INTRODUCTION

Probably the most well-studied cell is the erythrocyte. Glycolysis, almost uncontaminated by alternate metabolic pathways, accounts for almost 90% of its metabolism; and control mechanisms of glycolysis in the red blood cell are comparatively well understood (1). While it is true that lactate is both produced and consumed by liver, muscle, and perhaps other organs, a major contribution to blood lactate content in man is made by red and white blood cell glycolysis (2).

These facts and previous observations in this laboratory and others (3, 4) of decline of blood lactate concentration in acute, behaviorally induced rest status in man suggested that rate of whole blood metabolism may be affected by behavioral state and that, in particular, erythrocyte metabolism may decline in these acute hypometabolic states. If this were true, the well understood nature of the red blood cell makes it an excellent probe for in depth study of the metabolic changes underlying these and possibly other forms of behavior, since this cell is believed to typify, in many respects, the membrane (5), the metabolism, and the metabolic control (1, 6) of more advanced tissue such as nerve and muscle. Also, since oxygen transport to tissue is closely related to the metabolic status of the erythrocyte (1), it may directly participate in the hypometabolism characterizing these behavioral modalities.

In this report we study the effect of the practice known as the “Transcendental Meditation technique” (TM) on blood cell metabolism. This behavior was selected for study because acute change of blood lactate had been previously noted (3); other relevant physiologic and behavioral changes in this state are well characterized including hormonal, circulatory, and blood gas changes (3, 7); and prac-
tioners representing a large and relatively homogeneous group in terms of method and regularity of induction of a behavioral state are readily available for study (8).

METHOD

SUBJECTS—Twenty-four normal adults, ages 22–40, with at least six years of experience in this technique, all of whom were instructors of TM, were studied. These individuals regularly elicit this behavior twice daily.

PROCEDURE—As far as possible, subjects were observed at the same time between 12 and 1 P.M. in a comfortably seated arrangement. Subjects were postabsorptive since midnight. Uniform conditions of reasonably quiet, low-level lighting, and a constant, comfortable room temperature were maintained; these conditions approximate normal circumstances of TM practice. Previous to the day of the experiment, there were at least two accommodation periods (leads for electrophysiologic monitoring were attached) and the subject was asked to rest or practice TM in the laboratory situation (described in the next paragraph).

To minimize effect of testing and interaction of testing with practice, an experimental design similar to one recommended by Campbell and Stanley (9) was used—a design which does not employ a pretest period. Subjects were studied on two occasions, one week apart. On one occasion, the subjects were asked to close their eyes and practice TM for 45 minutes, followed by a recovery, eyes-open period of 30 minutes. On the other occasion, subjects were asked to read a "relaxing" selection of their own choice for 45 minutes, followed by a recovery, eyes-open period of 30 minutes.

Prior to the beginning measurements, an arterial catheter (1½" 20 G Longdwell) was placed percutaneously into a radial artery. The subject did not see insertion of the catheter, since his arm was placed through a slit curtain and the actual insertion was painless after local anesthesia was achieved with 1% xylocaine. After insertion, there was a 2½-hour wait period, during which the subject was free to move; this period was necessary because venipuncture is associated with large alterations in blood constituents such as plasma free amino and free fatty acid with return to basal levels generally assured after one hour (10).

To monitor occurrence of sleep, a unipolar electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG) were recorded during practice (and reading) periods. As indication of cognitive relaxation, phasic electrodermal response (EDR) was also measured (11). Eighteen ml arterial blood samples were taken every 15 minutes into heparinized syringes throughout practice and post-practice periods at 0, 15, 30, 45, 60, and 75 minutes for determination of rate of blood lactate generation, blood gases (Radiometer ABRL1 Blood Gas Laboratory), lactate concentration, glucose (12), and hematocrit.

Each of 16 ml samples of heparinized blood was immediately placed in ice water, and subsequently divided into two equal volumes; one was used to determine rate of total blood lactate production. From the second volume, most of the white cells were removed by three repetitions of centrifugation at 25° for 90 minutes and rate of lactate generation determined from slopes of best fit lines through lactate concentrations measured at 0, 30, 60, and 90 minutes (14). Data consisted of rates of lactate generation by whole blood samples, and by erythrocytes suspended in isologous plasma. Difference in the rates between whole blood and erythrocytes would then be attributed to white cells, because plasma itself has no glycolytic capacity (2).

ANALYSIS—Data were analyzed in an analysis of variance with groups and time as classification variables.

RESULTS

Figure 1 shows the most striking features of our data: rapid decline of blood glycolytic rate occurred in samples incubated at 37°; this effect was enhanced in samples incubated at 25°.

Also shown is a sharp decline in an additional series of samples incubated anaerobically (in capped
syringes) at 25°. Trends of change were significant during TM and differed from change during reading control. Change in samples free of white cells did not differ significantly from the whole blood measurements.

![Graph showing lactate generation during TM practice and reading control periods.](image)

**FIG. 1. PERCENT CHANGE OF RATE OF EITHER AEROBIC OR ANAEROBIC (CAPPED SYRINGES) LACTATE GENERATION (MEAN±S.E.) DURING AND AFTER EITHER TM PRACTICE OR READING CONTROL PERIODS.** Aerobic samples were incubated at 37° and 25°; anaerobic, at 25° only. Trends were significant during practice at both 37° and 25° ($p<.005$). Time mean glycolytic rates were 17.4 mg% (±2.0) at 37° and 9.6 mg%/hr (±1.9) at 25°. This graph depicts results in the long-term TM group.

Concentration of arterial lactate declined during TM; no significant variation in any group was noted of arterial PCO$_2$, PO$_2$, glucose, hematocrit, or pH (table 1). Rapid decline of oxygen extraction attributed to decreased muscle uptake was also observed in a related study (15) of forearm metabolism.

On the average, 80% of meditation time was spent in wakefulness, 17% in stage 1 sleep and 3% in stages 2 and 3 sleep. Standard correlation analysis did not reveal a relationship between total sleep time or sleep stage percentage and change of generation rate. Microsegment sleep analysis (total sleep and sleep stage percent at five-minute intervals) of seven subjects did not show a clear temporal relationship in a graphical comparison with generation rate change. Significant decrease of EDR occurred during the TM period. Partial correlation analysis (16) among blood glycolytic rate, lactate content, and oxygen extraction showed significant relationship between glycolytic rate and both lactate content and oxygen extraction (36% and 40% of variance of glycolytic rate, respectively).

From these data immediately arose the further question of the mechanism of the red cell effect—in particular whether the cell itself was altered in some way and/or whether inhibitory factors in plasma were responsible. Therefore, additional measurements, called the “red cell interchange experiment,” were performed in which six subjects demonstrating decreased red cell metabolic rate in the previous study were restudied in the same manner, except that to the separated erythrocytes of each sample (times 0, 15, 30, etc.), was added plasma from the time 0 sample, and glycolytic rate again measured. The time course of glycolytic rate in this interchange series was then compared to the normal time series plasma/red cell preparations studied before.

The rationale for this procedure was that presence of an inhibitory factor in the red cell itself would reflect in parallelism of the curves of glycolytic rate change in the interchange and original experiments. The results shown in figure 2 are consistent with alteration of the erythrocyte itself in the mechanism.

**TABLE 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 MINUTES</th>
<th>15 MINUTES</th>
<th>30 MINUTES</th>
<th>45 MINUTES</th>
<th>60 MINUTES</th>
<th>75 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.36±0.02</td>
<td>7.35±0.04</td>
<td>7.35±0.04</td>
<td>7.37±0.04</td>
<td>7.37±0.02</td>
<td>7.37±0.05</td>
</tr>
<tr>
<td>PCO$_2$</td>
<td>38.6±2.9</td>
<td>39.4±3.4</td>
<td>39.8±2.4</td>
<td>38.6±7.2</td>
<td>39.3±2.9</td>
<td>40.5±2.4</td>
</tr>
<tr>
<td>PO$_2$</td>
<td>102.0±8.0</td>
<td>99.0±9.5</td>
<td>99.0±10.0</td>
<td>102.0±8.0</td>
<td>106.0±7.4</td>
<td>106.0±4.8</td>
</tr>
<tr>
<td>Base Excess</td>
<td>-2.1±0.9</td>
<td>-2.5±0.6</td>
<td>-2.7±0.7</td>
<td>-2.6±0.8</td>
<td>-3.2±1.2</td>
<td>-3.4±1.4</td>
</tr>
<tr>
<td>Lactate (mg%)*</td>
<td>6.54±0.51</td>
<td>6.11±0.72</td>
<td>5.60±0.34</td>
<td>5.21±0.32</td>
<td>5.70±9.41</td>
<td>5.70±0.51</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>69.0±7.0</td>
<td>71.0±10.0</td>
<td>65.0±7.4</td>
<td>70.0±8.3</td>
<td>72.0±9.1</td>
<td>68.0±10.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.0±10.0</td>
<td>47.0±8.0</td>
<td>43.0±13.0</td>
<td>44.0±8.0</td>
<td>46.0±9.0</td>
<td>44.0±1.0</td>
</tr>
</tbody>
</table>

*Note: Values are means ± standard deviation.

*p < .05, significance of trend.
FIG. 2. RED CELL INTERCHANGE. Cells separated from each of times 0, 15, 30, 45, 60, and 75 minutes were resuspended in control 0 plasma and glycolytic rate measured (at 25° and 37°). Means ± standard error of percent change.

DISCUSSION

These results imply decreased blood cell metabolism accounted for mostly by decline of red cell glycolysis associated with this behavioral state. Sleep or drowsiness that may accompany TM does not appear to be related to the phenomenon. Although we do not know the cause, several possible mechanisms can be reduced in likelihood based upon the well characterized physiology of TM and of the erythrocyte.

Reduction of metabolism due to decreased transport of glucose is unlikely, since although glucose is the predominant substrate of red cell glycolysis and metabolism in tissue is regulated by substrate permeability, facilitated transport in the mammalian erythrocyte is 2–250 times greater than rate of glucose phosphorylation (1). Also, mean blood glucose levels were not altered during practice. The possible operation of several effectors at glycolytic control steps can be eliminated including a most powerful effector of red blood cell metabolism, pH, which cannot be responsible, since arterial blood pH did not change (table 1 and (3)); and weaker effectors such as blood Po2, PCO2, glucose, or hematocrit, which also do not vary (table 1 and (3)).

Other possible physiologic effectors range in size and complexity from small inorganic ions such as citrate, phosphate, and magnesium to hormones such as epinephrine and testosterone (1). Most investigated for their effect on red cell glycolysis, small ions may exert control, primarily at hexokinase (HK) and phosphofructokinase (PFK) steps of glycolysis. However, certain facts diminish the likelihood of significant role for them: the control strength of PFK and HK for these effectors are a sensitive function of pH (1); but as shown in figure 1, in vitro decline of glycolytic rate at 25° during TM was similar under both anaerobic and aerobic incubation conditions which differed considerably in mean pH (17, 18).

These considerations and data from the red cell interchange experiment, indicating persisting change of the erythrocyte itself in the mechanism, are consistent with the possible role of a larger effector that may bind to the erythrocyte, thereby accounting for apparent persistent change of the red cell itself. General endocrinological data support the possibility of a hormone-receptor interaction in the mechanism of the noted red cell effect of behavior—especially at the plasma membrane. They include the well-established existence of erythrocyte receptors at the red cell surface for polypeptide hormones such as insulin (19), and for catecholamines (20); the existence of an intact adenyl cyclase system (21); and demonstration of extracellular activation of erythrocyte glycolysis by inosine (22), cyclic AMP (21), epinephrine (1), and ion transport (22).

In spite of the several studies indicating the possible importance of erythrocyte-hormone interaction in physiology, specific modulation of red cell metabolism by known hormones under physiologic conditions remains to be demonstrated unequivocally. In TM the possible agency of several known hormones may be discounted because they do not change significantly, including testosterone, growth hormone, and prolactin (7), and epinephrine and norepinephrine (4). Also, while other investigators have reported controversial effects of behavior on the erythrocyte (23), specific change of glycolytic rate in an acute behavioral state is previously unknown. Therefore the mechanism of the effect described in this report is a unique and possibly important finding for many fields of research. The factor or factors responsible may have further significance for metabolism in general, since control mechanisms of red cell glycolysis are relevant for
control of glycolysis in general; the red cell plays a major role in transport of substances to and from the brain, heart, and muscle (1) and there apparently exists significant correlation between decline of muscle oxygen extraction and decline of erythrocyte glycolytic rate. Finally, demonstration of a chemical effector and/or specific tissue metabolic change in this behavioral rest state may contribute to future studies aimed at identification of its essential elements.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Dr. Thomas McManus, Departments of Physiology and Pharmacology, Duke University; and financial support from NIMH #MH29791 and American Heart Association #795117.

REFERENCES AND NOTES

11. All recordings were made on a Grass Model 7 polygraph. EEG records were scored blind according to sleep state criteria of Rechtschaffen and Kales (A. Rechtschaffen and A. Kales, eds. A manual of standardized technology, techniques, and scoring system for sleep stages of human subjects (Washington, D.C.: Public Health Service Publ. No. 204, Government Printing Office, 1968)). Percent time and sleep stage percent were computed for each five-minute interval for microsegment sleep analysis as well as for total practice period. Recording of eDR is as described by KILPATRICH, D. G. 1972. Psychophysiology 9: 219.
14. All lactate measurements were performed in duplicate by an automated (Technicon) procedure on 0.45 ml of blood delivered into 0.9 ml of ice cold perchloric acid according to an enzymatic method (HOACHELLA, N. J., and WEINHOUSE, S. 1965. Analytical Biochemistry 10: 304).
17. Mean pH of 90-minute incubations under 25°C aerobic conditions was 7.8, while anaerobic 25°C incubation averaged 7.2.