ABSTRACTS
ERNST R. JAFFÉ, M.D., Editor

P. Barkhan, M.D., London, England
T. H. Bothwell, M.D., Johannesburg, South Africa
T. E. Brittingham, M.D., Nashville, Tenn.
Jacque Caen, M.D., Paris, France
J. B. Chatterjeea, M.D., Calcutta, India
Pietro deNicola, M.D., Pavia, Italy
Ludvik Dormer, M.D., Prague, Czechoslovakia
A. J. Erslev, M.D., Philadelphia
H. Hugh Fudenberg, M.D., San Francisco
Katsuhiro Fukutake, M.D., Tokyo, Japan
Robert Goldstein, M.D., New York City
Ira Green, M.D., New York City
F. W. Gunz, M.D., Christchurch, New Zealand
Susanna R. Hollan, M.D., Budapest, Hungary
Harry S. Jacob, M.D., Boston, Mass.

ERYTHROCYTES

This investigation concerns a morphologic study of the changes in ribosome content and organization which occur during maturation of erythroid cells of the phenyldihydrazone-treated rabbit. In the intact cell, polyribosomes appear predominantly as rows of paired ribosomes. In immature cells, synthesizing protein at a relatively rapid rate, more than 99 per cent of the ribosomes are in the polyribosome configuration. As maturation proceeds, there is an orderly shift in polyribosome size toward smaller clusters and single ribosomes with an overall decrease in the cell content of ribosomes.


The intravenous injection of ethyl palmitate into mice resulted in necrosis of the spleen and, by depression of the phagocytic function of the liver and spleen, prolonged the survival of transfused human red cells. There is, in clinical medicine, a place for a simple, safe method for the widespread depression of phagocytic function. Ethyl palmitate is at present a rather crude experimental tool. It may well be that, in the future, some substance with a similar depressive action on the reticulo-endothelial system will provide a practical therapeutic measure in, e.g., acquired hemo lytic anemia.


Despite the widespread use of OsO₄ as a fixative for electron microscopy, few quantitative studies of cellular changes occurring during such fixation have been made. Before making a study of the fine structure of amphibian erythrocytes, it was desirable to measure the cellular changes resulting from OsO₄ fixation. The following measurements were made: the absolute amount of hemoglobin extracted during fixation; the effects of pH, ionic composition and tonicity upon the rate of hemoglobin extraction; and changes in the projected areas of the cells and nuclei during fixation, ethanol dehydration and embedding. Some of the conclusions were: erythrocytes were stabilized and hemoglobin extraction reduced when a fixative of low pH was used. Calcium ions completely suppressed extraction of hemoglobin and stabilized.
all erythrocytes, irrespective of the pH within the range used. The difference in concentration of sucrose did not have any significant effect upon the extraction of hemoglobin.—O. P. J.

**GROWTH AND DEVELOPMENT OF NEGRO INFANTS.**

**X. Fetal Hemoglobin in Sicklers and Non-sicklers During the First Two Years of Life.**


Among nonsickling Negro infants, mean fetal hemoglobin levels were 68 per cent at 2 weeks, 38 at 1 month, 30 at 2 months, 19 at 3 months, 5 at 4 to 7 months, 2 at a year and below 1 at 2 years. Approximately 10 per cent of infants had hemoglobin F levels between 2 and 4.7 per cent at 2 years. Among infants with sickle cell trait, no remarkable differences from the normal range were seen. In infants with sickle cell anemia, fetal hemoglobin levels appeared to be above the normal range after 9 months of age, but only a few determinations were reported.—J. B. S.

**DESCRIPTION OF A NEW, SLOW HEMOGLOBIN IN A FAMILY FROM CASERTA (Hb CASERTA).**


This new hemoglobin exhibited the following characteristics: migration rate identical with Hb F on electrophoresis at pH 8.6; agar gel electrophoresis at pH 6.2 and chromatography separated it from Hb A; it was present at a concentration not higher than 14 per cent; on fingerprinting, a new peptide was found between no. 18 and 19; anomalies of the beta chains were noted. In three carriers (mother and two children), an increase in the osmotic resistance and slight morphologic alterations in the erythrocytes were found, but there were no signs of thalassemia.—P. D. N.

**SUBSTITUTION OF TYROSINE FOR HISTIDINE (87) IN THE α CHAIN OF HEMOGLOBIN Miwate.**


Hemoglobin Miwate is a hemoglobin with an α chain anomaly and an abnormal α Tp-9 peptide. This abnormal peptide was isolated by one-dimensional high potential paper electrophoresis of the trypsic digest of the globin of Hb Miwate. Its chymotryptic digest yielded six smaller peptide fragments on fingerprinting. One spot was positive for tyrosine and negative for histidine. Amino acid analysis of the extract of this spot and of the other 5 spots has established that there is substitution of Tyr for His (87). It was concluded that Hb Miwate can be designated by the formula $\alpha_2^{87} \beta_4^A$.—K. F.

**EXPERIMENTAL ERYTHROBLASTOSIS FOETALIS IN RABBITS DUE TO INCOMPATIBILITY.**


The rabbit is one of the most convenient species for the experimental study of erythroblastosis fetoalis because important contributions to the knowledge of its blood groups have been made. In the present paper, typing sera prepared by Cohen were used to select rabbit does suitable for immunization with blood Factor A. Detailed data are given on two litters born to a single sensitized doe. There was no indication of erythroblastosis in 12 young born to un.injected does, but edema was seen in 2 of 23 A-compatible young born to does highly sensitized to other antigens.—O. P. J.

**NEW CONCEPT OF IRON METABOLISM.**


The abnormality of iron metabolism in iron deficiency anemia is considered by most hematologists to be due merely to a shortage of iron in the body. However, from results obtained in a series of clinical and experimental studies, it was presumed that iron incorporation into erythrocytes and distribution of iron are still under the control of another mechanism. Elevation of hemoglobin level following administration of iron, either orally or parenterally, is variable from case to case. The rate of elevation of hemoglobin by iron administration depends largely on the initial level of hemoglobin. The rate of elevation of hemoglobin is influenced by administration of protein anabolic hormone or adrenocorticosteroid hormone. In some cases, changes in hemoglobin level following menstrual hemorrhage are too large to be explained by simple loss of iron. Increased iron incorporation into erythrocytes and rapid disappearance of iron from plasma are observed after ad-
ABSTRACTS

ministration of protein anabolic hormone in phlebotomized rabbits. Iron distribution in the body is also altered by this treatment.—K. F.


Radioiron absorption in premature infants below the age of 10 weeks ranged between 7–74 per cent with a mean of 31. The percentage absorbed was inversely related to the total iron load and correlated significantly with the infant's growth rate. Wide variation in utilization of absorbed iron was noted. There was, however, a significant relationship between the quantity of iron incorporated into hemoglobin and the reticulocyte count, as well as the infant's weight gain.—J. B. S.


The authors studied the erythropoietic factor which presumably was released from an isolated hypoxic rabbit kidney when it was perfused with a modified Tyrode's solution. The factor was inactive when given subcutaneously to starved rats, but showed some erythropoietic activity when given intravenously. It could also be activated in vitro by preincubation with whole plasma or with the plasma alpha globulin fraction. The authors felt that the renal factor formed a complex with the protein fraction and that erythropoietin consequently can exist unbound and inactive or protein bound and active.—A. J. E.


Erythropoietin was found in urine from patients with refractory anemia and the specific erythropoietic activity was concentrated by flash evaporation and precipitated at high ethanol concentration. The erythropoietin was found to be quite stable and somewhat dialyzable. This latter observation has added support to the contention that erythropoietin may exist in a free and bound state.—A. J. E.


Erythropoietin titers in newcomers to Norococha at 4540 meters altitude were found elevated after 24 hours, but not after 72 hours or 10 days. When natives living at the high altitude were brought down to sea level, their plasma was found to suppress iron utilization and hemoglobin concentrations in normal rats. This suppressive effect of the plasma extract was first found after 24 hours at sea level. The data suggest the existence of an erythropoietic depressing factor and deserve to be confirmed and expanded.—A. J. E.


Fe59 uptake by red cells decreased markedly in rats starved 3 to 5 days. A factor which lowered Fe59 uptake was demonstrated in the serum of these starved rats. Although the decrease in Fe59 uptake in starved rats was thought to be influenced by the decrease in erythropoietin level, the effect of the inhibitor should be taken into consideration. It is suggested that the inhibitor does not act directly on erythropoiesis, but blocks the activity of erythropoietin at some point.—K. F.


In order to calculate the disappearance rate of erythropoietin in humans, 9 patients with disturbed erythropoiesis and high titers of erythropoietin in their plasma were transfused acutely until they had reached a level where they presumably did not produce any more erythropoietin. Erythropoietin levels were then followed by bioassay in polycythemic mice and the mean half-time of survival of erythropoietin was calculated to be 24 hours.

In the extensive literature dealing with Goldblatt's operation on dogs and rabbits, there has been no mention of polycythemia caused by renal anoxemia. The author of this paper tried to induce the Goldblatt kidney in 65 rabbits and studied the erythropoietin titer of urine, plasma and kidney extract. One of the animals developed polycythemia, but in no one could a heightened erythropoietin titer of plasma be demonstrated. Urine, however, showed higher erythropoietin titer and in some of the rabbits the kidney extract was found to have erythropoietic activity. The overall result appears to support the contention that local renal hypoxia will result in generation of erythropoietin.—A. J. E.


Benign familial polycythemia is described in 5 individuals in 2 families. Except for mild reticulocytosis in one patient and slightly depressed platelet counts in several patients, there were no unusual hematologic findings in blood or marrow. Liver chemistries were normal, as were red cell survival, arterial oxygen saturation and serum iron. Blood volume was normal or slightly increased, red cell mass was significantly increased and plasma volume significantly decreased. In one family, 3 consecutive generations appeared to have been involved, suggesting that genetic transmission was due to an autosomal dominant.—J. B. S.


A girl with hypoparathyroidism and moniliasis which were present since the age of 4 years and familial adrenal insufficiency which had become manifest when she was 8 years old was admitted with anemia at 10 years of age. Peripheral blood smear exhibited normochromic macrocytes and hypersegmented neutrophils. Bone marrow aspirate revealed moderate erythroid hyperplasia with many megaloblasts and with megaloblastoid changes in white cell precursors. Gastric biopsy revealed submucosal lymphocytic infiltration. Small intestine biopsy was normal. Serum B12 level was 50 µ Gm./ml. The Shilling test demonstrated 1.3 per cent excretion without intrinsic factor and 13.5 per cent excretion of Co60 B12 with intrinsic factor administration. Free gastric HCl was absent before and after histamine. Urinary FIGLU excretion was mildly increased. Antibodies against gastric tissue could not be demonstrated, but anti-adrenal and anti-intrinsic factor antibodies were found. Following vitamin B12 there was a prompt reticulocyte response to 19 per cent. On continuing B12 therapy, hemoglobin values have remained normal. Several other cases of pernicious anemia complicating one or another endocrinopathy have been reported. The possibility of an underlying immunologic disorder is discussed.—J. B. S.


Bone marrow samples from patients with pernicious anemia and with normoblastic erythroidpoiesis were incubated with cold deoxyuridine and then labeled with H3-thymidine. In 6 normoblastic marrows, deoxyuridine reduced H3-thymidine incorporation in erythroid precursors by 80–100 per cent. In 7 p.a. marrows, megaloblast uptake of H3-thymidine was reduced by 76 and 43 per cent in 2 marrows and was unaffected by deoxyuridine in the remaining 5 marrow samples. Five patients with p.a. were treated with 4.5–9 Gm. of thymidine continuously iv. over a period of 48–72 hours. In each case, this was followed by reticulocytosis. In 1 patient with a marked leuko- and thrombocytopenia, the leukocyte and platelet count became normal. Repeated bone marrow studies were done in 2 patients. Thymidine infusion was followed by
partial disappearance of megaloblasts and large numbers of intermediate erythroblasts and normoblasts were conspicuous. Two to 7 days later the marrow had reverted to its previous appearance. The data suggest that the vitamin B12-deficient human may be in a state of relative thymidine starvation and that vitamin B12 may be involved, probably indirectly, in the methylation of deoxyuridylic to thymidylic acid.—S.-A. K.


During the first 4 days of life, serum folic acid levels averaged 2 to 3 times higher than the levels found in healthy children and adults. By 2 weeks, the levels had fallen significantly below birth levels. They continued to fall until sometime between 1 and 2 months of age when the mean SFA activity was approximately half the normal adult level. A progressive rise then ensued and, after 3 months of age, SFA levels were comparable to those found in normal children and adults. During the second month, the mean SFA of infants whose birth weight was below 1700 Gm. was significantly lower than the mean activity present in the larger premature. A number of infants with very low SFA activity had significantly increased FIGLU excretion following histidine loading. Folinic acid injections resulted in normalization of FIGLU excretion in several infants. No remarkable hematologic response was noted in any of the infants given folic acid.—J. B. S.


The mean G6PD level in cord blood specimens of normal newborns was 8.9 units per ml. of red cells, in comparison to a mean adult level of 4 to 5.5 units. Venous blood specimens drawn during the first 4 days of life revealed an average activity of 7.1 units/ml. Somewhat higher values were obtained among infants with Rh-erythroblastosis. A miscellaneous group of sick infants and children was studied. As might be expected, G6PD levels 2 to 3 times greater than normal were present in many of the anemic patients, particularly when the anemia had a hemolytic component.—J. B. S.


Evidence was presented that H2O2 was formed in intact human erythrocytes after addition of primaquine, pamaquine, pentaquaine, isopentaquaine, hydroquinone, p-quinone, phenylhydrazine and menadione, but not after addition of chloroquine or resorcinol. Hydrogen peroxide generation by 8-aminoquinolines required the presence of oxyhemoglobin, but was not required for generation by phenylhydrazine. The authors concluded that these findings lend further support to the concept that drug-induced hemolysis represents a manifestation of H2O2 intoxication in vivo.—E. R. J.


An answer to the long-standing question of how sodium nitrite, a powerful reducing agent, oxidizes hemoglobin to methemoglobin has been proposed on the basis of the observation that H2O2 was formed during the reaction. The failure of nitrite to produce hemolysis in vivo in subjects with both normal and G-6-P dehydrogenase deficient erythrocytes was explained by the limited capacity of nitrite to generate H2O2. Hydrogen peroxide generation ceased after all oxyhemoglobin, a necessary cofactor, had been oxidized and nitrite itself could serve to detoxify intracellular H2O2.—E. R. J.


The presence of oxalacetate-kinase in human erythrocytes was suggested by the authors, based on the following properties for differentiation from pyruvate-kinase: different optimal buffer, stability, type of activity and resistance to denaturation. The relative activities were determined in several different buffers and in two cases of hemolytic anemia. Pyruvate-kinase activity usually was higher than oxalacetate-kinase activity.—M. J.

In acute normovolemic anemia there is a predictable increase in cardiac output and a decrease in peripheral vascular resistance. In order to find out if this was a response to a lowering of systemic oxygen transport and venous oxygen tension, dogs were rendered acutely anemic and then given 100 per cent oxygen. The oxygen breathing resulted in a return to normal of the mixed venous oxygen tension and of the systemic oxygen transport. Despite the supply of normal amounts of oxygen, no change occurred in the classical cardiovascular responses to the acute anemia. The conclusion was that the hemodynamic changes that follow acutely induced normovolemic anemia does not result from a reduction of total systemic oxygen transport or of mixed venous oxygen tension.—A. J. E.


Methemoglobinemia at a level of about 35 per cent in female mice, induced by intraperitoneal administration of sodium nitrite or p-aminopropiophenone, afforded a significant degree of protection against the lethal effect of sodium azide given 20 minutes after the methemoglobin-forming agent. Methemoglobinemia was associated with some prolongation of survival after fluoride intoxication, but not after cyanate, thiocyanate, selenate or borate administration. Formation of the azide-methemoglobin complex was demonstrated in vitro and was identified after in vivo trials. The authors emphasized that methemoglobinemia has been shown to protect only against known inhibitors of cytochrome oxidase.—E. R. J.


Armadillos, rabbits and mice were protected against death from parenteral administration of sodium sulfide by pretreatment with sodium nitrite or p-aminopropiophenone and survival of mice upon exposure to hydrogen sulfide vapors was prolonged. The effect was attributed to the formation of sulfmethemoglobin (not sulfhemoglobin!).—E. R. J.


A careful study of the lipid composition of erythrocytes obtained from healthy adults after an overnight fast revealed that total lipid phosphorus averaged 1.40 × 10⁻¹¹ mg. per cell. The average percentage distribution of phospholipids was: choline glycerophosphatides 30; sphingomyelin 24; ethanolamine glycerophosphatides 26; serine glycerophosphatides 15, and minor phospholipid components. The importance of complete extraction and of expressing results on a per cell basis were emphasized.—E. R. J.

Leukocytes


Comparable groups of patients with leukemic involvement of the central nervous system were treated by lumbar puncture alone, by intrathecal administration of methotrexate, or by radiotherapy. In all, 150 episodes occurring in 53 children were analyzed. Simple lumbar puncture effected a clinical response in approximately half the patients with a mean symptom-free interval of 2–3 weeks. The response rates to methotrexate injection and radiotherapy were 88 and 98 per cent respectively, with mean symptom-free intervals of 3.7 and 2.8 months. The differences between these 2 groups were not significant. Response to intrathecal methotrexate was almost immediate, whereas 3 to 4 days elapsed before remission was obtained following x-ray therapy. There were no untoward effects of methotrexate therapy, whereas significant alopecia following radiotherapy was frequent. For these reasons, the authors prefer methotrexate injection, unless isolated cranial nerve palsy is present. Where signs of meningitis accompany a peripheral seventh nerve paralysis, combined x-ray and
methotrexate therapy is recommended. The possible usefulness of prophylaxis with methotrexate is discussed.—J. B. S.


In cultured blood leukocytes from 2 patients with chronic myelocytic leukemia, a cell line with 47 chromosomes was found. In both cases, a small submetacentric member of the 17-18 group was lacking, whereas there were 2 supernumerary chromosomes of the 6 — x — 12 group. This karyotype was present only in Ph1-positive cells, but not all Ph1-cells showed the abnormal pattern. In one of the patients, the prevalence of the Ph1-47 chromosome cell line rose from 23 to 67 per cent within 6 weeks, suggesting a selective advantage of this line over the ordinary Ph1-line. —S.-A. K.


Among 694 patients with leukemia and other malignant systemic diseases, 35 (5 per cent) developed esophageal moniliasis. The main symptoms were pain on swallowing and obstruction to passage of food. The diagnosis was established by esophagoscopy. Radiologically, the esophagus showed irregular and ragged outlines with numerous indentations and protrusions. In about ¼ of the cases, the esophagus appeared radiologically normal. Candida albicans was cultured from 33 patients. A few therapeutic attempts with Nystatin were disappointing. Amphotericin B i.v. was recommended for treatment; of 29 courses, 23 were considered to be beneficial. No serious side effects were observed.—S.-A. K.


A murine leukemia virus which produces a high frequency of leukemic transformation in the thymus was injected into newborn Swiss mice. The injected mice were killed at varying time intervals and histological and chromosomal examinations were performed on the thymus. A nonproliferative preleukemic phase usually could be detected histologically. Gross chromosomal aberrations could not be detected during this preleukemic phase or even when frank early leukemia could be detected by histologic methods. The authors concluded that chromosomal alterations result from, rather than initiate, the leukemic transformation. If chromosomal alterations are causally related to the development of leukemia, these alterations are too subtle to be detected by current techniques.—I. G.


Newer developments emerging in the active field of thymus research have suggested a need for recognizing the existence of an appreciable content of nonlymphocytic components. This is especially true since the existence of a noncellular humoral factor responsible for activating the peripheral immune mechanism has been postulated. The procedures described in the present report apply the technics previously reported for the separation of salivary gland structural aggregates. One gram of thymus gland, fresh or frozen, from male guinea pigs of 250-400 Gm. weight, was minced in 12 ml. of a 70 per cent glycerol medium and disrupted for 10 minutes at high speed in a Vir-Tis "45" homogenizer. Drastic dispersion of the thymus gland lysed its thymocytes specifically and provided a neat and simple means for their elimination without the technical handicap of nuclear fibrillation or gelation. Separation of Hassall's corpuscles from the homogenate was based on their greater density which permitted their rapid sedimentation at low centrifugal acceleration, leaving the lighter stromal segments behind in the supernatant. It is hoped that the use of these isolated fractions, both of which predominate under the stresses of accidental involution and old age, can serve to clarify some of the unknown factors in thymus cell physiology. —O. P. J.


Leukocytic ingestion of Staphylococcus albus was studied in an in vitro system using glycogen-
induced rabbit peritoneal exudate leukocytes. The present study afforded more direct evidence for the role of heterophil granules in phagocytosis, confirmed the morphologic relationships involved and revealed by cytochemical means the presence of acid and alkaline phosphatases in the cytoplasmic granules and subsequently within phagocytic vacuoles. Each of 3 apparently distinct granule types evidently participated in this process.—O. P. J.


Polymorphonuclear neutrophilic granulocytes from healthy human donors were subjected to electron micrographic study after phagocytosis of rough pneumococcus in vitro. Three possible routes were found for the transfer of potentially bactericidal substances into phagocytosis vacuoles. First, granulo-vacuole coalescence which involved the discharge of granule contents from a cytoplasmic location to the interior of the vacuole. Second, small, bud-like extensions from the phagocytosis vacuoles into the surrounding leukocytic cytoplasm whereby cellular material might be transported to and from the vacuoles. Lastly, the finding of membrane-lined structures with diameters of 25–250 \( \mu \) inside the vacuoles suggested that organized cytoplasmic matter might contribute to the enzyme pool available for the destruction of intracellular matter.—O. P. J.


In 14 subjects exposed to benzene, a significant decrease in the osmotic resistance of leukocytes was found before any other blood alterations could be detected.—P. D. N.


The results of leukocyte enolase assays in 53 normal subjects and in 73 patients with a variety of leukemias and allied diseases are presented and the assay method is described. Leukocyte enolase activity was found to be low in either acute or chronic lymphocytic leukemia, as well as in lymphatic leukosarcomatosis. In 7 cases with acute myeloblastic leukemia with over 80 per cent myeloblasts and promyelocytes, the activity was either normal or elevated. In 1 case with chronic myelocytic leukemia in an acute blastic phase, 1 case with monocytic leukemia and 2 cases with erythroleukemia, the activity was found to be elevated. In cases with an increased percentage of neutrophils, such as chronic myelocytic leukemia, polycythemia vera, myeloid metaplasia and neutrophilic leukocytosis, the activity generally was elevated. Assay of leukocyte enolase activity may be of some diagnostic value in differentiating acute lymphoblastic from acute myeloblastic leukemia, provided blast cells predominate in the peripheral blood.—K. F.


Leukocyte enolase was partially purified from leukocytes of normal healthy individuals, a case with acute myeloblastic leukemia and 2 cases with chronic myelocytic leukemia. Kinetic studies revealed that there seemed to be essentially no difference in the pH curve, Michaelis constant, metal ion effect and electrophoretic pattern between enolase from normal leukocytes and that from leukemic leukocytes.—K. F.


The author reported the occurrence of small lymphocytes with “atypical nuclei” and the capacity of these cells to become Marshalkó plasma cells by mechanisms which appeared to arise from their nuclei. The present report represents one facet in a series of studies in search of an explanation of the occurrence and peculiarities of these specific lymphocytes with “atypical nuclei,” of the forces involved in their conversion to plasma cells, and of the significance of the specific reactions of their nuclei to staining with methylene blue followed by differentiation with alcoholic Lugol’s solution. Subcutaneous injections of various unrelated sub-
stances were given in the lower left abdomen or lower right back of albino mice and the inguinal nodes of the same side were used as the experimental areas. Increase in the number of lymphocytes with "atypical nuclei" in lymph nodes was elicited. These cells were considered to represent normal responses to normal nonspecific lymph drainage, i.e., to change in fluid volume of the tissue juices, per se, or to tissue products carried in them.—O. P. J.


Although the title of this paper does not indicate anything of interest to hematologists, the author did study macrophages, mast cells, eosinophils and lymphocytes in rat fetuses on the 14th day of gestation. Previous studies of cytoplasmic microtubules have been limited to plant and lower animal cells. The diameter of the microtubules was 220–260Å and their length was 2 to 3µ in epithelial cells. There was no evidence of a direct connection with the endoplasmic reticulum, Golgi membrane, nuclear envelope, or plasma membrane. They pursued an almost straight course and did not branch. In macrophages, microtubules were oriented at random, but they were sometimes more numerous in the peripheral part of the cytoplasm. In mast cells, eosinophils and lymphocytes, their number seemed much reduced. Perhaps they function in the transport of water and small molecules within the cytoplasm.—O. P. J.

HEMOSTASIS


In a review of the literature, the authors have found 43 cases of spontaneous epidural hematoma, 8 of which have been associated with coumarin anticoagulant therapy. Two additional cases associated with such therapy are reported. The first symptom is usually acute severe low back pain. It may begin after minor trauma or exertion, such as a Valsalva maneuver which is evoked by defecation or urination. The time interval from onset of pain until the appearance of motor weakness of the lower extremities has been as short as 20 minutes or as long as 10 days. In all the cases reported, the hematomas have been in the lower thoracic or lumbar region. The sequence of back pain, sensory changes, and muscular weakness progressing toward ultimate paraplegia should alert the clinician to the underlying condition, if the patient is on coumarin therapy. The hematoma may occur at levels of prothrombin time generally considered to be in the therapeutic range and in the absence of bleeding elsewhere. Prompt recognition and immediate surgical intervention are essential, if permanent cord damage is to be avoided.—R. G.


Actinomycin D inhibits vitamin K-induced formation of prothrombin, as measured by a one-stage prothrombin time, in chicks deficient in vitamin K. The administration of actinomycin D in doses which inhibit prothrombin formation also inhibits hepatic synthesis of RNA from ATP, as determined with adenine-8-C14. The results are consistent with the genetic action of vitamin K in inducing RNA formation for the synthesis of certain clotting proteins. The author proposes the following hypothesis: In the absence of vitamin K, a regulatory gene represses the activity of the operon concerned with the elaboration of the vitamin K-dependent clotting proteins (prothrombin and factors XI, X, IX and VII). Vitamin K may then act to derepress the operator by combining with the repressor molecule, thus freeing the structural gene components of the operon to elaborate their respective messenger RNA's which lead to synthesis of their respective proteins. The effect of actinomycin D in blocking the action of vitamin K is consistent with the known action of actinomycin in blocking DNA-dependent RNA synthesis.—R. G.

A boy with a history of severe epistaxes, easy bruising and bleeding following tooth extraction was found to have a prolonged bleeding time and a somewhat abnormal thromboplastin generation test. The abnormal TGT could be corrected with either normal serum or plasma. The bleeding time appeared to be the result of defective in vivo platelet adhesiveness. Fresh plasma infusion did not affect the bleeding time, but transfusion of platelets resulted in a normalization of both platelet adhesiveness and the bleeding time.

—J. B. S.


In a review of the literature, the authors found 36 cases which met their criteria for the diagnosis of factor VII deficiency and they added 3 more cases. In addition, 22 cases which were probably congenital factor VII deficiencies have been reported, but the published evidence was incomplete. Thirty-nine other cases reputed to be factor VII deficient probably were not, either because supplementary reports could not confirm the original impression or the laboratory data appeared more compatible with other diagnoses. The authors pointed out that the type of bleeding with factor VII deficiency is indistinguishable from that seen in factor V, X, VIII or IX deficiencies. Both sexes were affected about equally, but epistaxis and hemarthrosis were much more common in males. The 3 cases reported had lifelong bleeding tendencies. An 11-year-old boy had experienced recurrent hemarthroses of the right knee; a 13-year-old girl had hemorrhaged severely with each menstrual period; a 48-year-old man had bleeding after surgical procedures, such as dental extractions and hemarthroidecomy. Family studies confirmed previous reports that the heterozygous state is associated with a plasma concentration of factor VII of about 50 per cent of normal and that only the homozygous state is associated with marked deficiency and hemorrhagic manifestations, neither of which will respond to vitamin K therapy. The authors also confirmed previously reported findings of the transient effect of plasma therapy. They found that plasma concentrations of factor VII were negligible within 24 hours after plasma transfusion.—R. G.


Severe hereditary factor VII deficiency has been discovered in a propositus and 2 of his siblings. An extensive family study has confirmed previous reports that the disorder is transmitted by an autosomal "intermediate" gene which produces severe deficiency in the homozygote and partial deficiency in the heterozygote. The study brings out the unreliability of assessing the mode of transmission of a hereditary bleeding disorder by history alone, since many individuals with suggestive histories are found to be normal and others with severe deficiencies may not have a positive history. In addition, individuals who are heterozygous for the abnormal autosomal gene have partial deficiencies without a hemorrhagic diathesis. The authors point out the marked variability of the bleeding tendency seen in patients with severe factor VII deficiencies. The clinical course of the three patients which they report has been very mild in that bleeding attributable to impaired hemostasis has been minimal. The death of the propositus, due to pulmonary embolism, clearly establishes that isolated VII deficiency fails to protect against venous thrombosis.—R. G.


In a review of the literature, these authors find 52 cases which fulfill the necessary diagnostic criteria for isolated congenital factor VII deficiency and they add two cases of their own. Their family studies also are compatible with the genetic pattern of transmission by an autosomal gene with full clinical expression of hemorrhagic symptoms appearing only in the homozygous gene in which there is a marked deficiency of factor VII. In the heterozygous state, the level of factor VII is below normal, but there are no hemorrhagic manifestations. The decay of transfused factor VII has 2 components with respective half-lives of 35 and 300 minutes; the first component probably represents an initial rapid diffusion of the factor into the extravascular spaces and the second represents metabolism and back diffusion. These latter findings are in approximate agreement with previous
ABSTRACTS

reports. The decay half-life of autologous factor VII in normal subjects after Coumadin® administration is about 280 minutes. This also is in agreement with other reports. Treatment of factor VII deficient patients with plasma or plasma extracts is effective in controlling all types of spontaneous hemorrhage and in preventing hemorrhage associated with major surgical procedures. A successful course of long-term prophylactic plasma therapy is described.—R. G.


This patient with factor X deficiency and a bleeding tendency since the age of 5 had been reported previously by Brody and Finch to have improvement in both clinical and laboratory findings during pregnancy. They suggested that the increase in factor X levels might be related to the altered endocrine function during pregnancy. In the present report, norethynodrel, an analogue of progesterone, when given orally in a dose of 10 mg. per day, resulted in shortening of the prothrombin time from 800 seconds to 17 seconds within a week. Attempts at reducing the dosage to 5 mg. per day resulted in breakthrough bleeding. On a regimen of 10 mg. daily, the patient became asymptomatic, the prothrombin time was maintained at 16–20 seconds, but never reached normal levels, and the thromboplastin generation test reverted to normal. When the drug was discontinued, the patient remained asymptomatic for two months before hemorrhagic manifestations reappeared, at which time the prothrombin time and thromboplastin generation tests were markedly abnormal. Reinstatement of the drug resulted in rapid clinical improvement and correction of the coagulation defect.—R. G.


A 53-year-old male with hepatic amyloidosis was described. The prolonged prothrombin time was shown to be due to a factor X deficiency. The authors pointed out that of the 4 patients previously reported in the literature with acquired factor X deficiency, 3 had associated amyloidosis. The possibility of a circulating anticoagulant against factor X was ruled out in this case. The association of factor X deficiency and amyloidosis seems to be more than coincidental, suggesting that amyloid deposition has a specific effect on the synthesis, consumption or inactivation of factor X.—R. G.

PLASMA AND LEUKOCYTE FIBRINOLYSIS IN LEUKEMIAS. V. Boccardi and A. Grasso. From the University, Roma, Italy. Progresso med. 20:18–22, 1964.

Fibrinolysis was studied in plasma and with leukocytes from 15 cases of leukemia. Myeloid leukemia leukocytes exhibited marked spontaneous lytic activity with a low proactivator content and a high plasminogen level. In lymphoid leukemia, no spontaneous lytic activity was found and a high proactivator content (2.5 times higher than in normal controls) and a low plasminogen level were observed. Plasma fibrinolytic activity was below normal in lymphoid leukemia when leukocyte-rich plasma was studied, but it was increased in myeloid leukemia under the same conditions. In both forms, fibrinolysis in plasma was almost normal, if studied in leukocyte-poor plasma. No definite differences were found between acute and chronic cases.—P. D. N.


Traumatized rat omentum was used to demonstrate the development of platelet masses following agitation in platelet-rich plasma. In the absence of divalent cations, there was only platelet adhesion to connective tissue fibers with little evidence of platelets sticking to each other. In the presence of divalent cations, masses of platelets formed (this is referred to by the authors as cohesion) even in plasma adequately anticoagulated with heparin. Exposure of these platelet masses to thrombin produced greater compactness and stability. Human platelets and rat platelets behaved alike with the traumatized rat omentum. Platelets from 2 patients with von Willebrand's disease gave normal reactions, whereas platelets from a patient with thrombasthenia showed adhesion only. Washed connective tissue fragments and thrombin both caused clumping of human platelets, accompanied by release of serotonin and of adenine nucleotides, about one-third of which was ADP. Intermediate concentrations of connective tissue and thrombin caused platelet clumping without
the release of measurable amounts of serotonin and adenine nucleotides. ADP caused intense clumping of platelets, but did not cause release of serotonin or additional ADP. The authors suggested that the cohesion reaction was mediated by release of ADP. However, since ADP itself did not cause degranulation or release of serotonin or adenine nucleotides, it could not be responsible for the effects of thrombin or connective tissue. It was postulated that the lower concentrations of thrombin and connective tissue which caused platelet clumping without release of measurable amounts of nucleotides or serotonin liberated small amounts of ADP at the platelet surface. The cohesion reaction on the mesentry and platelet clumping produced by connective tissue fragments and ADP were inhibited by AMP and EDTA. The forces favoring adhesion are unknown. They were independent of cation concentration and were not altered by treatment of the platelets with neuraminidase which made the platelets less electronegative. Adhesion was prevented by prior treatment of the platelets with trypsin.—R. G.

**IN VITRO AND IN VIVO PLATELET ADHESIVENESS IN LEUKEMIAS IN RELATION TO ATP AND ADP CONTENT.** P. Ottaviani, F. Mandelli, L. Deriu and G. Bufano. From the University, Genova, Italy. Progresso med. 20:46–51, 1964.

In 7 cases of acute leukemia and 11 cases of chronic leukemia, a decrease of platelet adhesiveness in vitro and, particularly in acute cases, in vivo was found frequently. An increase of ATP and ADP in plasma with a high ATP/ADP ratio was also found frequently. Absence of ATP consumption was observed rarely.—P. D. N.


Platelet survival, determined with DFP³² labeling, was studied in 10 normal subjects and in 6 patients with vascular diseases. In thrombotic conditions, with and without inflammatory or degenerative vascular disease, the consumption of platelets was considerably increased above normal. Heparin reduced this difference.—P. D. N.


Serial platelet counts were performed in 28 premature infants during the first month of life. Duplicate capillary samples were obtained by heel puncture. All infants had platelet counts between 31,000 and 197,000 per mm.³ during the first 5 days. Among infants whose birth weight was below 1700 Gm., platelet counts fell to below 50,000 by 10 to 20 days of age and then rose to levels above 100,000 by the 5th week. In the larger prematures, platelet counts were above 100,000 after the 10th day.—J. B. S.

**MISCELLANEOUS**

**AUTOIMMUNITY AND AUTOIMMUNE DISEASES.** T. Kurayama. From the Faculty of Medicine, University of Tokyo, Tokyo, Japan. Jap. J. Clin. Hemat. 5:176–186, 1964.

Secondary disease in rats receiving bone marrow transplantation from normal rabbits was less than in rats receiving bone marrow transplantation from rabbits sensitized with egg albumin. Direct Coombs test, using anti-rabbit globulin serum, was positive in rats receiving a transplant from sensitized rabbits, but was negative in rats receiving a transplant from normal rabbits. A large amount of antigen suppressed antibody production. Therefore, a large amount of erythrocyte antigen may suppress the formation of anti-erythrocyte antibody by immune competent cells of transplanted rabbits, resulting in a negative Coombs test. However, transplanted immune competent cells of rabbits sensitized with egg albumin were able to produce anti-erythrocyte antibody, in spite of the presence of a large amount of antigen. These results suggested that antigenic stimuli may cause some changes in the antibody formation mechanism of immune competent cells to produce antibodies against a large quantity of other antigens. Some changes in the antibody formation mechanism in immune competent cells may occur in auto-immunization, because auto-antibodies are produced against a large amount of autoantigen. The possibility of changes in antibody formation due to prolonged sensitization or malignant neoplasms of lymphoid tissue was suggested. Injection of Ig labeled anti-rat liver antibodies into normal rats resulted in specific localization in the liver. The absorption of anti-liver antibodies with liver homogenate supernatant resulted in abolition of localizing antibody, leaving precipitin against liver homogenate supernatant unaffected. Activity of localizing antibody was not affected by absorption
ABSTRACTS

with supernatant, while the precipitin titer decreased markedly. When anti-liver antibodies were injected intravenously into normal rats, localizing in minutes, leaving precipitin against supernatant in 20 minutes, leaving precipitin against supernatant 20 minutes. The phenomenon was particularly pronounced.

Antibody disappeared from circulating blood with supernatant, while the precipitin titer decreased markedly. These results suggested that anti-liver antibodies contain antibodies produced against various antigens in liver homogenates. The antibody which localized in liver and exerts a cytotoxic effect is the antibody produced against liver homogenate supernatant as antigen. These antibodies may indicate the presence of autoimmunization in patients. However, they are not necessarily etiologic factors of the disease.—K. F.


The purpose of this series of papers is to re-examine with more modern and subtle techniques the mechanisms of delayed sensitivity. The authors, as others before them, used in vitro inhibition of migration of peritoneal exudate cells as the test system. PPD, ovalbumin and diphtheria toxoid specifically inhibited the migration of exudate cells of animals sensitized to these antigens. Serum antibodies could not passively confer inhibition of migration onto normal cells. Mixing sensitized living cells with normal cells conferred inhibition in the presence of specific antigen to the normal cells. As few as 2.5 per cent sensitized cells could exert this effect. Heat or cold killed sensitized cells were without effect. Finally, using 2,4-dinitrophenyl (DNP)-conjugated proteins, it was shown that carrier specificity was present in the inhibition of migration, i.e., cells sensitized to DNP guinea pig albumin would migrate normally in the presence of DNP bovine gamma globulin. This carrier specificity is also a prominent feature of delayed sensitivity in vivo. (Abstractor's note: This in vitro inhibition of migration is all very good. However, in vivo delayed sensitivity there appears to be, if anything, a stimulation of migration toward and a proliferation of blood borne cells at the site of antigen deposition. How to reconcile this apparent opposite behavior of cells under different conditions is not clear at the present state of our knowledge.)—I. G.


The exact mechanisms by which antigen elicits antibody synthesis are not clear. A detailed tracing of antigen molecules during this process would be helpful. The authors used ferritin, visible by electron microscopy, for this purpose. The primary response in rabbits was elicited by apoferritin (not seen by electron microscopy, but having the same antigenicity as ferritin). The secondary response was evoked by ferritin. The distribution of the ferritin in lymph nodes of immunized animals was then followed by immunofluorescent and electron microscopy. The principle findings were that ferritin was found in the nucleus and cytoplasm of hemocytoblasts, immature and mature plasma cells. As expected, ferritin also was found in the cytoplasm of sinus macrophages. In plasma cells, ferritin was most often associated with dense nuclear chromatin material and with ergastoplasm in the cytoplasm. The authors discussed the controversial nature of their findings. Other investigators did not find antigen in the nucleus or cytoplasm of immature or mature plasma cells after antigen administration.—I. G.


It has been reported that under appropriate conditions in a primary immunization to a given antigen, antibody of \( \gamma_1 \) (19S) macroglobulin class appears first and that of \( \gamma_2 \) (7S) type later. These findings suggest that different cells produce 2 different species of antibody or that the same cell produces 19S antibody first and then converts to the synthesis of 7S antibody. Using the Freund's adjuvant-diphtheria toxoid system, a study has been made of the relationship between the type of circulating antibody and the type of cells that synthesize or, perhaps, store antibody. The results indicate that in the spleen the plasma cell is associated with the production of 7S immune globulin and a large basophilic cell is associated with the production of 19S immune...
globulin. It is unlikely that these latter cells give rise to the mature plasma cell. This does not preclude the plasma cell arising from lymphocytes, but a sequential change from a large cell containing antibody to a mature plasma cell containing antibody has not been established.—O. P. J.


In addition to its already well-established functions of histamine, heparin and serotonin production, the mast cell apparently also plays a role in mineralization. The present report deals with the authors' interest in two forms of soft tissue calcification: calciphylaxis and calcergy (induced calcinosis). It has been reported previously that the mast cell plays an important role in certain types of soft tissue calcification. In mast cell calcification, lead acetate was injected intravenously and local calcification was produced by the injection of a mast cell discharger, such as polymixin or local calcification was produced by the injection of a mast cell discharger, such as polymixin or compound 48/80. The injection sites were studied after the following staining methods: von Kossa for demonstration of phosphate or carbonate; chloranilic acid for demonstration of calcium; and sodium rhodizonate for demonstration of lead. Mast cells in the treated area first showed degranulation without any mineralization, but this was soon followed by lead uptake on mast cell granules with secondary attraction of calcium, phosphate and, possibly, carbonate. Apparently, the mast cell granule is capable of metal binding. —O. P. J.


This experiment was begun as a histochemical study of the mast cell under experimental conditions. Sudan black and PAS technics were used to detect changes in lipid and carbohydrate content of the mast cell. Sudan black stained the mast cells only very slightly and the McManus periodic acid Schiff (PAS) reaction yielded variable results. However, when the mast cell was first stained with PAS and then stained with Sudan black, the mast cells gained an avidity for the Sudan dye which resisted extraction. Using the various components of the PAS technic, it was found that acid treatment of the section with either periodic or sulfurous acid produced the same result. Acetylated Sudan black without previous acid treatment of the section also stained the mast cells.—O. P. J.


Bone marrow smears from 33 patients with multiple myeloma were studied cytometrically. The cellular and nuclear area of myeloma cells was computed from diameter measurements and agreed surprisingly well with planimetric measurements on the same cells (correlation coefficients 0.96 and 0.95, respectively). Myeloma cells from patients with \( \gamma_1 \)-paraproteinemia were larger and had a less eccentrically located nucleus than those from cases of \( \gamma_2 \)- and \( \gamma_3 \)-paraproteinemia. —S.-A. K.


The distribution of blood group antigens in human tissues has been studied by using the mixed agglutination technic, the agglutination inhibition technic and, more recently, the fluorescent antibody technic. By these methods, the ABO(H) antigens have been shown to be widely distributed in normal tissues. However, the study of H antigen in normal and diseased tissues by labeled antibody technics has been limited by the availability of suitable anti-H serum. It has been shown that extracts of Ulex europaeus (Eurasian spiny shrub) seeds contain a protein that reacts with blood group O(H) antigen and, to a lesser extent, with A and B antigens. In the present study, the use of a fluorescein labeled extract of Ulex europaeus seed for demonstrating water soluble H antigen in tissue sections has been evaluated.—O. P. J.