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HUMAN ERYTHROCYTE MEMBRANE
UPTAKE OF PROGESTERONE AND CHEMICAL ALTERATIONS

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SUMMARY

The interaction of progesterone with hemoglobin-free membrane preparations from red cells obtained from blood of male and female donors has been investigated at time of collection of blood and after 42 days of storage. Alterations of some chemical components of such ghost membrane preparations have been analyzed. Progesterone shows a definite interaction with soluble protein components of the membrane, and the combining affinity is greater at 37 than at 4°. Differences are observed in the chemical composition and in the uptake of progesterone by membrane preparations obtained from fresh and 42-day-old blood of male and female donors. A great loss of phospholipid content and a significant loss of sialic acid occur in the membrane of red cells from blood of male donors after storage of 42 days under blood banking conditions.

INTRODUCTION

Several investigators have studied the binding of steroid hormones with proteins[1-3] or with subcellular fractions[4-6] in an attempt to elucidate the mechanism of action of such hormones. The interactions of steroid hormones with red blood cells have also been investigated in order to explore a possible role of erythrocytes in the metabolism and transport of steroids. VERMEULEN[7], in his studies on the transport of cortisol by erythrocytes, concluded that the steroid is adsorbed to the red cell surface. WILLMER[8] speculated that corticosteroids become attached to or packed into the phospholipid layer of cell membranes.

Previous studies[9] on the binding of progesterone and aldosterone with red blood cells of the rat have demonstrated that the uptake of progesterone by the erythrocytes is of the order of 70–85 % of the total steroid in contact with the red cells, whereas the uptake of aldosterone is insignificant. Further investigations[10] have shown that, when progesterone is added to stored human red blood cells, the loss of osmotic resistance and the spontaneous hemolysis during storage are greatly minimized as compared to red cells stored without addition of progesterone. Recently is has been reported[11] that the blood from female donors is able to survive the "storage lesions" better than the blood from male donors. These results have been attributed to the different endocrine systems in the human male and female and the ability to form structurally and pharmacologically different steroid hormones, which could interact with compartments of

the red cell membrane. In order to gain insight into the mechanism of action of progesterone in relation to erythrocytes preservation, it was considered of interest to study the interaction of progesterone with "hemoglobin-free" membrane of red cells prepared from blood of male and female donors at the time of collection of blood and after 42 days of storage. Analyses of several components of pure erythrocyte membrane preparations, free of intracellular content, were also made in an attempt to evaluate a possible progesterone effect on the alterations of the chemical composition of the membranes of the red cell during storage.

MATERIALS AND METHODS

Preparation of red cell membrane and other fractions

Units of blood were obtained from healthy male and female donors, 18–25 years of age. The female donors donated blood at 20–22 days after onset of menstruation. The blood was drawn into plastic bags containing 67.5 ml of acid–citrate–dextrose solution (National Institute of Health, Formula A) per 450 ml of blood. One half of each unit was used to prepare erythrocyte membrane and other cellular fractions at the time of collection of blood, the other half was stored at 2° in the original plastic bag, under sterile conditions, for a period of 42 days at which time red blood cell membranes were prepared. The preparation of hemoglobin-free ghost membrane was done according to the procedure of DODGE *et al.*[12]. The erythrocytes were isolated from freshly collected blood or from 42-day stored blood by centrifugation for 20 min at 1000 × *g*. The cells were washed twice and finally suspended in an equal volume of isotonic sodium phosphate buffer. The hemolysis of the cells and the washing of the ghosts was performed with sodium phosphate buffer, 30 imosM (ideal milliosmolarity, see ref. 12), pH 7.4, using a ratio of cells to buffer solution of approx. 1:30. After the third wash the ghosts were milky white and the supernatant was colorless or very faint pink.

Aliquots of fresh blood were used to prepare other cellular fractions. The non-hemoglobin constituents from erythrocytes were separated using the method described by HAUT *et al.*[13]. The removal of hemoglobin from red cell hemolysates was achieved by filtration through CM-Sephadex (C-50, coarse, Pharmacia Fine Chemicals) and the hemoglobin-free yellowish solution was concentrated by lyophilization.

The fraction designated as supernatant in Table II indicates a stroma-free, particle-free fraction obtained after centrifugation of red blood cell hemolysate at 100000 × *g*. The hemoglobin fraction was prepared from an aliquot of the freshly drawn blood according to the method of ROSSI-FANELLI *et al.*[14].

Chromatographic separation of membrane protein

In the interaction studies (see below) between progesterone and erythrocyte membrane, it was considered of interest to separate the cell membrane in various fractions and then determine the combining affinity of these fractions for the steroid. The procedure used for the chromatographic fractionation was slightly different from that described by MADDY[15]. An aliquot of the membrane suspension obtained from freshly drawn male blood and corresponding to about 40 mg protein was chilled to 0° in iced water. An equal volume of chilled *n*-butanol was added and the mixture, after shaking briefly and vigorously, was kept at 0° in ice for 30 min. Subsequent centrifugation at 27000 × *g* for 4 min separated the content into three phases, a butanol phase

containing lipid, an interphase consisting of a thin layer of insoluble protein and an aqueous phase containing soluble proteins. The lower aqueous phase was drawn into a cold hypodermic syringe and transferred to a dialysis bag. The protein solution was dialyzed for 24 h against buffered saline consisting of 0.25 M NaCl and 0.05 M phosphate buffer, pH 7.5; the outside liquid was changed 6 times during the course of the dialysis. Chromatographic separation of the soluble proteins was obtained using Sephadex G-100. The gel was first soaked in water, then suspended in the buffered saline mentioned above and finally packed into a column. An aliquot of the dialyzed protein solution (1 ml containing 20.0 mg protein) was introduced into the column, the column developed with buffered saline and the fractions monitored at 280 nm.

Disc electrophoresis of cell membrane

Electrophoresis was carried out in the Canalco Model 12 disc electrophoresis apparatus (Canal Industrial Corp., Rockville, Md.) using the standard 7.5 % polyacrylamide separating gel at pH 8.7 and the large-pore polyacrylamide stacking gel, both in the presence of 8 M urea, with the Tris–glycine buffer prepared according to the Canalco formulations. About 50 μl of membrane suspension, prepared from blood of male or female donors immediately after collection of blood or after 42 days of storage, were solubilized by adding a mixture of 50 μl of 5 % Triton X-100 in 8 M urea and 50 μl of 0.1 M β-mercaptoethanol. The suspension clarified immediately on shaking. Protein concentrations were adjusted so that each sample (50 μl) loaded on top of the polyacrylamide stacking gel contained about 250 μg protein. Electrophoresis was carried out at 5 mA per tube for 90–120 min at room temperature. The gels were extruded from the columns, fixed and stained in 1 % Amido Schwarz in 7.5 % acetic acid and then destained by electrolysis.

Chemical analyses

Stock solutions of water-dispersed membrane prepared from blood of male and female donors at the time of collection of blood and after 42 days of storage were used for several chemical assays. For the determination of the dry weight, the membrane suspensions were thoroughly mixed and 1-ml aliquots, in quadruplicate, were pipetted into aluminum planchets. These planchets had been dried at 70° for 24 h, allowed to cool in a desiccator, and weighed. The pipettes were washed with distilled water and the washings were also introduced in the aluminum planchets. The contents of the planchets were taken to near dryness by evaporation over N_2 and finally to dryness in a vacuum oven at 70° for 24 h and weighed on an analytical balance. The drying process was continued until constant weight was obtained. These stock solutions of known concentration were then used as a source of material for quantitative analyses.

Quantitative determination of protein was made by means of the microbiuret reagent described by ITZHAKI AND GILL[16], using crystalline bovine plasma albumin (Armour Pharmaceutical Co.) as standard. Lipid extraction was done on aliquots of the stock solutions using the chloroform–methanol procedure of WAYS AND HANAHAN[17]. Total phosphorus was measured by the method of BARTLETT[18]. In the absence of other phosphorus-containing molecules, the amount of phospholipids could be calculated from the amount of phosphorus present. Cholesterol was assayed by the method of ROSENTHAL *et al.*[19]. For the carbohydrate content, total neutral sugars were estimated by the anthrone reaction with the method of analysis developed by GOA[20], using an

equimolar mixture of mannose and galactose as standard. Sialic acids were determined by the thiobarbituric acid assay of WARREN[21]. Analysis of possible progesterone content in the membrane preparations from blood of male and female donors was done by the procedure described by ZENDER[22].

Interaction studies

The interaction of steroid with membrane preparations or with fractions of these membranes was studied by equilibrium dialysis[23,24]. The specific activity of the steroids was 1.8 mC/mg for [16-^{3}H]progesterone, 28 μC/μg for [7-^{3}H]cortisol and 700 mC/mmole for [1,2-$^3H_2$]corticosterone. Their radiochemical purity, tested by paper chromatography, was 96 % or higher. Aliquots of the various fractions to be tested, each corresponding to 5 mg protein, were brought to a volume of 5 ml and enclosed in individual dialysis bags. The bags were then placed in wide-mouthed jars and first dialyzed for 24 h at 4° with continuous agitation against four changes of 0.05 M phosphate buffer, pH 7.4, to equilibrate the various suspensions or solutions against the buffer. Each bag was then placed in individual jars and dialyzed against 10 ml of phosphate buffer containing 0.02 μg of radioactive steroid hormone for a period of 24 h at 4 or 37°, with continuous agitation. Previous experiments had shown that equilibrium conditions are reached in 24 h at 4 and at 37°. In these dialysis systems, penicillin was added in a concentration of 500 units/ml; this concentration did not affect the steroid binding. In every dialysis, the volume of the solution outside the bag was equal to twice the volume of the solution inside the bag.

At the end of the dialysis period, tests with 10 % aqueous solutions of sulfosalicylic acid showed that no protein had leaked into the outside fluid. The determination of tritium content of inside and outside solutions was done in an automatic Packard Tri-Carb liquid scintillation spectrometer as reported previously[25]. In all equilibrium dialysis studies the recovery of radioactivity was found to be 100 $\pm$ 5 %. The amount of radioactive material bound to the various fractions was calculated and expressed as per cent of the total activity (bound and unbound) in the individual dialysis bag and by the combining affinity[2] (C value) of the steroid for the fraction, determined by the expression: $C = [S]_{\text{bound}}/[S]_{\text{unbound}} \times [P]$; $[S]_{\text{unbound}}$ represents the concentration of the steroid in the outside solution at equilibrium, $[S]_{\text{bound}}$ is calculated as difference between total and unbound steroid inside the dialysis bag at equilibrium and [P] indicates the concentration of total protein inside the dialysis bag in mg/ml. All the determinations were done at least in duplicate and are based on direct measurement of the radioactivity in the inside and outside solutions. Protein concentration was quantitatively determined in each bag at termination of dialysis.

RESULTS

A comparison of the uptake of progesterone, corticosterone and cortisol by membrane of red cells obtained from fresh blood of male donors is shown in Table I. The results demonstrate that the uptake of progesterone is much greater than that of corticosterone or cortisol not only at 4 but also at 37°. The binding of the three steroids is expressed not only as uptake in per cent of total (bound and unbound) activity, but also as ng of steroid bound as well as combining affinity. In all three forms of expression it can be seen that corticosterone and cortisol display a very limited binding activ-

ity with practically no significant change at the two temperatures investigated, whereas progesterone is not only bound to a great extent by the red cell membrane, but the combining affinity at 37° is much greater than that observed at 4°. The data represent average values of determinations made on cell membrane preparations from blood of the same donor and of different donors; the range of the combining affinities are presented in the last column. When other fractions of the red cells are tested for their interaction with progesterone (Table II), at 4° the nonhemoglobin proteins show a very small uptake of the steroid but the supernatant and hemoglobin fractions do not demonstrate any binding at all. At 37° the interaction of nonhemoglobin proteins with

TABLE I

UPTAKE OF STEROIDS BY MEMBRANE OF RED CELLS PREPARED FROM FRESH BLOOD OF MALE DONORS

Equilibrium dialysis at 4 or at 37° for 24 h.

*Steroid**	*Uptake (% of total bag activity)***	*Bag activity**** *(counts/min)*	*Steroid bound (ng)*	*C value*	
				(Range)	*Av.*
At 4°					
Progesterone	26.5	1 760	2.15	(0.32–0.36)	0.35
Corticosterone	9.6	4 280	0.68	(0.07–0.14)	0.12
Cortisol	3.6	42 760	0.25	(0.03–0.07)	0.04
At 37°					
Progesterone	42.2	2 020	3.91	(0.69–0.79)	0.74
Corticosterone	7.2	4 210	0.61	(0.06–0.11)	0.08
Cortisol	5.5	43 320	0.38	(0.04–0.07)	0.06

* [16-^{3}H]Progesterone (0.02 μg, 4350 counts/min), [1,2-$^3H_2$]corticosterone (0.02 μg, 12000 counts/min), [7-^{3}H]cortisol (0.02 μg, 125200 counts/min).

** These values represent the uptake of radioactive steroid expressed as percent of total activity (bound and unbound) in the respective dialysis bag.

*** Total activity (bound and unbound) in the respective bag at the end of dialysis.

TABLE II

UPTAKE OF PROGESTERONE* BY FRACTIONS OF RED CELLS PREPARED FROM FRESH BLOOD OF MALE DONORS

Equilibrium dialysis at 4 or at 37° for 24 h.

Fraction	*Uptake (% of total bag activity)**	*Bag activity** *(counts/min)*	*Steroid bound (ng)*	*C value*
At 4°				
Non-Hb proteins	8.9	1541	0.63	0.14
Supernatant	No uptake	—	No binding	—
Hemoglobin	No uptake	—	No binding	—
At 37°				
Non-Hb proteins	10.6	1560	0.76	0.13
Supernatant	2.9	1479	0.20	0.05
Hemoglobin	3.5	1485	0.24	0.03

* For explanation, see Table I.

progesterone is the same as that seen at 4° and that of supernatant and hemoglobin fractions is of a very low affinity.

Since in these dialysis systems, 20 ng of progesterone per 5 mg membrane protein were used, it was important to know if under these conditions saturation of binding could have been achieved at both 4 and 37°. Fig. 1 shows the results of progesterone binding to cell membrane as a function of the concentration of the hormone with membrane protein content maintained constant. At both temperatures the amount of progesterone bound increases with the amount of steroid added to the system and no saturation of binding is achieved at the concentrations investigated. The uptake of progesterone at 37° is greater than at 4° at each concentration of the hormone and the slopes of the two curves are different, showing that, with increasing amount of steroid in the dialysis system, the difference between the progesterone bound at the two temperatures increases.

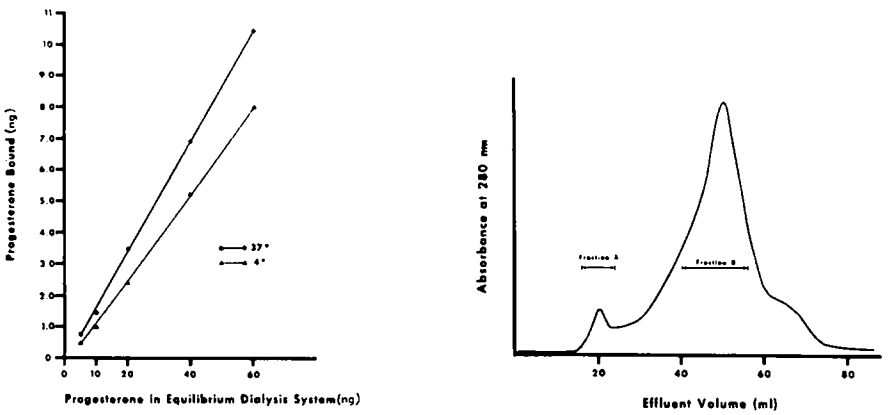

Fig. 1. Binding of progesterone to red cell membrane from fresh blood of male donors as a function of the hormone concentration. All samples contained 5 mg protein, suspended in 5 ml of phosphate buffer (pH 7.4), 0.05 M, and were dialyzed against 10 ml of phosphate buffer containing radioactive progesterone in the concentration indicated.

Fig. 2. Gel column chromatography of solubilized membrane of red cells from fresh blood of male donors. The protein solution was allowed to flow into the gel at the top of a 20 cm × 2 cm Sephadex G-100 column. The protein was eluted with buffered saline (0.25 M NaCl, 0.05 M phosphate buffer, pH 7.5). The absorbance of the effluent at 280 nm was recorded on a Moseley Autograph Model 680 absorbance recorder.

Gel column chromatography of solubilized membrane protein obtained after butanol extraction of cell membrane preparation from fresh blood of male donors is shown in Fig. 2. Two protein peaks were obtained, designated Fraction A and B, respectively. Fraction A represents a very small percentage of the total protein. These two fractions, the aqueous phase containing the unfractionated soluble protein and the whole unextracted membrane were tested for their binding affinity with progesterone in an equilibrium dialysis system at 37°; the results are shown in Table III. The data on the uptake of progesterone by the whole membrane duplicate very well the results seen in Table I. The solubilized protein in the aqueous phase shows an uptake greater than that for the whole membrane as demonstrated by the ng of steroid bound and by the combining affinity. After gel chromatography only Fraction B shows a

strong affinity for the hormone, whereas Fraction A does not interact with the steroid.

Table IV shows the uptake of progesterone at 4 and 37° by membrane of red cells prepared from fresh blood of male ($M\text{-}T_0$) and female ($F\text{-}T_0$) donors and of red cells from 42-day-old blood also of male ($M\text{-}T_{42}$) and female ($F\text{-}T_{42}$) donors. These results were obtained using several cell membrane preparations from blood of the same donor and of different donors (three male and three female donors); the range of *C* values for the various determinations are shown. The data for preparation $M\text{-}T_0$ are in good agreement with those obtained for a different membrane preparation shown in Table I. When fresh blood is used, a slight difference is observed in the binding of progesterone by the membrane preparations $M\text{-}T_0$ and $F\text{-}T_0$, where $F\text{-}T_0$ demonstrates an affinity for progesterone higher than that of $M\text{-}T_0$ preparation at both 4 and 37°. When 42-day-old blood is used the progesterone binding affinities of the membrane preparations $M\text{-}T_{42}$ and $F\text{-}T_{42}$ are significantly lower compared to the affinity of $M\text{-}T_0$ and $F\text{-}T_0$, respectively. However, the decrease observed in the steroid binding affinity for $M\text{-}T_{42}$ is larger than that for $F\text{-}T_{42}$, indicating a possible greater deterioration of the membrane of red cells from blood of male donors.

TABLE III

UPTAKE OF PROGESTERONE* BY WHOLE MEMBRANE AND MEMBRANE FRACTIONS FROM RED CELLS PREPARED FROM FRESH BLOOD OF MALE DONORS

Equilibrium dialysis at 37° for 24 h.

Fraction	*Uptake (% of total activity)**	*Bag activity** *(counts/min)*	*Steroid bound (ng)*	*C value*
Whole membrane	45.4	2080	4.35	0.77
Aqueous phase	49.0	2154	4.86	1.02
Fraction A	No uptake	—	No binding	—
Fraction B	54.0	2226	5.63	1.22

* For explanation, see Table I.

TABLE IV

UPTAKE OF PROGESTERONE* BY MEMBRANE OF RED CELLS PREPARED FROM FRESH OR 42-DAY-STORED BLOOD OF MALE AND FEMALE DONORS

Equilibrium dialysis at 4 or at 37° for 24 h.

*Membrane from***	*Temp.*	*Uptake (% of total bag activity)**	*Bag activity** *(counts/min)*	*Steroid bound (ng)*	*C value*	
					(Range)	*Av.*
$M\text{-}T_0$	4°	25.5	1747	2.05	(0.29–0.35)	0.32
	37°	44.1	2054	4.17	(0.68–0.74)	0.71
$F\text{-}T_0$	4°	27.4	1774	2.23	(0.35–0.40)	0.37
	37°	45.0	2072	4.29	(0.78–0.85)	0.82
$M\text{-}T_{42}$	4°	19.1	1662	1.46	(0.19–0.25)	0.22
	37°	31.3	1832	2.63	(0.38–0.43)	0.41
$F\text{-}T_{42}$	4°	23.4	1730	1.86	(0.28–0.35)	0.30
	37°	41.9	2012	3.88	(0.69–0.75)	0.72

* For explanation, see Table I.

** M, male donor; F, female donor; T_0, membrane from fresh blood; T_{42}, membrane from 42-day-old blood.

These changes are observed at the two different temperatures investigated.

Determination of progesterone content in the cell membrane preparations from blood of male and female donors did not show any detectable amount of this steroid, excluding thus the presence of endogenous hormone and ruling out the possibility of an exchange reaction with radioactive progesterone present in the dialysis system.

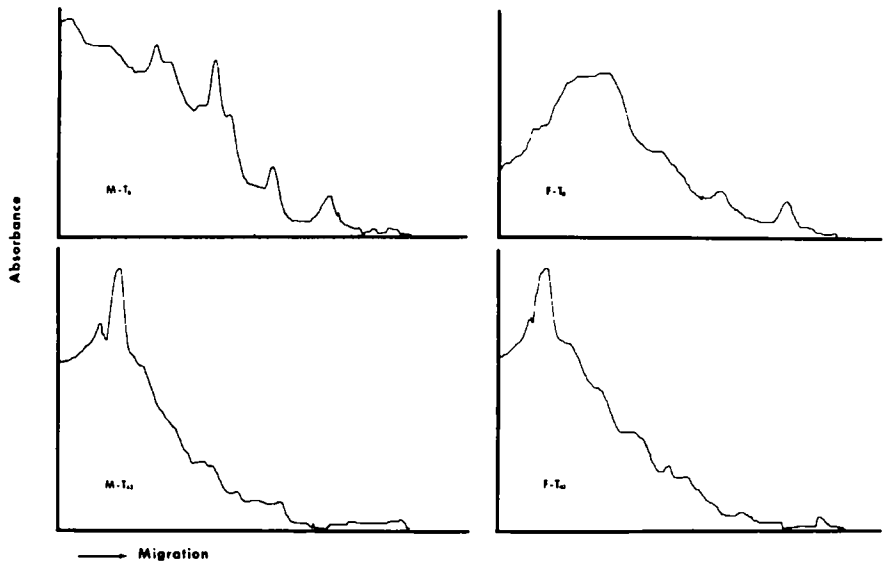

Fig. 3. Gel electrophoretic migration of solubilized membrane of red cells prepared from fresh or 42-day-old blood of male and female donors. Densitometry of the separating gel was obtained with the recording electrophoresis densitometer Model 542 of the Photovolt Corp.

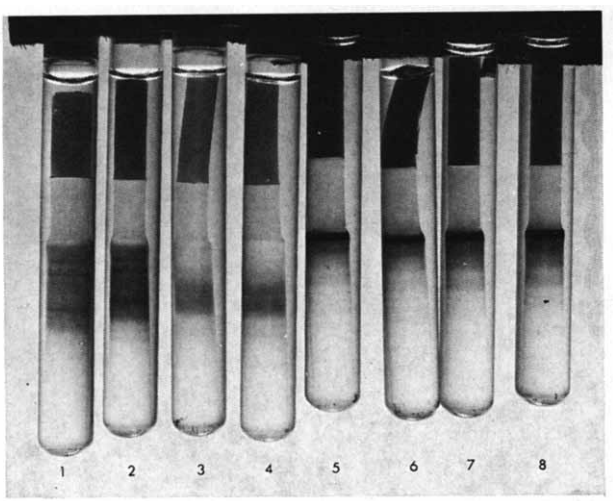

Fig. 4. Photograph of disc gels using solubilized membrane of red cells prepared from fresh or 42-day-old blood of male and female donors. 1 and 2, membrane from fresh blood of male donors; 3 and 4, membrane from fresh blood of female donors; 5 and 6, membrane from 42-day-old blood of male donors; 7 and 8, membrane from 42-day-old blood of female donors.

To explore possible differences in the chemical composition of the cell membrane from blood of male and female donors during storage, gel electrophoresis and chemical analyses were made. Figs. 3 and 4 show the gel electrophoretic migration and a photograph of disc gels, respectively, using solubilized membrane of red cells prepared from fresh or 42-day-old blood of male and female donors. These are typical patterns obtained under identical experimental conditions. Similar patterns were recorded with other membrane preparations from different donors. Striking differences can be observed not only between M-T_0 and M-T_{42} but also between F-T_0 and F-T_{42}. Furthermore, different patterns are obtained between membrane preparations M-T_0 and F-T_0 and also between M-T_{42} and F-T_{42}. These data would indicate a dissimilar composition of the membrane of red cells in the blood of male and female donors as well as a different alteration of the components of the membrane during the 42 days of storage. Table V shows analyses of some chemical components of erythrocyte membrane and changes which occur in these components during storage of blood. The preparations M-T_0 and F-T_0 demonstrate the same protein, cholesterol and total phosphorus composition. The content of neutral sugars and of sialic acid seems to be higher in M-T_0 than in F-T_0. After storing the blood for a period of 42 days the membranes of the red cell from blood of male donors are subjected to a great loss of total phosphorus and a significant loss of sialic acid, whereas the F-T_{42} membrane preparations do not show any change in the various chemical parameters investigated. The results obtained for erythrocyte membrane at T_0 are in agreement with the corresponding data reported in the literature[26,27].

TABLE V

CHEMICAL COMPOSITION OF MEMBRANE* OF RED CELLS PREPARED FROM FRESH OR 42-DAY-OLD BLOOD OF MALE AND FEMALE DONORS

Assays	T_0		T_{42}		% *Decrease*
	(*Range*)	*Av.*	(*Range*)	*Av.*	
	Male				
Protein (μg/mg dry wt.)	(475–525)	494.0	(430–505)	456.0	7.7
Cholesterol (μg/mg protein)	(458–502)	486.0	(460–495)	470.0	3.3
Phosphorus (μg/mg protein)	(34.7–38.5)	36.2	(22.8–26.1)	24.0	33.7
Neutral sugars (μg/mg protein)	(53.4–58.5)	56.1	(50.5–54.7)	52.3	6.8
Sialic acid (μg/mg protein)	(47.4–53.3)	49.0	(39.2–43.1)	41.1	16.1
	Female				
Protein (μg/mg dry wt.)	(490–546)	516.0	(480–530)	508.0	1.6
Cholesterol (μg/mg protein)	(464–510)	477.0	(452–490)	460.0	3.6
Phosphorus (μg/mg protein)	(35.2–39.9)	37.5	(36.4–40.4)	38.2	—
Neutral sugars (μg/mg protein)	(45.2–49.9)	48.3	(45.1–48.8)	47.8	1.0
Sialic acid (μg/mg protein)	(35.7–38.9)	36.8	(33.1–37.8)	35.7	3.0

*T_0, membrane from fresh blood; T_{42}, membrane from 42-day-old blood.

DISCUSSION

The interaction of various hormones with erythrocytes has been studied by several investigators. PETERSON *et al.*[28] have shown that 20–25 % of cortisol is present in the

red cell and BUSH[29] reported a rapid uptake of cortisol by suspensions of erythrocytes at 37–38°. Recently, binding of thyroid hormones to erythrocyte membrane has been reported[30]. Previous studies[9] have shown a great uptake of progesterone by the red cells of the rat suggesting an interaction of this steroid with the red cell membrane. The data presented in Tables I and II confirm this interaction. It appears that of the three steroids investigated only progesterone shows a definite and strong interaction with the membrane of the red cell and that this interaction does not occur with other fractions of the cell but only with the membrane. The data in Table I show also that the uptake of progesterone by the membrane is much greater at 37 than at 4°. In the binding studies of steroid hormones to plasma proteins, it has been observed that be combining affinity decreases with an increase of the temperature at which the interaction is determined. This is due to a higher dissociation constant and, therefore, an increase of the free energy of steroid–protein associations with rising temperature. However, the membrane of the red cell contains not only protein but also lipid and carbohydrate and the greater uptake of progesterone at higher temperature could be explained by increased penetration of the hormone or better accessibility of the binding sites or by a combination of these two phenomena.

Several investigators in various laboratories have been interested in the solubilization of the red cell membrane and in the characterization of its various components[27,31–33]. Gel column chromatography of solubilized membrane protein obtained after butanol extraction (Fig. 2) and determination of combining affinity of the various fractions (Table III) show that progesterone does not interact with the lipid components but only with a soluble protein fraction of the membrane and that this interaction increases with further purification of this fraction. Investigations for the isolation, eventual analysis and characterization of this soluble protein fraction are in progress.

It is well known that, when blood is stored under blood banking conditions, erythrocytes undergo numerous biochemical and structural changes designated collectively as "the storage lesion". Most of these changes in the red cells have been investigated, but there are very few reports which deal with changes occurring specifically in the membrane of the cell. It has been reported[34] that the red blood cell membrane contains 50–60 % protein, 35–45 % lipids and some 8–10 % carbohydrate with other important components represented by water and metal ions. Even slight changes in the physicochemical properties of the membrane have an important effect on the life span of the red blood cells. The electrophoretic patterns shown in Figs. 3 and 4 point out significant changes which occur during storage of blood in the membrane of red cells, obtained from blood of male and female donors. Furthermore, the chemical analysis in Table V shows that the membrane of cells obtained from blood of male donors sustain a loss of phospholipid and sialic acid components. Membrane lipid is composed entirely of free cholesterol and phospholipid. NYE AND MARINETTI[35] have shown that phospholipids play an important structural and functional role in the erythrocyte membrane. Sialic acid has been found to be a component of a glycoprotein located on the red cell membrane[36,37] and the negative charge on the erythrocyte surface is due to the carboxyl group of sialic acid[38–40]. The importance of sialic acid derives from the fact that a partial or complete loss produces several important changes on the erythrocytes and has a great influence upon its survival[39,41].

The differences observed in the chemical composition of cell membrane (Table V)

and in the uptake of progesterone (Table IV) by such cell membrane preparations obtained from fresh and 42-day-old blood of male and female donors could explain the results previously reported, *i.e.*, that the addition of progesterone to red blood cells helps to maintain the osmotic resistance and limits the *in vitro* spontaneous hemolysis of these cells[10] and that the blood obtained from female donors is able to survive the storage lesions better than the blood from male donors[11]. As stated in MATERIALS AND METHODS, female donors donated blood at 20–22 days after onset of menstruation. It has been very well documented in the literature[42–44] that, in the adult female, the highest plasma progesterone levels are found at about 20–24 days of the menstrual cycle. Also very little or no amount of progesterone has been found in the plasma of normal men. VAN DER MOLEN AND GROEN[43] have shown the mean progesterone levels in μg per 100 ml female plasma are 0.091 and 1.16 for the follicle and luteal phase, respectively, in a total of 22 observations. The same investigators have also reported mean progesterone level of 0.028 μg per 100 ml male plasma in 14 observations. It seems, therefore, that one basic difference in the blood of male and female donors used in these experiments is due to a great difference in the concentration of progesterone, which is very high in the blood of female and insignificant in the blood of male donors. It seems possible that progesterone by a strong interaction with a protein component of the red cell membrane prevents or minimizes the deterioration of some constituents of this membrane and consequently reduces the change in membrane permeability and thus extends the life of the cell during storage.

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