SYMPOSIUM: BINDING OF LIPIDS BY PROTEINS

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Binding of Steroids to Proteins[1, 2]

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Abstract

Steroid compounds form dissociable complexes of low binding energy with numerous proteins of different origin as can be demonstrated by various physicochemical procedures. This interaction has definite physiological consequences in case of the steroid hormones. The sites of attachment between Δ^4-3-ketosteroids and human serum albumin appear to be located at the alpha side of the steroid molecule. The affinity of interaction with serum albumins is increased by entrance of electron-repelling groups (alkyl) into the steroid, and decreased by electron-attracting groups (−OH; =O; halogen) ("polarity rule"). This rule is reversed in interactions with certain proteins which have a higher content of aliphatic hydroxyl groups. It was concluded from competition studies with higher fatty acids that the attachment of Δ_4-3-ketosteroids to serum albumin does not take place at the anion-binding sites. The SH-group of serum albumin is not involved in the interaction with testosterone.

The α_1-acid glycoprotein (orosomucoid) from human serum was found to have a particularly high binding affinity for progesterone. Removal of sialic acid results in a decrease of this binding affinity. Complex formation with the orosomucoid leads to physiological inactivation of progesterone.

A highly specific interaction occurs between the adrenocorticoid hormones and the corticosteroid-binding globulin (transcortin) in serum of human and other species. For a quantitative test, the endogenous corticosteroids have to be removed by dialysis at 37C. Cortisol, corticosterone and related hormones are bound by transcortin; aldosterone interacts with serum and transcortin-containing fractions more strongly than with albumin. The "transcortin" activity of rat serum increases after adrenalectomy and hypophysectomy; injection of corticosterone into adrenalectomized rats reverses this effect. The general increase of the total α-globulin fraction in adrenalectomized rats (15–16%) is smaller than the increase in "transcortin" activity (100%). The corticosteroid-binding serum proteins of different mammalian species (rat, rabbit, steer, horse) were found to be α-globulins. Their binding affinities towards different corticosteroids will be discussed.

Introduction

CHEMICAL BASIS and biological significance of interactions between steroid hormones and proteins have been of interest to my co-workers and me for a number of years (1). These interactions are considered important in any mechanism of action of these biocatalysts, for their transport in the circulating blood and for the regulation of their physiological activity, metabolic transformations and excretion.

The bonds that are operative in steroid-protein interactions are of a non-covalent nature; their energy is relatively low as can be seen in Table I where free energies of binding are listed for the interaction between several steroid hormones and serum albumin (See Table 8 in Reference 1). These binding energies are of a magnitude typical of hydrogen bonds or van der Waals forces; the complexes, therefore, are dissociable under conditions prevalent in the living organism.

[1] This work supported by Public Health Service research grants (AM-04040, AM-06369), a research career program award (5-K6-GM-14, 138) and a research contract of the Dept. of the Army, U.S. Army Medical Res. and Dev. Command (DA-49-193-MD-2263).

[2] For a review of the work on steroid protein interactions up to beginning of 1960 see (1).

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TABLE I

Approximate Free Energy of Binding of Steroids to Serum Albumin

ΔF° in kcal/mole steroid; pH 7.0–7.5

Steroid	BSA [a]	Human serum albumin		
	Solubility	Equilibrium dialysis	Spectrophotometry	Solubility
	37C	25 C	25C	37C
Testosterone	−6.2	−6.3	−6.2	−6.7
Progesterone	−6.1	−6.6	−6.5	−6.7
Cortexone	−5.8	−6.3	−6.2	−5.9
Cortisol	.....	−5.5	−5.5	.....

[a] Bovine serum albumin.

Interactions of Steroids with Serum Albumin

Investigation of the influence of the chemical structure of steroid hormones and other Δ^4-3-ketosteroids on interaction with serum albumin has demonstrated that entrance of electron-attracting groups (e.g., oxygen, halogen) weakens the complex, whereas entrance of electron-repelling groups (e.g., alkyls) results in a stronger interaction. This phenomenon is shown in Figure 1 where numerical values are given for the average percentage reduction of the UV-absorption coefficient for some 60 Δ^4-3-ketosteroids due to interaction with human serum albumin (2). Decreased binding to serum albumin with increasing number of polar groups in steroid hormones was first recognized by Samuels et al. (3).

Interaction according to this polarity rule occurs with serum albumin and many other proteins of blood serum and proteins from other sources. In contrast, there are proteins (e.g., lysozyme, chymotrypsin, trypsin) where the most polar Δ^4-3-ketosteroid is bound most firmly (1). This second type of protein

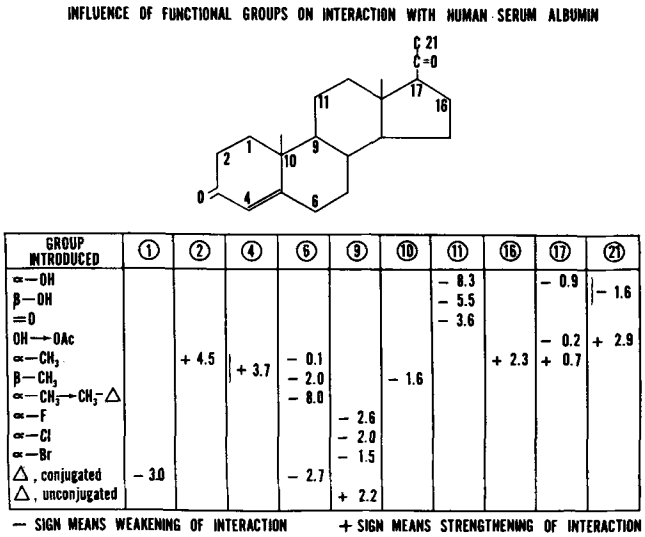


FIG. 1.

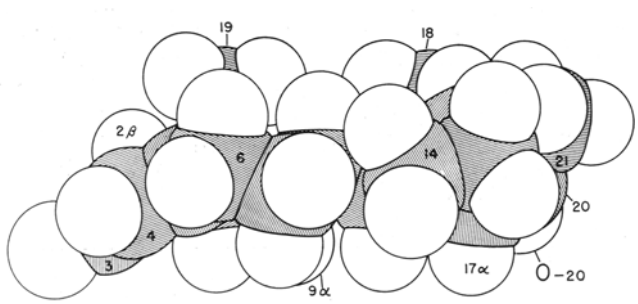


FIG. 2.

TABLE II

Combining Affinity (C) of Duo to Pentapolar Steroids [a] at pH 7.6, 4C

Steroid	O_n	=O	−OH	α_1-Acid glycoprotein C	Bovine submaxill. mucin C
Progesterone	O_2	2	0	18.0	0
Testosterone	O_2	1	1	11.0	<0.04
Cortexone	O_3	2	1	4.8	0
Corticosterone	O_4	2	2	1.7	<0.04
Cortisol	O_5	2	3	0.02	<0.02

[a] For definition of this polarity see reference (1).

appears to have a relatively higher number of aliphatic hydroxyl groups. It can be assumed that in the first type of steroid binding protein (e.g., serum albumin), van der Waals forces are mainly responsible for the interaction, whereas hydrogen bonds mediate the binding predominantly in the second type of protein (e.g., chymotrypsin).

The spectrophotometric investigation of the interaction of Δ^4-3-ketosteroids with human serum albumin has also provided information on the steric relationship between the two components of the complex. A comparison of a number of epimers has shown that the lower or α-side of this steroid nucleus is most likely to interact with the binding protein. A side view of the progesterone molecule (Fig. 2) shows that the α-side offers a relatively planar surface for a close interaction with the protein. Similar conclusions of α-side interactions have been drawn in other laboratories from entirely different points of approach (1).

The serum albumin molecule is quite unique in its very general binding affinity for a great number of ligands of different chemical structure. Although the chemical basis of such interactions is of definite interest, we were looking for types of protein binding which were more specific for steroid hormone molecules. In most cases to be discussed in this report, the binding affinity C was determined according to

$$C = \frac{S_{bound}}{S_{unbound} \times P} \text{ liter} \times \text{gm}^{-1} \qquad [1]$$

where S_{bound} and $S_{unbound}$ indicates the concn of bound and unbound steroid, respectively, and P the protein concn in gm/liter. These two steroid concn were determined by equilibrium dialysis; the procedure of multiple equilibrium dialysis (4) proved particularly useful for the comparison of different protein solutions under identical conditions. Radioactive steroid hormones were used for the determination of the bound and unbound portion of the interacting steroids.

Interaction of Progesterone with α_1-Acid Glycoprotein

A relatively high binding affinity was observed (4) between progesterone and the α_1-acid glycoprotein (5), a trace serum protein which is identical with orosomucoid (6). Table II shows the binding affinities of this glycoprotein for several steroid hormones. The highest affinity is seen for the complex with progesterone, the value being approx 15 times greater than that for the complex progesterone-human serum albumin (1). The C-values obtained with some other

TABLE III

Combining Affinity (C) of Progesterone with α_1-Acid Glycoprotein at pH 7.4, 4C

Species	C
Human	14.6; 14.3
Bovine	3.4; 3.9

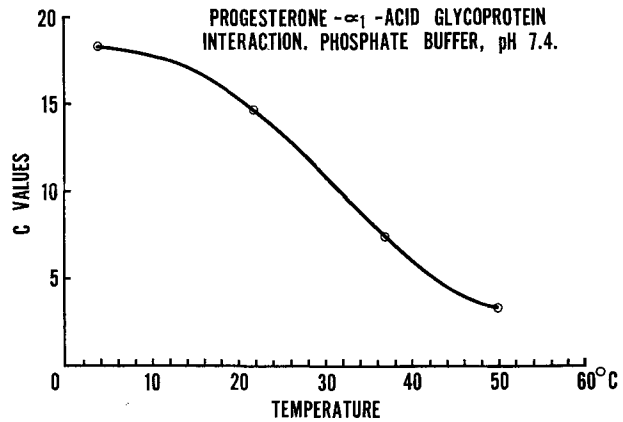


FIG. 3.

Δ^4-3-ketosteroid hormones indicate the validity of the polarity rule.

Some problems of the progesterone-orosomucoid interaction have been investigated recently by R. Carnighan in our laboratory. The α_1-acid glycoprotein is not unique for human serum; similar acidic glycoproteins have been observed as normal serum constituents in all mammalian species so far studied (7). The pure bovine orosomucoid (8) interacts also with progesterone (Table III), however, with a considerably lower affinity than its human counterpart.

The interaction between progesterone and human α_1-acid glycoprotein is highly dependent on temp, the association constant decreasing with rising temp (Fig. 3). In contrast, the dissociation of the progesterone-human serum albumin complex increases only slightly at higher temp as seen in Figure 4. It should be noted that the low binding affinities of orosomucoid and human albumin at the highest temp values in Figures 3 and 4 are not caused by denaturation effects; they have been found reversible in both cases.

The hydrogen ion concn also has a strong influence on the progesterone-orosomucoid interactions. Figure 5 shows that the highest binding affinity is obtained at pH 8, and that it declines steeply towards lower pH-values. This is again in contrast to the progesterone-albumin interaction where the influence of pH is less pronounced, and where the binding affinity increases with increasing pH up to approx pH 11 (1). The break in the curve in Figure 5 appears to be caused by the use of a different buffer in the higher pH range.

The number of binding sites for progesterone in human α_1-acid glycoprotein was determined by equilibrium dialysis, using different concn of progesterone-4-C^{14} at constant protein concn. Two samples of human orosomucoid were employed, prepared by a procedure based on the first precipitation step of

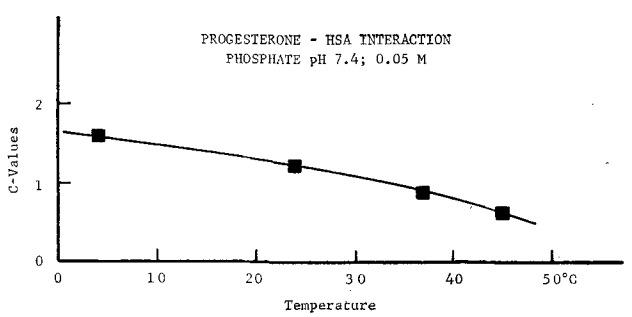


FIG. 4.

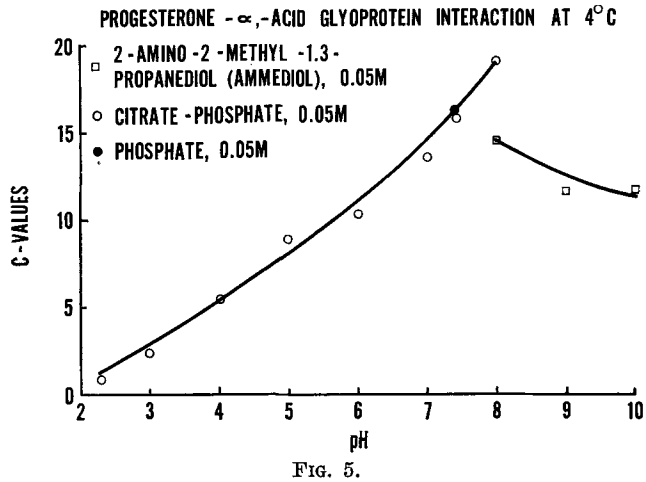


FIG. 5.

Michon (9) and the chromatographic techniques of Bezkorovainy and Winzler (10). One of these two preparations (I-117) had essentially the full progesterone-binding activity observed previously (4), the other one (I-103) had been deactivated to ca. 40% of its original binding activity as measured by the C-value. The data, obtained by equilibrium dialysis at pH 7.4, 4C, were evaluated by the graphic method of reciprocal plot, as well as by the Scatchard plot (11). Both procedures are based on the equation

$$r = \frac{n\,K\,(S)}{1 + K\,(S)} \qquad [2]$$

where r equals the number of bound steroid molecules/total number of protein molecules, n the number of binding sites for the steroid on each protein molecule, K the association constant, and (S) the molar concn of unbound steroid molecules. The assumption is made that the n binding sites are equiva-

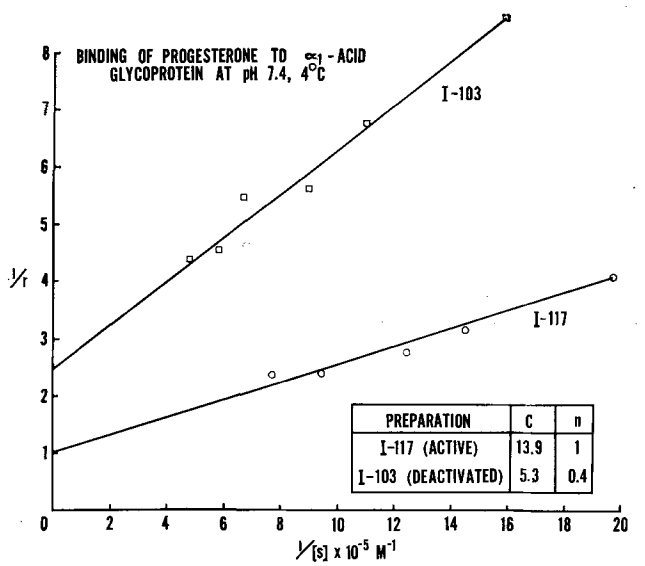


FIG. 6.

TABLE IV

Interaction Between Progesterone and α-Acid Glycoprotein at pH 7.4, 4C

Preparation	I-117	I-103
C-value	13.9	5.3
Association constant { Klotz / Scatchard	$8.1 \times 10^5 M^{-1}$ / 9.2	$9.7 \times 10^5\ M^{-1}$ / 8.1
Free energy of binding (for n = 1)	−7.5 kcal/mole	−7.5 kcal/mole

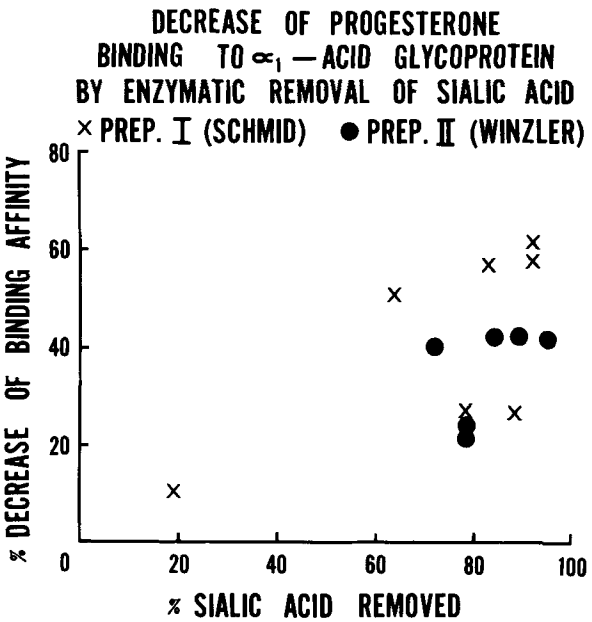


FIG. 7.

lent and independent, all having the same association constant K. If both sides of Equation 2 are inverted, one obtains

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nK\,(S)} \qquad [3]$$

The validity of Equation 3 can be tested by plotting 1/r vs. 1/(S); a straight line should be obtained. Figure 6 shows that Equation 3 holds for both orosomucoid preparations under the conditions used. The intercept on the ordinate gives the value 1/n, and the slope of the curve equals 1/nK. An approx value of n = 1 is obtained for the number of binding sites on the active preparation, I-117, whereas only 0.4 binding sites can be revealed/molecule of the deactivated glycoprotein (I-103). This would indicate that the deactivation process completely inactivates ca. 60% of the orosomucoid molecules; the remainder should then be expected to retain full binding affinity. The association constants, K, have been calculated by the reciprocal plot method of Klotz (11,12); Table IV shows that they are, within range of error, identical. Similar values have also been obtained by calculating the association constants by Scatchard's procedure (13). The free energy of binding for the binding site (n = 1) has the relatively high value of 7.5 Kcal/mole for both preparations. These results indicate that reactions that lead to deactivation of the orosomucoid do not effect a general decrease of the binding affinity,

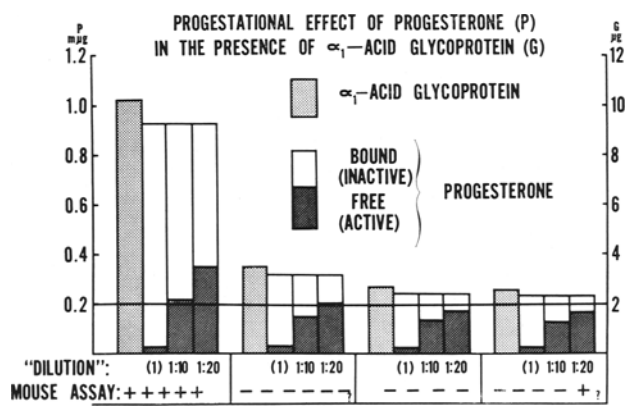


FIG. 8.

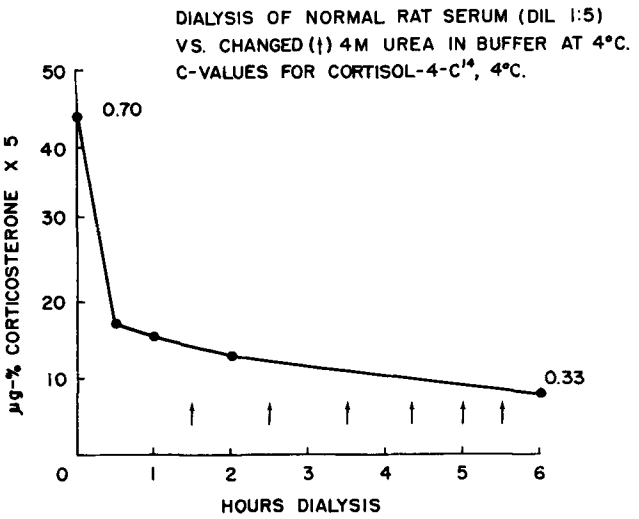


FIG. 9.

but rather complete inactivation of a portion of the glycoprotein molecules, leaving the remainder intact. Cause of the deactivation will be further studied; no differences between the preparations I-103 and I-117 could be detected in the sedimentation constants and in paper-electrophoretic behavior.

The α_1-acid glycoprotein molecule is characterized by the comparatively high content of ca. 12% sialic acid (*N*-acetylneuraminic acid (14). Partial removal of the sialic acid under the mild conditions of enzymatic hydrolysis resulted in a reduction of the binding activity of progesterone (Fig. 7); neuraminidase from clostridium perfringens (15) or from inactivated influenza virus was used in these experiments. It is not known whether the sialic acid is directly involved in the interaction with progesterone, or whether the removal of this constituent with its electronegative charge leads to conformational or other changes in the glycoprotein molecule which decrease the binding affinity for progesterone.

The biological activity of the hormone-protein complex is of considerable interest in any study of interactions between these components. In the case of the corticosteroid-binding globulin, it has been shown that complex formation with corticosteroid hormones effects inactivation *in vitro* and *in vivo* (15,16). The problem of biological activity of the progesterone-orosomucoid complex was studied utilizing the intrauterine test procedure of Hooker and Forbes (17).

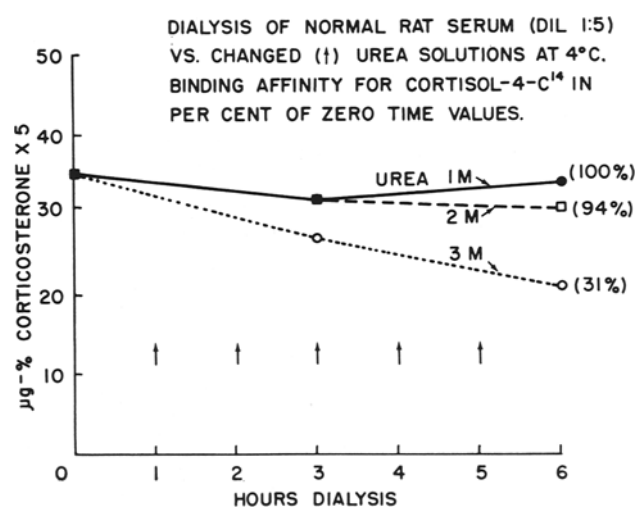


FIG. 10.

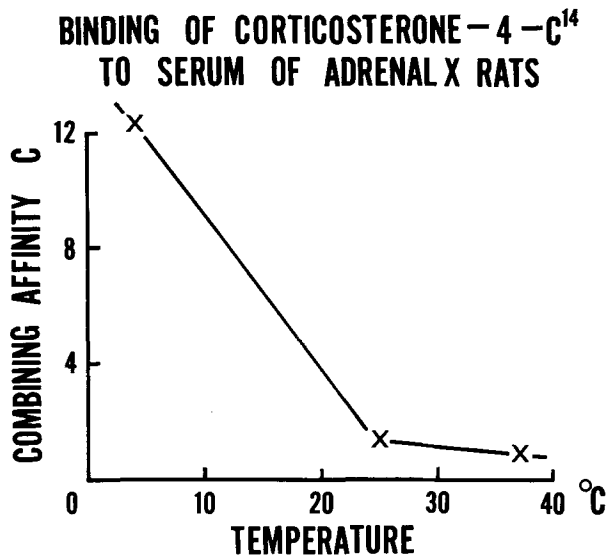


FIG. 11.

This method appeared particularly suitable since the test solution is injected directly into a ligated segment of the uterus horn of the mouse; the steroid-glycoprotein mixture does not enter into general circulation so that various possibilities of interfering reactions of the two components with other body constituents are avoided. Such a topical test therefore permits a simpler interpretation than a systemic assay procedure.

In cooperation with T. R. Forbes, it was found that interaction with orosomucoid leads to loss of progestational activity of progesterone (18). Results and interpretation of these experiments are illustrated in Figure 8 where the response of the mouse uterus segment is shown on the bottom line for four solutions consisting of mixtures of progesterone and α_1-acid glycoprotein. The amounts of glycoprotein and progesterone in these four solutions are shown in the four groups of bars, the dotted bars indicating the quantity of α_1-acid glycoprotein (G, right-hand ordinate), the total length of the light-and-shaded bars indicating the total amount of progesterone (P, left-hand ordinate). The three progesterone bars for each of the four injected solutions show what portions of the total progesterone, in the presence of the given

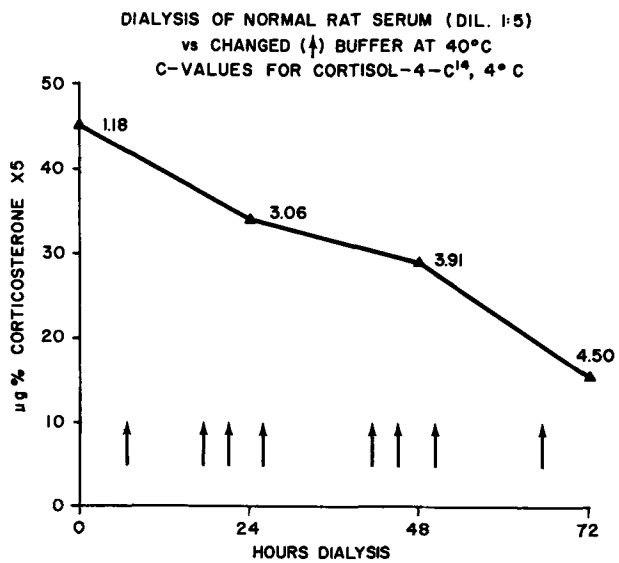


FIG. 12.

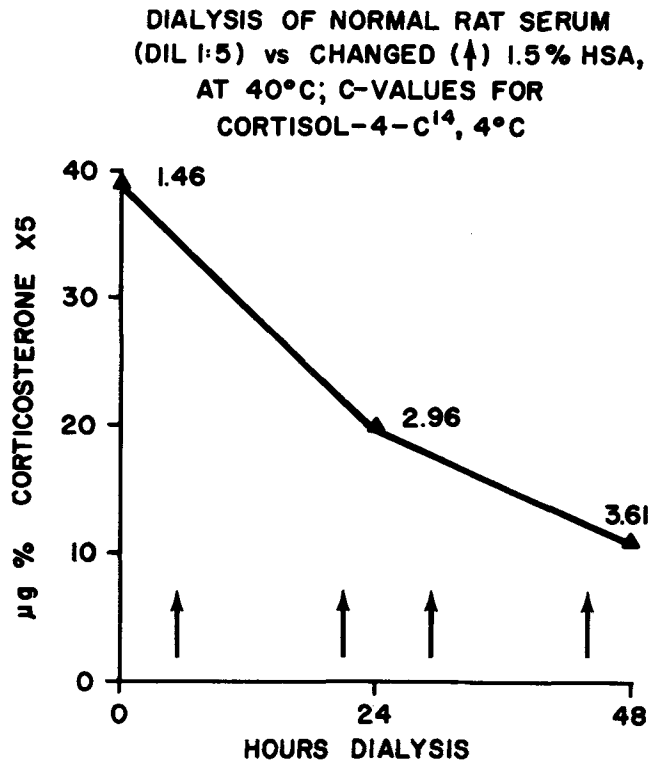
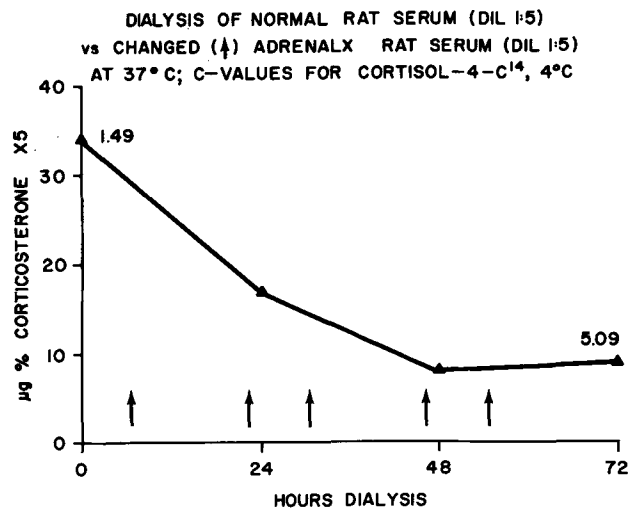


FIG. 13.



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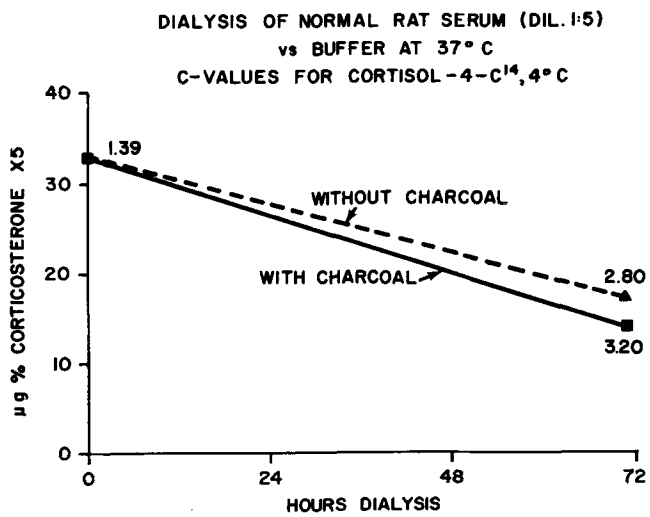

FIG. 15.

TABLE V

Removal of Corticosterone-4-C^{14} from Rat Serum by Dialysis vs. Buffer-Charcoal at 37C

Serum	Hours dialysis	No. of buffer changes	Corticosterone-4-C^{14} at		
			Start	End of exp.	
			μg-%	μg-%	% Removed
Normal	24	3	30.0	1.6	94.7
Adrenal X	48	5	30.0	1.7	94.4

quantity of the glycoprotein, is free (dissociated from the complex with the glycoprotein, shaded portion of bars) and what portion is bound to the glycoprotein (light portion of bars). The calculations for the bound and free portions of progesterone have been made for three dilutions as given on the second line from the bottom. The horizontal line at 0.2 mμg progesterone indicates the threshold amount of progesterone for positive test reactions. It is evident (bottom line) that the presence of the glycoprotein in the three assay solutions on the right-hand side prevents the test from being positive. The positive response of the left-hand solution has been explained by dissociation of the complex under the conditions of the test (18). The results of these assays have been interpreted by the assumption of a net "dilution" of not less than 1:10 and not more than 1:20; for details see reference (18). It should be noted that the dissociation of the progesterone-orosomucoid complex is considerably higher than that of the complex between cortisol and transcortin which has an association constant approx 1000 times greater than that of the progesterone-orosomucoid complex.

Interactions with the Corticosteroid-binding Globulin (transcortin)

The highest binding affinity between a steroid hormone and a protein has been observed so far between corticosteroids and a specific trace protein of blood serum, the corticosteroid-binding globulin (transcortin). This protein, independently discovered in human blood by Daughaday and by Bush in 1956

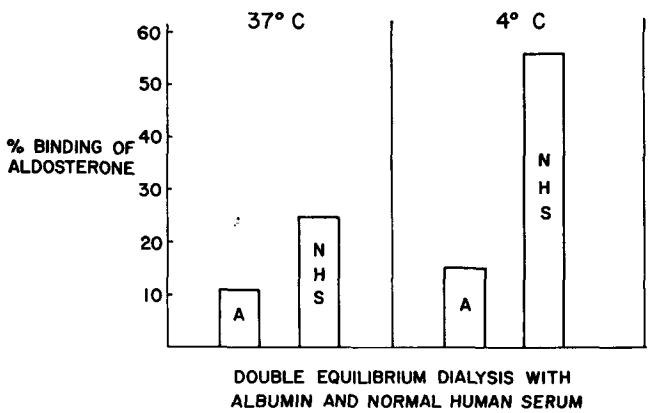

FIG. 16.

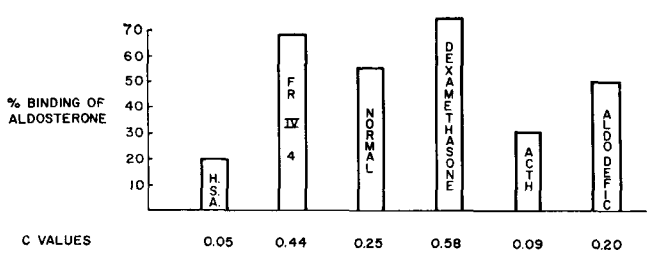

FIG. 17.

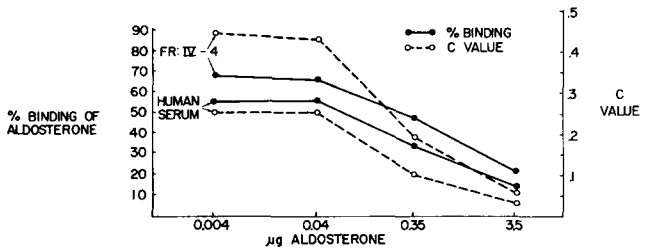

FIG. 18.

(1), has recently been isolated and characterized by Seal and Doe (19).

An inherent difficulty in the determination of the transcortin activity in serum or serum protein fractions is the presence of endogenous corticosteroids which occupy binding sites and thus interfere with the binding of radioactive corticosteroid which is added to the test system. In our studies on the corticosteroid-binding serum protein of the rat, we have used various procedures to remove the endogenous corticosteroids by dialysis. In early experiments, urea was added to the dialysis solution in order to facilitate dissociation and thus removal of the endogenous corticosteroid. Figure 9 shows that dialysis of diluted normal rat serum in the presence of 4 M urea resulted even at 4C in rapid removal of corticosterone (it should be noted that in the data in this and the following figures no blank values have been subtracted from the fluorometric readings in the corticosterone determinations; the lowest concn shown are those also found in adrenalectomized rats); however, the C-value decreased from 0.70–0.33 indicating inactivation of the corticosteroid-binding globulin. Reducing the concn of urea prevented this inactivation, but also the ready dissociation of the complex so that the endogenous corticosterone level did not decrease (Fig. 10).

The dissociation of the corticosteroid-transcortin complex is markedly increased at 37C (1), and this property has been utilized in the measurement of cortisol-binding activity in human plasma (20). We have found a similar decrease of the combining affinity between corticosterone and the corticosteroid-binding globulin of rat serum with higher temp (Fig. 11). This property was utilized in dialysis experiments at 37 and 40C. Figure 12 shows that dialysis of diluted rat serum at 40C vs. frequently changed phosphate buffer removed the endogenous corticosterone; at the same time, the C-values increased as would be expected as the binding sites become available to attach the radioactive test corticoid. Removal of the corticoster-

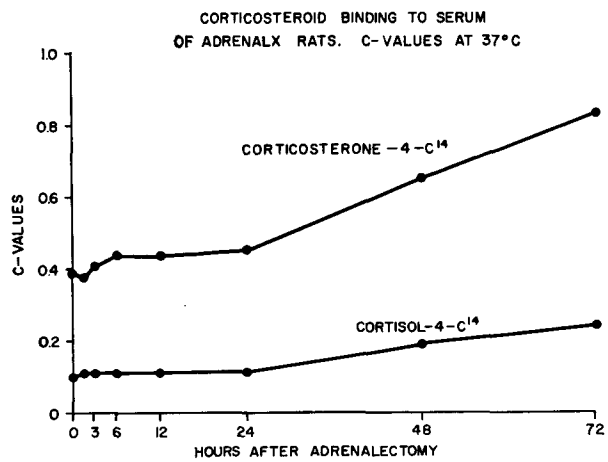

FIG. 19.

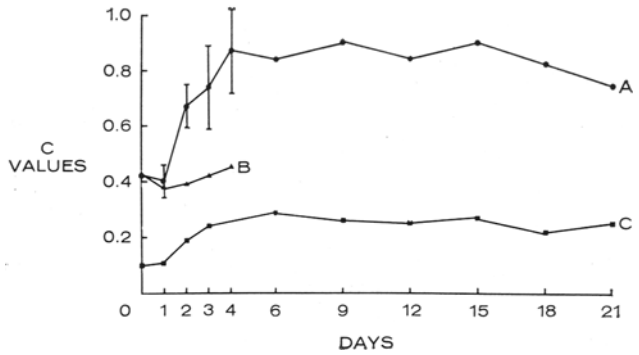

FIG. 20.

one is somewhat more rapid when a 1.5% solution of human serum albumin is used instead of buffer as outside solution (Fig. 13). Optimal removal of corticosterone was achieved by this technique in a dialysis vs. diluted serum of adrenalectomized rats which has unoccupied binding sites available, ready to take up the dialyzing corticosterone (Fig. 14). A more than three-fold increase of the C-value was observed. Table V shows that the removal of corticosterone-4-C^{14} added to serum of normal and adrenalectomized rats was essentially complete after 1 or 2 days' dialysis vs. charcoal-containing buffer at 37C. The effect of charcoal on the removal of corticosterone by dialysis at 37C, without change of outside solution, is seen in Figure 15.

Complete removal of endogenous corticosteroids by the above dialysis procedures is cumbersome and time consuming. The essential objective of elimination of interference from endogenous corticosteroids is achieved by equilibration of the various test samples at 37C in a multiple dialysis setup (4). This technique has been widely used in our laboratory; it gives reliable results, especially when a normal control serum from a large standard pool is included in each test. A different test procedure has been described by DeMoor et al. (20a) in which the plasma sample is overloaded with cortisol, and the protein-bound corticosteroid, including the endogenous portion, is separated from the unbound hormone by gel filtration over Sephadex G-50 and quantified fluorometrically. This clinically useful method determines binding capacity under the specific conditions of the experiment; it does not provide a physicochemically defined measure of the binding affinity.

Soon after the discovery of the corticosteroid-binding globulin it was observed that cortisol, corticosterone and other glucocorticosteroids are bound to this protein (1). It was of interest whether the mineralocorticoids, especially aldosterone, interact also with the transcortin system. Figure 16 shows that normal human serum has a higher binding affinity for aldosterone than solutions of crystalline human serum albumin (21,22); all solutions were adjusted to 5 mg protein/ml. The difference was particularly marked at 4C. An even higher binding capacity/mg protein was shown (Fig. 17) by Cohn's human plasma fraction IV-4 (22) which is known to contain corticosteroid-binding globulin (1). When the adrenocorticosteroid secretion was suppressed by administration of dexamethasone, the binding of aldosterone was increased further (Fig. 17) in a similar way as cortisol binding is augmented in such serum (23). On the other hand, if the cortisol level was elevated by administration of adrenocorticotropic hormone (ACTH), aldosterone interaction was reduced. These results indicate that aldosterone-binding macromolecules are present in the serum, and that cortisol competes with aldosterone for the binding sites. When the aldosterone-binding sites become saturated by increased amounts of the hormone, the binding affinity is decreased to a lower level, presumably that of albumin

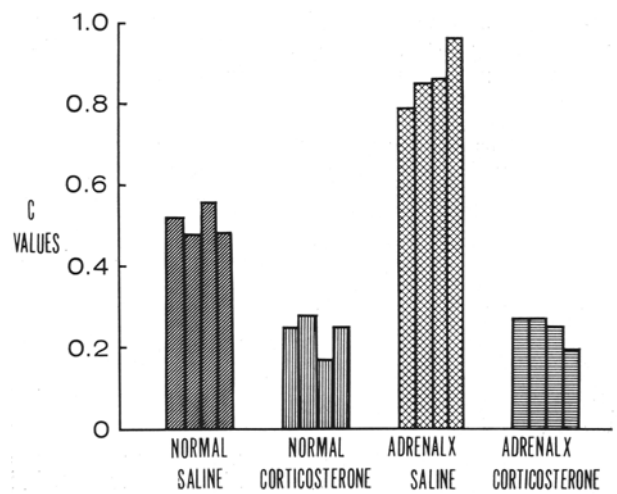

FIG. 21.

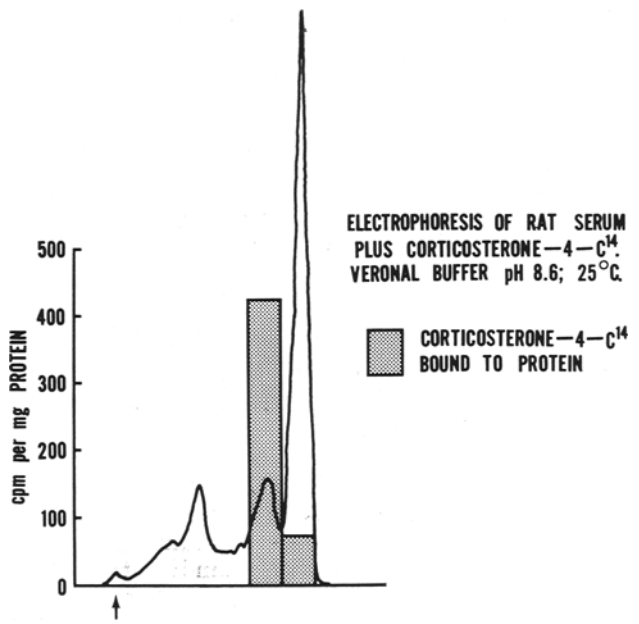

FIG. 22.

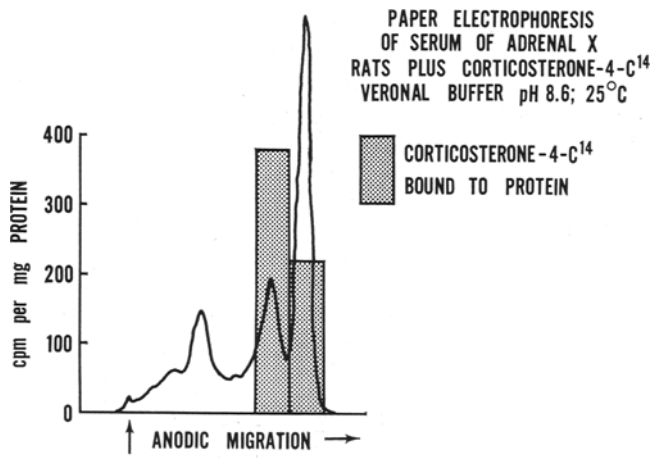

FIG. 23.

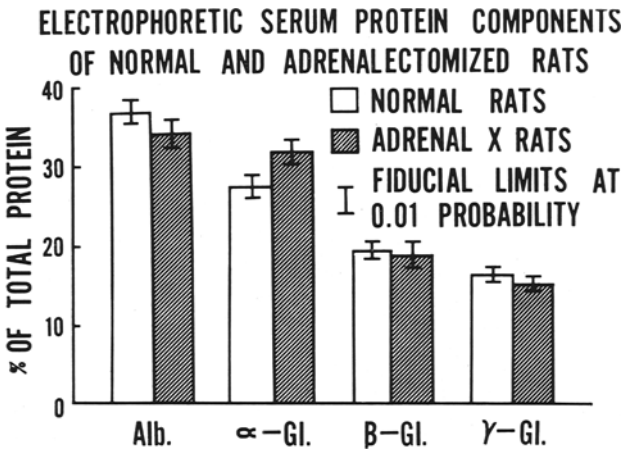

FIG. 24.

(Fig. 18). All these results are in accordance with the assumption that the aldosterone-binding macromolecule is identical with the cortisol-binding transcortin. Whether this is the case, or whether other closely related proteins are involved in the interactions can be clarified only with the isolated pure proteins (22).

When we dialyzed normal rat serum vs. serum from adrenalectomized rats, we noticed that at equilibrium more corticosterone was bound to the adrenalectomized than to the normal serum. The reason was found to be an increased "transcortin" (in strict usage, the term transcortin should be reserved to the corticosteroid-binding globulin of human blood) activity in the serum of the adrenalectomized rats (Fig. 19) which became statistically significant ca. 48 hr after adrenalectomy (23). The corticosteroid-binding activity continued to rise to the fourth day (Fig. 20) and stayed at the elevated level for at least three weeks (24). Strength of interaction with corticosterone (curve A in Fig. 20) and with cortisol (curve C) paralleled each other; the serum of sham-operated animals (curve B) did not differ from normal serum.

If the rise in corticosteroid-binding activity was caused by the low corticosterone level in the adrenalectomized rat, administration of the hormone should reverse the effect. Figure 21 shows that this was the case. Injection of massive doses of corticosterone into adrenalectomized rats reduced the C-values to ca.

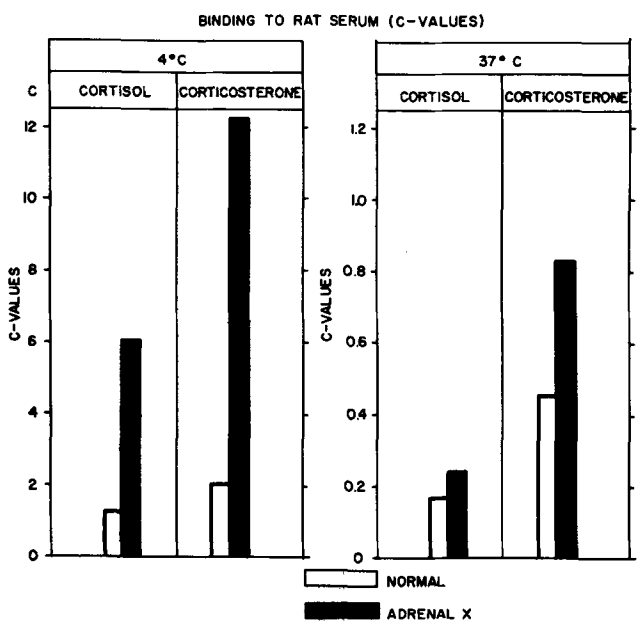

FIG. 25.

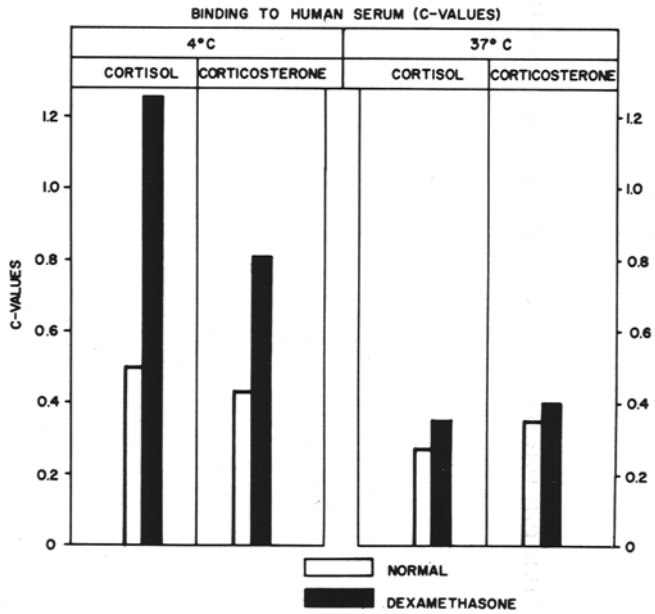

FIG. 26.

one-fourth. A similar low combining affinity was obtained after injection of equally high doses of corticosterone into normal rats (24).

The high corticosteroid-binding activity in the serum of adrenalectomized rats may well be part of the explanation of the prolonged biological half-life for corticosteroids in adrenalectomized rats (25,26) and mice (27). Increased binding to a serum protein would diminish the statistical chance for a corticosteroid molecule to interact with steroid-metabolizing enzymes, or to cross cell barriers and enter pathways of metabolism and excretion. The increase of corticosteroid-binding sites may be considered an attempt of the organism to preserve the vital hormone. Concerning the seeming paradox that increased transcortin activity results in lesser availability of unbound, i.e. physiologically active, corticosteroid it should be remembered that the affinity of the reactive tissue, or of the receptor sites, has to be high enough to detach the hormone from the binding serum protein in any case.

It seemed of interest to investigate whether the changes in the transcortin level of the adrenalectomized rat are reflected by changes in the composition of the serum proteins. By the elegant technique of equilibrium paper electrophoresis described by Daughaday (28) it could be demonstrated that the corticosterone-binding protein in serum of normal and adrenalectomized rats is an α-globulin (Fig. 22,23). Paper-electrophoretic analysis of sera from adrenalectomized

TABLE VI

C-Values for Cortisol (F) and Corticosterone (B)

Species	F/B [a] in blood	4C		37C	
		F	B	F	B
Human	10	0.92	0.77	0.32	0.39
Monkey	20	0.65	1.00	0.14	0.48
Rat	0.05	1.22	1.86	0.22	0.59
Rabbit	0.05	1.11	0.74	0.49	0.27
Sheep	20	0.27	0.25	0.17	0.18
Steer	1	0.29	0.26	0.15	0.13
Starling	–	1.34	1.24	0.20	0.26
Horse	–	0.51	0.26	0.26	0.17
Guinea pig	–	1.20	0.79	0.17	0.15
Dog	1	0.30	0.23	0.13	0.13
Pig	–	0.16	0.16	0.09	0.08
Cat	5	0.16	0.22	0.07	0.10

[a] Ratio of cortisol concn/corticosterone concn.

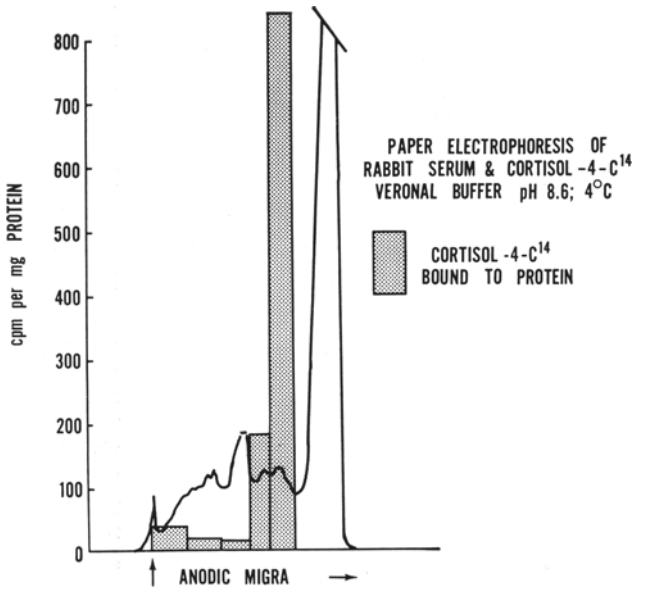

FIG. 27.

rats (Fig. 24) showed that the α-globulin component is the only one of the electrophoretic serum components that is significantly ($P = 0.01$) increased in comparison to normal serum (24). However, this increase is only ca. 15% above normal whereas the rise in the corticosteroid-binding activity is more than 100%.

It was apparent in Figures 19 and 20 that the transcortin system of the serum of adrenalectomized rats interacted more strongly with corticosterone than with cortisol. Figure 25 shows that the same relationship obtained for normal rat serum, at 4C as well as at 37C (23). Since corticosterone is the principal corticosteroid of the rat, in contrast to man who secretes cortisol as the main glucocorticoid, it was of interest to test this relationship in human serum. It was observed (Fig. 26) that in this case cortisol was bound more firmly than corticosterone at 4C, whereas this was not the case at 37C. Serum from a dexamethasone-treated subject behaved similarly. In further testing whether this specificity of the transcortin system for the species-specific corticosteroid was a general phenomenon, the C-values for corticosterone and cortisol were determined for a number of species. It is evident from Table VI that the binding ratio of the two corticosteroids has no general relationship to the ratio

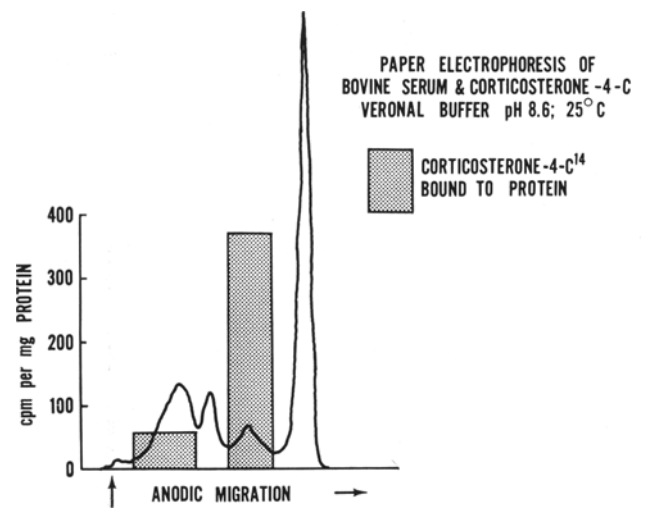

FIG. 28.

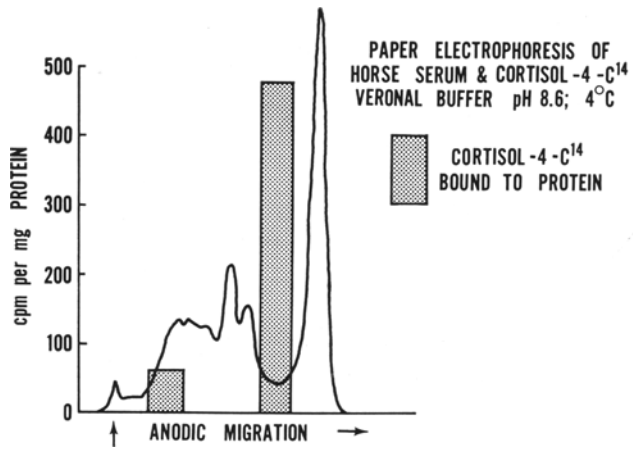

FIG. 29.

of these two adrenal hormones in the blood. A comparison of the pairs human-monkey, rat-rabbit and sheep-steer illustrates this clearly. Differences in the binding values of different species are considerable; we have also marked differences in different strains and in different individuals of supposedly the same strain. In all sera that we have analyzed by equilibrium paper electrophoresis so far, the high-affinity corticosteroid-binding protein belongs to the α-globulin component (Fig. 27–29).

In cooperation with F. DeVenuto, U.S. Army Medical Research Laboratory, Fort Knox, Ky., the continuous-flow paper electrophoretic technique was used to purify the corticosteroid-binding globulin of rat serum. The separated fractions were dialyzed against distilled water, lypophilized and their combining affinity to corticosterone determined by equilibrium dialysis at 37C. Figure 30 shows the amounts of protein obtained in the various fractions from 65 ml normal rat serum, and the C-values for each fraction. Surprisingly, the highest corticosterone-binding activity was observed in front of the albumin peak, and not in the α-globulin component as expected according to Figures 22 and 23. A similar experiment with human serum (Fig. 31) showed a normal location of the transcortin activity in the α-globulin region.

To explain this paradoxical behavior of rat serum which was consistently found in a number of experiments, the lyophilized fractions were subjected to paper-strip electrophoresis at various pH values. At

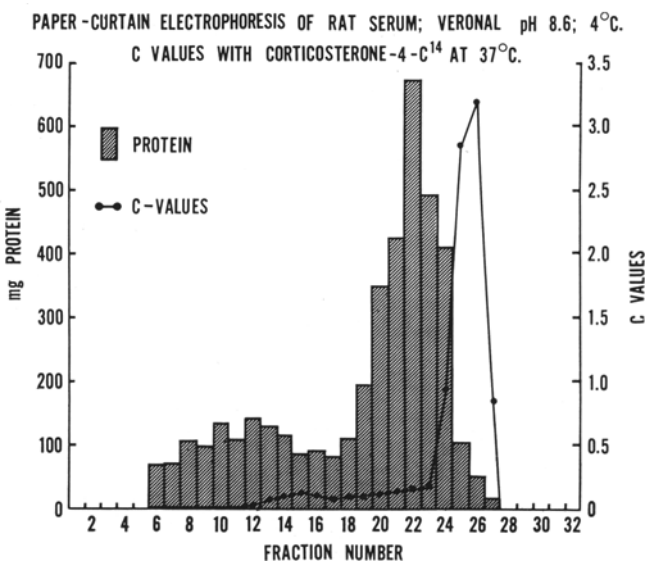

FIG. 30.

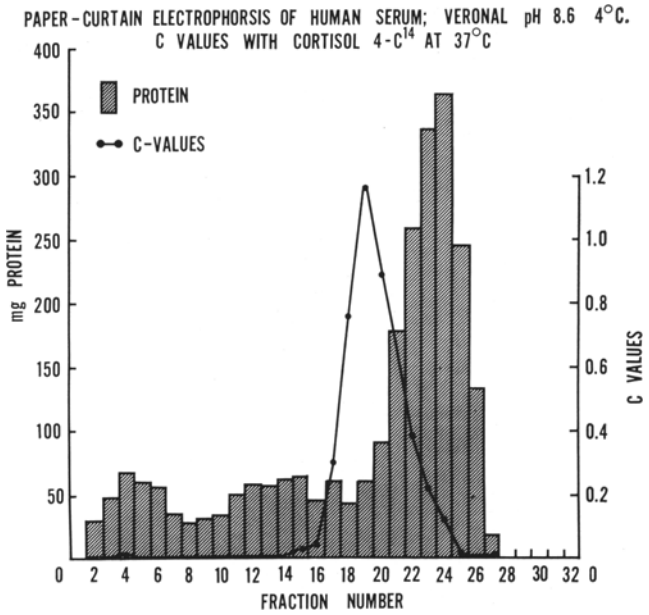

FIG. 31.

pH 8.6, the fractions 24, 25 and 26, obtained from the front shoulder of the albumin peak (Fig. 30) and containing the highest transcortin activity, migrated as α-globulin; fraction No. 24 had a small contamination of albumin, fractions No. 25 and 26 were free of albumin. This result restored the accordance with Figures 22 and 23. Paper-electrophoretic analysis of these fractions and whole rat serum at pH 7.8, 7.0, 5.5 and 4.0 revealed that a reversal of the relative electrophoretic mobility of the transcortin-containing α-globulin fraction and rat serum albumin occurs at about pH 7. A similar electrophoretic behavior has been observed for the α_1-acid glycoprotein which becomes a prealbumin at pH values of approx 7 and lower (29). In contrast, the transcortin-containing fraction isolated by continuous flow paper electrophoresis of human serum (Fraction 19 of Fig. 31) shows even at pH 4 an electrophoretic mobility slower than that of human albumin. The abnormal electrophoretic behavior of the transcortin-containing rat serum fraction on the paper curtain (Fig. 30) is interpreted as caused by a decrease of pH on the paper due to the low buffering capacity of the veronal buffer used (μ 0.02).

Although these results will have to be verified with the pure corticosteroid-binding globulins, they suggest characteristic differences in the physicochemical and biological properties of the transcortin systems of different species.

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Lipid-Protein Interactions[1]

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Abstract

Interaction of human serum albumin with phosphatidylserine was studied by turbidimetric methods. It was found that human serum albumin will bind phosphatidylserine. Analysis by the law of mass action for multiple equilibria has led to the conclusion that human serum albumin possesses a heterogeneity of binding sites for phosphatidylserine. The max number of sites and the respective affinity constants were calculated to be:

$K_1 = 2.0 \times 10^5$, $n_1 = 2$; $K_2 = 1.3 \times 10^3$, $n_2 = 30$

[1] Submitted in part as a thesis for the Ph.D. degree, University of Louisville. Carried out under the graduate training program of the U. S. Army Medical Research Laboratory.

Introduction

IN PRACTICALLY EVERY cell and tissue, complexes of protein with lipid may be found. Numerous studies have been carried out on protein-lipid interactions, including protein-phospholipid, protein-fatty acid, protein-steroid and protein detergent systems. In view of the occurrence of phospholipids in nearly all cells and the overwhelming evidence associating them with important biological phenomena such as the transport of various molecules across membranes (1–3), electron transport (4–7) and blood coagulation (8–14), the protein-phospholipid interactions would appear to be most interesting. However, progress has been slow in the study of the physiocohemical nature of the as-