Tissue Catecholamine Content of Cold-Acclimated Rats. (26485)

K. E. Moore,* D. N. Calvert,† and T. M. Brody (Introduced by M. H. Seevers)
Department of Pharmacology, University of Michigan Medical School, Ann Arbor

The role of the adrenal medulla in production of nonshivering thermogenesis was first reported by Cottle and Carlson(1). As a result of later work showing that norepinephrine (NE) had a very marked calorigenic action in cold-acclimated rats it was postulated that NE was the mediator of the nonshivering heat production in these rats(2).

Release of catecholamines in response to acute cold stress has been noted by several investigators(3). Catecholamine levels in the adrenal gland of rats during development of cold acclimation have been reported by Des Marais and Dugal(4), but the catecholamine content in other tissues of cold-adapted rats has not been previously studied. The effects of prolonged cold exposure on catecholamine content of rat brain, heart and adrenal are reported here.

Methods. Twenty-four female rats (Sprague-Dawley) were randomly divided into 2 groups. One group, mean weight 185 g (range 170-205 g), was placed in a cold room at 5-7°C. The other group, mean weight 174 g (range 165-195 g) remained in the animal colony at 25-27°C. After a period of 3-5 weeks, at which time a maximum metabolic response is obtained(5), the rats were sacrificed. The liver, kidney, heart and brain weights were recorded and the epinephrine (E) and NE content of the adrenal, heart and brain determined by a modification(6) of the fluorimetric method of Bertler *et al.* (7).

Results. At time of sacrifice both groups of rats appeared healthy. Those exposed to the cold had a mean weight of 215 g (range 205-235), those in the 25-27°C room, a mean weight of 198 g (range 180-220 g). Rates of increase in body weight for the 2 groups over the experimental period were not significantly different. However, whether presented as absolute organ weights or fractional organ weights $\left(\frac{\text{organ weight}}{\text{body weight}} \times 100\right)$, values of liver, kidney and heart of the cold-adapted animals were significantly greater than those of the control group (Table I). Brain weights of both groups were nearly identical. Since it has been shown(8) that brain weight is poorly correlated with body weight, fractional weights of brain are meaningless and therefore were not calculated.

Catecholamine content of the adrenal, brain and heart of normal and cold-adapted rats is recorded in Table II. Brain content of catecholamines is similar in both groups. Catecholamine content of the heart, when reported on a μg/g wet weight basis, is markedly lower in the cold-adapted rats. This difference is apparently due to hypertrophy of the heart muscle during the period of cold exposure since if the catecholamine content per heart is calculated, the μg of NE are: normal, 0.57 $\pm$ .12 and cold, 0.54 $\pm$ .16 and for E are: normal, 0.07 $\pm$ .01 and cold, 0.05 $\pm$ .03. The differences between these values are not significantly different (for NE, $P = .6$; for E, $P = .4$). The content of both E and NE in the adrenal of cold-adapted rats was markedly increased over the values for control rats.

Discussion. In contrast to the reports of others(9), we found no significant decrease in rate of weight gain in cold exposed rats. Our results might be explained by the fact that female rats were used in this study while most previous studies were carried out on male rats. That this sex difference may account for our findings is strengthened by the report of Baker and Sellers(10), who found no obvious weight difference between female rats in cold and warm environments for 45 days. In agreement with Heroux and Gridgeman(8) we found a very obvious hypertrophy of the liver, heart and kidney but

* Present address: Dept. of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, N. H.

† Present address: Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, Kan.

TABLE I. Organ Weights of Normal and Cold-Adapted Rats.

Organ	N	Absolute weights* Normal	Cold	P	Fractional weights† Normal	Cold	P
Brain	11	1.74 ± .09	1.74 ± .04	—	—	—	—
Heart	11	.62 ± .08	.85 ± .06	<.01	.31 ± .03	.40 ± .03	<.01
Liver	10	6.3 ± .8	8.9 ± .6	<.01	3.2 ± .34	4.1 ± .24	<.01
Kidney	10	.79 ± .11	1.10 ± .09	<.01	.40 ± .04	.52 ± .06	<.01

* Mean and stand. dev. of organ weights in g.
† *Idem* expressed as % of body wt.

not the brain of cold-adapted rats.

The catecholamine content of the brains from normal and cold-exposed rats was essentially the same. Catecholamine content of the heart was reduced in the cold-adapted rat when expressed as μg/g wet weight of the heart. This reduction however is apparently due to hypertrophy of the cardiac muscle. It would appear that the hypertrophy of the heart is not accompanied by a concomitant increase in the catecholamine content. A marked reduction of catecholamines by administration of reserpine produces changes in physiological responses of the heart, *e.g.* lowered heart rates(11). The slight reduction of catecholamines in the cold-adapted rats might in part be the cause of the reduced heart rates which have been reported in these animals(9).

In contrast to the reduction of catecholamines in adrenals of rats subjected to acute cold stress(13), it was found that upon chronic exposure to cold, adrenal catecholamine content was markedly increased. This confirms the work of Des Marais and Dugal (4), who, however, reported rather low initial values for E and NE so that their values for the cold-adapted animals were equivalent to normal values obtained by other workers. When calculated on the basis of μg amine/kg body weight, normal values, 148 μg E and 36 μg NE, are comparable to those reported by Hokfelt for female rats(14). When calculated on the same basis, values for the cold-adapted adrenals were 174 μg E and 54 μg NE/kg body weight. Although the increase of NE is more marked than that of E, the content of both amines was significantly increased over normal values.

The increased content of adrenal catecholamines is not necessarily accompanied by increased secretion of these amines. To determine if the development of nonshivering thermogenesis of rats is dependent upon an increased output of catecholamines from tissue stores one must measure the actual output of amines from the tissues, perhaps by measuring urinary excretion of the amines and their degradation products. Why the catecholamines are so markedly increased in the adrenal and not in the heart or brain of cold-adapted rats is not clear. The increased ascorbic acid content of the adrenals in cold-adapted rats may play some role in this increase, either by protecting against destruction of formed catecholamines or by stimulating the synthesis of these amines(15).

Summary. Exposure of female rats to temperatures of 5-7°C for 3-5 weeks resulted in hypertrophy of the liver, heart and kidney.

TABLE II. Catecholamines in Cold-adapted and Normal Rats.

		Adrenal (μg/adrenal pair)	Brain (μg/g)	Heart (μg/g)
Epinephrine	Normal	(11) 29.1 ± 4.7*	(11) .04 ± .01	(11) .11 ± .05
	Cold	(11) 37.4 ± 5.0	(10) .04 ± .02	(11) .06 ± .03
	P	<.01	≅.7	≅.02
Norepinephrine	Normal	(11) 7.0 ± 3.1	(11) .45 ± .11	(11) .94 ± .25
	Cold	(11) 11.6 ± 2.6	(10) .47 ± .08	(11) .64 ± .17
	P	<.01	≅.9	<.01

* Figures represent mean ± stand. dev. Numbers in parentheses represent No. of determinations.

In these rats the catecholamine content of the brain was unchanged, while that of the heart was reduced and the adrenal increased.

1. Cottle, W. H., Carlson, L. D., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 845.
2. Hsieh, A. C. L., Carlson, L. D., Gray, G., *Am. J. Physiol.*, 1957, v190, 247.
3. Von Euler, U. S., *Fed. Proc.*, 1960, v19, suppl. 5, 79.
4. Des Marais, A., Dugal, L., *Can. J. Med. Sci.*, 1951, v29, 90.
5. Hannon, J. P., *Am. J. Physiol.*, 1958, v192, 253.
6. Moore, K. E., Brody, T. M., *J. Pharmacol.*, 1961, in press.
7. Bertler, A., Carlson, A., Rosengren, E., *Acta physiol. scand.*, 1958, v44, 273.
8. Heroux, O., Gridgeman, N. T., *Can. J. Biochem. Physiol.*, 1958, v36, 209.
9. Heroux, O., Campbell, J. S., *ibid.*, 1959, v37, 1263.
10. Baker, D. G., Sellers, E. A., *ibid.*, 1957, v35, 631.
11. Maling, H. M., Cohn, V. H., Jr., Highman, B., *J. Pharmacol.*, 1959, v127, 229.
12. Heroux, O., Depocas, F., Hart, J., *Can. J. Biochem., Physiol.*, 1959, v37, 473.
13. Allen, J. M., *J. Histochem., Cytochem.*, 1956, v4, 341.
14. Hokfelt, B., *Acta physiol. scand.*, 1951, v25, suppl. 92.
15. Hoijer, D. J., *Fed. Proc.*, 1960, v19, suppl. 5, 90.

Received February 13, 1961. P.S.E.B.M., 1961, v106.

Metabolism of Thyroxine in Hyperthyroidism in Cattle.*† (26486)

B. N. PREMACHANDRA‡ AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

Mean biological half-life of thyroxine ($t_{1/2}$) in a group of 16 normal dairy cows was observed to be 2.47 days and a turnover rate of 28.4% per day(1). The present study was concerned with influence of hyperthyroidism on $t_{1/2}$ of thyroxine. Hyperthyroidism was defined in cattle in terms of 50 and 100% increase above their estimated winter thyroxine secretion rate (TSR).

Methods and materials. TSR was determined in late winter in lactating cows of several breeds by replacement technique(2). All animals were maintained in dry lot with free access to iodized salt. In each experiment 300 μc of carrier-free l-thyroxine-I^{131} was injected intravenously and blood samples taken from jugular vein each day following injection of hormone. Measurements of radioactivity of plasma were made in a National Radiac scintillation well counter (Model SA-2D) and conventional corrections were made for decay of isotope and for background. Disappearance of radioactivity in plasma from day to day followed an exponential course. In all experiments 4 g Tapazole/1000 lb body weight/day was administered to each animal to prevent recycling of I^{131} from thyroxine-I^{131} metabolism(3). Plot of disappearance of I^{131} from plasma was extraploted back to zero time (time of injection) and biological half-time ($t_{1/2}$) of thyroxine was determined graphically or from slope of regression line(1). $t_{1/2}$ represents time required by animal body to eliminate one-half of I^{131} in blood.

Eight animals, after they had attained peak milk production, were injected for several months with 1-thyroxine (T_4) subcutaneously daily, 50% in excess of estimated winter TSR, and its effect on milk production noted. $t_{1/2}$ of 1-thyroxine-I^{131} was determined during this period of hyperthyroidism. In 2 animals, (a Guernsey and Jersey-Holstein cross) the level of T_4 injection was further raised to equal twice normal estimated TSR of animal. $t_{1/2}$ of 1-thyroxine-I^{131}

*Contribution from Missouri Agri. Exp. Station. Journal Series No. 2255. Approved by Director.
† Aided-in-part by a grant from U. S. Atomic Energy Commission.
‡ Present address: Inst. of Exp. Pathology, Jewish Hosp., St. Louis, Mo.