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DEFECTIVE MICROTUBULE RELATED FUNCTIONS OF PHAGOCYTES IN GLUTATHIONE PEROXIDASE DEFICIENT RATS. Richard E. Harris, Laurence A. Boxer, Robert L. Baehner, Indiana University School of Medicine, J.W. Riley Hospital for Children, Department of Pediatrics, Indianapolis.

Peritoneal polymorphonuclear leukocytes (PMN) obtained from rats rendered glutathione peroxidase (GPX) deficient after an eight week selenium deficient diet exhibited defective chemotaxis and degranulation and enhanced spontaneous surface glycoprotein capping. To compare the phagocytic function of peritoneal macrophages (PM) to PMN, cells were obtained from the peritoneal cavity of rats and purified on a Ficoll-hypaque gradient. PMN and PM GPX activity after 10 weeks of diet was 7% and 15% of control respectively. Similar to the PMN, the PM exhibited negligible chemotaxis, elevated spontaneous capping of $28.6 \pm 6.3\%$ compared to $12.8 \pm 1\%$ for controls and impaired intracellular killing of *Staphylococcus aureus* 502A. Unlike the PMN, regurgitation of the lysosomal enzyme Beta-glucuronidase during phagocytosis was normal. The proportion of cytoplasmic depolymerized microtubules as determined by a radioactive colchicine binding assay was increased during phagocytosis in the GPX deficient PMN rather than decreased as observed in control. These studies show that rat PM rendered GPX deficient share certain functional abnormalities dependent upon intact microtubules but unlike PMN, degranulation appears less dependent on microtubules. Furthermore, in the GPX deficient PMN, microtubules appear to be susceptible to peroxidative damage during phagocytosis.

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α -THALASSEMIA SILENT CARRIER WITH HEMOGLOBINS S AND C. George R. Honig, R. George Mason, Larry Tremaine, and Loyda N. Vida. Northwestern University Medical

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The hematologic features of α -thalassemia trait include microcytosis, morphologic erythrocyte abnormalities, and deficient α -chain synthesis. With coexistent Hb S or Hb C trait a lower than usual percentage of the abnormal Hb is also found. Study of a family with genes for Hbs S and C and α -thalassemia disclosed a previously unrecognized syndrome. A 9 year old black child with Hb SC disease had persistent microcytosis and hypochromia with normal serum iron and ferritin levels. The α /non- α chain synthesis ratio from his blood reticulocytes was 0.69, supporting a diagnosis of Hb SC with α -thalassemia trait. The mother has Hb S trait with 32.7% Hb S, MCV 81, and normal red cell morphology; the father has Hb C trait with 38.1% Hb C, MCV 82, with typical Hb C trait red cell morphology. α /non- α chain synthesis ratios were 0.75 for the mother and 0.76 for the father, suggesting that both have mild α -thalassemia trait. These findings demonstrate that "silent carrier" α -thalassemia is also silent with respect to the percentage of Hb S or Hb C in the heterozygote. These results suggest that the genetic pattern of α -thalassemia in blacks may be similar to that of Far Eastern populations in spite of the milder hematologic expression of α -thalassemia in blacks.

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TIME SEQUENCE OF RBC PROTOPORPHYRIN (PP) ACCUMULATION IN LEAD POISONING. David Hart, Sergio Pionelli, Joseph Graziano, NYU Medical Center and NY Hospital-Cornell Medical Center.

Lead intoxication in children deranges the last step in heme synthesis and results in the accumulation in the RBCs of protoporphyrin in the form of the Zn-chelate (ZnPP); some patients however exhibit an increased level of unchelated PP (Free PP). To evaluate the hypothesis that Free PP accumulates first and is later converted to ZnPP, the sequence of accumulation of the two PP types was followed in lead poisoned animals. Total PP, ZnPP, and Free PP were quantitated by fluorescence spectrophotometry. Three different species were used: rats, guinea pigs and rabbits. In rats and guinea pigs, increase in total PP was prompt (within 10 days) but modest (3x normal); in rabbits the increase in total PP was more marked (10x normal). Initially in all 3 species there was a rapid increase in Free PP, with a subsequent increase in ZnPP. During Pb intoxication, the relative concentration of the two types of PP was measured in RBCs separated according to age on density gradients. There was 4 times more Free PP than ZnPP in the youngest RBCs, while the oldest RBCs contained 3 times more ZnPP than Free PP. After Pb administration was discontinued the predominant PP species was ZnPP and the concentration of Free PP was minimal. These data indicate that the effect of Pb on heme synthesis results in initial accumulation of Free PP which later chelates Zn to form ZnPP. Extrapolation of these data to humans suggests that when in children both Free PP and ZnPP are present, the exposure to lead is recent.

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UNBALANCED GLOBIN SYNTHESIS IN ANTI-LEPORE HEMOGLOBIN ERYTHROID CELLS. George R. Honig, Larry Tremaine, R. George Mason, and Loyda N. Vida. Northwestern

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The Lepore and anti-Lepore hemoglobins probably arose as a result of non-homologous crossing over of globin β and δ genes, with the formation of gene hybrids. Lepore ($\delta\beta$) variants produce a thalassemia-like disorder, whereas individuals with anti-Lepore ($\beta\delta$) hemoglobin typically are hematologically normal. Hb Lincoln Park is a $\beta\delta$ variant representing a crossover between β 22 and δ 50; in addition valine-137, in the δ segment, is deleted. This variant was found in a 3 year old boy, his mother and an uncle. None of these individuals had anemia nor microcytosis, but their reticulocyte counts were elevated (3.6 - 4.9%). Globin synthesis by peripheral blood reticulocytes was studied by determination of L-leucine- ^{14}C incorporation in vitro. The rate of synthesis of the $\beta\delta$ chain was significantly less than that of the normal β chain and was similar to that of the δ chain. Labeled α globin was recovered in early-emerging DEAE-Sephadex fractions, suggesting the presence of significant quantities of free α -chains. The total α /non- α globin synthesis ratio was 1.4 to 1.5, indicating a substantial excess of α chain synthesis. These findings may account for the elevated reticulocyte counts, and suggest that globin synthesis is unbalanced in this condition in the absence of a hematologic picture of thalassemia.

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LOCALIZATION OF THE SITE OF ACTION OF A SERUM INHIBITOR OF ERYTHROPOIESIS. Ronald Hoffman, LouAnn Maffei, Richard Weininger, Kim Ritchey, Nicholas Dainiak,

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Serum IgG inhibitors of erythropoiesis have been found in adults with acquired pure red cell aplasia but have not been described in children. We studied the mechanism of action of such an inhibitor in a 5 1/2 year old male with acquired erythroblastopenia and severe anemia of 4 months duration. After an unsuccessful trial of corticosteroids, normal erythropoiesis was established with the administration of Cytoxan (CT). When the patient's erythroblastopenic marrow cells were cultured in plasma clots, increased numbers of CFU-E and BFU-E derived colonies formed in the presence of erythropoietin. Addition of the patient's pretreatment whole serum both to erythroblastopenic autologous marrow and to allogeneic normal marrow cells suppressed CFU-E but not BFU-E derived colony formation. The specific inhibitor of CFU-E derived colony formation was present in the IgG fraction of the pretreatment serum. Whole serum obtained following the CT-induced remission no longer inhibited erythroid colony formation. Acquired pure red cell aplasia associated with a serum inhibitor occurs in the pediatric age group. Our findings suggest that this inhibitor is an IgG immunoglobulin which acts directly on the more mature CFU-E but not on the less differentiated BFU-E precursor.

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INHIBITION OF NEUTROPHIL PHAGOCYTOSIS BY HYDROCORTISONE IN VITRO. James R. Humbert and Josephine T. Lee

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Inhibition of several membrane-associated neutrophil (PMN) functions by corticosteroids has been suggested in previous reports. We evaluated the effect of hydrocortisone (HC) on phagocytosis by human PMNs with two widely utilized phagocytic assays. PMNs of adult volunteers were obtained by methylcellulose-hypaque separation. After a 30-minute pre-incubation with varying concentrations of HC, the cells were assayed either by Stoessel's Oil Red O method or by Tan's phagocytic-bactericidal method. Simultaneous control PMNs were pre-incubated with drug-free buffer. Trypan blue viability was satisfactory ($> 93\%$) with up to $2 \times 10^{-3}\text{M}$ HC. Phagocytosis and bactericidal activity toward *Staphylococcus aureus* were unperturbed using up to $2 \times 10^{-3}\text{M}$ HC. By contrast, in the Oil Red O assay a significant inhibition of phagocytosis was observed with $2 \times 10^{-3}\text{M}$ HC (mean % inhibition $\pm$ SE 14.5 ± 1.6) and with $2 \times 10^{-4}\text{M}$ HC (17.9 ± 4.2 , $p < 0.025$). With $2 \times 10^{-2}\text{M}$ HC profound inhibition of phagocytosis (76.1 ± 5.1) occurred; similar inhibition (60 ± 12.5) was observed in the phagocytic-bactericidal assay. At $2 \times 10^{-2}\text{M}$ concentration, however, HC caused severe morphological cell alterations with over 60% of the PMNs trypan blue positive. Bactericidal activity remained normal at any HC concentration. Our data indicate that 1) in vitro exposure to HC affects phagocytosis by PMNs 2) it does not impair bactericidal activity 3) the Oil Red O phagocytic assay is more sensitive than the phagocytic-bactericidal test in detecting moderate phagocytic defects.