se. The impaired respiratory burst reflects decreased activation of the HMP shunt probably through the mechanism of enzyme inhibition.

We are grateful to Mrs. Sheila Motoyama for her devoted technical assistance. We also want to thank Dr. Phyllis Bodel for her many helpful suggestions and comments. The crystalline chloramphenicol was generously provided by Park Davis & Co.

1. Baldridge, C. W. and Gerard, R. W., Am. J. Physiol. **103**, 235 (1933).
2. Strauss, B. S. and Stetson, C. A., J. Exptl. Med. **112**, 653 (1960).
3. Sbarra, A. J. and Karnovsky, M. L., J. Biol. Chem. **234**, 1355 (1959).
4. Cohn, Z. A. and Morse, S. I., J. Exptl. Med. **111**, 667 (1960).
5. Karnovsky, M. L., Physiol. Rev. **42**, 143 (1962).
6. Sbarra, A. J. and Karnovsky, M. L., J. Biol. Chem. **235**, 2224 (1960).
7. Malawista, S. E. and Bodel, P., J. Clin. Invest. **45**, 1044 (1966).
8. Baehner, R. L. and Nathan, D. G., Science **155**, 835 (1967).
9. Baehner, R. L. and Nathan, D. G., New Engl. J. Med. **278**, 971 (1968).
10. Holmes, B., Page, A. R., and Good, R. A., J. Clin. Invest. **46**, 1422 (1967).
11. Quie, P. G., White, J. G., Holmes, B., and Good, R. A., J. Clin. Invest. **46**, 668 (1967).
12. Selvaraj, R. J. and Sbarra, A. J., Nature **211**, 1272 (1966).
13. Cogan, R. H. and Karnovsky, M. L., Nature **204**, 255 (1964).
14. Weisberger, A. S., Armentrant, S., and Wolfe, S., Proc. Natl. Acad. Sci. U. S. **50**, 86 (1963).
15. Weisberger, A. S., Wolfe, S., and Armentrant, S., J. Exptl. Med. **120**, 161 (1964).
16. Follette, J. H., Shugarman, P. M., Reynolds, J., Valentine, W. N., and Lawrence, J. S., Blood **11**, 234 (1956).

Received Oct. 24, 1968. P.S.E.B.M., 1969, Vol. 130.

Growth Hormone Releasing Activity in the Hypothalamus of Rats Subjected to Prolonged Heat Stress* (33669)

MUKUND R. PARKHIE AND HAROLD D. JOHNSON
Department of Dairy Husbandry, University of Missouri, Columbia, Missouri 65201

Evidence for the existence of a neurohumor, designated as growth hormone releasing factor (GHRF), in the hypothalamic extracts of several animal species has been established and recently reviewed (1, 2). Hypothalamic control of the secretion of pituitary growth hormone (GH) is mediated by GHRF since crude hypothalamic extracts have been shown to stimulate the release of GH by the pituitary *in vitro* (3) and also *in vivo* (4). Highly purified preparations of pig GHRF have also been shown to cause depletion of the pituitary GH content in rats (5) with simultaneous increase in plasma GH. Such a response to GHRF represents an increased release of GH from the pituitary into the blood (6, 7).

Although several experimental investigations have produced evidence that a variety of nonspecific stimuli induce alterations in growth hormone secretion (8, 9), only a few studies (10) indicated variations in hypothalamic GHRF content due to stress.

Mueller and co-workers (10) found no significant difference from normal controls in growth hormone releasing activity (GHRA) of hypothalami, GH content in pituitaries, or GHRA of plasma in rats after 5 and 60 min subcutaneous injection of 0.5 ml of 10% formalin. No difference was produced in rats exposed to cold (4°) for 5 min. However, rats exposed to cold for 1 hr showed a significant increase in GHRA of the hypothalamus, with a simultaneous depletion of GH in pi-

* Contribution from Missouri Agr. Expt. Sta. Journal Series No. 5524 approved by the Director. Supported in part by Grant No. USPH UI 00116 10 9005125-2.

tuitaries, followed by increased GH content in the plasma.

Although the effect of low ambient temperatures on hypothalamic GHRA has been reported, no work has been reported on the hypothalamic GHRA of rats exposed to high ambient temperatures for long periods. The objective of this study is to report the effect of a high ambient temperature (35.6°) for varying periods on the hypothalamic GHRA.

Materials and Methods. Female rats of the Holtzman strain, weighing 200–250 g each, were maintained in a small animal climatic chamber for about a week prior to the beginning of the experiment. Each of the three experimental groups contained 16–18 rats, with 20 in the control group. The experimental groups were subjected to a high ambient temperature of 35.6° with 54% relative humidity for periods of 1, 24, or 168 hrs, respectively. The controls were kept at a constant temperature of 24°. All animals were fed Rockland rat pellets and received water *ad libitum*. After the period of exposure to heat, the animals were anesthetized under pentobarbital sodium and the hypothalami were dissected. Pentobarbital was preferred to ether since it does not alter hypothalamic GHRF (5). Two hypothalami were homogenized and extracted as described by Deuben and Meites (3).

Thirty-day-old rats of the same strain as experimental animals served as recipients of the hypothalamic extracts. Equal amounts (0.5 ml) of the hypothalamic extract were injected into the coccygeal vein of each animal. The pituitary homogenates were prepared as outlined by Pecile *et al.* (4). Each treatment group was represented by 8–10 recipient rats. Diluent controls (11), hereafter referred to as sham-treated, received the same volume (0.5 ml) of diluent (without hypothalamic extract) in which the hypothalami were extracted. The 22 rats used in the sham-treated group provided a reasonable comparison with the control group of 20 rats.

GH Assay. GH activity throughout all experiments was measured by the "tibia method" of Greenspan *et al.* (12). Only one strain of rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) was used throughout all experiments, as well as for the standard assay. The rats were hypophysectomized at 28 days of age and were shipped within 24–48 hr post operative. The hypophysectomized rats were fed a special recommended diet supplied by General Biochemicals, Chagrin Falls, Ohio (13) and received water *ad libitum*. A series of four consecutive daily injections began on postoperative day 14. The rats which gained or lost more than 7 g during the post operative pretreatment were excluded from the study (14). A three-point assay with threefold difference between the consecutive dosage levels was carried out with bovine growth hormone (NIH-GH-B12) as a reference standard. Statistical analyses were undertaken as outlined by Bliss (15). Duncan's new multiple range test as modified by Kramer for unequal replicates within treatments was applied to compare experimental groups within themselves and with normal control and sham-treated groups. Dunnett's procedure was used to compare all means with a normal control (16).

Results. Over a range of 40–360 micrograms (μg) of highly purified bovine growth hormone, a linear log dose relationship was found between GH (μg) and the change in tibial epiphyseal cartilage width (μ). The slope (246.36 $\pm$ 36.3) was very significantly different ($p<.001$) from zero, and the regression equation contained no statistically significant differences from linearity. The index of precision (lambda) was 0.1473 $\pm$ 0.0258. This lambda value for the standard assay from which GH potency of the unknown was estimated, compares favorably with lambda values reported elsewhere (17-19). The assays in which lambda values are less than 0.2 are considered as very precise (20).

The results of these experiments are summarized in Table I and illustrated in Fig. 1. Depletion of pituitary GH content by GHRA in the hypothalamus was evaluated in two ways: (a) from the mean epiphyseal cartilage width, and (b) by estimating the GH hormone content for these responses from the

TABLE I. Growth Hormone Releasing Activity in the Hypothalamus of Rats Subjected to Heat Exposures of Varying Duration.

Treatment[a]	AP equivalents/assay rat (total dose/4 days)	Growth hormone evaluation: Epiphyseal[b,c] width (μ; mean $\pm$ SE)	Growth hormone evaluation: Pituitary GH content mean[c,d] (μg)/1.33 AP	Significance of difference from normal controls[e]: Epiphyseal width	Significance of difference from normal controls[e]: GH content
Normal controls (20)	1.33	362 $\pm$ 17.3[f] (7)	80.12[f]	—	—
Sham-treated (22)	1.33	339 $\pm$ 24.5[f] (7)	71.31[f]	NS	NS
Heat exposed					
1 hr (18)	1.33	162 $\pm$ 10.4[g] (7)	11.90[g]	0.01	0.01
24 hr (16)	1.33	161 $\pm$ 13.3[g] (8)	12.18[g]	0.01	0.01
168 hr (18)	1.33	314 $\pm$ 21.2[f] (7)	61.24[f]	NS	NS

[a,b] Numbers in parentheses indicate the number of animals under each treatment group and number of hypophysectomized rats used for each assay, respectively.

[c] Means of epiphyseal width and pituitary GH content of treatment groups.

[d] Calculated from regression equation of standard assay and 1.33 AP represents total dose (0.333/day).

[e] Level of significance as judged by Dunnett's procedure for comparing all means with control in two tailed comparisons with confidence coefficient of 0.99.

[f,g] Means having the same superscript are not significantly different from each other as tested by Duncan's Multiple Range Test with Kramer's modification.

regression equation for the standard assay described above. It might be recalled here that increasing GHRA is indicated by decreasing pituitary GH content and in turn is reflected in a decrease in epiphyseal cartilage width. Exposure to heat stress (35.6°) for 1 and 24 hr very markedly enhanced GHRA of the hypothalamus causing highly significant ($p<.01$) depletion of pituitary GH content (Fig. 1). This was also accompanied by a marked reduction in the cartilage width (Table I) as compared to normal, sham-treated, and 168 hr heat-stressed animals. Such responses in heat-stressed animals approached normal levels at 168 hr. There were no significant differences in pituitary GH content of normal, sham-treated and 168 hr heat-stressed rats; although the levels in the latter were nonsignificantly lower than the normal. Similarly, there were no significant differences in the hypothalamic GHRA after heat exposures of 1 or 24 hrs.

Control rats kept at 24° were considered to show zero percentage decrease in pituitary GH content. Results of the experimental groups (right side) along with sham-treated (left side), as expressed in terms of percen-

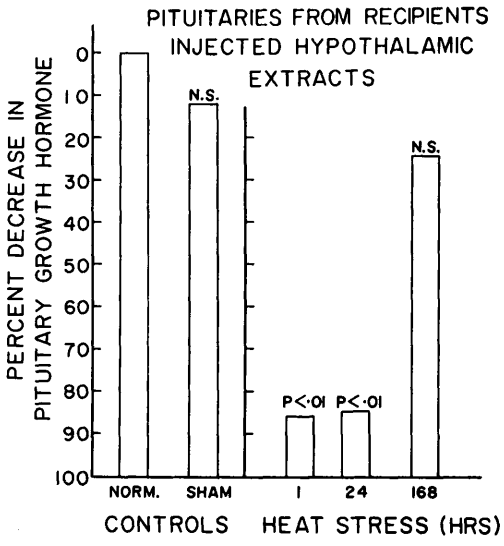

FIG. 1. Effect of heat stress (35.6°) on hypothalamic GHRA as measured by *in vivo* depletion of pituitary growth hormone: Increasing GHRA is indicated by decreasing pituitary GH content; hypothalamic GHRA from control rats kept at 24° is considered to show zero percentage decrease in pituitary GH; NS is nonsignificant from normal as judged by Dunnett's procedure (see text and Table I).

tage decrease in pituitary GH, are depicted in Fig. 1. Exposure of animals to a high ambient temperature for 1 and 24 hr resulted in an increased GHRA of the hypothalamus causing an 85% depletion of pituitary GH content as compared to normal. The depletion of pituitary GH in 168-hr heat-exposed and sham-treated groups was not significantly different from that in the normal group. This pronounced depletion of the pituitary GH content is of physiological significance although it was produced by the crude hypothalamic extract.

Discussion. These results conclusively show that heat stress for 1 and 24 hr causes significantly increased hypothalamic GHRF activity. Similar results were reported in rats on exposure to cold (10) and on insulin-induced hypoglycemia (11). In order to explain a similar behavior of hypothalamic GHRF to different stress stimuli, the role of glutathione as a common denominator of stress-induced phenomenon may be cited. A variety of stress stimuli, such as cold, scalding, anesthesia, hemorrhage, or epinephrine administration, caused a significant reduction in glutathione levels of blood and other tissues. Verma *et al.* (21) reported a significant reduction in brain and blood glutathione levels of rats subjected to heat stress (40°) for 1 hr. Apart from its physiological role in oxidation–reduction systems of a cell, glutathione is said to be responsible for keeping epinephrine and norepinephrine in their reduced states and maintains the activity of coenzyme A. Based on experimental evidence in hydra, Lenhoff (22) recently postulated that the receptor sites for peptide hormone(s) in vertebrates and for glutathione in hydra may have evolved in a similar manner.

The return of GHRF activity towards normal levels may be due (a) to relative unresponsiveness of cutaneous receptors to heat stimuli after exposure for 24 hr (b) to a gradual increase of a recently identified (23) growth hormone inhibiting factor (GHIF) by 168 hr or (c) to a feedback action of circulating GH and the hyperglycemic agents at the hypothalamic level. A variety of factors which alter GH fails to release hypothalamic GHRF. For example, various polypeptide hormones (24), central nervous system depressant drugs (5), thyroidectomy thiouracil feeding, and thyroxine injection at a dose equivalent to thyroxine secretion rate (25). The return to near normal levels, after 168 hr following initiation to stress, was reported for pituitary GH content following insulin-induced hypoglycemia and a variety of other stress stimuli (8). This situation can also be visualized to be due to what Prosser (26) described as compensatory acclimations to temperature changes. Such a trend was reported from our laboratory with the plasma corticosterone level in rats exposed to 34° (27).

Further work is necessary to correlate varying duration of heat stress with glutathione levels, with GHRF and GHIF activity, and with hyperglycemic circulating agents such as epinephrine, glucagon, and alpha 2-deoxyglucose, to further interpret these results.

Summary. The experiment investigated the effect of a high ambient temperature (35.6°) of varying duration on the hypothalamic content of growth hormone releasing activity (GHRA). These experiments consisted of five treatment groups: one group of normals at 24°, one sham-treated group receiving only diluent in which hypothalami were extracted, and three groups, each heat-exposed for 1, 24, or 168 hr, respectively. The GHRA was measured by *in vivo* depletion of pituitary growth hormone (GH) content in 30-day-old rats. Pituitaries of the recipient rats were assayed by the "tibia test" method for GH content. The standard assay was based on three doses of bovine growth hormone (NIH-GH-B12) with a threefold difference between consecutive levels. Growth hormone was evaluated by two parameters: (a) epiphyseal cartilage width, and (b) GH content of the unknown estimated from the response in terms of the reference standard. An exposure to heat for 1 or 24 hr resulted in an enhanced hypothalamic GHRA with a concomitant marked (significant at 1% level) depletion of pituitary

GH content and a return to near normal values by 168 hr post-exposure.

1. McCann, S. M. and Dhariwal, A. P. S., *in* "Neuroendocrinology" (L. Martini and W. F. Ganong, eds.), Vol. 1, p. 284. Academic Press, New York (1966).

2. Schally, A. V., Muller, E. E., Arimura, A., Saito, T., Sawano, S., Bowers, C. Y., and Steelman, S. L., Ann. N. Y. Acad. Sci. **148**, 372 (1968).

3. Deuben, R. R. and Meites, J., Endocrinology **74**, 408 (1964).

4. Pecile, A., Muller, E., Falconi, G., and Martini, L., Endocrinology **77**, 241 (1965).

5. Sawano, S., Arimura, A., Bowers, C. Y., and Schally, A. V., Endocrinology **81**, 1410 (1967).

6. Knobil, E., Physiologist **9**, 25 (1966).

7. Machlin, L. J., Harino, M., Kipnis, D. M., Phillips, S. L., and Gordon, R. S., Endocrinology **80**, 205 (1967).

8. Krulich, L. and McCann, S. M., Proc. Soc. Exptl. Biol. Med. **122**, 612 (1966).

9. Meyer, V. and Knobil, E., Endocrinology **80**, 163 (1967).

10. Muller, E. E., Arimura, A., Sawano, S., Saito, T., and Schally, A. V., Proc. Soc. Exptl. Biol. Med. **125**, 874 (1967).

11. Katz, S. H., Dhariwal, A. P. S., and McCann, S. M., Endocrinology 81, 333 (1967).

12. Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M., Endocrinology **45**, 455 (1949).

13. Shaw, J. H. and Greep, R. O., Endocrinology 44, 520 (1949).

14. Papkoff, H. and Li, C. H., *in* "Methods in Hormone Research" (R. I. Dorfman, ed.), p. 671. Academic Press, New York (1962).

15. Bliss, C. I., *in*, "Statistics in Bioassay," Vol. 1, p. 410. McGraw-Hill, New York (1967).

16. Steel, R. G. D. and Torrie, J. H., "Principles and Procedures of Statistics," p. 107. McGraw-Hill, New York (1960).

17. Meites, J. and Fiel, N. J., Endocrinology **77**, 455 (1965).

18. Grosvenor, C. E., Krulich, L., and McCann, S. M., Endocrinology **82**, 617 (1968).

19. Peckham, W. D., Hotchkiss, J., Knobil, E., and Nicoll, C. S., Endocrinology **82**, 1247 (1968).

20. Lorain, J. A. and Bell, B. T., *in* "General Principles of Hormone Assays," p. 1. Williams and Wilkins, Baltimore, Maryland (1966).

21. Verma, R. R., Khuteta, K. P., and Dandiya, P. C., Psychopharmacologia **12**, 170 (1968).

22. Lenhoff, H. M., Science **161**, 434 (1968).

23. Krulich, L., Dhariwal, A. P. S., and McCann, S. M., Proc. 49th Meeting, Endocrine Soc., Bal Harbour, Florida **1967**, 87.

24. Ishida, Y., Kuroshima, A., Bowers, C. Y., and Schally, A. V., Endocrinology **77**, 759 (1965).

25. Meites, J. and Fiel, N. J., Excerpta Med. **142**, 12 (1967).

26. Prosser, C. L., *in* "Molecular Mechanism of Temperature Adaptation," p. 351. Am. Assoc. Advance. Sci., Washington, D. C. (1967).

27. Kotby, S. and Johnson, H. D., Life Sci. **6**, 1121 (1967).

Received Nov. 1, 1968. P.S.E.B.M., 1969, Vol. 130.