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Corticosteroid-binding globulin in serum of western sitatunga (*Tragelaphus spekii*)

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Corticosteroid binding globulin (CBG) is a serum glycoprotein that binds with high affinity natural glucocorticoids and progesterone in several mammalian species [1]. It is commonly believed that physiological role of CBG consists in regulation of free cortisol or corticosterone levels in blood and thus their availability to the target cell receptors. Moreover intracellular transport of the hormone [2] and steroid transport from the side of release to blood circulation [3] has been suggested. Extensive studies with human CBG [4] showed that it consists of a single polypeptide chain of molecular mass of about 52000 Da [4], containing about 30% carbohydrate. One molecule of CBG binds one molecule of cortisol or progesterone with high affinity (K_a about 10^9 M^{-1}, at 4°C) [5]. In extension of the comparative studies to wild ruminants we have determined biochemical properties of CBG in blood serum of western sitatunga.

Blood, collected from jugular vein of four adult and one non mature female and one adult male sitatunga, was spun at 1500 × *g* for 15 min. Endogenous cortisol was removed from the serum by mixing with an equal volume of dextran-coated charcoal (3.75% Norit A, 0.375% dextran T70 in 20 mM Tris, pH 7.4, containing: 10 mM ammonium molibdenate, 5 mM mercaptoethanol, and 10% glycerol) [3]. The suspension was incubated at 4°C for 1 h and then centrifuged at 2500 × *g* for 15 min. The CBG content in blood serum was assayed by measuring of binding [^{3}H]cortisol. Aliquots of 400 µl (final dilution of serum 20%) were incubated with 50 µl [^{3}H]cortisol (final concentration, 2.5–40 nM) and either with 50 µl of buffer for determining total cortisol binding or 50 µl of unlabelled cortisol (final concentration, 2.5–40 µM) for measuring nonspecific cortisol binding at 4°C for 18 h. Free [^{3}H]cortisol was absorbed by the addition of 400 µl dextran-coated charcoal per assay tube for 30 min followed by 15 min centrifugation (2500 × *g* at 4°C). Radioactivity of 300 µl aliquots of the supernatants was counted in a Beckman scintillation counter. Scatchard analysis [6] was carried out by using a microcomputer LIGAND-PC program [7] to determine the maximum binding capacity (B_{max}) and the apparent dissociation constant (K_d) of these specific corticosteroid-binding proteins.

Chromatography on Sephadex G-100 column (0.9 cm × 30 cm) was performed at 4°C in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl, at a flow rate of approx. 0.5 ml per minute. For assessment of molecular mass, the same column was calibrated under identical conditions with pure preparations of standard proteins.

It has been shown that blood serum of all animals bound corticosteroids. Scatchard plots (Fig. 1) suggested that there was one type of binding site with high affinity, 0.05–0.32 × 10^9

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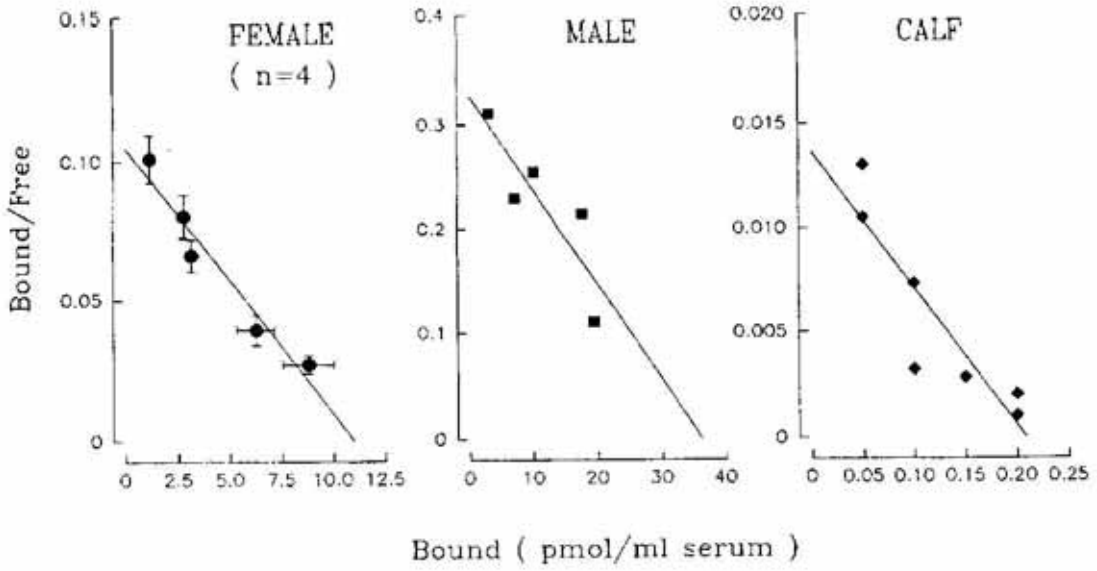

Fig. 1. Scatchard plots of the sitatunga blood serum CBG.

M^{-1}. Comparison of the association constants ($K_a = 1/K_d$; Table 1) of the CBG of sitatunga with those for other animals showed close similarity: K_a for human and animal CBG ranged from 0.007 to 1.0×10^9 M^{-1} [1, 3, 5, 8]. However, the data calculated from Scatchard plots (Table 1) demonstrated sex and age dependent differences in binding capacity (B_{max}) and equilibrium constants (K_d). Binding capacity of sitatunga CBG (0.2–36.3 pmol/ml) was significantly lower than in man and rodents (man 690 pmol/ml [1]; rat 800 pmol/ml [3]; mouse 720–4550 pmol/ml [9]). However, differences demonstrated in the present work between calf and adult sitatunga, and between male and female specimens were also noted by Facid *et al.* [9] in mice.

Relative molecular mass of CBG from either females or male sitatunga blood serum determined by gel filtration was 40000 ± 2000 (mean ± S.D., n = 4). Similar values of relative molecular mass were reported for CBC's from a variety of species (guinea pig 50000 [8]; monkey 54000 and 57000 [10]; man 52000 [4]), however larger proteins were also observed in man (M_r 110000 and 230000 [5]) and monkey (M_r 110000 [10]).

The results of comparative studies demonstrated that CBG of different animal species including wild ruminants showed the same molecular mass and corticosteroid binding capacity. The only difference consists in the number of CBG molecules in blood serum.

Table 1
Scatchard analysis of sitatunga blood serum corticosteroid binding globulin
B_{max} - binding capacity, K_d - dissociation constant.

	B_{max} (pmol/ml serum)	K_d (nM)
Male	36.3	22.1
Female (n = 4)	11.2 ± 3.0	22.5 ± 4.9
Calf	0.2	3.1

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