ABSTRACTS OF SPECIAL INTEREST


Although the phenomenon of erythrophagocytosis has been recognized and investigated for almost a century, the sequential steps in the transport of erythrocytes or of their fragments across the surface membrane of the macrophage are not well known, nor is it clear what events occur in the intracellular digestion of this material. Erythrophagocytosis as it occurs in the hemorrhagic fluid within the peritoneal cavity of rats bearing the Novikoff actes hepatoma has been investigated by electron microscopy. The white cell population of reaction cells consists primarily of macrophages whose pronounced phagocytic activity is probably responsible for the marked elimination of erythrocytes which generally occurs. The erythrocytes show a characteristic fragmentation which involves the formation of relatively short projections or buds on their surfaces. They accumulate selectively at the surface of the macrophages where they adhere to the plasma membrane. There is an increased opacity to electrons of the membranes in the immediate area of the erythrocyte fragments where such fragments make contact with the surface of the phagocyte. This may represent an alteration in the physico-chemical properties of the surface layer. The underlying physico-chemical alteration is probably analogous to that which appears to occur in amoebae where the first step in pinocytosis may involve protein binding and an alteration of the physical state of the plasmalemma. Erythrocytes or fragments induce pseudopod formation leading to phagosome formation. There was no indication of any connection between phagosomes or their contents and any other cell organelles. They were intensely stained in the acid phosphatase procedure.—O. P. I.


The RH₀ (D) antigen content of 199 RH₀ (D) positive, Rh⁻ (C) negative blood donors was measured by ¹³¹I uptake from radioactive iodinated RH antibody. A bimodal distribution was found and family studies revealed the lower antigen content to represent heterozygotes. Homozygotes appeared to have 1.6 times as much antigen.—R. E. R.

SEROLOGICAL DIFFERENCE BETWEEN OLD AND YOUNG CELLS. F. Stratton, P. H. Renton and
A non-blood-group-specific antibody was found in the serum of a woman with anemia secondary to Hodgkin's disease. Further testing showed that the antibody was most active against cells suspended in serum albumin at 37°C, which were agglutinated within 2 minutes. Stored cells were found to be more strongly agglutinated than fresh ones, and the strength of agglutination increased with the period of storage of the cells. Cells aged in vitro, obtained by differential centrifugation of fresh blood, were agglutinated more strongly than younger cells from the same sample. The authors describe some other properties of the antibody, and suggest that the red-cell surface difference between young and old cells revealed by this serum may play a part in the normal process by which effete cells are removed from the circulation. They suggest that the feeble nature of the agglutination of the patient's own fresh cells by the serum may have been due to an increased rate of elimination of aged cells from her circulation.—R. M. H.

**DERMAL LOSS OF IRON IN HEALTHY INDIAN MEN.**


The average value for iron in insensible perspiration from an average area of 769 sq. cm. during an 8 hour period was found to be 2.1 μg. Based on this calculation, the loss of iron through insensible perspiration would average about 0.13 mg. per day. The average iron content of "cell rich" and "cell free" sweat was found to be 1.15 ± 0.52 mg. and 0.34 ± 0.16 mg. per liter respectively. It was concluded that in tropical countries under conditions of profuse sweating associated with desquamation of epithelial cells, significant amounts of iron could be lost from the body.—J. B. C.

**ANTIFIBRINOLYTIC ACTIVITY AND METABOLISM OF Σ-AMINOCAPROIC ACID IN MAN.** Inga Marie Nilsson, A. Sjoerdma and J. Waldenström. From the Department of Medicine, University of Lund, Sweden. Lancet 1:1322-1328, 1960.

Σ-aminocaproic acid (EACA) was given to two patients with clinically significant fibrinolysis, one patient with an increased plasma level of plasminogen activator but no symptoms of bleeding, and four subjects with no abnormality of coagulation. The pathological fibrinolyis was temporarily abolished in the first two patients, with good clinical effect; in the third patient, the plasma level of activator was much reduced. Metabolic studies in the other four subjects showed that absorption of EACA from the intestinal tract was rapid and essentially complete, and that EACA was rapidly excreted in the urine, about half being recovered in the urine within 12 hours. The plasma half-life appeared to be of the order of 1-2 hours; doses of 3-6 Gm. were needed every 4-6 hours in order to maintain an effective blood level.—R. M. H.

**HEMOSTASIS**

**HEMATOLOGIC AND COAGULATION STUDIES IN VARIOUS ANIMAL SPECIES.** P. Didelheim, K. Hattori and J. Lewis. From the University of Pittsburgh School of Medicine, Pittsburgh, Pa. J.Lab.& Clin.Med. 53:866, 1959.

This paper is of particular interest because it is a rather comprehensive study on the coagulation mechanisms of 8 mammal (human, monkey, cow, sheep, dog, cat, rabbit, raccoon, and opossum) and 2 avian (chicken and duck) species, and includes a study of species specificity of tissue thromboplastin and the thrombin fibrinogen reaction. Fowl showed large thrombocytes instead of platelets which were nucleated cells and similar in appearance to lymphocytes. Brain tissue thromboplastin showed a definite species specificity only in the opossum, chicken and duck. However, there was considerable variation in the prothrombin time of various animal plasmas with different thromboplastins. For example, human brain thromboplastin clotted human plasma in 13.8 seconds, bovine in 22.5 seconds and rabbit in 13.8; whereas bovine brain clotted human plasma in 43 seconds, bovine in 19 seconds and rabbit in 25.2. There was a similar, although much less marked, variation in the thrombin fibrinogen reaction when species were mixed, with the exception that opossum thrombin clotted only opossum fibrinogen although opossum fibrinogen was clotted slowly by all thrombins and chicken fibrinogen could be clotted only by chicken thrombin although the latter clotted all fibrinogens. It is obvious that when coagulation tests are dependent upon a tissue thromboplastin and thrombin fibrinogen reaction (prothrombin time, one stage procedures for prothrombin, proaccelerin, factors VII and X) and the usual reagents are used comparative studies on various species can be misleading. This paper emphasizes the importance of using homologous systems in study.
of blood coagulation mechanisms. By the usual one stage technics, low levels of prothrombin and proconvertin (VII and X) were found in opossum, chicken, duck, sheep and cow. Employing homologous systems the prothrombin-proconvertin concentration among the mammalian group with the exception of the opossum did not vary greatly. The chicken showed very low levels. Other findings were (1) negligible difference between clotting time in glass and silicone tubes in chicken and duck; (2) negligible clot retraction in fowl blood; (3) when tested by their corrective effect on human plasmas with a deficiency of AHF, PTC, or Hageman factor, cow and sheep plasmas exhibited very high levels of AHF, whereas chicken and duck plasma appeared to be totally deficient in this factor; (5) Hageman factor levels were fairly equal in all the mammals tested, but there appeared to be no Hageman factor activity in duck or chicken plasma. These latter plasmas also exhibited no “glass factor activity.” PTA was not tested for. (6) Thromboplastin generation of chicken and duck, even when using homologous substrate, was negligible.—R. G.


Preparations of proconvertin rich fractions were prepared from normal human citrated (ACD) plasma by aluminum hydroxide adsorption, elution with phosphate buffer, and precipitation of the protein in the eluate at pH 5.6 with 40 per cent ethanol at -5 C. The final preparation when compared to normal plasma contained 35 to 88 times the proconvertin cativity, 6 to 30 times the prothrombin plus Stuart activity, 3.6 to 8.6 times the Stuart activity and negligible PTC activity. Such preparations were administered intravenously to human patients, and the rise in and the rate of fall of proconvertin activity in the recipients’ plasma determined. It was found that in individuals who were deficient in proconvertin irrespective of the cause, disappearance curves with 2 exponential components were found, whereas in a normal subject a single exponential component having a half-life of 230 minutes was found. In mildy deficient and one moderately deficient congenital hypoproconvertinemic patients, the first component had a half-time of 18 to 30 minutes, and the second of 210 to 240 minutes. In 4 patients with severe congenital deficiency, one patient with severe liver disease and one normal subject receiving oral Warfarin, the first component had a half-time of 11 to 17 minutes and the second 85 to 120 minutes. Intravenous administration of such preparations to 2 patients with congenital hypoproconvertinemia resulted in prompt cessation of bleeding.—R. G.

THE ACETYLATION OF THROMBIN. R. H. Landa

Purified thrombin which had high clotting activity also exhibited high esterase activity. A method is described for the acetylation of thrombin which resulted in loss of the clotting action of the thrombin; but the esterase activity, as measured by the hydrolysis of TAME, was doubled. This product, which does not activate fibrinogen, does lyse fibrin. Evidently amino groups are not necessary for the esterase or lytic activity. It is suggested that thrombin with clotting activity (thrombin C) is a dimer of the basic structure of the esterase and lytic active material (thrombin E).—R. G.


With the use of the rabbit assay as a quantitative measure of the serum thrombotic accelerator (STA) activity of human serum, the STA activity of normal serum, at a dose of 1.32 ml. of serum per Kg. of rabbit, was compared with that of sera obtained from patients with the following known heredofamilial coagulation defects: factor V, factor VIII, factor XIII, factor IX, factor X, Hageman factor and PTA. Sera from patients with factor IX or Hageman factor deficiency were essentially devoid of STA activity, and sera from patients with PTA deficiency appeared to have significantly less STA activity than normal. The sera obtained from patients deficient in factors V, VII, VIII or X contained normal STA activity.—R. G.


Thromboplastic and fibrinolytic activities of the 3 layers of the aortic wall were estimated in vari-
ABSTRACTS

ous animal species. The results varied widely between species; the rabbit aorta was found to be low in fibrinolytic activity but had higher thromboplastic activity in both intima and media than any other species tested except man. It is suggested that these findings partly explain the difficulty in producing lesions resembling human atherosclerosis in experimental animals.—R. M. H.


The first author is a hemophiliac who has repeatedly observed symptomatic improvement of his bleeding state following the ingestion of peanuts, peanut flour, or a concentrate prepared from peanut flour. Relief of symptoms, which has also been obtained in 3 other hemophiliacs, was not correlated with shortening of the clotting time of either capillary or venous blood. No other coagulation tests were carried out, but it is stated that tests on hamsters suggest that vasoconstriction is enhanced by ingestion of the concentrate.

—R. M. H.


Ten ml of a 1 per cent solution of protamine sulfate were active in hemoptysis, which ceased immediately after injection. Protamine had no preventive action, but in repeated hemoptyses repeated injections were effective. The mode of action seems to be activation of the clotting system within the lungs.—H. M.


The concentration and the formation of taurine in human platelets has been followed. It has been found that the level of free taurine in platelets is rather stable and amounts to about \(7.8 \times 10^{-8}\) mg. per platelet. It is possible that taurine may be formed in platelets and that cysteine is the precursor. Cysteinosulphonic acid is probably an intermediary in the process.—E. K.


The phospholipids of human platelets and red cells were isolated and identified utilizing refined chromatographic technics (silicic acid column chromatography and silicic acid-impregnated paper chromatography). The phospholipids of platelets and red cells were found to be very similar and in each case were just less than 70 per cent of the total lipid. The identified phospholipids were phosphatidyl ethanolamine, phosphatidyl serine, lecithin, sphingomyelin and inositol phosphatide. The red cell lipids contained significantly more phosphatidyl serine and less lecithin than the platelet lipids. It was calculated that there was 1.25 mg. of platelet lipid and 26 mg. of red cell lipid in 10 ml. of whole blood. The coagulation activity of the various phospholipids was assayed in vitro by measuring the effect of the lipid on prothrombin conversion of platelet poor plasma and on thromboplastin generation. Within the limits of resolution of the chromatographic system only phosphatidyl serine exhibited coagulant activity in these 2 systems. Its activity was enhanced by the presence of lecithin so that the coagulant activity of complete platelet lipid extract was reproduced by employing only the amounts of phosphatidyl serine and lecithin contained in the whole lipid extract. Phosphatidyl ethanolamine, inactive alone, showed some activity when combined with lecithin, and a lesser activity when combined with sphingomyelin. Saturation of the double bond with hydrogen, hydroxylation and epoxylation of the fatty acid double bonds, and acylation of the free amino groups of the activity fractions resulted in loss of their coagulant activity. Phosphatidyl serine could not be identified by any method in carefully prepared red cell and platelet free plasma.—R. G.


The granulomere and the hyalomere of platelets were isolated, and the fractions were tested for their clotting activity in vitro; sections were investigated by electron microscopy. Platelet factors 1 and 3 are localized in the granulomere; platelet factors 2 and 4 are found in the hyalomere.—H. M.

Studies were made on four patients with thrombasthenia. The bleeding times were always prolonged. The platelets were normal in number or slightly increased, and they showed anisocytosis and an increased diameter with some giant forms. By electron microscopy the platelets showed increased numbers of vacuoles and blurred structures in the mitochondria. There was impaired agglutination and retraction. By thombelastography there was a diminution of me and a prolongation of k. The content and liberation of platelet factor 3 was normal as was the heparin tolerance test. Twenty enzymes of carbohydrate metabolism were tested. Glyceraldehydephosphate-dehydrogenase and pyruvate kinase were reduced to 20 to 35 per cent of normal. Glucose consumption and the formation of lactic acid were reduced, and ATP was found to be very low. The in vitro addition of Mg++ and ATP produced normal retraction. It appears that "irretractility" in thrombasthenia is caused by an enzyme defect of the platelets. In addition, marked augmentation of monoaminodicarbonic acids and a diminution of taurine were found.—H. M.


Pulmonary megakaryocytes in the vascular bed were found in 101 of 103 premature and term infants, and in 17 of 21 fetuses. The average number of megakaryocytes was 2 to 3 per square centimeter of tissue. The youngest fetus in which a megakaryocyte was seen in a lung blood vessel was approximately 2½ months old. These findings are not surprising in view of the fact that anatomiists, including histologists and embryologists, have known for many years that these cells appear quite early in human embryonic development and may be present in scattered areas throughout the organism. The present authors suggest that hypercoagulability of the blood may be produced following stressful stimuli with the increased action of the right ventricle acting on the megakaryocytes and thereby increasing the number of platelets.—O. P. J.

ABSTRACTS


The authors described in their previous papers the appearance of desoxyribonucleic acids in the cytoplasm of megakaryocytes and blood platelets in numerous cases of idiopathic thrombocytopenia and in pancytopenia of undetermined pathogenesis. This phenomenon is not observed in healthy persons or in symptomatic thrombocytopenias. In the present study it was reported that in five cases of "spontaneous" thrombocytopenia and in five patients with pancytopenia showing a positive Feulgen reaction in the blood platelets, nuclear material progressively disappeared following splenectomy.—E. K.


The serum of the healthy mother of a child with neonatal thrombocytopenic purpura was shown, by means of the mixed antiglobulin reaction, to contain an antibody active against the platelets of the father and those of 10 normal subjects, but not against the mother’s own platelets. Leukocyte antibodies were demonstrated by means of the same technic, but the serum contained no demonstrable antibodies against the father's red cells.—R. M. H.


Using the lysis of bovine fibrin clots with added human plasminogen as an indication of activator activity and the hydrolysis of casein to demonstrate plasmin activity, the authors have re-investigated clot lysis by cells in tissue culture. Their results showed that the supernatant of some cell cultures released an "activator" of plasminogen.
ABSTRACTS

The supernatant both activated plasminogen and was also proteolytic in the absence of plasminogen with others; neither activator nor proteolytic activity was present in the rest. Since some of the tissue cultures tested were those derived from monkey kidney, 83 lots of poliomyelitis vaccine were tested and about one-half were found to contain varying amounts of activator and/or proteolytic activity. The authors do not believe the amounts of proteolytic activity present are clinically significant because of the abundance of inhibitors in vivo, but they believe that the production of these enzymes may be helpful for the further identification and classification of the cultures. The activator described above resembled tissue activator since it was not proteolytic in the absence of serum and could be dissolved with KSCN. Differential centrifugation showed that the activator was in the microsome fraction, and repeated decanting of the cell supernatant from growing cultures suggested that the release of activator was a metabolic process, since a very small fraction of the total activity obtained could be found in the supernatant of extracted cells. An inhibitor of proteolysis was also found in some of the culture supernatants.—A. I.


Since human plasminogen is the plasma precursor of both activator and plasmin, when activated by the bacterial product streptokinase, and because proactivator does not seem to be present in the plasminogen of some animal species, purification studies of this plasma protein assume special practical and fundamental importance. The authors of these fine papers purified plasminogen (prepared by the Kline procedure) 3 to 4 times, with a resulting product which is virtually homogeneous by various physical techniques, including determination of sedimentation constant, diffusion constant, partial specific volume, intrinsic viscosity and electrophoretic mobility. The calculated molecular weight of this material was 83,000 with an axial ratio of 9 to 16 for an unhydrated prolate ellipsoid and 16 to 22 for an unhydrated oblate ellipsoid. The highly purified material had the same ratio of proactivator to plasminogen that is found in plasma, suggesting that both activities reside in the same molecule. In addition, during the activation of plasminogen by trypsin a decrease in proactivator activity was shown to occur as the plasmin increased. These data fail to support the contention that plasmin accounts for proactivator activity, but taken with the evidence mentioned above, does favor the one-molecule dual-function concept. Preliminary purification of the plasminogen was performed by the Kline technic; this was followed by 3 stages of column chromatography on CM cellulose resin, 2 with decreasing pH in formate buffer, and one with increasing pH in a formate-citrate buffer, with $10^{-1}$ M lysine added. All fractions contained small amounts of contaminating plasmin activity, and the purest fractions contained the least amount of plasmin.

—A. I.


Purified bovine thrombin which had both clotting and esterase activity when acetylated was practically devoid of clotting activity but maintained or even increased its esterase activity. This material (Thrombin-E) was also found to be fibrinolytic. This material was administered intravenously to dogs without causing any intravascular coagulation. The white count and platelet count dropped, and there was an increase in the blood sugar level. These alterations disappeared within 24 hours. Evidence of increased fibrinolysis was also found. No actual data on the fibrinolytic activity or other changes produced are presented in this paper.—R. G.


The authors have made a courageous attempt to determine the effect of a plasmin preparation, Actase (containing both SK and SK-plasmin) on 32 patients with deep thrombophlebitis. The control group was composed of an additional 30 patients. The experimental group was selected by the alternate case study method. The general conclusion, that the short-term response to this
therapy was favorable, was supported by an evaluation which was primarily clinical in its orientation, and which included: pain and tenderness, edema, abnormality in leg size, and duration of: bed rest, anticoagulant therapy and hospital stay. No mention was made of an attempt to determine the amount or kind of proteolytic activity induced in vivo, except to state that the dosage was based upon criteria which showed "detectable plasma fibrinolytic activity in 90 per cent or more of patients." Generally, a single 3 hour infusion was given. Since previous investigators have shown that "older" thrombi are more resistant to clot lysis, patients with deep thrombophlebitis of more than 10 days were excluded from the series. The number of episodes of pulmonary embolism was also determined for each group (4 in the controls and 1 in the experimental). In evaluating the high incidence of pulmonary embolism, the authors disclose the startling fact that 28 per cent of their patients had suffered an embolus preceding the institution of therapy. In conclusion, it would seem that clinical investigations of this problem are exceedingly difficult to evaluate; that the clinical variation implicit in a comparatively small series of patients, the anti-inflammatory effect of the enzyme preparations, the statistical problems attendant upon the alternate case study method, and the variation in amount and kind of proteolytic activity induced, are just a few of the variables which would discourage all but the most hardy.—A. J.


A patient is reported with unilateral renal hemorrhage and metastatic carcinoma of the prostate, associated with hypofibrinogenemia and fibrinolysis. Ten injections of phosphorylated stilbene (Honvan) in doses of 250 mg. daily i.v. suppressed fibrinolysis. Attention is called in the discussion to the possibility of unilateral hematuria as the only clinical manifestation of a hemorrhagic diathesis.—L. D.

ERYTHROCYTES


The effect of the pituitary gland on the formation of an erythropoietic principle by the kidney ("kidney factor") was investigated, using reticulocytosis as evidence of increased erythropoiesis. Hypophysectomized rats showed a much poorer reticulocytosis after bleeding than did normal rats; the degree of reticulocytosis was increased by ACTH administration. Serum from hypophysectomized, bled rats produced a reticulocytosis in normal mice, but not in nephritic mice. Reticulocytosis could be produced in nephritic mice by injecting serum from adrenalectomized mice, or from mice previously treated with ACTH. Cortisone-treated mice, on the other hand, showed a diminished degree of reticulocytosis after bleeding, and their serum produced a reticulocytosis in normal mice, but not in nephritic mice. The author concludes that "kidney factor," as distinct from erythropoietin, is produced in the intact kidney following stimulation by ACTH by a direct extra-adrenal mechanism.—R. M. H.


To answer the question as to whether increased liberation of reticulocytes is caused by a decrease in blood pressure or by anemic anoxia, dogs were subjected to a series of bleedings unaccompanied by a decrease in blood pressure. It was demonstrated that in such cases the "bone marrow barrier" functions normally. To answer the question as to whether the fall in blood pressure acts directly upon the receptors of the blood-forming system, upon other receptors or directly upon the central nervous system, a series of experiments was undertaken in which venous and arterial circulations of pairs of dogs were crossed. On the basis of these experiments, it is concluded that reflex reticulocyte liberation following acute 40 per cent blood loss originates in receptors of the cranial blood vessels.—E. K.


The present study explores the sodium and potassium exchanges, and glucose usage, in rabbit cells prior to hemolysis after various treatments with rose bengal. This dye photosensitizes red blood cells so that they are hemolyzed when exposed to light (photodynamic action). It appears likely that the acceleration of cation exchange...
with photosensitization is the result of cell surface alteration rather than to an effect on cell metabolism.—O. P. J.


The authors investigated the antihemolytic activity of serum against hemolysis by soaps of higher fatty acids. They showed that serum contains two different components with an inhibitory effect, one of which is cholesterol α-lipoprotein. They also found that the inhibitory effect of cholesterol is markedly potentiated by the presence of plasma proteins. Removal of the α-lipoprotein inhibitor does not reduce serum antihemolytic activity. The presence of the antihemolytic factor was identified in the precipitate of the cold-antibody factor after the removal of lipids from the serum by Bloor’s method. The solution was called “fraction D”) and it was found that it contained serum mucoprotein and partly denatured albumin. Preliminary findings showed changes in fraction D antihemolytic activity in patients suffering from carcinoma.—L. D.


Antisera produced in rabbits against antibodies from patients with acquired hemolytic anemia of the cold-antibody type were shown by the double diffusion technic in agar gel to react specifically with the sera containing the antibodies against which they had been prepared. Cross reactions with other pathologic sera were abolished by absorption of the rabbit antisera with normal human serum. These findings are in accord with the hypothesis that cold-antibody formation represents the synthesis of abnormal proteins rather than a normal immune response.—R. M. H.


The distribution of ABO and Rh(D) blood groups in a series of 127 patients with acquired hemolytic anemia, in each of whom a positive anti-human globulin test was obtained, did not differ significantly from that found in a control series of 6000 subjects.—R. M. H.


The serum of a group O, Rh-positive woman, at least 3 of whose children showed severe hemolytic disease, was shown to contain a weak anti-c and anti-f of higher titer. An eluate from the cord red cells of her fourth child also contained anti-f. These findings suggest that the hemolytic disease was due to anti-f. No explanation can be offered as to why the mother responded preferentially to the ‘weaker’ of the two antigens concerned.—R. M. H.


In 17 of 25 patients with rheumatoid arthritis, the red cell life span (Ashby) was shortened; in several the life span was 18 to 34 days. Episodes of anemia were found to be due to combined hemolysis and marrow aplasia. A normal life span of transfused red cells was obtained only when steroids were given in much higher doses than are necessary in the treatment of the arthritis.—H. M.

HEMOLYTIC ANEMIAS AND VERDOGLINEMIA FOLLOWING USE OF ANALGESICS CONTAINING PHENACETIN. B. Wiedermann ad R. PodivImk. From the First Medical Department, University Olomouc, Czechoslovakia. Vint.lék. 6:149, 1960.

Hematologic consequences of chronic intoxication caused by analgesics containing phenacetin are presented in 5 patients. All patients presented verdoglohinemia with the typical “grey cyanosis,” 4 patients had hemolysis and in 1 female patient there was severe leukopenia. One patient had mild iron deficiency, and in 2 patients hyper-sideremia was present. In one patient in whom splenectomy had been performed, a large number of very coarse Heinz’s bodies was found. Daily and total doses of analgesics were not great as compared with those described by other authors, indicating the importance of individual
factors in manifestations of chronic intoxication caused by analgesics containing phenacetin.—L. D.


A patient is described who presented on two occasions with megaloblastic anemia while being treated for epilepsy with large doses of phenobarbitone. There was no evidence of vitamin B12 deficiency, and intestinal function appeared normal. Plasma clearance of folic acid was very rapid on the first admission, when the anemia was severe, and within normal limits on the second admission. Treatment with folic acid (30 mg daily) produced an optimal hematologic response but on each occasion was associated with the precipitation of frequent minor epileptic attacks.—R. M. H.


Iron metabolism was studied in a series of patients with various anemias. (1) Idiopathic acquired hemolytic anemia: Six cases had a very short plasma T1/2, i.e., between 15 and 36 minutes. The iron incorporation curves showed between 93 and 23 per cent uptake. Mean life of red cells was 17 to 49 days. The theoretical equilibrium between the destruction and the production of the red cells was estimated by relative hemoglobin formation and destruction. (2) In three patients with Mediterranean anemia the authors demonstrated rather high iron clearance (8 to 18 times the normal), which was accompanied by a low incorporation of iron into hemoglobin (maximum 19 per cent and minimum 9 per cent). Mean life of erythrocytes was somewhat shortened, to about 60 days. This anemia seems to be more related to difficulty in production of hemoglobin than to hemolysis. In a patient with sickle cell anemia it was established that the plasma iron clearance is accelerated and iron incorporation is deficient. Mean life of red cells was extremely shortened. A case of sickle cell trait presented no deviation from normal values. Two of 4 patients with hereditary spherocytosis presented a considerable shorter plasma T1/2 and increased iron incorporation, so that the production compensated for destruction. A patient with nonspherocytic hemolytic anemia was in spontaneous remission. The indices obtained indicated a red cell production twice that of destruction. (3) Pancytopenic cases were subdivided in 2 groups according to marrow cellularity. Four patients with cellular marrow showed decreased iron incorporation in spite of adequate erythroid precursors. In the cases of aplastic anemia the iron incorporation was much slower than in the former group. In every case of pancytopenia a component of increased destruction was encountered, with an appreciable shortening of the mean life of the red blood cells. (4) Paroxysmal nocturnal hemoglobinuria: In 4 cases it was established that iron clearance was accelerated, the incorporation of iron was normal, and the survival of the red cells was shortened. (5) Some secondary hemolytic anemias were also studied. The red cell survival was normal in one case of hemolytic anemia associated with disseminated lupus erythematosus in remission. The erythrocyte survival time was very short in another case before treatment. In this case the iron incorporation was normal. In hemolytic anemia associated with primary amyloidosis, there was a shortening of the mean life of the red cells and normal iron incorporation.—M. J.

GENETIC BASIS OF THE THALASSAEMIA DIS EASES.


By analogy with known mutations in one or other polypeptide chain of adult hemoglobin the hemoglobinopathies, the authors suggest that, in its typical form, thalassemia might be due to a mutation of either the α or the β gene, giving rise to a new hemoglobin which is nevertheless indistinguishable from hemoglobin A by electrophoresis. Such a mutation might either result in amino acid substitution at one point in the peptide chain, or lead to total suppression of one chain. This hypothesis would explain the selective suppression of the formation of hemoglobin A, but not that of hemoglobin S, C or E, in double heterozygosity. It would also imply that— if the same α gene control α-chain formation in both fetal and adult hemoglobin—persons homozygous for severe α-chain thalassemias should not be found, as severe deficiency of hemoglobin F in utero would be fatal. Further, all patients with thalassemia major should be either homozygous...
for a β-chain defect, or doubly heterozygous for α-chain and β-chain defects. If hemoglobin A₂ possessed α-peptide chains which were controlled by the same 'α-chain' gene as those in hemoglobin F and hemoglobin A, mutations in either the α-chain or the β-chain would allow the formation of different levels of hemoglobin A₂. This substitution hypothesis is discussed fully with a genetic analysis of published families with sickle cell-thalassemia and with hemoglobin-H disease, and should be read in the original. The observed facts appear to support the concept that thalassemia may result from either of the 2 defects postulated, affecting the α and β genes, respectively. An alternative hypothesis—that thalassemia could be caused by defects in the units which connect genetically significant desoxynucleic acid in the chromosome—would also fit the observed facts; the substitution hypothesis is, however, preferred, as it is based on fewer assumptions.—R. M. H.


Hb-F was obtained from cord blood; Hb-A was separated by electrophoresis in starch. The "pure" Hb-F was then again investigated in starch gel and there were found 3 fractions (Hb-F₁', Hb-F₁", and Hb-F₂). The latter migrates faster, as is the case of Hb-A fraction Hb-A₁. Possibly Hb-F₁ and Hb-A₁ are products of normal aging, and it may be that different Hb-F types are normal Hb-F fractions.—H. M.


Because more rapid hemolysis occurs in whole blood collected in sequestrene as compared to ACD-preserved blood after 16 days' storage, sequestrene appears unsuitable as an anticoagulant for routine transfusion practice. More rapid decrease in activity of some coagulation factors (prothrombin, factor V and factor VII complex) can be demonstrated in sequestrene-treated blood.
—L. D.

LEUKOCYTES

THF HISTOCHENICAL DEMONSTRATION OF TRACE METALS IN LEUKOCYTES. W. F. McNary, Jr.

From Boston University School of Medicine, Boston, Mass. J.Histochem.& Cytochem. 8:124, 1960.

The presence of trace metals in leukocytes has been reported by several authors. New analytical methods designed for the colorimetric determination of trace metals have appeared, and the application of these methods to biological material in the form of histochemical tests offers several technics to demonstrate minute amounts of metal. Metallic elements were found only in the leukocytes (human and rat) belonging to the myeloid series of cells. The neutrophil was found to contain Zn⁺⁺ and small amounts of Cu⁺⁺; the eosinophil, on the other hand, was found to contain large amounts of Cu⁺⁺ and smaller amounts of Zn⁺⁺. Basophils were not reported because they could not be identified. Mg⁺⁺ and Mn⁺⁺ were present in both the neutrophil and the eosinophil while Co⁺⁺ was found only in the eosinophil. Iron in either the ferric or ferrous state was not demonstrated in any of the leukocytes in this investigation.—O. P. J.


In blood smears stained with iodine, 1 to 2 per cent of normal rabbit and rat leukocytes show mahogany-brown granulation. After the injection of different pyrogenic endotoxins, more "iodine-positive" leukocytes are seen. Extracts of various gram-negative organisms are active. Within 2 or 4 hours after the injection of these reagents, the number of iodine-positive leukocytes reaches its maximum. This phenomenon is observed both in vivo and in vitro. The same phenomenon may be elicited by using phenol water extracts from urine and pleural exudates. It is noteworthy that the urine from patients with leukemia had greater activity than did the urine from healthy subjects. The appearance of iodophilic leukocytes may be an expression of toxic damage, but it may also represent increased activity of the cells in preparation for increased phagocytic activity.—H. M.
DIFFERENTIATION OF HUMAN LEUICEMIC LEUKOCYTES.-R. H. B., identical with that found in the nuclei of normal leukocytes studied, the DNA distribution was increased either in the amount of DNA in many malignant neoplasms, suggesting an increase in the number of chromosomes. In a group of aplastic anemias without myeloid metaplasia. Scores between 100 and 200 were seen in polycythemia, plasmacytoma, lymphohgranulomatosis, chronic lymphatic leukemia and in acute leukemia. Normal values were seen in hypersplenism, pernicious anemia, rheumatic diseases and in iron deficiency anemia. Very low values or absence of any activity were found in chronic myeloid leukemia. In aplastic anemia there were some cases with very low values or completely negative activity, so there may arise some difficulties in the differentiation from myeloid leukemia. The authors feel that these low values might be an expression of a preleukemic state. The same might be true for cases of sideroblastic anemia in which a terminal change into leukemia and erythroleukemia is not uncommon. In leukemoid reactions, scores above 200 are usually found.—H. M.


The amount of Feulgen-stained DNA was measured, with the use of the integrating microdensitometer, in the nuclei of leukocytes of cases of leukemia and related diseases. In 2 cases of subacute lymphatic leukemia, 1 of acute monocytic leukemia and 1 of reticulosarcoma, the DNA distribution in nuclei of cells in the bone marrow and lymph nodes resembled that found in many malignant neoplasms, suggesting an increase either in the amount of DNA per chromosome or in the number of chromosomes. In other cases, including all the chronic leukemias studied, the DNA distribution was identical with that found in the nuclei of normal cells.—R. M. H.


In order to determine whether or not cells which appear to be undifferentiated in leukemic blood are capable of maturation, tissue culture was used as an approach to this problem. Leukocytes from patients with subacute granulocytic and subacute monocytic leukemias and healthy individuals were studied in cultures maintained according to Osgood's gradient method. The observations reported would appear to indicate that undifferentiated myeloid and monocytic leukemia cells are capable of differentiating into characteristic cell types specific for the leukemia involved.—O. P. J.


Multiple chromatin appendages, indistinguishable from the single sex chromatin appendages of other species, were observed in individual neutrophils from both male and female mice. Apparently there is a relationship between the intranuclear "sex" chromatin described by Barr (1959) and the chromatin appendage of the neutrophil.—O. P. J.


One of the handicaps in trying to study basophils for their development, cytology and ultrastructure, cytochemistry and function has been the lack of a method which would yield appreciable numbers of these cells in the blood and bone marrow. It has been known for some time (Michels, 1938) that repeated subcutaneous injections of foreign proteins and other antigens would increase blood basophils in the guinea pig and rabbit. In the present report, it was shown that the daily injection of 1 ml. fresh citrated horse plasma into guinea pigs produced the most regular and rapid response. The average number of basophils could be increased from 43 to more than 800 per cubic millimeter of blood and from 15,000 to about 145 per cubic millimeters of bone marrow.—O. P. J.

When animals are subjected either to ionizing radiation or the administration of a nitrogen mustard derivative, there is a rapid fall in the number of circulating lymphocytes and rapid atrophy of the lymph nodes, spleen, Peyer’s patches and thymus. Following this there are two recovery peaks. However, it is not known what proportion of the lymphocytes at any stage are dead, damaged or normal; nor is it known whether the cells that appear during the recovery peaks are ordinary small lymphocytes or represent a new population of cells. The authors have approached this problem by utilizing the technic of determining cytoplasmic solid concentration by cell refractometry. One special advantage of this method is that it is capable of dealing with a large number of cells. Changes in cytoplasmic solid concentration may be the result of one or more of 3 basic causes, viz., change in cytoplasmic volume, change in solid content, and change in cell type. The studies, which were carried out on CBA adult mice for 28 days, showed the first fall in the concentration to be the result of primary damage to the cytoplasm which caused swelling, and that the remaining changes may be related regenerative processes in the lymphoid system.—O. P. J.

The observation that cells of the reticuloendothelial system may contain lipid droplets has led to the suggestion that these cells are in some way concerned in lipid metabolism. Experiments with rats were carried out to study the uptake of various lipid particles by reticuloendothelial cells, and the ability of these cells to dispose of ingested lipids. They are taken up first by cells in the lymph sinuses but are later stored predominantly in the medullary cords and cortical follicles. Fine suspensions of cholesterol and cholesteryl oleate are taken up readily and are stored in slowly diminishing amounts for at least 4 months. Chylomicra appear to be relatively resistant to uptake by reticuloendothelial cells. Triglyceride remains in the glands for only a few days unless the particles coalesce to form relatively large extracellular droplets, when it is removed more slowly.—O. P. J.

Compensatory Hypertrophy of the Spleen.
A Study of Splenic Growth. G. R. Cameron and K-S Rhee. From University College Hos-

The spleen in albino rats and mice can undergo compensatory hypertrophy by means of a reaction like that encountered in splenic autografts. Subtotal splenectomy in mice and rats is followed by compensatory hypertrophy of the spleen. This is the outcome, in the first instance, of reticulum cell proliferation followed by the formation of venous sinuses, fresh white pulp and red pulp production and even a limited amount of hematoipoiesis. A kind of accelerated developmental time table is thus adhered to. Autolysing splenic cells liberate thermolabile substances that encourage the migration of leukocytes from blood vessels.—O. P. J.

Miscellaneous
A Method for Avoiding Centrifugation in the Imbedding of Suspensions in Poly-
methacrylates. D. Danon and Y. Markov-

Blood cells, blood platelets, bacteria and virus particles are difficult to imbed for electron microscopy because the required centrifugation is a handicap. This paper describes a method of imbedding these suspensions in polymethacrylates in which the suspending medium is gradually changed by dialysis.—O. P. J.

Experimental Production of Bone Marrow Hypoplasia by ImmunoLogic Means (A Pre-

Antisera against leukocytes of guinea pigs were produced in rabbits. Such antisera when injected into guinea pigs produced variable changes in blood and bone marrow, depending mainly on the dose, route and frequency of administration. Three ml. of serum injected subcu-
taneously was found to produce marked bone marrow hypoplasia with a myeloid-erythroid ratio of 0.74:1. —J. B. C.

Serological Studies in 5 Cases of Acute Acci-
dental Radiation. The Effect of Homo-
logous Marrow Transfusion. A. Eyquem et
In 5 patients accidentally exposed to acute irradiation, the disappearance of properdin could be shown by inhibition of hemolysis. Properdin reappeared after bone marrow transfusion.—G. M.

**UNSPECIFIC INTERACTIONS BETWEEN SERUM AND TISSUE SECTIONS IN THE FLUORESCENT-ANTIBODY TECHNIC FOR TRACING ANTIGENS IN TISSUES. H. Mayerburch.** From Institute of Histology and Embryology, University of Graz, Graz, Austria. J. Histochem. & Cytochem. 7:427, 1959.

Fluorescein isocyanate-labeled sera for the immunohistologic technic react unspecifically with tissue components. This reaction does not occur if tracers other than fluorescein derivatives are used, e.g., 1-dimethyl-aminonapthalene-5-sulfonic-acid. A second factor for unspecific reactions is an adsorption of labeled antiserum on frozen sections of fixed or unfixed tissues obviously not containing any antigen. It can be shown that the unspecific reactions occur through an interaction of serum with tissues on the basis of electrostatic adsorptions.—O. P. j.


Antivascular activity of human serum was determined by its ability to produce an endothelial cytotoxic lesion on intradermal injection into guinea pigs. The following results were obtained:

<table>
<thead>
<tr>
<th>Antivascular activity</th>
<th>No. Positive</th>
<th>No. Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Disseminated lupus erythematosus</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Henoch-Schonlein purpura</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Negative results were obtained in 5 patients with acute blastic leukemia, 3 with pancytopenia, 3 with myelofibrosis, 2 with hepatitis, and 1 each with chicken pox hypersplenism, acute monocytic leukemia, rheumatic fever, acute glomerulonephritis and reticulum cell sarcoma. Evidently, the antivascular activity found was nonspecific and did not contribute to an understanding of any disease studied.—M. J.


Pathologic sera sometimes show, by immunoelectrophoresis, a weak precipitin (gamma globulin X), which is usually obscured by normal gamma globulin. Its demonstration is facilitated by pretreating antiserum with normal gamma globulin or normal serum, neither of which contains gamma globulin X. Four hundred sera, gathered in Belgium, the Belgium Congo and West Germany, were tested for the presence of gamma globulin X. In healthy persons (86 Europeans and 13 Bantu-Negroes) gamma globulin X was absent. The abnormal protein was found in patients with inflammatory disease and malignancies. It was indentified in hepatic pus and pleural exudates. In all sera showing gamma globulin X, C-reactive protein was demonstrable. Gamma globulin X can be removed from sera by pretreatment with anti-CRP-serum or with pneumococcal polysaccharides, but not by pretreatment with zymosan. Anti-CRP-sera when used in immuno-electrophoresis instead of immune sera gave a precepitin band similar to that of immune serum. Thus it seems probably that gamma globulin X and C-reactive protein are identical. This was further confirmed by studies with isolated gamma globulin X obtained from a pleura exudate.—H. M.


Serum is incubated with inulin at room temperature and after centrifugation at 1 C. and washing of the inulin-properdin complex, the nitrogen content is estimated by the method of Conway. Properdin (µg. of nitrogen/ml. of serum) was 12.5 ± 3.9 for man; 27.6 ± 13.5 in rats; and about 30 in ruminants.—H. M.


Normal properdin titers were found to be 5 to 12 units/ml. serum. In 9 of 10 splenectomized
SUCCESSFUL SPLENECTOMY AFTER REPEATED BACTEREMIA IN SUBACUTE BACTERIAL ENDOCARDITIS. F. Cernik, J. Rehov and J. Groh. From the First Medical Department, University Hradec, Kralove, Czechoslovakia. Cas. lek. ces. 99:330, 1960.

The authors describe a case in which healed subacute bacterial endocarditis was followed by reinfection with a virulent streptococcus after 5 years. Within the next 6 years, bacteremia caused by the same micro-organism developed 3 more times without clinical manifestations of bacterial endocarditis, each episode lasting up to several weeks and being associated with fever and splenomegaly. Treatment with antibiotics proved successful only temporarily. The infection was finally brought under control by splenectomy. During 2½ years after splenectomy the blood cultures have been sterile, and the patient is back at work.—L. D.


Two patients are described in whom hematologic features dominated the clinical picture in primary amyloidosis. In one there was "autoimmune" hemolytic anemia; in the other thrombocytopenic purpura with platelet agglutinins. Neither responded to hormone therapy or splenectomy.—M. J.


Three cases of gargoylism (Pfaundler-Hurler's disease or Ellis disease) are reported. The authors have observed 2 types of cellular inclusions: (a) some of large size in the cytoplasm of histiocytes; (b) some small ones in the cytoplasmic vacuole of plasma cells (Buhot's cells).—G. M.


During the course of a series of studies in tissue proteins of various rat organs, it became necessary to identify individual plasma proteins that may be present in organ sera either as contaminants or as cellular products. Such identification requires both biophysical and serologic data on as many serum proteins as possible and the use of specific antisera. The present designation of serum proteins as albumin, alpha (with subscripts), beta and gamma globulins is unsatisfactory for general application to sera other than human. There is a possibility that a number of "albumins" exist in rat serum. There is also a component sedimenting faster than gamma globulin in addition to the macroglobulin observed.—O. P. J.
Possible Sites of Macroglobulin Synthesis:
A Study Made with Fluorescent Antibody.

This paper describes experiments on the localization of macroglobulins by the fluorescent antibody technic in the tissues of 2 patients, one showing Mikulicz's syndrome and the other suffering from myeloma. The first patient had the macroglobulin in large quantities in mature and immature plasma cells in the bone marrow. It was considered that the macroglobulin was a product of a plasmacytosis reactive to the pathologic process in the patient's parotid gland. The second patient had both a macroglobulin and a myeloma globulin in his serum. The fluorescent antibody studies suggested that these two abnormal proteins originated in different plasma cells in the bone marrow, which were, however, morphologically identical with orthodox staining. Neither protein could be demonstrated in frozen sections of other tissues obtained at autopsy.
—G. C. de G.


This is an excellent review article. It gives an account of the clinical aspects and pathology of macroglobulinæmia and of physiochemical studies and antibody properties of macroglobulins. The author states that "The underlying pathological process in macroglobulinæmia is a proliferation of those elements of the mesenchymal tissues responsible for the synthesis of immune globulins. Both lymphocytes and plasma cells may participate in this process, but the predominant cell type is of lymphocytic origin. The antigencic and physiochemical individuality of macroglobulins and other paraproteins has led to the supposition that they are products of clones of genetically identical cells within the antibody-producing system. Such a clone may arise through a mutation process, and ultimately assume frankly neoplastic properties, although occasionally macroglobulinæmia may continue to be the sole evidence of the existence of the aberrant clone."—G. C. de G.

The Value of Splenectomy in the Treatment of Essential Pulmonary Hemosiderosis.

Analysis of results obtained in 18 patients with essential pulmonary hemosiderosis shows that splenectomy (or splenectomy and steroid therapy) prolongs the life of the patients.—S. R. H.


Eighteen patients received blood transfusions from donors presenting a positive complement fixation test for Chagas's disease, the transfused blood having previously been treated with gentian violet in a concentration range of 0.25 to 0.50 mg. per liter. It was not possible to demonstrate transmission of the parasitosis, after careful parasitologic and serologic search. In a previous study (1955) the authors verified that in 4 of 16 patients, transfused with blood giving a positive complement fixation test for Chagas's disease, post-transfusional infection developed.
—M. J.


Of the various conditions associated with eosinophilia, one clinical entity, called eosinophilic lung or tropical eosinophilia, is characterized by persistent hypereosinophilia and chest symptoms responding favorably to treatment with organic arsenicals. Knowledge of the underlying pathologic changes in eosinophilic lung has been limited because the condition is benign. A complete pathologic study of an adult Ceylonese man who died of encephalopathy following treatment with neoarsphenamine showed the presence of foreign-body granulomas in the lungs. No granulomas were found in the liver, but there was portal infiltration with leukocytes similar to those found in liver biopsy specimens from cases of filariasis.—O. P. J.