ABSTRACTS

THEODORE H. SPAET, M.D., Editor

ABSTRACTS OF SPECIAL INTEREST

PROLIFERATIVE ACTIVITY OF BLAST CELLS IN ACUTE LEUKEMIA. C. Astaldi and C. Mauri. From the Medical Department, Pavia, Italy. Rev. belge Path. 23:70, 1953 (sic).

Several previous reports on low mitotic indices in bone marrow of acute leukemia are quoted. Bone marrows from five patients with acute leukemia were cultured with colchicine ("Stathmoskine test"), and mitotic counts were made every 2nd hour. After 16 hours, basophilic erythroblasts had 21 per cent mitoses, leukemic blasts 0.8 per cent. The authors concluded that acute leukemia is "fundamentally an aplastic disorder resembling agranulocytosis and erythropenia," and that treatment with cytostatics may aggravate the disease. (Abstractor's comment: This early paper is reviewed now because recent results by Cronkite et al. support this concept.)—P. G. R.


Rabbit peritoneal histiocytes and lung macrophages maintained in a sterile observation chamber were observed frequently to form cytoplasmic bridges between one another. BCG bacteria were observed passing from one histiocyte to another, suggesting that cytoplasmic flow between histiocytes was taking place. In addition, the author believes that he found labeled RNA in the bridges, suggesting that RNA may be transferred from one cell to another.—T. E. B.


A female infant with mongolism undergoing open-heart surgery received pooled donor blood from 10 healthy adult males. Blood was obtained from the patient at various time intervals after surgery, and the leukocyte suspensions were placed in tissue culture. One hundred chromosomal plates were then counted from each culture preparation. It was shown that significant numbers of viable donor leukocytes were present in the recipient's circulation 3 hours after surgery was completed but not at 24 hours or longer. It is suggested that in patients who are to receive an exchange transfusion, the use of pooled blood from normal donors of the opposite sex provides one with a method for following the survival of donor leukocytes in the recipient. This technic may be useful in studying the etiology of the lymphocytic-splenomegaly syndrome occurring after open-heart surgery.—T. E. B.
PERSISTENT MITOSIS OF TRANFUSED HOMOLOGOUS NUCLEATED CELLS

Three children with acute lymphoblastic leukemia were each transfused with more than $10^{11}$ nucleated cells obtained from the peripheral blood of patients with chronic myelocytic leukemia. All donor patients were shown to have the Philadelphia chromosome in most marrow cells which could be examined, and the Philadelphia chromosome was then used as a label for the cells of donor origin. The recipient patients had mild hypogammaglobulinemia and were receiving intensive antileukemic treatment at the time of transfusion. Donor cells in large numbers were found in the blood and marrow of the recipients for several weeks after transfusion, thus apparently demonstrating the presence of a replicating homograft of transfused leukocytes. The recipients showed a dramatic increase in peripheral granulocytes for as long as 5 weeks after transfusion. No definite evidence of "homologous" disease in the recipients was seen either clinically or at necropsy. Interestingly, the leukocyte alkaline phosphatase of the cells of donor origin seemed to rise sharply to normal or elevated values after replication of the injected cells in the recipients.—T. E. B.


Autoimmune disease has been transmitted to normal mice by neonatal replacement of the normal thymus with a thymus derived from the "autoimmune" strain. Such mice had a positive Coombs test, and their serum contained free antibody against ficin-treated red cells as well as antinuclear factor. Neonatal exchange grafting of thymus glands from newborn normal mice into the "autoimmune" strain did not prevent the onset of the disease; nor did neonatal thymectomy in the "autoimmune" strains prevent the development of the disease.—I. C.


Marrow aspiration showed megaloblastic hemoipoiosis in 46 of 72 patients who presented with abruptio placentae. All but one of the 73 cases of abruptio placentae gave a "positive" Figlu test. Of 22 patients whose antepartum hemorrhage was found to be due to placenta previa, only four gave a "positive" Figlu test, and two were megaloblastic. The work confirms the high incidence of megaloblastic anaemia in pregnancy in cases with abruptio placentae described by Coyle and Geoghegan (Proc. Roy. Soc. Med. 55:764, 1962).—I. C.


A simple, specific and sensitive method for the assay of the intrinsic factor (IF) content of human gastric juice is described. It is based on the observation that the ability of IF to promote the transfer of vitamin $B_{12}$ to normal serum or plasma (Millar: Arch. Biochem. 72:8, 1957) is abolished by the addition of an antibody to IF. One unit of IF was designated as that amount of IF which mediated the specific uptake of 1 $\mu$g. of Co$^{57}$-B$_{12}$ to serum or plasma. Following histamine stimulation, normal subjects excreted between 4000 to 18,000 units of IF in 1 hour. Patients with pernicious anaemia excreted 0 to 200 units. Patients with histologically proved gastric atrophy excreted between 400 to 1000 units of IF. Methods for the detection and titration of the antibody to IF are described. This antibody, which was a 7S $\gamma$-globulin, was present in the sera of 57 per cent of patients with pernicious anaemia, and was entirely absent from a series 87 control sera.—I. C.


Although it has been shown that amniocentesis may be the cause of significant transfer of fetal cells to the mother, no evidence was found that this procedure in any way modified the severity of hemolytic disease of the newborn in subsequent pregnancies. There was no rise in antibody titer following the procedure.—I. C.

ERYTHROCYTES

ANGIOTENSIN II AND ERYTHROPOIESIS. Y. C. Bil-

There has been increasing interest in the juxtaglomerular apparatus and its relationship to the production of erythropoietin. In order to study this relationship, polycythemic mice and hypophysectomized rats were injected with synthetic angiotensin II and with angiotensin II made from a crude extract of hog kidney. There was no increase in the erythropoietic activity following these injections, although the assay animals were responsive to concentrated plasma erythropoietin.

—A. J. E.


Basic data are presented on mice rendered polycythemic by discontinuous hypoxia and used for the assay of erythropoietin. The hypoxia was produced in a cylindrical decompression chamber in which the animals were kept at 0.5-0.4 atmosphere pressure. It was found that the 0.4 atmosphere pressure was far more effective in increasing the hematocrit and depressing the iron incorporation. After a total of 80 hours of exposure to 0.4 atmosphere, the 24-hour iron utilization had reached a constant level of about 0.4 per cent. The following schedule was used: exposure of mice to 0.4 atmosphere for 100 to 110 hours, approximately 2 weeks in the chamber, return to normal pressure on day 0, administration of test material on days 3 and 4, radioiron injection on day 5, blood sampling 24 hours later. With this assay method, reproducible values were obtained with an excellent lag-response relationship for erythropoietin standard A. As an aside, it was shown that incubation of erythropoietin in dog plasma for 5 hours did not affect the potency, indicating the stability of the factor in plasma.—A. J. E.


A thorough study of the erythropoietin level in cord blood, in blood from normal infants, from erythroblastotic infants, from anemic infants and from infants with cyanotic heart disease is presented. The assays were carried out in mice with transfusion polycythemia, with 4-day iron utilization as an index of erythropoietin titer. Although the results showed a large standard of error of the mean, there was a clear-cut difference between the higher titer in the cord blood and the very low titer during the first weeks of life. The infants with erythroblastosis, anemic or hypoxic hypoxia all had high titers of erythropoietin. It was concluded that the regulatory mechanism for erythropoiesis through erythropoietin is intact during the last part of intrauterine life and in the neonatal period. The results support the well-accepted theory that the postnatal fall in red cell production is due to an increased supply of oxygen in the tissues.—A. J. E.


Sheep erythropoietin was added to a red blood cell-Ringer mixture, and this mixture was used to perfuse rat livers according to the method of Miller et al. (J. Exper. Med. 94:431 1951). The titer of erythropoietin was measured with a fasting rat assay and the titers were found to be exactly the same before and after the perfusion. It was concluded that exogenous erythropoietin is not inactivated by the perfused rat liver.—A. J. E.


Using the Coulter counter for frequency distribution of red cell size, differential centrifugation for separating mixed cell population, and iron-deficient rats, the authors showed that erythropoietin does not affect the size distribution curve of iron-deficient rats even when given in high doses. When iron was administered, it resulted in a macrocytic response when given in large doses, and a normocytic response when given in low doses. These observations are used to support the theory that erythropoietin is involved in controlling the rate of hemoglobin synthesis. When the concentration of hemoglobin reaches a critical level, it supposedly triggers a feedback mechanism which shuts off further division. If erythropoietin results in accelerated hemoglobin synthesis,
the shut off will be early, few divisions will take place and macrocytosis will ensue. If there is an iron deficiency, the shut off will be late, more divisions will take place and a microcytosis will ensue.—A. J. E.


Primary erythrocytosis is characterized by a significantly elevated hemoglobin and red blood cell count and the absence of leukocytosis and thrombocytosis. The diagnosis depends upon demonstration of an absolute increase in total circulating red cell mass unassociated with any of the known causes of secondary polycythemia. The authors describe four cases, three of whom were non-identical twins, and their father. The grandfather had a mild erythrocytosis but no real increase in red cell mass. The erythrocytosis in the four cases was quite pronounced, with hematocrits between 60 and 70 per cent. The spleen was slightly enlarged and there was a mild thrombocytopenia. In one case there was a duplication of the right kidney but no evidence of obstructive anomalies. All other studies were normal. Erythropoietin was tested in one case and found to be normal. With the three children in this report there are now on record 19 cases of probably primary erythrocytosis in children occurring in 13 families. The authors summarize the data and emphasize that primary erythrocytosis, despite the high hematocrit, is almost completely asymptomatic, and specific therapy is not recommended. The characteristic mild thrombocytopenia is of interest but unexplained.—A. J. E.


An interesting case report of a 58-year-old man with hemochromatosis who simultaneously developed explosive polycythemia and a hepatoma. Although this association has been found frequently in Hong Kong by McFadzean (Blood 13:427, 1958), it had not been observed previously among the 108 cases of hepatoma autopsied in the Mallory Institute of Pathology. The cardiac output of this patient was elevated and it did not rise further when challenged with exercise. The increased oxygen requirements were met simply by doubling the extraction of oxygen from the blood. After phlebotomy, the cardiac output was diminished, and it increased normally after exercise. The possibility that the hemachromatosis was caused by long-standing alcoholism, poor diet and increased iron intake, rather than by an inborn error in iron metabolism, is discussed.—A. J. E.


The urinary excretion of Figlu was measured after an oral dose of 15 gm. of histidine in a series of 40 normal subjects throughout pregnancy. The mean excretion before the 16th week of pregnancy was 21 mg. and this declined to a mean of 14 mg. at 20 weeks, 10 mg. at 30 weeks, 7 mg. at 35 weeks and 8 mg. at 39 weeks. Many subjects failed to excrete Figlu at all after the 30th week. The excretion rose again in the puerperium. The factors responsible for the declining Figlu excretion were slow intestinal absorption of histidine, urinary loss due to a lowered renal threshold, increasing utilization for protein synthesis and transport across the placenta.—I. C.


In two years, 31 cases of infantile megaloblastosis were recognized at one hospital; most of them were of nutritional origin. Many had originated with diarrheal diseases, two had mucoviscidosis. All were anemic but some only mildly. One had a low serum B12; most of the others rapidly recovered following improvements in diet and oral administration of folic acid. Megaloblastosis is being recognized much more frequently than in former years. This is partly owing to greater awareness and more frequent bone marrow studies, but there may also be a genuine increase in the condition because formerly many infants used to receive prophylactic “liver cocktails” or “shotgun” hematinics, which may have prevented some cases. Possibly the widespread use of broad-spectrum antibiotics has also been instrumental, by
causing changes in the intestinal bacterial flora and thus malabsorption and folic acid deficiency. —F. W. G.


A 6-year-old girl receiving anticonvulsant therapy with phenobarbitol and primidone developed severe macrocytic anemia accompanied by leukopenia and thrombopenia. Bone marrow revealed megaloblastic erythroid hyperplasia. Serum folic acid was greatly reduced and serum ascorbic acid was slightly below normal. Folic acid therapy resulted in a fall in serum folic acid, but anemia did not recur.—J. B. S.


In an effort to sort out some of the factors reported to influence absorption of dietary iron, groups of male rats were placed on different diets for 40 days. They were saturated with iron by intramuscular injection, and were then treated in several different ways before being given the test dose of FeSO₄ by intragastric tube. Rapid and marked changes in iron absorption occurred in many instances and the direction of change could not be predicted from the nature of the original diet. The authors feel that, in addition to the level of erythropoiesis and other well-recognized factors, changes in the intestinal luminal milieu play an important part, although these cannot be fully identified at the present stage.—C. R. M.


Absorption of oral iron was abnormally high in all of seven patients with hepatic cirrhosis, despite the fact that six of these patients had a normal or elevated serum iron. However, there was poor utilization of the absorbed iron. The addition of hog pancreas reduced the amount of iron absorbed.—I. C.

Thin-Layer Chromatography of Bile Acids with Special Reference to Separation of Keto Bile Acids. T. Usui. From Tottori University School of Medicine, Yonago, Japan. J. Biochem. 54:283, 1963.

Thin-layer chromatography was used for the separation of keto bile acids. Improved solvent systems described in this paper are: benzene-acetic acid (80:20) for free bile acids; ethyl acetate-acetic acid-methanol (70:10:20) for conjugates; benzene-acetic acid (70:30) for keto bile acids. Spots on the thin-layer plate can be sensitively developed by spraying with a mixture consisting of phosphomolybdic, acetic and sulfuric acids. Also keto bile acids can be detected by a technic in which the plate is sprayed in advance with methanolic sodium brohydrate reagent. The C-3 epimers, reduced-hydrochloric and isoreducedhydrochloric acids can be separated by the use of benzene-acetic acid (70:30).—K. F.


With a neat but difficult technic, transfer of bilirubin across the placenta was studied in two pregnant Rhesus monkeys. Bilirubin—C14 was infused into a fetal limb vein, and maternal bile was collected from an externalized cannulated common bile duct. Significant amounts of the injected C14 appeared in the maternal bile, both as labeled bilirubin and as unidentified labeled bilirubin derivatives.—J. B. S.


Eleven per cent of 624 male Negro infants showed evidence of G-6-PD deficiency by the dye discolorization test. Thirty of these infants and 30 random non-reactors were studied with daily bilirubin levels during the 1st week. No differences in the mean and range of serum bilirubin levels were noted. The authors conclude that although occasional infants may have severe jaundice resulting from G-6-PD deficiency, this defect does
not commonly produce neonatal hyperbilirubinemia.—J. B. S.


The changes in erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) and acetylcholinesterase (AChE) activity were studied in healthy full term infants. Normally, G-6-PD shows increased activity, and AChE shows decreased activity during early infancy. Before the end of the 1st year, G-6-PD has decreased, and AChE has increased so that both are essentially equal to normal adult levels. Between 3 and 9 weeks of age, the levels of G-6-PD and AChE were significantly lower that at birth or at 10–17 weeks. These changes may result from differences in the age distribution of the circulating red cells, and suggest that reduced erythropoiesis just after birth results in a relative increase in aged red cells at 1–2 months of age.—J. B. S.


The rate of disappearance of transfused Heinz-body-containing erythrocytes was studied in five healthy subjects, eight premature infants, and four patients following splenectomy. The damaged erythrocytes were no longer present in the circulation of the healthy controls 20 hours after injection. The rate of disappearance of damaged red cells was considerably slower in the prematures and was somewhat slower in the splenectomized patients. The authors suggest that these findings may be related to a decreased activity of the premature infant's reticuloendothelial system.—J. B. S.


Thirty patients with acute and subacute lead poisoning were studied. The glutathione content of erythrocytes was found to be 40 ± 9 mg. the stability test 35 ± 10 mg., the level of glucose-6-phosphate-dehydrogenase 233 ± 3 units/10^9 erythrocytes. In subjects exposed to lead, the values were 47 ± 11 mg., 4 ± 8 mg., and 245 ± 33 units respectively. The difference between these values and those of normal subjects (57 ± 7 mg.; 47 ± 4 mg.; 278 ± 12 units) was found to be statistically significant.—P. d. N.


Penetration of d-ribose and d-fructose into normal and favic erythrocytes is identical. The same is true for d-arabinose. A higher concentration of d-xylose was observed in favic than in normal erythrocytes. Glucose seemed to exhibit a similar behavior, although other factors may influence the results.—P. d. N.


Using two immunization technics, neutralizing antibodies were produced in rabbits against human-erythrocyte G-6-PD. Definite differences in the amount of cross-reaction between the antisera obtained and the enzymes from different sources were observed. The neutralizing antibodies appear to be specific since they are present in the γ-globulin fraction and they do not inhibit other enzymes such as LDH and 6-phosphogluconic dehydrogenase. The data obtained by immunologic method suggests the presence of an inactive form of G-6-PD in enzyme “deficient” subjects which is immunologically similar to the normal enzyme.—H. H. F.
The pedigree of a family in which a variant of this pattern was found is illustrated.—I. C.


Red cells obtained from mice surviving irradiation frequently reacted with antiglobulin sera. This was not considered to indicate a breakdown of immune-tolerance, but to be due to a persistent reticulocytosis shown by these animals.—I. C.


Ninety-six subjects, aged between a few hours and 4 years, including full term newborn infants, immatures, newborn infants with severe jaundice, patients with hemolytic disease due to Rh incompatibility, those with hemorrhagic disease, hemolytic, constitutional jaundice, and Cooley’s disease were studied as to reticulocyte level and Coombs test. The latter was negative when reticulocytes were below 20 per cent and positive when they were above this value.—P. d. N.

**Modified Direct Anti-Globulin Test for Diagnosis of Haemolytic Disease of the Newborn Due to ABO Incompatibility.** J. Ducos. From Hôpital Purpan, Toulouse, France. Lancet 2:1170, 1963.

It is suggested that a positive Coombs test can be obtained with cord blood from infants affected by hemolytic disease due to anti-A if these cells are treated with papain and then allowed to react with an antiglobulin serum. Newborn red cells first treated with papain appear to react like untreated adult red cells to anti-A and to antiglobulin sera.—I. C.


An acute hemolytic anemia with a strongly positive Coombs test and free antibody of 7-S type in the serum is described in a 2½-month-old infant. The anemia could not be controlled with steroids nor with splenectomy, but removal of the thymus gland brought hemolysis under control: no further transfusions were required in the subsequent 6 months. The Coombs test became negative and the infant appears to be developing normally. The thymus gland weighed 3 Gm. and showed virtually complete disappearance of cortex, most of the tissue being medullary in character.—I. C.


There appeared to be a correlation between the blood groups of patients with rheumatoid arthritis, rheumatoid spondylitis, disseminated lupus erythematosus, etc., insofar as there was an excess of patients with the Rh antigen D in this group. No correlation was found between antigens C and E or the ABO groups.—I. C.

**Hemolytic Anemia Secondary to Stibophen Therapy.** M. V. V. de Torregrosa, A. L. Rosando, and E. Montilla. From San Juan Medical Hospital, San Juan, P. R. J.A.M.A. 186:182, 1963.

Two cases of hemolytic anemia secondary to stibophen therapy are described. Six have previously been reported. Of the total of eight patients, five died after stibophen therapy. The mechanism responsible for the hemolytic process is apparently the development of antibodies against a combined stibophen red-blood-cell antigen. When the serum of one patient was tagged with fluorescein, and incubated with the patient’s red blood cells and stibophen, antibodies attached to the patient’s red blood cells, is shown by the fluorescent technic.—H. H. F.


Study of the effects of bacterial O and Vi antigens on survival of Cr51-labeled erythrocytes in the rabbit revealed that injection of red blood cells modified by either antigen were rapidly hemolyzed in immune, but not in nonimmune rabbits. The degree of hemolysis in immune animals

The reactions of two components of hemoglobin obtained from body-wall tissue of Ascaris lumbricoides with ethyl-isocyanide and potassium cyanide were studied. The affinities for ethyl-isocyanide and potassium cyanide were 9 x 10^{-7} M for A1, 3.9 x 10^{-5} M for A2 and 9.5 x 10^{-2} M for A1, 5.0 x 10^{-2} M for A2, respectively. A1 hemoglobin exhibits the converse situation. Both hemoglobins do not show the Bohr effect in ethyl-isocyanide equilibrium. The property of the reaction site of hemoglobins with cyanide is almost the same as that of the reaction with ethyl-isocyanide.—K. F.


Hemoglobin was purified from perienteric fluid of Ascaris lumbricoides, and its spectral data are presented in this paper. The hemoglobin combines with ethyl-isocyanide or cyanide to form a stable ferrous hemoglobin complex. The hemoglobin seems to be identical with the minor component of body-wall hemoglobins of Ascaris lumbricoides as shown by its spectral and functional properties.—K. F.

Hemoglobins in Mice. Segregation of Genetic Factors Affecting Electrophoretic Mobil-

The segregation of genetic factors affecting electrophoretic mobility and solubility of hemoglobins was observed in back-crosses involving five different inbred strains of mice. At least two genetic factors affect solubility of carbonmonoxyhemoglobin in phosphate solutions. One is identical or closely linked to the locus for hemoglobin pattern (Hb), the other (s), called solubility (sol), segregates independently of Hb. There is no observable difference in hemoglobin solubility between mice of strains FL/Re and C57BL/6J at Sol under conditions described, though a-chain peptide patterns differ in these strains—H. H. F.


Fetal erythrocytes persist to a minor degree in most children up to the age of 1 year and may still be detected in some children up to the age of adolescence.—I. C.


The effect of pyrexia on the fate of red cells with the sickle cell trait was investigated by labeling these cells with Cr51 and inducing pyrexia by an injection of TAB vaccine. This procedure resulted in an increase in radioactivity over liver and spleen, the result of a transient increase in the rate of destruction of the labeled red cells.—I. C.


Comparison of children with sickle cell anemia with their healthy siblings revealed no differences in intellectual function.—J. B. S.


The subject was a healthy male of a West Indian family of West African stock. The blood findings were normal. Similar findings were present in a maternal aunt.—I. C.


A new form of drepanocytic anemia was observed in two sisters from the province of Caserta. The simultaneous presence of two anomalies (Hb S and Hb Lepore) was documented and confirmed by study of the relatives.—P. d. N.

LEUKOCYTES


The activity of dihydrofolate reductase (the enzyme responsible for the conversion of folate and dihydrofolate to tetrahydrofolate) is about 30 times greater in lysates of chronic myelocytic or acute leukemic cells as compared to normal and chronic lymphocytic leukemic cells. Elsewhere it has been shown that the activity of leukocyte dihydrofolate reductase is significantly inhibited by aminopterin and amethopterin. The authors also measured the activities of formate-activating enzyme, N5, N10-methylene tetrahydrofolate dehydrogenase, serine hydroxymethylase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase in normal and leukemic leukocyte lysates and found much less striking differences than were noted for dihydrofolate reductase. Both the latter and formate-activating enzyme were partially purified from leukocytes and some of their properties studied.—T. E. B.


Human leukocytes are shown to contain both
ABSTRACTS

A TD transhydrogenase catalyzing the exchange of hydrogen between TPNH and DPN or between DPNH and TPN, and a DD transhydrogenase catalyzing the exchange of hydrogen between DPN molecules alone. The pH optima, relative activities, and effect of various inhibitory substances were studied in the two enzymes as obtained from lyzed leukocytes. It has been previously shown that acute and chronic lymphocytic leukemic cells contain higher amounts of DPN than do other leukocytes. It is now found that acute and chronic lymphocytic leukemic cells have 3-4 times more TD transhydrogenase activity than do normal leukocytes.—T. E. B.


Thymidylate synthetase catalyzes the reaction, deoxyuridylicate + NAD + thymidylate + dihydrofolate, thymidylate being a key precursor of DNA. Thymidylate synthetase has been partially purified from lyzed leukocytes and some of its properties characterized. The reaction catalyzed by thymidylate synthetase may be the rate-limiting step in the biosynthesis of DNA. No thymidylate synthetase activity could be detected in normal or chronic lymphocytic leukemic leukocytes, but it was easily demonstrable in chronic myelocytic leukemic cells and in the leukocytes of two non-leukemic patients with marked leukocytosis.—T. E. B.


One patient with chronic granulocytic leukemia, one with acute myeloblastic leukemia, one with polycythemia vera, and one with chronic granulocytic leukemia in blast crisis received tritiated thymidine intravenously. Four to 14 per cent of blasts in the marrow were labeled. Initially, only the last mentioned patient had labeled blasts peripherally; later, all had. Labeled cell percentages in blood reached a maximum after 36-48 hours and then declined. It is concluded that no release from the marrow took place in cells undergoing DNA synthesis or mitosis, or of cells capable of mitosis in three of the four patients. Labeled blasts disappeared from the blood in a random fashion with a half-time of about 23 hours. The estimated generation time for blasts was 45-80 hours and their DNA synthesis times were 3.3-7.5 hours. It is concluded that these leukemic blasts did not proliferate more rapidly than normal granulocytopenic cells.—P. G. R.

LYMPHOSARCOMA IN CHILDREN. B. Jones and W. C. Klingberg. From the St. Louis Children's Hospital, St. Louis, Mo. J. Pediat. 63:11-20, 1963.

An analysis of 43 cases of childhood lymphosarcoma and a review of recent literature is presented. The cases were well distributed throughout childhood with only a slight increase in incidence at 3 to 5 and 9 to 11 years of age. Seventy per cent of the patients were males. Peripheral lymph nodes, particularly the cervical nodes, were involved most commonly. Second in order were nodes within the abdomen. Mediastinal adenopathy was also a frequent finding. Thirty per cent of the children developed a leukemic phase, often within 3 months of onset. The course of the disease was slightly longer in these patients than in those not developing leukemia. Half the children were dead by 6 months after onset and only 10 per cent survived longer than 15 months.—J. B. S.


Remissions were obtained in 16 of 19 cases. These included "complete" remissions in two patients with lymphosarcoma, and remission in two of three patients with a monocytic type of acute leukemia. Partial or complete (one case) remissions were obtained in five of six cases with Hodgkin's disease. Results were unsatisfactory in chronic leukemia and myeloma. Toxic effects, hematological and gastrointestinal, were frequent.—I. C.


An infant with mongolism presented with hepatosplenomegaly and a leukemoid white count
LEUKOCYTE ANOMALY, MENTAL RETARDA
tion, and Dwarfism in a Family with Abnor
mals Chromosomes. D. K. Briggs, C. W. Stimson
and J. Vinograd. From New York University
Medical Center, New York, N. Y. J. Pediat. 63:

A morphologic abnormality of the leukocytes
characterized by marked hyposegmentation, and a
greatly elevated leukocyte alkaline phosphatase
activity, were present in a boy with dwarfism,
mental retardation and deaf-mutism. Neither par
tents nor siblings displayed the leukocyte anomaly,
but the paternal grandmother presented sex
chromosome mosaicism of the type XO/XX/XXX,
and had no leukocyte sex chromatin.—J. B. S.

Studies on the Role of the Thymus in Im
munobiology. Reconstruction of Immunologic
Capacity in Mice Thymectomized at Birth.
A. P. Dalmasso, C. Martinez, K. Sjodin
and R. A. Good. From the University of Min
nesota School of Medicine, Minneapolis, Minn.

The immunologic competence of spleen cells of
mice increases to 35 days of age and beyond.
Thymectomy at any point along this continuum of
development produces "immunologic arrest," for
the peripheral lymphoid tissues of such mice do
not seem to show significant changes in activity as
the animals mature further. Injections of cell-free
extracts of spleen and thymus tissue did not affect
the immunologic reactivity of neonatally thynectom-
mized mice. Allogeneic thymus grafts are able
to restore the immunologic capacity of neonatally
thymectomized mice. Such animals are chimeric,
the immunologically competent cells of their
peripheral lymphoid tissues being chiefly of host
origin though sometimes having a significant donor
component. When allogeneic adult spleen tissue
was transplanted to neonatally thymectomized
mice, less restoration of immunologic competence
occurred, but it was attributable almost entirely
to donor cells. It is suggested that the deficiencies
of neonatally thymectomized mice reflect a deficit
of peripheral lymphoid cells, that cells of thymic
origin can "peripheralize" to the spleen and lymph
nodes, but that in addition a grafted thymus
probably has a distinct effect on the cells of the
host.—T. E. B.

Immune Response and Mitosis of Human
Peripheral Blood Lymphocytes in Vitro. K.
Hirschhorn, F. Bach, R. L. Kolodny, I. L.
Firschein and N. Hashem. From New York Uni-
versity School of Medicine, New York, N. Y.

Phytohemagglutinin, by mechanisms which are
not understood, causes almost all cultured lympho-
cytes to agglutinate, divide, and produce γ-globul-
in. Most cells are transformed into large lympho-
cytes, some resembling plasma cells. In lympho-
cytes from a patient with congenital agamma-
globulinemia phytohemagglutinin produced the
usual increase in mitoses, but there was no evi-
dence of γ-globulin production. When specific an-
tigens were added to cultures of lymphocytes from
sensitized individuals, only a minority of the cells
seemed to undergo morphologic transformation,
divide, and produce γ-globulin.—T. E. B.

Preservation of Antibody-Producing Cells at
Low Temperatures: A Method of Storage
That Allows Complete Recovery of Acti-
From Oak Ridge National Lab., Oak Ridge,

Previous studies on low-temperature preservation
of mammalian cells capable of differentiation and
sustained proliferation have not included rigorous
estimates of retention of functional capacity. Meth-
ods for prolonged storage at low temperatures,
and recovery of antibody-producing cells from
spleens of preimmunized mice were investigated.
A method was devised which permits the cells to
be stored for at least 135 days and presumably
much longer with no decline in their capacity to
synthesize antibodies upon subsequent culture.
The method consists of suspending cells in 10 per
cent dimethyl sulfoxide, decreasing the tempera-
ture of the suspension gradually to -50 C., and
then storing the suspension at -196 C. The extent
to which antibody-producing cells are damaged
by certain other freezing procedures was evaluated.
—T. E. B.
ABSTRACTS


These compounds inhibited antibody formation following injections of serum albumin to rats. The development of delayed hypersensitivity was also suppressed.—I. C.


The authors show that lymphocytes previously exposed to phytohemagglutinin were relatively resistant to x-ray and to nitrogen mustard. The surviving cells were mostly those that had undergone transformation to “lymphoblastoid” cells.—I. C.


Intraperitoneal injections into mice of synthetic polylysine (molecular weight about 2600) or polyglutamic acid regularly inhibited hepatic phagocytosis of subsequently injected Cr51-labeled sheep red cells. This inhibition increased somewhat with increasing total dose of polyamino acid. The inhibition seemed to have largely disappeared about 1 week after injections were discontinued. It is not known how polyamino acids interfere with phagocytosis. The interference with phagocytosis might explain the previously reported depression of formation of antibodies in mice treated with polylysine.—T. E. B.


Oxidative decarboxylation of the ketoacids of the branched-chain amino acids valine, leucine and isoleucine, is deficient in maple syrup urine disease. Normal leukocytes can decarboxylate these ketoacids. Using branched-chain amino acids labeled with C14 at the carboxyl-carbon as substrates, transamination and oxidative decarboxylation were assessed in the leukocytes of five patients. Transamination was normal but decarboxylation was absent or greatly reduced.—J. B. S.


The authors describe 10 alcoholic patients who responded to acute bacterial infections with severe temporary leukopenia. One total white count of 200 per cu. mm. was noted. At least three patients had marked transient thrombocytopenia as well. Marrow aspirates examined during the leukopenic stage of the infections regularly showed decreased total cellularity with virtual absence of granulocytes more mature than myelocytes. Hematologic recovery within several days occurred in all patients. None of the patients had palpable spleens, and none had overt cirrhosis. Five other patients, healthy except for alcoholism, appeared to have decreased marrow granulocyte reserves when tested with intravenously injected endotoxin. —T. E. B.

**HEMOSTASIS**


Using an anti-fibrinogen serum and a fluorescent technic, “fibrinogen” was demonstrated in platelets before and after washing, and after enzyme treatment. Similar material was present in the cytoplasm of narrow megakaryocytes. It is suggested that fibrinogen forms part of the platelet structure.—I. C.


Platelet labeling with Cr51 was done in 18 cases of ITP, nine of congestive splenomegaly and four of hereditary spherocytosis, with the main aim of deciding if surface scanning over the spleen would predict successful splenectomy. Platelet life span was low in all cases of ITP, but excess radioactivity over the spleen was found in normals as well as in ITP patients. It appeared possible that this excess in ITP might eventually be found to
be significantly greater than that in normals, provided more patients were studied. In congestive splenomegaly, there was reduction of platelet life span, as well as a low yield of platelets before and a higher yield after splenectomy; this, together with excess radioactivity over the spleen, suggested sequestration of platelets by the enlarged organ. In hereditary spherocytosis, too, the evidence was in favor of sequestration having taken place. —F. W. C.

**NATURE OF PROTHROMBIN BIOSYNTHESIS: PRE-PROTHROMBINAEMIA IN VITAMIN X-DEFICIENCY.**


Using a modified thrombotest (Owren) the authors found a difference in the behavior of plasmas with lowered clotting factors II, VII, IX and X derived from patients with hepatic damage as opposed to patients with vitamin K deficiency. They suggest that the data could be explained by the presence of an inhibitor in vitamin K deficiency which is active in both the intrinsic and extrinsic coagulation systems. This inhibitor might be a precursor of prothrombin normally present only in hepatic cells. —I. C.


It is suggested that normal plasma contains two components lacking in the plasma of patients with von Willebrand’s syndrome. One factor stimulates factor VIII activity and the other shortens the bleeding time. The latter factor is absent from serum. —I. C.

**ANTIFIBRINOLYTIC PROPERTIES OF KUNITZ Pancreatic INHIBITOR.** A. Gibelli and F. Soardi. From the University, Pavia, Italy. Gazz. interna. med. chir. 67:2105-2116, 1963.

Antifibrinolytic activity of Kunitz pancreatic inhibitor was demonstrated at various concentrations both in vitro and in vivo in rabbits given incompatible blood and human fibrinolysis. Patients with liver cirrhosis were successfully treated with the inhibitor, which was able to reduce considerably their fibrinolytic activity. The fibrin plate method (heated plates, euglobulin precipitate, with and without the added activator) was used. —P. d. N.


The mechanism whereby ATP inhibited the coagulation of blood was investigated. Increasing concentrations of ATP produced an increasing prolongation of the clotting time and increasing depression of prothrombin consumption. Prothrombin time was prolonged by ATP and this was corrected by adsorbed rabbit plasma which was rich in fibrinogen and factor V. Since the thrombin time was also prolonged by ATP, its action appeared to be on fibrinogen. —I. C.


Blood changes during the course of 100 cases of cardiac surgery with extracorporeal circulation are reviewed. Severe hypothermia was used in 77 cases and moderate hypothermia in another nine. A careful personal and family history was taken in all cases. Unexplained prolonged bleeding time preoperatively was restored to normal by steroid therapy for 1 week before operation. Heparin neutralization was of prime importance in ensuring postoperative hemostasis, and residual heparin was found in 25 cases. Evidence for defibrination and fibrinolysis was sought for at the end of perfusion and was present in about 20 cases. Seven cases required fibrinogen, and five epsilon-aminocaproic acid to stop hemorrhage. A fall in platelets was the rule. The use of rheomacrodex decreased the severity of all these complications other than the thrombocytopenia. Five patients developed homologous serum jaundice 2 to 3 months after perfusion, and three died. It would be interesting to know the overall mortality in this series. —I. C.

**STUDY OF BLEEDING TIME AND INTENSITY IN CAISSON WORKERS IN CONNECTION WITH WORKING ACTIVITY.** G. Turazza and M. Palmieri. From the University, Pavia, Italy. Lavoro Umano 15: 420-429, 1963.

The bleeding intensity (amount of blood lost in bleeding time) was studied by means of the method of de Nicola and Candura (Hémostase 1:113,
1961). Increased values were observed, even when the standard bleeding time was normal, and even in cases where only early signs of caisson disease were present.—P. d. N.


Mild degrees of impairment of the coagulation mechanism appear in children with Cooley's anemia, sometime after the seventh birthday. These changes are similar to those found in liver disease of other etiology. No correlation was found between these changes, and the not infrequent appearance of epistaxis in this group of patients. —J. B. S.

**MISCELLANEOUS**


Normal newborn infants were given 1.5–2.5 mg. of oral prednisone or 1.5 mg. parenteral ACTH Mg./Kg. day 10 days starting from the first day of life. Blood and bone marrow were not significantly different from controls.—P. d. N.


Three cases of bone marrow hypoplasia were related to chronic exposure to kerosene. Kerosene was used either as a poultice for lumbar pain; as kerosene and sugar taken by mouth (sugar in a teaspoon was saturated with kerosene, ignited and, after the flame had disappeared, the charred sugar was ingested); or as a massage. As with all other cases of bone marrow suppression, definite proof could not be established. However, the authors discuss other exposures and conclude that kerosene appeared to be the most logical etiologic agent. Kerosene contains extremely small amounts of aromatic compounds including benzene, but it was suggested that kerosene in itself may be responsible because of its potency as a fat solvent. In all three cases, the history of kerosene exposure was elicited only after a prolonged and thorough history.—A. I. E.


Among 35 children with SLE were seven with a history of seizures, and treatment with anticonvulsants. Signs and symptoms, as well as laboratory data in these patients were typical of SLE. Remission could be induced by adrenocortical steroids. Following cessation of therapy with the anticonvulsant implicated (trimethadione and/or a hydantoin), clinical remission was sustained, although in several patients some laboratory abnormalities persisted. Two of the seven children died, one of hemorrhage, possibly steroid-induced; the other patient developed severe renal involvement. This child had continued to take trimethadione for a year after the onset of symptoms of SLE. The previously reported statistics suggesting that SLE in children had a rapidly fatal course were not corroborated, nor could a strong correlation be seen between long-term steroid therapy and prognosis.—J. B. S.

**ANTIBODY DEFICIENCY SYNDROME WITH INCREASED GAMMA(A) (GAMMA1M) GLOBULINS AND ABSENT GAMMA(A) (GAMMA2A) GLOBULINS AND GAMMA(A) (G) GLOBULINS.** C. C. Huntley, J. W. LaFofty and A. Lyerly. From the Bowman Gray School of Medicine, Winston-Salem, N. C. Pediatrics 32:407–415, 1963.

A boy with increased susceptibility to bacterial infections demonstrated the serum protein abnormalities described in the title. The institution of monthly gamma-globulin injections resulted in clinical improvement, and in a significant reduction in the gamma-globulin concentration.—J. B. S.


The incidence of viral hepatitis and its relation to gamma-globulin prophylaxis has been investigated in 21,000 Scandinavian soldiers serving in the United Nations Emergency Force in the Middle East. In
the inoculated group of 9,800 soldiers, the hepatitis incidence was 0.10 per cent; in the non-inoculated group of 11,000 soldiers, the incidence was 1.14 per cent. The investigations indicate that a dose of about 0.04 ml./Kg. body weight gives a satisfactory protection of adults. This is only one-third the dosage which hitherto has been recommended. Immunity seems to last about 5 months in agreement with results of previous investigations.—H. H. F.


Autoradiography was accomplished with C14 labeled glycine. In the bone marrow of patients with aplastic and hypoplastic anemia there was marked reduction in the number of labeled cells of the erythroblastic and leukoblastic series, both in the first hours and in the subsequent period of incubation. These data confirm the cell defects in these patients.—I. K.

BONE MARROW APLASIA ASSOCIATED WITH ANTERIOR HYPOPITUITRISM. S. Perugini, G. Fontana, P. L. Prati and V. Silingardi. From the University, Modena, Italy. Minerva med. 54:2851-2863, 1963.

Five cases are reported. Hypopituitism was total and severe in two cases, and partial in three. Hyporegenerative anemia was present in all cases, and was associated with leukopenia in four, and with thrombocytopenia in three. Specific hormonal treatment proved to be effective.—P. d. N.


Eighty-three diabetics who did not appear to have any other disease were investigated. Their sera contained complement-fixing antibody to thyroid in 17 per cent of cases (controls 4 per cent) and to gastric mucosa in 22 per cent of cases (controls 8 per cent). The authors suggest that some of these patients have gastritis, which may progress to pernicious anemia.—I. C.


Gamma-globulin human groups Gm and Inv were studied on 46 aliquots of blood kept and stored under different conditions. The bloods were sterile or non-sterile, frozen at -20 C. during night and left at room temperature each day for 21 days, kept at laboratory temperature, hemolyzed or normal, and some sent airmail. No differences in the y-globulin phenotypes were found irrespective of treatment.—G. M.


Twenty of 30 patients with urinary tract infection showed a significant rise in the appearance of urinary white cells after an injection of 25 mg. of iron-sorbital. It is suggested that this is related both to the urinary excretion of iron-sorbital as well as to the tendency of iron to localize in infected tissues.—I. C.


The authors describe a new method for determining cobalt in urine. Its advantage over older technics consists in the ability to remove interference by the staining reagent, a-nitroso-b-naphthol. Cobalt per 24-hours of urine was determined in 25 healthy subjects, including 10 men and 15 women. An average of 6.1-0.9 y of cobalt were excreted with a range of 0.85 to 15.6y. Urinary cobalt excretion had no relation to sex. —J. K.