Effect of Age, Sex, Hypophysectomy and Gonadectomy on Plasma Corticosterone Levels and Adrenal Weights Following the Administration of ACTH and Stress[1]

The presence of the pituitary is generally considered to be necessary both for the development of high blood pressure and cardiovascular lesions following the administration of desoxycorticosterone (DOCA). On the other hand, DOCA hypertension may be produced in hypophysectomized rats (HALL et al.[2]). This apparent discrepancy was attributed to an inherent sex difference since the latter workers had used female rats in their studies.

GLENISTER and YATES[3] have reported a sex difference in corticosterone metabolism in the rat. Thus, following stress and ACTH administration, female rats responded with higher plasma corticosterone levels than did male rats (KITAI[4]). The latter author interpreted this finding as a manifestation of greater sensitivity of the female pituitary to stress with respect to ACTH release. This argument, however, may hardly be invoked in hypophysectomized rats which are obviously deficient in their gonadal secretions. Yet, in vitro studies (TROOP and POSSANZA[5]) have shown differences in corticosteroid synthesis even in gonadectomized rats. Furthermore, SELYE et al.[6] and SELYE and COLLIP[7] have shown that the administration of estrogen to hypophysectomized rats did not affect adrenal weight while it did so in intact rats. Similar results were obtained by SUCHOWSKY[8].

The present studies were undertaken to investigate adrenal responsiveness in terms of plasma corticosterone and adrenal weight (a) in hypophysectomized male and female mature and immature rats, and (b) male and female immature gonadectomized rats.

(a) *Response of hypophysectomized male and female mature and immature rats to ACTH.* 100 male and 100 female Sprague Dawley rats were treated in the following manner: Groups 1–5 consisted of immature (50–60 g body weight) rats and were hypophysectomized 48 h prior to injection. Group 1 served as non-injected controls and were sacrificed at 0 h. The animals of groups 2–5 were injected with 2 I.U. ACTH (Acthar-Gel) and sacrificed by decapitation at intervals of $^1/_2$, 1, 3, and 6 h respectively. Blood was collected in heparinized beakers, centrifuged, and plasma collected and frozen. The adrenals were excised and weighed. Plasma fluorescent material was determined by the method of ZENKER and BERNSTEIN[9]. Groups 6–10 consisted of mature rats (200–220 g body weight) and were treated in an analogous manner.

Table I shows that the administration of ACTH to *immature* hypophysectomized female and male rats (groups 1–5) revealed no sex difference in the secretory pattern of corticosterone. This is reflected also in the almost identical weights of the adrenal glands. Administration of ACTH to *mature* rats (groups 6–10), however, resulted in striking changes. $^1/_2$ h after injection, the female rats showed a highly significant elevation of plasma corticosterone when compared with the male animals; this difference became negligible after 1 h, only to be highly significantly greater again at the 3 h interval. At all intervals of sacrifice, the adrenal glands showed the well-known sex difference in weight.

(b) *Response of male and female immature gonadectomized rats to bilateral tibial fracture.* 50 male and 50 female immature Sprague-Dawley rats were gonadectomized shortly after weaning (body weight 50–60 g). When they reached maturity (200–220 g body weight) they were divided into 5 groups each and treated in the following manner: Group 1 (male) and Group 6 (female) served as zero time controls; these animals were killed by decapitation and blood collected as described above (a). Groups 2 (male) and 7 (female) were anesthetized with ether and their tibiae and fibulae fractured bilaterally. 30 min thereafter, they were sacrificed and treated like the animals of groups 1 and 6. Group 3 (males) and group 8

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Table I. Plasma corticosterone (μg/100 ml)

Time of sacrifice (h)	Immature rats Group No.	Males	Females	Mature rats Group No.	Males	Females
0	1	4.5* ± 0.60	3.1 ± 0.45	6	2.4 ± 0.13	2.9 ± 0.61
0.5	2	25.2 ± 1.13	26.3 ± 1.55	7	21.1^a ± 1.86	34.2^b ± 2.32
1	3	35.0 ± 2.10	36.8 ± 4.46	8	34.5 ± 2.01	37.3 ± 2.17
2	4	43.3 ± 2.62	48.7 ± 4.09	9	38.7^c ± 1.80	52.3^d ± 2.44
3	5	37.9 ± 3.46	41.2 ± 3.02	10	36.7 ± 1.81	31.9 ± 3.33
			Adrenal weight (mg)			
0	1	9.9 ± 1.16	12.0 ± 0.40	6	20.1^e ± 0.83	28.6^f ± 1.54
0.5	2	11.1 ± 0.30	13.2 ± 0.57	7	19.6^g ± 0.79	30.4^h ± 0.69
1	3	10.8 ± 0.32	12.1 ± 0.44	8	19.5i ± 0.78	30.8^j ± 1.48
3	4	10.7 ± 0.45	10.9 ± 0.24	9	19.1^k ± 0.41	31.0^l ± 0.81
6	5	10.2 ± 0.45	12.6 ± 0.48	10	20.7^m ± 0.66	29.0^n ± 0.70

* Mean ± S.E.M. [a] vs [b], [c] vs [d], [e] vs [f], [g] vs [h], [i] vs [j], [k] vs [l], [m] vs [n]: $p < 0.001$.

Plasma corticosterone levels and adrenal weights of hypophysectomized immature and mature male and female rats. Groups 1 and 6 were non-injected controls, Groups 2–5 and 7–10 were injected with ACTH and sacrificed at the times indicated.

(females) were treated similarly but sacrificed 1 h after fracture; group 4 (males) and group 9 (females) were sacrificed 3 h, and groups 5 (males) and 10 (females) were sacrificed 6 h after tibial fracture. At sacrifice, the adrenals were also excised and weighed.

Table II shows that the mean baseline level of plasma corticosterone was identical in male and female gonadectomized rats. $^1/_2$ h after tibial fracture no sex difference was observed in the mean plasma corticosterone levels. At 1 h and thereafter, however, mean levels were significantly greater in the female than in the male rats. The lesser response of the male castrated rats is reflected also in the adrenal weights.

The above results confirm the findings of others (COURRIER et al.[10], KITAI[4]) regarding the greater sensitivity of the female adrenal gland to exogenous and perhaps also endogenous ACTH. This difference, however, was found in mature rats only.

The greater size of the female adrenal gland (HATAI[11,12]) has suggested the existence of a difference between male and female adrenal function also. Thus, ovariectomy resulted in adrenal atrophy (ANDERSEN and KENNEDY[13], FREUDENBERGER and HASHIMOTO[14]) and replacement with estrogen in such animals prevented this (CARTER[15]). While studies on plasma corticosterone levels had been reported for intact male and female rats[4], and had shown greater levels in the females, no such studies have been performed in gonadectomized rats. In the present study, there was no evidence of a sex difference in the baseline level of plasma corticosterone of non-stressed, gonadectomized rats. This agrees with the in vitro findings of TROOP and POSSANZA[5] that male and female rat adrenals produced similar quantities of corticoid in incubation for 2 h. Our findings further suggest that gonadectomy does not affect the inherent sex difference in terms of plasma corticosterone in response to stress. This is in accordance with the findings on intact males and females made by KITAI[4] and in castrated males and spayed females in in vitro studies after the addition of ACTH (TROOP and POSSANZA[5]).

Thus, the responsiveness of the adrenal gland is not dependent on gonadal function since if it were, gonadectomy would affect this pattern. Under the circumstances the best interpretation for our findings is KITAI's[4] concept of greater responsiveness of the female adrenal gland to ACTH.

Table II. Plasma corticosterone (μg/100 ml)

Time of sacrifice (h)	Group No.	Male rats	Group No.	Female rats
0	1	9.1[a] ± 1.35	6	8.0 ± 1.50
0.5	2	56.8 ± 2.37	7	62.8 ± 4.30
1	3	60.2[c] ± 6.34	8	86.2[d] ± 2.53
3	4	57.3[e] ± 3.70	9	78.5[f] ± 3.35
6	5	53.5[g] ± 3.35	10	73.5[h] ± 2.43
		Adrenal weight (mg)		
0	1	53.9 ± 1.45	6	56.6 ± 1.88
0.5	2	44.5[i] ± 1.53	7	55.0[j] ± 1.84
1	3	43.5[k] ± 1.03	8	52.8[l] ± 2.92
3	4	47.9[m] ± 1.11	9	55.3[n] ± 1.45
6	5	48.0[o] ± 1.49	10	56.8[p] ± 2.56

[a] Mean ± S.E.M. [c] vs [d], [k] vs [l], [o] vs [p] = $p < 0.01$; [e] vs [f], [g] vs [h] [i] vs [j], [m] vs [n] = $p < 0.001$.

Table II. Plasma corticosterone levels and adrenal weights of male and female rats that were gonadectomized shortly after weaning. Groups 1 and 6 served as unstressed controls. Groups 2–5 and 7–10 were subjected to bilateral tibial and fibular fracture and sacrificed at the times indicated.

Zusammenfassung. Injektion von 2 IE ACTH in hypophysektomierte unreife Ratten beiderlei Geschlechts: kein Geschlechtsunterschied des Corticosteron-Plasma-Spiegels (CPS). Gleiche Behandlung von weiblichen hypophysektomierten, geschlechtsreifen Ratten: $^1/_2$ h nach der Injektion bereits höherer CPS. Kastrierte, weibliche unbelastete Ratten zeigten keinen Unterschied in der Höhe des CPS gegenüber kastrierten, unbelasteten männlichen Ratten. Nebennieren-Belastung solcher weiblicher Ratten durch bilaterale Fraktur der Tibiae und Fibulae: höherer CPS 1, 3 und 6 h nach Fraktur. Die Befunde deuten darauf hin, dass die weibliche Nebenniere eine grössere Empfindlichkeit für ACTH-Wirkung hat.

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DISPUTANDUM

The Influence of the Thymus on Radiogold Clearance

MILLER was the first to point out the primary role of the thymus in immunity[1]. Numerous experiments have since confirmed that thymectomy of new-born mice or thymectomy of adult animals combined with whole-body irradiation or cytostatic agents results in immunological deficiency[2]. On the other hand, the connection between thymus and phagocytosis is obscure. Therefore, it is not known whether phagocytosis is caused by a central ner-

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