## Suppression of Erythropoiesis by Administration of Deoxycorticosterone and Corticosterone

It has been reported that the steroids, testosterone[1] and estradiol[2], as well as some non-steroid hormones[3,4], are capable of influencing red blood cell production and release (erythropoiesis). Suggestions have been made that testosterone influences the production of erythropoietin, the hormone which controls erythropoiesis[5]. The report presented here deals with the erythropoietic effects of the mineralocorticoid, deoxycorticosterone (4-Pregnen-21-ol-3, 20-Dione) and the glucocorticoid, corticosterone (4-Pregnen-11$\beta$, 21-Diol-3, 20-Dione). These 2 adrenal cortical steroids exert a pronounced influence on a number of processes which may primarily or secondarily affect oxygen requirements and thus, signal for an elevated titer of erythropoietin.

*Materials and methods.* Female mice of the ICR strain were used throughout this study; weights ranged from 18 to 30 g. On Day–1 mice received 10.0 mg of corticosterone or deoxycorticosterone in 0.1 cm³ peanut oil. These s.c. injections were repeated the following day (Day 0) for a total dosage of 20 mg, referred to as 10 mg × 2. Control mice were injected with peanut oil (0.1 cm³) on each of the 2 days. At daily intervals post-steroid treatment, approximately 0.5 µc of radioactive iron citrate ($^{59}$Fe), specific activity greater than 10 mc/mg, was injected. The injection volume was 0.1 cm³. 24 h after $^{59}$Fe, heart blood was collected using heparin as the anticoagulant; blood cells were rinsed with chilled 0.9% saline. Percentages of $^{59}$Fe incorporated into the hemoglobin of harvested peripheral red blood cells was calculated relative to the amount of $^{59}$Fe activity injected. The percentages of reticulocytes and hematocrit were determined from peripheral samples by standard methods.

*Results and discussion.* With reference to times between the second steroid dosage and $^{59}$Fe injection, deoxycorticosterone-treated mice show a gradual decrease in $^{59}$Fe incorporation percentages appearing to begin about 7 days following the second mineralocorticoid injection. Maximum reduction is manifest after approximately 8 days. Following the depression phase, $^{59}$Fe incorporation gradually increases, re-establishing comparable control $^{59}$Fe uptake values at about 11 days (Figure 1).

Corticosterone induces an abrupt and early depression of $^{59}$Fe incorporation (open circles of Figure 2). Recovery from suppression induced by corticosterone appears to begin after 2 days and is complete or nearly complete 4 days post-corticosterone 10 mg × 2.

The percentage of reticulocytes of deoxycorticosterone- and corticosterone-treated mice is shown in the Table; administered dose was 10 mg × 2 for both experimental groups. On harvest days 9, 10, and 11, the percentages of reticulocytes are significantly different between control and deoxycorticosterone-treated mice; reduction in the number of peripheral reticulocytes appears to follow the depression of $^{59}$Fe incorporation. For corticosterone-treated mice, however, the only significant differences between experimental and control percentage reticulocytes is on the 3rd day post-corticosterone.

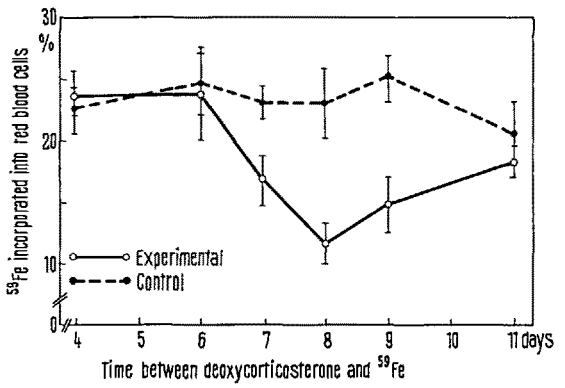


Fig. 1. Effect of deoxycorticosterone on erythropoiesis of female mice. Days refer to times of $^{59}$Fe injection. Vertical lines represent standard errors of the means.

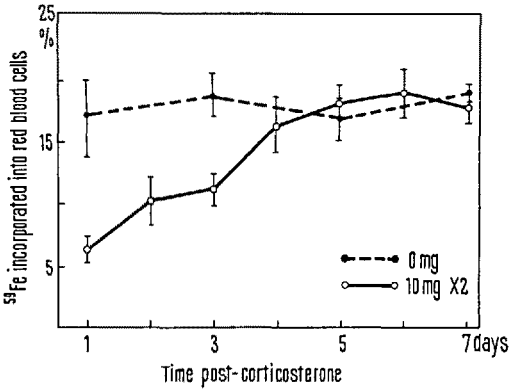


Fig. 2. Erythropoietic response of corticosterone-treated and control (0.0 mg) female mice. Radioiron was administered on the days indicated. Vertical lines from the mean incorporation values represent standard errors.

Percentage reticulocytes of female mice treated with deoxycorticosterone and corticosterone

| Group and time[a] | | Percentage reticulocytes |
|---|---|---|
| Corticosterone[b] | 2 | 2.8 ± 0.6[c] (4) |
| | 3 | 2.2 ± 0.2 (4) |
| | 4 | 3.0 ± 0.2 (5) |
| Deoxycorticosterone[b] | 8 | 2.4 ± 0.2 (4) |
| | 9 | 1.7 ± 0.2 (6) |
| | 10 | 2.8 ± 0.3 (3) |
| Control | 2 | 3.4 ± 0.3 (4) |
| | 3 | 4.0 ± 0.7 (2) |
| | 4 | 4.1 ± 0.2 (4) |
| | 8 | 3.8 ± 0.1 (5) |
| | 9 | 3.5 ± 0.1 (4) |
| | 10 | 3.2 ± 0.1 (6) |

[a] Interval (days) between the second steroid injection (Day 0) and harvest of cardiac blood for peripheral blood smears. [b] Total dose administered: 10 mg × 2. [c] Standard error of the mean. Parenthetical numbers refer to number/group.

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No significant differences in hematocrit percentages were detected among corticosterone, deoxycorticosterone, and control groups. Such a lack of significant differences indicates that in this system the suppression by the steroids tested is not due to a hemolytic effect and is not associated with peripheral blood hemodilution following in the wake of sodium retention.

The time course for the erythropoietic depressor effect of deoxycorticosterone approximates that for the maximum acceleratory effect on erythropoiesis by testosterone[5]. In contrast, the abrupt corticosterone-induced depression of $^{59}$Fe incorporation is seen much earlier. If days are required for an alteration of peripheral red blood cell mass associated with changes in the production of erythropoietin, then it is conceivable that corticosterone affects the bone marrow directly, rather than the kidney or the liver's production of erythropoietic precursor substance. It is possible that erythroid cellular progression may be blocked at some stage(s), the total process may be 'turned-off', or the overall rate of progression may be reduced. The former possibility might not be the most favorable postulate, since it could be argued that if a cellular stage is blocked then there would be a damming-up of cells which are progenitors of the cells of the blocked stage; thus, when the depressor effect is alleviated one might observe an erythropoietic overshoot, i.e., a greater $^{59}$Fe incorporation percentage in relation to controls. Erythropoietic recovery from the effect of corticosterone is relatively gradual, and no overshoot is detected[6].

*Résumé.* Les stéroïdes surrénaux, déoxycorticostérone et corticostérone font diminuer l'érythropoïèse chez la souris femelle. La suppression produite par le corticostérone a lieu plus tôt et est plus grande que celle qui résulte du traitement au déoxycorticostérone.

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## Enhancing Effect of *Bordetella pertussis* and Propranolol on Experimental Immune Hemolytic Anemia

Pertussis-vaccinated mice and rats display a heightened susceptibility to a wide variety of pharmacological, immunological, and physical stresses and stressor agents[1,2]. The $\beta$-adrenergic blocking agent, propranolol, shares with *Bordetella pertussis* the capacity to enhance sensitivity to several of these stressors[3–5], and it has been postulated that the sensitization effect of *B. pertussis* is primarily mediated through blockade of $\beta$-adrenergic receptors of the autonomic nervous system[6,7].

The observation, among others, that pertussis-inoculated mice have decreased blood sugar[7] and increased immunoreactive insulin levels[8] has led to the proposal of an inverse relationship between the glycemic state of a host and its susceptibility to a wide array of stressful stimuli[9,10]. In addition to their hypoglycemic effects, both *B. pertussis* and insulin are capable of inducing hypersensitivity to the pharmacological mediators, histamine and serotonin[7]. Both increase susceptibility to immediate[9], and delayed-type[11,12] hypersensitivity states, as well as to the reaction elicited by both carbohydrate[9,13,14] and non-carbohydrate[3,15] anaphylactoid agents. Additionally, we have found that insulin, like *B. pertussis*, can heighten the susceptibility of mice to bacterial endotoxins[16] as well as to the physical stress of hypoxic decompression[17]. Propranolol, which potentiates the hypoglycemic action of insulin[18,19], also has been shown to share some of these sensitizing properties[3–5].

Recently ADAMKIEWICZ et al.[20] reported that when mice are injected with rabbit antimouse erythrocyte serum, a complex hemolytic anemia syndrome develops. The symptoms include erythropenia, splenomegaly, hypoglycemia and death. These workers have demonstrated that the intensity of this syndrome is inversely proportional to blood sugar levels[20]. Hypoglycemia elicited either by fasting or by the injection of insulin rendered mice more suceptible to the lethal effect of antimouse erythrocyte serum[20]. Conversely, hyperglycemia induced by injection of either glucose or alloxan exerted a protective effect[20].

In view of the lengthening list of mouse-sensitizing properties shared by insulin, *B. pertussis*, and propranolol, we considered it of interest to determine whether the latter two agents could, like insulin, enhance the

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