ABSTRACTS OF SPECIAL INTEREST


A new variant of one of two forms of carbonic anhydrase present in human erythrocytes has been demonstrated upon starch gel electrophoresis of hemolysates prepared from erythrocytes obtained from four Micronesian subjects. The variant, CA-Ic, appeared to be allelic with CA-Ia and CA-Tb. Preliminary studies of four pedigrees suggested that the variant was under the control of a single autosomal gene.—E. R. J.


Various tissues of C57BL mice were injected intraperitoneally into newborn Balb/c mice. C57BL placental cells, composed chiefly of trophoblastic cells, were found to produce runt disease; C57 BL liver and disrupted placental cells were ineffective. Isologous adult Balb/c spleen cells could prevent this reaction. C57BL x Balb/c F1 placental cells were incapable of producing disease in newborn Balb/c animals. Participation of maternal leukocytes were excluded as a source of this phenomenon. The authors conclude that placental cells, probably trophoblastic, are capable of mediating immunologic reactions; they speculate that these trophoblastic cells do not normally react against the mother animal because they are exposed to maternal antigens very early and thereby develop tolerance. What this phenomenon has to do with the mother tolerating the placenta remains unsettled.—I. G.


An HN2 resistant guinea pig cell strain was selected in vitro by cloning technics. This new cell line was compared with the original cell line as to x-ray sensitivity. No difference in susceptibility to radiation damage between the HN2 resistant and HN2 sensitive cell lines was noted. The author infers that the primary events in HN2 damage and radiation damage may be different, even though later biological manifestations may be similar.—I. G.
ERYTHROCYTES


Experiments performed with intact human erythrocytes demonstrated that the passive transfer of Na and K was higher at pH 8 and pH 7 than at pH 7.5, and that the passive transfer was increased by ouabain that presumably inhibited active transport. Glucose utilization was also inhibited by ouabain and the degree of inhibition was directly correlated with the changes in Na and K concentrations. At pH 7, glucose utilization was inhibited 50 per cent and lactic acid was directly correlated with the changes in Na. These studies indicated that the permeability of the erythrocyte membrane was probably altered during incubation at different pH’s and they provided indirect evidence for a mechanism for active transport of Na and K linked to glucose metabolism and the ouabain-inhibitable ATPase.—E. R. J.


An impressive series of studies was performed with morphologically intact, homogeneous membranes, essentially free of hemoglobin, prepared by dialysis of human erythrocytes against increasingly hypotonic sodium chloride solutions. These membranes were unable to utilize glucose, even when ATP or ADP was added, or ribose, inosine or adenosine. The pentose of ribose-5-phosphate was utilized and ketopentoses were formed. Activity of the following enzymes that are involved in carbohydrate metabolism was demonstrated in these membranes: glyceraldehyde phosphate dehydrogenase, aldolase, phosphoribosylamine, nucleoside phosphorylase, phosphoglycerate kinase, transketolase, and phosphoketopentose epimerase. Activity of the following enzymes was not detectable: phosphoribomutase, glucokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and triosephosphate isomerase. Membrane enzyme levels were lower than the levels in hemolysates prepared by osmotic lysis as usually performed, but by no fixed amount. Since the enzymes required for the generation of ATP were found in these membranes, it was suggested that the organization of the erythrocyte membrane was ideally suited for phosphate, sodium and potassium transport. —E. R. J.


Liberation of orthophosphate from phosphatidic acid by ghosts prepared from human erythrocytes was demonstrated to be magnesium-dependent and was stimulated by sodium and, to a lesser extent, by potassium and lithium. The activity of the phosphatase was of the same order of magnitude as that of diglyceride kinase. The authors had previously suggested that sodium transport is coupled to renewal of phosphate in phosphatidic acid and that this renewal is dependent upon the phosphatidic acid cycle (diglyceride kinase plus phosphatidic acid phosphatase). The present investigation provided evidence compatible with the hypothesis that the phosphatidic acid phosphatase might also be a part of the Na+ + K+ dependent, ouabain-inhibitable ATPase.—E. R. J.


Ghosts, prepared from human erythrocytes, were assayed for their ability to synthesize phosphatidic acid by the three known pathways. The rate of synthesis of the diglyceride kinase reaction was 40 times greater than the rate of synthesis from monoglyceride, ATP, CoA and fatty acids, and about 2500 times greater than the rate of synthesis from alpha-glycerophosphate, ATP, CoA and fatty acids. It was found that the diglyceride kinase activity in ghosts was as high as the Na+ + K+ dependent, ouabain-inhibitable ATPase and
it was suggested that the diglyceride kinase might be a component of the ATPase system.—E. R. J.


An approximately 350-fold purification of phosphoglycerate kinase from human erythrocytes, with a yield of 29 per cent, is described.—E. R. J.


Blood (erythrocyte) GSH concentrations were determined with a stable, water-soluble reagent, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) that reacted with sulfhydryl compounds to produce a relatively stable yellow color. After initial spectrophotometric standardization, GSH standards were no longer required. The method did not appear to be influenced by temperatures between 0 and 40 C., the amount of DTNB (between one-half and twice as much as was used routinely), pH between 5.7 and 8.3, the amount of blood between 0.1 and 0.5 ml., or incubation of blood with acetylphenylhydrazine (small correction necessary if results of high accuracy required). Only three solutions were required, and the values obtained were in excellent agreement with those obtained with the nitroprusside method. As with the nitroprusside method, other sulfhydryl compounds reacted with the DTNB reagent and yielded extinction coefficients essentially identical with those observed with GSH.—E. R. J.


Procedures for the isolation of lactic dehydrogenase were detailed. The immunologic and kinetic characteristics of the enzyme obtained from human erythrocytes were similar to those of the enzyme isolated from human heart tissue, but differed from those of the enzyme isolated from liver and from a hepatoma.—E. R. J.


Intact and hemolyzed human red cells were incubated with trypsin and chymotrypsin. Trypsin reduced the red cell acetylcholinesterase content by about half; chymotrypsin destroyed it completely. Trypsin (but not chymotrypsin)-treated cells responded to acid hemolysis (Ham test) in the same way as those of patients with paroxysmal nocturnal hemoglobinuria (PNH). The authors conclude that acetylcholinesterase activity is superficially placed on the red cell membrane and that the red cell membrane in PNH may be structurally abnormal.—F. W. G.


The enzyme activity per red cell mass was studied in 41 healthy people and 162 patients, most of them severely anemic. Five enzymes were measured: glucose-6-phosphate dehydrogenase (G-6-PD), glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), aldolase (ALD), and acid phosphatase (PHOSPH). Increased levels of red cell enzyme activity were found in vitamin B_{12} deficiency anemia, chronic hemorrhagic anemia, iron deficiency anemia, and various types of hemolytic anemia. A further increase was noted in vitamin B_{12} deficiency and in iron deficiency during the early phase of remission after treatment. When compared to normals, the GOT rose more than the other enzymes in the cases mentioned above with the exception of megaloblastic anemia, where ALD rose to higher levels. In some cases of myeloma, leukemia and lymphoma, subnormal enzyme levels were noted. In azotemia with severe anemia, the G-6-PD and ALD levels were increased. The enzyme activity of the red cell population is correlated to the mean age of the red cells. The enzyme content of young cells is high, and the activity decreases during the aging process.—C. W.
radioiron was incorporated into ferritin and heme. Similar results were obtained in vivo using rat marrow; incorporation of both ferritin and heme was enhanced by phenylhydrazine and depressed by bacterial endotoxin. After continued incubation, ferritin Fe$_{59}$ declined and heme iron rose slightly. The authors conclude that ferritin plays an active role as an intermediary between iron originating from plasma and heme synthesized by marrow and reticulocytes. Ferritin has a very rapid turnover releasing its iron as the protein is degraded; the iron released is either incorporated into heme or returned to the serum.—R. O. W.

THE PLASMA-TO-CELL CYCLE OF TRANSFERRIN.

$^{59}$Fe-labeled transferrin has selective affinity for the surfaces of immature red blood cells; its uptake is roughly proportional to its degree of saturation with iron; transferrin molecules that have yielded their iron to the reticulocyte are replaced on the cell surface by iron-laden transferrin molecules. Transferrin may be responsible for the agglutinability of immature erythrocytes.—R. O. W.


To support the hypothesis that transferrin is not kinetically homogenous, donor plasma was first labeled with Fe$_{55}$; after several hours plasma was withdrawn from the donors and tagged in vitro with Fe$_{59}$ as a second label. When this doubly labeled plasma was injected into hematologically normal recipients the 50 per cent clearance rate of Fe$_{59}$ was slower by 1-3 hours than the Fe$_{55}$ clearance rate. The significance of this "second component" is not known. It has little relevance to the "labile pool" concept which is based on a 50 per cent clearance rate of approximately 7 days.—R. O. W.


The authors describe the preparation of highly purified (possibly homogeneous) human intrinsic factor, which is a vitamin B$_{12}$ binder and appears to have a molecular weight of approximately 93,000. About 30 μg. of this preparation are fully active in the Schilling test. This binder "S" accounts for most of the vitamin B$_{12}$ binding capacity of gastric juice. In addition, gastric juice contains two other binders, "T" and "R." The former appears to be the peptic digestion product of "S." It also possesses intrinsic factor activity. Binder "R" seems to be unrelated to intrinsic factor. The purification procedure is described in detail. The following steps were used: chromatography on CM-cellulose, DEAE-cellulose, DEAE-sephadex, CM-sephadex, gel filtration on sephadex G-200 and G-75, and finally electrophoresis on pevikon. Throughout the procedure all fractions were assayed for intrinsic factor activity. This extensive work brings up the purity of human intrinsic factor to the same level as that described by Broner and Davison for hog intrinsic factor, but has the advantage of not including preparative free electrophoresis and ultracentrifugation.—C. W.


A 13 year old child with thalassemia major developed a megaloblastic crisis with increasing transfusion requirements after two severe episodes of epistaxis. In addition to elevated urinary FIGLu excretion, a severely megaloblastic blood picture was found. The response to folic acid therapy appears to be definitive with reversion of the hematologic findings to pre-crisis levels. Increased need for folic acid in hemolytic syndromes and a possible absorptive defect for this vitamin are considered to play an important role in the development of megaloblastic states in such patients. Any additional stress on the erythropoietic system (i.e., blood loss, infection, pregnancy) will increase the likelihood of precipitating such an event.—A. I. C.


A total of 179 cases of megaloblastic anemia consisted of 95 of pernicious anemia, 38 of nutritional anemia, 15 anemias of pregnancy, 14 post-gastrectomy anemias, and a few others. The
ABSTRACTS

When tocopherol supplements were administered; R. J.
... they require extension and explanation.—E.


Minimal increases in hemolysis of erythrocytes exposed to H2O2, slight decreases in apparent total erythrocyte life span (90 or 95 per cent Cr51 life span), and decreased plasma tocopherol concentrations were observed in subjects maintained on a tocopherol-deficient diet for about 1 week, but seriously ill patients may need initial folic acid and B12 treatment. Twenty-three of 24 PA patients but only 9 of 14 with nutritional anemia maintained on tocopherol-depleted monkeys, 5 had deficient intrinsic factor absorption, 5 nutritional deficiency of folic acid, and 4 intestinal malabsorption.—F. W. G.


A review of the author's studies on the effect of thiamine deficiency on transketolase activity in rat and human erythrocytes. Transketolase, with thiamine pyrophosphate as a cofactor, is involved in the conversion of pentose phosphate to sedoheptulose phosphate. Decreased activity of the enzyme system may be determined by increased accumulation of pentose phosphate upon incubation of erythrocytes with glucose, by decreased formation of C14O2 from glucose-2-C14 by intact cells and by decreased formation of hexose when ribose-5-phosphate is incubated with hemolysates. Partial restoration of activity occurs upon addition of thiamine in vitro. These assays of transketolase activity as a measure of thiamine deficiency on a cellular level appear to be specific and more sensitive than the methods previously used to determine deficiencies of this vitamin. Alterations in erythrocyte transketolase activity precede other manifestations of thiamine deficiency and further evaluations of these technics are warranted.—E. R. J.


The erythrocytes of young rabbits that had been maintained on a vitamin E-deficient diet for 3 to 4 weeks contained 50 per cent more GSH than did the erythrocytes of control rabbits. The specific activity of GSH isolated from the erythrocytes probably was not significantly different in the two groups of animals ½ to 12 hours after the intraperitoneal injection of glycine-1-C14. Specific activities of unbound glycine in plasma and whole blood were approximately the same in the two groups. These findings were interpreted to suggest that the rate of incorporation of glycine into erythrocyte GSH was higher in the vitamin E-deficient, dystrophic rabbits. The physiologic significance of the inverse relationship between erythrocyte GSH and vitamin E remains unknown.—E. R. J.


The fatty acid patterns of total phospholipids and of individual phosphatide classes of erythrocytes from subjects fed diets with known fat contents were studied in detail. Phospholipids of erythrocytes were remarkable for their high content of palmitic (16:0) and arachidonic (20:4) acids, and the ratios of oleic (18:1) and linoleic (18:2) acids in plasma and erythrocytes were strikingly dissimilar in subjects eating ad libitum. Variations in kind and amount of dietary fat produced only small changes in amounts of the major erythrocyte lipids and in distribution of phospholipid classes. However, alterations in dietary fat did change the fatty acid composition of the phospholipids after 4 to 6 weeks. A linear relationship was observed between the linoleic acid content of the diet and the linoleic acid content of erythrocyte phospholipids. Studies in which corn oil or triolein served as the sole sources of dietary oil or triolein served as the sole sources of dietary

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fat indicated that the incorporation of new fatty acids did not occur, primarily, during erythropoiesis in bone marrow. Exchanges of fatty acids or intact phospholipids must have occurred between mature erythrocytes and precursor pool(s), and the exchange may have been analogous to that demonstrated for free cholesterol. The rate at which fatty acid alterations occurred in erythrocytes was slower than that for plasma lipids, but more rapid than for adipose tissue. It was suggested that determinations of erythrocyte fatty acid patterns might be useful indexes of adherence to prescribed diets in large-scale, long-term feeding studies in man.—E. R. J.


The extent of synthesis of porphobilinogen and porphyrins from δ-aminolevulinic acid (ALA) in hemolysates prepared from erythrocytes obtained from 16 normal subjects, 9 children with iron deficiency anemia and 11 children with plumbism was determined. ALA dehydrase activity, responsible for the conversion of ALA to porphobilinogen, was not significantly different in erythrocytes of iron deficiency anemia, but was markedly decreased in erythrocytes of lead poisoning. This decreased activity in erythrocytes of plumbism could be enhanced greatly by the addition of GSH to the incubation medium, but GSH had no effect on ALA dehydrase activity of normal cells. Porphyrin synthesis in vitro was greatest in those cells with the lowest initial porphyrin concentration. Normal erythrocytes were capable of greater uroporphyrin and coproporphyrin synthesis than were cells of lead poisoning. The capacity for porphyrin synthesis was not influenced in either type of cell by the addition of GSH. Erythrocytes of iron deficiency had a lower capacity for uroporphyrin synthesis compared with normal cells, suggesting decreased activity of porphobilinogen deaminase or isomerase. Significant protoporphyrin synthesis was never observed. These studies demonstrated deficient ALA dehydrase activity in erythrocytes of plumbism, perhaps due to inactivation of sulfhydryl groups, and decreased capacity for porphyrin synthesis from porphobilinogen even when ALA dehydrase activity was restored with GSH. They did not, however, prove that the activity of ALA dehydrase was rate-limiting in the erythrocytes of plumbism.—E. R. J.


Two patients with cutaneous manifestations of porphyria and three normal subjects were studied to determine the effects of natural and artificial light with wavelengths greater than 320 mp upon porphyrin metabolism. An increase in urinary and fecal excretion of uroporphyrin occurred when the two patients were exposed to light. There was some decrease in urinary and fecal excretion of coproporphyrin and a decrease in fecal protoporphyrin and deuteroporphyrin concentrations after the exposure period. No changes in urinary, fecal or erythrocytic porphyrins were noted after exposure of control subjects to light. Concentrations of uroporphyrin were higher in the skin biopsies taken from the patients than in similar biopsies obtained from the control subjects, but there were no differences in the concentrations of coproporphyrin and protoporphyrin. The concentrations of uroporphyrin in erythrocytes, urine and feces of the two patients appeared to be higher during summer months than during March and May, although erythrocyte porphyrins were not affected by exposure to light during the experimental period. The authors suggested that the exposure of patients with cutaneous porphyrin to light of the wavelengths studied evoked photodermatitis, although neither patient manifested cutaneous lesions during the study, and altered their porphyrin metabolism with increased production of porphyrins.—E. R. J.


By modifying the original column chromatographic procedure that had been used to identify free bilirubin, pigment I and pigment II in icteric sera, it was possible to show that pigment I consisted of free bilirubin and pigment II. That the manipulations involved probably were not responsible for the findings was indicated by the observation that similar treatment of pigment II yielded only minute amounts of free bilirubin. It was suggested, therefore, that pigment I, previously thought to be a monoglucuronide, consisted of a complex or aggregate of free bilirubin and pigment II, the diglucuronide. However, the existence of bilirubin monoglucuronide was not excluded.—E. R. J.
ABSTRACTS


Free (unconjugated) or conjugated bilirubin-

\[ \text{C}^{14} \]

was instilled into the intestinal tract of rats with external bile fistulas. Bile, urine and feces were assayed for radioactivity, and the amount of labeled bilirubin excreted in the bile was measured. Four to 40 per cent of administered free and conjugated bilirubin was excreted during 8 to 75 hours after infusion of labeled pigment into the duodenum, whereas only traces appeared in the urine, and most of the radioactivity remained unabsorbed. Excretion of unconjugated bilirubin began within 30 minutes, reached peak values during the first few hours and declined asymptotically over the next 15 to 40 hours. Excretion of conjugated bilirubin occurred within 30 minutes, but the early phase was distinctly slower than with unconjugated pigment and persisted for up to 24 hours with little change in rate. Treatment of rats with oral neomycin failed to alter the pattern of absorption and excretion of labeled pigment. Evidence was presented to indicate that unconjugated bilirubin was probably not altered chemically prior to absorption, whereas conjugated bilirubin was hydrolyzed. With rat and hamster everted gut sacs, transfer of unconjugated bilirubin into serosal fluid could not be demonstrated, but gross absorption of bilirubin on mucosal surfaces was noted. Studies in vivo indicated that bilirubin absorption occurred throughout the small and large bowel.—E. R. J.


Studies on the rate of reactivity of reactive -SH groups in oxy and deoxyhemoglobin were carried out to provide further evidence for the conformational changes taking place in this protein on oxygenation. Only the -SH group in position 93 in the \( \beta \) chain is reactive. Reactions of this -SH group with p-mercuribenzoate at pH 7 were identical and instantaneous in both oxy and deoxyhemoglobins. However, N-ethyl maleimide reacted more slowly and at different rates with the -SH groups in the two forms of hemoglobin whereas iodoacetamide reacted with the -SH groups of oxyhemoglobin and not at all with deoxyhemo-

globin. Hb H, however, has eight reactive -SH groups with iodoacetamide whether oxygenated or not. These observations lend strong support to the concept that configurational changes take place on deoxygenation and that interchain forces bear an important relationship to the Bohr effect and heme-heme interaction.—A. I. C.


Conformational changes in biologically active proteins result when reactions take place with smaller molecules. Oxygenation of hemoglobin is known to be accompanied by a variety of altered physical and chemical properties which suggests such configurational alterations. It has been postulated that the heme groups of deoxy hemoglobin are brought closer together than in the oxygenated molecule which accounts for the sigmoid shape of the oxygen dissociation curve and for the concept of "heme-heme interaction." More direct evidence for this concept has been obtained in recent years by determining the reactivity masked -SH groups on deoxygenation and alterations in interchain bonding with modification of the vinyl side chain of heme. In order to test this hypothesis further, oxy- and deoxyhemoglobins were dissociated into half molecules in 2M sodium chloride. Dissociation was complete in the case of oxyhemoglobin but incomplete with the deoxy compound, suggesting stronger bonds between \( \alpha \) and \( \beta \) subunits in the deoxy compound.—A. I. C.


Additional studies on the order of tryptic peptides in rabbit hemoglobin are presented. These findings, taken in conjunction with previous studies on the order of these peptides on the basis of their rate of label incorporation, provide further evidence for the stepwise sequential addition of amino acids to a polypeptide chain growing from the NH\( _2 \)-terminal to the COOH-terminal end.—A. I. C.

Measurement of In Vivo Survival of Red Blood Cells by Means of Starch Block Hemoglobin Electrophoresis. F. A. Restrepo

Red cell survival times were studied in 10 patients by means of starch block electrophoresis of hemoglobin solutions using combinations of hemoglobin types which permitted suitable separations. Six subjects had normal red cell survival times, four abnormal. \( \Gamma_2 \) studies done simultaneously on six of the subjects corroborated these findings. The authors discuss the uses and limitations of this method in the study of red cell survival times.—A. I. C.


Umbilical cord plasma hemoglobin levels, determined by a sensitive method giving values of less than 0.58 mg. per 100 ml. in the normal, yielded concentrations of plasma hemoglobin 10 to 20 times that found in the mother’s plasma. Plasma hemoglobin was normal in 22 of 30 mothers’ samples, the remaining having levels of up to 1.5 mg. per 100 ml. By contrast, 26 of the 30 infants exhibited elevated values (up to 11.0 mg. per 100 ml.). To account for these findings, the authors ruled out artifacts of collection, confusion with Inyoglobins as well as evidence for known hemolytic mechanisms. In view of the observation of higher umbilical cord hemoglobin concentrations in arterial than in venous plasma, the authors suggest that the placenta clears plasma hemoglobin probably by means of diffusion into the maternal circulation. A positive correlation of plasma levels with infant hemoglobin levels suggested an inability on the part of the fetus to deal with extravascular red cell destruction because of the immaturity of the reticuloendothelial system. These observations may also explain the low levels of haptoglobins seen in newborns as well as the moderate decreases noted in some patients during the latter part of pregnancy.—A. I. C.


Hybrid formation between donkey and C57/B1 mouse hemoglobin was carried out by the technic described and four hemoglobin species were found. The identity of these species was elucidated by tagging the readily available \(-SH\) groups of the \( \beta \) chain of the two original hemoglobins by \( \text{C}^{14} \)-labeled agents as well as by chain separation in starch-gel at pH 1.8. Oxygen equilibria on each of the four hemoglobins was carried out and it was demonstrated that not only is the Bohr effect associated with the \( \beta \) chain, but also that the latter determines the over-all oxygen affinity. The inconsistency of these findings with reports from the literature on the Bohr effect in \( \text{Hb} \ H (\beta_4) \) is explained on the basis of the altered ionic environment of certain acid groups in the \( \beta_4 \) tetramer.—A. I. C.


Linkage studies for genes controlling the \( \alpha \) and \( \beta \) chains of mouse hemoglobins were carried out. Genetic differences in \( \alpha \) chain structure segregated independently from genetic differences in \( \beta \) chain structure. The two non-\( \alpha \) (\( \beta \)) chains found in diffuse mouse hemoglobin are felt to bear a relationship similar to the \( \beta \) and \( \delta \) chain found in human hemoglobins. Complicating the situation in mice is the finding that two \( \beta \) chain units occur in some strains, while a single \( \beta \) chain occurs in others.—A. I. C.


The author experienced difficulty in relating gestational age to \( \text{Hb} \ F \) content of the fetus when using several modifications of the alkali denaturation technic for \( \text{Hb} \ F \) determination. An adaptation of the IRC-50 method of Allen, Schroeder, and Balog (J. Am. Chem. Soc. 80:1628, 1958), using a phosphate buffer, pH 7.02 at 5 C, to elute the fetal pigment is presented. A high degree of internal consistency was noted and a good correlation between \( \text{Hb} \ F \) content and gestational age was found in a study of 172 infants at delivery. The method is not applicable as described to the quantitation of \( \text{Hb} \ F \) since approximately 10 per cent adult hemoglobin and a variable percentage of non-heme proteins appear in the \( \text{Hb} \ F \) fraction from IRC-50. Nevertheless, the author suggests that the easy reproducibility and appar-
ent good correlation with gestational age make this technique an excellent one for such studies.—A. I. C.


A slight modification of the acid elution staining technique of Hb F containing red cells, first proposed by Betke, was utilized to study 25 women pre- and postpartum for the presence of circulating fetal erythrocytes. One woman had a positive test prepartum, 10 were positive postpartum. The low prepartum incidence of fetal red cells in the maternal circulation is at variance with reports in the literature and is explained on the higher degree of specificity of the procedure described. The use of carbazochrome salicylate to suppress placental red cell transfer is mentioned.—A. I. C.


Evidence for the transplacental passage of fetal and maternal cells was obtained by the use of high titered fluorescent antibody. Minor transplacental red cell populations were found at the time of birth in 2 of 27 mothers and in 8 of 22 infants. Two weeks later, only 2 infants and only 1 mother continued to manifest the minor erythrocyte population.—A. I. C.


The distribution of Hb F and the erythrocytes of various hematologic disorders was studied by the means of a modification of the acid elution technique of Betke. In normal individuals and in patients with hemoglobinopathies having less than 2.8 per cent Hb F by the alkaline denaturation technique, no positive cells were seen although an occasional erythrocyte appeared to be lightly stained. In a patient with homozygous hereditary persistence of fetal hemoglobin and 100 per cent alkaline-resistant hemoglobin, all erythrocytes were deeply stained. In 13 heterozygotes for this anomaly, with 18 to 32 per cent alkaline-resistant hemoglobin, all red cells stained positively for Hb F by the acid elution method. A similar distribution of Hb F was seen in S-\(\beta\) heterozygotes, C-\(\beta\) heterozygotes and in a patient with heterozygous \(\beta\) thalassemia-Hb F. Heterogeneous distribution of fetal hemoglobin-containing cells was seen in blood films from patients with sickle cell anemia, sickle cell-Hb D disease, thalassemia, sickle cell-thalassemia and Hb C-thalassemia diseases and aplastic anemia. This heterogeneity indicated that some cells contain considerable amounts of Hb F, while others were virtually devoid of this material in contrast to the uniform distribution of the fetal pigment in the conditions mentioned above. Studies of Hb F distribution in fetal erythrocytes demonstrated a shift from a relatively homogeneous population in a 300 Gm. fetus to a double population at birth and shortly thereafter, consistent with the appearance of increasing amounts of Hb A. These findings indicate that individual red cells can synthesize both adult and fetal hemoglobin but that the distribution of these hemoglobins in the red cell population is not uniform. In diseases in which elevated levels of Hb F occur, the hemoglobin was heterogeneously distributed among the red cells except in the presence of the anomaly "hereditary persistence of fetal hemoglobin" where uniform distribution was regularly found.—A. I. C.


Oxygen dissociation curves were determined on 51 cord blood specimens. Some degree of correlation between the pO_2 required to produce half saturation and the level of Hb F (in the range of 50 to 85 per cent) was obtained leading the authors to conclude that the hemoglobin composition is one of the parameters influencing the position of such curves. Points of divergence between these studies and two others taken from the literature are discussed.—A. I. C.


The effect of various procedures on the sickling of Hbs A plus S containing red cells was studied. Washing 10 times in sodium or potassium chloride abolished sickling in wet-sealed preparations left at room temperature for 24 hours but did not
interfere with the development of a positive sickle cell test on the addition of sodium metabisulphite or of vitamin C. KCN also abolished sickling in the wet-sealed preparation but did not interfere with the vitamin C or sodium metabisulphite tests, while potassium thiocyanate had no effect whatsoever on the phenomenon. Methemoglobinemic or sulfmethemoglobinemic cells failed to sickle even with strong reducing agents, while sulfhemoglobinemic cells retained their ability to sickle. Red cells from a patient with thalassemia-Hb I disease, which sickled poorly in 2 per cent metabisulphite and significantly in 4 per cent metabisulphite, underwent sickling when in the methemoglobinemic phase with added bisulphite. Vitamin C would not induce sickling in these red cells. The authors discuss several aspects of the reduction of hemoglobin in the production of the sickling phenomenon, such as carbonic anhydrase levels and the presence of abnormal hemoglobin pigments. The mechanism for sickling in thalassemia-Hb I cells appears to depend on another as yet undetermined mechanism.—A. I. C.

**THE CONJUNCTIVAL SIGN OF SICKLE-CELL DISEASE.**


A pathognomonic conjunctival sign of symptomatic sickle cell disease is said to consist of "multiple, short, comma-shaped or curlicued capillary segments" which appear to be isolated from the vascular network by vessels devoid of blood. These findings, best seen with a slit lamp, occur predominantly in the bulbar conjunctiva toward the lower fornix. It is necessary to distinguish these changes from beaded capillary flow or mild sludging which may occur in other conditions as well. Furthermore, the typical picture described above rarely occurs in asymptomatic sicklers. Of 74 sickling patients with symptomatic disease, 93 per cent had diagnostic conjunctival changes, 4 per cent were questionable and 3 per cent were negative. In normal controls and clinically asymptomatic sickling disorders, 96 per cent lacked this sign while in 4 per cent the examination was indiscernive. The author stresses the reliability of this parameter and the response of the conjunctival sign to changing or variable clinical courses (i.e., positive in symptomatic thalassemia-Hb S disease, negative during asymptomatic phases). At present, no specific mechanism is suggested although the phenomenon may be related to the rate of blood flow.—A. I. C.


An 8 year old girl with sickle cell anemia complicated by cholelithiasis is described. The rare occurrence of gallstones in children, and their relative frequency in sickle cell anemia, suggest the need for considering the latter diagnosis in any Negro child with cholelithiasis.—A. I. C.

**PROLONGED SURVIVAL IN SICKLE CELL DISEASE.**


The histories of a total of 19 patients with sickling disorders (10, SS; 6, SC; 3, S-Thal.) living beyond the age of 30 were reviewed. The oldest patient with sickle-cell anemia was 59. In spite of classical laboratory and roentgenographic evidence of sickle cell anemia, these patients reached adulthood without the usual physical stigmata and debilities of the anemia. The disorders in the adult patients with sickle cell-Hb C and sickle cell-thalassemia diseases were milder in character. The oldest patient, 66, suffered from sickle cell-thalassemia disease. The authors ascribe the prolonged survival to the lack of hemolytic and thrombotic crises.—A. I. C.

**AN EVALUATION OF SOME ROENTGENOGRAPHIC SIGNS IN SICKLE CELL ANEMIA AND ITS VARIANTS.** J. R. Reynolds. From the University of Texas Southwestern Medical School, Dallas, Texas Southwestern Medical School, Dallas, Texas Southwestern Medical School, Dallas, Texas. South. M. J. 55:1123-1128, 1962.

Radiologic findings in the mandible and skull of patients with the sickling disorders are reviewed. These changes result primarily from narrow hyperplasia which leads to a diminution of supporting trabeculae and from the effects of bone infarction. Roentgenographic studies of the mandible revealed that the bony alterations were more easily defined in this region than in the skull because of the short object to film distance and the lack of overlying soft tissue. Changes noted included dissolution of the fine trabeculae of the medullary bone and thinning of the cortical bone leading to increased radiolucency. The corticies of the inferior margin of the mandibular body and of the ramus were extremely thin. By contrast, the remaining coarse trabeculae and the lamina

No correlation between the incidence of cold injury in the Negro and his hemoglobin type was noted, in spite of an attractive hypothesis relating such phenomenon to the presence of Hb S.
—A. I. C.


The effect of sickling on the viscosity of blood from patients with the sickle cell phenomenon was studied. The pH of the system affected the rate of increase in viscosity with decreasing oxygen content in a manner analogous to the effect of the same variable on per cent of sickled cells. A close relationship of flow time (as a measure of blood viscosity) to per cent sickled cells was evident and the nature of this relationship depended on the pH of the system. The presence of non-sickling red cells in the test mixture delays the increase in flow time and limits its maximum level to a value which can be predicted from the percentage of non-sickling cells present. These studies were conducted in parallel with experiments designed to test the effect of partial replacement transfusion therapy on the clinical course of patients with severe sickle cell anemia. Five patients, followed for up to 6 years, received replacement blood therapy at 6- to 8-week intervals and were noted to have a reduction in disability, discomfort and the need for hospitalization. Three were strikingly improved, one patient not being hospitalized during the 6 years she has been under treatment, another for 4 years on this regimen. Crises which occur on this program are less frequent than before therapy, milder, of shorter duration and tend to appear shortly before the next scheduled transfusion. Two patients were only moderately improved. The authors conclude that in selected patients a regimen of partial exchange transfusion may prevent the appearance of severe sickle cell crises and that the mechanism may be related to the lowered viscosity potential of sickle cell anemia blood containing up to 30 or 40 per cent normal (Hb A) red cells.—A. I. C.


A young woman with sickle cell-thalassemia disease was observed in the course of two separate pregnancies. Painful crises, with associated anemia, were observed during intervals of both pregnancies, but the anemia was not associated with signs of severely increased hemolysis. The authors correlate the increased sedimentation of oxygenated blood, the evidence for sludging and the lack of signs of excess hemolysis with a hypothesis that crises result from the stagnation of clumped oxygenated erythrocytes with increased sickling occurring in areas of such stagnation. The particular predilection for patients with the sickling disorders to develop severe manifestations during pregnancy may relate to the known increase in the sludging phenomenon during such times.—A. I. C.


The authors studied the hemoglobin types of 524 pregnant and 304 non-pregnant Negro females by paper electrophoresis. They noted a remarkably low incidence of sickle cell hemoglobin in the two groups: 4.8 per cent and 3.6 per cent,
respectively. Hb C incidence was 1.5 per cent and 3.3 per cent in the two groups. Spontaneous abortions occurred approximately 4 times as often among heterozygous carriers of Hbs S or C compared to those with Hb A (25 per cent vs. 7 per cent). Although the small number of cases studied leaves some doubt as to the significance of these figures, the authors mention several possible explanations for their findings.—A. I. C.


Roentgenographic findings in 15 patients with sickle cell-Hb C disease are presented. Except for a lower incidence, findings in this syndrome do not differ significantly from those seen in sickle cell anemia, 40 per cent of the author's series showing neither osseous nor visceral roentgenographic abnormalities. The author stresses the importance of aseptic necrosis of the femoral head in patients with the sickling disorders.—A. I. C.


Extensive retinal pathology was noted in a 30 year old Negress and treated by fever therapy. The subsequent development of a hemolytic crisis led to the diagnosis of sickle cell-Hb C disease. The authors emphasize the importance of searching for abnormal hemoglobins in Negro individuals with obscure retinopathy.—A. I. C.


A Negro family in which each of three brothers had four distinct types of hemoglobin is described. The patients were hematologically normal except for the presence of morphologic changes in the red cells (target cells, aniso- and poikilocytosis and some microcytoses). These hemoglobins were identified as A, C, Ga and a hybrid hemoglobin C/Ga. Hybridization experiments proved that the amino acid abnormality in the C fraction was in the β chain, that in the G fraction in the α chain. In addition, the expected A2 variant was found, with the structural formula $\alpha^G\delta^A$, in members of this sibship who were heterozygous carriers of Hb C. Hb G was found to be identical on peptide mapping with Hb $C_{Philadelphia}$ in which the abnormality has been shown to be $\alpha^A^B$. These observations add further support to the common genetic origin of the α chains in hemoglobin and to the concept that random recombination of dimers is involved in the last stage of hemoglobin synthesis.—A. I. C.


Clinical jaundice in a 9 year old girl presumed to have thalassemia minor was investigated by studying the kinetics of bile pigment formation. It was noted that sufficient ineffective erythropoiesis was present to account for most, if not all, the bile pigment secretion resulting in the jaundice. By following the incorporation patterns of C14-labeled glycine into stercobilin and hemoglobin-heme, and allowing for utilization of the label, it was possible to estimate a minimal daily hemoglobin turnover of at least 14.5 Gm., over 75 per cent of which was destroyed in situ. These findings demonstrate that chronic hyperbilirubinemia of the retention type can result primarily from intramarrow hemolysis while peripheral hemolysis is only moderately severe.—A. I. C.


Three subjects with thalassemia presented atypical genetic patterns for this syndrome. In two, typical thalassemia was associated with an unusually prominent level of Hb F, while in the third, these findings were present in association with Lepore hemoglobin. The authors review the literature relative to levels of Hbs A2 and F in the thalassemia syndromes. A2 levels have been reported to be persistently elevated in the majority of patients with thalassemia minor, while most laboratories note normal values for this minor hemoglobin fraction in the major form of the disease. Hb F levels in thalassemia minor, however, are less uniform, but the majority of investigators report less than 5 per cent Hb F in such patients. Patients with morphologic findings consistent with thalassemia, but having Hb F values greater
ABSTRACTS

than 5 per cent, were usually noted to have normal levels for Hb A\textsubscript{2}. These findings are discussed in the light of speculations concerning the relationship of thalassemia to abnormalities in polypeptide chains synthesis. Although no supporting evidence is presented, the authors conclude that in the three pedigrees reported, thalassemia major results from a defect of \beta-chain synthesis or of a combined deficiency of \beta and \delta chain synthesis.

—A. I. C.

LEUKOCYTES


The authors address themselves to the question as to whether the lymphatics can be sealed by x-irradiation. Rabbits were given varying doses of irradiation, 3000 r, 6000 r and 8000 r to a 4 cm. x 6 cm. pelvic field. Animals were sacrificed 1 week later and the lymphatics were injected with latex. No evidence of a break in the continuity of lymphatic vessels were found. However, at the higher dose ranges the lymphatics became tortuous and rigid. Postirradiation uteri removed from 30 women treated previously by intracavity irradiation were also examined. Lymphatic channels in these uteri were intact except in areas of frank necrosis. Conclusion: x-irradiation does not produce lymphatic blockage.—I. G.


By electrophoretic partition in agar, leukocytes were found to contain two proteases. One of them (cathepsin E) migrated towards the anode and exhibited its maximum activity at pH 2.5; the other (cathepsin D) migrated to the cathode and had maximum activity at pH 3.5. These enzymes were separated chromatographically on DEAE cellulose. Ninety per cent of cathepsine D was contained in the nuclear fraction, whereas 50 per cent of cathepsin E was contained in the nuclear fraction; the rest was found in the granules and cytoplasm.—L. D.

HEMOSTASIS

ON THE EFFECT OF MONOAMINOXIDASE INHIBITORS ON BLOOD COAGULATION AND ON THE ACTIVATION OF ANTICOAGULANTS. P. Hrdina and V. Kovářík. From the University of Bratislava, Czechoslovakia. Čas. lék. česk. 102:574-579, 1963.

The monoaminoxidase inhibitors (iproniazid, SKF-385 B and methylene blue) significantly potentiated the hypoprothrombinemic effect of Tromexan and phenylindandione in rats. Ephedrin in the doses used lacked this effect. Iproniazid and methylene blue slightly increased the prothrombin time in normal rats.—L. D.

MISCELLANEOUS


Why reduced heme pigment molecules react with oxygen to become oxygenated in one instance and in another instance to become oxidized continues to be an unanswered question. The results of the present investigation have suggested the possibility that heme can dissociate from reduced myoglobin and still remain physically associated with apoprotein in aqueous solution. The dissociated heme can then be auto-oxidized to hemein which will recombine with apoprotein to form metmyoglobin. The hypothesis is compatible with the finding that rates of auto-oxidation increase with decreasing partial pressures of oxygen.—E. R. J.


Antisera prepared to twice recrystallized myoglobin contained multiple antibodies which cross-reacted with serum and extracts of liver and kidney. After absorption, two or three lines of reaction were obtained between the sera and recrystallized antigen by immunelectrophoretic technics. The major line presumably is due to the reaction of myoglobin with the antiserum while the origin of the other two lines remains in doubt. Different forms of myoglobin, breakdown products of myoglobin or unrelated contaminating antigens are suggested as possibilities.—A. I. C.

CHANGES IN PROPERDIN LEVEL AFTER BLOOD LOSS IN VOLUNTEER BLOOD DONORS. J. Sourek and S. Sutko. From the Institute of Hematology
ABSTRACTS


Properdin levels were determined before and following the loss of about 500 ml. blood in a group of 28 volunteer blood donors with a large number of donations to their credit, at intervals of 2, 24, 48 and 72 hours, and then at weekly intervals during the course of 1 month. The reaction to the loss of the above-mentioned quantities of blood manifested itself by an increased properdin level on the 2nd to 6th day after donation, followed by a reduction in the level at the end of the 3rd week, to below the lower normal limits for the summer period. An occurrence of higher than normal values were demonstrated only in the spring.—L. D.


Six patients with normal blood counts received total body radiation ranging from 225 to 1500 r. Three of these patients subsequently received autologous bone marrow and one patient received homologous bone marrow. The rate and degree of depression of the cellular elements were roughly proportional to the dose of x-irradiation. Depression of reticulocyte count was an early and especially sensitive indicator of radiation effect on the marrow. Two of three patients receiving autologous bone marrow had slightly earlier recovery of bone marrow than was expected. Homologous marrow was completely ineffective in this regard.—I. G.


Thirty mongrel dogs were given autologous bone marrow 24 hours after receiving 600 r. The marrow was obtained by multiple bone marrow puncture and preserved with 20 per cent glycerol or 20 per cent dimethyl sulfoxide. Eleven of these 30 dogs survived for more than 30 days. These had received more than 2.5 billion cells. Thirteen dogs receiving less than 2.5 billion cells died. Dimethyl sulfoxide was as good as glycerol as a marrow protective agent.—I. G.

CYTOCHEMICAL EXAMINATION OF BONE MARROW IN LEAD POISONING. A. David. From the Department of Industrial Medicine, Prague, Czechoslovakia. Casop. lék. česk. 102:69-72, 1963.

Cytochemical analysis of normoblasts in chronic lead poisoning confirmed older findings of the presence of ribonucleic acid in the basophilic stippling, and an increase of non-hemoglobin iron. A new finding is the presence of polysaccharides with a positive PAS reaction. The polysaccharide differs partly from glycogen and is usually in granular form. It has no relation to the basophilic stippling, but is found significantly more frequently in sideroblasts. Other cytochemical deviations were not observed (reaction to lead, lipids, desoxyribonucleic acid, SH-groups). Supravital staining with Janus green and neutral red demonstrated an increase in mitochondria and neutral red staining vacobles in normoblasts.—L. D.


In severe anemia of various types and in acute leukemia, the usual laboratory methods were used to investigate renal function. In anemia, changes in renal function were rare. In the leukemias, proteinuria and hematuria, decreased concentrating ability, and increased NPN levels were common. Rarely there was also a decrease in creatinine clearance. In 3 of the 120 cases there was renal damage leading to uremia.—L. D.