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# EFFECT OF CULTURE AT SUBOPTIMAL TEMPERATURE ON DEHYDROGENASE ACTIVITY OF BLOOD MONOCYTES FROM HEALTHY SUBJECTS AND LEPROSY PATIENTS

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The structure of the energy metabolism of macrophages and their precursors (monocytes) largely determines the functional properties of these cells [4]. The study of the mononuclear phagocytic system in leprosy is particularly important because the agent of this disease is an obligate parasite of macrophages. Functions of blood monocytes, precursors of macrophages, have been studied intensively in recent years. It has been found that blood monocytes of patients with the lepromatous type of leprosy differ from monocytes of healthy subjects and patients with other chronic diseases in their ability to carry out phagocytosis and lysis of certain bacteria [5], in their interaction with lymphocytes [3, 10], their ability to reduce nitro-blue tetrazolium (nitro-BT test) [8], and their activity in endocytosis [9].

The aim of the present investigations was to study the effect of culture at suboptimal temperatures on oxidative metabolism of blood monocytes from healthy subjects and patients with leprosy. Existing views on the affinity of *Mycobacterium leprae* for the coldest parts of the body served as the starting point: Skin lesions as a rule begin on the lobes of the ears and the dorsal surfaces of the hands and feet [2], the skin temperature on lepromatous lesions is lower than that of evidently healthy skin, in mice *M. leprae* reproduces most intensively in the foot pads [11], and the nine-banded armadillo, which has a relatively low body temperature, has been shown to be a promising animal for reproduction of experimental leprosy [7]. Information on the effect of low temperatures on enzyme activity of blood monocytes of man and animals could not be found in the accessible literature. There are reports [6] that lowering the temperature to 32°C inhibits (compared with 35-37°C) the response of lymphocytes to mitogenic stimulation. According to the authors cited, lowering the temperature of some areas of skin (of the limbs, for example) is a factor which causes insufficiency of cellular immunity, and this mechanism may be the reason why infection is localized in cooled areas of the body.

## EXPERIMENTAL METHOD

Heparinized venous blood was obtained from 15 healthy blood donors and 20 patients with the lepromatous type of leprosy, in the stage of active disease. Mononuclear cells were isolated by fractionation in a Ficoll—Isopaque gradient (Pharmacia, Sweden). The monocytes were incubated on coverslips in medium 199 in Leighton's tubes for 48 h at 37 and 25°C. This last temperature was chosen because of evidence to show that multiplication of various cells

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in animals and man can take place if the culture temperature falls to 25°C [1]. After incubation and rinsing in Hanks' solution a monolayer consisting chiefly of monocytes remained on the coverslips. Eight coverslips were obtained from each healthy donor and each leprosy patient: four were incubated at 37°C and four at 25°C. Control coverslips were stained by the Romanovsky–Giemsa method, and histochemical tests were carried out on three coverslips for succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PDH). The intensity of the enzyme reaction was assessed with the SMP-01 scanning microscope-photometer (Opton, West Germany). Measurements were made in monochromatic light with a wavelength of 540 nm, that usually adopted for photometry of formazan reactions, and the thickness of the probe was 1 μ. Photometry was carried out in each case on 25 to 50 cells and the parameter of total extinction of an individual monocyte was determined. The results were processed on the "Wang-720 C" computer linked to the microscope-photometer. To determine the relative activity of the different stages of biological oxidation, the relative percentage content of dehydrogenases was calculated (total extinction of SDH + LDH + G6PDH was taken as 100%). Coefficients characterizing the ratio of aerobic to anaerobic oxidation (coefficient I = SDH/LDH) and the relative importance of the pentose phosphate shunt [coefficient II = (SDH + LDH)/G6PDH] also were calculated.

## EXPERIMENTAL RESULTS

High activity of the pentose phosphate shunt was observed in healthy human monocytes cultured at 37°C, in which it accounted for up to 50% of relative activity. Activity of glycolysis (31%) and the Krebs' cycle (18.7%) was weaker. The ratio of aerobic to anaerobic oxidation showed some predominance of the latter (coefficient I = 0.73). The relative importance of the pentose phosphate shunt was high (coefficient II = 1.23). Lowering the culture temperature to 25°C was accompanied by a marked change in ratio between activities of the key dehydrogenases in normal human monocytes. This was reflected in a considerable increase (almost twofold) in LDH activity and a small decrease in SDH and G6PDH activity. Coefficient I was reduced by two thirds (from 0.73 to 0.25), indicating considerable predominance of glycolysis over aerobic oxidation. The sharp increase in coefficient II (from 1.23 to 3.03) reflected a fall in the relative importance of the pentose phosphate shunt.

Glycolytic activity (LDH) was most marked in blood monocytes from patients with the lepromatous type of leprosy, and activity of the pentose phosphate shunt (G6PDH) was rather lower. The relative activity of SDH, marker enzyme of the Krebs' cycle, was only 12%. Coefficient I revealed definite predominance of glycolysis over aerobic oxidation, whereas coefficient II showed a relatively low contribution of the pentose phosphate shunt to oxidation–reduction processes in the monocyte.

Comparison with values obtained in healthy subjects at the same culture temperture (37°C) shows that in active lepromatous leprosy there is a marked increase (more than twofold) in

TABLE 1. Values of Dehydrogenase Activity of Monocytes from Healthy Blood Donors and Leprosy Patients, Cultured at Different Temperatures (M ± m)

| Culture temperature, °C | Enzyme | Healthy blood donors | | Leprosy patients | |
|---|---|---|---|---|---|
| | | total extinction | content in percent | total extinction | content in percent |
| 37 | SDH | 17,10±4,15 | 18,74±4,13 | 19,05±2,41 | 12,92±1,03 |
| | LDH | 36,07±7,75 | 31,04±4,86 | 74,49±3,47* | 51,42±0,26* |
| | G6PDH | 51,37±6,13 | 50,24±3,47 | 51,45±2,19 | 35,63±0,86* |
| | SDH/LDH (coefficient I) | 0,73±0,05 | | 0,24±0,02 | |
| | (SDH + LDH)/G6PDH (coefficient II) | 1,23±0,11 | | 1,84±0,09 | |
| 25 | SDH | 12,43±2,38 | 12,91±2,76 | 19,88±1,18 | 12,01±1,16 |
| | LDH | 70,49±9,68 | 58,58±3,88 | 86,20±3,28 | 52,70±1,92 |
| | G6PDH | 34,40±4,67 | 30,42±2,86 | 57,57±3,69* | 35,11±1,85 |
| | SDH/LDH (coefficient I) | 0,25±0,04 | | 0,23±0,03 | |
| | (SDH+LDH)/G6PDH (coefficient II) | 3,03±0,52 | | 1,83±0,10 | |

*P < 0.05 compared with the control.

LDH activity whereas G6PDH activity was reduced by one third. This change in the activity of the key dehydrogenase abruptly disturbs the balance of the monocyte's energy metabolism in patients with leprosy, bringing it closer to that observed in healthy human monocytes cultured at a suboptimal temperature (25°C). Meanwhile, lowering the culture temperature for monocytes from leprosy patients had virtually no effect on activity of the key dehydrogenases of the monocyte. This was shown both by analysis of absolute values and when the relative activity and coefficients I and II were calculated (Table 1).

It can be tentatively suggested that the fall of temperature was a stimulating factor causing certain changes in the energy balance of the blood monocyte, reflected in the predominance of glycolysis, with LDH activity as its marker. Blood monocytes from leprosy patients had values of enzyme activity which were apparently already stimulated by the lower temperature. Similar results for monocytes of leprosy patients were obtained by the use of the nitro-BT test [8].

These investigations thus yielded comparative photometric data on the effect of the culture temperature on oxidative metabolism of blood monocytes from normal subjects and patients with leprosy.

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