inhibitor of NAD⊕, potentiated the inhibitory powers of ADP when used in physical combination indicating that another one of the points that NAD⊕ uses for attachment to the LDH had been partially blocked. The same was true for the mixture of ADP with niacinamide and D(−)-ribose.

The K_i for adenosine was fairly high; however, if a phosphate group was joined to the 5′ position covalently to yield the adenosine-5′-PO_4 the K_i decreased to $^1/_3$ of its value. The placement of a covalently bonded phosphate in the 3′ position of adenosine reduced the K_i of adenosine to only $^1/_2$ of its original value. This indicates that the phosphate of a nucleotide is a required portion for bonding to the enzyme; in addition there are steric requirements for maximum binding since both isomers do not have equal K_i values.

Ringold (7) recently theorized that there is an interaction between the quaternary nitrogen of the NAD⊕ and the basic nitrogen of the imidazole ring from histidine at the active site of LDH. With this idea in mind we can see why niacinamide, without a quaternary nitrogen, is not a catalytic species, nor is it very strongly bound to the active site of the enzyme.

One can conclude that the individual components of NAD⊕ are not very strongly bound to LDH, especially in the case of ribose or phosphate alone. However, the amount of inhibition increases as one systematically joins these portions together covalently and the products more closely resemble NAD⊕. This is expected since they become more effective in preventing the complete binding of NAD⊕ to LDH which apparently is a necessary prerequisite for enzymatic activity.

SUMMARY AND CONCLUSIONS

The K_m value for NAD⊕ and the K_i values for the component parts of NAD⊕ as inhibitors of the LDH catalyzed conversion of lactate to pyruvate were determined in 0.1 *M* Tris buffer at pH 8.8.

One finds that the individual component parts of NAD⊕, especially the phosphate and D(−)-ribose, were very poor inhibitors of the coenzyme; however, as the component parts were joined together covalently to make the nucleoside, nucleotide, nucleotide phosphate, *etc.*, their inhibitory powers increased because of greater binding at the active site thus preventing the normal binding of NAD⊕ to LDH.

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Alterations in Hyaluronidase Activity and Serum Protein Electrophoretic Patterns After Chronic Heat Stress

By M. M. CLAY* and M. W. ADLER

The effects of a hot environment upon plasma proteins and dermal hyaluronidase spreading activity was studied in rats. Hyaluronidase activity decreased after 1 day of heat, increased after 4 and 16 days, and again decreased after 48 days. Swimming for 1.5 hr. after each period of heat stress resulted in inhibition of hyaluronidase spreading activity with the exception of the 48-day period when activity increased. Swimming caused adrenal gland hypertrophy in unstressed but not in heat stressed rats. The relative concentration of plasma albumin increased during heat stress.

WITTERSFORS *et al.* (1, 2) have reported hypoalbuminemia with increased extravascular albumin distribution in some patients with gastric carcinoma. The authors suggested increased vascular permeability as a mechanism for loss of circulating albumin. Enhanced as well as diminished dermal connective tissue permeability to colloidal particles has been reported after stress and cortisone administration by this laboratory (3–5). The above work and that of Menkin (6) show a role for glucocorticoid hormones in regulation of vascular as well as connective tissue permeability.

The authors studied the effects of stress upon circulating proteins of rats. They also attempted to determine whether changes in serum proteins accompanied changes in dermal connective tissue permeability. For the first study environmental heat was selected as the stress and intradermally injected India ink and hyaluronidase–India ink were selected as indicators of connective tissue permeability to colloidal particles.

Received April 25, 1966, from the College of Pharmacy, Columbia University, New York, NY 10032
Accepted for publication February 20, 1967.
Presented to the Pharmacology and Biochemistry Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
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EXPERIMENTAL

Male albino Wistar strain rats initially weighing 90–120 Gm. were used. Food and tap water were supplied *ad libitum* at all times. Unless otherwise indicated, the rats were maintained at 24 ± 2°

Animal Treatment—Five groups of 24 rats each were maintained at 32 ± 2° for 1, 4, 16, 32, or 48 days in a ventilated semidark chamber. Each group was then divided into three equal subgroups. Dermal permeability was assayed 2 hr. and 24 hr. after removal from the heat in groups *A* and *B*, respectively. Twenty-four hours after heat stress, the remaining rats of group *C* were forced to swim for 1.5 hr. in water at 25 ± 1°. After swimming,

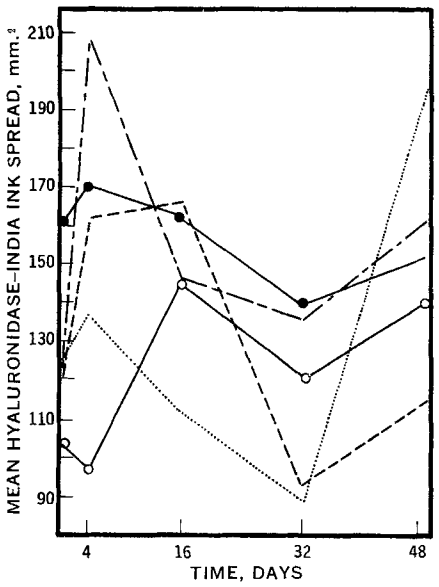

Fig. 1—Effect of stress on the spread of hyaluronidase–India ink indicator solution. Group A, *heat stress; group* B, *heat stress, followed by 24 hr. recovery; group* C, *heat stress, recovery and 1.5 hr. of swimming; group* A′, *nonstressed controls; group* C′, *nonstressed controls, 1.5 hr. of swimming. Key:* – – –, *group* A; — · —, *group* B; ····, *group* C; ●, *group* A′; O, *group* C′.

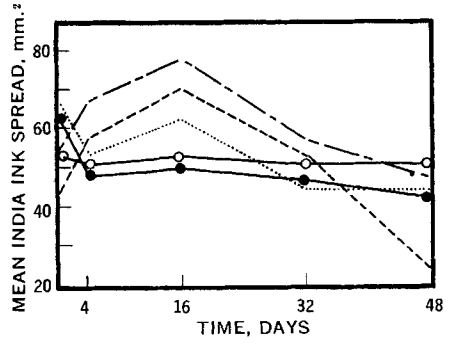

Fig. 2—Effect of stress on the spread of India ink. Group A, *heat stress; group* B, *heat stress, followed by 24 hr. recovery; group* C, *heat stress, recovery, and 1.5 hr. of swimming; group* A′, *nonstressed controls; group* C′, *nonstressed controls, 1.5 hr. of swimming. Key:* – – –, *group* A; — · —, *group* B; ····, *group* C; ●, *group* A′; O, *group* C′.

the rats were gently wiped and kept for 2 hr. in a draft-free cage maintained at 27°. Dermal permeability was then assayed. For each group of 24 treated rats there were 16 control rats of equal initial age and body weight. After 1, 4, 16, 32, or 48 days, dermal permeability was assayed in control rats, group *A′*. The remaining eight control rats, group *C′*, were treated in the same manner as group *C* above.

Assay of Dermal Permeability—Dermal permeability was assayed by the method of Clay and Nelson (7). A volume of 0.05 ml. of each indicator

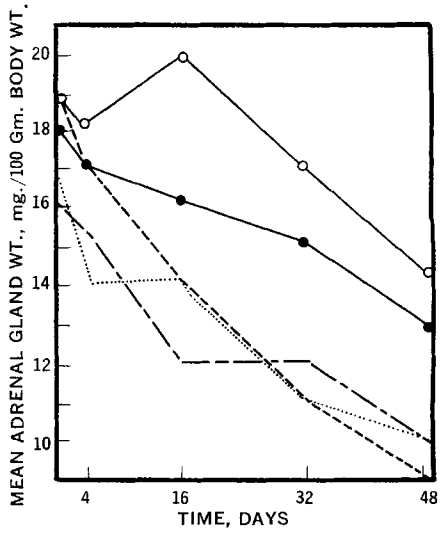

Fig. 3—Effect of stress on adrenal gland weight. Group A, *heat stress; group* B, *heat stress, followed by 24 hr. recovery; group* C, *heat stress, recovery, and 1.5 hr. of swimming; group* A′, *nonstressed controls; group* C′, *nonstressed controls, 1.5 hr. of swimming. Key:* – – –, *group* A; — · —, *group* B; ····, *group* C; ●, *group* A′; O, *group* C′.

solution was injected into the skin of the flank of rats lightly anesthetized with ether. The indicator solution contained 17% by volume of India ink and 0.9% NaCl or the same solution with 150 N.F. units of hyaluronidase[1] added. The area of spread of the India ink in the skin was calculated by substituting the largest and widest dimensions of the ink spot in the formula for an ellipse. Measurements were made 1 hr. after injection under light ether anesthesia. Immediately after measurement of ink spread, the rats were weighed, decapitated, and blood collected. Adrenal glands were removed, dissected free of fat, and weighed on a Christian Becker analytical balance.

Serum Protein Electrophoresis—The blood was permitted to stand for 20 min. The coagulated blood was centrifuged for 10 min. The sera of selected groups were pooled for electrophoretic analysis by means of the Perkin-Elmer model 38 Tiselius electrophoresis apparatus. Pooled sera were diluted 1:3 with barbital buffer of pH 8.6 which was 0.1 *M* with respect to sodium diethyl barbiturate and 0.015 *M* with respect to diethyl barbituric acid, with an ionic strength of 0.1. Each sample was dialyzed in a bag of Visking tubing against the barbiturate buffer for 24 hr. at 1.5°. A volume of 2 ml. of the dialyzed diluted serum was subjected to electrophoretic analysis. The electrophoretic patterns obtained by the scanning method of Longsworth (8) were projected, magnified, traced, and the areas under the peaks measured by means of a planimeter.

RESULTS AND DISCUSSION

Connective Tissue Permeability—Spread of the hyaluronidase–India ink indicator solution in the control rats of the *A′* groups did not vary signifi-

[1] The authors thank G. D. Searle and Co. for the supply of hyaluronidase, trademarked Alidase.

TABLE I—SERUM ELECTROPHORESIS DATA

Treatment	% Total Protein				
	Albumin	α1-Globulin	α2-Globulin	β-Globulin	γ-Globulin
4 Days heat stress	40.7	22.7	7.8	18.0	8.7
24 hr. recovery	50.9	16.2	7.2	16.4	6.7
Swimming	53.6	11.7	7.3	21.6	4.4
32 days heat stress	58.3	9.7	6.1	12.0	5.5
24 hr. recovery	51.0	16.2	10.3	16.4	5.2
Swimming	45.0	25.2	5.7	12.9	7.5
48 days heat stress	69.8	[a]	8.5	15.0	4.8
24 hr. recovery	58.6	14.2	7.6	14.0	5.3
Swimming	66.3	[a]	9.8	16.5	7.2
Control	51.2	13.1	6.0	20.5	9.0
Swimming	53.6	11.7	7.3	21.6	4.4

[a] Values included with α2-globulin, as peaks were indistinct. *Note:* totals do not equal 100% due to presence of unidentified components.

cantly during the 48 days of the study (Fig. 1). The acute stress of swimming caused spread to decrease at each time interval ($P = <0.05$, in each case), accompanied by increased adrenal gland weights in the control rats of the C' groups.

Heat stress (group A) resulted in decreased hyaluronidase spreads compared to control levels (group A') at each time interval except 16 days when the spreads were approximately equal. The spreads were decreased as follows: 1 day, (−) 47.6 mm.^{2}; 4 days, (−) 10.7 mm.^{2}; 32 days, (−) 48.1 mm.^{2}; and 48 days, (−) 38.4 mm.^{2}. The differences were significant only at 32 and 48 days ($P = <0.01$). At 32 and 48 days adrenal gland weights decreased from control levels ($P = <0.01$). (Fig. 3.) Clay and Sinai (4) reported that rats maintained at 12° also showed decreased spreads accompanied, however, by increased adrenal gland weights. Hellman *et al.* (9) reported no change in urinary output of 17-hydroxycorticosteroids in human subjects exposed to heat stress. There was, however, an increase in aldosterone secretion.

Figure 1 shows that hyaluronidase–India ink spreads generally increased 24 hr. after removal from the heat (group B) compared to heat stress levels (group A). The differences were, however, not significant. The acute stress of swimming (Group C) caused decreased spreads at the 4, 16, and 32-day intervals compared to the 24-hr. recovery levels ($P = <0.05$ in each case). After 48 days of heat stress, however, swimming resulted in an increase in spread of 40% ($P = <0.05$) and 70% ($P = <0.01$) compared to 24 hr. recovery and heat stress levels, respectively. Swimming caused a similar rebound in spread after only 4, 8, or 16 days of cold stress (4). In this laboratory a single subcutaneous injection of cortisone acetate (5 mg./Kg. body weight) has also caused a rebound in spread in heat and cold stressed rats. In the present study, the changes in spread produced by swimming after 32 and 48 days of heat stress were not accompanied by significant alterations in adrenal gland weight. Fediay and Clay (5) have reported that the combined stress of cold and restriction of movement for 14 days resulted in increased spread, adrenal gland weight, and plasma corticosterone levels.

Spread of the India ink solution not accompanied by hyaluronidase (Fig. 2) failed to show significant differences among the various treatments.

Serum Electrophoresis—In the data presented in Table I, only a single pattern in serum protein composition can be related to dermal permeability changes. Serum albumin rose from 41% after 4 days of stress to 58% and 70% after 32 and 48 days of stress, respectively. Figure 1 shows that hyaluronidase–India ink spread in the skin fell after these time intervals. Decreased connective tissue permeability to colloidal particles might act to restrict movement of albumin out of the vascular system.

The remaining changes in serum protein composition cannot be related to alterations in connective tissue permeability.

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