptiFlour scintillation liquid).

2.5. Determination of hormone affinity to cells and albumin

For each hormone the following equilibrium analyses were carried out at 38°C: in a 2 ml vial 50 μl of medium A containing a small amount of ^{3}H-labeled hormone ($\approx$ 3 kBq) was added to the following 3 solutions and suspensions: 1: 950 μl medium A, 2: 475 μl medium A and 475 μl packed cells, 3: 475 μl medium D and 475 μl packed cells. The mixtures were

gently shaken for 10 min to equilibrate the hormone between medium and cells. The hematocrit was determined by centrifugation of samples from each vial (in a Hematocentrifuge HC 102, Heraeus Christ Gmbh, Osterode am Harz, Germany). The vials were centrifuged for 5 min, and samples of the supernatants were analyzed by liquid scintillation counting. A similar set of analyses were performed with resealed ghosts, using medium B only.

2.6. Determination of rate constants and apparent permeability

The time dependency of extracellular activity of radioactive tracer derived from a population of cells can be expressed as:

$$A(t) = A_{t=\infty}(1 - e^{-kt+b}) \quad (1)$$

if the cells only contain tracer in a single compartment. Here k is the rate constant for the unidirectional release of tracer, $A_{t=\infty}$ is the extracellular activity at infinity and b is defined as

$$b = \ln\frac{A_\infty - A_{t=0}}{A_\infty}.$$

In this study we have fitted this function to our data using non-linear regression. Each curve-fit has been submitted to a χ^2-test; all fitted graphs with $p < 80\%$ have been excluded from the material. From the remaining estimates of k we have calculated a weighted mean for each group of experiments, weighing each k with the inverse of its variance:

$$\bar{k} = \frac{\sum_{i=1}^{n} k_i\left(s^2(k_i)\right)^{-1}}{\sum_{i=1}^{n}\left(s^2(k_i)\right)^{-1}} \quad (2)$$

Accordingly, the variance of $\bar{k}$ has been computed as:

$$\mathrm{var}(\bar{k}) = \frac{\sum_{i=1}^{n}\left(\left(k_i - \bar{k}\right)^2\left(s^2(k_i)\right)^{-1}\right)}{\sum_{i=1}^{n}\left((1-n^{-1})\left(s^2(k_i)\right)^{-1}\right)} \quad (3)$$

In experiments with intact red cells the calculation of apparent permeabilities, P_{app} (μm s^{-1}), were carried out as:

$$P_{app} = k\frac{V}{A_m} = k\frac{f'}{\rho_{aq}A'_m} \quad (4)$$

where ρ_{aq} is the specific weight of water (10^{-3} kg cm^{-3}), f' is the ratio of the mass of water to the mass of dry matter in the red blood cells (determined by drying packed cells at 100°C for 24 h), and A'_m is $4.4 \cdot 10^4$ cm^2 g^{-1} (the surface area of $3.2 \cdot 10^{10}$ erythrocytes, which contain 1 g of dry solids under 'physiological' conditions). In experiments with resealed ghosts the mean cell volume of the cells was determined with a Coulter counter (Coulter Electronics. Dunstable Beds., UK), and $V \cdot A_m^{-1}$ (cm) was computed assuming that the mean membranearea of a ghost is $142 \cdot 10^{-8}$ cm^2 (the same as for a RBC).

3. Results

3.1. Equilibrium analyses

The equilibrium: H + RBC ⇄ H-RBC has the equilibrium constant:

$$K_1 = \frac{[\text{H-RBC}]}{[\text{H}][\text{RBC}]} \quad (5)$$

where [H] is the concentration of free hormone in the entire suspension, [H-RBC] is the concentration of bound hormone and [RBC] is the concentration of binding-sites in the erythrocytes. As long as the reaction is reversible and far from saturating the binding-sites of the cells, the ratio of bound to unbound hormone in the cells, $R = [\text{H-RBC}]\,[\text{H}]^{-1}$, remains constant. R can be computed from:

$$R = \frac{\frac{A^{ref}}{A} - (1 - \text{hct})}{r_{H_2O,RBC}\,\text{hct}} - 1 \quad (6)$$

where A^{ref} and A are the activity concentrations in the cell-free reference vial and in the supernatant from a suspension with hematocrit = hct; $r_{H_2O,RBC}$ is the ratio between the volume of cell water and total cell volume. Similarly, one can compute R for the hormone distribution in ghosts. The computation of K_2, the constant for binding between hormone and human albumin, is based on:

The activity of labeled free extracellular hormone, A^{fec}, and albumin-bound labeled hormone can be calculated as:

$$K_2 = \frac{[\text{H-alb}]}{[\text{H}][\text{alb}]} \quad (7)$$

can be calculated as:

$$A^{fec} = \frac{A^{ref} - A(1 - \text{hct})}{1 + R}\,\frac{1 - \text{hct}}{\text{hct} \cdot f} \quad (8)$$

leading to the calculation of K_2:

$$K_2 = \frac{A^{alb}}{A^{fec}[\text{alb}]}(l \cdot \text{kg}^{-1}) \quad (9)$$

where f is the fractional cell water volume, and [alb] is the extracellular concentration of albumin, measured

Table 1
Distribution constants for the equilibria between free hormone and hormone bound to erythrocytes (R_{RBC,H_2O}), resealed ghosts (R_{MEM,H_2O}) and human albumin (R_{alb,H_2O}) at 38°C and pH 7.2

Hormone	R_{RBC,H_2O}	R_{MEM,H_2O}	R_{alb,H_2O} (4% albumin)
Estrone	8.7±0.6	2.5 ±0.7	10.8±1.0
Estradiol	10.5±0.3	2.4 ±0.2	21.7±2.1
Estriol	6.5±0.7	0.77±0.09	6.6±0.5
Progesterone	8.6±0.6	3.7 ±0.2	27.4±2.2
Testosterone	5.2±0.3	0.9 ±0.2	13.8±0.9

The constants were calculated from data obtained by liquid scintillation counting of the supernatants from centrifuged suspensions of erythrocytes and ghosts in saline media with or without 1% human albumin (see details in text). The data represent mean±95% confidence intervals.

in kg l^{-1}. Multiplying K_2 with a extracellular albumin concentration of 0.04 kg l^{-1} results in a R-value for distribution of extracellular hormone between albumin and the aqueous phase at physiological conditions. The constants of distribution between bound and unbound hormone for the three different systems, R_{RBC,H_2O}, R_{MEM,H_2O} and R_{alb,H_2O}, are listed in Table 1.

3.2. Flow-tube experiments

A total of 50 preparations of erythrocytes and 11 preparations of resealed ghosts were used in 352 flow tube experiments. Of these, 252 flow-tube experiments were used in the calculations of rate constants and apparent permeabilities. The remaining experiments were excluded on the following grounds:

(1) Experimental errors occurred, e.g., leakages in the tube or inappropriate choice of flow-rate in an experiment, leading to a useless time-resolution (typically the first one or two times a hormone was tested).

(2) There was significant difference between experimental data and the fitted model at a 20% confidence level, found by χ^2-testing.

The included experiments on erythrocytes were carried out as follows: 122 in media with one hormone in physiological concentration (media A1–A5), 37 in media with one hormone in unphysiological high concentration (media A6–A10), 28 in medium with all five hormones in physiological concentrations (medium C). The 65 included experiments on ghosts were all performed using media with a single hormone in physiological concentration (media B1–B5). The rate constants, half times and apparent permeabilities of the five hormones in erythrocytes and resealed ghosts are listed in Tables 2 and 3. As can be seen in Figs. 1–5 there is a very fast release of labeled hormone from resealed ghosts. In fact, it is only possible to determine a rate constant for the release of estriol. For the other hormones we have calculated the lowest possible limit for the value of k using the approximation that it takes

Table 2
Kinetic data for the release of ^{3}H-labeled hormone from erythrocytes at 38°C and pH 7.2

Hormone	Medium	n	k (s^{-1})	$T_{1/2}$ (ms)	P_{app} ($\mu m\ s^{-1}$)
Estrone	A3	15	91±14	7.6± 1.2	41 ± 6
	A8	6	140±25	5.0± 0.9	59 ±11
	C	5	147±35	4.7± 1.1	67 ±16
Estradiol	A1	16	84±14	8.3± 1.4	37 ± 6
	A6	4	125±39	5.6± 1.7	63 ±20
	C	5	104±23	6.7± 1.5	47 ±10
Estriol	A2	27	4.7± 0.4	148 ±13	2.1± 0.2
	A7	8	6.1± 0.6	114 ±11	2.7± 0.3
	C	5	4.7± 0.5	147 ±15	2.0± 0.2
Progesterone	A4	11	149±37	4.7± 1.2	66 ±16
	A9	8	126±41	5.5± 1.8	56 ±18
	C	7	108±24	6.4± 1.4	53 ±10
Testosterone	A5	53	164±22	4.2± 0.6	73 ±10
	A10	8	224±30	3.1± 0.4	100 ±14
	C	6	221±27	3.1± 0.4	103 ±10

The experiments were performed by employing the continuous flow-tube method. The data represent mean±95% confidence intervals calculated from n determinations.

6 half-times to reach 98% of equilibrium between cellular and extracellular activity of a diffusing agent. A t-testing of the results shows that there is a significant higher k for estriol release from ghosts than from erythrocytes ($p = 7.1 \cdot 10^{-14}$!). We conclude that this also is the case for the other hormones, since the release of hormone from the ghosts is so fast that the time resolution of the flow-tube method is too low. For four of the hormones we find a significant faster release from erythrocytes when using hormone in unphysiological high concentrations: estradiol (p = 0.0092), estriol ($p = 0.0012$), estrone ($p = 0.00054$) and

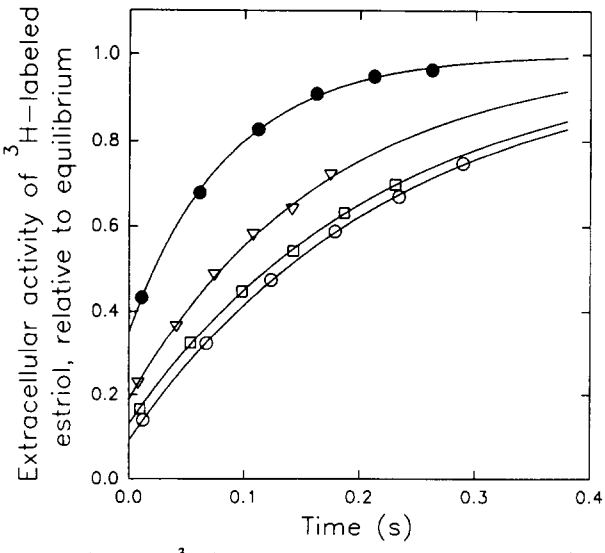

Fig. 1. Release of ^{3}H-labeled estradiol from erythrocytes (open symbols) and resealed ghosts (filled symbols) using various media: •, A1; ▽, A6; □, C; ○, B1.

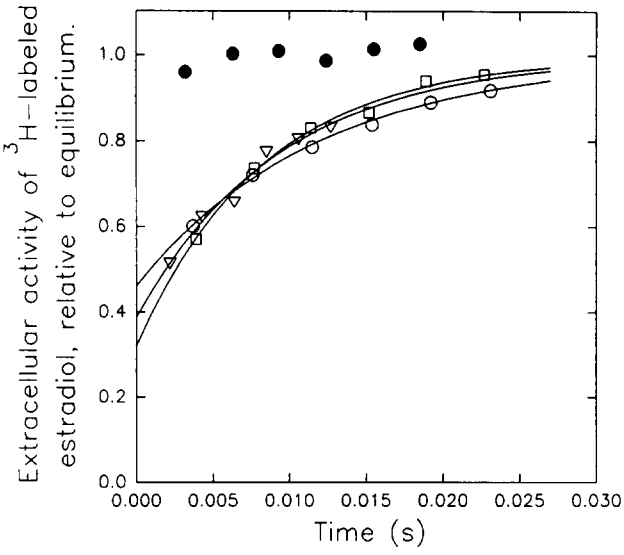

Fig. 2. Release of ^{3}H-labeled estriol from erythrocytes (open symbols) and resealed ghosts (filled symbols) using various media: ●, A2; ▽, A7; □, C; ○, B2.

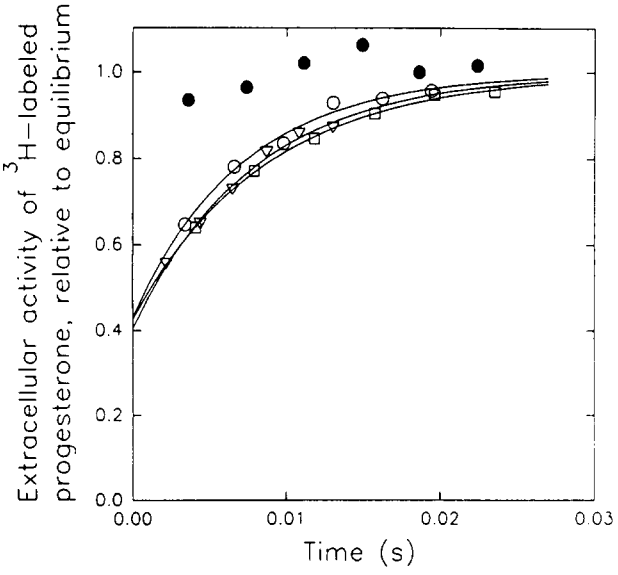

Fig. 4. Release of ^{3}H-labeled progesterone from erythrocytes (open symbols) and resealed ghosts (filled symbols) using various media: ●, A4; ▽, A9; □, C; ○, B4.

testosterone ($p = 0.0022$). For progesterone we found no significant difference between release rates at the two concentrations ($p = 0.36$). In experiments with a medium containing all 5 hormones the release-rates are not altered significantly for three of the hormones: estradiol ($p = 0.077$), estriol ($p = 0.92$) and progesterone ($p = 0.090$). We determined a significant faster release of estrone ($p = 0.00045$) and testosterone ($p = 0.0016$).

4. Discussion

4.1. The binding of hormones to cellular structures

The present study shows that human erythrocytes contains a conspicuous amounts of bound steroid sex hormones under equilibrium conditions, which is indicated by an equilibrium between the free hormone concentrations of the intracellular water phase and the

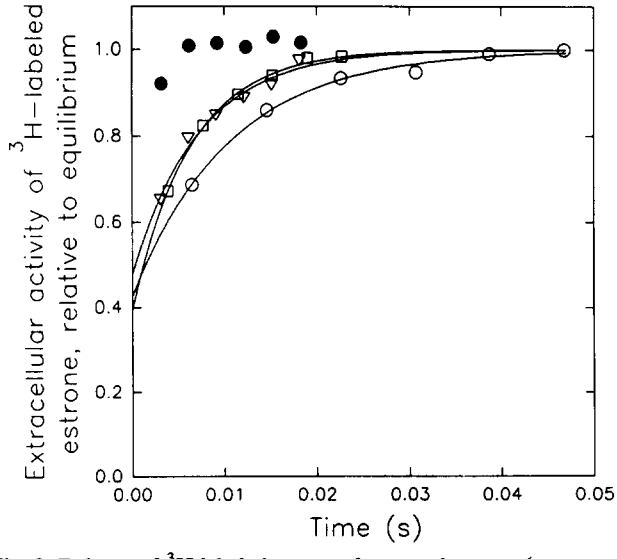

Fig. 3. Release of ^{3}H-labeled estrone from erythrocytes (open symbols) and resealed ghosts (filled symbols) using various media: ●, A3; ▽, A8; □, C; ○, B3.

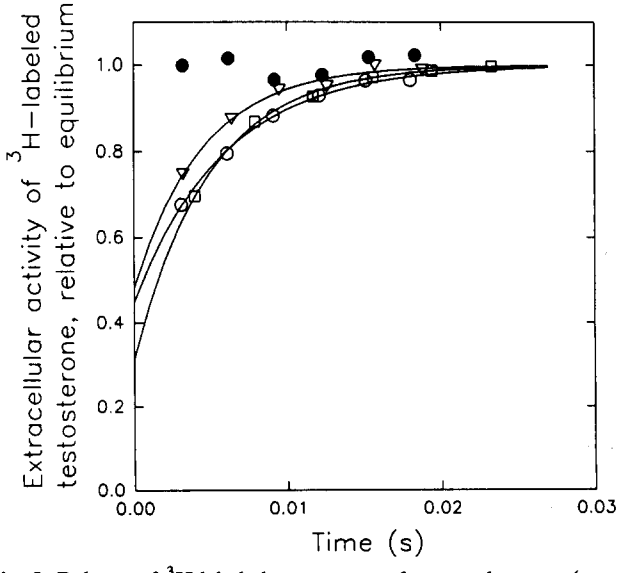

Fig. 5. Release of ^{3}H-labeled testosterone from erythrocytes (open symbols) and resealed ghosts (filled symbols) using various media: ●, A5; ▽, A10; □, C; ○, B5.

external medium. Our results show a ratio of bound over free sex hormone concentration in intact red cells of 5–10 that is a somewhat more narrow range than the range of 8–18 determined by Moll and Brahm [4]. Our comparison of experiments with intact red cells and resealed red cell ghosts shows that the hormones bind to cytosolar components as well as to membrane associated structures. Because hemoglobin is the predominant intracellular component of the cell solids ($\approx 33\%$ (w/w) of the cell, $\approx 95\%$ of the cytosolar solids), it is likely that binding of hormones to hemoglobin accounts for the main part of the cytosolar binding-capacity for the sex hormones.

The binding experiments with resealed red cell ghosts whose content of hemoglobin has been reduced by $> 98\%$ indicate that the membrane and any membrane related components remaining after the ghosts preparation procedure also bind the hormones with a substantial affinity. This is clearly illustrated by the R_{MEM,H_2O} values of Table 1 that express the distribution between the membrane phase and the cell water for the different hormones. Since the structural components amount to but few percent of the total cell mass, the R_{MEM,H_2O} values indicate that the relative distribution of hormone per kg of the two compartments varies between ≈ 100–1000, the sequence of increasing distribution being estriol < testosterone < estrone < estradiol < progesterone. Our results on distribution of steroid hormones in a biological system show an overall agreement with partition coefficient studies of organic solution/water systems [10].

Which membranous structures are involved in binding of the hormones is not known. The lipophilicity of the hormones suggests that they may bind to the lipid phase of the membrane. However, their ability to bind to plasma proteins and hemoglobin also makes the membrane proteins likely candidates.

4.2. *The kinetics of radioactive labeled hormone efflux*

Our results concordantly show that the unidirectional efflux of radioactive labeled hormone is much faster in resealed ghosts than in intact erythrocytes. When intracellular binding is not taken into account the kinetics for a unidirectional efflux of a solute in a closed two-compartment system under the given experimental conditions should, however, give the opposite result. For the same water soluable solute the efflux rate is faster in red cells than in ghosts because the cell water volume is less in red cells ($\approx 66\%$ of 90 fl) than in ghosts ($\approx 98\%$ of 90 fl) with no hemoglobin. The reason for this is given by the relation between the membrane permeability (P, cm s^{-1}), the rate coefficient (k, s^{-1}), and the ratio of cell water volume to cell membrane area (VA_m^{-1}, cm): $P = kVA_m^{-1}$ (see, e.g., Fig. 1 in [11]). This well-known fact of kinetics may, however, as in the present study be concealed if intracellular binding of the transported solute is substantial. The faster efflux rates in experiments with resealed red cell ghosts in the present study raises the question whether the ghost membranes in fact were resealed. We used the standard procedure of preparing ghosts, and the results with estriol (Fig. 2) suggest that the ghosts were indeed resealed, because equilibrium was not reached within the first 200 ms. Further, transport studies with intact red cells and resealed ghosts of other labeled solutes, such as ^3H_2O and ions that are dissolved primarely in the water phases, support that resealed ghosts indeed are made by the used preparation procedure [9,11].

Hence, we are left with the problem of explaining the different rate constants in the two types of cells by means of kinetics of hormone association, dissociation and transition. Since intact red cells show higher affinities for the hormones than ghosts, and since ghosts do show affinity for the hormones, one must conclude that we observe at least a three-compartment system in our flow-tube experiments. On the other hand, the curves of hormone release are very robust to χ^2-testing, which means that the monoexponential time course to which we have fitted the data seems well chosen. We therefore will restrict our search of possible models to the ones that will result in a monoexponential time course, even though the system basically is polyexponential of nature.

The crucial question is whether the rate constants of dissociation from various compartments in the cells are higher or lower than the rate constant of transition. If one assumes that all dissociation constants are higher than the transition rate, and if all binding-sites are located inside the cell, the result will be a situation of a constant ratio between bound and unbound hormone inside the cell, which in turn leads to a true monoexponential time course where one estimates a relative low rate constant of transition compared to a system in which no binding is present. The obvious reason for this is that an increased intracellular binding corresponds to an increase of the volume/area ratio of the cell as long as the dissociation from binding is a very fast process compared to transition through the membrane. In such a situation the real rate constant of transition will be linked to the observed by: $k_{mem} = k_{obs}(1 + R)$, where R is the ratio of bound to unbound hormone in the cell. From this value of k_{mem} it is possible to compute a true membrane permeability of the hormone.

A very fast transition rate compared to the dissociation rates from one or more binding sites, will lead to a polyexponential time course of release, but one might observe a monoexponential time course anyway. If the first measurement of extracellular activity takes place after a rapid initial release from all fast releasing

compartments one will only observe the monoexponential time course of the slowest releasing compartment. But, in such asituation the first measurement of activity must have a relative high value compared to the activity at equilibrium, because substantial amounts of the total load of labeled hormone already has been released at this time. It is noteworthy that in such a model it is possible that some of the fast releasing compartments are intramembranous binding sites or extracellular binding sites in the glycocalyx. Hence, in this situation it becomes impossible to exactly compute the membrane permeability.

It appears that the latter is the most likely of the two models. By inspecting the curves of release from intact cells we find that the activity curves of estradiol, estrone, progesterone and testosterone intercept with the activity axis at an average of 50% of the maximum. The tracer trapped between the cells constitutes only approximately 2% of the total amount, which together with the effect of slower linear velocity in the mixing chamber than in the rest of the system can account for a intercept of some 5–10% of maximum extracellular activity. Hence, there seems to be a rapid release of hormone before one observes the first activity after 2–3 ms. This observation is in accordance with the second of the two above mentioned models, but not with the first. A further argument in favor of model 2 is that the slow-released estriol shows higher intercepts with the activity axis in experiments on ghosts than in experiments on intact cells. From the R_{MEM,H_2O} value one can calculate that $\approx 40\%$ of total labeled hormone is restricted to the cell membrane in a ghost (the rest in the cell water and trapped between cells), whereas membrane bound estriol only constitutes $\approx 10\%$ in an intact cell. Assuming that hormone from the membranous compartment is released fast, one will expect that the intercept in a ghost experiment lies close to 40% of equilibrium activity, while the intercept in a red cell experiment should be some 10% of equilibrium. These predicted values show good concordance with the values found in the experiments on estriol.

With estriol as the only clear exception (cf. Fig. 2) the radioactive labeled hormones were equilibrated across the membrane of the resealed ghosts before the first sample was taken approximately 2 ms after mixing was initiated. Assuming that the transport process under the given conditions is not influenced by any intracellular binding, and that the efflux follows a monoexponential course (cf. the section below: validity of our monoexponential model), we can obtain an indirect estimate of the minimum membrane permeability. Equilibrium after two milliseconds thus indicates that over six half times have passed ($\approx 98\%$ equilibration), that is the half-time, $T_{\frac{1}{2}} \leq 0.3$ ms. For 'normal' resealed red cell ghosts the permeability becomes ≥ 600 μm s^{-1} (cf. Table 3). Clearly, the measurements show

Table 3
Kinetic data for the release of ^{3}H-labeled hormone from resealed ghosts at 38°C and pH 7.2

Hormone	Medium	n	k (s^{-1})	$T_{1/2}$ (ms)	P_{app} ($\mu m\ s^{-1}$)
Estradiol	B1	6	> 925	< 0.75	> 600
Estriol	B2	24	12 ± 1	55 ± 5	8.0 ± 0.7
Estrone	B3	12	> 925	< 0.75	> 600
Progesterone	B4	12	> 925	< 0.75	> 600
Testosterone	B5	12	> 925	< 0.75	> 600

The data for estriol represent mean ± 95% confidence intervals, calculated from n determinations. The other values were determined by using the observation that equilibrium between cellular and extracellular isotope was reached before the first filtration site, and assuming that 6 half-times are required to reach this equilibrium, the minimal values of k were computed for the other hormones.

that the efflux of these hormones is much faster than can be determined by means of the present version of the continuous flow tube system. The equilibrium values obtained in < 2 ms further show that unstirred layers in the flow tube system may be minimal, in accordance with a previous study with aliphatic alcohols showing that the equivalent unstirred layers are 1–2 μm [12].

Independent on how the hormones are distributed in the cells, and how fast they permeate through the membrane our experiments show that all labeled hormone leaves the intact cells within 1 s. This is true for all the five hormones, even the relatively slow-released estriol. Since the average time a red cell spends in a capillary is ≈ 1 s we conclude that the total sex hormone content in a red cell is delivered during a capillary passage. We therefore believe that red cells serve as a temporary circulatory reservoir of sex hormones (and perhaps of other steroids), thereby increasing the bloods capacity for these hormones. In this way the red cell plays a role similar to the role of albumin [7], namely as a fast exchanging dynamic store of steroids. The values in Table 1 suggest that the hormone restricted to red cells makes up as much as 15–35% of total exchangeable hormone in the bloodstream: if we assume a hematocrit of 43%, an albumin concentration of 4% and a water fraction in the erythrocytes of 67%, we find that the cellular fractions of total exchangeable hormone are: estrone: 30%, estradiol: 20%, estriol: 35%, progesterone: 15%, and testosterone: 15–20%.

4.3. Validity of our monoexponential model

One might argue that the use of a monoexponential model for the release of hormone is hazardous, since we most likely may have at least 3 compartments containing hormone (membrane, hemoglobin and cytosol). On the other hand the monoexponential model is the best choice as long as we do not know the number of hormone-containing compartments that re-

lease hormone during a flow-tube experiment. For instance, the observation that release is faster when one raises the concentration of hormone suggests that a compartment is saturated, so that a faster release of hormone becomes dominant. This would of course match better with a multi-compartment model, but it will also affect a monoexponential model by dragging the curve towards a faster initial raise in extracellular activity, or a higher intercept with the activity-axis.

In the study by Moll and Brahm [4] a monoexponential course was assumed, and the calculation was based upon a linear regression analysis including an equilibrium sample obtained at 'infinite' time. This approach to determine efflux rates requires that a 'true' equilibrium is attained. If the radioactive labeled solute that is transported does not equilibrate completely between the two compartments, adjustments may be necessary (cf. [9]). A more advanced method of fitting the curve to the data, not using the equlibrium sample is the χ^2-testing. Since the 'infinity' sample for hormone transport may not represent the equilibrium sample to be used for the linear regression analysis, we have chosen to apply the χ^2-testing using the collected efflux samples.

As the monoexponential model is very robust to χ^2-testing, we for simplicity, therefore, accept as a working hypothesis that a monoexponential time course of tracer flow may fit to the data.

5. Conclusions

The primary goal of this work has been to evaluate the permeability of a biological membrane to some sex steroid hormones, and to estimate whether cellularly bound hormone contributes to the amount of hormone that may be extracted during a capillary passage. We conclude that the release of hormone from the RBC is a very fast process. The longest half-life for release is 148 ms for estriol. After 6 half-times (≈ 0.9 s) the cells have lost $> 98\%$ of their contained hormone. This means that the cells can liberate almost the whole content of their hormone during an average capillary passage of ≈ 1 s. As a consequence one may regard the red cell as a transporter of sex hormones in a manner similar to that of albumin and sex hormone binding globulins. Our findings suggest that it is more accurate to monitor the level of hormone in whole blood than in plasma alone, since the capacity for hormone transport is increased when cells are present. It is, however, necessary to perform further experiments to establish the magnitude of this capacity.

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