Weight losses and urine osmolarity in the hypophysectomized-adrenalectomized albino rat

by

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It is a well known fact that the interruption of the hypothalamus-hypophyseal tract, removal of the hypophysis or removal of the neurohypophysis alone, induces the excretion of a large volume of diluted urine Also, in several cases, animals with diabetes insipidus produce slightly hypertonic urine (7).

In this study, hypophysectomized-adrenalectomized rats were found to be near normal in regard to urine osmolarity.

MATERIAL AND METHODS

ANIMAL PREPARATION: Hypophysectomized female albino rats (Sprague-Dawley strain, Hormone Assay Laboratories, Inc., Chicago, Illinois) were used in this study. On the date of hypophysectomy the rats ranged in weight from 210 to 240 g. One week after operation, bilateral adrenalectomy was performed in all rats (18). Immediately after adrenalectomy, tap drinking water was replaced with a 1% NaCl solution, thus enabling the rats to replenish their salt losses.

WEIGHT LOSS STUDY FOLLOWING HYPOPHYSECTOMY AND ADRENALECTOMY: The rats were housed in pairs in a room maintained at a constant temperature of 27 C. Food consisting of regular Purina Rat Chow and drinking fluid were offered *ad libitum*. At several critical points, the rats were weighed as follows:

1.—On the day of hypophysectomy.

2.—One week after hypophysectomy and immediately prior to adrenalectomy.

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3.—One week after adrenalectomy.
4.—Three weeks after adrenalectomy.
5.—Four weeks after adrenalectomy.
6.—Dead animals, at least 15 days after adrenalectomy.

VARIABILITY IN URINE OSMOLARITY: From 20 animals, 12 were randomly chosen and for five consecutive days their electrolyte excretion was studied. Paired rats were placed in metabolism cages (Microchemical Specialties Co., Berkeley, California) for 3 hours under dim light conditions. In an attempt to minimize circadian variability (5), the experiments were performed at the same hour of each day.

After each collection period, urine volume was measured, and since rate of flow was low, urine samples from two cages were pooled, representing urine from four rats. The osmolarity determinations were made immediately after the urine collection by means of an Advanced Osmometer (Model 31 LS, Advanced Instruments, Inc., Newton Highlands, Massachusetts).

RESULTS

The weight losses of a cumulative record of 15 rats are shown in Table 1. After hypophysectomy and adrenalectomy, body weight steadily decreased from 219.66 g in the preoperative animals, to 180.41 g for weeks after adrenalectomy. There were large differences among the critical points selected (Table 1). Statistical Anova analysis of these data (15) are presented in Table 2, F value is highly significant. A further study on the mean differences among the rat weight losses by means of Keul's test (15), D = 7.94 detected that of the 15 different possible comparisons among means, all were different, except for two comparisons: a) weight one week after hypophysectomy, vs. weight one week after adrenalectomy; and b) weight three weeks after adrenalectomy, vs. weight four weeks after adrenalectomy.

In the above cases there are no significant differences. A brief look at the body weight of dead animals, as compared with the preoperative animals, shows that individuals in this group died when the weight losses were about 25%. Eight weeks after initial operation, 12 hypophysectomized-adrenalectomized rats still lived; thier mean body weight of 182.50 g shows a slight increase, as compared to the mean body weight four weeks after adrenalectomy and during the following month. No animals died during the first week, five died during the following days and four became sick.

VARIABILITY IN URINE FLOW AND ELECTROLYTE EXCRETION: Results from the urine analysis during five consecutive days are illustrated in Table 3. Since the rate of urine flow was highly variable from day to day, ranging from 0.753 cc/hr/4 rats, to 1.687 cc/hr/4 rats, those measurements of electrolyte

TABLE 1

Cumulative weight losses (g) in a group of 15 hypophysectomized-adrenalectomized female albino rats

Rat N°	On day of hypophysectomy	One week after hypophysectomy (prior to adrenalectomy)	One week after adrenalectomy	3 weeks after adrenalectomy	4 weeks after adrenalectomy*	Dead animals**
1	225	205	205	165	(165)	165
2	208	200	190	195	190	155
3	209	200	195	165	(155)	160
4	216	210	205	190	190	155
5	222	215	215	185	175	160
6	215	205	200	185	175	160
7	215	215	205	195	180	155
8	222	205	195	185	190	165
9	231	215	215	190	175	175
10	223	210	205	185	(160)	160
11	228	210	205	195	195	175
12	226	205	190	180	165	165
13	212	195	190	180	170	170
14	224	215	205	180	170	
15	219	200	195	190	185	
$\overline{X}$	219.66	207.00	201.00	184.33	176.00	163.07
s	6.94	6.49	8.28	9.41	12.13	6.93

* Weight in parenthesis indicates death of animal.

** Death occurring two weeks post-adrenalectomy, no effect on cumulative animal study.

excretion, dependent upon the rate of urine flow, are similary affected. Mean values of the osmolarity and urine flow were: 965.33 m0sm/1 and 1.252 cc/hr/4 rats respectively.

TABLE 2

Anova study of the cumulative weight losses (g) in a group of 15 hypophysectomized-adrenalectomized albino rats

Source of variation	degrees of freedom	sum of squares	mean squares	F ratio
Among	5	29.319	5.864	90.94*
Error	79	5.094	64.48	

* Highly significant

Anova tests were performed and the results are shown in table 4. The F ratios proved to be highly significant for the urine flow, but not for the urine osmolarity. The high osmolarity values of the endocrinectomized rats is of interest. A group of ten normal albino rats of similar weight, drinking a 1% NaC1 solution, had a urine osmolarity of 1.119 m0sm/1.

TABLE 3

Urine flow and osmolarity in 12 hypophysectomized-adrenalectomized female albino rats during five consecutive days

Days	urine flow (cc/hr/4 rats)	urine osmolarity (m0sm/1)
1 $\bar{X}$ =	0.797	1,040
2 $\bar{X}$ =	0.753	1,069
3 $\bar{X}$ =	1.687	929
4 $\bar{X}$ =	1.500	917
5 $\bar{X}$ =	1.577	912
$\bar{X}$ =	1.252	965.33
s =	0.44	81.00

DISCUSSION

WEIGHT LOSSES: The survival period for operated rats is influenced by post-operative care. Sucrose in the drinking water has been recommended for hypophysectomized animals and a diet high in NaC1 for the adrenalectomized rat (8). Also, it has been shown that adrenalectomized laboratory rats fed diets containing NaC1 remained in good health and maintained their body weights for a period of 70 to 90 days (13). In this experiment only salt

TABLE 4

Anova study of urine flow and osmolarity in a group of 12 hypophysectomized-adrenalectomized rats

Variables under study	SS days / d.f. days	SS error / d.f. error	MS days / MS error	F and significance
Urine volume (cc/hr)	2.434 / 4	1.014 / 10	0.609 / 0.101	6.03*
Urine osmolarity (m0sm/1)	0.52 / 4	0.38 / 10	0.0130 / 0.0038	3.42 NS

* Highly significant.

was added to the drinking water and the body weights of the operated rats were not maintained at control (pre-operative) weights, as compared with the initial body weight (Table 1).

The removal of the hypophysis also greatly affects survival time and body weight. In the mouse there is no weight gain and the total body weight is reduced (3).

It seems, according to these data, that apparently the combined endocrinectomy is much more severe and that the animals suffer a considerable weight loss. Therefore, in experiments of more than one week duration with endocrinectomized rats, frequent weight checks must be made to adjust the dosage if replacement therapy is to be administered. It is a well established fact (4, 17) that adrenalectomized animals lose weight and also their capacity to tolerate stress. This could account for the death of several animals in this study.

VARIABILITY IN OSMOLARITY: The urine osmolarity of the endocrinectomized rats is very high. The production of slightly hypertonic urine was expected in this particular case, but in order to get o figure of 969 m0sm/1, the presence of ADH (Antidiuretic hormone) in the circulating blood was required, according to the new theories of urine concentration (1, 10).

ADH elaborated by neurosecretory cells of hypothalamus (supraopticus and paraventricular nuclei) is transported by means of the hypothalamus hypophyseal tract and stored in the neurohypophysis, were it is released by a variety of stimuli (9, 14).

The technique of hypophysectomy by means of the parapharingeal approach permitted removal of the whole gland by breaking the pituitary stalk, and yet, the rats were producing hyperosmotic urine. Because these endocrinectomized rats were drinking a slightly hypertonic saline solution, one would expect that the osmolarity of plasma would be elevated above normal, thus stimulating the osmoreceptor in the hypothalamic nuclei, resulting in an increased production and release of vasopressin. Somewhat similar results have been obtained in experimental diabetes insipidus after lesions of the pituitary stalk (11).

In 1951 STUTINSKY (16) reported that following complete removal of the hypophysis in rats, stainable neurosecretory material accumulated at the place of the incision, forming "some sort of neurohypophysis". This was confirmed later (2) by means of histological studies which showed reorganization of the neurohypophysis and the disappearance of the neurosecretory material when the rats were given a hypertonic saline solution. Also, the ADH activity, 30 days after operation, was only slightly lower than that of control (12).

This study supports the above reports in terms of reorganization of the neurohyphysis or release of the ADH directly from hypothalamic nuclei into the blood stream. However, we also have to take into account that the absence of adrenal glands favors the excretion of larger amounts of salt (6) which contribute to increase urine osmolarity.

SUMMARY

Body weight loss in doubly endocrinectomized albino rats was higher than in adrenalectomized or hypophysectomized rats alone. Frequent body weight checks to adjust the dosages on a per weight basis for chronic experiments are suggested.

The urine osmolarity in these rats was hypertonic, 965.33 m0sm/1, and close to that of normal rats: 1.119 m0sm/1. This finding is explained by reorganization of the neurohypophysis following hypophysectomy and ADH release into the blood stream directly from hypothalamic nuclei stimulated by a slightly hypertonic plasma.

RESUMEN

Se estudió la pérdida de peso en ratas albinas hipofisectomizadas y adrenalectomizadas, sufriendo en promedio una pérdida de peso de 40 g a las cuatro semanas de operadas. En estudios crónicos se aconseja pesar con frecuencia a los animales para ajustar las dosis.

La osmolaridad de la orina fue relativamente elevada para ratas en estas condiciones experimentales, siendo su promedio 965.33 m0sm/1 en comparación con la de ratas normales que fue de 1.119 m0sm/1. Se discute la posible reorganización de la neurohipófisis después de la hipofisectomía o la liberación directa de hormona antidiurética desde los núcleos hipotalámicos para explicar la elevada osmolaridad de la orina.

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