α-Succinimidoglutarimide and Derivatives.—These were obtained from the corresponding anhydrides by imidation either with urea or with ammonium carbonate according to the following general directions.

(*a*) *Urea Method.*—A mixture of the anhydride (2 moles) and urea (1 mole) was heated in a pressure bottle to 170–180° in an oil bath for 15 min. during which the fused mixture was shaken from time to time. The cooled, hard glossy mass was dissolved in boiling 50% aqueous ethanol. On cooling, the imide separated in a nearly pure state. Recrystallization from ethanol–water mixture afforded the pure imide.

(*b*) *Ammonium Carbonate Method.*—The anhydride (3 moles) and ammonium carbonate (4 moles) were mixed and heated gently to a state of quiet fusion in a flask fitted with an air condenser. The heating and occasional shaking were continued until effervescence ceased and a homogeneous melt was obtained (total heating time about 1 hr.). After being cooled, the hard glossy mass was dissolved in boiling alcohol from which the imide separated on cooling. (See Table II.)

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Stress Effects on Hyaluronidase Activity

By MICHAEL M. CLAY and CLAIRE R. SINAI

Rats maintained in a cold environment for 2 to 16 days showed decreased dermal hyaluronidase spreading activity, which was not accompanied by adrenal hypertrophy. Stress-free conditions for 24 hr. after cold caused a return toward prestress hyaluronidase spreading activity in rats treated with cold for 2, 4, and 8 days but not 16 days. Swimming for 1 hr. caused an increase in spreading activity in rats treated for 4, 8, and 16 days but a decrease at 2 days. These changes were followed by adrenal hypertrophy 24 hr. later. The results obtained with cold and cold followed by forced swimming are in agreement with those reported elsewhere with heat and heat followed by forced swimming, respectively.

SEVERAL STUDIES have shown that glucocorticoid hormones and stress decrease diffusibility of colloidal particles through connective tissue (1–3). This effect has been attributed to a decreased concentration or alteration in connective tissue hyaluronic acid (4).

Enhanced spread of a hemoglobin–hyaluronidase solution has been reported by Hayes and Baker (5) in the skins of rats treated for 37 days with glucocorticoid hormones. To account for the increased spread, the authors suggested that the substrate, hyaluronic acid, was altered structurally or reduced in amount. However, Clay and Nelson (6) reported that rats stressed with heat for as long as 4 months failed to demonstrate increased hyaluronidase spreading activity. Clay and Nelson reported further that a single injection of cortisone acetate (7) or forced swimming for 2.25 hr. (8) increased hyaluronidase spreading activity in rats previously stressed with heat for 7 weeks.

The following study was undertaken to determine whether prolonged cold treatment followed by forced swimming would duplicate the effects of heat and swimming described above.

METHODS

Seventy-two male Wistar rats were bred in our animal quarters. They were assigned randomly to nine equal groups.

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Dermal diffusibility was assayed in a control group by injecting a hyaluronidase–India ink solution into the skin. These rats were sacrificed 24 hr. later. Four groups of rats were maintained in a cold chamber for 2, 4, 8, or 16 days. Dermal diffusibility of hyaluronidase–India ink was assayed in each rat 24 hr. before and 2 hr. after cold treatment. They were sacrificed 24 hr. after removal from the cold. The remaining four groups were treated with cold for 2, 4, 8, or 16 days, and dermal diffusibility was assayed 24 hr. before and 2 hr. after treatment. However, 24 hr. after removal from the cold, dermal diffusibility was assayed again. Two hours later, the rats were forced to swim for 1 hr. in water maintained at 25 ± 1°. Two hours later, a fourth assay of diffusibility was made. The rats were sacrificed 22 hr. later.

Dermal diffusibility was assayed by the method of Clay and Nelson (6). A volume of 0.05 ml. of an indicator solution was injected into the skin of the flank. The solution contained 150 U.S.P. units of hyaluronidase, 17% India ink, and 0.9% sodium chloride in distilled water. The area of spread was calculated by substituting the longest and widest dimensions of the ink spot in the formula for an ellipse. Measurements were made 22 or 24 hr. after injection under light ether anesthesia.

During cold treatment, the rats were maintained in an environment at 8 ± 2° in individual wire mesh cages (10 in. in diameter, 5 in. high) in a dark ventilated chamber. Free access to food and water was provided.

At all times other than during cold treatment the

TABLE I.—EFFECT OF COLD ON DERMAL INK SPREAD AND ADRENAL GLAND WEIGHT

Duration Cold Treatment	Body Wt., Gm. Initial	Body Wt., Gm. Final	Area of Ink Spread, mm.² 24 Hr. Before Cold	Area of Ink Spread, mm.² 2 Hr. After Cold	Adrenal Wt.[a]
Control	262 ± 8[b]	...	261 ± 15	...	11.6 ± 2.4
2 days	284 ± 12	279 ± 14	261 ± 21	143 ± 15	12.5 ± 1.9
4 days	320 ± 11	300 ± 14	301 ± 42	179 ± 14	12.7 ± 2.0
8 days	342 ± 14	329 ± 17	374 ± 28	148 ± 8	12.0 ± 2.2
16 days	358 ± 16	318 ± 18	411 ± 49	140 ± 13	12.5 ± 2.5

[a] Milligrams/100 Gm. body weight for two adrenal glands. [b] Mean values with ± standard error.

TABLE II.—EFFECT OF COLD, RECOVERY, AND SWIMMING ON DERMAL INK SPREAD AND ADRENAL GLAND WEIGHT

Duration Cold Treatment, Days	Body Wt., Gm. Initial	Body Wt., Gm. Final	Area of Ink Spread, mm.² 24 hr. Before Cold	2 hr. After Cold	24 hr. After Cold	2 hr. After Bath	Adrenal Wt.[a]
2	267 ± 10[b]	156 ± 15	296 ± 14	116 ± 19	186 ± 15	86 ± 8	16.2 ± 2.3
4	262 ± 11	261 ± 12	253 ± 20	143 ± 11	185 ± 12	258 ± 36	15.7 ± 2.3
8	313 ± 9	302 ± 16	351 ± 16	142 ± 11	202 ± 25	349 ± 34	13.4 ± 1.8
16	330 ± 14	313 ± 18	377 ± 21	154 ± 16	131 ± 15	307 ± 25	15.1 ± 2.8

[a] Milligrams/100 Gm. body weight for two adrenal glands. [b] Mean values with ± standard error.

rats were maintained on wood shavings in solid-bottom cages, three per cage and at 25 ± 2°.

Hearts, kidneys, and adrenal glands were removed and weighed shortly after the rats were sacrificed.

Statistical treatment of the data was based upon the Student *t* test.

RESULTS

Effects of Cold.—Dermal spread of the ink decreased after 2, 4, 8, and 16 days of cold by 46, 41, 62, and 66%, respectively ($p = < 0.01$ in each case), from pretreatment levels (Table I). Mean adrenal weights of the cold-treated rats did not differ significantly from the control value. No consistent pattern of change was evident in the heart and kidney weights.

Effects of Cold, 24-hr. Recovery and Forced Swimming.—Dermal spread of the ink decreased after 2, 4, 8, and 16 days of treatment by 61, 44, 59, and 64%, respectively ($p = < 0.01$ in each case), from pretreatment levels (Table II). Twenty-four hours after removal from the cold, spreads had increased by 60%, ($p = < 0.01$), 27%, ($p = < 0.05$), and 41%, ($p = < 0.05$) in the 2-, 4-, and 8-day treated groups, respectively. However, the rats treated for 16 days showed a further decline of 15%, which was not significant. In rats treated with cold for 2 days, swimming caused a decrease in area of spread of 53% ($p = < 0.01$) from the recovery period level. However, the groups treated for 4, 8, and 16 days showed increases of spread from 24-hr. recovery levels after swimming of 34%, ($p = < 0.05$), 73%, ($p = < 0.01$), and 134%, ($p = < 0.01$), respectively. Mean adrenal weights were all higher than the value for the control group as well as the groups treated with cold alone. No consistent pattern of change was observed in the heart and kidney weights.

DISCUSSION AND SUMMARY

Failure of the adrenal glands to hypertrophy after cold treatment suggests that hormone secretion was near normal levels. In an earlier study, Denison and Zarrow (9) reported that environmental cold caused a marked eosinopenia, associated with elevated glucocorticoid hormone secretion, in rats after 6 hr. Eosinopenia was followed by a rise to normal levels at 24 hr., levels maintained for the remainder of the 90-day treatment. The continued low level of hyaluronidase spreading activity throughout the cold treatment, after circulating glucocorticoid levels are presumed to have returned to normal, points to a role of factors other than rate of glucocorticoid hormone secretion.

Twenty-four hours after removal from the cold environment, there was an increase in hyaluronidase spreading activity in rats treated with cold for 2, 4, and 8 days but not 16 days. No explanation is available for the difference in response.

Ink spread after forced swimming decreased again in the rats treated with cold for 2 days. The sharp rise in spread after forced swimming in the remaining groups points to a qualitatively different dermal reaction. The changes observed after forced swimming were followed by adrenal hypertrophy. A rise in hyaluronidase spreading activity also was reported in rats forced to swim after prolonged heat treatment (8).

It may be concluded that prolonged maintenance at 8 ± 2°, like the heat treatment of normal rats, depresses hyaluronidase spreading activity presumably accompanied by normal circulating glucocorticoid hormone levels. However, changes in dermal reactivity occur after 2 days of cold, so that spreading activity increases when the animal is subjected to a new stress, *i.e.*, forced swimming. The phenomenon may occur in connective tissue throughout the body and in response to combinations of stress other than those described herein.

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