tentatively suggested that the additional energy of respiration is utilized for nonphosphorylating oxidation and is slowly dissipated in the dissues in the form of heat.

Further investigations are needed for a complete explanation of the physiological significance of the correlation between respiration rate and oxygen supply. The conditions under which this relationship was observed were far from physiological. Although it has been reported that a similar relationship was observed in response to an increase in the coronary flow under physiological conditions [5, 6, 14], these reports require verification. However, it is clear even now that the view that oxygen consumption is independent of supply is not true under all conditions. Oxygen consumption may depend on oxygen supply when the tissue oxygen consumption is unchanged.

LITERATURE CITED


1. Yu. S. Alyukhin, Fiziol. Zh. SSSR, 61, 749 (1975).
2. Yu. S. Alyukhin, Fiziol. Zh. SSSR, 64, 1605 (1978).
3. Yu. S. Alyukhin, Fiziol. Zh. SSSR, 65, 67 (1979).
4. G. Arnold, F. Kosche, E. Miessner, et al., Pflüg. Arch., 299, 339 (1968).
5. R. Gorlin, N. Brachfeld, P. Bopp, and C. McLeod, J. Clin. Invest., 37, 898 (1958).
6. R. Gorlin, N. Brachfeld, J. V. Messer, and J. D. Turner, Am. Intern. Med., 57, 698 (1959).
7. D. E. Gregg, in: Circulation. Proceedings of the Harvey Tercentenary Congress, London (1958), p. 163.
8. B. Grubb and G. E. Folk, Jr., J. Comp. Physiol., B-128, 185 (1978).
9. I. Gutmann and A. W. Wahlefeld, in: Methods of Enzymatic Analysis, New York (1974), p. 1464.
10. C. R. Honig, J. L. Fierson, and C. N. Nelson, Amer. J. Physiol., 220, 357 (1971).
11. W. Lochner, G. Arnold, and E. R. Müller-Ruchholtz, Amer. J. Cardiol., 22, 299 (1968).
12. J. R. Pappenheimer, J. Physiol. (London), 99, 182 (1941).
13. J. Piiper, P. E. Di Prampero, and P. Cerretelli, Pflüg, Arch., 311, 312 (1969).
14. M. Tauchert, Basic Res. Cardiol., 68, 183 (1973).
15. F. Verzar, J. Physiol. (London), 44, 243 (1912).


## MYOCARDIAL METABOLISM ON INDUCTION AND PROLONGATION OF ARTIFICIAL HYPOBIOSIS


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Disturbances of cardiac activity occupy a leading place among disorders of the vital functions of animals and man exposed to deep hypothermia [3, 6, 7, 10]. Basic factors in the pathogenesis of these disturbances are changes in energy metabolism of the myocardium [8, 10]. Mobilization of energy-yielding substrates and stimulation of the initial stages of their utilization under the influence of cold on the body are largely determined by the activating influence of the sympathicoadrenal system [6, 10]. Meanwhile it has been shown that preliminary exhaustion of the catecholamine reserves leads to depression of metabolism and to a corresponding decrease in heat production in response to cooling. This, in turn, had a beneficial effect on the production of long-term stable hypothermia, to which the name artificial hypobiosis has been given [11].


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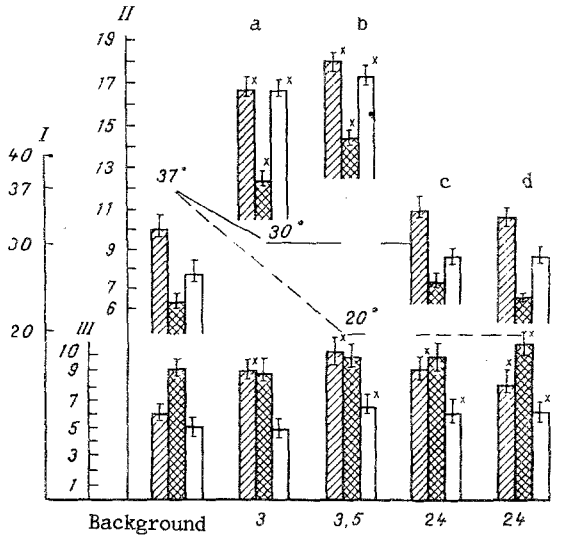


Fig. 1. Glucose and UEFA concentrations in total lipids in blood and myocardium in course of artificial hypobiosis. Abscissa, time (in h); ordinate: I) $T_b$ (in °C); II) glucose concentration (in mM); III) UEFA concentration (in %/g lipid); oblique shading, arterial blood, cross-hatching, venous blood. a) Cooling to 30°C (3 h); b) cooling to 20°C (3.5 h); c) prolongation of hypobiosis at $T_b$ = 30°C (24 h); d) prolongation of hypobiosis at $T_b$ = 20°C (24 h). Asterisk indicates that differences relative to background are significant ($P < 0.05$).

The aim of the present investigation was to study the time course of the principal substrates of energy metabolism (unsaturated esterified fatty acids (UEFA) and glucose) and the fatty acid spectrum of the myocardium and blood, using a model of artificial hypobiosis (depression of activity of the catecholaminergic systems of the body) [12].

## METHODS

Experiments were carried out on 122 noninbred male rats weighing 180-220 g. Artificial hypobiosis was created by injection of rausedil (0.4 mg/kg) 18-20 h before the experiment and 5-hydroxytryptophan (5-HTP) in a dose of 100 mg/kg 1 h before the experiment, followed by fixation of the animals, which were placed in a heat chamber at an ambient temperature ($T_{amb}$) of 5°C until their body temperature ($T_b$) had fallen to 30 or 20°C [12]. Hypobiosis was maintained for 24 h when $T_{amb}$ was 18-19°C. $T_b$ was measured in the rectum at a depth of 3.5 cm every 30 min by a TPÉM-1 electrothermometer until it stabilized at the desired level. Tests were carried out on intact animals (initial level), 18-20 h after premedication with rausedil, at the moment when $T_b$ fell to 30°C for 24 h under the temperature condtions were recorded on the ÉÉGP-4-02 electroencephalograph. Blood was taken under pentobarbital anesthesia (30 mg/kg) by catheterization of the common carotid artery (arterial blood — A) and of the right atrium through the jugular vein (venous blood — V). The blood glucose concentration was determined by Nelson's method [9] and UEFA in the blood and tissues was determined by Duncombe's method [2]. Considering the importance of the degree of saturation of UEFA for increasing the resistance of cell membranes to a low $T_b$, the fatty acid composition of the total lipids was analyzed by gas-liquid chromatography on the Tsvet'-5 chromatograph [2]. The results were subjected to statistical analysis.

## RESULTS

Placing the animals, when immobilized after premedication with rausedil and 5-HTP, in the heat chamber led to a fall of $T_b$ to 30°C (Fig. 1) in 3 ± 0.4 h (series I). It will be clear from Fig. 1 that in this period an increase was observed in the blood glucose con-

TABLE 1. Changes in HR, RR, and $T_b$ in Artificial Hypobiosis

| Parameter | Initial level | 18 h after injection of rausedil | Series of experiments | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| $T_b$, °C | 37,8±0,09 | 37,4±0,2 | 30,2±0,45* | 20,8±0,9* | 29,4±0* | 20,2±0,3* |
| HR, beats/min | 494±23 | 408±16* | 278±24* | 160±15* | 261±25* | 126±25* |
| RR, cycles/min | 110±13 | 92±10* | 82±9* | 58±4* | 82±5* | 45±2* |

Legend. *P < 0.05 compared with initial level.

TABLE 2. Fatty Acid Spectrum of Total Lipids of Arterial Blood (A), Myocardium (M), and Venous Blood (V) in course of Artificial Hypobiosis (M ± m)

| Series of experiments | Tissue | Fatty acid | | | | | |
|---|---|---|---|---|---|---|---|
| | | palmitic (16:0) | palmito-oleic (16:1) | stearic (18:0) | oleic (18:1) | linoleic (18:2) | arachidonic (20:4) |
| Initial level | A | 29,96±0,70 | 4,63±0,30 | 18,36±0,30 | 23,42±1,20 | 24,26±1,10 | 5,34±1,40 |
| | M | 14,87±1,30 | 2,23±0,12 | 19,80±1,20 | 17,41±0,60 | 30,75±2,40 | 4,95±0,31 |
| | V | 22,98±1,22 | 4,71±0,42 | 21,27±0,80 | 23,35±0,51 | 22,02±2,32 | 2,75±1,70 |
| 18 h after injection of rausedil I | A | 32,23±0,70 | 0,82±0,31* | 21,65±0,30* | 21,88±1,20 | 13,88±1,11* | 7,32±1,40* |
| | M | 12,90±1,31 | 3,13±0,12* | 21,38±1,20 | 10,38±0,60* | 13,80±2,40* | 3,33±0,30* |
| | V | 30,14±1,20* | 2,37±0,40* | 27,52±0,80* | 23,87±0,52 | 11,00±2,31* | 7,08±0,70* |
| II | A | 27,85±0,4** | 2,77±0,90*** | 21,95±0,6* | 31,16±0,5*** | 15,16±1,2* | 1,10±0,9*** |
| | M | 15,59±0,60 | 4,35±0,1*** | 26,34±0,6*** | 23,47±0,2*** | 24,69±0,9*** | 8,02±0,4*** |
| | V | 25,95±0,30*** | 1,72±0,20* | 23,00±0,30** | 27,90±0,40*** | 19,55±1,20*** | 1,85±0,34*** |
| III | A | 32,47±2,40- | 3,21±1,20*** | 24,22±1,20*** | 25,70±1,30*** | 12,43±0,70* | 1,82±1,20*** |
| | M | 18,90±1,40*** | 0,82±0,30*** | 27,31±0,80*** | 24,62±3,60*** | 21,87±2,40*** | 8,06±2,20*** |
| | V | 27,13±1,30* | 5,16±0,60** | 19,02±2,20** | 31,29±0,90*** | 15,35±1,20*** | 1,98±0,90*** |
| IV | A | 31,22±0,73 | 4,04±0,33** | 26,65±0,30*** | 28,02±0,22*** | 9,89±0,70*** | 0,28±0,11*** |
| | M | 14,46±0,10 | 0,90±0,04*** | 29,03±1,80*** | 12,72±0,80* | 29,54±2,60** | 16,43±1,10*** |
| | V | 32,32±1,30* | 2,56±0,72* | 23,26±0,34** | 25,98±0,52* | 14,15±1,20* | 1,15±0,45*** |
| | A | 29,98±3,90 | 1,80±0,48* | 25,64±2,09*** | 28,03±2,30*** | 14,77±1,50* | 1,84±0,70*** |
| | M | 24,67±1,20*** | 1,88±0,40** | 28,98±0,60*** | 23,72±0,90*** | 21,53±2,40*** | 6,09±0,20*** |
| | V | 28,02±1,60* | 3,36±0,60* | 26,85±0,90* | 26,16±1,56*** | 13,99±0,80*** | 1,78±0,30*** |

Legend. Fatty acid content in total lipids shown (in %). *P < 0.05 compared with initial level, **P < 0.05 compared with results obtained 18 h after injection of rausefil, ***) combination of significances indicated by one and two asterisks.

centration, which was greater in venous than in arterial blood (by 2 and 1.5 times respectively), thus causing disappearance of the arteriovenous difference. The glucose level in the myocardial tissue also rose to twice its original value. Meanwhile the UEFA concentration rose only in arterial blood, and this was accompanied by a significant increase in the arteriovenous difference for UEFA.

In the experiments of series II $T_b$ of the rats reached 20°C 3.6 ± 0.3 h after they had been placed in the heat chamber. Under conditions of such deep hypothermia the glucose level continued to rise relative to its initial value. The blood UEFA level increased even more, and the arteriovenous differences was almost doubled. Incidentally, despite the considerable difference of $T_b$ in the rats of series I and II, there was no significant difference in the changes in the substrates tested between animals of these two groups, with the exception of the glucose concentration in the myocardium ($P < 0.05$).

The results are evidence that, despite preliminary exhaustion of the catecholamine reserves, in the early stages of development of hypobiosis release of the principal energy-yielding substrate is activated, although to a lesser degree than is characteristic of the thermoregulatory response to acute cooling in intact animals [8, 14]. In this case, however, the response was not accompanied by tachycardia or tachypnea but, on the contrary, significant (by more than half) slowing of the heart rate (HR) and respiration rate (RR) was observed (Table 1). The changes observed can probably be explained by the sharp fall of the catecholamine levels and by hypometabolism, which correlates positively with $T_b$ [11, 13].

After hypobiosis had been maintained for 24 h (series III and IV) the glucose concentration in the myocardium and arterial and venous blood fell, although it remained a little above the initial level, and the arteriovenous difference was preserved (Fig. 1). Despite the marked bradycardia, reflecting the degree of hypometabolism [11], the UEFA concentration in the myocardium and arterial and venous blood, and also the arteriovenous difference

for UEFA remained high. Differences between parameters in series III and IV, just as in the early stages of hypobiosis, were not significant. The data suggest that during prolongation of hypobiosis at temperatures of between 30 and 20°C the importance of fatty acids in the energy supply of the myocardium increases. This can evidently be explained on the grounds that oxidation predominantly of fatty acids enables energy production (necessary to maintain adequate cardiac activity) to be intensified due to activation of the flow of acetylcoenzyme A (AcCoA) aimed at maintaining the essential level of metabolites of the Krebs cycle [4, 13].

Analysis of the fatty acid spectrum of lipids given in Table 2 showed a significnat change after rausedil even during normothermia. A decrease in the concentrations of palmito-oleic and linoleic acids accompanied by a significant increase in the concentrations of stearic and arachidonic acids in arterial and venous blood, and a significant increase in the palmitic acid concentration in venous blood were observed. In the myocardium the palmito-oleic acid level rose, whereas levels of oleic, linoleic, and arachidonic acids fell. Later, levels of linoleic and palmito-oleic acids were maintained at lower than initial values in the tissues studied in animals of all series (except series I). The relative percentage concentration of oleic acid, on the other hand, was raised in all series except III, in which prolongation of hypobiosis at $T_b$ = 30°C caused a deficiency of this acid in the myocardium. By contrast with acute hypothermia [8, 14], hypobiosis was characterized by an increase in the concentrations of saturated fatty acids (stearic and palmitic), which was observed even during normothermia after injection of rausefil. At the same time, the myocardial tissues contained a high concentration of arachidonic acid, which is necessary to increase the stability of cell membranes at a low $T_b$ [5]. Meanwhile a sharp fall was observed in its blood level (on many chromatograms arachidonic acid could not be identified at all), evidently due to its involvement in protaglandin synthesis [1].

The induction and maintenance of artificial hypobiosis at $T_b$ = 30-20°C, after preliminary depression of activity of the sympathiocoadrenal system, are thus accompanied by an increase in the contribution of UEFA to the energy supply of the myocardium and by changes in the fatty acid composition of myocardial and blood lipids, which differ from those observed in acute hypothermia.

LITERATURE CITED


1. I. S. Azhgikhin, in: Prostaglandins [in Russian], Moscow (1978), pp. 6-84.
2. E. K. Alimova et al., Textbook of Methods in Biochemistry [in Russian], Rostov-on-Don (1973).
3. G. A. Akimov, N. V. Alishev, V. A. Bershtein, and A. V. Bukov, Whole-Body Cooling [in Russian], Leningrad (1977).
4. M. N. Kondrashova and E. I. Maevskii, in: Regulation of Energy Metabolism and the Physiological State of the Organism [in Russian], Moscow (1978), pp. 5-15.
5. E. M. Kreps, Lipids of Cell Membranes [in Russian], Leningrad (1981).
6. E. V. Maistrakh, in: Physiology of Temperature Regulation [in Russian], Leningrad (1984), pp. 181-222.
7. V. M. Pokrovskii, Yu. R. Sheikh-Zade, and V. V. Vovereidt, The Heart in Hypothermia [in Russian], Leningrad (1984).
8. I. K. Prostov, in: Proceedings of the 2nd All-Union Congress of Pathophysiologists [in Russian], Vol. 21, Tashkent (1976), pp. 121-122.
9. M. N. Prokhorov and Z. N. Tupikova, Large Textbook of Practical Carbohydrate and Lipid Metabolism [in Russian], Leningrad (1965).
10. B. A. Saakov, in: Pathological Physiology of Extremal States [in Russian], Moscow (1973), pp. 237-268.
11. N. N. Timofeev, Artificial Hypobiosis [in Russian], Moscow (1983).
12. N. N. Timofeev, V. V. Ivleva, et al., Inventors' Certificate No. 1091213 (USSR), Otkrytiya, No. 17 (1984).
13. A. J. Liedtke, Prog. Cardiovasc. Dis., 23, 321 (1981).
14. E. Masoro, Physiol. Rev., 46, 67 (1966).