Serum Corticosteroid Binding Following Thermal Injury[1] (36257)

R. F. MORTENSEN, A. A. JOHNSON, AND KARL EURENIUS
(Introduced by G. H. Mudge)

United States Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

A high plasma concentration of 17-hydroxycorticosteroids is associated with burns in both laboratory animals and man (1, 2). Since only the unbound or albumin-bound corticosteroid is thought to be biologically active (3, 4), an increase in the level of corticosteroid-binding globulin (CBG), an alpha-globulin, might protect thermally injured laboratory animals or man from the effects of a high plasma corticosteroid concentration. By measuring the binding activity of serum proteins, an assessment of the relative amounts of bound and unbound corticosteroids can be made. A simple adsorption method was employed to measure both the total corticosteroid-binding capacity (CBC) and the fraction of the total binding capacity due to (CBG). Albumin-binding capacity was not measured since it is not saturable under these experimental conditions (3, 5). Changes in the binding capacity are directly related to the availability of the two serum proteins for binding: albumin, which has a high capacity, but low affinity for corticosteroids; or CBG, which has a low capacity, but high affinity with an association constant 10^5 greater than that of albumin (3–6).

Materials and Methods. Cleanly shaven, male, Sprague-Dawley rats (200–300 g) received a standardized third-degree scald burn over 30% of their body surface area following sodium barbital anesthesia (1 mg/25 g ip) (7) and were immediately resuscitated with 10 ml of physiological saline ip. Human sera from 33 adult human male burn patients (10–70% total body surface area burned) were collected during the early morning by venipuncture. Five patients were serially monitored. Rat serum was collected by cardiac puncture following mild ether anesthesia. All sera were stored frozen.

Cortisol or corticosterone levels were determined by modifications of a fluorometric technique (8) using daily standardization curves. Serum albumin and total protein determinations were performed on the autoanalyzer (9) using crystallized bovine serum albumin (Sigma Chemical Co.) as a standard. ACTH was obtained from Armour Pharmaceuticals.

Corticosteroid-binding capacities were determined by slight modifications of the method of Trapp and West (10) based on the separation of protein-bound steroid from unbound steroid by selective adsorption with dextran-coated florisil, DCF, prepared as described by Trapp and West (10). To 0.5 ml of freshly prepared 0.1 *M* phosphate KCl buffer (pH 7.4) containing tritium-labeled cortisol or corticosterone, were added either 0.25 μg of unlabeled cortisol or corticosterone for rat studies, or 0.05 μg of unlabeled cortisol for human studies, and 100 μl of test serum. Labeled cortisol-1,2-^{3}H (sp act, 44 Ci/mmole) and corticosterone-1,2-^{3}H (sp act, 50 Ci/mmole) were purchased from New England Nuclear Corp. at greater than 97% purity. Unlabeled cortisol and corticosterone (Sigma Chemical Co.) were dissolved in absolute ethanol and diluted with the buffer solution to less than 1% ethanol. Each serum sample was examined in triplicate tubes. The mixture was allowed to equilibrate

[1] In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

for 18 hr at 4°; 155 mg of DCF adsorbent was then added, and the tubes were shaken for 60 min at 4°. This amount of DCF removed 96% of the cortisol from serum free solutions and did not alter the protein concentration of the supernatant when sera was present. After a 2 min centrifugation at 1000 *g*, 100 μl of supernatant was immediately withdrawn and assessed for radioactivity. The radioactive samples (100 μl) were added to a 10 ml scintillation cocktail containing solubilizer (Beckman BBS-3) and fluor (Beckman Fluoralloy) in toluene. Samples were counted on a scintillation counter to a 1% statistical error at 40% efficiency using internal standardization.

CBC was calculated from the formula:

$$\text{CBC } (\mu g/100 \text{ ml of sera}) = \text{Fs} \frac{\text{cpm } 100\ \mu l \text{ of supernate} \times 6}{\text{cpm total mixture}}$$

Where Fs = combined steroid concentrations of the serum and the buffered steroid solutions (μg/100 ml). The heat-denaturable corticosteroid-binding globulin (CBG) capacity was calculated (6, 10) from the difference in binding capacity between unheated serum and heated serum (60° for 25 min).

A twofold increase in the rat serum corticosterone level altered the CBC by only 3% because of the high corticosteroid concentration in the binding assay buffer (50 μg/100 ml). A similar increase in the human serum cortisol level would result in a 17% error in the CBC value since only 10 μg of cortisol/100 ml of buffer was used. Proportionally less cortisol was used in the human serum assay than with rat serum because of the lower magnitude of total binding.

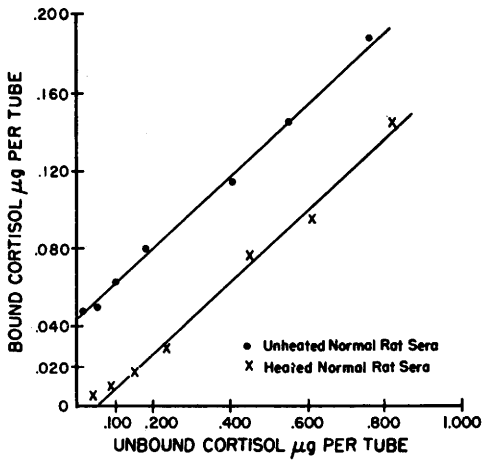

FIG. 1. Cortisol binding by rat serum: Serum (100 μl) and adsorbent (155 mg, DCF) were kept constant. Serum was heated to 60° for 25 min to denature CBG. Total volume of the mixture was 0.6 ml.

Results. Figure 1 shows the total binding (CBC) of cortisol by a constant amount (100 μl) of either unheated or heated rat serum as a function of cortisol concentration. The heated serum demonstrates binding by albumin or other weak cortisol-binding serum proteins which is unaffected by temperatures that denature CBG (11, 12). Thus, the CBG fraction of the total binding is the constant difference between these lines. These nearly parallel lines clearly demonstrate that the CBG capacity does not vary over this range of cortisol concentration and that the high affinity CBG binding (unheated serum) is noticeable at low cortisol concentrations.

These results for rat serum are similar to those previously reported for human serum (10). The CBG binding as measured by the *y*-intercept (0.043 μg) is greater than that seen in human serum (0.024 μg) and agrees well with a reported CBG value of 0.045 μg for rat serum (5). The difference between the lines at 0.250 μg cortisol (the cortisol concentration used in the assay) is 0.043 μg also. The slope of the line for unheated serum ($m = 0.186$) is less than the slope reported for human serum ($m = 0.233$) (10) and reflects the different association constants of rat ($K_a = 0.3 \times 10^9$) and human ($K_a = 0.6 \times 10^9$) serum at 4° (13). The same experiment with corticosterone, the native rat corticosteroid, replacing cortisol resulted in a slightly higher CBG capacity (48.5 μg/100 ml), in agreement with a reported value of 52.0 μg/100 ml (5).

Increases in both CBC and CBG capacity were observed following the standarized scald-burn injury to rats. A maximum increase in the total binding capacity (CBC) was seen 4 days postburn (Fig. 2). This rise in total binding capacity is accounted for by

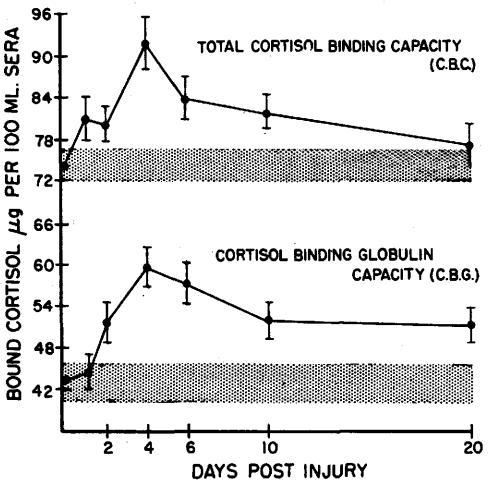

FIG. 2. Binding capacities of the corticosteroid-binding fractions of rat serum following thermal injury: (shaded area) control values ± SEM; (vertical bars) means ± SEM.

the twofold increase in the CBG capacity. Control levels of binding were determined on rats receiving sodium barbital anesthesia without subsequent injury. The non-CBG-binding activity was slightly increased the first day following injury and slightly decreased 20 days postburn.

Corticosterone levels were determined on all rat serum samples, since a small correction was necessary for the endogenous steroid level of the serum used in the binding assay. An immediate rise in the serum corticosterone level was observed in the burned rats (55.8 μg/100 ml) (Fig. 3). Following a drop in this initial level, the serum corticosterone levels peaked about the six day postburn; and the level remained elevated even at 20 days postburn. No CBG rise was observed in unburned animals despite the high corticosterone level (38 μg/100 ml) seen 1 hr after ip sodium barbital. The rise in CBG seen in burned animals coincided with the rise in serum corticosterone levels.

The chronic administration of adrenocorticotropin (ACTH, 8 USP units im daily for 5 days) failed to increase either CBC (80.0 μg/100 ml) or the CBG fraction (41.0 μg/100 ml), although the corticosterone level rose to 20 μg/100 ml serum.

Total protein and albumin concentrations (globulin by difference) were measured on all serum samples to determine any effects on the binding capacity by hemoconcentration and plasma protein evascularization as reported following burn injury (14). All serum protein concentrations dropped significantly and immediately following injury (Table I). Globulin concentrations returned to normal on the second postburn day and subsequently rose to elevated levels from days 2 to 20 postburn. To insure that the increase in binding capacity by CBG, and alpha$_1$ globulin (4, 5), was not just a reflection of the increased synthesis of all globulins or the initial postburn concentration of serum proteins, a ratio of CBG capacity to globulin concentration was calculated. With this correction, a twofold increase in CBG activity was still seen by postburn day 4. The ratio of non-CBG binding/g of albumin/100 ml of sera remains relatively constant and correlates with serum albumin levels.

Human sera from burn patients was also assayed for both CBC and CBG activity. Both CBC and CBG capacity were slightly lower during the first 20 days following burn

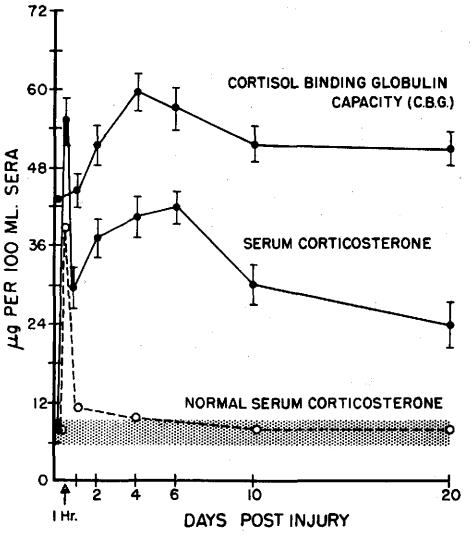

FIG. 3. Serum corticosterone levels and CGB capacity in the rat following thermal injury: (- - -) the serum corticosterone levels in anesthetized, unburned rats; (shaded area) normal serum corticosterone levels ± SEM; (vertical bars) means ± SEM.

TABLE I. Serum Protein Levels and Specific CBG Capacity Following Thermal Injury in the Rat.[a]

	Control (16)	Postinjury 1 hr (9)	1 days: 1 (9)	2 (9)	4 (14)	10 (11)	20 (9)
Total protein (g/100 ml of sera)	5.66 ± 0.12	4.62 ± 0.13	4.63 ± 0.11	4.18 ± 0.06	5.85 ± 0.20	6.05 ± 0.09	6.07 ± 0.13
Globulin (g/100 ml of sera)	3.20 ± 0.08	2.95 ± 0.17	2.90 ± 0.11	3.38 ± 0.07	3.78 ± 0.17	3.80 ± 0.04	4.32 ± 0.12
CBG capacity/g of globulin	4.1		4.8	6.4	7.8	5.8	4.9

[a] Data are mean ± SEM for albumin, globulin, and total protein. Numbers in parentheses are number of rats tested each day postburn.

injury (Table II). During the remaining course of the patient's recovery, these binding capacities returned to the levels of the nonburned subjects with an increasing percentage of the CBC due to the CBG activity (Table II). Individual patients were assayed at various times following their burn injury with no significant alterations in binding capacities noted; for example, in one patient, at 10, 14, 28, and 36 days following a 44% total body surface burn, CBC values of 32.0, 32.8, 38.8, and 30.7 (μg of cortisol bound/100 ml of sera) and CBG levels of 9.3, 13.0, 16.0, and 11.9 (μg of cortisol bound/100 ml of sera) were noted. Increased extent of burn [percentage of the total body surface area burned (% TBS)] was associated with both slightly lower CBC and CBG activities and an increasing fraction of the total binding due to CBG binding (Table II).

Serum cortisol levels in these patients were measured and as expected were significantly elevated immediately following injury (1, 15), and only returned to normal levels 40 plus days postburn (Table II). The extent of the injury was correlated with increased levels of serum cortisol (1, 15).

Discussion. Cortisol-binding capacities for serum proteins as measured by adsorption methods have previously been correlated with equilibrium dialysis and gel filtration techniques (10–12) so that comparisons of values obtained by the latter methods might be made. Normal male rat CBG values of 40.0, 45.0, and 50 μg/100 ml of serum have been reported (5, 16). Estrogen treatment (16, 17), adrenalectomy (15, 18), and gonadectomy (16, 17) have been shown to increase CBG in male rats from 50 μg/100 ml to 100, 80, and 130 μg/100 ml, respectively. We have now shown an increase in CBG activity following a third-degree burn injury (Fig. 2) to the degree seen in hypophysectomized male rats (65 μg/100 ml) (5, 15). The time sequence for this CBG response (maximum level at 4 days, Fig. 2) contrasted to a time period of 10 days for a maximum response in rats following adrenalectomy and castration (male rats only) (15).

In our experiments, the rise in serum corti-

TABLE II. Serum Cortisol Binding and Serum Cortisol Concentrations in Human Burn Patients.[a]

	N	Cortisol (μg/100 ml of sera)	(μg Cortisol bound/100 ml of sera) CBC	CBG
Control	(4)	17.3 ± 1.1	36.3 ± 1.0	19.0 ± 0.8
Days				
5–20	(14)	38.9 ± 2.7	28.4 ± 2.2	11.9 ± 1.5
21–30	(9)	42.0 ± 4.4	35.6 ± 1.7	14.4 ± 1.9
31–40	(11)	29.5 ± 1.8	30.5 ± 1.1	15.2 ± 1.0
41–90	(13)	21.5 ± 2.4	28.6 ± 2.0	17.8 ± 2.0
% TBS				
6–30	(15)	29.7 ± 2.4	35.7 ± 2.0	17.8 ± 2.1
31–50	(19)	32.5 ± 3.0	32.8 ± 1.3	14.2 ± 1.2
51–70	(13)	35.6 ± 3.9	29.6 ± 1.8	11.4 ± 1.1
All burn samples	(47)	32.5 ± 1.8	32.8 ± 1.1	14.6 ± 1.0

[a] All values ± SEM; N = Number of serum samples tested in each group. % TBS = percentage of total body surface area burned.

costerone concentration paralleled the CBG rise (Fig. 3). This relationship of a serum steroid level and CBG binding is similar to that seen in pregnant (5, 17) and estrogen-treated rats (3, 5). The chronic stress of cold exposure has been shown to be related to increased protein-bound corticosteroids in rats (19). These investigators also found most of the corticosteroid to be unbound following the acute, transitory stress of anesthesia, similar to our observations of anesthetized control animals. The chronic administration of ACTH did not alter CBG which is in agreement with the results of Gala and Westphal (18) but in contrast to the decrease observed by Acs *et al.* (20) following a similar treatment. Adrenalectomized rats with adequate corticosteroid replacement exhibited a lower CBG level (21). There is evidence for increased alpha globulin (CBG is an α_1-globulin) synthesis in the rat following a burn (22).

Gala and Westphal (17) have proposed that TSH is responsible for inducing the synthesis of CBG in the rat. Increased binding activity following any experimental manipulation of the rat may be due to the removal of a steroid that would normally compete for binding to CBG. This is a possibility in this experimental system, since a very high metabolic rate is observed following thermal injury.

No significant changes in binding capacities were noted in the sera of burn patients. In contrast to the injured rat, the burn patients' serum cortisol concentration exceeded the total binding capacity (CBC) of that sera as measured under nonphysiologic conditions (Table II, Fig. 3). This "excess" cortisol was seen up to 30 days postinjury (Table II). The human's failure to respond to thermal injury with an increased CBG binding activity like the rat may be related to the differences of response between these species observed after various endocrine gland reactions (18, 20, 23). The presence of large amounts of cortisol in excess of CBC in burn patients' sera implies either unprotected hypercorticalism (3) or weak binding of excess cortisol to other serum glucocorticoid-binding components which have not yet been characterized (3–5).

Summary. An adsorption technique has been adapted to measure both the total corticosteroid-binding capacity (CBC) and corticosteroid-binding globulin (CBG) capacity of rat serum following thermal injury. A maximum increase in the rat serum CBC was seen 4 days following a third-degree burn. This increase in CBC activity was accounted for by a twofold increase in the activity of the CBG fraction. Changes in corticosterone

levels paralleled alterations in the CBG capacity. Increased levels of CBG were still indicated when CBG capacity was corrected for changes in serum globulin levels following a burn. The binding by non-CBG serum elements remained relatively constant postburn.

In contrast to the rat, no significant alteration in CBC or CBG capacity occurred in human burn patient serum. As a result, the level of cortisol in excess of CBC was as much as 5 times normal in the early postburn period and remained elevated up to 40 days after injury. It is concluded that the human, unlike the rat, does not respond with an increase in serum corticosteroid-binding activity following the stress of a severe burn.

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