Effect of Steroids on Serum Sialic Acid Values in the Canine[1] (35142)

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Researchers have reported that the glycoprotein structure, *per se*, is (1) required for maintenance of biological activity of some compounds, (2) required for some of the organism's protective systems, and (3) associated with stress conditions. The carbohydrate moiety of glycoproteins is made up of different ratios of amine sugars, neutral sugars, and neuraminic acid (sialic acid) derivatives (1, 2). Many compounds such as FSH (3, 4), gonadotropins (5, 6), erythropoietin (7), prothrombin, ceruloplasmin, and gamma globulin (1) contain sialic acid (SA) which is essential for biological activity.

The surface SA of red blood cells forms an integral part of the influenza virus-cell hemagglutination process. Hemagglutination does not occur when SA has been removed from the glycoprotein on the cell (8, 9). Bacteria such as *Mycoplasma pneumoniae* also attach to the sialic acid moiety on either the red blood cells or epithelial cells of the respiratory tract (10).

The α-glycoprotein levels increased in tissues of animals injected with estrogen (11) and in blood of traumatized animals injected with cortisol (12). HeLa cells which were grown in tissue culture with hydrocortisone contained more sialic acid than did the hydrocortisone-free cells (13). Bodgen *et al.* (14) demonstrated that the α-2-glycoprotein fraction could be used as an index for the anti-inflammatory activity of the glucocorticoids.

Cellular characteristics (15), such as mobility in electric field, membrane-bound antigenic properties, and the transport of material in and out of the cell, are all influenced by the presence of intact membrane glycoprotein. Gottschalk (4) reported that serum sialic acid values are elevated in disease conditions. Apparently, sialic acid is essential in many biological processes. Therefore, the elucidation of some factors associated with the control of systemic sialic acid is necessary to understand the involvment of this compound. With the evidence of estrogen controlling the α-2-glycoproteins (11) and the fact that serum sialic acid is greatly elevated in some disease conditions, an experiment was conducted to determine the effect of the steroids, hydrocortisone and dexamethasone, on serum sialic acid values.

Materials and Methods. Design. Twenty-nine healthy, mongrel dogs weighing 8.0–11.0 kg were used. Preinjection jugular blood samples (10 ml) were collected for packed-cell volume and sialic acid analyses. Hydrocortisone acetate[2] or dexamethasone[3] were administered intramuscularly daily during the experiment. The average dosage levels for each of the experiments included in this study are listed in Table I.

Blood samples were collected prior to the initial steroid injections and on the third and fifth day after the initial injection. In Experiments B, E, and G, blood samples were not drawn at designated intervals (see Table I). Because injected dosage was the same in Experiments B, C, and E, the three experiments were combined (BCE). Packed-cell

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[2] Hydrocortone acetate = hydrocortisone acetate 25 mg/cc. Merck, Sharp, and Dohme, Division of Merck and Company, Inc., West Point, Pennsylvania.

[3] Azium = dexamethasone (2 mg/cc). Schering Corporation, Bloomfield, New Jersey. (9-α-fluoro-16-α-methylprednisolone).

TABLE I. Influence of Exogenous Steroids on Canine Sialic Acid.

Experiment	Serum sialic acid (mg/100 ml) Pretreatment	Day 3 post treatment	Day 5 post treatment	Dosage (mg/kg body weight/day)	Injected steroid (im)
A	60.4 ± 2.4 (4)[a]	57.8 ± 8.2 (4)	65.0 ± 8.0 (3)	1.10	Hydrocortisone
BCE	47.8 ±11.3 (12)	33.0 ± 2.2* (4)	71.7 ±12.0***(12)	3.14	Hydrocortisone
D	53.0 ± 5.5 (4)	56.7 ± 3.7 (4)	72.0 ± 6.8** (4)	2.42	Hydrocortisone
F	39.6 ± 6.6 (5)	71.8 ±12.8**(5)	65.0 ± 7.7***(5)	0.086	Dexamethasone
G	61.7 ± 3.9 (4)	77.2 ± 4.4**(4)		0.156	Dexamethasone

[a] Number of animals in each group.

* 0.05, ** 0.01, and *** 0.001 level of significance—value of mean above or below preinjection mean.

volumes and serum sialic acid concentrations were determined on each blood sample. The intramuscular injection of steroids did not significantly alter the packed-cell volumes during this experimental period.

Sialic acid analyses. Serum (0.5 ml) was diluted in glass-stoppered centrifuge tubes with 9.5 ml of trichloracetic acid (TCA): saline mixture (500 ml 10% TCA + 400 ml saline). The serum:TCA solution was hydrolyzed in a 80° bath for 1 hr. After hydrolyses, the samples were centrifuged, and the supernatant fluid was removed and frozen. Sialic acid analyses for Experiments A and B were conducted by the manual method of Warren (16) and for Experiments C, D, E, F, and G by the automated Delmotte (17) modification of the Warren procedure. Standards for sialic acid were prepared from *N*-acetylneuramic acid.[4]

A standard curve was plotted from solutions representing 20, 40, 60, 80, 100, and 120 mg/100 ml concentrations. Serum samples were diluted to assure recording of the unknowns in the linear portions of the curve between 40 and 80 mg/100 ml.

The Student *t* test was used to compare the serum sialic acid values of the third and fifth day with preinjection values.

Results and Discussion. As summarized in Table I, the steroids significantly increased the serum sialic acid concentration, and this agrees with the work of Bodgen and Gray (12, 14) who demonstrated an increase in the α-2-glycoprotein complex by hydrocortisone. However, this work demonstrates that a specific carbohydrate moiety associated with the glycoprotein molecules can be altered by steroid hormones. According to Drill (18), dexamethasone has about 30 times more anti-inflammatory activity than hydrocortisone. Bodgen *et al.* (19) reported that the α-2-glycoprotein determination was valid in evaluation of the anti-inflammatory activity of the glucocorticoids. Therefore, hydrocortisone and dexamethasone should produce identical responses but with proportional dosages. This potency ratio was reflected in the serum sialic acid response to a reduced dosage of dexamethasone; however, the physiological response occurred earlier than with hydrocortisone. From these data, it seems plausible to relate the level of serum sialic acid concentrations to increased circulating blood glucocorticoids. Thus, in stressed conditions, such as disease, the elevated serum sialic acid values are associated with increased steroid production.

Summary. Daily injections of hydrocortisone and dexamethasone significantly increased the serum sialic acid in dogs. Dexamethasone, a steroid with 30 times more glucocorticoid activity than hydrocortisone, produced a significant response at a much lower dosage than hydrocortisone.

[4] *N*-Acetylneuraminic acid. Calbiochem, Los Angeles, California.

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