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## ABSTRACT

The activity of five liver enzymes involved in amino acid metabolism was found to be markedly increased in cold-exposed rats. However, the activity of three of the enzymes, arginase, glutamic-oxalacetic and glutamic-pyruvic transaminase, was increased only as a result of a cold-induced higher protein intake. In contrast, the activity of tryptophan pyrrolase and tyrosine alpha ketoglutaric transaminase was increased by cold per se. The data demonstrate that both substrate-induced and cold-induced enzymatic changes occur in cold-exposed animals.

## PUBLICATION REVIEW

  
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Director of Research

# EFFECT OF PROTEIN INTAKE AND COLD EXPOSURE ON SELECTED LIVER ENZYMES ASSOCIATED WITH AMINO ACID METABOLISM

## SECTION 1. INTRODUCTION

It is well established that animals exposed to cold for a prolonged period of time undergo certain enzymatic changes associated with an increased thermal demand in the cold environment.

Major changes in the activity of specific enzymes involved in carbohydrate metabolism of cold-exposed rats were reported by Hannon and Vaughan (1960, 1961) and Vaughan et al (1961). These authors found that the cold exposed rat has an increased activity of liver glucose-6-phosphatase, glucokinase, malic dehydrogenase and an increase in the rate of pyruvate formation from 3-phosphoglycerate. The rate of oxidation of the Krebs cycle substrates, succinate, isocitrate, citrate, alpha-ketoglutarate, fumarate and malate were also found to be markedly increased as a result of a chronic cold exposure (Hannon, 1958). In addition, a significant increase in the activity of liver succinic dehydrogenase, cytochrome oxidase, and of DPNH- and TPNH-cytochrome c reductase of cold-exposed animals were reported by Hannon (1960) and Reynafarje and Chaffee (1960). More recently, it was reported from this laboratory that the activity of liver arginase, glutamic-oxalacetic and glutamic-pyruvic transaminase in cold exposed rats fed a low protein diet was significantly higher than that in the comparable animals kept at room temperature (Klain et al, 1962).

However, it has been observed that the cold exposed animals increase appreciably their food intake in order to satisfy the energy needs for both heat production and growth. Since, in the final analysis, the elevated energy metabolism is the result of an increased rate of oxidation of proteins, carbohydrates and fats, it can be assumed that the augmented flow of substrates passing over certain enzymes in the cold-exposed animals will increase the rate of synthesis of these enzymes, and thus alter their activity, according to the hypothesis proposed by Spiegelman (1946).

The present experiments were conducted to study effects of diet and cold exposure on five liver enzymes associated with amino acid metabolism in the rat and to examine the possibility that increased activities of some enzymes in cold exposed animals could be substrate-induced rather than an effect of cold per se.

## SECTION 2. EXPERIMENTAL

Male Sprague-Dawley rats, ranging in weight from 165 to 200 grams, were used in all experiments. The control group (Group 1), kept at 25° C, was given the experimental diet on an ad libitum basis. The cold-exposed rats were kept in a cold room at 7° C. One group of the cold-exposed rats (Group 2) was also fed on an ad libitum basis, while the other two cold-exposed groups (Groups 3 and 4) were pair-fed with the warm controls. However, in order to meet a higher energy demand in cold, the animals in Groups 3 and 4 were offered an unrestricted amount of either sucrose or fat, respectively, as soon as they consumed the daily portion of the experimental diet. Individual wire cages were used throughout the experiment.

The experimental diet consisted of 20% crude casein, 72% sucrose, 4% corn oil, and 4% U. S. P Salt mixture No. II. The vitamin mixture supplied 2,000 units of vitamin A, 222 units of vitamin D, 11.1 mg of  $\alpha$ -tocopherol, and the following mg per 100 gm of diet: ascorbic acid, 100; inositol, 11.1; choline chloride, 166.5; menadione, 5; p-aminobenzoic acid, 11.1; niacin, 10; pyridoxine hydrochloride, 2.22; riboflavin, 2.22; thiamin hydrochloride, 2.22; Ca-pantothenate, 40.3; also 44  $\mu$ g of biotin, 200  $\mu$ g of folic acid and 3  $\mu$ g of vitamin B<sub>12</sub>.

At the end of 28 days the animals were sacrificed by decapitation, the liver was immediately excised, chilled in chipped ice and assayed for arginase, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT), in experiment 1, and for tryptophane pyrrolase (TP) and tyrosine-alpha-ketoglutaric transaminase (TKGT), in experiment 2.

Arginase determination was carried out according to the procedure of Brown and Cohen (1959) as used by Klain et al (1962). GOT and GPT were measured by following the oxidation of DPNH with a Beckman DK recording spectrophotometer according to procedures similar to those used by Wroblewski and La Due (1956) and La Due et al (1954).

The activity of TP was determined according to the procedure of Knox and Auerbach (1955) and estimations of TKGT were made according to the method used by Lin and Knox (1957). All enzyme assays were carried out on duplicate samples, involving five or more animals.

Aliquots of tissues were analyzed for nitrogen content by acid digestion and the nesslerization procedure. Since there was no difference in the nitrogen content of livers, the enzyme activities were expressed in units per gram of fresh tissue rather than per unit of nitrogen.

### SECTION 3. RESULTS AND DISCUSSION

The data obtained in these studies are summarized in Table I. The mean weight changes for the 28-day experimental period indicate that the amount of protein consumed by the pair-fed animals met their needs for the maximal rate of growth (Groups 3 and 4 vs 2).

It will be noted that the activity of arginase, GOT and GPT was markedly increased in the cold-exposed, ad libitum-fed animals (Group 2 vs 1) and remained unchanged in the pair-fed groups (Groups 3 and 4 vs 1). This effect can be apparently related to a higher protein intake per se since the animals in Group 2 consumed over 44 per cent more of the complete diet than those in the pair-fed groups. There is ample evidence in the literature demonstrating that activity of certain enzymes involved in protein metabolism can be readily affected by the quantity of protein consumed. Thus, arginase activity increases on high protein diets and, conversely, decreases on low protein diets (Mandelstam and Yudkin, 1952; Ashida and Harper, 1961; Rosenthal et al, 1950). Similarly, Rosen et al (1959) reported an increase in GOT and GPT activity in livers of both intact and adrenalectomized rats fed high protein diets, showing that protein per se was primarily involved in this metabolic adaptation.

In contrast, the activity of TP and TKGT was uniformly increased in all three cold-exposed groups, regardless of the dietary treatment. Thus, the most important conclusion to draw from the foregoing data is that both substrate-induced and cold-induced enzymatic changes occur in the cold-exposed animals. Cold-induced adaptive changes in the enzyme activity may be assumed to be mediated by adrenocortical hormones, since it has been established that cold-exposed animals produce more corticosteroids than comparable animals kept at room temperature (Heroux et al, 1959; Schonbaum, 1960, Sobel et al, 1960). In addition, Thomson and Mikuta (1954) found that the activity of the mammalian liver TP was greatly increased after the injections of glucocorticosteroid hormones, and Knox and Auerbach (1955) presented evidence that adrenalectomized rats developed an increased activity of the liver TP after the intraperitoneal injections of tryptophan. More recent studies of Civen and Knox (1959) showed that the inducing effect of tryptophan and hydrocortisone given together was the sum of their separate action. Similarly, the activity of TKGT can be stimulated in vivo by injecting hydrocortisone or tyrosine or by a mixture of these components (Lin and Knox, 1957; 1958; Litwack and Diamondstone, 1962).

Since the activity of TP and TKGT in Group 2 was not altered as a result of a higher protein intake, it appears that the increased intake of the specific substrates was not adequate to increase the activity over that induced by cold.

TABLE I

WEIGHTS, FOOD INTAKES AND ACTIVITIES OF SELECTED ENZYMES  
IN WARM AND COLD EXPOSED RATS

	Treatment Group			
	1	2	3	4
	Warm	Cold Ad Lib	Cold -- + Sugar	pair Fed + Fat
<b>Experiment 1</b>				
Average $\Delta$ BW (gm) <sup>1</sup>	120 $\pm$ 3.1	107 $\pm$ 2.9	104 $\pm$ 3.0	107 $\pm$ 2.5
Average daily intake of complete diet (gm)	17.4	25.1	17.4	17.4
Average daily sucrose intake (gm)	-	-	6.4	-
Average daily fat intake	-	-	-	3.1
Arginase <sup>2</sup>	41.6 $\pm$ 1.4	53.5* $\pm$ 1.7	43.2 $\pm$ 1.2	40.4 $\pm$ 2.4
Glutamic-oxalacetic transaminase <sup>3</sup>	194 $\pm$ 7.3	245* $\pm$ 9.2	190 $\pm$ 12.1	209 $\pm$ 5.2
Glutamic-pyruvic transaminase <sup>4</sup>	35.3 $\pm$ 2.6	47.6* $\pm$ 3.5	31.1 $\pm$ 3.5	41.2 $\pm$ 2.6
Tryptophan pyrrolase <sup>5</sup>	1.9 $\pm$ 0.1	2.8* $\pm$ 0.2	2.8* $\pm$ 0.1	3.0* $\pm$ 0.2
<b>Experiment 2</b>				
Average $\Delta$ BW (gm) <sup>2</sup>	118 $\pm$ 4.2	114 $\pm$ 3.1	108 $\pm$ 4.4	109 $\pm$ 4.7
Average daily intake of complete diet (gm)	17.8	25.7 $\pm$	17.8	17.8
Average daily sucrose intake (gm)	-	-	6.1	-
Average daily fat intake (gm)	-	-	-	3.9
Tyrosine-alpha-ketoglutaric transaminase <sup>6</sup>	58.1 $\pm$ 3.6	72.3* $\pm$ 2.8	75.4* $\pm$ 5.1	69.2* $\pm$ 4.8

<sup>1</sup> Average value of 10 animals  $\pm$  standard error<sup>2</sup> mM urea gm liver/hr<sup>3</sup>  $\mu$ M DPN/gm liver/hr<sup>4</sup>  $\mu$ M DPN/gm liver/hr<sup>5</sup>  $\mu$ M kynurenine/gm liver/hr<sup>6</sup>  $\mu$ M p-hydroxyphenylpyruvate/gm liver/hr

\* Difference from warm groups - (p &lt; 0.05)

The data indicate that the enzyme activity in the pair-fed groups was not altered, regardless of whether sugar or fat was offered as an additional source of energy in cold.

#### REFERENCES

1. Ashida K. and A. E. Harper. Metabolic adaptations in higher animals. VI. Liver arginase activity during adaptation to high protein diet. *Proc. Soc. Exper. Biol. Med.* 107:151, 1961.
2. Brown, G. W., Jr. and P. P. Cohen. Comparative biochemistry of urea synthesis. I. Methods for the quantitative assay of urea cycle enzymes in liver. *J. Biol. Chem.* 234:1769, 1959.
3. Civen, M. and W. E. Knox. The independence of hydrocortisone and tryptophan induction of tryptophan pyrrolase. *J. Biol. Chem.*, 234:1787, 1959.
4. Hannon, J. P. Respiration of rat liver homogenates following prolonged cold exposure. *Proc. Soc. Exper. Biol. Med.* 97:368, 1958.
5. Hannon, J. P. Effect of prolonged cold exposure on components of the electron transport system. *Am. J. Physiol.* 198:740, 1960.
6. Hannon, J. P. and D. A. Vaughan. Effect of prolonged cold exposure on the glycolytic enzymes of liver and muscle. *Am. J. Physiol.* 198:375, 1960.
7. Hannon, J. P. and D. A. Vaughan. Effect of exposure duration on selected enzyme indexes of cold acclimatization. *Am. J. Physiol.* 200:94, 1961.
8. Heroux, O., E. Schonbaum and A. Desmarais. Adrenal and thyroid adjustments in white rats during seasonal and thermal acclimation. *Fed. Proc.* 18:264, 1959.
9. Klain, G. J., D. A. Vaughan and L. N. Vaughan. Interrelationships of cold exposure and amino acid imbalances. *J. Nutrition*, 78: No. 3, (in press) 1962.

10. Knox, W. E. and V. H. Auerbach. The hormonal control of tryptophan peroxidase in the rat. *J. Biol. Chem.* 214:307, 1955.
11. LaDue, J. S., F. Wróblewski and A. Karmen. Serum glutamic-oxalacetic transaminase activity in human acute transmural myocardial infarction. *Science* 120:497, 1954.
12. Lin, C. C.E. and W. E. Knox. Adaptation of the rat liver tyrosine alpha-ketoglutarate transaminase. *Biochim. et biophys. acta* 26:85, 1957.
13. Lin, C.C.E. and W. E. Knox. Specificity of the adaptive response of tyrosine alpha-ketoglutarate transaminase in the rat. *J. Biol. Chem.* 233:1186, 1958.
14. Litwack, G. and T. I. Diamondstone. Nonspecific stimulation of tyrosine alpha-ketoglutarate transaminase activity in vivo. *J. Biol. Chem.* 237:469, 1962.
15. Mandelstam, J. and J. Yudkin. Effects of variations in dietary protein upon hepatic arginase of rat. *Biochem. J.* 51:681, 1952.
16. Reynafarje, B. and R. Chaffee. Pyridine nucleotide-cytochrome c reductases in acclimatization to cold. *Proc. Soc. Exper. Biol. Med.* 103:225, 1960.
17. Rosen, F., N. R. Roberts and C. A. Nichol. Glucocorticosteroids and transaminase activity. I. Increased activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis. *J. Biol. Chem.* 234:476, 1959.
18. Rosenthal, O., C. S. Rogers, H. M. Vars and C. C. Ferguson. Arginase, adenosinepyrophosphatase and rhodanase levels in the liver of rats. *J. Biol. Chem.* 185:669, 1950.
19. Schonbaum, E. Adrenocortical function in rats exposed to low environmental temperatures. *Fed. Proc.* 19 (Suppl. 5):85, 1960.
20. Sobel, H., M. Sideman and R. Arce. Steroid excretion by guinea pigs exposed to cold for prolonged periods. *Am. J. Physiol.* 198:1107, 1960.

21. Spiegelman, S. Nuclear and cytoplasmic factors controlling enzymic constitution. Cold Spring Harbor Symposia on Quant. Biol. 11:256, 1946.
22. Thomson, J. F. and E. T. Mikuta. Effect of total body x-irradiation on the tryptophan peroxidase activity of rat liver. Proc. Soc. Exper. Biol. Med. 85:29, 1954.
23. Vaughan, D. A., J. P. Hannon and L. N. Vaughan. Interrelationships of diet and cold exposure on selected liver glycolytic enzymes. Am. J. Physiol. 201:33, 1961.
24. Wróblewski, F. and J. A. LaDue. Serum glutamic-pyruvic transaminase in cardiac and hepatic disease. Proc. Soc. Exper. Biol. Med. 91:569, 1956.

<p>Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. Rpt. AAL-TDR-62-61. EFFECT OF PROTEIN INTAKE AND COLD EXPOSURE ON SELECTED LIVER ENZYMES ASSOCIATED WITH AMINO ACID METABOLISM. January 1963. 7 p. incl table. 24 ref.</p> <p>Unclassified Report</p> <p>The activity of five liver enzymes involved in amino acid metabolism was found to be markedly increased in cold-exposed rats. However, the activity of three of the enzymes, arginase, glutamic-oxalacetic and glutamic-pyruvic transaminase, was increased only as a result of a cold-induced higher protein intake. In contrast, the activity of tryptophan pyrrolase and tyrosine alpha ketoglutaric transaminase was increased by cold per se. The data demonstrate that both substrate-induced and</p>	<p>1. Proteins 2. Metabolism 3. Exposure 4. Amino Acids 5. Enzymes 6. Rats</p> <p>Project 8238-03 Klain, G. J., D. A., Vaughan, L. N. Vaughan Available from OTS In ASTIA collection</p>
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