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Influence of Adrenal Cortex on Synthesis of a_2-AP Globulin of Rat Serum.* (31339)

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The appearance of a new protein in the serum of the albino rat during the acute phase of inflammatory states has been reported by several investigators(1). The protein which we have designated a_2-AP (acute phase) globulin(1) is a carbohydrate-containing euglobulin of high molecular weight(1,2) and is synthesized by the liver(3). Since noxious stimuli which evoke the appearance of the protein also cause enhanced levels of corticosteroids (4,5), it was of interest to determine the influence of the adrenal glands on the formation of the protein. In the present study, the effects of adrenalectomy and of cortisol administration on the synthesis of a_2-AP globulin were investigated.

Materials and methods. Young, adult, male rats of Sprague-Dawley origin, weighing from 360 to 400 g, were housed singly in suspended-type wire cages. They were maintained on Purina laboratory chow and tap water. Adrenalectomized rats were supplied with isotonic saline. Paired-feeding was not deemed necessary, since it has been demonstrated that the response of the acute phase reactants of rat serum to the phlogogenic agent used in the present study is not affected by nutritional status(6).

Bilateral adrenalectomy was performed under ether anesthesia employing a dorsal approach. Two incisions were made and the wounds were closed with 2 stitches and sutures each. Sham-operated rats were subjected to the same surgical procedures with the exception that the adrenals were left intact. All rats were allowed to rest 10 to 14 days following surgery.

Thirteen of the groups listed in Table I were injected by the intramuscular route with cortisol† or with pyrogen-free saline. Daily injections were made for 4 days and the rats were bled on Day 5. An inflammatory response was induced in 11 groups by subcutaneous injection of 0.5 ml of a sterile solution of spirits of turpentine, N.F., in corn oil (v/v) at the midline of the back. The rats were anesthetized with ether prior to challenge. Animals were bled 48 hours later by cardiac puncture under ether anesthesia. For the groups which received hydrocortisone or saline, the turpentine challenge was made on Day 3 of the treatment schedule.

The relative concentration of a_2-AP globulin in each of the samples with respect to a standard reference serum was determined by the single radial immunodiffusion procedure described by Fahey and McKelvey(7). The standard was a pooled serum obtained from 25 male rats which had been injected 48 hours previously with 1.0 ml of the turpentine in corn oil solution described above. The concentration of a_2-AP globulin in this serum was arbitrarily established at 100 units per 100 ml. Preparation of antiserum against the protein for use in the immunodiffusion analysis has been previously reported(1). Hematocrit values were determined in Wintrobe tubes. Analysis of the data was made by standard statistical methods employing the t test(8).

A 3-letter code was used to designate the

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† Cortef® (hydrocortisone), Upjohn Co., Kalamazoo, Mich.

TABLE I. Effects of Adrenalectomy and Cortisol Administration on Synthesis of a_2-AP Globulin.

Group	No. rats	Surgical status	Daily treatment	Turpentine challenge	Final wt (g)*	Hematocrit (%)*	a_2-AP globulin (units/100 ml)†
IOO	19	Intact	None	—	384 ± 3.9	47 ± .5	0
ISaO	8	"	0.25 ml pyrogen-free saline	—	386 ± 3.5	44 ± .8[a]	0
IC_2O	8	"	0.3 mg cortisol/100 g b.w.	—	359 ± 4.4[a]	47 ± .6	2 ± .5[b]
$IC_{20}O$	10	"	3.0 mg cortisol/100 g b.w.	—	332 ± 4.2[a]	49 ± .7	5 ± 1.2[b]
IOT	10	"	None	+	373 ± 5.4	48 ±1.0	91 ± 8.9
IC_2T	9	"	0.3 mg cortisol/100 g b.w.	+	359 ± 9.4[a]	46 ± .4	50 ±10.4[b]
$IC_{20}T$	10	"	3.0 mg cortisol/100 g b.w.	+	334 ± 4.5[a]	53 ±1.0[a]	11 ± 3.9[b]
AOO	8	Bilateral adrenalectomy	None	—	369 ± 4.0[a]	42 ± .7[a]	0
ASaT	10	*Idem*	0.25 ml pyrogen-free saline	+	389 ±10.1	47 ±1.2	8 ± 3.8[b]
AC_1T	8	"	0.15 mg cortisol/100 g b.w.	+	388 ± 8.0	51 ±1.5[a]	39 ± 8.0[b]
AC_2T	10	"	0.3 mg cortisol/100 g b.w.	+	355 ± 9.7[a]	49 ±1.9	105 ±10.8
AC_4T	8	"	0.6 mg cortisol/100 g b.w.	+	347 ±11.3[a]	45 ±2.6	53 ±10.9[b]
$AC_{20}T$	12	"	3.0 mg cortisol/100 g b.w.	+	344 ± 8.5[a]	48 ±1.5	17 ± 5.7[b]
SSaT	14	Sham-operated	0.25 ml pyrogen-free saline	+	393 ± 8.2	50 ±1.1[a]	56 ±14.2[b]
SC_2T	10	*Idem*	0.3 mg cortisol/100 g b.w.	+	367 ±11.4[a]	53 ±1.4[a]	50 ± 9.2[b]
$SC_{20}T$	12	"	3.0 mg cortisol/100 g b.w.	+	338 ±11.8[a]	46 ±1.5	15 ± 4.6[b]

Findings include standard error of mean.

* Significant differences from normal values are indicated; [a] $P = <.01$.

† Significant differences from Group IOT are indicated; [b] $P = <.01$.

16 groups; the 3 letters indicate surgical, treatment and challenge status, respectively.

Results and discussion. Results of the experiment are summarized in Table I. The higher dosages of cortisol (C_2, C_4, C_{20}) caused an abrupt decline in weight, irrespective of the surgical status of the group. A loss of weight also occurred in all groups challenged with the phlogogenic agent. Hemoconcentration as estimated by hematocrit values was observed in some of the groups treated with cortisol. Hemodilution occurred in the adrenalectomized control group (AOO) and the intact group injected with saline (ISaO). The humoral response of the groups to the experimental procedures was of such nature that interpretation of the results was not obscured.

Since a_2-AP globulin is not present in normal rat serum results for all groups were compared with Group IOT. The protein could not be detected in the intact group administered saline (ISaO). Conversely the daily injection of the same volume of cortisol (IC_2O, $IC_{20}O$) stimulated the formation of a small amount of the globulin. It is believed that the particulate nature of the hormone preparation caused enough additional tissue injury to exceed the threshold required for synthesis (6). Turpentine challenge of intact rats (IOT) evoked the formation of a high level of circulating a_2-AP globulin. Treatment of intact rats with cortisol caused a significant suppression of the response to challenge which was especially pronounced in the group given the pharmacologic dosage ($IC_{20}T$).

The protein could not be detected in the serum of adrenalectomized rats 10 days after surgery (AOO). The a_2-AP globulin response to challenge was significantly impaired in the adrenalectomized group (ASaT). Replacement therapy with the lowest dosage of cortisol partially restored the response (AC_1T). Doubling the dosage (AC_2T) produced a response equal to that of the intact control group (IOT). Higher levels of cortisol were inhibitory (AC_4T, $AC_{20}T$).

The concentration of a_2-AP globulin in sham-operated animals treated with saline (SSaT) was significantly less than that of the intact control group (IOT). The cause of the reduced response in a group with functional adrenals is not readily apparent. This experiment was repeated with similar results which accounts for the larger number of rats in the group. Suppression of the response was observed in sham-operated groups administered cortisol (SC_2T, $SC_{20}T$). The difference between groups SSaT and SC_2T was not significant.

The administration of cortisol to intact, sham-operated, and adrenalectomized rats

may have multiple effects on the response of a serum protein fraction to tissue injury; (a) Regulatory, (b) Permissive, (c) Homeostatic (anti-inflammatory), (d) Catabolic, (e) Inhibitory (anti-anabolic), (f) Stimulatory (anabolic), (g) No effect. It is apparent that the possible modes of action are not mutually exclusive and that more than one effect may be operative in a single animal. In interpreting the results, the parameters of surgical status, hormonal dosage, and degree of tissue injury must be evaluated.

It has been postulated that substances released from injured or necrotic cells stimulate the synthesis of a_2-AP globulin(1). A comparison of the results for the intact, treated groups ISaO, IC_2O, $IC_{20}O$ confirms the observation(6) that a certain threshold of tissue injury must be attained before an acute phase response will occur. Whether the dramatic effects of adrenalectomy (ASaT) and replacement therapy (AC_1T, AC_2T) on a_2-AP globulin concentrations represent regulation or the permissive effect of Ingle (9) may be partly a semantic problem. A certain degree of regulation is implicit in the permissive concept. We prefer to interpret the results as a classic example of the permissive theory. This conclusion is at variance with that of Bogden *et al*(10) whose preliminary report prompted the initiation of the current study.

The highest dosage of cortisol significantly reduced the concentration of acute phase globulin, irrespective of the surgical status of the group ($IC_{20}T$, $AC_{20}T$, $SC_{20}T$). Homeostatic, inhibitory or catabolic action could explain the observed results. An anomalous finding was the lack of statistical difference in the levels of the protein between turpentine-challenged ($IC_{20}T$) and unchallenged ($IC_{20}O$) groups administered the pharmacologic dosage of the hormone.

The interaction of surgical status and hormonal level on the a_2-AP globulin response to challenge was best delineated with the C_2 dosage. This level of cortisol was adequate replacement therapy for the adrenalectomized group (AC_2T) but inhibitory for the intact (IC_2T) and sham-operated groups (SC_2T). Twice this dosage (AC_4T) yielded results similar to those of the IC_2T and SC_2T groups.

Several recent reviews have been concerned with the possible mode of action of glucocorticoids without arriving at any consensus (4,11,12). Regardless of the biologic mechanism involved, the data presented above indicate that the a_2-AP globulin response to tissue injury is highly sensitive to adrenal cortical influence.

Summary. The effects of cortisol administration on the a_2-AP globulin response to tissue injury were studied in adrenalectomized, sham-operated, and intact rats. Adrenalectomy significantly reduced the response to challenge. The response could be partially or completely restored, or suppressed depending upon the dosage of the hormone. It was concluded that the adrenal cortex has a permissive role in the synthesis of a_2-AP globulin of rat serum.

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