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}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 exhypoxic 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^{51} 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_3T in vitro and autoradiographs prepared. Electron microscopy was also done on the same specimen. The percent of uninucleated normoblasts labeling with H_3T indicated normal DNA synthesis. However, only 2% of the binucleated and none of the multinucleated polychromatophilic normoblasts labeled with H_3T, 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 intramedullarly 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^{32} phospho-

lipid kinetic studies were performed serially at intervals post ligation. Osmotic fragility studies show progressively increasing red cell sensitivity to osmotic lysis following ligation. Morphologically, a striking increase in "burr cells" is noted within 24–48 hrs. Elevation of erythrocyte membrane and plasma lecithin levels are noted by 3–4 days post ligation reaching maximal values near 40% of total membrane phospholipid by one week. Injection of P^{32} at varying intervals from 2–16 days post ligation show the specific activity of phospholipid phosphorus in both red cells and plasma to be higher in the bile duct ligated animals than in controls. Rise in plasma P^{32} activity preceeds that of red cell and is maximal at 24 hrs. Red cell labelling shows a progressive rise over a 3–4 day period. Despite the quantitation difference of specific activity in experimental and control groups, the ratio of red cell specific activity: plasma specific activity is similar in both groups. These results suggest that the qualitative and quantitative alterations of erythrocyte phospholipid are directly related to the plasma phospholipid alterations.

NEONATOLOGY

Factors influencing predisposition to serious illness in low birth weight infants. LEONARD GLASS, NORMA KOLKO, and HUGH E. EVANS (Intr. by Gilbert W. Mellin). *Columbia Univ. Harlem Hosp. Ctr., N. Y., N. Y.*

Both retrospective and prospective studies of low birth weight infants born at Harlem Hospital and discharged to their mothers showed high rates of serious illness requiring rehospitalization during the first 9 months of life to be related to specific socio-medical factors. These factors were utilized in forming the following weighted prognostic index:

Failure of mother to receive prenatal care	2	0
Absence of father from home	1	0
Receipt of public assistance	1	0
Other children in the home	1	0

Thus a maximum score of 5 would indicate an infant at highest risk, and a minimum score of 0 lowest risk. In both series, infants scoring 4 and 5 had three times the rehospitalization rate of those infants who scored 0, 1 and 2 ($p < 0.05$). Infants scoring 3 occupied an intermediate position. By prospective assignment of a score to each low birth weight infant, those at highest risk of inadequate follow-up care and rehospitalization may be identified prior to discharge from the nursery so that intensive medical, nursing and social services can be directed toward this high-risk group.

Hepatitis-associated antigen: A possible relationship with premature delivery. ELIZABETH M. SMITHWICK, ELEANOR PASCUAL, and SUAT CHENG GO. *Downstate Med. Ctr., Brooklyn, N.Y.*

Preliminary observations in 271 pregnancies indicated a high incidence of hepatitis-associated antigen (HAA) in mothers delivered at a large metropolitan hospital. On analysis, the frequency of HAA appeared to be higher in mothers of premature infants and these infants had a poor survival rate. The present study was designed to check these findings and to determine the incidence of HAA and the outcome of pregnancy in mothers with viral hepatitis. The outcome of pregnancy in apparently healthy HAA+ women was also analyzed. *Mothers of premature and fullterm infants.* Ninety mothers of infants weighing 2000 gms or less were studied. The controls were 90 mothers of the nextborn fullterm infants. Three of the 90 mothers of prematures were HAA+; 3 of their 4 infants (1 set of twins) died. On the other hand, only 1 of 90 mothers of infants >2000 gms was HAA+; her infant survived. The overall 2.2% incidence is identical to that of the preliminary observation. *Mothers with clinical hepatitis.* Eight pregnant women with hepatitis were studied. Five of them were HAA+. Four of their 5 infants were premature; 3 of the prematures died. The infants (2 fullterm, 1 premature) of the negative mothers survived. *HAA+ mothers, apparently healthy.* A total of 7 mothers, HAA+ at delivery and with no history of hepatitis, were studied. Five of their infants (1 set of twins) were premature; 4 died. The 3 fullterm infants survived.

The data suggest that pregnant women who are HAA+, with or without hepatitis, tend to deliver prematurely and that their infants, if premature, have a high mortality rate.

Identification of the high-risk infant from placental phase microscopy. AVROY FANAROFF, SILVIO ALADJEM, F. LANE FRANCE, and MARSHALL KLAUS. *Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio.*

Fresh placental tissue was studied by phase contrast microscopy following 125 normal and complicated pregnancies. 76 infants were full-term, 29 premature, 11 small for gestational age, and 9 from insulin-dependent diabetics. The fetal outcome was correlated with the placental score determined by grading pathological features in the 1) syncitium (hypoplasia, hyperplasia); 2) stroma (edema and intravillous hemorrhage); and 3) vascularity of the villus (congestion, ischemia and avascularity). A total score of 0 indicated normal features for gestational age. Significant correlation was observed between placental score, fetal mortality and morbidity. The mortality was 52% (11/21) with placental scores 6 or above; whereas only 2 of 104 infants with scores below 6 died ($p < .001$). The table below shows results in infants below 37 weeks.

N	Survived	Score range	Mean score	Mean gest. age	Mean weight	Weight range (gm)
14	14	0–5.9	2.6	34.1	2029	1550–2820
15	4	6–23	11.1	31.0	1564	880–2180

19 of the 21 infants with scores of 6 or above presented with problems of extra-uterine adaptation including asphyxia, anemia, respiratory problems, in contrast to 12 of 104 with scores below 6 ($p < .001$). All infants with severe hyaline membrane disease (arterial PO_2 <50 mm. Hg. in 100% O_2) had scores above 6 and demonstrated placental vascular changes with syncitial hypoplasia. Phase microscopy of the placenta is a simple (ten minute) procedure and appears to be helpful in predicting fetal outcome.

Increased skin permeability in preterm infants. RICHARD L. NACHMAN, and NANCY B. ESTERLY. *Univ. of Illinois Coll. of Med., Ohio State Univ. Med. Sch.* (Intr. by Irving Schulman).

Localized cutaneous blanching of preterm neonates following the topical application of a 10% solution of Neo-Synephrine attests to the permeability of immature skin. Skin permeability was evaluated in 18 healthy infants between 30 to 40 weeks of gestational age. The response consisted of speckles or islands of