in the reported findings on the effect of PHA on thymic cells. However, the variability in findings can be eliminated to a considerable extent if we consider the age, species and the percentage of the thymic cells that react.

The present finding that PHA caused transformation of thymic cells of the human fetus is in agreement with observations of Bain and Gauld(5,6) and of Wilson(7). Thymic cells obtained from children and adults, on the other hand, did not respond to PHA according to several studies(8,9,10). In contrast, Claman(11) reported that 20% of the surviving thymic cells took up $H^3$-thymidine after 3 days of incubation with PHA. In comparison, about 70% human blood lymphocytes were transformed by PHA.

The discrepancy between the studies on post natal human thymic cells may be quantitative. All 3 studies are in apparent agreement that human thymic cells show a reduced capacity to react as compared to blood lymphocytes. Fuprthermore, Winklestein and Craddock(8) mention a slight increase in morphologically enlarged cells. It can be concluded that PHA caused a transformation of neonatal human thymic cells but produced little or no effect on thymic cells of children and adults.

*Summary.* Thymic cells from newborn rats and mice and from 2 human fetuses were killed by incubation with prednisolone (10 μg/ml) for 1 day. In contrast, thymic cells of newborn rabbits were resistant to the hormone. Thymic cells from young rabbits acquired increasing sensitivity with age and the cells from 12-week-old or older rabbits were nearly all killed by prednisolone. Lymphocytes from the spleen and appendix of rabbits were moderately sensitive to the cytocidal action of prednisolone. Many thymic cells from the human fetus were transformed by PHA into atypical lymphoblastoid cells but very few thymic cells from neonatal and adult rabbits were transformed.

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## Growth Hormone-Releasing Activity in the Hypothalamus and Plasma of Rats Subjected to Stress.* (32228)

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It has recently become clear that a variety of non-specific stimuli can induce alterations in growth hormone (GH) secretion in the rat(1,2). In addition to hypoglycemia(3,4), large doses of epinephrine, vasopressin, or exposure to cold result in a depletion of pituitary GH(1,2). By contrast, other types of stimuli, *i.e.*, injections of formalin and histamine, or laparotomy with exposure of the internal organs, induced only a slight but not significant depletion or rather elevation of

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pituitary GH. Evidence for the existence of a growth hormone releasing factor (GRF) in the hypothalamus of animals of different species has been recently reported(5-10). This hypothalamic GRF has been purified and separated from other releasing factors(11,12). It appeared worthwhile to study whether the increased secretion of GH during stress involved increases synthesis and/or release of hypothalamic GRF. This paper reports the study of the effect of stress on pituitary GH content and on hypothalamic and plasma GRF activity in the rat.

*Materials and methods.* Sprague-Dawley female rats weighing 130-140 g were used as experimental animals. Some rats were hypophysectomized 1 week before the experiment by the method of Tanaka(13). The stresses given to the animals were 1) subcutaneous injection of 0.5 ml 10% formalin and 2) exposure to cold environment, 4°C. In order to examine whether any changes in blood glucose levels were involved in the regulatory mechanism of GH secretion, they were measured with a Technicon Auto Analyzer using a modification of the method of Hoffman(14).

*GH assay.* Animals were decapitated at different time intervals (Tables I, II). Their anterior pituitaries were removed, weighed to the nearest 0.01 mg on a microtorsion balance, pooled by groups, and homogenized in 0.9% saline. The homogenates were kept frozen until the time of assay. GH activity of the samples was measured by the "tibia test" method of Greenspan *et al*(15). Hypophysectomized assay rats were obtained from Hormone Assay Laboratories, Chicago, Ill., or from Charles River Breeding Laboratories, Brookline, Mass. Six to eight assay rats were used for assaying each sample for GH. Pituitary extracts equivalent to 0.20 or 0.25 mg of pituitary per assay rat were administered each day for 4 days. This dose of pituitary extract was equivalent to approximately one-fifth of the anterior pituitary gland. On the fifth day, the width of the epiphysial cartilage was measured. Ovine growth hormone (NIH-GH-S7) was used as reference standard. GH potencies of the pituitary homogenates were calculated either by 3-point or 4-point assay, according to Finney(16). Significance of differences in epiphysial cartilage width was tested by Student's t test or by factorial analysis.

*Test of hypothalamic GRF activity.* GRF activity of stalk median eminence extracts of experimental rats was evaluated as follows: Acid extracts of the stalk median eminence (SME) were prepared by the method reported previously(6). Thirty-day-old Sprague-Dawley female rats were used as recipient animals. They were given the equivalent of 2.5 SME in 1.0 ml of saline by intracarotid injection under ether anesthesia. The control recipient rats were injected with 1.0 ml saline only. Fifteen minutes after injection the recipient rats were decapitated. GH activity of their anterior pituitaries was measured as described above. The depletion of pituitary GH in recipient animals was used as an index of GRF activity contained in the SME extract injected.

*Test of plasma GRF activity.* Blood was collected from the trunk of intact or hypophysectomized rats, some of which were injected with 10% formalin subcutaneously 1 hour before or exposed to cold for 1 hour. Blood from each group of 5-6 rats was pooled in a chilled heparinized plastic tube and the plasma separated by centrifugation. The pooled plasma samples were kept frozen until immediately before injection. One ml of the plasma was injected into the carotid artery of recipient rats. GRF activity of the plasma was determined in a way similar to that for hypothalamic GRF.

*Results. Formaldehyde.* As shown in Table I, a subcutaneous injection of 0.5 ml 10% formalin did not affect the pituitary GH content 5 minutes or 60 minutes after the injection. (Groups 2 and 3 *vs* 1). Similarly, formalin did not modify the GRF content of the hypothalamus 5 minutes and 60 minutes after its administration (Table II, groups 3 and 4 *vs* 2). A slight increase of blood sugar was observed in the group of animals treated with formalin 60 minutes before (Table II, group 4).

*Cold.* No significant depletion of pituitary GH level resulted from exposure to cold for 5 min. However, a considerable decrease of pituitary GH content was observed when the

TABLE I. Effect of Formalin and Cold on Pituitary Growth Hormone Activity.

| Group | Treatment of animals* | Width of epiphysial cartilage,† $\mu$ (mean ± S.E.) | Growth hormone potency, $\mu$g GH/mg pit. | p value *vs* Group 1 |
|---|---|---|---|---|
| 1 | —— | 236 ± 3.9 | 81.5 | — |
| 2 | Formalin 10% 5 min (.5 ml/rat sc) | 226 ± 4.2 | 66.6 | NS |
| 3 | Formalin 10% 1 hr (.5 ml/rat sc) | 232 ± 3.1 | 75.0 | NS |
| 4 | Cold exposure 4°C | 238 ± 6.7 | 82.5 | NS |
| 5 | Cold exposure 4°C 1 hr | 207 ± 4.3 | 41.6 | .001 |
| | GH standard 30 $\mu$g | 204 ± 6.1 | — | — |
| | " " 120 $\mu$g | 288 ± 3.5 | — | — |

* 16 animals per group were used.
† 0.8 mg of anterior pituitary tissue administered in four days.

TABLE II. Growth Hormone Releasing Activity of Stalk Median Eminence (SME) Extracts of Rats Exposed to Formalin and Cold Stress.

| Group | Treatment of SME donor animals‖ | Material administered to recipient animals | Width of epiphysial cartilage,§ $\mu$ (mean ± S.E.) | Blood glucose (mg/100 ml) |
|---|---|---|---|---|
| 1 | —— | Saline (1.0 ml/rat) | 261 ± 7.3 | — |
| 2 | —— | 2.5 SME | 228 ± 6.0** | 81.5 ± 4.3 |
| 3 | Formalin 10% 5 min | 2.5 " | 236 ± 6.6* | 92.0 ± 6.9 |
| 4 | " 10% 1 hr | 2.5 " | 233 ± 5.5** | 106.2 ± 9.4*** |
| 5 | Exposure to cold 5 min | 2.5 " | 232 ± 2.7** | 85.9 ± 6.7 |
| 6 | " " " 1 hr | 2.5 " | 256 ± 7.6†‡ | 73.4 ± 4.7 |

* p .05 *vs* group 1.
** p .01 *vs* group 1.
*** p .05 *vs* group 2.
† NS (not significant) *vs* group 1.
‡ p .02 *vs* group 2.
§ 1 mg of anterior pituitary tissue administered in 4 days.
‖ 16 rats were used per group.

cold exposure was prolonged to 60 minutes (Table I, group 5 *vs* 1). Hypothalamic GRF activity did not change at 5 minutes (Table II, group 5 *vs* 2), but had almost completely disappeared at 60 minutes (Table II, group 6 *vs* 2 and 1). During the exposure to cold, blood sugar levels did not change significantly.

*GRF activity in plasma.* In the last series of experiments we investigated whether GRF activity appeared in the peripheral blood of rats when its activity disappeared from the hypothalamus. Plasma GRF activity of intact rats was determined after they were exposed to cold for 60 minutes. We previously reported that GRF activity was not detected in the plasma from rats hypophysectomized for one week, although it was found in the plasma from animals hypophysectomized 3 months previously(17). We thought that it would be interesting to study whether exposure to cold induces an increase of GRF to a detectable amount in the peripheral blood of rats hypophysectomized one week previously. To evaluate the actual magnitude of pituitary GH depletion which would be induced by the plasma from rats exposed to cold, the pituitary GH of the recipient rats was assayed at two-dose levels. As shown in Table III, neither injection of plasma from intact untreated rats nor from intact rats treated with formalin induced depletion of pituitary GH content of recipient rats (Groups 2 and 3 *vs* 1). By contrast, the injection of plasma obtained from intact rats exposed to cold for one hr induced a significant depletion of pituitary GH (Group 4 *vs* 1, 2, 3), indicating the presence of GRF in the plasma. Similarly, plasma from untreated hypophysectomized rats or from formalin-injected hypophysectomized rats did not induce depletion of pituitary GH content (Groups 5 and 6 *vs* 1). On the other hand, a significant depletion resulted from the administration of plasma from hypophysectomized animals exposed for one hr to a cold environment (Group 7 *vs* 1, 5, 6). An elevation of blood glucose levels was noted

TABLE III. Growth Hormone-Releasing Activity in 1 ml of Plasma from Intact or Hypophysectomized (Hypox) Rats Exposed to Formalin and Cold Stress.

| | Treatment | AP§ equivalents assay rats, total dose/4 days (mg) | Width of tibia cartilage, μ (mean ± S.E.) | Potency, μg GH/mg pit. | 95% fiducial limits |
|---|---|---|---|---|---|
| 1 | Saline | .80 | 257 ± 6.8 | 105.4 | 95.3–122.3 |
| | Saline' | .20 | 223 ± .9 | | |
| 2 | Plasma from intact rat | .80 | 266 ± 6.8* | | |
| 3 | Plasma from intact rat treated with formalin | .80 | 260 ± 2.3** | | |
| 4 | Plasma from intact rat exposed to cold | .80 | 241 ± 5.1*** | 79.3 | 13.3–113.4 |
| | Plasma from intact rat exposed to cold' | .20 | 213 ± 2.3 | | |
| 5 | Plasma from hypox rat | .80 | 276 ± 7.6** | | |
| 6 | Plasma from hypox rat treated with formalin | .80 | 258 ± 5.6**† | | |
| 7 | Plasma from hypox rat exposed to cold | .80 | 234 ± 6.0‡ | 69.7 | 49.8–89.1 |
| | Plasma from hypox rat exposed to cold' | .20 | 201 ± 1.8 | | |
| 8 | GH standard 80 μg | | 256 ± 7.6 | — | — |
| | " " 20 μg | | 217 ± 2.7 | — | — |

* NS (not significant) *vs* saline.
** NS *vs* plasma from intact rat.
*** P .02 *vs* plasma from intact rat.
† NS *vs* plasma from hypox rat.
‡ P .01 *vs* plasma from hypox rat.
§ Anterior pituitary.

Footnote: Although the 95% fiducial limits for pituitary content of GH overlapped when calculated against the GH standard, a direct comparison of the GH levels, using the level in the pituitaries of saline injected animals as 100%(18,19) showed that the potencies of the pituitaries from normal rats exposed to cold were only 71% of the control values. The variance ratio F(18,19) indicated a significant difference between these samples, and the upper fiducial limit was smaller than 100% (88%).

in the hypophysectomized animals (55 ± 5.2 mg/100 ml) after treatment with formalin (94 ± 3.6 mg/100 ml) or exposure to cold (86 ± 4.4 mg/100 ml).

*Discussion.* Simultaneous determinations of hypothalamic GRF and pituitary growth hormone content in rats clearly demonstrated that depletion of pituitary GH content caused by cold exposure for 1 hour was accompanied by disapperance of GRF activity from the hypothalamus. This implies that this stress released pituitary GH by triggering the discharge of hypothalamic GRF. Cold exposure for 5 minutes, or injection of formalin, did not affect the pituitary GH content or hypothalamic GRF activity. In keeping with these results in the rat are the observations of Horton and Lebovitz(20), in the human, who reported that a non-specific stimulus, such as the administration of pseudomonas endotoxin, stimulated GH secretion *via* the hypothalamus.

Release of hypothalamic GRF induced by exposure to cold for 1 hour resulted in the appearance of this neurohumor in the peripheral plasma both in intact rats and in rats hypophysectomized 1 week previously. It is interesting to note that the magnitude of depletion of pituitary GH effected by injection of plasma from cold-stressed, hypophysectomized animals seemed to exceed that induced by plasma from intact rats subjected to the same stress. This finding may suggest that a larger amount of GRF appeared after cold exposure in the peripheral blood in hypophysectomized rats than in intact rats. It may be possible that synthesis and release of hypothalamic GRF are already enhanced one week after hypophysectomy, even though GRF activity in the peripheral blood does not reach a detectable level. An effective stimulus like cold stress or insulin injection readily increases plasma GRF to a detectable concentration in these animals. Re-

cently, Katz *et al* reported that insulin-induced hypoglycemia leads to a discharge of GRF from the hypothalamus of the hypophysectomized rat and to its appearance in the plasma(21). In intact animals, insulin hypoglycemia failed to evoke the appearance of GRF in plasma(21). In the present studies, cold exposure for 1 hour was effective in inducing the appearance of GRF activity in the peripheral blood not only in the hypophysectomized rats but also in the intact rats. This finding may be explained by the greater effectiveness of a cold environment than of insulin injection in evoking GH release in the rat(1).

Since it has been reported that TSH(22) and ACTH(23) interfere with the assay of GH by the "tibia test," it cannot be excluded that variation in pituitary TSH or ACTH could account for part of our findings. However, the changes in SME and plasma GRF activities which accompany changes in pituitary GH make this unlikely.

*Summary.* Experiments were performed in the rat to determine whether the increased secretion of growth hormone (GH) during stress involves augmentation of synthesis and/or release of hypothalamic GH-releasing factor (GRF). Two different stimuli were used as stress: injection of formalin, which was previously shown to be ineffective in inducing GH release; and cold exposure, which evoked depletion of pituitary GH. Exposure of rats to cold (4°C) for 1 hr resulted in a significant depletion of pituitary GH, which was accompanied by disappearance of hypothalamic GRF and appearance of GRF activity in plasma. Cold exposure for 5 minutes was ineffective. Injection of 10% formalin did not induce pituitary GH depletion, or affect hypothalamic and plasma GRF activity. These results indicate that some stressful stimuli, such as cold exposure, capable of releasing GH, induce this effect *via* the hypothalamus; other stresses, like formalin injection, which do not release GH, exert little effect on the GH-release mechanism of the hypothalamus.

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