Pantothenic Acid Deprivation and Thermal Behavior of the Rat

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PANTOTHENIC acid appears to be vitally implicated in the capacity to withstand stress, and its withdrawal provokes disturbances in a wide range of functions.[1] One of the ways in which a deficiency of this vitamin may be manifested is in structural deterioration and functional impairment of the adrenal glands,[1-6] a result due at least in part to the fact that most of the pantothenate in the tissues is bound as coenzyme A (CoA), essential to the synthesis of steroid hormones.[7] CoA is also essential to a great number of metabolic processes, such as fatty acid breakdown and carbohydrate metabolism, and its versatility as a biocatalyst has been amply demonstrated. Further evidence for its critical role in stress resistance comes from the finding that large doses of pantothenate appear to sustain tolerance to cold stress,[1] while deficiencies impair the ability to survive such stress.[1-8]

Another way in which the function of pantothenic acid may be studied is to observe its behavioral effects. Behavioral indices are useful because they provide a picture of the total organism at work, a condition which is sometimes necessary to reveal certain subtle changes produced by internal chemical states. One variant of such a condition is provided by a scheme in which the intensity of a stress may be diminished by an appropriate response on the part of the organism. In the present study, a situation was used in which the frequency of such a response provided an index of the impact of a cold stress.

METHOD

Apparatus

A device commonly used by psychologists to measure the effects of different variables upon frequency of responding is the Skinner box.[9] As typically used, it consists of a cabinet which contains a lever or bar that can be pushed by an animal, and a means for the delivery of a "reward". In the most simple program, continuous reinforcement, a reward is given for each press of the lever.

The Skinner box provides, for certain purposes, an excellent means with which to gauge the physiologic state of an organism. For example, the rate of responding with food reinforcement is directly related to the length of time that the animal has been deprived of food (up to the point where the debilitation induced by prolonged starvation supervenes).

In this laboratory, the Skinner box has been modified* as shown in Figure 1, with the reinforcement for pressing a plastic lever provided by the activation of an infrared heat lamp while the animal is exposed to a low ambient temperature. Since this arrangement (unpublished data) leads to reliable differences in response rate as a function of temperature, it was expected that it would evoke different rates of responding from animals differentially affected by cold stress. The present data are based on an ambient box temperature of 0° C and a heat reward provided by a 250-watt infra-red

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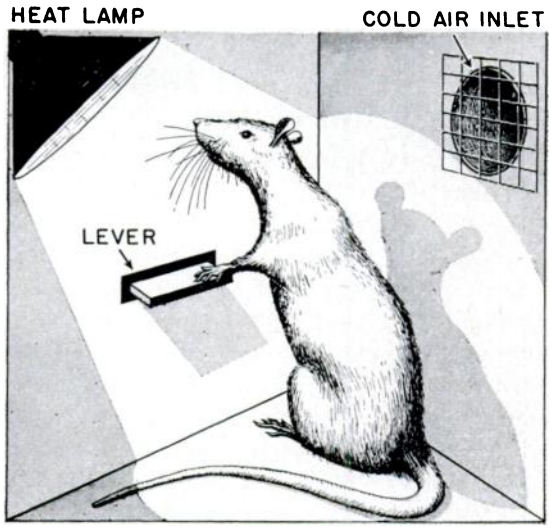

Fig. 1. Sketch of the modified Skinner Box using heat reinforcement.

heat reflector bulb which was activated for 10 sec by each lever pressing. The lamp extended to within 5.5 in. of the floor of the chamber, which measured 10 x 10 x 11 in.

Procedure

Sixteen male albino rats of the Sprague-Dawley strain, approximately seven months old, were used as subjects. After preliminary training in the Skinner box with food reinforcement and, later, heat reinforcement, half the animals were placed on a pantothenate-deficient diet and half on a pantothenate-supplemented diet and allowed to feed ad libitum.* Calcium pantothenate (Merck) was given to the supplemented group as a drinking solution in a concentration of 1 mg/10 cc. Each animal in this group was given 20 cc of the solution each night and received no other liquid the following day until it had been consumed. Drinking tubes with curved lips were used to prevent spilling of the solutions. This procedure insured that each animal in the group received 2 mg of calcium pantothenate daily.

Both groups of animals were then given practice in the Skinner box for four weeks, four hours per week. The coats were clipped at the beginning of the investigation and maintained in that condition throughout.

At the end of this time, no significant differences in response rate were evident. Indeed, no overt signs of pantothenate deficiency could be observed and weight gains and food consumption, as shown in Table I, were not significantly different, although a trend in the expected direction seems apparent. Moreover, all of the animals appeared to be less active than usual.

TABLE I

Mean Weight Gain from First to Fifth Week and Food Consumption for Fifth Week for Pantothenate-Deficient and Pantothenate-Supplemented Rats

		Weight gain g	Food consumption g
Supplemented	Mean	35.00	77.00
	S. D.	±17.29	± 9.80
Deficient	Mean	29.75	73.63
	S. D.	±22.21	±12.04

It was then hypothesized that the pantothenate deficiency was being compensated for, to some degree, by the unlimited intake of other nutrients. Beginning with week five, the animals were placed on a continuous reinforcement schedule for one hour daily. After six experimental days on such a schedule, a three-day starvation period was inaugurated. The day following this period, access to food was permitted for 30 minutes. An intervening day of starvation preceded another 30-minute feeding period and this schedule was continued to the end of the experiment. The investigation was ended when the group of pantothenate deprived animals had become so severely impaired physically that some died, making the reliability of further observations on the survivors questionable.

RESULTS

The results are summarized in Figure 2, which illustrates the conjoint effects on response rate of starvation and pantothenate deprivation. Prior to the starvation regimen the pantothenate-deficient group responded at only a slightly higher rate, but the diver-

* The basic diet was approximately the same as that used by Ershoff[3] and was supplied by General Biochemicals, Inc.

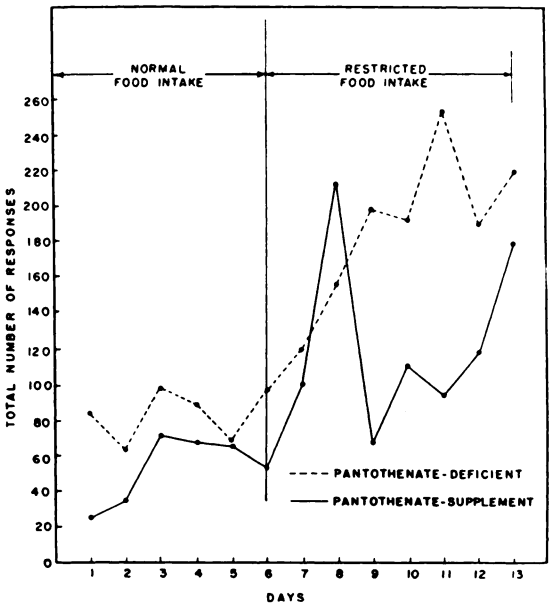

Fig. 2. Total number of responses per day for pantothenate-deficient and pantothenate-supplemented groups throughout the 13-day experimental period.

gence became greatly pronounced during food restriction. The greater frequency at day eight for the pantothenate-supplemented group was due to but two animals and is the only "impurity" in the trend. Table II gives

TABLE II

Mean Daily Response Rate and Variability Among Animals for Normal and Restricted Food Intake Periods and Differences Between Periods

		Normal	Restricted	Difference
Pantothenate-Supplemented	Mean	6.60	15.71	9.11
	S. D.	±6.00	±4.70	±4.02
Pantothenate-Deficient	Mean	10.44	23.61	13.20
	S. D.	±7.40	±10.77	±7.32

the mean and standard deviation for both periods and for the differences between periods.

Evaluation of the results by the analysis of variance and some supplemental procedures lead to the following statements:

(1) The starvation regimen produces a significant increase in response rate.

(2) The two groups differ significantly during the restricted period, the deprived group making significantly more responses.

(3) The deficient animals show somewhat greater variability.

DISCUSSION

These data demonstrate that what might be called the thermotactic drive can be intensified by at least two kinds of dietary restrictions. One is a reduction in total food intake. The other is a reduction in intake plus deprivation of pantothenic acid, the latter adding an extra increment of intensity.* It is analogous to the finding that hungry rats respond more frequently (with food reinforcement) than sated rats.

Another feature that deserves comment is the change in variability produced by the experimental treatment. Such a result is often obtained in experiments which expose groups of animals to different treatments. Its significance is not understood, but it appears so often in biologic research that it warrants more attention from experimenters, particularly because it indicates that the treatment has produced a significantly different population.

The fact that no effects related to pantothenate deprivation appeared until intake restriction was superimposed is an important aspect of the data. Ershoff[8] has noted that prolonged pantothenate deprivation leads to a decline in food intake and suggests that forced feeding may help to overcome deprivation-induced symptoms, although he believes that reduced intake alone cannot be adduced as the only factor involved. The present results tend to support his hypothesis along with that of Beznák and van Alphen,[10] who contend that the symptom-poor picture of pantothenate deficiency obtained by recent investigators is due to their use of a uni-deficient diet, while earlier researchers used a multi-deficient diet.

It would probably be a mistake to suppose that the effects of inanition and pantothenate deprivation are additive. The data presently

* A second experiment, this time using chronic rather than acute undernutrition, also demonstrated differential effects due to pantothenic acid deprivation. Statistical tests indicate that these effects are independent of weight and food intake changes.

available[1,3,7,8] indicate that both conditions derange a wide variety of functions and interact in a rather complex fashion. For example, they produce adrenal impairment via two different avenues: hypersecretion of ACTH (inanition) and insufficiency within the adrenals themselves (pantothenate-deficiency). Also, as a component of CoA, pantothenic acid influences the operation of many metabolic processes. The response to pantothenate surfeit, moreover, seems to depend on the general nutritive state of the animal. Dumm and Ralli,[4] using unilateral adrenalectomy as a stress, found that large supplements of pantothenate increased adrenal cholestrol levels in rats fed a 16 per cent protein diet but not in those fed a 22 per cent protein diet. Granados and Verzár,[5] also working with adrenalectomized rats, reported that supplements of pantothenate prolonged survival only if adequate quantities of fat and the B-vitamins were present in the diet. Thus, it seems that the role of pantothenic acid can be defined only as a function of the nutritional status in other respects.

SUMMARY

After preliminary training in the Skinner box, during which they learned to activate a heat lamp by pressing a lever, sixteen albino rats were separated into two equal groups. One was put on a pantothenate-enriched diet (2 mg daily). The other was put on a pantothenate-deficient diet. The frequency of heat-reinforced responses in an ambient temperature of 0° C was recorded for a period of four weeks, during which the animals were in the Skinner box for four hours per week. When differences in response rate did not appear at the end of this time, both groups were submitted to a starvation diet. This resulted in a marked increase in frequency of responding for both groups, with the rate for the pantothenate-deprived group being significantly greater. It was concluded that food and pantothenate-deprived animals are more sensitive to cold than animals deprived only of food.

REFERENCES

1. Ralli, E. P., and Dumm, M. E.: Relation of pantothenic acid to adrenal cortical function. *Vitamins and Hormones* 9: 133, 1953.
2. Casentini, S., Libretti, A., and Tusini, G.: Sul quadro isotochimico e sul contenuto in colesterlo e acido ascorbico dei surreni di animali carenti in acido pantotenico e tratti o no con ACTH. *Arch. Internat. Pharmacodyn. Therap.* 92: 217, 1952.
3. Cowgill, G. R., Winters, R. W., Schultz, R. B., and Krehl, W. A.: Pantothenic acid deficiency and the adrenals: some recent experiments and their interpretation. *Internat. Ztschr. Vitaminforsch.* 23: 275, 1952.
4. Dumm, M. E., and Ralli, E. P.: Influence of ascorbic acid, pantothenic acid and protein on the resynthesis of adrenal cholesterol after stress. *Fed. Proc.* 13: 38, 1954.
5. Granados, H., and Verzár, F.: The influence of pantothenic acid and other B-vitamins on the adrenalectomized rat. *Internat. Ztschr. Vitaminforsch.* 24: 403, 1952.
6. Beare, J. L., Beaton, J. R., and McHenry, E. W.: Anterior pituitary growth hormone administration in B vitamin deficiencies in the rat. *Endocrinology* 55: 40, 1954.
7. Novelli, G. D.: Metabolic functions of pantothenic acid. *Physiol. Rev.* 33: 525, 1953.
8. Ershoff, B. H.: Comparative effects of pantothenic acid deficiency and inanition on resistance to cold stress in the rat. *J. Nutrition* 49: 373, 1953.
9. Skinner, B. F.: *The Behavior of Organisms.* Appleton-Century-Crofts, New York, 1938.
10. Beznák, A. B. L., and van Alphen, G. W. H. M.: Cardiac automatism and choline acetylation in rats on thiamine and pantothenic-acid-deficient diets. *Canad. J. Biochem. Physiol.* 33: 867, 1955.