Increased Corticosteroid Binding ("Transcortin") Activity in Adrenalectomized and Hypophysectomized Rats. (27380)

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The intravascular half-time of cortisol administered to adrenalectomized rats has been found prolonged when compared with that observed in intact control animals(1,2). Similarly, the passage of cortisol into the gut of adrenalectomized mice is slower than in normals(3). It was of interest to investigate whether this prolonged retention of corticosteroid hormone in the blood of adrenalectomized animals might be causally related to quantitative changes in the activity of the specific corticosteroid-binding protein system, "transcortin" (for references, see (4)).

The commonly employed *in vitro* assay of transcortin by equilibrium dialysis(4) does not determine directly the concentration of the binding protein. Rather, experimental conditions being controlled, an apparent binding activity is measured which is a product of 3 variables: concentration of the binding protein, concentration of corticosteroids endogenous to the material being tested, and amount of radioactive corticosteroid added to test the system. Nothing else being changed, variations in endogenous steroid concentration will leave more or less binding sites available for association with the radioactive corticosteroid, and thus result in changes in apparent binding activity. In single dialysis experiments at 4°C, it has been found previously in this laboratory that the serum of adrenalectomized rats has a much greater apparent transcortin activity than sera of normal or sham-operated controls(4). When partial equilibrium of endogenous corticosteroids occurred between control and adrenalectomized sera, using "multiple equilibrium dialysis"(5) at 4°C, this difference became less pronounced. The significance of these differences in binding activity was uncertain since they might have been due simply to the great disparity in endogenous corticosteroid concentrations between control and adrenalectomized sera. In the present study this uncertainty was eliminated by permitting complete equilibration of endogenous corticosteroids between normal and post-adrenalectomy sera, using a dialysis system at 37°C (6). At 37°C the dissociation constant for the corticosteroid-transcortin complex is greater than at 4°C, thus allowing complete equilibration under the conditions employed.

Materials and methods. Serum was obtained by aortic puncture under ether anesthesia from male Sprague-Dawley rats averaging 300 g body weight. Post-adrenalectomy and post-hypophysectomy sera were taken 3 days after operation. The sera of 30 to 200 rats were obtained for each normal and post-adrenalectomy pool and 12 rats were used for each post-hypophysectomy pool. All sera were diluted 1 +4 with Na-phosphate buffer, pH 7.6, μ 0.1, and streptomycin was added to a final concentration of 10 μg/ml, an addition which does not affect the binding values. For equilibration of endogenous corticosteroids, 35 ml of one diluted serum (*e.g.*, normal serum) were enclosed in a dialysis bag and dialyzed at 37°C for 72 hours, with gentle shaking, against 35 ml of the other diluted serum (*e.g.*, post-adrenalectomy or post-hypophysectomy serum). Corticosterone concentrations of the diluted sera before and after the equilibration were determined fluorometrically(7). In this procedure, no attempt was made to correct for "background" fluorescence, the usual values thus obtained for adrenalectomized and hypophysectomized rats being between 4 and 10 "μg corticosterone" per 100 ml undiluted serum and for normal rats (actually acutely stressed normals due to blood sampling technic) between 25 and 45 "μg corticosterone" per 100 ml undiluted serum.

For determination of binding activity before and after equilibration at 37°C, serum protein concentrations were determined by a biuret technic and adjusted with buffer to 5

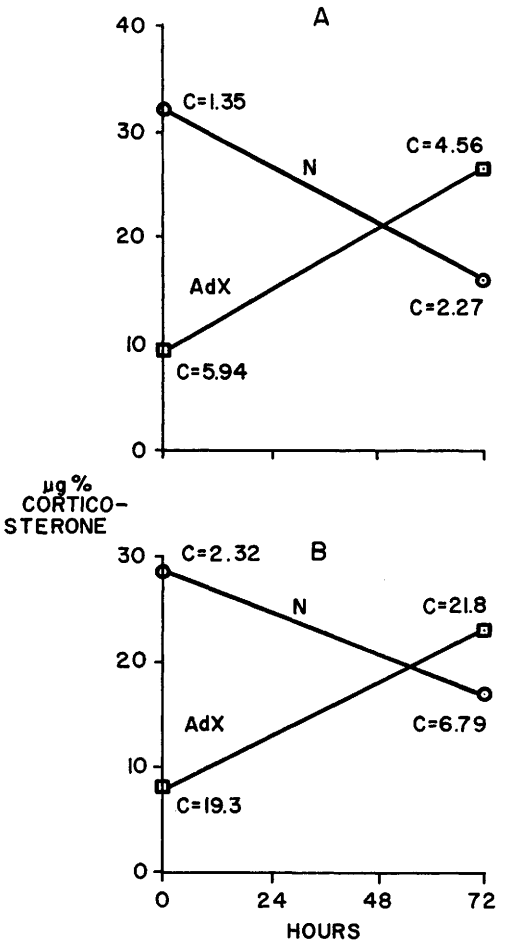

FIG. 1. Equilibration of diluted normal (N) *vs* diluted adrenalectomized (AdX)-rat serum for 72 hr at 37°C. Binding values (C) obtained by equilibrium dialysis for 48 hr at 4°C *vs* buffer containing (upper Fig. A, Exp. UW-46) 5.2 mμg cortisol-4-C^{14} or (lower Fig. B, Exp. UW-21) 9.6 mμg corticosterone-4-C^{14}/ml at start of dialysis. All corticosterone values expressed as μg/100 ml of undiluted sera. Lines in the figures connect the points for initial and final corticosterone concentrations and do not indicate rates of equilibration.

mg protein per ml. Ten ml of each adjusted serum were then dialyzed for at least 48 hours at 4°C against 20 ml of buffer containing approximately 0.2 μg cortisol-4-C^{14} (39,000 cpm/μg) or corticosterone-4-C^{14} (42,250 cpm/μg).* The technics for determination of C^{14} in a gas-flow counter have been described(8). The binding values were expressed as

$$C = \frac{S_{bound}}{S_{unbound} \cdot [P]} g^{-1} l,$$

where bound and unbound steroid (S) may be given as percent, concentration or counts per minute, and where [P] equals total serum protein concentration in g/l(4). The C-values of control sera in these experiments remained unchanged after incubation at 37°C for at least 96 hours. The procedures for equilibration of endogenous corticosteroids at 37°C were all performed in duplicate, as was each determination of both binding activity and corticosterone concentration.

Results. Fig. 1 and 2 show representative results which have been consistently found in a number of experiments. It is evident in all 3 types of experiments shown that equilibration of the endogenous corticosteroids, consisting mainly of corticosterone(9), leads to an increase in corticosterone concentration of the initially corticoid-depleted sera of adrenalectomized and hypophysectomized rats. This increase goes beyond the arithmetical mean of the starting concentrations, indicating higher binding affinity for the corticosteroids in sera of the operated animals. The subsequent transcortin assays confirm this conclusion: in spite of a higher corticosterone concentration accumulated in these

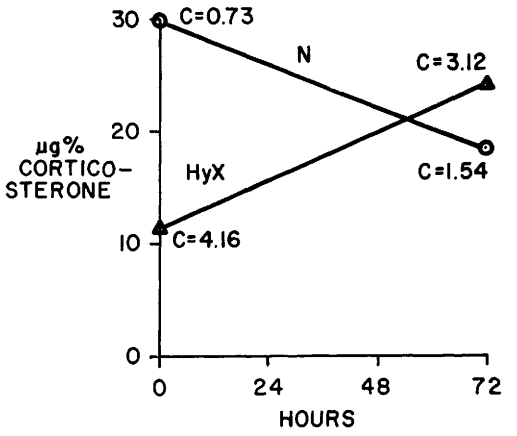

FIG. 2. Equilibration of diluted normal (N) *vs* diluted hypophysectomized (HyX)-rat serum for 72 hr at 37°C (Exp. UW-49). Other conditions as in Fig. 1; outside buffer contained 9.4 mμg cortisol-4-C^{14}/ml at start of dialysis.

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TABLE I. Binding Affinity (C) of Rat Serum and Human Serum for Cortisol and Corticosterone.

Serum	Cortisol-4-C^{14} mμg per ml buffer at start	C 4°	C 37°	Corticosterone-4-C^{14} mμg per ml buffer at start	C 4°	C 37°
Rat, normal		1.20	.16		2.10	.46
Pool May '61	9.2			11.0		
31 "μg % corticosterone"		1.25	.17		1.98	.45
Rat, adrenalectomized,		6.57	.26		10.68	.82
Pool Mar '61	9.3			12.0		
8 "μg % corticosterone"		5.50	.22		13.65†	.84
Human, normal (Ni)		.65	.28		.53	.34
	9.8			11.0		
10 μg % 17 OHCS*		.62	.25		.53	.33
Human, normal (Cha)		.48	.25		.45	.33
	9.3			11.0		
19 μg % 17 OHCS*		.53	.28		.41	.36
Human, normal (Cha) after 0.5 mg		1.26	.35		.82	.41
dexamethasone p. o. q. 6h x 9	10.0			12.0		
0 μg % 17 OHCS*		1.23	.35		.80	.38

* 17-hydroxy corticosteroid values determined by Peterson modification(11) of Porter-Silber procedure.

† It should be noted that the range of error for the C-values becomes larger with higher binding affinities due to reduced accuracy in determination of the low "unbound" C^{14} concentration in the outside solution after equilibrium dialysis.

sera during the equilibration at 37°C, their binding values, C, for additional radioactive cortisol or corticosterone are at least twice those of the control sera. These results leave no doubt that the serum concentration of binding sites or their affinity for cortisol or corticosterone is higher in adrenalectomized and hypophysectomized rats than in normal animals.

Discussion. Comparison of the C-values recorded in Fig. 1 shows that the binding affinity of rat serum is considerably higher for corticosterone than for cortisol. This holds true for normal as well as for adrenalectomized animals. Additional experiments in which C-values for cortisol-4-C^{14} and corticosterone-4-C^{14} were determined in pooled sera of normal or adrenalectomized rats, simultaneously at 4°C and at 37°C, yielded similar results (Table I). These observations are not in accord with other reported findings of higher binding affinity for cortisol than for corticosterone in rat plasma(10).

When normal human serum was used in analogous studies, no distinct preference for association with corticosterone could be demonstrated. Table I shows that there is little difference between the binding affinities of the 2 steroids for the transcortin system in normal human serum (see also 12), and that the influence of temperature on binding affinity is less pronounced than in rat serum. However, when human serum was obtained after treatment of a normal individual with dexamethasone so that the serum was depleted of endogenous corticosteroids, the binding affinity at 4°C was definitely greater for cortisol than for corticosterone. The exact influence of albumin or other serum proteins on these binding values for human serum, especially at 37°C, remains to be clarified.

If teleological considerations are permitted, the increased corticosteroid-binding activity under conditions of corticosteroid depletion in the rat may suggest an attempt by the organism to preserve these vital hormones from metabolism and excretion. The higher binding affinity of rat serum for corticosterone in relation to cortisol would seem to indicate a possible specificity for the corticosteroid typical of the particular species. The bind-

ing characteristics of human and rat serum for "physiological" quantities of cortisol and corticosterone demonstrate distinct differences in the "transcortin" systems in the blood of the two species.

Summary. Equilibrium dialysis experiments with pooled sera of rats demonstrate that the binding activity of the specific corticosteroid-binding protein ("transcortin") is higher in adrenalectomized and hypophysectomized animals than in normal controls. Rat serum at both 4°C and 37°C has a higher binding affinity for corticosterone than for cortisol, whereas in human serum at 4°C, cortisol appears to be bound more firmly than corticosterone. The "transcortin" systems of man and rat, therefore, show distinct differences.

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Chemotactic Activity and Phagocytosis of Eosinophils.* (27381)

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Neutrophils and mononuclear phagocytes have been shown to respond chemotactically to bacteria or bacterial products, but move in a random manner in the presence of injured tissue or tissue breakdown products(1). In contrast, the large accumulation of eosinophils following reexposure to antigen(2,3,4) suggests a chemotactic reaction in sensitized tissues(5,6). The present study utilizes *in vitro* preparations of inflammatory exudates to determine the factors which result in this eosinophil response.

Materials and methods. Adult C57 Bl/6 mice were immunized by 6 weekly subcutaneous injections of fluid tetanus toxoid (Lederle). Approximately 30 days after the last immunizing injection, the mice were injected intraperitoneally with 0.2 ml of 5 Lf tetanus toxoid in isotonic saline. At daily intervals, during a 10-day period, animals were killed by cervical dislocation, and autopsies performed. WBC pipets were used to withdraw from the peritoneal cavity sufficient fluid to permit total and differential cell counts, as well as preparations for living cells. The living preparations consisted of a drop of peritoneal exudate flattened between a cover glass and microscope slide and sealed with a mixture of paraffin and beeswax(7). The cells were maintained at 37°C and observed under phase microscopy for as long as 3 hours. In a few instances, the exudate was incubated for 12 or 24 hours and then examined. Zeiss lenses were used on a Unitron inverted microscope with Kohler type illumination.

Results. Observations and photographic recordings were made of more than 200

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