ABSTRACTS

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ABSTRACTERS

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ERYTHROCYTES


It was shown previously (see Blood 15:431, 1960) both in vitro and in experimental animals that use of glutamic acid decreased the amount of methemoglobin produced by sodium nitrite. This could either be due to inhibition of methemoglobin formation or to promotion of conversion of methemoglobin back to hemoglobin. Accordingly, rats were injected with sodium nitrite and after 20 minutes with sodium glutamate or saline as a control. Blood collected after another 20 minutes showed no difference between test and control methemoglobini levels. In another experiment nitrite was preceded by glutamate and here the methemoglobin level was considerably lower but not as low as when the two substances were injected simultaneously. It was also found that other amino acids did not have this effect whereas glutamate was also effective against other methemoglobin forming substances. Thus glutamate possesses a specific inhibitory action on methemoglobin formation.—J. J. B.

THE INFLUENCE OF METHEMOGLOBINEMIA ON THE DEVELOPMENT OF HEINZ-BODIES. L. Magos.


Pretreatment of erythrocytes with nitrite-compounds reduces the development of Heinz-bodies after the cyanide-peroxide test.—S. R. H.


Three age groups of subjects were studied: less than 25 years, 26 to 65 years, and over 65 years. The transaminase values in the red cells exhibit a progressive, considerable and parallel diminution with increasing age. The true cholinesterase activity increases with maturation and decreases in old age to values which are lower than in young subjects. There is poor correlation between red cell and serum values which diminish only slightly in old age.—P. d. N.

The same case material was used as in the previous report. A progressive increase of cholesterol to adult age and a moderate, subsequent diminution were observed. The free fraction of cholesterol was increased in all cases; in old subjects the esterified fraction did not exhibit any modification. The reaction of the cholesterol-esterase activity was mostly hydrolytic in young subjects, but different behavior was observed in older ages with primarily an esterase mechanism.—*P. d. N.*


The concentration of erythrocytic GSH, before and after incubation with acetyl-phenylhydrazine, was studied in 353 subjects from Sardinia, 146 of which were unselected, 37 affected by favism, 62 relatives without favism, and 108 normal controls. In the subjects with a history of favism and in the healthy relatives the GSH content was significantly lower than in controls. After incubation there was a marked instability of GSH in 97 per cent of the subjects with favism and in 32 per cent of the relatives. In 3 per cent of the subjects with favism and in 21 per cent of the relatives there was intermediate instability of GSH. Males rarely exhibited an intermediate instability of GSH. The incidence in the general population is 12 per cent. In males it is of 24 per cent and in females of 3 per cent. Among the latter 23 per cent of the subjects exhibited an intermediate instability of GSH. Favism affects 8 per cent of the same population. This means that 39 per cent of the subjects with the erythrocytic defect are not sensitive to the hemolytic effect of vicia fava.

—*P. d. N.*


The erythrocyte life span was measured by the Cr*51* method, normal T/2 being 29 days. In 13 patients misusing phenacetin, without renal insufficiency, the average T/2 was normal, but in 5 it was reduced to 20–23 days. In 11 patients abusing phenacetin, with renal insufficiency, the average T/2 was 18 days. In addition 10 patients suffering from renal insufficiency but not using phenacetin were investigated and a normal T/2 value found (31.9 days).—*C. W.*


Separation of the α and β chains of human hemoglobin has been achieved by countercurrent distribution using 2-butanol-1/2 aqueous dichloroacetic acid.—*A. I. C.*


A technic for rapid agar electrophoresis, utilizing a paper backing, is described. Excellent resolution of serum proteins and hemoglobins in 20 to 30 minutes occurs. The method is particularly convenient for solution and densitometry manipulations.—*A. I. C.*


The amino acid analyses of horse globin, as well as its α and β chains, have been determined on Amberlite IR-120 resin. The values found correspond to a postulated molecular weight of approximately 64,000.—*A. I. C.*


Oxygen equilibrium curves of human, horse and sheep hemoglobins were determined on dilute solutions of hemoglobin in urea solutions of different molarity. A progressive effect on the position of these curves was noted. The effect could be reversed by dialyzing the solutions free of urea. The increased affinity for oxygen with increasing urea molarity is discussed in terms of the known splitting effects of urea on hemoglobin and of theories of heme-heme interaction.—*A. I. C.*

**FETAL BLOOD STUDIES. XVII. THE OXYGEN-DISSOCIATION CURVE OF THE POSTMATURE FETUS.** H. Prystowsky, A. Hellegers and P. Bruns. From The University of Florida College of
Oxygen dissociation curves of six postmature fetuses differed in no significant fashion from those of normal full term infants. The shift to an adult type of pattern must therefore be completed in the postnatal period. A lack of correlation of the percentage of Hgb F as determined by the alkali denaturation procedure and the position of the oxygen dissociation curve was noted.—A. I. C.


As a part of a study of physiologic adaptations to low oxygen tension, the oxygen dissociation curves on adult and fetal llamas were determined. The curves were found to be shifted far to the left with half saturation at an oxygen tension of about 20 mm. The fetal curve was positioned slightly further to the left, but the difference was not striking. The oxygen tension in the fetal umbilical vein was found to be slightly higher than the oxygen tension of the uterine vein, a finding which supports the concept that the fetal and the adult newborn types of Pattern must therefore be completed in their placental capillaries thereby creating a counter-flow effect.—A. E.


No significant difference was noted in the survival time of sickle cell trait and normal blood stored under routine blood bank conditions for 17 days. After 3 weeks storage, it may be possible to detect a small percentage of relatively fragile erythrocytes in sickle cell trait blood which failed to appear in normal blood. Sickle cell trait blood is felt to provide no additional risk of hemolysis to the recipient and may be used in a routine fashion.—A. I. C.


Angioid streaks occurred in 6 per cent of eyes of 69 patients with sickle cell disease (S-S, S-C). The authors review the literature, clinical features and pathology of angioid streaks as well as the general funduscopic findings of sickle cell disease. They suggest that the primary pathology depends on a defect of the elastic tissue, primarily in Bruch's membrane. Possible etiological mechanisms, including genetic considerations, are discussed.—A. I. C.


The first reported case of sickle cell-Hgb D disease in a Negro is described in detail. A summary of the clinical and hematologic findings of this syndrome, based on six reported cases of sickle cell-Hgb D disease, is presented. Erythropoietic data suggested a relative insufficiency of erythropoiesis in addition to the mild hemolytic process in this individual.—A. I. C.

CLINICAL AND HEMATOLOGICAL MANIFESTATIONS OF HEMOGLOBIN CS DISEASE IN CHILDREN. A. H. Tuttle and B. Koch. From the University of Tennessee College of Medicine, Memphis, Tenn. J.Pediat. 56:331–342, 1960.

Eighteen children with sickle cell-Hgb C disease are described. Their ages ranged from nine months to fifteen and one half years at the time of their last observation. Clinical and hematologic findings are detailed. Crises were in general less frequent and less painful than in sickle cell anemia. Hepatomegaly and splenomegaly were frequently observed. Bone changes were less striking than in sickle cell anemia, although two patients had aseptic necrosis of the femoral head, and one had extensive osteomyelitis. The body habitus was relatively normal. The hematologic findings were those of a moderately severe hemolytic process with periods of varying degrees of severity of the anemia.—A. I. C.


A hemoglobin variant with the same electrophoretic and chromatographic mobility as Hgb G, found in a Negro woman and her five children, is described. The clinical and hematologic findings were unremarkable. Unusual features included the following: the abnormal hemoglobin is the major component when determined by free electrophoresis; a discrepancy was noted in quantitat-
ing this pigment by different techniques of separation; the abnormal hemoglobin was present in each of this woman's five children—A. I. C.


Vitamin B12 binding appears to be an essential property for intrinsic factor activity. Vitamin B12, once bound to intrinsic factor, does not exchange freely with unbound vitamin.—V. II.


In both man and rat the absorption percentage decreases with increasing doses of vitamin B12 given together with homologous intrinsic factor. In gastrectomized rats the absorption after a preceding loading dose was again normal at a time when orally administered vitamin B12 could be detected only in the intestinal wall; not in the blood, liver, kidneys and spleen. Hence, the absorption is not determined by the saturation of an intracellular B12 acceptor. In the rat, absorption of vitamin B12 bound to intrinsic factor was found to be impaired in an intestinal loop which had been exposed to intrinsic factor. It is concluded that there is an acceptor on the surface of the small intestinal wall with affinity for the intrinsic factor end of the B12-intrinsic factor complex.—C. W.


The vitamin B12 level per unit of normal plasma was about half that per unit of normal red cells (see also N. Kato, J. Vitaminol. 4:226-234, 1958); in vitamin B12 deficiency the red cells retain a more nearly normal amount of the vitamin than does the plasma. Similar findings have been reported elsewhere (V. Herbert in The Megaloblastic Anemias, New York, Grune & Stratton, 1959). Such findings suggest that red cell vitamin B12 levels may be a valuable parameter of tissue stores of the vitamin. This may be especially true when the patient has recently received a single injection of vitamin B12, which raises the serum level preferentially. We have found such a patient to have a normal serum vitamin B12 level, but a low red cell level.—V. II.


The disappearance time of intravenously injected radioactive vitamin B12 was found to be slow in pernicious anemia in relapse (as found by Mollin, Pittney, Baker, and Bradley in Blood 11: 31, 1956), and also in remission. This latter finding suggests the existence of a "serum B12" transferase," which is diminished or absent in patients with pernicious anemia. Further studies in patients in remission are required to confirm the validity of the data presented. As the authors state, "Whether this postulate will withstand the test of further experimentation remains, of course, to be determined." (See also the authors' paper on the same subject in Blood 15:646, 1960.)—V. II.


The biologic half-life of vitamin B12 in the normal human liver averages one year, with individual variations of from 5 to 35 months. Damaged liver cells may not only release their vitamin B12 content into the blood stream, but also be unable to accept the vitamin. Both these factors may play a role in producing the high serum vitamin B12 levels usually seen with marked hepatic damage.—V. H.


Earlier investigations by Nyberg have shown that the vitamin B12-absorption is impaired in all carriers of fish tapeworm, anemic as well as nonanemic, and that the tape worm anemia is a true vitamin B12-deficiency state. The present investigations were an attempt to elucidate the mechanism of the disturbed B12 absorption in
worm carriers. The in vivo studies were performed with the urinary excretion technic of Schilling, and indicate that the worm either interferes with the B12-intrinsic factor (IF) complex or has a blocking effect on the intestinal mucosa. The in vitro studies supported the first hypothesis. Radioactive B12 bound to human gastric juice or to hog IF concentrate was incubated with tape worm. The results showed that the worm was capable of splitting the B12-IF complex. Most of the liberated activity was taken up and bound by the worm in a nondialysable form. The binding capacity of gastric juice for vitamin B12 was highly reduced after incubation with pieces of tapeworm. From the experiments in vitro it was concluded that the fish tapeworm is able to interfere with the absorption of vitamin B12 either by attacking the B12-IF complex as such or by preventing the binding of the vitamin to IF.—C. W.

VITAMIN B12 IN PREGNANCY AND THE PUH-PERIUM.

The mean serum vitamin B12 level decreases progressively during pregnancy, and at delivery the mean cord blood levels are strikingly higher than in maternal blood. (Both of these findings have been noted by others mentioned in authors' bibliography.) Some patients with megaloblastic anemia in pregnancy may have primary deficiency of vitamin B12 rather than of folic acid, as manifested by low serum vitamin B12 levels and hematologic response to administration of this vitamin. —W. H.

SELECTIVE VITAMIN B12 MALABSORPTION AND PROTEINURIA IN YOUNG PEOPLE. A SYNDROME.

Severe megaloblastic anemia was found in two young patients, a boy, 11, and a woman, 22 years old. Both of them had suffered from anemia of varying degree since early childhood. In both cases proteinuria was found, but no signs of renal insufficiency. The B12 content of the blood was low, and the Schilling test revealed impaired absorption in both cases, but both free hydrochloric acid and intrinsic factor activity were found in the gastric juice. There were no signs of a general malabsorption syndrome or steatorrhea.—C. W.


The histological pattern of biopsy specimens from the gastric mucosa was found to be identical in severe atrophic gastritis and pernicious anemia. In many nonanemic subjects with atrophic gastritis the vitamin B12 level of the serum was low, the B12 absorption impaired, and the uropepsin excretion low. It is concluded that chronic atrophic gastritis may lead to deficient secretion of intrinsic factor and thereby possibly to pernicious anemia.—C. W.


Oral epithelium was examined in 12 cases of pernicious anemia (PA) and in 15 cases of hyposiderotic anemia. In all the patients suffering from PA and in 10 with hyposiderotic anemia changes in the epithelium were noted. Multinuclear cells were found and the number of large granulated cells and of cells with large nuclei was increased. The changes were reversed with appropriate therapy.—E. K.


A concise survey of the radioactive isotopes of cobalt (atomic mass numbers 56, 57, 58, and 60) successfully employed for labeling vitamin B12. Includes data on specific activity, decay schemes, detection sensitivity, and maximum permissible concentrations.—V. H.


A concentrated filtrate of erythropoietic plasma from experimentally anemic sheep was injected into carp yearlings. The results, based on counts of erythrocytes and erythroblasts in the circulating blood, show that: 1. The filtrate promoted erythropoiesis in animals belonging to a different class, viz., in fishes: 2. Per body weight doses four
times higher than given earlier to mammals (rabbits and mice) increased the number of red cells in the circulating blood of fishes by roughly 20 per cent, that is, gave increases comparable to those obtained in other experimental animals; 3. Single doses of more than 0.5 ml. killed a notable percentage of the fishes. Four repeated injections had the same effect and failed to stimulate further erythropoiesis.—E. K.


Bleeding can stimulate erythropoiesis in irradiated mice (500 r). The response is, however, diminished in irradiated animals even when the blood picture has returned to normal some months after the irradiation. Definite disturbances of erythropoiesis and of iron metabolism persist for several months, but gradually disappear.—G. M.


In the urine of a patient suffering from porphyria cutanea tarda the following compounds were isolated: isomer I and III of coproporphyrin, uroporphyrins, Waldenström's porphyrin, and 7-carboxyl porphyrin; trace porphyrins most probably 1, 2, 3 and 5-carboxylic porphyrin were detected but not isolated. In the raw esters of porphyrins, nonporphyrin pigments were found and they were classified as di-pyrrol compounds. —E. K.


In 1955, a man, 35 years old, was examined for normochromic anemia; peculiar gigantic erythroblasts, some of them multinucleated, were found in the bone marrow. In 1957, a young woman was admitted for anemia. She was a niece of the man and revealed a similar blood and bone marrow picture. The family was thoroughly investigated and 13 cases were found among 45 people examined. The anemia is chronic. It does not respond to therapy, but as far as is known it has not caused death. Increased percentage of fetal hemoglobin was found in all of 8 patients examined and in 5 of 8 healthy members of the family. The bone marrow picture is typical with giant erythroblasts resembling those in some cases of Di Guglielmo's disease.—C. W.


The author describes a rare form of the blood group antigen A. Red cells of the propositus were agglutinated by some anti-A+B (group O) sera but not by anti-A (group B) or anti-B (group A) sera. The propositus serum contained anti-A1 and anti B. In saliva neither A nor B substance was present. Mother was of group O, father was unknown. The findings are similar to those of the A* group, described in 1953 by Fischer and Holm.—E. K.


On the basis of studies on 152 patients, most of whom had hematologic disorders, it is concluded that the Coombs test may fail to detect positive cells unless serial dilutions of reagent are used.—E. K.

**LEUKOCYTES**


Refined extracts were prepared in the manner described in the previous abstracts from human and animal tumors. The extracts were injected to Drosophila melanogaster larvae. The presence of tumors in the larvae 24 hours after inoculation is believed to indicate the presence of a tumor-producing factor in the extracts. An attempt is made to correlate the results obtained from the larvae with those obtained in mice.—P. G. R.

Extinction dilution titrations of tumor viruses, based on the number of animals dying from a certain virus dilution, are difficult and consume large numbers of animals. Assays based on a measurable, continuously varying parameter of infection clearly represent an improvement. Serial dilutions of spleen suspensions from mice infected with the Friend leukemia agent were injected to Swiss mice. The hosts were killed and their spleens weighed. Two weeks after inoculation the regression of the log spleen weight in the hosts on the dilution of virus was 0.285 ± 0.003. Reasonable estimates of virus titer could be made and reproduced on the basis of spleen weight responses to a single dilution of virus.—P. G. R.


In chickens with erythroleukemia and in control chickens the pick-up of mitosis in the bone marrow, the penteose- and deoxypentose nucleic acid phosphorus concentrations, and the DNase activities in the bone marrow were studied. It was shown that at 2 and 4 hours after colchicine poisoning, fewer mitotic cells pile up in leukemic bone marrows than in the controls. At 8 hours after the colchicine, the mitotic frequency in the leukemia bone marrow was significantly higher than the average mitotic frequency in all erythroblast maturation stages in the controls. However, the mitotic index in the basophilic erythroblasts from the controls at this time seems to be close to that found in the leukemia cells. It is also shown that the RNAP/DNAP ratio is clearly higher in all leukemia bone marrows than in either normal controls or control animals with regenerating bone marrow. The DNase activity also seems to be higher in the leukemia bone marrows. The authors have previously found that there is often, although not always, a good correlation between the variations in the proliferative activity and the DNase activity. They therefore conclude that “the virus-induced leukemic cells must be considered to possess a marked and frequently manifested proliferative capacity.”—P. G. R.


Radiophosphorus uptake in the spleen was studied and leukemic mice compared to controls. Leukemic mice had an increased phosphorus uptake. Radiation decreased the uptake. Steroids did not affect phosphorus uptake, although the spleens stopped growing. The main increase in radioactivity was found in RNAP.—P. G. R.


Tumor virus purification is difficult because of the small quantities of agent in gross amounts of tumor tissue. Previous purifications of tumor viruses have used tissues where high virus concentrations were fortunately found, such as cotton tail rabbit warts (rabbit papillomatisis) or chicken plasma (avian myeloblastosis virus). Rottino and his group have used a purification procedure previously employed to extract a tumor-inducing factor from Drosophila melanogaster. The supernatant fluid obtained after high-speed centrifugation of tissue homogenate, serum, or ascites fluid is used. After mild acid precipitation of proteins, the active principle is twice adsorbed on calcium phosphate gel, from which the refined extract is re-eluted. Extracts were procured from human lymphosarcomatis lymph nodes, spleens, and lymph nodes from patients with Hodgkin's disease. Buffy coats from patients with myelogenous leukemia, serum or ascites fluid from patients with Hodgkin's disease, and tissues from AK and C3H (f) mice with induced leukemia were also used. The refined preparations were injected into newborn C3H (f) hosts. Control animals were inoculated with buffer or other chemicals included in the extraction procedure. Extracts from mice induced tumors in 60 out of 85 hosts. Parotid, mammary and adrenal carcinomas, osteogenic sarcomas and myxomas were found. Human extracts induced tumors in 29 out of 228 hosts. Parotid and mammary carcinomas, fibrosarcomas and one leukemia were found. None of 435 controls developed tumors after 2 to 300 days. If the present results are confirmed, they represent one of the few systematically successful attempts
to transmit human tumors to experimental animals.—P. G. R.


The author succeeded in transferring mouse leukemia of the myeloid type with cell-free media to mice and rats. The virus was successfully grown on the chorio-allantoic membrane of the embryonated hen’s egg. It is very sensitive to high temperature (65 C.), formalin, ether, desoxycholic acid, but less sensitive to trypsin, glyceral and ultraviolet rays. It is greatly resistant to DN-ase, RN-ase and lipase. RNA and DNA from leukemic tissue are completely inactive. The virus could be demonstrated by electron microscopy in ultrathin sections of lymph nodes as round particles of 60 to 90 nm diameter, with characteristic inner structure. Splenectomy almost completely inhibits the leukemia-inducing activity of the virus. Latter has specific antigenic properties and is inactivated by heterologous antiserum. The virus is produced continually by the leukemic cells, even in tissue cultures, since the filtered culture medium is highly leukemogenic. The author attempts to offer a uniform conception to explain oncogenesis.—S. R. H.


Cell-free filtrates were prepared from the brains of four patients who died of acute leukemia. Fourteen male volunteers received five doses each of 1 cc. filtrate subcutaneously. Six to ten weeks later a pool of serum was obtained from the volunteers. 3.5-cc. doses of this serum protected AKR mice from getting leukemia. The leukemia in the mice was induced with cell-free brain filtrates from four other patients who died of acute leukemia. Human control serum did not protect the mice.—P. G. R.


One of the authors received multiple intravenous injections of leukemic blood from a patient with chronic myelocytic leukemia. A leukoagglutinin appeared in the author’s serum, which was reactive with leukemic leukocytes from the donor, other chronic leukemic leukocytes, and with leukocytes of almost all normal persons. Ten injections of the author’s serum to one of the donors who died of chronic myelocytic leukemia had no unequivocal therapeutic effect. While the leukocyte antibodies found in the present study may well be similar to those frequently found after ordinary transfusions, the factor described in the previous abstract to protect mice against leukemia may conceivably be a virus antibody similar to those found in experiments with avian myeloblastosis, chicken erythroblastosis, and rabbit papilloma.—P. G. R.


Previous studies from this group have shown that when Fe59- or Cr51-labeled red cells are injected into rats with lymphosarcomas or other tumors, much of the radioactivity is recovered in the tumor. The authors assume that this is due to hemorrhage. The present study shows that tissue iron from extravascularly injected erythrocytes is reutilized only slowly. Both normal tissues, lymphosarcomas, and other tumors retain 60–80 per cent of the iron thus injected for several months. Bleeding into tumors and loss of iron may explain, in part, anemia in some animals or patients with tumors.—P. G. R.


The effect of a single treatment with heparin on tissue cultures of rat thymus, liver, spleen and lymph nodes has been studied. Thymus has been found to have the strongest affinity, and a single treatment with heparin ensured continuous migration of metachromatic cells for 20 days. Thymic tissue is essential for the synthesis of heparin: detached single cells are not capable of that function. The liver, too, synthesizes heparin, but produces no mast cells. The spleen and...
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CARBOHYDRATE METABOLISM IN THE LEUKOCYTES.

Polymorphonuclear leukocytes obtained from rabbits by peritoneal irritation were incubated with C14 labeled substrates (acetate, pyruvate, lactate, glycerol) in vitro. The amount and distribution of incorporation of C14 into glycogen and lactic acid were then studied as part of an attempt to assess the role of the various carbohydrate pathways in the over-all metabolism of the leukocyte. The authors found that very small amounts of the three-carbon units were incorporated directly into the glucose of glycogen and suggested that this incorporation occurred via a reversal of the pyruvic kinase reaction. The labeling of the six carbons of the glucose unit of glycogen was found to be highly asymmetric with most of the C14 activity incorporated into C-4, -5, and -6 of the hexose unit of glycogen. The transaldolase exchange reaction was suggested as a possible explanation for the observed asymmetric labeling.—T. E. B.


Tissue cultures made from the buffy coat of rabbit blood were found to be sometimes capable of proliferating as fibrocytes and of forming moderate amounts of hydroxyproline, an amino acid found in quantity only in connective tissue fibers. The authors felt they had excluded the possibility of contamination of their cultures by cells not in the circulating blood and concluded that mononuclear cells of the blood are capable of transforming into functional fibrocytes.—T. E. B.


Two bacterial pyrogens studied were found to increase the in vitro rate of glycolysis and of oxygen consumption of leukemic leukocytes, but to have no significant effect on nucleic acid synthesis by the leukocytes. Because of the ubiquity of bacterial pyrogens the possibility of metabolic effects produced by them must be considered in any experiment involving separated leukocytes.—T. E. B.


Single doses of prolactin increased the relative level of the neutrophiles and eosinophiles and decreased that of lymphocytes.—E. K.

INVESTIGATIONS ON GRANULopoIESIS IN CASES OF LEUKOCYTOSIS. K. Scheinberg. From the Medical Academy, Zährze, Poland. Arch.Immun i Terap. Doświad. 8:117, 1960.

In a study of 9 patients, the following types of leukocyte response were observed: 1. leukopenic (average results in a group of three healthy men after an injection of 2 ml. of Delbet’s vaccine intramuscularly). 2. Prolonged and distinct leukocytosis (average results computed from a group of 4 patients with purulent disease). 3. Leukemoid reaction (one patient with a long-lasting purulent pleuroporcariditis). One case of chronic myeloid leukemia was investigated by the same methods, assuming that chronic myeloid leukemia might be considered as an extreme phase of the process. Absolute numbers of all kinds of granulocytic cells were calculated in the peripheral blood and bone marrow. Mitotic indices and mitotic coefficients after incubation with demecolcin were then calculated for each kind of dividing cell, enabling the author to calculate by his original method the duration time of mitosis and interphase. Thus one can evaluate qualitatively mitotic activity (numbers of generation in 48 hours), mitotic coefficient (number of cells entering division), and the speed of maturation for every dividing cell. The results for every phase were compared.

The data showed that in an early stage of leukocytosis the mitotic coefficient of older cells was increased and the behaviour of the younger cells was rather passive. Acceleration of matura-
tion was a further mode of reaction, and increase of mitotic activity, especially in young cells was a late form of bone marrow response. In cases, where pathological stimulation continued, mitotic activity was arrested in myeloblasts and promyelocytes, maturation also stopped, and only the myelocytes exhibited their enormously increased mitotic activity for compensatory purposes. Failure of that last defense-mechanism led directly to agranulocytosis. The stab-cell may normally be considered a "brother" and not "mother" cell in relation to polymorphonuclears. In pathological conditions, however, the stab-cell may be considered as a mother-cell of polymorphonuclears in conditions of an exceedingly stimulated granulopoietic state of the bone-marrow. Chronic myeloid leukaemia is not a final phase of the disease.-E. K.


Rats receiving lethal x-ray dosage, when treated with zymosan, show considerably accelerated regeneration after the critical fall in leucocyte count. The authors conclude that zymosan treatment may be effective in other diseases with leucopenia.—S. R. H.


The pattern of leucocyte exudation in patients with ulcerative colitis was studied by the skin window technic. Thirteen of 19 patients with ulcerative colitis showed an abnormal pattern consisting of an excessive number of basophilic leukocytes in the exudate.—T. E. B.


According to mortality statistics based on death certificates, 25 deaths from apparently drug-induced agranulocytosis occurred in Denmark from 1951-1957. Two cases of agranulocytosis due to urethane and x-rays respectively were included in this figure. The incidence of drug-induced lethal agranulocytosis in Denmark thus was 0.7 per million per year.—S. A. K.

HEMOSTASIS


The peak of thrombin generation in whole blood was delayed by 4-6 minutes in 10 adults following the ingestion of 1 Gm. magnesium with 50 Gm. peptone. The amount of thrombin formed was the same as in the controls. The effect lasted for up to 6 hours, and for 3 hours when 15 Gm. of glutamic acid was given instead of peptone. One-stage prothrombin time and thromboplastin generation were unaffected. Higher dosage of magnesium did not increase the effect, nor was there any evidence of a cumulative effect of repeated doses. A child aged 3 years with sickle cell anemia and frequently repeated crises remained free of symptoms for 4 weeks while receiving 1 Gm. magnesium per day; it is suggested that this effect may have been partly due to a reduced coagulability of the blood. Two patients with ischemic heart disease were also given magnesium with a resulting slight increase in alkali reserve. No coagulation studies on these patients are reported.—R. M. H.


Surprisingly close resemblance between the carp (cyprinus carpio L.) clotting system and the clotting system in mammals was found.—E. K.


This article contains an interesting and thought-provoking discussion of the authors' view that thrombin is the activator of prothrombin. New data are also presented. (1) Protamine sulfate (0.1 per cent), like 25 per cent sodium citrate,
depresses esterase activity of biothrombin but not its ability to clot fibrinogen. It also effectively converts prothrombin to thrombin. (2) When prothrombin is added to thrombin, and TAME added at once, TAME hydrolysis is delayed for a few minutes and then proceeds at the same rate as in the absence of prothrombin. However, if prothrombin and thrombin remain together for 30 seconds before addition of TAME, the lag is shorter and hydrolysis then proceeds much more rapidly. These experiments are interpreted as evidence for the presence of a prothrombin-thrombin complex with formation of additional thrombin. (3) Prothrombin free of Ac-globulin plus platelet factor 3 and calcium generates only a small amount of thrombin, the remaining prothrombin becoming inactive. Addition of a small amount of purified thrombin starts rapid generation of thrombin. Thrombin which has lost its clotting activity but retained its esterase activity cannot do this, nor can it convert plasma Ac-globulin to serum Ac-globulin.—M. B. Z.


Solutions of purified lyophilized bovine prothrombin containing 5150μ/ml in M/15 phosphate buffer, pH 6.0 or 7.2 were kept at 4°C. Prothrombin activity, measured by bioactivation, increased to about 7000 μ/ml between the third and eighth days, dropped sharply to about 1000 μ/ml until the thirteenth day, then returned abruptly to the original level. Thrombin only appeared later; traces were evident on the twenty-eighth day and amounts up to 1059 μ/ml a month later. Similar changes occurred with prothrombin which had not been lyophilized when it was dissolved in buffer, neutral distilled water or 0.9 per cent NaCl. Addition of thrombin had previously been shown to produce similar effects in about two days.—M. R. Z.


Using a three-stage reaction system, it was found that Ac-G. was necessary to obtain prothrombin conversion in the presence of serum-eluate, AHF, platelet factor 3 and calcium. Serum Ac-G., or plasma Ac-G. plus a trace of thrombin caused a faster rate of prothrombin activation than plasma Ac-G. Serum Ac-G. was as effective when added at stage 2 (i.e., with the prothrombin) as it was when added to the stage 1 reagents.—M. B. Z.


As little as 1.5 μg. ml protamine sulfate prolonged partial thromboplastin time. Concentrations between 2 and 7.5 μg./ml. decreased rate of blood thromboplastin formation and higher concentrations also affected yield. Addition of 50 μg. ml. protamine to fully formed blood thromboplastin had virtually no effect. When protamine was added to substrate plasma, over 25 μg./ml. was necessary to prolong clotting time: when blood thromboplastin was added. However, when tissue thromboplastin was used (prothrombin time), prolongation was noted with as little as 2 μg. ml. No greater effect was observed after preincubation of protamine with either plasma or tissue thromboplastin. Concentrations between 15 and 63 μg./ml. shortened thrombin-fibrinogen time; and 125 μg. ml. precipitated fibrinogen. —M. B. Z.


Generation of thromboplastin was much decreased in the presence of 50 μg. protamine/0.8 ml. mixture. Addition of protamine after maximal thromboplastic activity was generated had much less effect, and as much as 25 μg. could be added to each substrate tube without any effect. Increasing the concentration of plasma was ineffective but increasing the concentration of serum almost completely prevented the inhibitory effect of protamine. Serum from patients with severe PTC deficiency could not prevent inhibition by protamine, and the effectiveness of serum from patients with milder degrees of PTC deficiency varied in proportion to severity of defect. Combining 50 μg. of protamine with 25-33 μg. of heparin before adding it to the thromboplastin generating mixture prevented its inhibitory effect. When protamine was added at time of recalcification, and heparin 3 minutes later, or when

Plasma decalcified with oxalate or a cation-exchange resin (Dowex 50) after adsorption by 2.5 per cent bentonite (hydrated aluminum silicate) retains only prothrombin from among the various coagulation factors (plus traces of Hageman factor and PTA). It has lost its fibrinogen and factors V, VII, VIII, IX and X. The prothrombin of such plasma is electively adsorbed by a 5 per cent solution of Baker tricalcium phosphate and the citrated eluate can be concentrated by precipitation of the prothrombin at the isoelectric point (pH 5.2) or by alcoholic precipitation (25 per cent ethanol). The preparations of prothrombin thus obtained have an average activity of 600 units per milligram of proteins. Their coagulating and electrophoretic properties are evidence of the high purity of these preparations which are stable in the lyophilized form.—G. M.


Hageman factor is primarily activated by surfaces; activated Hageman factor reacts with PTA forming a third prothromboplastic factor. In the absence of the Hageman factor PTA is adsorbed but not activated by surfaces. Activated Hageman factor causes pseudo-activation of PTC and pro-convertin. Activation of Hageman factor accelerates fibrinolysis. Thrombin is strikingly adsorbed by silica containing surfaces which activate Hageman factor.—G. M.


Antihemophilic globulin activity was found to be high in blood collected in siliconized bottles and stored for less than 24 hours. Proaccelerin levels were sometimes found to be even higher than in the control samples. This was probably due to contamination with traces of accelerin.

Bank blood collected in glass bottles and stored for 5-7 days showed a mean AHG activity of 55 per cent, but individual bottles had activity as low as 30 per cent. Blood stored for 12-14 days had a mean activity of AHG of 35 per cent. Proaccelerin activity was 75 per cent up to two weeks of storage.—W. J. M.


The study is based on 292 cases of hemophilia, 109 of whom were examined by the author personally. The number of living hemophiliacs in Finland is estimated at 1:13,000-14,000 males, and the male livebirth ratio of hemophiliacs at 1:8,500. The severity of the coagulation defect was assessed by determination of AHG, factor IX, prothrombin consumption index, recalcification time of platelet-poor plasma, and coagulation time. Twenty-five per cent of the examined patients had hemophilia B. Among 89 cases of hemophilia A, 67 were classified as severe, 11 as fairly severe, and 11 as mild. The corresponding figures in hemophilia B were 9, 0, and 20, respectively. The clinical features were well correlated with the laboratory classification. A circulating anticoagulant was found in one patient. One family included a female bleeder with symptoms and laboratory signs of hemophilia A. Data on the localization of bleeding and causes of death are given. Roughly, the life expectancy of the series as a whole was 17 years although lately the prognosis has probably improved. The effective fertility in hemophiliacs was low, 0.236 for A-hemophiliacs and 0.776 for B-hemophiliacs. The mutation rate per chromosome per generation was estimated at 3.2 × 10⁻⁵ for the A-hemophilic gene and at 0.2 × 10⁻⁵ for the B-hemophilic gene. Pedigrees of the families studied are included.—S. A. K.


Assays for anti-hemophilic globulin (AHG) were carried out on fresh plasma, frozen fresh plasma, freeze-dried fresh plasma and freeze-dried Cohn's fraction I. Frozen fresh plasma was found to retain about 80 per cent of the original AHG
activity after storage at −40 C. for 2 months and approximately 70 per cent activity was present in freeze-dried fresh plasma, provided the freezing process was modified by the reduction of freezing to a minimum. In contrast freeze-dried Cohn’s fraction 1 exhibited only an insignificant amount of AHG activity. Both frozen fresh plasma and freeze-dried fresh plasma were shown to be effective in raising the levels of AHG in hemophilic subjects. In addition frozen fresh plasma was used successfully in four subjects submitted to major surgery. It was given in an initial dosage of 30 ml./Kg./day for at least three days followed by smaller amounts until about the eighth postoperative day.—T. H. B.


The fourth reported case of coexistent hemophilia and parahemophilia is described. Hemophilic platelets are deficient in antihemophilic activity, parahemophilic platelets are deficient in proaccelerin (V) activity, and either defect is abolished by incubating the platelets with normal plasma. The defective thromboplastin generation test in parahemophilia is accentuated if deficient platelets and substrate plasma are used.—M. B. Z.


Chemical characterization was performed on a nontoxic anticoagulant prepared from the glycerophosphatide fraction of brain. The active material is probably an unsaturated phosphatidylserine. —M. B. Z.


A patient with low factor V (prothrombin time about 50 sec.) and normal prothrombin consumption time had no platelet factor I activity unless platelets were incubated in normal plasma. A second patient with a lifelong moderately severe history of bleeding had a long bleeding time, 2–3+ tourniquet test and clot retraction, normal clotting and prothrombin times and abnormal prothrombin consumption time. Plasma fibrinogen was 304 mg. per cent but fibrinogen determination on clotted supernatant of frozen and thawed platelets (6,000,000/ cu.mm.) was 33 mg. per cent compared to a control of 107 mg. per cent.—M. B. Z.


Platelets were preserved in a medium composed of: 10 ml. of original plasma, 15 ml. of 0.85 per cent NaCl, 1 Gm. of glucose and 1 ml. of 5 per cent disodium salt of EDTA, at +4 C. and at −20 C. Their morphological and functional states were assessed by enumeration, electron microscopy, determination of clot retraction ability, serotonin content and thromboplastin formation. After 20 days at +4 C. the number of platelets was down to 50 per cent as was also the thromboplastic function (though the latter was nearly normal after 10 days); clot retraction ability was absent after 8 days; serotonin level of platelets declined slowly and regularly. After 2 months at −20 C. the thromboplastic function was almost 100 per cent though platelet count was down to 25 per cent as was also serotonin. It was interesting that the clot retraction ability disappeared in 2 days. The electron microscope showed slight granularity of the chromomere after 10 days but even after a month general changes were not marked at either temperature. The stored platelets were transfused into two groups of patients: with hypoplastic and aplastic anemia and with i.d.opathic thrombocytopenic purpura. Both groups suffered from hemorrhage and a profoundly disturbed hemostasis. After several platelet transfusions spread over several days bleeding stopped in nearly all cases and the hemostatic picture returned almost to normal. However, neither platelet count nor clot retraction were improved. Neither group showed any change in bone marrow. The studies show that platelets stored in the medium described preserve their morphology and thromboplastic function for at least a month and they can be used effectively clinically. The clot retraction ability is lost early during storage but is not essential for the preservation of normal hemostasis.—J. J. B.

THE NONPROTEIN PRODUCTS OF PLASMA COAGULATION. S. I. Cohen. From the Department of

Amino acids and peptides were determined by paper chromatography before and after recalcification and clotting of human platelet-rich resin plasma. Significant increases in free arginine and glutamic acid were observed after clotting. In one of the three donors, aspartic acid also increased. Other amino acids remained unchanged. Although reaction between purified human fibrinogen and thrombin resulted in appearance of two peptides corresponding in position to fibrinopeptides A and B described by Bettelheim, human blood serum revealed only one peptide in the position of fibrinopeptide B.—M. B. Z.


Fifteen ml. of plasma or its derivatives were separated into 32 fractions using barbital buffer pH 8.6, ionic strength 0.02 and a special cooling technic. Good recovery of clotting factors was usually observed. Fibrinogen, profibrinolysin, Hageman factor, PTA and "glass factor" appeared in the α globulin area. The latter three had similar distributions. Proacceleton appeared in the albumin area. Prothrombin, proconvertin, PTC, AHF, antifibrinolysin, antithrombin and heparin cofactor spread over the β, α2, α1 and early albumin areas. The latter two activities had similar but not identical distribution. There was no evidence of separation of proactivator and profibrinolysin.—M. B. Z.


Plasma fibrinogen and fibrinolytic activity were determined in pregnant and nonpregnant African women. Plasma-fibrinogen levels rose significantly during the early months of pregnancy and remained elevated into the puerperium. Plasma fibrinolytic activity decreased during the early months of pregnancy and remained low till the second stage of labor. There was a significant rise to control levels within 24 hours post partum. It is suggested that endocrine changes during pregnancy may be responsible for these effects.—R. M. H.


Twelve patients were given a diet for one week containing 44 per cent of calories as animal fat. For the next 4 weeks an isocaloric amount of soybean oil was substituted for the animal fat. The soybean oil diet was accompanied by a decrease in serum cholesterol, phospholipid, and total lipid levels. The maximal decrease was observed in total cholesterol level. There was no change in iodine number, in coagulability or fibrinolysis under the influence of this diet.—E. K.


Acute hemorrhagic shock was induced in dogs. Coagulation factors were estimated before and during evipan anesthesia before and during severe irreversible shock. Two periods were distinguished: With the onset of shock only minimal clotting changes were observed; in the later stages there were decreased prothrombin, fibrinogen, factors V and VII; increased sensitivity to heparin in vitro; and no acceleration of the euglobulin fibrinolysis time on activation of the plasma antithrombin.—E. K.


Plasma fibrinolytic activity was measured by two methods in a series of normal males. In all, there was a spontaneous increase in activity over a period of 3 hours during the morning, with a return to a low titer by the following day. The morning increase was greater in 17 men aged 20–24 than in 10 men aged 39–50, and the average level of fibrinolytic activity was higher in the younger men.—R. M. H.