PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 588–592 (1975)

Erythrocyte 2,3-Diphosphoglycerate Concentrations in Hibernating, Hypothermic, and Rewarming Hamsters (38589)

GEORGE E. TEMPEL AND X. J. MUSACCHIA

Dalton Research Center and Department of Physiology, University of Missouri, Columbia, Missouri 65201

The major pathway of carbohydrate metabolism in mature mammalian erythrocytes has been shown to have a shunt for the production of 2,3-diphosphoglycerate (2,3-DPG), a pathway that does not yield ATP. 2,3-DPG has been shown to reduce the oxygen affinity of hemoglobin solutions (1, 2), and hence, alter the position of the dissociation curve. This finding gave impetus to investigations which examined the adaptive significance of changes in 2,3-DPG concentration. For example, residents of high altitudes have higher 2,3-DPG concentrations than sea level counterparts, accounting for a reduced hemoglobin affinity for oxygen (3). Association of the functional state of the erythrocyte with its metabolic status, as well as reported alterations in the dissociation curve from blood of hibernators (4) has prompted investigations in the area of depressed metabolism. In hibernating 13-lined and golden mantled ground squirrels, Burlington and Whitten (5) demonstrated a significant decrease in red cell 2,3-DPG content. They suggested that reduced 2,3-DPG levels play a role in periodic arousal, increasing the affinity of hemoglobin for oxygen. Such a shift in the oxygen dissociation curve might prove adaptive in a winter burrow where oxygen is limited (6).

The present study was undertaken to examine 2,3-DPG concentrations in another species of hibernator, the hamster, *Mesocricetus auratus*. Alterations reported in the ground squirrel, if of adaptive significance, should also be found in another species of hibernator. In addition, since decreased temperature *per se* effects a pronounced leftward shift of the oxyhemoglobin dissociation curve, the physiologic consequence of a depression in 2,3-DPG concentration is questionable. To be of adaptive value, alterations in 2,3-DPG concentration would have to persist in the aroused, normothermic animal where its effect would not be masked by temperature. Accordingly, rewarmed animals were studied. Finally, helium-cold hypothermic animals were examined both to provide further information on this state of depressed metabolism as a model of hibernation, and to gain insight into possible mechanisms underlying the depression in synthesis of 2,3-DPG.

Materials and Methods. Adult male and female golden hamsters weighing 100–140 g were used in this study. Normothermic control animals were taken directly from our closed colony, lightly anesthetized with halothane, and bled by transthoracic cardiac puncture. Hibernation was induced in an age-matched group by 6–8 wk of exposure to an ambient temperature (T_a) of 7°. Hibernators were bled by cardiac puncture after approximately 48 hr of hibernating torpor as determined by the placement of cedar chips on the dorsum of the animal. The hamster's rectal temperature was noted at the time of sacrifice to ensure that all animals were truly hibernating, and not arousing. Deep hypothermia, with comparable rectal temperatures (7–9°) was induced in a third group by the helium cold method of Fischer and Musacchia (7), and a modification thereof. In brief, the animals were exposed to one of two gas mixtures of helium and oxygen (helox) (80% He:20% O_2; or 90% He:10% O_2) at T_a 0°. After reaching a rectal temperature (T_{re}) of 6–8°, hamsters were removed from the helox environment and transferred to a room air environment at T_a 7°. After 12 hr and 24 hr at T_{re} 7°, a single 2 ml blood sample was obtained from each animal by cardiac puncture. Hamsters which were hibernating or hypothermic were rewarmed by placing them at $T_a \approx 22°$. Blood samples were similarly obtained by cardiac puncture under light halothane anesthesia both immediately upon rewarming ($T_{re} \approx 37°$) and after approximately 2 hr of normothermia.

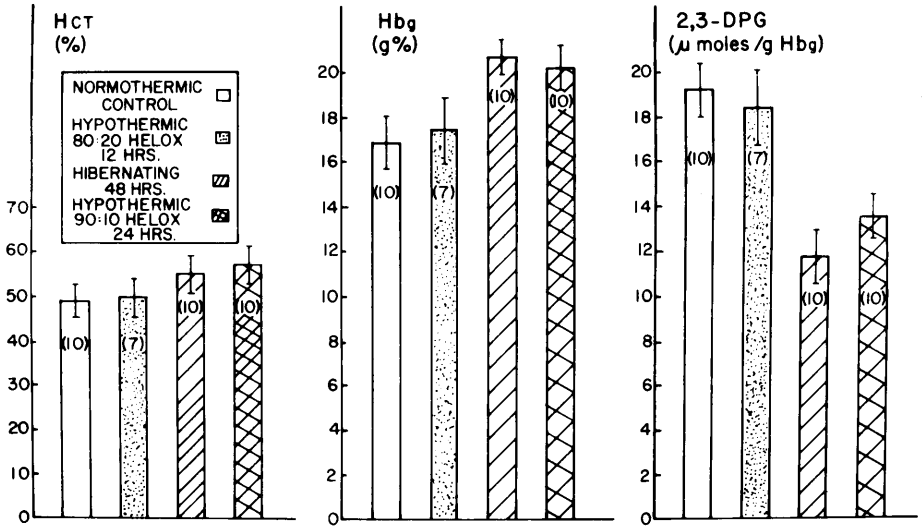

FIG. 1. Hematocrit, hemoglobin, and red cell 2,3-DPG of normothermic golden hamsters compared with hibernators, and hypothermic animals. Hypothermic hamsters were sampled after 12 and 24 hr at T_{re} 7°. Hypothermia was induced in the former by 80:20 helox and cold, and in the latter by 90:10 helox and cold.

The blood samples obtained from the various experimental groups were analyzed for hematocrit, hemoglobin, and 2,3-DPG. Hematocrits were determined by the micro method, and hemoglobin concentration was assessed as cyanmethemoglobin at 540 nm using a Beckman Spectrophotometer, Model DB-G. A protein-free filtrate was obtained by cold trichloroacetic acid precipitation, stored at 0° for 1 day, and analysis was made by the colorimetric determination of inorganic phosphate produced by the action of 2,3-DPG phosphatase (Sigma).

Intergroup comparisons of the data were made using the nonparametric Mann–Whitney test (8).

Results. The data comparing normothermic control, hibernating, and hypothermic hamsters are summarized in Fig. 1. Hematocrits, although not significantly different ($P > 0.05$), reflect the alterations observed in hemoglobin concentration for hibernating, hypothermic and rewarming groups. In normothermic animals hemoglobin and 2,3-DPG values were 16.9 ± 1.2 g% and 19.2 ± 1.2 μmoles/g Hgb, respectively. Hypothermia of 12 hr duration induced by exposure to 80:20 helox and cold did not significantly affect hemoglobin or 2,3-DPG concentrations ($P > 0.05$). By contrast, both hibernation for 48 hr and hypothermia for 24 hr induced by 90:10 helox and cold exposure effect a marked increase in hemoglobin as well as a marked decline in 2,3-DPG levels. Hemoglobin increased approximately 20% in both groups, to 20.7 ± 0.8 and 20.2 ± 1.0 g% for hibernators and hypothermic hamsters, respectively. The decrease in 2,3-DPG concentration in the hibernator to 11.7 ± 1.2 μmoles/g Hgb, was a 39.1% decrease from control values. The hypothermic hamsters demonstrated a 33.9% decrease to 13.5 ± 1.0 μmoles/g Hgb. Although both groups demonstrate the same trend, hamsters hypothermic for 24 hr showed 2,3-DPG concentrations approximately 15% greater than the group hibernating for 48 hr.

2,3-DPG levels were measured in rewarmed hibernating and hypothermic hamsters at various time periods after reaching normothermic body temperatures. These data are summarized in Table I. Rewarming hibernators and hypothermic hamsters sacrificed immediately upon reaching a stable core temperature did not differ significantly

TABLE I. HEMATOCRIT, HEMOGLOBIN, AND ERYTHROCYTE 2,3-DPG OF REWARMING HAMSTERS.[a]

	Hematocrit %	Hemoglobin g%	2,3-DPG μ moles/g Hgb
Hibernating; 48 hr Normothermic 15 min	48.0 ± 4.9 (5)	17.5 ± 1.5 (5)	17.2 ± 1.0 (5)
Hypothermic; 24 hr Normothermic 15 min	49.8 ± 2.3 (8)	17.4 ± 1.0 (8)	16.9 ± 0.8 (8)
Hypothermic; 24 hr Normothermic 2 hr	45.1 ± 2.2 (8)	15.3 ± 0.8 (8)	17.5 ± 1.3 (8)

[a] Mean ± SD (N).

($P > 0.05$). In both groups hemoglobin concentration had returned to control levels with values of 17.5 ± 1.5 (5) and 17.4 ± 1.0 (8) g% for rewarmed hibernating and hypothermic animals, respectively; erythrocyte 2,3-DPG concentrations increased to 17.2 ± 1.0 in the former, and 16.9 ± 0.8 μmoles/g Hgb in the latter. Although this represents a notable increase, these values remain about 10% less than those in the control animals. Rewarmed hypothermic hamsters sacrificed after 2 hr at a stable core temperature demonstrated a 2,3-DPG concentration of 17.5 ± 1.3 μmoles/g Hgb, a value not significantly different from the groups sacrificed immediately upon rewarming ($P > 0.05$).

Discussion. The data from this study reflects the hemoconcentration reported for the hibernating hamster (9–11) with approximately a 20% increase in hemoglobin concentration. Hamsters hypothermic for 24 hr showed a similar increase while 12 hr of hypothermia produced no detectable effect as had been previously reported (12).

2,3-DPG concentrations reported for control animals were consistently higher than values previously reported for the hamster (13). In the present experiments, standard determinations were run with each assay and the data are highly reproducible. We have no explanation for the difference between the values herein obtained and those of Meyerstein and Cassuto (13). However, the alteration reported for the hamster parallel changes noted in the golden mantled ground squirrel by Burlington and Whitten (5).

In the regulation of oxygen transport and delivery to the tissue, 2,3-DPG interacts with several other variables. In the metabolically depressed animal ($T_{re} \approx 7°$), decreased temperature *per se* effects a marked leftward shift of the dissociation curve. This has been shown for the hibernating ground squirrel (14) and hedgehog (15), as well as for the helium-cold hypothermic hamster (12). The influence of temperature on the oxygen carrying capacity of the blood might therefore, be expected to play the predominant role in the animal with a reduced core temperature. However, a role for alterations in DPG has been suggested by Burlington and Whitten (5). These investigators suggest that the depression in 2,3-DPG observed in hibernating golden mantled and 13-lined ground squirrels plays a role in periodic arousals. In the aroused animal at $T_{re} = 37°$ a reduced 2,3-DPG concentration would increase the affinity of hemoglobin for oxygen in a burrow where the P_{O_2} may be reduced.

If alterations in 2,3-DPG concentration were of adaptive advantage to a hibernator, one might expect the 45.5% and 48.0% decrease reported in the hibernating golden-mantled and 13-lined ground squirrels respectively (5), both to occur in other species of hibernators and to persist in the aroused animal ($T_{re} \approx 37°$) where the temperature effect on the affinity of hemoglobin

for oxygen does not predominate. This study has shown a similar decrease (29.1%) in 2,3-DPG concentration in the hibernating hamster, as well as a 32.9% decrease in the 24 hr hypothermic animal. It is noteworthy that the depression in 2,3-DPG concentration persists for some 2 hr in the aroused animal although rapid changes occur during arousal to values approximately 10% less than controls. It can be speculated that the reported 10% decrease in hamster 2,3-DPG would decrease the P_{50} by approximately 1 mmHg, a decrease similar to that reported for the woodchuck (16). Although possibly an adaptation to a reduced P_{O_2} environment, the small size of the change suggests that it is of little functional advantage to the animal.

A comparison of the 24 hr hypothermic hamster with the 48 hr hibernator shows a 15% greater concentration of 2,3-DPG in the former. In hibernation various changes occur in intermediary metabolism of adaptive significance. For example, the primary energy substrates during hibernation are lipids as shown by a respiratory quotient very close to 0.7 (17). A greater efficiency for some liver mitochondrial enzymes in cold exposed and hibernating hamsters has also been demonstrated (18). In an investigation of alterations in intermediary metabolism in hibernation, Brock (19) suggests that adaptive changes occur before or during hibernation which result in the maintenance of red cell high energy phosphates at low *in vivo* temperatures. Although metabolic adaptations undoubtedly exist in hibernators, comparison of the experimental hypothermic group with natural hibernators suggest a cold suppression of glycolysis. Thus, the higher concentration of 2,3-DPG reported for the hypothermic animals may be explained by their shorter stay at a reduced body temperature (24 hr compared to 48 hr for the hibernator).

In both the hibernating and hypothermic animals, reduced levels of 2,3-DPG might be anticipated. Cold suppression of phosphoglyceromutase and diphosphoglycerate phosphatase, the enzymes involved in anabolism and catabolism respectively, would occur in both metabolically depressed groups. It is, however, possible that 2,3-DPG stores are reduced through the mainstream of glycolysis via the Rapoport–Luebering shunt as has been suggested by Larkin (20).

Summary. Hematocrit, hemoglobin and erythrocyte 2,3-DPG concentrations were examined in normothermic control, hibernating, and helium-cold hypothermic hamsters. Hematocrit was not significantly different ($P > 0.05$) between groups, but did reflect alterations reported for hemoglobin. Hemoglobin concentration did not change from control values during 12 hr at T_{re} 7°; however, approximately a 20% decrease occurred in hibernators (48 hrs) and animals hypothermic (24 hr). 2,3-DPG concentrations declined 39.1 and 33.9% from control values in the hibernating and 24 hr hypothermic groups, respectively. No change was observed in animals hypothermic for 12 hr. Both parameters were studied in the aroused animal. Hemoglobin returns to control values immediately after the animals reached a stable $T_{re} \approx 37°$. Although 2,3-DPG levels increased during arousal, they were still 10% lower than control values in both metabolically depressed groups. 2,3-DPG remained approximately 10% less than controls in rewarmed hypothermic animals studied 2 hr after reaching stable $T_{re} \approx 37°$. The data are discussed in terms of cold depression of erythrocyte glycolysis.

The authors wish to thank Janet Burnett for her excellent technical assistance.

Research was supported by Grant No. NGL 26-004-021, Supplement 9, 10, from the National Aeronautics and Space Administration, and the Dalton Research Center.

1. Chanutin, A., and Curnish, R. R., Arch. Biochem. Biophys. **121,** 96 (1967).
2. Benesch, R., and Benesch, R. E., Biochem. Biophys. Res. Commun. **26,** 162 (1967).
3. Eaton, J. W., Brewer, G. J., and Grover, R. F., J. Lab. Clin. Med. **73,** 603 (1969).
4. Bartels, H., Schmelzle, R., and Ulrich, S., Resp. Physiol. **7,** 278 (1969).
5. Burlington, R. F., and Whitten, B. K., Comp. Biochem. Physiol. **38A,** 469 (1971).
6. Studier, E. H., and Procter, J. W., J. Mammol **52,** 631 (1971).

7. Fischer, B. A., and Musacchia, X. J., Amer. J. Physiol. **215,** 1130 (1968).
8. Mann, H. O., and Whitney, D. R., Ann. Math. Statist. **18,** 50 (1947).
9. Suomalainen, P., and Granstrom, T., Exp. Cell. Res. Suppl. **3,** 335 (1955).
10. Lyman, C. P., Weiss, L. P., O'Brien, R. C., and Barbeau, A. A., J. Exp. Zool. **136,** 471 (1957).
11. South, F. E., and Jaffay, H., Proc. Soc. Exp. Biol. Med. **98,** 885 (1958).
12. Volkert, W. A., and Musacchia, X. J., Amer. J. Physiol. **219,** 919 (1970).
13. Meyerstein, N., and Cassuto, Y., Comp. Biochem. Physiol. **41A,** 297 (1972).
14. Musacchia, X. J., and Volkert, W. A., Amer. J. Physiol. **221,** 128 (1971).
15. Clausen, G., and Ersland, A., Resp. Physiol. **5,** 221 (1968).
16. Harkness, D. R., Roth, S., and P. Goldman. Comp. Biochem. Physiol. **48A,** 591 (1974).
17. Lyman, C. P., and Chatfield, P. O., Physiol. Rev. **35,** 403 (1955).
18. Chaffee, R. R., Oxidative and Phosphorylating Enzyme Systems in Cold Acclimated and Hibernating Mammals (Ph.D. Dissertation). Harvard University, Cambridge, Massachusetts (1957).
19. Brock, M. A., *in* "Mammalian Hibernation III" (Fischer, K. C., Dawe, A. R., Lyman, C. P., Schonbaum, E. and F. E. South, eds.), p. 409. Oliver and Boyd, Edinburgh and London (1967).
20. Larkin, E. C., Comp. Biochem. Physiol. **45A,** 1 (1973).

Received July 19, 1974. P.S.E.B.M. 1975, Vol. 148.