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Anti-Granuloma and Anti-Edema Properties of Corticosterone in Hyper- and Hypothyroid Rats. (32177)

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Hypothyroidism may reduce anti-inflammatory properties of glucocorticoids(1,2), although this evidence has been challenged (3,4). Thyroid hormones may increase thymus weight(5) and aggravate hypersensitivity reactions to egg white or dextran(6,7), yet hyperthyroidism is accompanied by ACTH-release(5,8), adrenal hypertrophy and elevated plasma corticosteroids(8)—events which would seemingly favor reduced inflammatory potential and thymolysis.

In an attempt to resolve these conflicting views, antigranuloma, anti-edema and thymolytic properties of corticosterone were studied simultaneously in hyper-, hypo- and euthyroid rats. Participation of endogenous hormones was evaluated by comparing intact, thyroidectomized, adrenalectomized and hypophysectomized rats.

Materials and methods. One hundred and sixty-two male rats (CD® strain) weighing 120-130 g initially were purchased from Charles River Breeding Laboratories in 4 basic groups: intact, thyroidectomized ("TX"), adrenalectomized ("ADX", salt maintained) and hypophysectomized ("HYPOX").* Two and one-half weeks after operation, each basic group was divided into 4 subgroups (10-11 rats each) and treated as follows: 1. no treatment (control); 2. corticosterone, 30 mg/kg s.c. in 0.2 ml sesame oil daily; 3. thyroglobulin (Proloid® W1744, Lot EM-3) at level (300 μg % iodine) equivalent to 0.15% USP thyroid in ground chow diet; 4. combination of corticosterone injections and thyroid diet. Treatments were continued for 9 days. Two cotton pellets (35 mg each) were implanted s.c. on day 1. On day 8, each rat received plantar injections of 0.1 ml 0.85% saline (right front paw) and 0.1 ml 1% ovalbumin (left front paw). At 4 hours circumference of control (saline) and edematous (ovalbumin) paws were compared and results expressed as the difference $\pm$ SE. On day 9, rats were killed with ether and cotton pellet granulomas (after oven drying to constant weight), adrenals and thymus were weighed to the nearest 0.1 mg. Results were presented as mean values $\pm$ SE and probability of significance of differences was determined in Fischer's "t" table.

Results. Despite large differences in body weight gain, dry granuloma weight was similar in intact, Tx, Adx or Hypox control rats, and corticosterone inhibited granuloma formation similarly in each of these groups (Table I). Thyroid feeding depressed granuloma weight in Tx rats, but did not influence granuloma formation in other groups nor modify effects of corticosterone on granulomas (Table I). Increases in circumference of edematous paws after egg white injection were similar to control in Adx or Hypox rats but less ($p<0.05$) in Tx rats (Table I). Corticosterone partially prevented circumferential increase in edematous paws of intact,

* Completeness of hypophysectomy was checked at autopsy by exploration of the pituitary area for possible remnants and by observation of testicular size.

Adx or Hypox rats, but did not significantly alter paw size in Tx rats (Table I). Thyroid feeding enhanced paw edema formation ($p<0.01$) in Hypox rats, and prevented full anti-edema activity of corticosterone in intact or Hypox rats (Table I).

Corticosterone suppressed adrenal weight in intact, but not Tx or Hypox rats, and this effect was not altered by thyroid feeding (Table I). Tx depressed adrenal weight whereas thyroid induced adrenal hypertrophy in Tx rats (Table I). Thymus weights were significantly reduced in intact, Tx, Adx or Hypox rats treated with corticosterone (Table I). Although thyroid feeding increased thymus weight in Adx or Tx rats, it did not prevent the thymolytic effects of corticosterone in any basic group (Table I).

TABLE I. Anti-Granuloma, Anti-Edema and Thymolytic Properties of Corticosterone in Intact, Thyroidectomized, Adrenalectomized or Hypophysectomized Rats With or Without Thyroid Diets.

Operation	Treatment: Thyroid, .15% in diet	Treatment: Corticosterone 30 mg/kg s.c.	No. rats	Body wt gain, g	Adrenal wt, mg/100 g	Thymus wt, mg/100 g	Dry granuloma, mg ± SE	Change in circumference of edematous paw, mm ± SE
None	—	—	10	+114	18.3 ± .7	241 ± 16	99 ± 6	6.6 ± .4
"	+	—	10	+ 90*	19.9 ± 1.1	243 ± 17	93 ± 4	7.3 ± .2
"	—	+	10	+ 48	11.6 ± .5	66 ± 12	40 ± 5	3.7 ± .2
"	+	+	10	+ 63	10.9 ± .5	104 ± 17	50 ± 7	5.2 ± .3
Tx	—	—	10	+ 57	14.9 ± .5	242 ± 23	105 ± 4	5.2 ± .5
"	+	—	10	+ 70	27.2 ± .4	342 ± 24	91 ± 4	6.2 ± .7
"	—	+	10	— 6	15.1 ± 1.0	40 ± 7	38 ± 2	4.3 ± .4
"	+	+	10	+ 7	13.5 ± .7	49 ± 8	36 ± 2	4.8 ± .4
Adx	—	—	10	+ 71	—	356 ± 24	109 ± 4	7.1 ± .3
"	+	—	9†	+ 51	—	437 ± 10	101 ± 5	9.2 ± 1.1
"	—	+	10	+ 30	—	73 ± 9	38 ± 1	5.5 ± .4
"	+	+	10	+ 27	—	98 ± 10	40 ± 3	5.5 ± .3
Hypox	—	—	10	+ 2	9.0 ± .4	187 ± 21	108 ± 5	6.0 ± .2
"	+	—	10	+ 11	9.6 ± .3	245 ± 20	103 ± 6	8.7 ± .3
"	—	+	11	— 7	8.9 ± .4	43 ± 4	37 ± 1	4.0 ± .2
"	+	+	11	— 9	7.9 ± .3	42 ± 8	37 ± 1	5.7 ± .3

* Underlined values significantly different from control in each major group ($p<.05$–$p<.01$).
† One rat died in this group.

Discussion. Granuloma weights were similar in all basic control groups, suggesting that metabolic derangements resulting from Tx, Adx or Hypox had not seriously impaired fibroblastic proliferation around cotton pellets. In contrast, paw edema formation was impaired by Tx, in agreement with Bois and Selye(3). Granuloma pouch exudation is likewise impaired by hypothyroidism(3,9), possibly because of decreased skin histamine(9).

Anti-cotton pellet granuloma activity of corticosterone was similar in intact or endocrinectomized groups, and was not influenced by thyroid. Corticosterone-induced thymolysis likewise was not significantly affected by thyroid treatment, despite confirmation of the thymotrophic effects of thyroid(5). In contrast, anti-edema properties of corticosterone depended upon endocrine status of the animals. Thus corticosterone reduced paw edema formation in intact, Adx or Hypox, but not Tx rats, and thyroid treatment opposed anti-edema activity of corticosterone in intact or Hypox rats. Since antigranuloma and thymolytic activity of corticosterone in the *same rats* was *not* influenced by thyroid, the results cannot be explained simply on a basis of thyroid-accelerated catabolism of the steroid. Results in Hypox rats suggest, instead, direct and opposite actions of thyroid and corticosterone on edema formation. Thyroid enhanced, whereas corticosterone opposed, development of edema. Combined treatment had a cancelling effect, resulting in a degree of edema similar to control. Thyoid hormone is known to inhibit histamine catabolism(6), whereas glucocorticoids deplete body histamine stores(10). Also, thyroid reduces the volume of distribution of corticosteroids(8), and might conceivably exclude them from certain tissues.

Thyroid favors hypophyseal release of ACTH(8, for discussion) yet insufficient endogenous glucocorticoid was produced in intact thyroid-treated rats to inhibit granuloma formation. However, a small but significant reduction in granuloma weight was observed in thyroid-treated Tx rats. Concomitant adrenal hypertrophy indicated ACTH release in these rats. When the pituitary-adrenal axis had been destroyed by Hypox or Adx, thyroid did not influence granuloma formation.

Fibroblastic encapsulation and edema formation thus differ basically in their response to hormones: Granuloma formation proceeds relatively independently of endocrine or metabolic status of the animal and is specifically inhibited by pharmacological doses of glucocorticoids; both edema formation and its inhibition by corticoids are modified by thyroid state. We thus agree with Domenjoz *et al*(1) and Stenger *et al*(2) that hypothyroidism may interfere with observation of anti-inflammatory effects of corticoids upon experimental edema. We likewise agree with "conflicting" reports of Bois and Selye(3) and Trnavsky (4) that hypothyroidism does not interfere with the anti-inflammatory effects of corticoids when cotton pellet granulomas are studied. Simultaneous evaluation of both parameters in the same animals is therefore recommended to avoid confusion over what is meant by the term "anti-inflammatory."

Summary. 1. The anti-granuloma, anti-edema and thymolytic properties of corticosterone were studied in intact, thyroidectomized, hypophysectomized or adrenalectomized rats with or without 0.15% thyroid in their diets. 2. Terminal granuloma pellet weights were not much influenced by the operative procedures or by thyroid feeding despite large differences in body weight gain of the various groups. Growth of granulomas was inhibited by corticosterone to a like degree in all groups regardless of endocrine gland ablation or induction of hyperthyroidism. 3. There were no significant differences in the thymolytic effects of corticosterone in the various groups including those in which thyroid feeding alone induced thymic hypertrophy. 4. To the contrary, both the degree of paw edema formed in response to plantar injection of ovalbumin and the prevention of this edema by corticosterone, depended upon other endocrine factors. Thus no significant anti-edema effects of corticosterone were observed in thyroidectomized rats. Thyroid treatment likewise antagonized the anti-edema effects of the steroid in intact or hypophysectomized rats. 5. These data favor the view that cotton pellet granulomas form fairly in-

dependently of the hormonal environment of the animal and are inhibited specifically by glucocorticoids. On the other hand, edema formation and its inhibition by glucocorticoids are definitely influenced by the level of circulating thyroid hormone.

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Infantile Manipulation and Aggregate Living in the Rat: Effect of Electric Shock on Blood Histamine Level.* (32178)

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There is ample evidence that histamine injection results in pituitary-adrenal activation(1,2,3). As an outgrowth of these studies, it has been proposed that endogenous histamine participates in the response to stressful stimulation(4,5). Despite the voluminous literature on histamine now available, it appears that this notion has not been adequately tested(6).

The present study was undertaken to investigate the effects of differential neonatal experience and post-weaning group living on the concentration of blood histamine in the rat following noxious stimulation administered in early adulthood. It employed a reliable chemical method to measure blood histamine levels in animals raised under four different living conditions, then later subjected to electric shock.

Methods. Subjects comprised 20 litters of Sprague-Dawley rats§ culled to 10 pups each at birth. Half of the neonates were manipulated during the period 1 to 7 days of age, inclusive. In this procedure, pups were carefully removed from the nest and placed in separate, cardboard containers for 3 minutes each day. All animals were weaned at 21 days of age and assigned by sex to one of 4 experimental categories. These were: (a) Infantile manipulation followed by post-weaning isolation; (b) No infantile manipulation followed by post-weaning isolation; (c) Infantile manipulation followed by post-weaning group living (4 to 6 rats per cage); (d) No infantile manipulation followed by post-weaning group living (4 to 6 rats per cage).

In the tenth week of life, all animals were subjected to electric shock. Shock parameters were 1.0 ma delivered through a grid to the feet for 30 seconds. At time zero, and at 5-, 10-, and 20-minute intervals following initiation of shock, representative animals were sacrificed by cervical spinal separation. With prior practice, it was possible to obtain a blood sample, containing arterial and venous components, within 10 seconds following removal of the animal from its cage. This permitted the investigators to secure

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§ Pregnant, primiparous, cesarian-derived females were secured from the Charles River Breeding Laboratories, North Wilmington, Mass.