TRANSCORTIN: A CORTICOSTEROID-BINDING PROTEIN OF PLASMA *†

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(Submitted for publication September 19, 1958; accepted October 30, 1958)

The general field of the binding of steroids and their conjugates to human plasma proteins has recently been reviewed by the authors (1). Evidence was presented that Fractions IV-1, IV-4 and V (obtained by the Cohn fractionation procedure) were mainly responsible for the binding, and hence transport, of the unconjugated steroids. The binding of conjugates of steroids to the plasma protein was also shown to be considerable. Furthermore, evidence was obtained that cortisol and corticosterone were avidly bound by a "special" protein in human plasma. This conclusion was based on the fact that cortisol and corticosterone were bound to unfractionated human plasma much more strongly than determinations of binding to alcohol-fractionated human plasma proteins had led us to expect. In fact, these two corticosteroids were bound at least as strongly as estrone and estradiol to plasma diluted 1:4 or 1:40 [see Table VII of (1)]. The existence of such a "special" protein has been independently described by Daughaday (2–5). At first he attributed all the binding to albumin; that observed in Fraction IV-4 (α-globulin) was assigned to its content of albumin (6, 7). Later (2), using the same techniques but employing a radioactive label for assay instead of a chemical determination, he concluded that there was in the α-globulin fraction of human plasma from cortisone-treated patients (but not normal subjects) a protein low in capacity but high in affinity for corticosteroids. This is in contrast to albumin which has a high capacity but low affinity for corticosteroids (1). In subsequent papers (3–5) he concluded that normal subjects also possess corticosteroid-binding protein.

Some time ago Roberts and Szego (8) concluded that Fraction III-0 was important in the binding and transport of estrogens in human blood, a finding which was challenged by Bischoff, Stauffer and Gray (9). In our studies we were unable to demonstrate the binding of estrogens to Fractions III-0 and recent work by Bischoff and Stauffer (10) is in agreement with these findings, pointing to albumin as the major protein concerned with the transport of estrogens.

In this paper we wish to present further data pointing to the existence of a special protein (or proteins) with great affinity for the binding of cortisol and corticosterone. By analogy to transferrin, the iron-binding protein of plasma, we have named the corticosteroid-binding protein "transcortin." Additionally, evidence will be presented that the concentration of this protein is considerably increased during the third trimester of pregnancy. Data will also be shown which indicate the lack of such a protein for the binding of estrogens in human plasma, from both normal and pregnant subjects.

METHODS AND MATERIALS

Equilibrium dialysis was performed according to methods previously reported (1). Since previous studies indicated that undiluted plasma bound 99 per cent of the C^{14}-corticosteroid added, dilution of plasma gave results which made differences in the binding among the various steroids more pronounced and easier to quantitate. It was also established that the presence or absence of stabilizers in human serum albumin (HSA) did not affect the binding of the various steroids to HSA or plasma, a finding observed by Bischoff and Stauffer for the estrogens (10). Hence, for the majority of the experiments salt-poor HSA supplied in solution by the American Red Cross was used. Free electrophoresis showed the HSA to be homogenous. It was also shown that when plasma and HSA were diluted with phosphate buffer (pH 7.4, 0.05 M), the binding of cortisol did not differ from that observed when 0.15 M saline was used for dilution. The method, therefore, consisted of placing 2 ml. of plasma di-

* An abstract of part of this work appeared in the J. clin. Invest. 1958, 37, 928.

† This investigation has been supported in part by a grant (A-1240) from the National Institutes of Health, United States Public Health Service.

luted to 10 ml. with 0.15 M saline, in 23/32" NoJax Visking casing. The casing was placed in a 125 ml. Erlenmeyer flask containing 29 ml. of either 1 per cent HSA (diluted with saline) or saline and 1 ml. of radioactive steroid dissolved in saline. The flasks were rocked slowly for 72 or more hours at 4° C. to assure equilibration of these steroids. Small amounts of penicillin were added to each flask (10,000 units) following studies which indicated the antibiotic did not interfere with the binding of any of the steroids studied.

Blood (30 to 50 ml.) was collected in 0.5 ml. of heparin (4 mg. per ml.) and the erythrocytes separated from the plasma (use of serum did not alter the results). The blood samples were obtained in the late morning or early afternoon from normal pre-menopausal subjects and pregnant women. The plasma was used the day on which the blood was drawn.

C^{14}-labeled steroids were added in amounts ranging from 0.25 to 0.5 μg. per flask (*ca.* 2,000 cpm). Carrier steroid was added dissolved in small amounts of ethanol, the concentration of the alcohol never exceeding 0.1 per cent. Both the carrier and radioactive steroids were 99 per cent or more pure as judged by infrared spectroscopy and/or paper chromatography. Deoxycorticosterone acetate and cortisone acetate were converted to the free alcohols by incubation with acetyl cholinesterase or by chemical hydrolysis.

Following dialysis the contents of the cellophane bag and of the flask were separately extracted three times with two volumes of chloroform. The chloroform extracts were dried by air, plated on steel planchettes and counted in a windowless gas flow counter. The weight of the samples was negligible and no correction was necessary for self-absorption of radioactivity. The percentage of binding of steroids to protein was determined by the following Equation *1*):

$$1) \qquad \% \text{ bound} = 100\left(1 - \frac{D \cdot V_R}{R \cdot V_D}\right)$$

where R and D are the amounts of radioactivity present inside and outside the dialysis casing, respectively, and V_R and V_D are the corresponding volumes.

The recovery of the added C^{14}-steroids ranged from 85 to 100 per cent. No binding of the steroid occurred to the glassware or to the dialysis casing. In several experiments it was shown that under the conditions of the dialysis the added C^{14}-cortisol was recovered unchanged as judged by paper chromatography. The complete procedure was performed in triplicate and the results represent the average of the three determinations which seldom varied more than 3 percentage points from the average.

Certain samples were submitted to continuous flow paper electrophoresis on the Spinco Model CP apparatus using Schleicher and Schuell No. 470 paper. Two buffer systems were used: barbital buffer, 0.02 M, pH 8.6 and acetate buffer, 0.02 M, pH 5.2. A potential of 660 to 800 V. was applied, producing a current flow of 40 to 60 ma. Usually 25 ml. of serum was equilibrated against the buffer overnight and the small amount of sediment formed centrifuged down before application of the serum to the paper at the rate of *ca.* 1 ml. per hour. The volumes of the 32 fractions varied; each tube was diluted to 15 ml. with buffer. The optical density at 280 mμ was determined using a Beckman Model DU spectrophotometer. Protein analysis was performed using a Spinco Model R paper electrophoresis apparatus. The binding of 4-C^{14}-cortisol was determined on each fraction by means of equilibrium dialysis. For this purpose 5 ml. of each fraction diluted with 5 ml. of saline was used. After dialysis the samples containing barbital buffer were adjusted to pH 9 to 10 to avoid the extraction of diethylbarbituric acid, which, if present, gave significant weight to the samples.

The optical density was converted to concentration using a separate factor, based on Kjeldahl determinations kindly performed by Mr. Kenneth Buchwald of this Institute, for each protein fraction. From this the amount of binding per mg. of protein was calculated.

RESULTS

Earlier work (1) showed that undiluted plasma bound trace amounts of added C^{14}-labeled cortisol and corticosterone essentially quantitatively. Therefore, in order to be able to demonstrate increases in extent of binding, dilution of the plasma was necessary. Dialyses of diluted plasma (1 : 5 with 0.15 M saline) against 0.15 M saline showed, however, only minor differences in the binding of the various classes of steroids. We therefore sought to cancel the effect of the albumin on binding by dialyzing against a concentration of albumin equal to that in diluted plasma. Since the albumin concentration in the plasma is usually approximately 5 Gm. per 100 ml., dialyses of plasma diluted 1 : 5 were performed against 1 Gm. per 100 ml. HSA.

TABLE I

Demonstration of corticosteroid-binding protein other than albumin in human plasma

C^{14}-Steroid	% Bound*
Cortisol	89, 88, 82
Corticosterone	72, 78
11-Deoxycorticosterone	77
Cortisone	62
Pregnane-3,11,20-trione	63
Progesterone	20, 15
Δ^4-Androsten-11β-ol-3,17-dione	18
Testosterone	11, 10
Estrone	0, 0
Estradiol	0, 0
Estriol	12

* Dialysis of plasma from clinically normal individuals diluted 1:5, against 1 per cent HSA. Each number is the average of triplicate determinations done with plasma from three subjects.

TABLE II

Demonstration of a corticosteroid-binding protein other than albumin in human plasma *

Subject	Condition	Dialyzed against	Steroid†	% Bound
Pe.	Pregnant	Saline	F	95
Pe.	Third trimester	1% HSA	F	94
Cr.	Third trimester	Saline	F	94
Cr.	Third trimester	1% HSA	F	93
Cr.	Third trimester	Saline	B	93
Fa.	Third trimester	Saline	F	96
Fa.	Third trimester	1% HSA	F	94
Fa.	Third trimester	Saline	B	94
Ke.	Third trimester	Saline	F	95
Ke.	Third trimester	1% HSA	F	96
Py.	Third trimester	Saline	F	95
Py.	Third trimester	1% HSA	F	94
Wa.	Normal female	Saline	Estriol	77
Wa.	Normal female	1% HSA	Estriol	0

* Dialysis of plasma diluted 1 : 5.
† F, cortisol; B, corticosterone.

The results of a series of such experiments with various steroids are shown in Tables I and II. Binding varies from zero for the estrogens to 80 to 90 per cent for cortisol (Table I). The same results were obtained for estrone and cortisol by dialysis in the opposite direction. As Daughaday (4) observed, slight alterations in the cortisol molecule decrease the ability of the molecule to bind to transcortin. Because the binding of cortisol to transcortin is so much stronger than that to albumin, dialysis against albumin usually lowered the amount of binding by only one or two percentage points as compared to dialysis against physiological saline (Table II). On the other hand, the value for estriol dropped to zero, indicating binding exclusively to albumin.

From Tables I and II it can be seen that corticosterone binds nearly as effectively as cortisol.

TABLE III

Competition experiments with cortisol (F) and corticosterone (B)

C^{14} Steroid	Carrier F	% Bound*	Carrier B	% Bound*
F		61		
	1 μg.	44	1 μg.	49
	3	22	3	31
	5	15	5	30
B				86
	1	62	1	67
	3	59	3	61
	5	57	5	59

* This is the per cent of the radioactive steroid bound to pooled plasma proteins from normal subjects diluted 1:5 and dialyzed against 1 per cent HSA.

Competition experiments were performed to determine if the sites of binding of the two steroids were identical. The results shown in Table III demonstrate that cortisol is more effective than corticosterone in inhibiting the binding of radioactive cortisol whereas these two steroids are nearly equally effective in inhibiting the binding of radioactive corticosterone.

The binding curve of cortisol to plasma is shown in Figure 1. This was obtained by determining the binding of C^{14}-cortisol with the addition of 0 to 10 μg. of carrier cortisol. From the equilibrium equation for association of cortisol with plasma protein (Equation *2*),

$$2) \qquad K = \frac{(Pr \cdot C)}{(Pr)\ (C)}$$

the following equation can be derived:

$$3) \qquad \frac{1}{(Pr \cdot C)} = \frac{1}{K\ (Pr_t)\ (C)} + \frac{1}{(Pr_t)}$$

where (Pr_t) is the total concentration of protein, (Pr) and (C) are the concentrations of unbound protein and cortisol, respectively, and $(Pr \cdot C)$ is the concentration of protein-bound cortisol. A plot of $1/(Pr \cdot C)$ *versus* $1/(C)$ will be a straight line if the equilibrium constant K is the same for all sites (homogeneity of sites). Deviation of the binding curves from linearity reflects the heterogeneity of the sites for cortisol. From the intercept of the y axis, it was calculated that (Pr_t) was 3.2×10^{-7} M per L. and 1.3×10^{-7} M per L. for the pregnancy and normal plasmas, respec-

tively. These values are actually the concentration of sites. The protein concentration cannot be determined unless n, the number of sites, is known.

When one-half of the sites are occupied, *i.e.*, $(Pr_t)/(Pr \cdot C) = 2$, then $K_{av.} = 1/(C)$. This value is 3.0×10^7 L. per M at 4° C. for both cases. Since the corresponding value for HSA is 0.5×10^4 L. per M (1), it is apparent that transcortin binds cortisol approximately 6,000 times more firmly than does albumin.

It would be advantageous to have an easier measure of the concentration of transcortin. Efforts to devise a measure have been thwarted by the presence of endogenous corticosteroids bound strongly enough to defy removal except under those circumstances which denature proteins. An alternative is to determine the amount of additional cortisol required to reduce the binding of a tracer amount of cortisol to an arbitrary level, say 50 per cent, or the decrease in binding produced by the addition of a fixed amount of cortisol, say 1 μg. The latter appears to be a more sensitive index as well as a more practical one. It should be realized, however, that this measures the difference between two variables, the level of transcortin and the level of corticosteroids.

Table IV compares such values for normal and

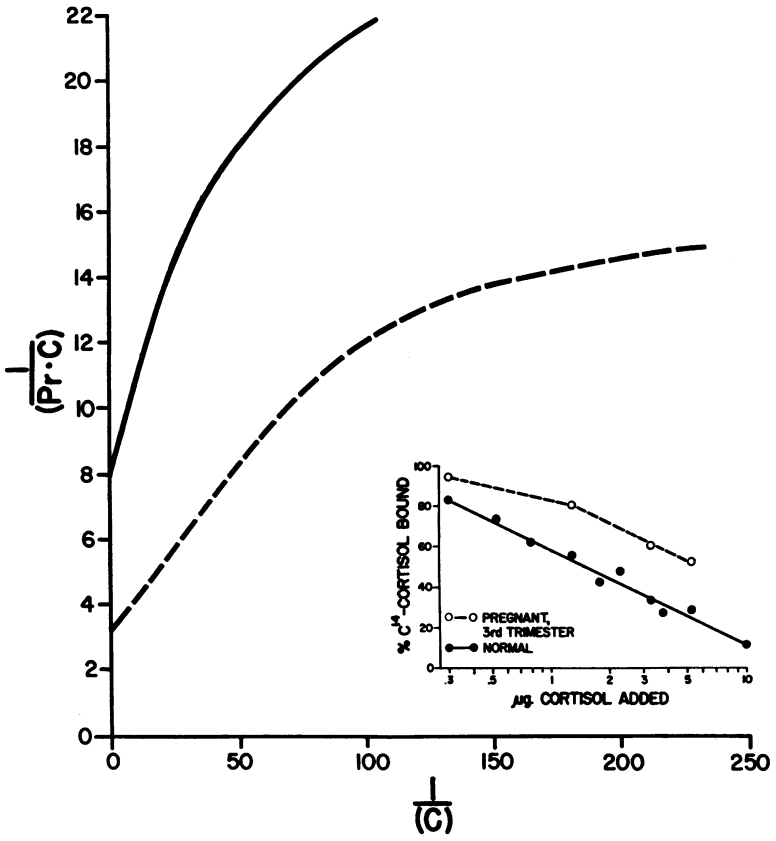

FIG. 1. THE BINDING CURVE OF CORTISOL

To 0.3 μg. of C^{14}-cortisol in 0.15 M saline was added 0 to 10 μg. of carrier cortisol. This was dialyzed against plasma diluted 1:5 with saline. The insert shows the data plotted as per cent C^{14}-cortisol bound *vs.* the amount of cortisol present. Each point is an average of determinations on three to six serums. Of more theoretical interest is the larger plot, which is calculated from the smaller one. The reciprocal of the concentration of bound cortisol is plotted against the reciprocal of the concentration of unbound cortisol, both expressed as L. per $M \times 60^{-6}$. The solid and dashed lines denote plasmas obtained from normal and pregnant (third trimester) subjects, respectively.

TABLE IV

The decrease in binding of 0.3 μg. of C^{14}-cortisol upon the addition of 1.0 μg. of cortisol *

State	No. of subjects	% Bound before 1 μg.	Decrease in % bound after 1 μg.
Normal	6	82 ± 11.5†	26 ± 6.0
Pregnancy, third trimester	12	96 ± 2.0	13 ± 3.7
Pregnancy, second trimester	9	93 ± 1.6	20 ± 3.6
Pregnancy, first trimester	3	87 ± 5.5	21 ± 1.7

* Dialysis of plasma diluted 1:5 with saline against either saline or 1 per cent HSA.
† Average ± standard deviation.

for pregnant subjects in each trimester. As would be expected from Figure 1, plasma from pregnant women showed a progressively smaller decrease in binding as pregnancy progressed than did those from nonpregnant women. Since the corticosteroid level in the plasma of pregnant women is known to increase with the duration of pregnancy (11), we interpret this finding as an indication of increased transcortin levels during this period of pregnancy.

Because transcortin exhibits electrophoretic mobility, does not dialyze, and generally exhibits the characteristics expected of a protein, it has been assumed that it is a protein. As an additional check on the validity of this assumption, a sample of partially purified transcortin was digested overnight at 37° C. with trypsin at pH 7.5. The ability of transcortin to bind cortisol was completely lost.

The preceding dialysis was performed in the cold room. To answer questions regarding the

TABLE V

Effect of temperature on the binding ability and stability of transcortin *

Procedure	% Bound Expt. 1	Expt. 2	Expt. 3
Equilibrium dialysis at 4° C.	85	90	
Equilibrium dialysis at 37° C.	61	73	
Stand at 37° C. for 72 hrs.; then equilibrium dialysis at 4° C.	87	88	
Stand at 37° C. for 72 hrs.; then cortisol added and equilibrium dialysis at 4° C.	87	87	92
Stand at 4° C. for 72 hrs.; then cortisol added and equilibrium dialysis at 4° C.	86		94

* Two ml. of normal plasma diluted to 10 ml. with saline dialyzed against 30 ml. of 0.15 M NaCl and 0.3 μg. of C^{14}-cortisol.

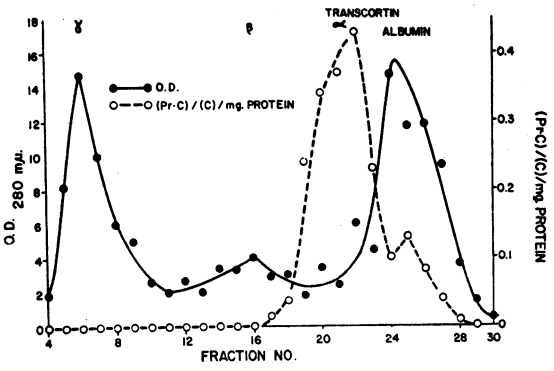

FIG. 2. CONTINUOUS FLOW PAPER ELECTROPHORESIS OF 25 ML. OF NORMAL HUMAN PLASMA IN 0.02 M BARBITAL BUFFER, pH 8.6, 660 VOLTS, *CA.* 50 MA.

The right-hand ordinate is the ratio of bound to unbound cortisol per mg. of protein.

effect of temperature on binding and stability of transcortin, the experiments outlined in Table V were performed on normal plasma. Elevation of the temperature produced a definite decrease in the amount of binding of cortisol, indicating a positive enthalpy of binding. This effect was not observed in the binding of cortisol and other steroids to human serum albumin (12). Transcortin is stable *in vitro* at 37° C. for three days, for no decrease in binding was observed upon return of the system to 4° C. as compared to the original determination or to a system which had remained at 4° C. throughout the experiment. Similar experiments on plasma from pregnant subjects led to the same conclusion.

Pooled blood-bank plasmas, collected in acid-citrate-dextrose (ACD) solution and stored in a refrigerator at 4° C., for various ages (fresh to four weeks old) showed essentially the same binding capacity. Freezing of these plasmas, or any other plasma, at −10°C. for several weeks did not affect their binding capacity for cortisol.

Continuous flow paper electrophoresis effects a considerable purification of transcortin and demonstrates that it is quite acidic. In Figure 2 are shown the results of electrophoresis of normal serum in barbital buffer at pH 8.6. The transcortin has migrated as an α-globulin. In Figure 3 is shown the result of electrophoresis of normal serum in acetate buffer at pH 5.2. It should be noted that the serum in this case was applied in the center instead of the left quarter point. The β- and γ-globulins have moved toward the cathode

while the α-globulins, albumin and transcortin have moved toward the anode. Transcortin has the mobility of an α-globulin. This result is in accord with that of Daughaday (5).

Fractions 22 through 26 inclusive (Figure 3) were pooled after withdrawal of one-third of the volume of each tube for determination of binding. After dialysis against two changes of distilled water to remove buffer, lyophilization yielded 10 mg. of dry residue, indicating a 150-fold protein concentration over the original plasma. Further purification and characterization are being continued.

Transcortin is also present in varying degrees in the blood of animals (Table VI). The dog appears to have very little transcortin while rabbits, guinea pigs, rats and perhaps alligators have substantial concentrations.

DISCUSSION

It is apparent from the data of our experiments and from those published by Daughaday (4) that human and some animal plasmas contain a protein (or proteins) with a great avidity for the binding of cortisol. We have named this protein transcortin with the full realization that it may actually constitute a system of several proteins rather than a single one and that under abnormal conditions the changes in the binding capacity for cortisol may not be related necessarily to changes in transcortin concentrations. Nevertheless, we believe that the name simplifies communication

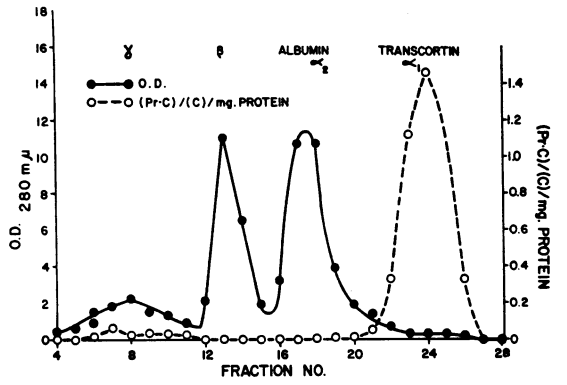

FIG. 3. CONTINUOUS FLOW PAPER ELECTROPHORESIS OF 25 ML. OF NORMAL HUMAN PLASMA IN 0.02 ACETATE BUFFER, PH 5.2, 800 VOLTS, 40 TO 60 MA.

The right-hand ordinate is the ratio of bound to unbound cortisol per mg. of protein.

TABLE VI

Binding of cortisol to animal plasma *

Amount of carrier	Rabbit	Dog	Guinea pig	Rat	Alligator
0 μg.	91†	39	83	86‡	93
2	60	20	70	63	
5	48	12	61	49	
10	36		52	40	
20		8	38		

* Plasma diluted 1:5 dialyzed against saline.
† Corticosterone, 82 per cent.
‡ Corticosterone, 75 per cent.

and does not commit any particular protein (glycoprotein, lipoprotein, and so forth) as being the cortisol-binding protein until rigid proof of the identity is established. In agreement with Daughaday (5) we believe transcortin to be associated with the α-globulin area in the electrophoretic separations.

Since no specific method exists for determining the concentration of transcortin, recourse was made in our experiments, as well as in those of Daughaday (4, 5), to the ability of human plasma to bind cortisol *in vitro* during equilibrium dialysis. The estimate of the transcortin binding capacity under these conditions is only an approximation. The reasons for this statement are as follows. Although transcortin binds cortisol most avidly, it appears that it can also bind corticosterone, 11β-hydroxyandrostenedione and other steroids to a lesser extent. In addition, it would be difficult to deny, until definite evidence is presented, that transcortin does not bind conjugates and metabolites, both unknown and known, of many other steroids. Since the determination of the concentration of these various steroid metabolites is at the moment impossible, it is apparent why the determination of the binding capacity of human plasma for cortisol remains an approximation. Nevertheless, the reproducibility and constancy of the binding capacity of transcortin under the conditions of our experiments offer at present the best method for the quantitative estimation of transcortin concentration.

The results of the competition experiments are in accord with the hypothesis that there are two types of sites at which corticosterone and cortisol may be bound. For example, at one type of site cortisol may be bound strongly and corticosterone weakly so that the former would have a greater

inhibiting effect on the binding of C^{14}-cortisol. At the second type of site both steroids would then be bound with an intermediate strength; otherwise, the first type of site would bind nearly all the cortisol.

Using undiluted plasma, Daughaday (13) failed to show any differences between the binding of normal and pregnant subjects. On the other hand, with the use of diluted plasma and the addition of carrier cortisol we were able to show a definitely increased transcortin capacity in the plasmas of pregnant women during the third trimester. Daughaday's failure (13) to show differences between normal and pregnant plasmas was due to the high equilibrium constant of transcortin combined with the use of a high protein concentration. The increased levels of transcortin during the third trimester of pregnancy are even more pronounced in light of the possibility that the greatly increased levels of other steroids (progesterone and its derivatives, corticosteroids, and so forth) infringe on the capacity of transcortin to bind the added labeled and carrier cortisol. The identity of equilibrium constants for both normal and pregnant plasma indicates that no qualitative changes in transcortin have occurred as a result of pregnancy. In agreement with Daughaday (4) we believe that at so-called physiological concentrations of cortisol in plasma it is bound preponderantly by transcortin. It should be pointed out, however, that equilibrium must exist between transcortin-bound cortisol, cortisol present in solution and albumin-bound cortisol. Thus transcortin, like albumin, would function in the transport of cortisol through the vascular system.

Although some of the concepts to be presented are merely heuristic, we believe that they can be of help in furthering the identification and study of the significance of transcortin. We propose that transcortin-bound cortisol may be for all practical purposes biologically inactive, whereas that cortisol which is not bound to transcortin is physiologically active. Thus, when the cortisol concentration exceeds the transcortin capacity, marked physiological effects will appear. This is seen following the administration of pharmacological doses of cortisone or cortisol or during increased adrenocortical activity (stress, adrenocorticotropic hormone [ACTH] administration). The lack of real evidence of hyperadrenocorticism during pregnancy, in spite of the elevated concentrations of cortisol, can best be explained on the basis of increased transcortin concentrations. On the other hand, it is possible that in some patients with Cushing's syndrome having normal levels of corticosteroids in the plasma, the disease may be due to the decreased concentrations of transcortin, resulting in an increased concentration of nontranscortin-bound cortisol with resultant increased physiological effects. The levels of transcortin in this and in other conditions in which adrenocortical function have been implicated as a factor are now under study in our laboratory. It will also be interesting to ascertain the role of transcortin in the regulation of ACTH secretion and whether transcortin-bound cortisol is metabolized in a fashion similar to that which is not bound to transcortin. These studies await the purification and ultimate isolation of transcortin, so that it can be administered with or without cortisol.

Our failure to demonstrate a protein-binding system, akin to transcortin, for the estrogens corroborates the findings of Bischoff and associates (9, 10) and indicates the lack of existence of a special protein for the binding of these steroids as postulated by Roberts and Szego (8). These authors indicated that Fraction III-0 of Cohn was mainly responsible for the binding of the estrogens. Our data and those of Bischoff and co-workers (9, 10) point towards albumin as the protein system primarily responsible for the binding and transport of estrogens. Indeed, the data of the present study indicates that albumin plays a paramount role in the binding, and hence probably transport, of such steroids as progesterone, testosterone and some of the other androgens.

SUMMARY

There is in human plasma of normal and pregnant subjects, as well as that of a number of animals, a protein having a high affinity for cortisol ($K = 3.0 \times 10^7$ L. per M) but which is present in low concentrations (1.3×10^{-7} M per L.). We propose to call this protein transcortin. Competition experiments showed that cortisol and corticosterone are bound at two types of sites. At one, cortisol is definitely bound more strongly

than corticosterone, while at the second, they are approximately equal in strength of binding. Estrogens, androgens and progesterone are bound weakly or not at all by transcortin. A method of measuring transcortin concentration is described. Using this method it was found that transcortin levels increase during pregnancy. A speculative hypothesis was advanced to explain the physiological significance of transcortin. Basic to this hypothesis is the assumption that corticosteroids bound to transcortin are biologically inactive. The concentration of corticosteroids in the plasma must, therefore, exceed the effective concentration of transcortin before physiological, and especially pharmacological, effects of the corticosteroids become evident. Transcortin is stable for at least one week in plasma or in dilute solution at either 4 or 37° C. It is destroyed by incubation with trypsin. Continuous flow paper electrophoresis at pH 5.2 separates transcortin from the bulk of the plasma proteins, effecting a 150-fold purification. It is believed to be an α-globulin.

ACKNOWLEDGMENTS

We wish to acknowledge the cooperation of Dr. Robert Patterson of the Buffalo Medical Group, who supplied us with plasma from pregnant subjects; the generous donation of human serum albumin by the American Red Cross and of C^{14}-labeled corticosteroids by the Endocrinology Study Section of the National Institute of Health; and the valuable technical assistance of Miss Jean Rae Schubarg, Mr. Lawrence Beecher and Mr. Elek Karsay.

ADDENDUM

After submission of our manuscript an interesting article by Dr. Ian E. Bush (Ciba Foundation Colloquia on Endocrinology, 1957, 11, 263) on "The Physicochemical State of Cortisol in Blood" came to our attention. This article describes experiments pointing to the existence of a cortisol-binding protein which becomes saturated at the upper level of the normal range for plasma cortisol concentrations.

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