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Effect of Hypobaric Hypoxia on Levels of Reduced Glutathione and 2,3-Diphosphoglycerate in Rabbit Red Blood Cells

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Introduction

Since the discovery by Benesch and Benesch[2] and Chanutin and Curnish[5] that red cell organic phosphate compound 2,3-diphosphoglycerate (DPG) is an important modifier of hemoglobin function, it is now well appreciated that the level of DPG is important in the regulation of tissue oxygenation in many animal species.[4,8] It is also recognised now that the rapid decrease in the affinity of hemoglobin for oxygen in human subjects exposed to hypobaric hypoxia is due to increase in DPG in the red cells.[7,8,9]

Although the effects of hypobaric hypoxia on mice, rats and guinea pigs have been extensively investigated.[1,6,20] We are aware of only one report on red cell glycolysis and DPG levels in rabbits.[6] This report does not give values for DPG prior to hypobaric exposure. The present communication describes the results of an experiment designed to study on the changes in red cell metabolism in rabbits exposed for 30 consecutive days to a barometric pressure of 380 mm Hg.

Materials and Methods

Six mature healthy New Zealand rabbits were used. They were kept in a hypobaric chamber for two weeks prior to the period of hypoxic stress to obtain normal pre-experimental values. The barometric pressure was then reduced to 380 mm Hg for 10 hrs per day for 30 consecutive days. Measurements were continued for 70 days after termination of the decompression schedule.

Blood was collected from the ear vein and heparin was used as an anticoagulant. Packed cell volume (PCV), hemoglobin concentration (Hb Conc.) and red cell numbers (RBC No.) were determined by standard laboratory procedures. Levels of reduced glutathione (GSH) were measured by the 5,5-dithiobis(2-nitrobenzoic acid) method of Beutler, Duron and Kelly[3]. DPG levels were determined by an enzymic method (Sigma Technical Bulletin No. 35-UV) with a slight modification that the protein-free filtrate was prepared by mixing 1.0 ml blood to 1.0 ml cold 12% (W/V) trichloroacetic acid. DPG levels are expressed as μM/gHb.

Results

The results are shown in Fig.1. The PVC increased from a mean value of 39% to 68%; Hb conc. from 14.4g% to 23.3g% and the RBC number from $5.6 \times 10^6/mm^3$ to $9.4 \times 10^6/mm^3$ during the period of hypobaric hypoxia. Hemat-

ological values began to decline soon after decompression ceased and reached their pre-experimental levels within 55 days.

The levels of GSH increased from an average of 6.1 μM/gHb to 9.2 μ M/gHb by day 24 of hypobaric exposure. After decompression ceased, there was a further rise in the level of GSH for a period of about 20 days after which the level declined. However, even after 70 days, when the experiment was terminated, GSH levels were slightly higher (7.5 μM/gHb) compared to pre-experimental values of 6.1 μM/gHb.

There was a small rise in red cell DPG levels from 26.0 μM/gHb to 29.6 μM/gHb soon after the animals were exposed to high altitude. The levels were then maintained during the period of exposure and reached pre-experimental values soon after the animals were taken out from the decompression chamber.

Discussion

An increase in circulating red cell mass is an early hematopoietic response in animals exposed to high altitude. This was also shown in the present experiment (Fig.1) in confirmation with a previous report on the rabbit.[6]

A rise in GSH level from 6.1 μM/gHb to 9.2 μM/gHb represents a signficant rise and is similar to that observed by 0'Dea and Agar in sheep under similar conditions (unpublished data) but is considerably smaller compared to the rise in rabbits after phlebotomy by Yu and Agar[21] Since this rise parallels the rise in PVC, Hb conc. and RBC number (Fig.1), it may reflect true GSH levels in the young red cells. However, the finding of a second peak of GSH in animals soon after they were taken out from the decompression chamber is hard to explain. A similar peak of GSH was also observed in rabbits recovering from anemia induced by phelbotomy by Yu and Ager[21] representing the complexity of GSH regulation in young and mature red cells. It was suggested by Shojania et al.[16] that the red cell glycolytic intermediates should be expressed per ml red cells rather than per gHb (because of differences of hemoglobin in young and old red cells). Although this system is not universally accepted and, in fact, most workers prefer to use values per gHb, calculating GSH values per ml red cells gave identical curve to that of per gHb (Fig. 1). We are therefore unable to explain the second peak of GSH at a time when red cell number, PCV and Hb concentration were steadily declining. The second peak of GSH cannot be explained on the basis of differential distruction of red cells of different hemoglobin content as proposed by Shojania et al.[16]

It has been shown that activities of several glycolytic enzymes of the red cells is increased in rats and man at high altitude.[11,17,18] However, the most remarkalbe change is a significant rise in the level of red cell DPG which results in a displacement of the oxyhemoglbin dissociation curve to the right favouring the release of oxygen to tissues.[1,7,9,20] The increase in red cell DPG level has been attributed to; (i) a change in glycolysis due to increase in intracellular pH; (ii) increased DPG production to compensate for the greater affinity and increased binding to Hb in blood of reduced oxygen content.[8]

Form the results presented in Fig.1, it is evident that the changes in PVC, Hb conc., RBC number and GSH levels in rabbits exposed to high altitude were similar to those found in several mammalian species including man, mice, guinea pig and rat examined under similar conditions. However, the most important result of this study is the finding that there was very little rise in DPG levels in rabbits during exposure to reduced barometric pressure.

Mammals have been classified into two groups on the basis of the effect of DPG on the oxygen transport functions of Hb. The first group, containing the majority of mammalian species tested, has abundant red cell DPG and hemoglobins with a high affinity for oxygen though this is markedly reduced in the presence of DPG.

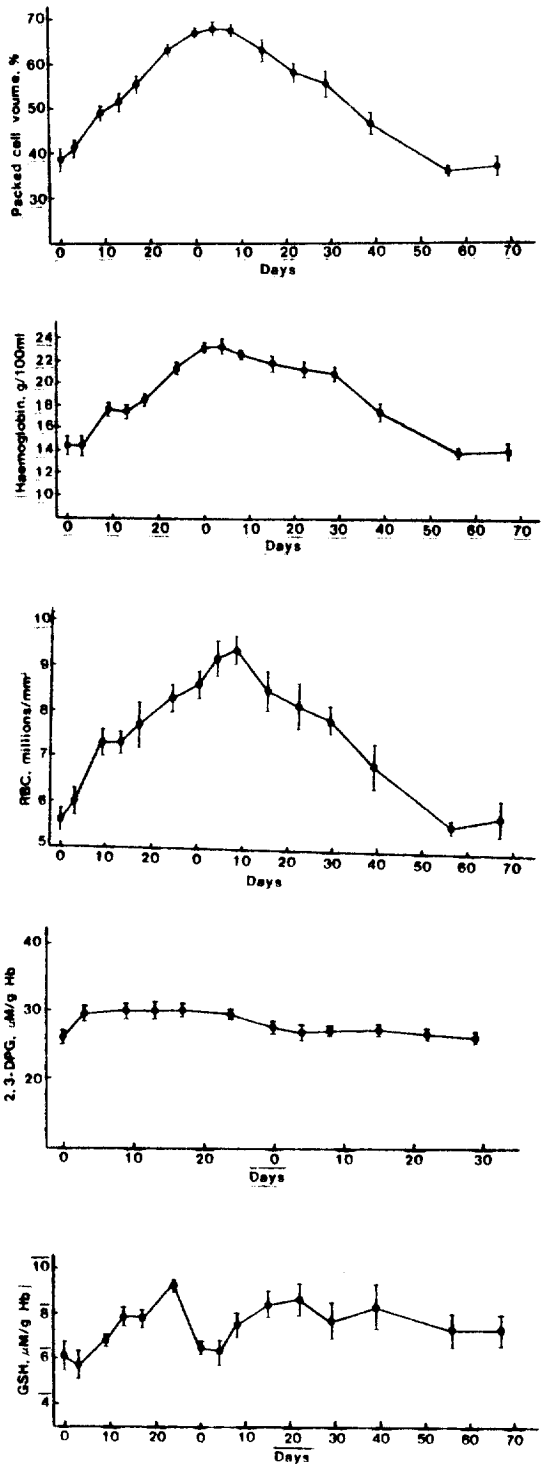

Fig. 1. Effect of hypobaric hypoxia on packed cell volume, haemoglobin, RBC number, and the levels of GSH and DPG in the rabbit red blood cell.

The second group includes sheep, goat, cattle, and cat and is characterized by low levels of red cell DPG and hemoglobins of low oxygen affinity which do not react strongly with DPG[4]. Since rabbit falls in the first group, it was expected that rabbits exposed to reduced baromtric pressure would show a marked rise in red cell DPG. This is not evident from Fig.1 which shows only a marginal rise in the level of this compound. Whether this level would compensate for the hypobaric hypoxia is not known. Our previous results on changes in the red cell level of DPG in anemia produced by acetyl-phenylhydrazine were also in conclusive by Yu and Agar[21]. In fact, several reports on levels of DPG during anemia in rabbits are conflicting. For example, Ohyama and Minakami[13] reported that the content of DPG is lower in rabbit reticulocytes than that in mature red cells, while Shojania, Israels and Zipursky[16] found a short term rise followed by a rapid fall during the maturation time of the circulating reticulocytes. It has also been shown that reticulocyte count is not always a reliable parameter for the age of a cell suspension in the rabbit[10] and that a fraction of the new red cells formed during recovery from anemia have an abnormally short life span.[12] It may also be mentioned here that a rise in red cell DPG level in anemia in man is not a universal phenomena; whereas there is a direct relationship between the degree of anemia and the red cell DPG concentration in iron deficiency anemia, and megaloblastic anemia, this does not hold true for several other types of anemias.[15]

It appears, therefore, that the type of red cells produced during anemia and hypobaric hypoxia are different and that there are also differences in the red cell metabolism during these conditions. Confirmation of this view is derived from the observations of Perry[14] and O'Dea and Agar (unpublished data) in sheep. In fact, using an improved electrical sizing method, Valet, Franz and Lauf[10] have shown three red cell populations in newborn sheep and have

stressed the importance of "cellular replacement" rather than the classical view of "celluar matutation". Further studies are needed to explain the role of DPG in the red cells of rabbits exposed to high altitude.

Conclusion

Effect of hypobaric hypoxia on PCV, Hb conc., RBC no. and red cell GSH and DPG was examined in the rabbit. There was significant rise in all the parameters measured except DPG which did not change during and/or after the hypobaric hypoxia.

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低氣壓이 家兎赤血球 GSH 및 2,3— DPG에 미치는 영향에 대하여

유 창 준

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抄 錄

低氣圧이 家兎의 赤血球용적, 血色素농도, 赤血球數, 赤血球 GSH 및 DPG에 끼치는 영향에 對하여 연구 하였다. 赤血球 DPG만 제외하고 모든 관찰수치는 增加되었다.