phenomena, could point to a more direct effect of INH on the axon. The time-course of onset of PNCV changes obtained in this study is consistent with the described Wallerian type of axonal degeneration[17,20]. The demyelinating neuropathies do not give such an early and small (less than 20% of control values) decrease in PNCV. Moreover, recent work[7,8] clearly shows that a large dose of this compound, administered either in one single dose or in several smaller doses (quantitatively similar to those employed by us), produces a multifocal localized action on the axon itself indicating a special susceptibility of the axon to this drug.

1 Acknowledgment. The authors wish to express their thanks to Mr C. Avellaneda for his expert technical assistance and to Dr P.C. Croghan for reading the manuscript.
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0014-4754/84/040378-03$1.50 + 0.20/0
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Lipid peroxidation in rabbit reticulocytes[1]

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Summary. Lipid peroxides in rabbit erythrocytes and plasma were determined while anemia was induced by daily bleeding. They increased as reticulocytes increased and returned to normal with the morphological transformation to mature cells.

Peroxidation of polyunsaturated fatty acids of the cell membrane is considered to be mediated by active oxygen species, and the rate of malondialdehyde formation has been used as an index of this reaction[2]. In the course of maturation, young erythrocytes undergo a number of metabolic changes including loss of membrane components, such as total lipid, cholesterol and phospholipids[3,4], a gradual decrease in a number of enzymes[3,5-8], and a decline in mitochondrial activity[9]. Reticulocytes are also known to consume much more oxygen than mature cells[10], with a resultant production, presumably, of larger amounts of oxygen metabolites. It is postulated that a peroxidation reaction may be working during the decomposition of the membrane structures, and that elevated malondialdehyde levels may be associated with reticulocytes. The purpose of this article is to demonstrate such changes in reticulocytes and in plasma during the course of anemia due to blood loss in rabbits.

Materials and methods. The method of induction of reticulocytosis in rabbits and the preparation of reticulocyte specimens have been described elsewhere[11]. To determine lipid peroxide, erythrocyte suspensions in saline were adjusted to contain 10 g of hemoglobin/100 ml and treated in ice by sonication with a Heat Systems Cell Disruptor Model W-225R (Ultrasonics, Inc., Plainview, NY) for 60 sec at 60 W. $^1/_{10}$ ml of the hemolysate was used without further centrifugation in a final volume of 0.5 ml of 17 mM N-hydroxyethyl-piperazine-N'-ethanesulfonic acid, pH 7.4 (HEPES) buffer. Plasma and erythrocyte lipid peroxides were measured by the fluorometric method of Yagi[12], using thiobarbituric acid, and expressed in terms of malondialdehyde (nmol/ml plasma or g hemoglobin) using tetraethoxypropane as a standard. As the average percent difference in erythrocyte counts in relation to hemoglobin was small, i.e., 470 ± 21 (mean $\pm$ 1 SD) $\times 10^4$ RBC/mm^3 throughout the experiment, the lipid peroxide value was expressed only as nmol MDA/g hemoglobin. The procedure of sonication itself did not affect the MDA value which was the same before and after this treatment. Every specimen was assayed in triplicate.

Results. The general trend of changes in reticulocyte count during the course of daily bleeding was as follows: the count, which usually remained less than 2% for the first 4-5 days, rose rather abruptly on days 6-8 to 30-35%, then fell to below the original level during the next 3-4 days.

Figure 1 illustrates the changes of lipid peroxide levels in erythrocytes and plasma during the course of daily bleeding for 7 days and for the next 8-10 days. As is clear from figure 1, changes of the lipid peroxide values in both erythrocytes and plasma paralleled those of the reticulocyte count, showing peak values on days 7 und 8 and subsequent return to their initial levels after daily bleedings were discontinued. The peak values were 1.34 and 1.52 times the initial value for erythrocytes and plasma, respectively. The lipid peroxide values on days 7 and 8 were the only ones significantly different from the original values. Although

the mean value of plasma lipid peroxide on days 7 and 8 was obviously higher than on days 5 and 6, the difference was not significant. Lipid peroxide values for erythrocytes were determined in 3 fractions of a given specimen; a reticulocyte-rich fraction, the original specimen, and a reticulocyte-poor fraction (the original specimen from which reticulocytes had been removed by the method of Kimura et al.[13]). As illustrated in figure 2, lipid peroxide levels were higher in reticulocyte-rich fractions than in the other two; and as the anemia progressed, the basal peroxide values gradually increased regardless of the reticulocyte count.

Discussion. The present study shows that concurrently with an increase of reticulocytes in the erythrocyte population, the lipid peroxide level rises, and it then falls to the normal level as the cells mature. This pattern is accompanied by a similar change in plasma lipid peroxide levels. When the erythrocyte specimens were separated into 2 or 3 fractions depending on the number of reticulocytes, the reticulocyte-rich ones yielded higher lipid peroxide values, as expected. However, with further bleeding, the basal lipid peroxide values increased even when the percentage of reticulocytes remained the same. This finding suggests a more rapid turnover of reticulocytes to mature cells with increasing anemia. Reticulocytes in the peripheral blood are short-lived and lose their reticulum in about 24 h. Although freshly recruited mature cells are no longer morphologically qualified to be counted as reticulocytes, it is apparent that they still retain some characteristics of the preceding stage. Hence, the above observation appears to reflect the increasing population of such newly transformed cells, presumably with a still higher lipid peroxide content than normally mature cells. Consequently, the increased lipid peroxide content may be associated with the process of erythrocyte maturation. The present finding is compatible with observations of Goldstein et al.[14] who showed increasing levels of fluorescence in red cell extracts to be indicative of in vivo red cell lipid peroxidation in phenylhydrazine-treated rabbits. The rapid normalization of elevated lipid peroxide levels in reticulocytes appears to be indicative of a similarly quick morphological and functional transformation of reticulocytes to mature cells.

An increase of plasma lipid peroxide levels may be due in part to lipid peroxide release from tissues rendered temporarily hypoxemic by acutely induced anemia. Regarding the relationship between lipid peroxidation and tissue hypoxia, Yamamoto et al.[15] recently demonstrated a significant increase of lipid peroxides in rat brain in which ischemia had been produced by bilateral ligation of the common carotid arteries. This increase in the tissues was accompanied by an elevation of serum lipid peroxide levels. During such an incomplete metabolic combustion, a possible leakage of electrons from the regular transport system may cause an increased radical production in the cellular constituents and an increase of lipid peroxide generation. However, it is unknown to what extent, if any, lipid peroxide emitted from cells influences the elevation of plasma lipid peroxide levels.

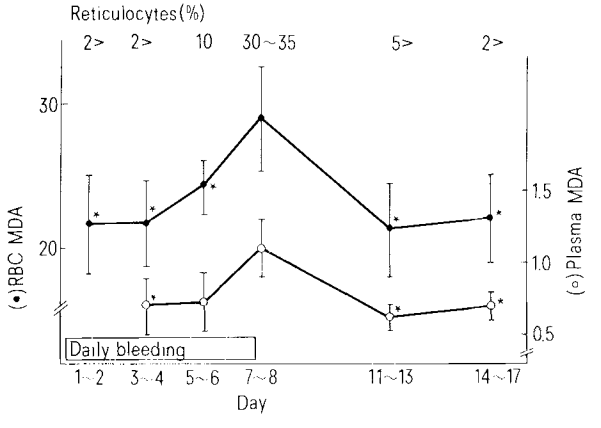

Figure 1. Changes of lipid peroxide levels in rabbit erythrocytes (●) and plasma (○) induced by blood-loss anemia. Each value represents triplicate determinations of 4–5 specimens on days indicated on the abscissa and expressed as a mean ± 1 SD. Lipid peroxide values of both erythrocytes and plasma on days 7–8 of bleeding were significantly higher than those marked with asterisks ($p < 0.05$). Statistical evaluation was by Student's t-test.

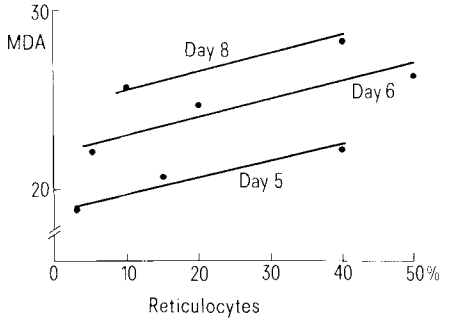

Figure 2. Changes of erythrocyte lipid peroxide levels in relation to reticulocyte counts according to the day of bleeding. Ordinate indicates malondialdehyde (MDA) expressed in nmol per g hemoglobin.

1 This work was supported in part by a grant-in-aid from the Japan Medical Research Foundation and a grant-in-aid (project No. 56570363) from the Ministry of Education, Science and Culture of Japan.

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