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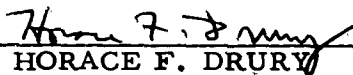
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ABSTRACT

The changes in the protein metabolism in animals exposed to cold are discussed. Work of the authors has shown that a moderate cold exposure is an effective agent in correcting and overcoming amino acid imbalances in the rat, and that both substrate-induced and cold-induced enzymatic changes occur in cold-exposed animals.

PUBLICATION REVIEW



HORACE F. DRURY
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ALTERATIONS OF PROTEIN METABOLISM DURING COLD ACCLIMATION*

One of the phenomena associated with a chronic cold exposure is a greater capability of the exposed animal to produce heat. Since, in the final analysis, the elevated energy metabolism is the result of an increased rate of oxidation of carbohydrates, fats and proteins, it can perhaps be assumed that exposure to cold will alter some metabolic pathways or some specific reactions. Such metabolic changes indeed have been found, particularly in the metabolism of carbohydrates and fats, and we hope that this discussion will add something of value to our concepts of the alterations which take place in the metabolism of proteins.

As early as 1938 it was reported, on rather inconclusive evidence, that protein metabolism was unaltered by cold exposure (Dontcheff and Schaeffer, 1938). Other reports, however, indicate the contrary, as that published in 1950 by Hoberman who reported that amino acid and protein catabolism is accelerated in fasting cold-exposed rats. Williams et al (1950), using fasting rats exposed to -5°C for six hours, reported a marked decrease in plasma proline followed by methionine, threonine, arginine, and lysine. In contrast, the plasma levels of leucine, phenylalanine, and valine were found to be markedly increased. These workers also found a decrease in the level of liver histidine and a slight increase in valine and threonine levels. No significant changes were detected in free amino acid levels in the muscle.

Mefferd et al (1958) and Hale and Mefferd (1958) found that cold-exposed rats excrete more alanine, valine, serine, threonine, glycine and glutamic acid than their warm counterparts.

Rangneker and Dugal (1958) observed that phenylalanine disappeared from the plasma and urine of rats exposed to 2°C for 72 hours, and tyrosine, although still present in traces in the plasma, disappeared from the 24-hour urine samples. Similar observations on the disappearance of tyrosine from the blood of dogs were made by Greene and Johnston (1942). These findings would, therefore, indicate a more rapid conversion of these two amino acids to their metabolic products in the cold-exposed animals.

* Presented at the International Symposium on Temperature Acclimation, Leiden, The Netherlands, September 5 to 7, 1962.

It has also been demonstrated that animals subjected to cold and given 10% glycine in the diet exceed their controls in ability to maintain liver glycogen during fasting (Todd and Allen, 1960). Incorporation of 5% threonine, histidine, alanine or serine into the high glycine diet had no additional action on the glycine effect (Todd, 1961). On the other hand, incorporation of 1% glutamic acid to the diet failed to prolong survival of rats exposed to cold (Dugal and Therien, 1952).

Effect of cold exposure on two enzymes directly involved in the protein metabolism has been reported. Thus, Schayer (1960) found a marked increase of histidine decarboxylase in mice exposed to 2° C for six hours, and recently Vaughan et al (1962) found a higher activity of xanthine oxidase in the liver and kidney of rats exposed to 7° C for four weeks.

Levels of liver protein and nonprotein sulfhydryl compounds also have been found to be affected by cold (Register and Bartlett, 1955; Register et al, 1959). The former were increased in cold-exposed rats regardless of whether they were maintained on a complete or a methionine-deficient diet. In contrast, cold-exposed animals on an adequate diet showed a decrease in nonprotein sulfhydryl levels, while those on a methionine-deficient diet showed an increase in nonprotein sulfhydryl levels.

It also has been reported that rabbits exposed to 3° C for 20 days showed a striking decrease in serum beta globulin and apparently a significant rise in gamma globulin (Waugh, 1952). Similar changes in electrophoretic patterns of plasma proteins as a result of continuous exposure of rabbits to cold were found by Sutherland et al (1956; 1958). Shields et al (1960), using rats exposed to 4° C for 40 days, found a marked decrease in the albumin fraction of the plasma, decrease in the gamma globulin and increase in the alpha₂ fraction. Recently, Trapani and Campbell (1959) reported that the disappearance of passively administered antibody is more rapid in rabbits at -15° C than at 18° C, thus indicating faster protein turnover in the cold. From the work of Trapani (1960) it is apparent that cold-exposed animals exhibit changes in the electrophoretic distribution of the plasma proteins, an increase in the total mass of circulating protein, an increase in protein turnover, and an apparent decrease in the immune response. Furthermore, Hannon and Young (1959) reported that one month's cold exposure of rats resulted in a significant decrease in plasma protein levels.

Excretion of nitrogenous compounds other than amino acids was also increased in the cold regardless of whether food intake was equalized (Lathe and Peters, 1949; Ingle et al, 1953) or whether the rats were allowed to feed ad libitum (Hannon and Young, 1959; Treichler and Mitchell, 1941; You et al, 1950). Cold-exposed rats excrete more urea, allantoin, creatine, (Young and Cook, 1955; Mefferd et al, 1958; Hale and Mefferd, 1958), creatinine (Treichler and Mitchell, 1941; Selye, 1946; Chinn et al) and uric acid (Chinn et al).

During the last year we conducted a series of experiments dealing with amino acid metabolism of rats exposed to 7° C for a period of three to four weeks. Some of the results are presented in this paper.

There are a number of reports in the literature indicating increases in enzyme activities of cold-exposed animals. However, some of these increases can be assumed to be substrate-induced by virtue of the increased general flow of metabolites over certain pathways, since the cold-exposed animals increase appreciably their food intake. To test this hypothesis, we set up an experiment in which the cold-exposed animals were allowed to eat only as much protein as their warm mates, while their extra energy requirement was obtained from carbohydrate or fat. The diet employed consisted of 20% casein, 72% sucrose, 4% corn oil and was supplemented with adequate amounts of vitamins and 4% minerals. After a 4-week cold exposure, the rats were sacrificed and all of the livers assayed for specific enzymes involved in amino acid metabolism. The data obtained are summarized in Table I.

It will be noted that the animals in Group 2 consumed over 44% more food than the warm rats in Group 1. Furthermore, the results of the arginase assay indicate that the enzyme activity was not affected directly by the cold stress (Group 1 vs Groups 3 and 4). In contrast, the arginase activity was markedly increased (by 29%) in Group 2, apparently as a result of a higher protein intake. The same is also true of the glutamic-oxalacetic and glutamic-pyruvic transaminase. These data demonstrate that the increased activities of arginase and of both transaminases are related to a higher protein intake per se.

In contrast, the activities of tryptophan peroxidase and tyrosine-alpha-ketoglutaric acid transaminase were found to be increased directly by the cold stress and not by an increased amount of food consumed. These cold-induced adaptive changes are probably mediated by adrenocorticoids.

These data thus clearly demonstrate that some increases in enzyme activity of cold-exposed animals are substrate-induced rather than a direct effect of cold.

Effect of cold exposure on several selected metabolites associated with the urea cycle is shown in Table II.

It will be noted that the arginase activity of the cold-exposed animals was again higher than that of the warm controls, apparently as a result of a higher protein intake in cold. However, the arginine added to the casein diet had no additional effect on arginase activity.

TABLE I

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

	1	Treatment Group			4
		2	3	Pair-fed Cold	
	Warm	Ad. Lib.	+ Sugar	+ Fat	
Average Δ BW (gm)	122.0 $\pm$ 3.1 ¹	107.0 $\pm$ 2.0	104.0 $\pm$ 3.0	101.0 $\pm$ 2.5	
Average daily intake of complete diet (gm)	17.4	25.1	17.1	17.1	
Average daily sugar intake (gm)	-	-	6.4	-	
Average daily fat intake	-	-	-	3.1	
Arginase ²	41.6 $\pm$ 1.4	53.5 $\pm$ 1.7*	43.2 $\pm$ 1.2	40.4 $\pm$ 2.4	
Tryptophan peroxidase ³	1.9 $\pm$ 0.1	2.8 $\pm$ 0.2*	2.8 $\pm$ 0.1*	3.0 $\pm$ 0.2*	
Glutamic-oxalacetic transaminase ⁴	194.0 $\pm$ 7.3	245.0 $\pm$ 9.2*	190.0 $\pm$ 12.1	209.0 $\pm$ 5.2	
Glutamic-pyruvic transaminase ⁵	35.3 $\pm$ 2.6	47.6 $\pm$ 3.5*	31.1 $\pm$ 3.5	41.2 $\pm$ 2.6	
Tyrosine-alpha-ketoglutaric ⁶ transaminase	58.1 $\pm$ 3.6	72.3 $\pm$ 2.8*	75.4 $\pm$ 5.1*	69.0 $\pm$ 4.8*	
1 Average value of 10 animals $\pm$ standard error		4, 5 μ M DPN/gm/liver/hr			
2 mM urea formed/gm liver/hr		6 μ M p-hydroxyphenylpyruvate/gm/liver/hr			
3 μ M kynurenine/gm/liver/hr		* Difference from warm group (p<0.05)			

TABLE II

EFFECT OF COLD EXPOSURE AND/OR SUPPLEMENTAL L-ARGININE ON
SELECTED METABOLITES ASSOCIATED WITH THE UREA CYCLE

Metabolite		Treatment			
		20% Casein ¹		20% Casein + 4% L-Arginine HCl	
		Warm	Cold	Warm	Cold
Arginase	Liver (mM urea/gm/hr)	35.2 ± 2.1 ²	44.8 ± 1.9	34.9 ± 1.4	45.2 ± 2.3
	Kidney (mM urea/gm/hr)	3.1 ± 0.12	4.8 ± 0.21	3.6 ± 0.18	4.6 ± 0.19
Arginine	Liver (μg/gm)	6.3 ± 0.08	6.4 ± 0.06	25.4 ± 0.14	37.4 ± 0.14
	Kidney (μg/gm)	26.6 ± 1.11	28.1 ± 0.09	62.8 ± 1.21	70.3 ± 1.42
	Plasma (μg/100 ml)	255.1 ± 5.6	268.1 ± 8.9	442.5 ± 12.4	460.9 ± 9.3
	Urine (μg/24 hrs)	20.1 ± 1.2	39.4 ± 1.3	150.2 ± 2.5	269.3 ± 2.8
Guanido- acetic acid	Liver (μg/gm)	31.9 ± 1.3	46.3 ± 1.1	49.1 ± 1.4	68.3 ± 1.2
	Kidney (μg/gm)	41.2 ± 0.9	50.7 ± 2.2	49.2 ± 2.1	72.5 ± 1.8
	Plasma (μg/100 ml)	140.2 ± 4.2	148.3 ± 3.8	179.5 ± 1.6	203.5 ± 3.2
	Urine (μg/24 hrs)	71.8 ± 2.5	92.9 ± 1.8	150.4 ± 2.3	229.6 ± 4.6
Urea	Plasma (mg/100 ml)	72.1 ± 4.2	126.8 ± 3.8	83.6 ± 2.9	158.4 ± 4.6
	Urine (mg/24 hrs)	155.2 ± 6.8	240.8 ± 8.6	341.2 ± 10.2	504.7 ± 14.8

¹ Composition of the complete diet given in text

² Standard error of the mean for 10 rats

TABLE II

EFFECT OF COLD EXPOSURE AND/OR SUPPLEMENTAL L-ARGININE ON
SELECTED METABOLITES ASSOCIATED WITH THE UREA CYCLE

Metabolite		Treatment		20% Casein + 4% L-Arginine HCl	
		20% Casein ¹		Warm	Cold
Arginase	Liver (mM urea/gm/hr)	35.2 ± 2.1 ²	44.8 ± 1.9	34.9 ± 1.4	45.2 ± 2.3
	Kidney (mM urea/gm/hr)	3.1 ± 0.12	4.8 ± 0.21	3.6 ± 0.18	4.6 ± 0.19
Arginine	Liver (µg/gm)	6.3 ± 0.08	6.4 ± 0.06	25.4 ± 0.14	37.4 ± 0.14
	Kidney (µg/gm)	26.6 ± 1.11	28.1 ± 0.09	62.8 ± 1.21	70.3 ± 1.42
	Plasma (µg/100 ml)	255.1 ± 5.6	268.1 ± 8.9	442.5 ± 12.4	460.9 ± 9.3
	Urine (µg/24 hrs)	20.1 ± 1.2	39.4 ± 1.3	150.2 ± 2.5	269.3 ± 2.8
Guanido- acetic acid	Liver (µg/gm)	31.9 ± 1.3	46.3 ± 1.1	49.1 ± 1.4	68.3 ± 1.2
	Kidney (µg/gm)	41.2 ± 0.9	50.7 ± 2.2	49.2 ± 2.1	72.5 ± 1.8
	Plasma (µg/100 ml)	140.2 ± 4.2	148.3 ± 3.8	179.5 ± 1.6	203.5 ± 3.2
	Urine (µg/24 hrs)	71.8 ± 2.5	92.9 ± 1.8	150.4 ± 2.3	229.6 ± 4.6
Urea	Plasma (mg/100 ml)	72.1 ± 4.2	126.8 ± 3.8	83.6 ± 2.9	158.4 ± 4.6
	Urine (mg/24 hrs)	155.2 ± 6.8	240.8 ± 8.6	341.2 ± 10.2	504.7 ± 14.8

¹ Composition of the complete diet given in text² Standard error of the mean for 10 rats

TABLE II

EFFECT OF COLD EXPOSURE AND/OR SUPPLEMENTAL L-ARGININE ON
SELECTED METABOLITES ASSOCIATED WITH THE UREA CYCLE

Metabolite		20% Casein ¹		Treatment		20% Casein + 4% L-Arginine HCl
		Warm	Cold	Warm	Cold	
Arginase	Liver (mM urea/gm/hr)	35.2 ± 2.1 ²	44.8 ± 1.9	34.9 ± 1.4		45.2 ± 2.3
	Kidney (mM urea/gm/hr)	3.1 ± 0.12	4.8 ± 0.21	3.6 ± 0.18		4.6 ± 0.19
Arginine	Liver (µg/gm)	6.3 ± 0.08	6.4 ± 0.06	25.4 ± 0.14		37.4 ± 0.14
	Kidney (µg/gm)	26.6 ± 1.11	28.1 ± 0.09	62.8 ± 1.21		70.3 ± 1.42
	Plasma (µg/100 ml)	255.1 ± 5.6	268.1 ± 8.9	442.5 ± 12.4		460.9 ± 9.3
	Urine (µg/24 hrs)	20.1 ± 1.2	39.4 ± 1.3	150.2 ± 2.5		269.3 ± 2.8
Guanido- acetic acid	Liver (µg/gm)	31.9 ± 1.3	46.3 ± 1.1	49.1 ± 1.4		68.3 ± 1.2
	Kidney (µg/gm)	41.2 ± 0.9	50.7 ± 2.2	49.2 ± 2.1		72.5 ± 1.8
	Plasma (µg/100 ml)	140.2 ± 4.2	148.3 ± 3.8	179.5 ± 1.6		203.5 ± 3.2
	Urine (µg/24 hrs)	71.8 ± 2.5	92.9 ± 1.8	150.4 ± 2.3		229.6 ± 4.6
Urea	Plasma (mg/100 ml)	72.1 ± 4.2	126.8 ± 3.8	83.6 ± 2.9		158.4 ± 4.6
	Urine (mg/24 hrs)	155.2 ± 6.8	240.8 ± 8.6	341.2 ± 10.2		504.7 ± 14.8

¹ Composition of the complete diet given in text

² Standard error of the mean for 10 rats

The levels of free arginine in the liver, kidney and plasma of the animals on the basal diet were not affected by the cold exposure. In contrast, the cold-exposed animals excreted more arginine in the urine than the controls. As expected, arginine concentration in the tissues of the animals receiving supplemental arginine was markedly increased over those fed the unsupplemented diet. The levels of urea both in the plasma and the urine were also markedly increased as a result of the cold stress, regardless of the dietary treatment.

It will be noted that the level of guanidoacetic acid in the liver, kidney, and the urine of the cold-exposed animals was higher than in the controls, regardless of whether or not the diet was supplemented with arginine. This is interesting since guanidoacetic acid is a precursor of creatine and creatinine. These two metabolites are known to be excreted at higher levels in cold than at room temperature (Young and Cook, 1955; Chinn et al).

We have also found that a moderate cold stress is an effective agent in correcting and overcoming amino acid imbalances. An amino acid imbalance may be defined as any change in the proportions of the amino acids in a diet that results in an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid.

From the recent work concerning the effects of amino acid imbalances, it is obvious that the main obstacle in studying these phenomena is the refusal of animals to consume voluntarily adequate amounts of the imbalanced diet, which in turn causes a major difficulty in interpreting and extrapolating the experimental data. In an effort to overcome this problem, and thus to approach the normal physiological state of the animal, such techniques as forced-feeding (Deshpande et al, 1958), spaced-feeding (Kumta et al, 1958), or insulin injections (Spolter and Harper, 1961) have been used as means of inducing a higher food intake. However, these techniques proved either unsatisfactory or even detrimental to the animal and the results obtained may be subjected to an alternative explanation.

In view of the finding in this laboratory by Vaughan and Vaughan (1957; 1959; 1960; 1961) that appetite of cold-exposed rats, while characteristically depressed in vitamin deficiencies, was simultaneously stimulated by the low environmental temperature, it seemed to us advantageous to use cold exposure as a tool to induce voluntarily a higher intake of imbalanced diets in studying amino acid imbalances in the rat. The results of one experiment dealing with leucine toxicity and amino acid imbalances are given in Table III.

TABLE III
EFFECT OF COLD EXPOSURE AND AMINO ACID IMBALANCE
ON GROWTH AND FOOD CONSUMPTION OF RATS

Group	Diet	Δ W gm/3 weeks		Food Intake gm/day	
		Warm	Cold	Warm	Cold
1	9% Casein ¹	61.1 $\pm$ 2.4 ²	50.8 $\pm$ 6.3	11.9 $\pm$ 0.39	17.3 $\pm$ 0.23
2	9% Casein + 5% L-leucine	-7.1 $\pm$ 3.0	-3.9 $\pm$ 3.2	7.9 $\pm$ 0.32	13.8 $\pm$ 0.15
3	6% Fibrin	42.8 $\pm$ 2.8	39.6 $\pm$ 3.8	11.7 $\pm$ 0.45	17.1 $\pm$ 0.15
4	6% Fibrin amino acid mixture ³ -histidine	22.7 $\pm$ 2.7	39.0 $\pm$ 2.7	8.6 $\pm$ 0.28	16.7 $\pm$ 0.31

¹ Plus sucrose, supplemented with adequate amounts of vitamins and minerals, and containing 4% corn oil

² Standard error of the mean for 7 rats

³ Complete amino acid mixture consisted of: DL-methionine, 0.4%; DL-phenylalanine, 0.6%; L-leucine, 0.4%; DL-isoleucine, 0.4%; DL-valine, 0.7%; L-lysine HCl, 0.6%; L-arginine HCl, 0.2%; L-tryptophan, 0.2%; DL-threonine, 0.4%; L-glutamic acid, 1.0%; and L-histidine HCl, 0.4%.

The data show that an addition of 5% L-leucine to the casein basal diet caused a severe retardation of growth and appetite depression both in the warm and the cold groups. It should be noted that despite a highly toxic level of leucine in the diet, the cold-exposed animals increased their food consumption almost 75 per cent over the corresponding warm group, thus also increasing the absolute daily intake of this amino acid from approximately 395 to 690 mg. However, such an increment in leucine intake had no additional adverse effect on growth.

A similar effect of cold on food intake and growth can be seen in Group 4, in which an amino acid imbalance was induced by adding an amino acid mixture lacking histidine to the fibrin basal diet. This imbalanced diet caused a considerable decrease both in food intake and the rate of growth of the warm rats.

In contrast, the cold-exposed animals readily consumed the imbalanced diet and, consequently, grew as well as the controls (Group 3).

An imbalance produced by omitting isoleucine from the amino acid mixture was studied in another experiment shown in Table IV. Again, the lack of isoleucine (Group 2) caused a pronounced growth retardation and a depression of appetite in the warm group. However, these adverse effects were overcome to a great extent by the cold exposure. The higher food intake of the cold-exposed animals is reflected in the higher levels of the amino acid nitrogen both in the plasma and in the urine.

Incorporation of 0.4% DL-methionine and 0.6% DL-phenylalanine in the fibrin diet (Group 3) produced a severe imbalance in the warm group. On the other hand, these two amino acids failed to produce a growth depression and a decrease in food consumption in the cold-exposed animals, despite the fact that their absolute intake was increased by 90 per cent. It should be pointed out that rats forced-fed a similar diet by other investigators (Deshpande et al, 1958) died within two or three days, apparently due to their inability to metabolize the diet efficiently. In contrast, the cold-exposed animals not only tolerated a higher intake of the two imbalancing amino acids but grew as well as the controls.

The leucine-isoleucine antagonism, in which an excess of dietary leucine can act as an anti-metabolite of isoleucine, was studied in the experiment shown in Table V.

When either isoleucine (Group 2) or isoleucine and valine (Group 4) were omitted from the amino acid mixture, a severe growth depression occurred in the two warm groups. Furthermore, an omission of leucine together with isoleucine (Group 3) or leucine together with isoleucine and valine (Group 5) restored normal growth, showing that an excess of leucine is responsible for some metabolic alteration affecting appetite, which in turn leads to a depression in growth. This phenomenon was completely overcome by the cold exposure. The cold-exposed animals were able to increase food intake and performed as well as either of the controls (Group 1). As expected, the complete amino acid mixture added to the fibrin basal (Group 6) supported an excellent rate of gain.

Effect of cold exposure and the leucine-isoleucine antagonism on three selected enzymes is given in Table VI.

It will be noted that the activity of the two transaminases and of the arginase was higher in the cold controls than in the warm controls, possibly as a result of an increased food intake in the cold. An omission from the amino acid mixture of either isoleucine alone (Group 2), or isoleucine plus leucine (Group 3) or isoleucine plus valine (Group 4) resulted in a uniform increase in the activity of all three enzymes. The enzyme activity in this case was not, however, further affected by the environmental temperature.

TABLE IV

EFFECT OF COLD EXPOSURE AND AMINO ACID IMBALANCE ON GROWTH, FOOD CONSUMPTION AND URINARY AND PLASMA AMINO NITROGEN OF RATS

Group	Diet	Δ W gm/3 weeks		Food Intake gm/day		Urinary NH ₂ -N mg/24 hours		Plasma NH ₂ -N mg/100 ml	
		Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold
1	6% Fibrin	42.4±1.5 ¹	50.8±5.8	12.2±0.58	19.0±0.72	3.99±0.21	8.89±0.25 ³	7.81±0.32	10.77±0.46 ³
2	6% Fibrin + amino acid mixture ⁴ -DL-isoleucine	19.3±3.0	36.7±4.3	9.5±0.32	17.6±0.67	18.98±0.36 ²	25.78±0.41 ²	9.34±0.36 ²	10.68±0.27
3	6% Fibrin +0.4% DL-methionine +0.6% DL-phenylalanine	28.4±4.6	58.4±3.5	10.4±0.42	19.8±0.14	10.88±0.62 ²	16.12±0.57 ²	8.44±0.29	10.26±0.35

¹ Standard error of the mean for 7 rats

² Difference from fibrin group ($p < 0.05$)

³ Difference from warm group — same diet ($p < 0.05$)

⁴ Same as in Table 3

TABLE V
EFFECT OF COLD EXPOSURE AND THE LEUCINE-ISOLEUCINE
ANTAGONISM ON GROWTH AND FOOD CONSUMPTION

Group	Diet	Δ W gm/3 weeks		Food Intake gm/day	
		Warm	Cold	Warm	Cold
1	6% Fibrin	35.8 ± 1.4^1	41.3 ± 5.2	13.4 ± 0.38	18.2 ± 0.53
2	As 1 plus amino acid mixture ² -isoleucine	18.7 ± 3.3	34.5 ± 6.0	11.7 ± 0.52	18.0 ± 0.79
3	As 1 plus amino acid mix -isoleucine -leucine	37.2 ± 2.2	44.5 ± 4.3	12.8 ± 0.54	18.9 ± 0.64
4	As 1 plus amino acid mix -isoleucine -valine	18.4 ± 2.6	35.6 ± 3.0	11.7 ± 0.46	17.7 ± 0.59
5	As 1 plus amino acid mix -isoleucine -leucine -valine	32.8 ± 6.6	39.6 ± 7.0	14.5 ± 0.87	19.3 ± 0.57
6	As 1 plus complete amino acid mixture	85.7 ± 5.4	76.3 ± 5.0	15.1 ± 0.36	20.8 ± 0.22

¹ Standard error of the mean for 7 rats

² Same as in Table 3

TABLE VI
EFFECT OF COLD EXPOSURE AND THE LEUCINE-ISOLEUCINE
ANTAGONISM ON GOT, GPT AND ARGINASE ACTIVITIES

Group	Diet	Activity ($\mu\text{M}/\text{min}/100 \text{ gm BW}$)					
		Transaminase		Glutamic-pyruvic		Arginase	
		Glutamic-oxalacetic		Warm	Cold	Warm	Cold
1	6% Fibrin	510 $\pm$ 54 ¹	753 $\pm$ 54 ²	129 $\pm$ 9.7	171 $\pm$ 8.0 ²	526 $\pm$ 29	652 $\pm$ 38 ²
2	As 1 plus amino acid mix -isoleucine ⁴	718 $\pm$ 35 ³	856 $\pm$ 57	194 $\pm$ 10.3 ³	229 $\pm$ 30.2	692 $\pm$ 36 ²	718 $\pm$ 45
3	As 1 plus amino acid mix -isoleucine -leucine	788 $\pm$ 55 ³	814 $\pm$ 73	195 $\pm$ 9.3 ³	196 $\pm$ 17.9	721 $\pm$ 42 ³	695 $\pm$ 45
4	As 1 plus amino acid mix -isoleucine -valine	698 $\pm$ 66 ³	829 $\pm$ 86	191 $\pm$ 20.2 ³	191 $\pm$ 18.5	678 $\pm$ 29	701 $\pm$ 31

- 1 Standard error of the mean for 7 rats
2 Difference from warm group — same diet ($p < 0.05$)
3 Difference from fibrin group ($p < 0.05$)
4 Same as in Table 3

Although the basic mechanism underlying the phenomena presented herein remains to be determined, the results of these experiments indicate that a moderate cold stress is an effective agent in correcting and overcoming amino acid imbalances. A warm rat, which suffers a severe metabolic disorder when it consumes a diet containing an imbalanced amino acid mixture can effectively metabolize and utilize it for tissue synthesis when exposed to cold. It has been suggested that consumption of an imbalanced diet either increases the breakdown of tissue proteins or that an imbalanced diet is not properly utilized for the protein synthesis. From the data presented here it would appear that the cold-exposed animals are able to use an imbalanced diet for the formation of tissue proteins. Cold-exposed animals could possibly accomplish this step by catabolizing preferentially the imbalancing portion of the amino acid mixture and utilizing it for heat production. The remaining balanced portion of the mixture could then be effectively utilized for the protein synthesis.

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