

The concentration of methemoglobin increases as red cells age in normals and in patients with congenital methemoglobinemia (CMHb), yet the activity of NADH diaphorase (ND) (Scott assay) does not decrease with age. A specific assay for methemoglobin reductase (MHR) (J. Lab. Clin. Med. 72:339, 1968) permits reassessment of this phenomenon. Red cells were separated by centrifugation at 100,000 g for 1 hour. The top and bottom 10% were harvested and mean ages determined by glutamic oxaloacetic transaminase (GOT) activity. The age stability of ND activity was confirmed. The MHR activity of old cells (bottom layer) was 15% lower than that of younger (top layer) cells in normal individuals. In one family with CMHb and two fast migrating isoenzyme variants of ND, the activity was only slightly reduced (12%) whereas MHR activity was markedly reduced (40 to 90%) in the old cells compared to young cells. An unrelated female with CMHb and a slow migrating variant of ND was previously reported to have "pseudo-mosaicism" on the basis of heterogeneous distribution of methemoglobin between her younger and older cells (NEJM 275:397, 1966). Her older cells actually had greater activity of ND than did her younger cells. In contrast, the MHR activity of her older cells was only 10% of her younger cells. Thus, the normal age-lability of MHR can account for the accumulation of methemoglobin as erythrocytes mature. Exaggeration of this tendency due to structural modifications in the enzyme molecules may account for methemoglobinemia in patients carrying variant isoenzymes.

Relationship between erythropoietin (EP) and erythropoiesis in chronic inflammation. JOHN LUKENS. *Univ. of Missouri, Columbia, Mo.* (Intr. by Calvin Woodruff).

The anemia associated with chronic inflammation results from failure of the erythroid marrow to increase its production sufficiently to compensate for a modest shortening of red cell survival. This defect in erythropoiesis was characterized by examining the quantitative relationship between EP production and erythropoietic response in rats with adjuvant-induced polyarthritis. EP production was measured by exposing rats to 0.5 atm. for 6, 9, 12, or 15 hours. The immediate post-hypoxic EP levels (assayed in post-hypoxic polycythemic mice and expressed as percent RBC  $^{59}\text{Fe}$  incorporation per ml. plasma) was as follows for groups of 5 rats:

	0 Hr	6 Hr	9 Hr	12 Hr	15 Hr
Control	1.5 ± 0.7 ( $\bar{x}$ ± SEM)	9.3 ± 3.7	9.8 ± 2.5	10.4 ± 3.1	12.8 ± 2.2
Adj. disease	0.6 ± 0.04	5.8 ± 1.6	3.5 ± 0.9	3.1 ± 1.2	3.4 ± 1.6
"p"	>0.2	>0.4	<0.05	<0.05	<0.01

That the decrease of biologically active EP in adjuvant disease (AD) plasma was not due to an EP inhibitor was demonstrated by failure of AD plasma, 1) to compromise the biologic activity of sheep EP, or 2) to suppress the erythropoietic response of ex-hypoxic mice to 10 hours of hypoxia. Finally, the responsiveness of the marrow to EP was quantitated in rats in whom endogenous EP was suppressed. Exogenous EP elicited a linear dose-response curve which did not differ for control and AD rats. These data suggest that the disturbance of erythroid homeostasis in chronic disease results from a relative insensitivity of EP elaboration to erythropoietic stimuli.

Toxic effect of lead on erythrocyte membranes. D. GRANT GALL, PATRICIA USHER, and ROBERT KLEIN. *Boston Univ. Sch. of Med., Boston, Mass.*

Lead has been reported to poison many enzyme systems including the Na+K dependent ATPase essential to maintaining normal membrane potentials. The present study was designed to measure the toxic effects of lead on membrane transport in human erythrocytes resulting from possible changes in ATPase activity. Na flux and membrane Na+K dependent ATPase were measured in erythrocytes of patients with lead poisoning and in normal erythrocytes exposed in vitro to lead in concentrations of 50-200 micrograms/100 ml. We have not been able to confirm the presence of decreased ATPase activity in patients with mild lead poisoning (i.e. no encephalopathy and blood concentrations of lead between 60 and 90 micrograms/100 ml.). However, we have demonstrated a markedly increased passive Na leak in both erythrocyte ghosts and intact cells. Active outward Na transport was also increased perhaps as a compensatory mechanism. In vitro exposure of intact red cells to lead has produced similar increases in membrane permeability to Na. In addition, when lead is incorporated in vitro into erythrocyte ghosts inhibition of active transport can be demonstrated. The mechanisms producing the two different effects of lead on membrane transport are separable and may be dose dependent. Thus lead affects the membrane in addition to any possible enzyme injury.

Autoradiographic and electron microscopic studies of marrow in congenital dyserythropoietic anemia. K. Y. WONG, GEORGE HUG, and BEATRICE C. LAMPKIN. *Univ. Cincinnati, and Children's Hosp. Research Found., Cincinnati, Ohio.*

A 12 year old Caucasian girl with congenital anemia and episodic jaundice was studied. Hemolysis was not present as evidenced by a normal Cr<sup>51</sup> red cell survival time. Congenital dyserythropoietic anemia type II (Heimpel) was diagnosed after finding a positive acidified serum test of the circulating red cells and marked erythroid hyperplasia with erythroblastic multinuclearity in a bone marrow aspirate. A bone marrow specimen was labeled with H<sub>3</sub>T in vitro and autoradiographs prepared. Electron microscopy was also done on the same specimen. The percent of uninucleated normoblasts labeling with H<sub>3</sub>T indicated normal DNA synthesis. However, only 2% of the binucleated and none of the multinucleated polychromatophilic normoblasts labeled with H<sub>3</sub>T, indicating decreased DNA synthesis in these forms. By electron microscopy, excessive membrane structures forming invaginations or cisternae and encompassing the circumference of the cell in varying degrees were seen in the majority of the normoblasts. The nuclear membrane appeared normal. Despite the abnormality, nuclear extrusion was noted in these cells. Small cisternae were also found at the periphery of the cell in about 1-2% of the mature erythrocytes. These findings are suggestive that the cells with more severe structural abnormalities and/or decreased DNA synthesis are destroyed intramedullarily and the circulating red cells are derived from a less abnormal portion of precursors.

Erythrocyte membrane alterations in experimental biliary obstruction. ROBERT C. NEERHOUT. *UCLA Sch. of Med., Los Angeles, Calif.*

To further clarify the erythrocyte abnormalities reported in patients with biliary atresia, a study was performed utilizing the bile duct ligated rabbit as a model. Hematologic parameters, membrane and plasma lipid determinations and P<sup>32</sup> phospho-