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EFFECTS OF THERMAL ACCLIMATION ON THE LIPID METABOLISM IN THE EARTHWORM *LAMPITO MAURITII*

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Abstract—1. Changes in the total lipid and phospholipid content, the degree of unsaturation of lipids, the lipase activity, the levels of oxaloacetic, acetic and formic acids, the ketone bodies and the cholesterol content, on thermal acclimation of the tropical earthworm, *Lampito mauritii*, were studied.

2. The total fat content, the formic and oxaloacetic acid levels decreased significantly on acclimation to a decreasing temperature ($P < 0{\cdot}0001$, 0·01 and 0·05 respectively).

3. The unsaturation of lipids, the enzyme lipase activity, the levels of acetic, β-hydroxy butyric and acetoacetic acids and cholesterol showed a significant increase on cold temperature adaptation. The t values were 5·947, 5·917, 3·297, 8·354 3·015 and 12·70 respectively.

4. The percentage increase in the levels of phospholipids and acetone on acclimation to cold temperature was +10·9 and +75·8 respectively.

5. The adaptive significance of the constituents studied is discussed.

INTRODUCTION

RECENTLY, detailed studies have been carried out on the compensatory changes in the metabolism of carbohydrates and proteins on thermal acclimation in some poikilotherms (Rao, 1961, 1962, 1963a, b; Rao & Ramachandra, 1961; Rao & Saroja, 1963; Raghupathiramireddy & Rao, 1963; Saroja & Rao, 1965; Das & Prosser, 1967). The present study is an attempt at an understanding of the compensatory variations in the lipid metabolism following thermal acclimation in the hope that this may fill some of the existing gaps in our understanding of the compensatory changes that occur in the metabolism of poikilotherms following persistent temperature changes. The purpose of the present paper is to provide a picture of the sequence of events occurring in lipid metabolism on thermal acclimation by the detailed analysis of several changes such as those in the total lipid content, the unsaturation of lipids, the lipase activity, in the levels of oxaloacetic, acetic, formic, β-hydroxy butyric acid and in the ketone bodies.

MATERIALS AND METHODS

The experiments were conducted on the common earthworm *Lampito mauritii*. They were collected from the same locality periodically and kept in the laboratory in glass troughs under a small quantity of tap water with blotter pulp. The troughs were covered with wire sieves to prevent the worms escaping.

For the experimental acclimation, worms were kept at cold (20°C), warm (35°C) and normal (28 ± 1°C) temperatures for more than 15 days to get them completely acclimatized to the respective temperatures. They were fed with blotter pulp to clear out the gut contents.

The following methods were used to estimate the different constituents. Tissues were never preserved and the estimations were always made with fresh material.

For estimation of the *total lipid content*, the worm was weighed after blotting the moisture with filter paper; it was then cut into pieces and dried in a hot air oven at 110°C for 2 days. The dried material was weighed, powdered and placed in a centrifuge tube. Total lipids were extracted from this powder with 5 ml of an ether–ethanol (1 : 1) mixture. The extract was separated after centrifugation and transferred into an Erlenmeyer flask. The centrifuge tube with the residue was dried and weighed. The difference between the weight of the powder and that after extracting the lipid was taken as the weight of the fatty material extracted with ether–ethanol. Extracted fats were expressed as percentage values of unit dry weight of the worm.

For analysis of the *iodine number* (as a measure of the unsaturation of lipids), the contents of the Erlenmeyer flask used in the above experiment were titrated with sodium thiosulphate according to the Hanus iodobromide method as described by Winton & Winton (1947).

Lipase activity was calculated by the method of Colowick & Kaplan (1955a).

Phospholipid content was estimated by the column chromatographic method using activated silicic acid, developed in the Department of Biology, North Western University, Evanston, Illinois (Habibullah, 1965, personal communication).

The study on formic, acetic and β-hydroxy butyric acids was carried out following the paper chromatographic method of Mukherjee (1959). Body fluids were utilized for the analysis.

Quantitative studies of acetone and acetoacetic acid in the body fluids after deproteinization were carried out by the salicylaldehyde colorimetric method of Cnoka (1916) as described by Milton & Waters (1955) and the colorimetric method of Milton & Waters (1955) respectively.

The oxaloacetate level in the body fluids was calculated by the method of Colowick & Kaplan (1955b).

The cholesterol content in nervous tissue was analysed by the colorimetric method as given in *Micro Analysis in Medical Biochemistry*, Churchill, London, 1964, p. 83.

For measurement of the respiratory quotient (R.Q.) the acclimated and normal animals were left in a measured quantity of tap water for 30 min with the surface of the water covered with liquid paraffin to prevent the exchange of gases at the surface of the water. Samples of water taken before and after the experiment were analysed for changes in oxygen and carbon dioxide by the Scholander & Roughton (1943b) and the Scholander *et al.* (1947) microgasometric techniques respectively. The ratio of carbon dioxide eliminated to oxygen absorbed from the samples analysed was taken to be the respiratory quotient.

RESULTS AND DISCUSSION

Table 1 summarizes the statistical analysis of the data. The percentage fat content decreased on decreasing acclimation temperature. Earlier studies (Kodama & Pace, 1963) in hamsters and in rats (Steiner & Cahill, 1964) had revealed similar results, showing that such changes in lipid content on acclimation to low temperature are adaptive in their significance and would influence the metabolism at a cellular and organismic level.

Studies on *L. mauritii* (Saroja & Rao, 1965) showed that glycogen and protein content increases on acclimation to low temperature. If the glycogen stores and proteins are to be synthesized on decreasing the acclimation temperature, the only

TABLE 1—CHANGES IN THE PERCENTAGE FAT CONTENT, IODINE NUMBER, LIPASE ACTIVITY, PHOSPHOLIPID CONTENT, FATTY ACIDS, OXALOACETATE CONTENT, KETONE BODIES, AMOUNT OF CHOLESTEROL AND RESPIRATORY QUOTIENT IN NORMAL AND ACCLIMATED EARTHWORMS, *L. mauritii*

Constituents measured	20°C cold acclimated	28 ± 1°C controls	35°C warm acclimated	Level of significance between 20 and 35°C
1. Percentage fat content (calculated to dry weight)	11·19 ± 2·3 (21)	12·41 ± 2·4 (16)	14·12 ± 2·45 (13)	$t = 15·72$ $P < 0·001$
2. Iodine number	46·66* (46)	30·95* (26)	25·47* (26)	$t = 5·95$ $P < 0·01$
3. Lipase activity (lipase activity units/g wet wt.)	286·25* (25)	119·20* (17)	104·38* (17)	$t = 5·92$ $P < 0·01$
4. Percentage phospholipids	5·58 ± 0·83 (9)	5·31 ± 0·69 (10)	5·06 ± 0·73 (9)	+10·9†
5. Acetic acid (mg)/ 100 ml of body fluids	35·20 ± 11·4 (19)	36·25 ± 7·4 (17)	33·89 ± 15·6 (17)	$t = 3·3$ $P < 0·01$
6. Formic acid (mg)/ 100 ml of body fluids	23·80 ± 7·5 (18)	28·03 ± 7·2 (17)	32·32 ± 10·4 (16)	$t = 3·99$ $P < 0·01$
7. Oxalo acetate (μg)/ 100 ml of body fluids	249·4 ± 243·1 (18)	359·5 ± 63·7 (11)	415·9 ± 129 (14)	$t = 4·77$ $P < 0·05$
8. β-Hydroxy butyric acid (mg)/ 100 ml of body fluids	56·23 ± 7·5 (18)	58·56 ± 15·6 (9)	48·42 ± 7·8 (19)	$t = 8·35$ $P < 0·001$
9. Acetone (mg)/ 100 ml of body fluids	15·31* (14)	19·33* (7)	8·71* (14)	+75·88†
10. Aceto-acetic acid (mg)/ 100 ml of body fluids	507 ± 175·8 (11)	305·4 ± 49·4 (8)	246·8 ± 47·2 (7)	$t = 3·02$ $P < 0·01$
11. Cholesterol (mg)/ gram weight of nerve tissue	31·47 ± 10·9 (13)	23·51 ± 11·3 (7)	16·95 ± 8·1 (18)	$t = 12·7$ $P < 0·001$
12. Respiratory quotient (R.Q.)	0·7345 (4)	0·9953 (6)	0·8490 (4)	

All values are mean ± S.D. Numbers in parentheses are the number of observations.

* Standard deviation is not calculated as the values are weight dependent.

† Values represent the percentage change.

metabolite (in the absence of feeding) left to supply the energy need is the endogenous fat. Moreover, there is considerable evidence in the literature which shows that on decreasing the acclimation temperature the metabolic rate is enhanced. If the metabolic rate and the glycogen and protein stores were to be increased, then endogenous fat would be mobilized and utilized preferentially to supply the augmented energy needs.

Unsaturation imparts a low melting point and high fluidity to the corresponding glyceride because of the presence of the double-bonded or triple-bonded carbon in the middle of the chain. It can be supposed that this feature has a role in the mechanism of thermal adaptation and acclimatization (Belehradek, 1963).

On decreasing the acclimation temperature, though unsaturation increases, imparting a higher level of reactivity and fluidity to the triglycerides, these more active triglycerides are apparently not utilized as they are required to maintain the specific liquid–crystalline state of the cellular membranes in order to permit normal functioning at a lower acclimation temperature (Johnston & Roots, 1964). Likewise, Kodama & Pace (1963) presumed that on cold acclimation highly saturated triglycerides are utilized preferentially, rendering the remaining fats more unsaturated, possibly for the maintenance of a specific liquid–crystalline state of the cellular membranes.

The significant increase in lipase activity on decreasing the acclimation temperature may be presumed to be due to the stimulation of intracellular lipolysis, probably by the activation of lipolytic enzyme systems (Hollett, 1964). It can also be conceived that on low temperature acclimation the "fat mobilizing substance" (FMS) primarily mobilizes fatty acids from adipose tissue by directly augmenting the lipolytic action (Cahill *et al.*, 1961).

The increase in the phospholipid content on low temperature acclimation may presumably be due to the fact that the precursors, glycerol and α-glycerophosphate, may be available in surplus so as to favour its synthesis. From the preceding findings it is seen that on cold acclimation hydrolysis of lipids occurs at a rapid rate thus providing fatty acids and glycerol. In all probability this glycerol may act as a precursor for phospholipid synthesis.

The decrease in formic acid on acclimation to a decreased temperature may be due to its greater extent of oxidation in order to meet augmented metabolic needs and to allow the desired increase in unsaturation.

The increase investigated in the acetoacetic acid on decreasing the acclimation temperature is presumably due to the fact that the precursor, acetyl CoA, may be available in surplus (produced owing to a higher rate of hydrolysis of triglycerides and an increased β-oxidation of fatty acids on cold adaptation) so as to favour its increased production. The significant decrease in the levels of acetone and β-hydroxy butyric acid on cold acclimation compared to controls may be due to the fact that though the acetoacetic acid content increases, it may not have been reduced to β-hydroxy butyric acid or decarboxylated to acetone, or the precursor acetyl CoA may have been diverted towards the production of acetylcholine or cholesterol. In support of this view an increment in the levels of these two substances (Ach

and Cholesterol) was observed on cold acclimation (Saroja, 1962; Smith & Hoijer, 1962).

The results of the present study revealed that the oxaloacetate content was less on lowered acclimation temperature. This decrease is explained as follows. On acclimation to low temperature a greater amount of acetate (produced owing to augmented β-oxidation and also from carbohydrate metabolism) enters the TCA cycle. This condenses with the oxaloacetate produced from the carbohydrate metabolism to form citrate. Consequently, by the time the cycle completes its course, some of it may be lost and the replenished amount may be taken to be less in quantity. Thus with such repeated operations of the cycle, more and more of the oxaloacetate may be lost, thus leading to a lesser amount of oxaloacetate than could be estimated.

The reason for the enhancement of the level of cholesterol in the nervous tissue on decreasing the acclimation temperature may be owing to the fact that cold exposure could probably act as a stimulus for its synthesis from its precursors (Smith & Hoijer, 1962). It can be suggested that the cholesterol thus synthesized may act as the main if not the only precursor for the synthesis of a corticosteroid-like hormone, which may in turn presumably be responsible for the mobilization of fat from depot tissues, to meet the augmented metabolic needs on cold adaptation.

The decrease of R.Q. on exposure to low temperatures may be interpreted to mean that on acclimation to low temperatures, the metabolite having a greater lipid content is most probably metabolized, as the R.Q. of fats in general has a value approximately 0·7.

GENERAL CONCLUSIONS

Thus, knowing the multiple changes occurring in the lipid metabolism in the compensatory direction, it would be of interest to consider the factors that initiate these changes and once initiated how these changes are regulated and co-ordinated in the organism.

The likely sequence of events may be suggested to be as follows:

1. It may be presumed that the thermal stress (lowering in the temperature) resulting in decreased metabolism arouses the central nervous system and triggers the release of one or more humoral agents (perhaps from the activated neuro-secretory cells) into the body fluids (Rao & Saroja, 1963).

2. One of the released humoral agents may be a fat mobilizing substance (FMS) which, once released into the body fluids, might mobilize preferentially the saturated triglycerides.

3. The mobilized saturated triglycerides may now be suggested to undergo hydrolysis by the increased lipolysis. The increase in lipolysis either may be owing to an increased enzyme protein synthesis or may be owing to an increase in ascorbic acid content or both. As an alternative possibility it can also be supposed that the presumed neurohumoral control system responsible for the mobilization of fat depots may perhaps act by stimulating intracellular lipolysis, most probably by the activation of lipolytic enzyme systems within the cell.

4. Thus by the increased lipase action when adequate amounts of fatty acids and glycerol are produced, these may be presumed to undergo further operating steps. Of the two, glycerol may be suggested to be utilized for the increased synthesis of phospholipids. This in turn may have the role of not only assuring the functional integrity of the mitochondria, but may also have a part in increasing lipolysis and in accelerating β-oxidation of released fatty acids.

5. Thus, when the β-oxidation is increased owing to increased phospholipids and also owing to increased production of fatty acids (by increased lipolysis), it is permissible to suggest that acetyl CoA is produced in a surplus amount. This acetyl CoA may in turn be utilized for the production of cholesterol, the precursor for the adrenocorticotropic-like hormone which is responsible for the mobilization of depot fats.

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Key Word Index—Temperature acclimation; fat mobilization; unsaturation of lipids; lipolysis; metabolic rate; thermal stress; adaptive significance; humoral agents; saturated triglycerides; stimulus; oxidation; neurohumoral control system; *Lampito mauritii*.