ABSTRACTS

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ABSTRACTS OF SPECIAL INTEREST


The action of sera and lymphocytes from kidney homografted dogs on tissue cultures from the second kidney of the donor was studied. Sera alone taken from animals after rejecting the graft showed no cytotoxic action on the cultures. Living lymphocytes from the immunized recipients, however, regularly produced cytotoxic lesions in renal cultures from the corresponding donors but not in cultures from other dogs. The cytotoxic effect seemed to depend on the coincident presence of immune serum from the recipients. Interestingly, living lymphocytes from the recipients were not found to be obviously toxic for white cell suspensions obtained from the peritoneal fluid of the donors.—T. E. B.


Tritiated pteroylglutamic acid was found to be cleared from plasma of normal subjects as would be expected if it were not tritiated. As indicated by the study of Anderson, Belcher, Chanarin, and Mollin (Brit. J. Haemat. 6:439, 1960), and the present authors, tritiated folic acid may prove especially useful in studying the absorption of folic acid.—V. H.


Since bilirubin in the plasma circulates bound to albumin, and unbound bilirubin is free to escape into the tissues, an attempt was made to increase the amount of circulating bilirubin, prior to exchange transfusion. Twelve-and-a-half Gm. of salt-poor albumin were administered intravenously to erythroblastic infants before, and again in the middle of, an exchange transfusion. The total amount of bilirubin removed from the body by the exchange transfusion was then measured. It was found that significantly greater amounts of bilirubin were removed from the albumin-treated infants than from a group of control infants. It is suggested that the administration of albumin before and during the first exchange transfusion, by increasing the amount of bilirubin removed, will result in a decreased need for multiple exchange transfusions.—J. R. S.

An instance of severe, glycogenesis is reported, possibly related to a total absence of glucose-6-phosphatase. Both indirect and direct observations supported this hypothesis; hypoglycemia was not affected by adrenaline or glucagon and hyperlactacidemia was present after a galactose load; study of some liver tissue showed absence of the enzyme. At the age of 20 months a hemorrhagic tendency led to finding of a prolonged bleeding time, disturbance of platelet thromboplastin formation and a low “serum platelet activity.” These results were confirmed on many occasions during the illness; in the absence of thronbopenia they must represent functional disturbance of the platelets. The association of thrombopathy and glycogenesis is not a chance one; a hemorrhagic tendency has often been reported with glycogenesis. The thrombopathy does not seem to be dependent on the hypoglycemia, or on the acidemia or hyperlipemia. Possibly a platelet enzyme deficiency is associated with the absence of glucose-6-phosphatase.—G. M.


A technic is described for producing very pure fibrinogen with 100 per cent recovery. A fibrinogen-tanin complex is formed, and the fibrinogen is displaced by polyvinylpyrrolidone (Plasdone). The technic was developed as a by-product in the preparation of antihemophilic factor from human plasma.—G. M.

HEMOSTASIS


A method is described for the purification of bovine fibrinogen by means of repeated precipitations with sodium sulfate till 97 per cent clottable protein was obtained. On cooling to 0°C a portion of the fibrinogen remained in solution (cold soluble fraction) and a portion sedimented (cryo-fibrinogen). The two fractions did not differ in their clotting ability, specific rotation, viscosity or carbohydrate content. Cryofibrinogen, however, showed a stronger tendency to sedimentation. Another feature of cryofibrinogen was the ease with which it could be solubilized upon addition of the cold soluble fraction. Since ionic strength of the solvent (saline) influenced the cold precipitation, the author suggests a difference in the distribution of the electric charges on the surface of molecules of the two fibrinogen fractions.—J. J. B.


Bovine fibrinogen and fibrin-monomer were photo-oxidized in a Warburg apparatus by means of ordinary electric light at 26°C, with methylene-blue as photosensitizer. Oxygen consumption was 28.8 μmole of protein. After 12.5 min. of photo-oxidation at pH 8.2–8.6 fibrinogen or the monomer lost its clotting ability upon thrombin addition. At pH 5.1–5.2, however, a normal clot was obtained though with considerable delay. Quantitative determinations of certain amino-acid residues showed that following photo-oxidation at pH 8.2–8.6 tyrosine did not change, histidine content decreased by 14 per cent and tryptophan by 13 per cent. At pH 5.1–5.2, however, only tryptophan content was reduced. The photo-oxidized fibrinogen displayed the same electrophoretic mobility and sodium sulfate precipitation properties as native fibrinogen. Thus, following photo-oxidation, fibrinogen and the monomer lose their polymerizing property at alkaline pH only. Since tryptophan is affected at both pH values but histidine only at alkaline pH, it is possible that histidine forms part of the “active site” of the fibrinogen molecule and all of its units must be intact for polymerization into fibrin to occur.—J. J. B.


Epsilon aminocaproic acid has been shown to be an excellent inhibitor of plasminogen activation. The authors have made a systematic study of aliphatic amino acids in an attempt to determine the approximate molecular configuration which favored epsilon aminocaproic acid as an inhibitor. The compounds tested were: beta-
ABSTRACTS

alanine, gamma-aminobutyric acid, putrescine, delta-aminovaleric acid, delta-aminolevulinic acid, L-glutamic acid, ornithine, L-arginine, cadaverine, epsilon aminocaproic acid, norleucine, lysine, and omega-aminocaproic acid. The in vitro testing system was essentially that of a clot lysis system testing for both activator and plasmin, respectively. Residual plasmin in inhibited samples was also tested on heated fibrin plates. The results showed that epsilon aminocaproic acid was by far the most potent amino acid tested, exhibiting moderate activity at 10⁻³ M, delta-aminolevulinic acid and omega-aminocaproic acid had significant activity at 5 x 10⁻³ M, gamma-aminobutyric and lysine at 10⁻² M and putrescine, cadaverine and ornithine at 5 x 10⁻² M. Even at this latter concentration, no inhibitor activity was found with the other compounds tested. None of the substances was an anti-plasmin agent as measured by the procedures described. Thus, the most potent of the amino acids tested were those with a 4-8 carbon chain length and the amino group in the omega position.—A. J. J.


One of the most difficult problems in the purification of plasminogen has been that of obtaining a reproducibly pure product. The yields have also varied markedly, depending upon the initial fraction III from which the plasminogen has been purified. The authors have investigated the use of cellulose ion-exchange chromatography of plasminogen, starting with a fraction III paste which was dialysed against sodium phosphate buffer at pH 7. The precipitate contained 12 to 15 per cent of the protein in the starting material and had a specific caseinolytic and fibrinolytic activity which was 4 to 5 times that of the fraction III. The yield of plasminogen in this step was about 60 per cent. When this partially purified plasminogen was put on a cellulose column in the presence of 0.01 M lysine, the eluate fibrinolytic activity was not adsorbed to the DEAE-cellulose but appeared in the front. Elution was performed step-wise, with buffers of increasing acideity from pH 9 to pH 4. The yield in this step was approximately 80 per cent of the plasminogen with 20 per cent of the protein. The increase in purity was 18-24 as compared to fraction III, and the overall yield from fraction III was about 50 per cent. When these fractions were tested by the caseinolytic method of Mullertz and the fibrinolytic method of Christiansen, no significant separation occurred between the plasminogen and proactivator activity ratios of the two fractions.—A. J. J.


Contamination of thrombin and fibrinogen with plasminogen, the precursor of the fibrinolytic enzyme plasmin, has always presented technical difficulties in the measurement of fibrinolytic activity. Thus, the contaminating plasminogen nearly always contributed activity of its own to the thrombin and fibrinogen in the assay systems. This was particularly objectionable when attempting to use highly purified reagents in highly purified systems with a very short lysis time. These investigators have used the reducing agent, 2-mercaptoethylamine to inactivate the contaminating plasminogen in thrombin. Since most previous methods for doing this have resulted in marked loss of clotting ability, this particular method is unique in that there is little diminution in clotting ability following removal of the plasminogen. They also found that the thrombin was partially denatured by this treatment, therefore much more susceptible to changes in pH. However, treatment with iodoacetamide after dialysis of the mercaptoethylamine prevented some of this instability. The authors speculate that the most likely explanation of the effect is the splitting of one or more disulphide bonds essential for the activity of the plasminogen molecule. Finally, it was felt that mercaptoethylamine treatment did not interfere with the fibrinolytic activity of thrombin itself, since both treated and untreated thrombin lysed the purified clots in 5 hours, in high concentration, in the absence of streptokinase.—A. J. J.


The effect of plasmin on complement and the mechanism of this effect has been disputed for some years. Lepow and Pillemer, in 1954, showed that plasmin affected complement and seemed to inactivate C₁. The action of plasmin on C₁ is clarified, somewhat, in the present study. It does not, however, bring conclusive evidence to bear on the effect of plasmin itself, or the effect of the
antigen-antibody reaction with SK. In general, streptokinase and plasmin depleted C₁ and increased the activity of C₂, C₃ and C₄, when human plasma was incubated with complement components. The rate of fall in C₁ was about equal to the rate of fall of the complement in whole plasma. In an attempt to elucidate this further, the authors asked themselves whether the decreased activity of C₁ was due to consumption by direct proteolysis of plasmin or consumption of this component due to activation of the other complement components. Therefore, R₁ sera was incubated at 37°C with plasmin and the activity of the single components was investigated. The activity of all components C₂₋C₄ showed no change, even after 6 hours incubation. This seemed to indicate that C₁ was essential for the activation of C₂₋C₄. Experiments with whole plasma and defibrinated plasma suggested further that albumin acted as a "competitive substrate," regarding the effect of plasmin on complement. Thus, on using streptokinase alone, a rapid inactivation of all components occurred in the defibrinated plasma. The authors ascribe this to the antigenic character of streptokinase which presumably might account for the consumption of C during the antigen-antibody reaction. However, earlier studies in the reviewer's laboratory failed to indicate a depressing effect of the SK antigen-antibody reaction on complement. In summary, the results confirm that the component attacked by plasmin is C₁. It is assumed further that a change occurred in C₁ which allowed the combination of plasmin and C₁ to activate C₂, C₃ and C₄. During this process it was itself inactivated, causing a decrease in the activity of the whole complement, C. — A. J. J.


In the course of a series of experiments using fibrin plates, the authors found that bile, when tested on the fibrin plate, appeared to contain a heat resistant activator. Further investigation, however, showed that the observed lysis was not due to plasminogen activation, but to simple solution of fibrin by, formed in the absence of calcium. Fibrin i, formed in the presence of calcium, was not dissolved by bile but was susceptible to two plasminogen activators. To discover what substance in bile was responsible for lysis of unheated fibrin, solutions of several bile salts, sodium taurocholate, sodium glycocollate and sodium dehydrocholate were tested and were found to lyse the unheated fibrin plates. The lytic property of bile salts was also stable at 100°C. Since these substances are biologic detergents, synthetic detergents were also tested and found to be capable of solution of the fibrin. Bile salts and synthetic detergents, however, uniformly failed to activate the proteolytic activity of plasminogen when tested by the caseinolytic technique. Dialysis of lysed clots to remove the bile salt or detergent caused the clot to reform just as dialysis of urea from urea soluble clots causes the clot to reform. This demonstrated that the fibrin had been dissolved but not digested. It is suggested that fibrin i rather than fibrin j should be used to test for plasmin and plasmin activator activity. — A. J. J.


The Symposium was held in the Spring of 1960. Included in it were articles by Koller, Fearnley, Kline, Norman, Clifton and many others. The physiology of fibrinolysis was discussed during the first half of the conference. This portion of the meeting was marked by the wide diversity of methods used, and a concerted attempt was made to emphasize and utilize the most widely accepted, available methods. The second portion of the meeting was concerned with the evaluation of clot lysing agents in the treatment of thrombotic disease. The sense of the meeting was perhaps best summarized by Dr. Sokal and the panel members who met to discuss this subject in the meeting: "We believe that a great deal of research remains to be carried out before the place of fibrinolytic therapy in medical practice can be established. Investigations so far have been designed to define the situations in which fibrinolytic agents might be effective, and not to evaluate the clinical usefulness of fibrinolytic therapy." Dr. Sokal also estimated that in two to four years we will have some idea whether fibrinolytic therapy is useful in coronary thrombosis. — A. J. J.


In 20 cats heterogenous hemolytic shock was produced by injection of 10 ml. human type B blood. The following conclusions were drawn.
from the experiments: (1) fibrinolysis in diluted plasma was activated, (2) fibrinolysis in euglobulin fraction of plasma was activated, (3) plasminogen activity in plasma was decreased, (4) plasma antiplasmin activity showed great variations, (5) prothrombin time of plasma was prolonged, (6) plasma thrombin time was lengthened. The presence of a potent plasmin inhibitor in cat erythrocytes was demonstrated. The authors suggest that plasma antiplasmin activity variations were due to the decrease of plasma antiplasmin level plus liberation of erythrocyte inhibitor incident to hemolysis.—E. K.


The effectiveness of the activators presently used for human therapeutic use, such as streptokinase and human “fibrinolysin” has been determined by measurements taken on peripheral blood. The authors have attempted to evaluate the effect of ischemia during this sampling procedure. Fibrinolytic activity was usually measured by a modified standard clot lysis test with plasma euglobulin and bovine substrate; in a few patients fibrinolytic activity was also measured by the method of Biggs and McFarlane. The normal euglobulin lysis time by this method was greater than 24 hours. Thus, when venous occlusion was produced by application of a rubber tourniquet for 5 minutes, 12 of 15 human patients demonstrated some increase in fibrinolytic activity. When a sphygmomanometer cuff was used instead of the rubber tourniquet and the cuff was inflated to diastolic pressure for 5 minutes, the same pattern of increased activity was seen in 7 of 10 patients. Finally, a third group of patients was studied and the vessels were occluded by a cuff for 20 minutes. In this series, 8 of 9 developed some increase in activity, as measured by the Biggs and McFarland method. These studies are at some variance with the work of Kwaan and McFarlane who have shown that the arrest of arterial blood flow to an extremity results in increased fibrinolytic activity in that extremity after the tourniquet is released. It is assumed that these differences arise from technical variations in the procedures used. In conclusion, the authors suggest that blood specimens used in the study of fibrinolysis be obtained from free flowing venous blood.—A. J. J.


An artificial substrate for factor V assay is prepared by incubation of a mixture of Russell's viper venom, cephalin and normal plasma. In this mixture the proaccelerin is first converted to accelerin which is then destroyed. After four hours of incubation at 37° C. the substrate is ready for use and can be stored at -20° C. The test is performed by adding test plasma to the substrate and incubating for 2 minutes. During the incubation the proaccelerin of the test plasma is converted to accelerin. At the end of 2 minutes calcium is added and the clotting time determined. Clotting times are converted to per cent factor V activity by means of a correlation graph prepared by serial dilutions of normal plasma. The authors claim the system has three principal advantages: it does not require congenitally deficient plasma; it provides a substrate with predictable and reproducible characteristics after only 4 hours of incubation, and it permits complete conversion of proaccelerin to accelerin and therefore permits complete measurement of all the factor V activity in any test sample.—R. G.


The thromboplastin generation test was modified by using 1:50 dilution of adsorbed plasma with a 1:10 dilution of serum and a soybean phosphatide preparation instead of platelets. With this retarded thromboplastin generation test, it was found that 22 of 69 patients with thrombotic arterial and venous disease had accelerated formation of thromboplastin. It appeared that the acceleration was due to changes in the adsorbed plasma and not in the serum. The end point was reached 1 to 3 minutes faster in these patients than in the normal control and the yield of thromboplastin was frequently greater in the patients' test than in the control. Accelerated formation of thromboplastin was found in: 10 of 18 cases with acute arterial occlusion, one of 15 patients with occlusive arterial disease without history of acute arterial occlusion, 3 of 8 patients with thrombogenic ulcers, 7 of 17 patients with idiopathic thrombophlebitis, but only 1 of 4 patients with secondary thrombophlebitis. No
Evidence of acceleration was noted in 7 patients with miscellaneous arterial disease. Neither ganger nor recent thrombus formation seemed to account for the findings. Serial studies of several patients suggested that acceleration of the thromboplastin formation may be temporary (a few weeks) or permanent (at least a year). The authors suggest that the acceleration may be due to (1) pre reaction in vivo of certain first phase coagulation factors. (2) presence of an undefined accelerator in plasma, (3) an excess of one of the normal factors involved and (4) a relative lack of a normally present inhibitor. No mention was made of any control series.—R. G.


Thrombin generation in platelet-rich and platelet-“free” plasma of four patients with paroxysmal nocturnal hemoglobinuria was found to be the same as in normal platelet-rich and platelet-free plasma. This was true for oxalated, ACD or native plasma. PNH platelets were found to have normal clot retraction activity. The findings provide no evidence for the hypothesis that by virtue of a stromal defect similar to that of the PNH erythrocyte, the PNH platelet is lysed by the same plasma system that hemolyzes the red cell. The authors also suggest that the thrombocytopenia frequently encountered in PNH could result from the liberation of a non-hemolytic thromboplastic activity of destroyed red cells or from the liberation of thromboplastic-like activity from hemolyzed red cells.—R. G.


In an effort to explain the high red cell fallout of the clotted blood in polycythemia vera, platelet plasma clots from normal individuals and polycythembia vera patients were sectioned and stained at various intervals during coagulation and clot retraction. Great disorganization of the platelet-fibrin network was observed in the polycythemia vera clots when compared with the normal clots. In the polycythemia clots there was great irregularity in the fibrin network and numerous holes without any visible fibrin strands were noted. It is postulated that red cells could easily slip through these openings in the fibrin network. The authors believe that this defect is inherent in the platelet, although they note that it might also be due to an abnormal fibrinogen.—R. G.


Plasma AHG levels were measured by the Pool-Robinson Assay in 35 obligatory carriers of hemophilia A (daughters of hemophiliacs, mothers of 2 or more hemophilic sons, or mothers of one hemophilic son who had at least one other close male relative with hemophilia) and in 30 normal women. AHG levels did not vary with the phase of the menstrual cycle. In the normal women a mean AHG value of 92 per cent of the reference plasma was found (range 52 to 133 per cent). The carriers had a mean AHG value of 58 per cent (range 22 to 135 per cent). One carrier with 22 per cent AHG had experienced clinically significant bleeding. Seven carriers with AHG levels between 30 and 40 per cent had not bled abnormally. The distribution of AHG values in the carrier series suggested that AHG levels low enough to predispose to bleeding should occur in about 20 carriers per 1000. Approximately one-half the carriers had AHG levels below 60 per cent; many carriers had levels well within the normal range. AHG did not increase with age. Probability calculations indicate that AHG assay by this method will detect with reasonable accuracy (4 chances out of 5) about 75 per cent of the true carriers in a potential carrier population and about 60 per cent of the normal members in a potential carrier population. However, a potential carrier even with a very high AHG level still has one chance in five of being a true carrier.—R. G.


Two patients with PTC deficiency were treated during bleeding episodes with small transfusions of serum. It was the opinion of the author that their clinical response was more prompt and prolonged than when they had been treated with whole blood or fresh frozen plasma on previous occasions. The transfusions of serum were followed by improvement in thromboplastin generation, as measured in the TGT, which was more
ABSTRACTS

Pronounced than when equivalent amounts of plasma were given. The author also felt that the effect of serum transfusion on the TCT could be detected for as long as 17 days after the serum transfusion, whereas the effect of plasma could not be demonstrated after 24 hours. The data presented only partially substantiate these conclusions.—R. G.


The hemostatic effect of Premarin, a complex of estrogen sulphates from the urine of pregnant mares, was tested in various ways. No effect was demonstrable on the results of any of 9 tests of hemostatic function within 3 hours of the intravenous injection of 20 mg. of premarin to 8 normal subjects, on the bleeding-time or tourniquet test in patients with various hemorrhagic disorders, or on the blood loss during and after prostatectomy in 31 patients, as compared with 40 others who did not receive the drug. In a small group of patients with epistaxis, premarin was no more effective than an intravenous placebo in controlling the bleeding.—R. M. H.

DICUMAROL THERAPY: SOME EFFECT ON PLATELETS AND THEIR RELATIONSHIP TO CLOTTING TESTS. A. A. Murphy and J. F. Mustard. From the University of Toronto, Toronto, Canada. Circulation Res. 8:1187, 1960.

Tests for platelet clumping and platelet adhesiveness were done on normals and on patients receiving dicumarol. In addition, clotting times, prothrombin times and a modified thromboplastin generation test were done, and a statistical analysis performed to determine which of these tests correlated most closely with changes in the platelet clumping and adhesiveness. The authors assume that platelet adhesiveness or clumping is a good index of in vivo coagulability. Their findings show that dicumarol therapy reduces platelet clumping and adhesiveness. Of the three coagulation tests done the clotting time was the most efficient for evaluating these platelet findings and the prothrombin time was least efficient, although in about one third of the patients the prothrombin time was as good an index of platelet clumping as the clotting time. It is pointed out that while thromboplastins of 2 different sources may give congruous results in untreated patients, consider-

Most antiglobulin sera of animal origin react with the γ-globulins present on platelets and leukocytes from cases of disseminated lupus erythematosus (DLE), and of pancytopenia, as well as with those fixed on the platelets of certain cases of idiopathic thrombocytopenic purpura (ITP). However, one antiglobulin serum was found which did not react with platelets from ITP. The authors therefore conclude that the γ-globulins detected on platelets of DLE and pancytopenia, and those associated with ITP, have a different structure, even if they both probably are autoantibodies.—G. M.


Ten allergic patients were given rather large doses of the specific allergen intracutaneously. All patients got a marked local reaction, and the platelet count decreased considerably during the first hour after injection. However, when the test was repeated after an intravenous injection of heparin, the local reaction was much milder, and no platelet drop was observed. It is concluded that heparin is able to protect the platelets and prevent the release of 5-hydroxytryptamine.—C. W.


This paper reports three cases of the syndrome “giant hemangiomata with thrombocytopenia.” The first occurred in an infant with a malignant hemangioendothelioma invading the muscles of the tongue and neck, and was fatal. In the second the tumour responded to X-ray therapy, and the patient was sufficiently recovered in one month to be discharged from hospital. The third patient failed to improve after X-ray therapy, and was finally cured by amputation of the limb. The question of X-ray therapy is discussed, and a plea is made for early excision of large cavernous angiomata before thrombocytopenia has developed.—G. C. de G.


Twenty-five patients with Schönlein-Henoch’s syndrome were tested for a possible relation to bacterial infections. Eighty-four per cent of the patients had a history of infection: 64 per cent with β-hemolytic streptococcus, 20 per cent with staphylococcus aureus. Non-bacterial sensitization (food, drugs) as the etiologic factor could not be proved with certainty in this group of patients.—L. D.

LEUKOCYTES


Investigations have shown that glycolysis occurs at the site of, and probably influences the dynamics of, the inflammatory reaction. The following work was undertaken to establish a basis for a metabolic approach to the study of certain immune and allergic mechanisms associated with inflammation. Data, obtained from inducing a peritoneal inflammation in male guinea pigs weighing 500–800 Gm., indicate that both blood and exude leukocytes from animals in which inflammation of a critical intensity is present show a glycolytic activity that differs quantitatively from that of normal blood leukocytes. The metabolic change is one favoring the overall economy of the animal since such a change involves the metabolic system which seems to supply the energy necessary for phagocytosis.—O. P. J.


Good quality, air-dried preparations from unattached leukocytes handled in suspensions were obtained by modifying existing technics in order to provide well-spread chromosomes in one focal plane during metaphase. These give an adequate mitotic yield for the short-term cultures of leuko-
cytes and the same technic may be used for other cells which do not adhere to a glass surface in culture.—O. P. J.

**ABSTRACTS**


Human γ-globulin was prepared by cold alcohol fractionation, characterized by free electrophoresis, and labeled with I\(^{131}\). Four patients with a β-globulin type of myeloma cleared the labeled γ-globulin from the blood significantly more slowly than 4 patients with the γ-globulin type of myeloma.—P. G. R.

**Turnover of Autologous and Homologous Labeled Gamma-Globulin in Multiple Myeloma.** S. W. Lippincott, S. Korman and W. L. Hughes. From the Medical Department, Brookhaven National Laboratory, Upton, New York. Arch. Path. 70:467–475, 1960.

Gamma globulins isolated from patients with multiple myeloma and labeled with I\(^{131}\) were given intravenously to 6 patients with β-globulin type myeloma and 8 with γ-globulin type. Like normal γ-globulin, the myelomatous γ-globulin or its label is cleared more slowly from the blood stream of the former group than from that of the latter.—P. G. R.


Thirty four cases are reported, one of them a patient with plasma cell leukemia and 262,000 white cells per cu.mm. in the blood, 99 per cent of them plasma cells. In this case the β-globulin fraction was increased. The other cases were typical multiple myelomas. Ten were men and 24 women, with average age 54 years. Serum electrophoresis revealed a typical γ-globulin peak in 12 cases and β-globulin in 12 cases. In two cases the peak occurred between a and β, and in four cases increased amounts of α\(_2\)-globulin were found. The average survival time was 10.7 months from the diagnosis and 18.7 months from the onset of symptoms. The women lived significantly longer than the men. There was no correlation between the electrophoretic pattern and the prognosis, but the survival time was shorter in cases with renal involvement on the first admission.—C. W.


Fluorescent antibody and cytochemical methods were used to study the intranuclear material present within the lymphocytoid plasma cells of three patients with Waldenström's Macroglobulinemia. Both nuclear and cytoplasmic localization of macroglobulin was noted. Intranuclear fluorescence coincided with the presence of intranuclear PAS positive protein. The observations support the concept that circulating macroglobulin and intranuclear protein of lymphocytoid plasma cells are closely related and may be identical. The evidence suggests that macroglobulin is formed in lymphocytoid plasma cells and not the typical plasma cell (as previously suggested by Curtain).—H. H. F.


Since recently highly complicated electronic scanning devices have been introduced, it was considered worthwhile to re-evaluate the older method of light diffraction as a means of size determination. Cells to be measured are best prepared as a cytologic smear, made in the customary way or by a spray technique. Slides prepared in this way were illuminated by a collimated beam of monochromatic light of 495 m\(\mu\) wavelength. Fraenhofer type diffraction patterns were obtained. Mixtures of cells of different sizes, as in malignant material, pose a more complicated problem than that presented by blood from leukemic patients.—O. P. J.


Starch block electrophoretic mobility and alkali denaturation rate of hemoglobin obtained from fowl erythroleukemia cells did not differ from normal fowl erythrocyte hemoglobin.—P. G. R.

**Serum Lactic Dehydrogenase in Myeloproliferative Diseases and Neoplasia of Lymp-**
ABSTRACTS

The activity of serum lactic dehydrogenase (LDH) in myeloproliferative diseases and neoplasia of lymphoid tissue was studied and compared with groups of other blood dyscrasias accompanied by alterations of bone marrow activity. The activity of LDH in the serum was conspicuously raised in the majority of chronic myeloses. LDH was also frequently elevated in untreated polycythemia vera and in the myelofibrotic syndrome. LDH was markedly increased in acute leukoses. LDH was increased in one case of chronic lymphadenosis associated with a rise of lymphoblasts. Neither lymphogranuloma nor myeloma showed increased LDH activity even in the advanced stage. In a varied group of various marrow depressions, serum LDH was found elevated in about half the cases. Increased values of LDH found in megaloblastic and some hemolytic anemias indicate that serum LDH elevation is a nonspecific phenomenon, incidental to increased proliferative activity of the myeloid and erythroid series, not related to malignancy. Lymphoid neoplasia usually failed to influence serum LDH activity.—L. D.


This paper reports six patients who had been treated with phenylbutazone for rheumatism and who developed leukemia. (Acute 1 case, myeloid 3 cases, lymphatic 2). The interval and dose varied from 3 weeks and 10 Gm. to 4 years and some hundreds of grams; in one case there seemed to be a strong sequential relationship between phenylbutazone and the development of myeloid leukemia. Three of these patients had suffered from spondylitis and the remainder had osteoarthrosis, but the possible association between leukemia and the rheumatic diseases is not considered.—R. M. H.


Of 1,548 children who died in England and Wales with acute leukemia during 1943-58, 8 were found to have been at Queen Charlotte's Hospital; only one of these had been exposed to antenatal irradiation, compared with 25.3 per cent of all babies born at the hospital. From these figures, the incidence of leukemia in children born at the hospital is calculated to be 1 in 1,808 for non-irradiated cases and 1 in 4,291 for irradiated cases. Although the figures are too small for proper statistical evaluation, they do not support the theory that leukemia is induced by antenatal irradiation.—R. M. H.

Incidence of Leukaemia after Exposure to Diagnostic Radiation in Utero. W. M. Court Brown, R. Doll and A. Bradford Hill. From the

This paper reports an investigation of the incidence of leukemia in some 40,000 liveborn children known to have been irradiated in utero in eight hospitals between 1945 and 1956. Women irradiated during pregnancy in these hospitals were identified in the hospital records, and the subsequent deaths from leukemia of the children of these pregnancies were discovered by comparing their names with those on death certificates. Tests of the accuracy of this procedure showed that it may have underestimated the true incidence of leukemia deaths by some 2–3 per cent. Nine children in the series had died of leukemia before the end of 1958; the expected number, calculated from the death-rates for England and Wales, was 10.5. There was no apparent correlation between the dose of irradiation or the stage of pregnancy at which it was given; few children, however, were irradiated during the first few months. The findings are discussed in detail in relation to reports of previous surveys and the authors conclude that, while the evidence on the leukemogenic effect of irradiation in utero is conflicting, the case in its support has not been established.—R. M. H.


This paper reports a survey, based on numerous questionnaires, of the incidence of leukemia in 60,000 patients (representing over 220,000 patient-years) who had been treated with radioiodine for thyrotoxicosis in the United Kingdom, Austria, Canada, and the U.S.A. Eighteen of these patients are known to have developed leukemia, compared with an expected 21 cases (range of 14–24) calculated from the natural leukemia rates appropriate to the age and sex distribution of the populations under investigation; the number of cases, 13, with acute leukemia is higher than would have been expected, although not strikingly so; the intervals between radioiodine treatment and the diagnosis of leukemia, however, are consistent with a fortuitous incidence. The author concludes that the information available at present gives no evidence of leukemogenesis by radioiodine, although this cannot be excluded, as the total number of cases of leukemia might greatly exceed those so far known to have occurred.—R. M. H.


Chick embryos were inoculated with folic acid and purine-analogs, alkylating agents, etc. In 48 hour old chick embryos, multiple abnormalities were found, most frequently in the neural tube. Authors hope to elucidate pathogenesis of congenital malformation and usefulness of antineoplastic in provoking abortion.—P. G. R.


Six-substituted purines were given to 33 adults with chronic granulocytic leukemia. Twenty-six had received previous treatment (for up to 9 years) and 5 were in blastic stage. Starting doses of 2 mg. 6-MP or TG or 15 mg. 6CP, all per Kg. and daily, were found to be most adequate. Maintenance therapy was found to be better than intermittent. Sixteen patients had good, 9 fair response. Only 3 of 10 patients with initially poor clinical condition (including blastic stage) improved. Remissions lasted 2–69 months. In 5 patients treatment was stopped because of hematologic depression; 4 of these subsequently responded to alkylating agents. One third of patients had icterus. Authors conclude that Myleran should be first choice, 6-MP used when patient stops responding to Myleran.—P. G. R.

Augmentation of Therapeutic Efficacy of 3'-5'-Dichloroamethopterin Against an Antifolic-Resistant Variant of Leukemia (L1210-M46R). From the National Cancer Institute, Bethesda, Md. Cancer Res. 20:1066–71, 1960.

Halogenated-foiic-acid-antagonist-treated ADBA mice with L1210 leukemia become immune to reinoculation of both L1210—leukemia cells and to folic acid antagonist resistant substrains (M46R). Even when M46R is inoculated 7 days after L1210—prior to the development of immunity—M46R becomes amenable to treatment with a folic acid antagonist.—P. G. R.

The author noted a very favorable therapeutic effect of Leukeran in patients suffering from mycosis fungoides. In one patient with severe symptoms, enlarged nodes and the release of atypical elements into the blood stream, a single course of treatment with Leukeran was followed by complete remission which has continued for 16 months. In another patient a remission lasting 7 months was observed, and repeated courses of Leukeran treatment were effective. In other patients the period of observation is not yet sufficiently long to permit an evaluation of the results.

Treatment of mycosis fungoides was begun by the author in March of 1958. In the literature the author did not find any previous similar reports, and these preliminary results can be considered the first of their kind.—L. D.

ERYTHROCYTES


It has been known for some time that amphibian erythrocytes will segregate or concentrate certain dyes in a cluster of granules near each pole of their ellipsoidal nucleus. This so-called "segregation apparatus" has been likened to the vacuum "apparatus" has been likened to the vacuum of Parat and the Golgi bodies. Blood cells from the heart of Necturus maculosus, fixed and prepared for electron microscopy, revealed many new details in the elements making up the "segregation apparatus." The granules appeared as relatively dense, transversely laminated short rods, surrounded by clear vacuoles. The fine structure of the rods (0.25 x 1.0μ) constituting the "segregation apparatus" is unlike that of the Golgi apparatus, nor does it appear exactly like that of other cytoplasmic inclusions. Probably they represent organelles that are characteristic of fish and amphibian erythrocytes, or some organelle that has undergone change with the maturation of the erythrocyte.—O. P. J.


Hemoglobin is usually considered to be restricted to the cytoplasm of the vertebrate red cell. (See Sondhaus and Thorell, Blood 16:1285–1297, 1960.) However, during the course of an investigation into the effects of chemical interference with RNA metabolism on hemoglobin synthesis in the early chick embryo, the author obtained some evidence that hemoglobin may be found within the nucleus. Presence of hemoglobin in the embryonic erythroblast was demonstrated histochemically by using o-dianisidine (3,3'-dimethoxybenzidine) as the H-donor for the pseudoperoxidase activity. The absence of deeply stained nuclei in the early stages of hematopoiesis and their increasing number as development proceeds, and the amount of hemoglobin increases, suggests a developmental sequence in which the synthesis of hemoglobin is initiated in the nucleolar region from which it passes through the nucleus into the cytoplasm. Whether nuclear synthesis precedes cytoplasmic synthesis in this system has not been established, although the evidence presented suggests that it does.—O. P. J.


The paper reports the results of studies comparing the values obtained for the total dry mass of individual human erythrocytes by interference microscopy and by x-ray microradiography with those for the mean corpuscular hemoglobin (MCH) obtained from determinations of erythrocyte count and hemoglobin. The mean difference between mean total dry mass per cell and MCH represents the mass of dry material other than hemoglobin in the red cell. This supports Ponder's view that other protein, in addition to hemoglobin, is present in the interior of the red cell, possibly in the form of an internal framework. —O. P. J.

ABSTRACTS

The effect of phenylhydrazine and related redox compounds on human erythrocytes and hemoglobin solutions has been studied. The authors find that the first change in hemoglobin on exposure to phenylhydrazine in vitro is the appearance of a compound with the spectroscopic characteristic of methemoglobin. This finding disagrees with those reported earlier by Warburg (Biochem. Ztschr. 242:170, 1931), but is in agreement with studies published recently in this Journal (Blood 16:1723, 1960). Next, a fast-moving component of hemoglobin on electrophoresis and column chromatography appeared. Shortly thereafter, a group of brownish-green compounds of varying solubility, referred to, for the sake of convenience, as "sulfhemoglobin" appeared. Finally, denatured products of hemoglobin with properties like those of Heinz bodies were found. The authors consider this process to be closely analogous to changes occurring in the in vitro aging of red cells and suggest that Heinz body formation may represent greatly accelerated aging of hemoglobin.—E. B.


Normal human washed red cells, when incubated at 37° in the presence of 2 x 10⁻²M sodium fluoride, were observed to become crenated at first, then spherical: at 6 hours almost all the cells were smooth spheres. The fluoride was removed by washing, and the cells were then incubated further in the presence of glucose, inosine and adenine; after 2 hours the cells had reverted to either a biconcave disc or a shallow cup form. This disc-sphere-disc transformation could be repeated several times. The adenosine triphosphate content of the cells was estimated, and it was found that when the level fell below two-thirds of the original value, the cells became spherical. At 6 hours the nucleotide had decreased to 20-25 per cent of the initial level. The resumption of a disc or bowl shape was shown to be accompanied by resynthesis of nucleotide. The progressive change of shape from disc to sphere in stored blood was also shown to be accompanied by a fall in nucleotide; again, the disc shape could be restored when materials for nucleotide resynthesis were supplied. This work provides further strong evidence that maintenance of the disc shape in red blood cells depends upon the regeneration of adenine nucleotides.—R. M. H.


In a variety of tissues, enzymes have been described which catalyze the conversion of free amino acids into corresponding aminoaoyl adenylates. In the present paper, the presence of such an enzyme system in human erythrocytes is described for the first time. An assay system for the detection of amino acid activating enzymes (AAAE) in erythrocytes is described. Hemolysate is incubated with buffer, MgCl₂, ATP, P³² pyrophosphate, an amino acid mixture, and sodium fluoride. After incubation, the radioactivity of ATP, isolated by absorption on charcoal, is determined. The level of the AAAE in erythrocytes from patients with macrocytic and microcytic hypochromic anemias was markedly increased as compared with normal values. A significant decrease in activity was observed in red cells obtained from patients with polycythemia vera. Differential osmotic hemolysis studies showed no correlation between red cell age and level of AAAE activity. The normal role of AAAE in other tissues is believed to be the activation of amino acids preliminary to the biosynthesis of proteins. It is entirely possible, therefore, that this enzyme system represents in erythrocytes a non-functional rudiment carried over from earlier stages of erythropoiesis.—E. B.


The authors have measured the following enzymes in the erythrocytes, bone marrow, and in peripheral blood and bone marrow plasma: phosphohexoisomerase, aldolase, lactic dehydrogenase, malic dehydrogenase, isocitric dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. As shown previously, plasma enzymes are greatly elevated in the megaloblastic anemias. However, only a modest increase in peripheral blood erythrocyte enzymes is found. In contrast, a marked increase was found in the marrow enzymes in megaloblastic anemias compared with blood loss anemia. Similarly, the bone marrow plasma showed considerably higher levels of released serum of enzymes than did the periph-
eral blood plasma. It is suggested that the high plasma levels of enzyme of carbohydrate metabolism which occur in megaloblastic anemia originate in the bone marrow. The authors have also studied plasma and erythrocyte enzymes in various hemoglobinopathies and in thalassemia minor. Substantial elevations of several of the enzymes were observed.—E. B.


X-irradiation in doses of 500, 700 and 1000 r was administered to 3 groups of rabbits. Determinations of carbonic anhydrase were carried out within the first 24 hours after radiation, then at several day intervals for 4 weeks or up to the death of the animal. All rabbits exhibited reduction in carbonic anhydrase activity during the first 24 hours after irradiation. No correlation could be established between dose and degree of anhydrase inactivation. To elucidate the mechanism of this phenomenon, the blood hemolysates were irradiated in vitro; no decrease of enzyme activity was found.—E. K.

HAEMOGLOBINURIA AMONG ADULT NIGERIANS DUE TO GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY WITH DRUG SENSITIVITY. H. M. Gilles, and A. C. Ikeme. From the Liverpool School of Tropical Medicine, England. Lancet 2:889–891, 1960.

Three cases of acute hemolytic anemia with hemoglobinuria are described, all occurring in young adult male Nigerians. In one case, hemolysis followed ingestion of mothballs: the other two patients had been taking tablets containing aspirin, phenacetin, and caffeine. The red blood cells of all three patients showed low glucose-6-phosphate dehydrogenase activity, as demonstrated by a rapid screening test. In only one of the patients were a few malarial parasites found. The authors suggest that, in Nigerians, some of the cases of hemoglobinuria commonly diagnosed as “black-water fever,” may in fact be drug-induced hemolytic anemia in subjects with this enzyme deficiency.—R. M. H.


A method is described for the determination of the blood methemoglobin content. Plasma is removed from red cells which are lysed with a saponin solution. Stroma is removed by centrifugation. Optical density readings are made at 630 millimicra before and after the addition of cyanide and a reading is made at the same wavelength on an aliquot of hemolysed red cells which has been treated with ferricyanide. Finally, a determination of the total hemoglobin is made. Advantages claimed by the authors for this method include the fact that it is not necessary to compute the extinction constant for calculating the amount of methemoglobin and that interference from turbidity due to stromal proteins and lipid substance in the plasma is eliminated. On the other hand, it must be pointed out that the well-known method of Evelyn and Malloy, especially when modified by the addition of a small amount of saponin, has given highly satisfactory results in most laboratories, including that of this reviewer, and requires fewer manipulations.—E. B.


A saline extract of normal rabbit kidney has erythropoietic activity in rats, as demonstrated by its ability to influence the number of blood reticulocytes and marrow erythroblasts. Spleen, liver and lung extracts, prepared in the same manner, have no effect at all.—G. M.


This paper presents the results of an investigation of the validity of a Cr51 study not exceeding 10 days. Cr51 activity is plotted logarithmically against a linear time scale, starting 48 hours after the injection of labelled cells. The initial early loss of Cr51 is disregarded, and the subsequent percentage loss per day is calculated by treating it as a single exponential over the following eight days. Accuracy was increased by bunching the samples at both ends of the study. It was found that in normal subjects, the rate of Cr51 loss did not exceed 4.25 per cent per day; patients with undoubted hemolysis, on the other hand, showed rates of loss varying from 5.5 per cent to 19 per cent per day. The authors consider that, using
their standard disposition of blood samples, rates of daily Cr\textsuperscript{51} loss higher than 5 per cent can be regarded as abnormal although the mean cell life cannot be reliably calculated. While this method is of value when only a short period of study is possible, it may well give misleading results in those cases of mild hemolytic anemia where survival figures are at first within the normal range, but later fall below it, or when a dual population of red cells is present. When the mean cell life is suspected of being only slightly shortened, the period of study should be extended.—R. M. H.


Two kinds of Fe-porphyrin complexes can be distinguished: ionic complexes with large numbers of unpaired electrons present, such as hemoglobin and myoglobin, and covalent complexes with no or few unpaired electrons such as oxyhemoglobin and ferrocytochrome C. The authors have calculated the energies and types of occupied and empty molecular orbitals in the two types of ferroporphyrins. Ionic ferro- and ferriporphyrins have low HOMO and LEMO-energies and should be good electron donors or acceptors. Covalent ferroproteins have high energies, and are good donors of electrons, whereas ferriproteins have a low LEMO energy and are thus mainly electron acceptors. A shuttle of cytochromes between the ferrous and ferric states would thus explain their electron-carrying function. Total iron electric charges are 0.520 e in ionic complexes, 2.472 e in covalent ferrous complexes. (Abstractors note: Low charge in iron-porphyrin such as hemin and hematin may explain Mossbauer effect recently demonstrated in these substances).—P. G. R.


The penetration of iron into the red blood cell and its utilization for heme synthesis have been studied by incubation of human erythrocytes with plasma containing tagged iron. The entry of iron into the cells does not seem to depend upon its further utilization for hemoglobin production, since, in certain cases, good penetration has been found in old cells. Likewise the phenomenon does not appear to depend upon the total quantity of iron present in the incubation medium, or upon the degree of siderophilin saturation. It is not an equilibrium reaction, since the non-heme red cell iron does not come out of the erythrocyte when the medium contains unsaturated siderophilin. There seems to exist a peculiar plasma activity, dependent in some way upon the proteins, which favors the penetration of iron into the red cell, and then the synthesis of hemoglobin.—G. M.

**DETERMINATION OF BLOOD COBALT LEVEL IN CHRONIC POST HEMORRHAGIC ANEMIAS.** K. Wysoki, A. Smoczkiwiczowa, W. Mizgalski and R. Kabza. From the School of Medicine, Poznan, Poland. Polski Tyg. Lek. 15:1899, 1960.

The level of cobalt in plasma and red blood cells of patients with chronic post-hemorrhagic anemia was investigated. The cobalt content was estimated by the chromatogram-spectrographic method based on Thier's technic. It has been found that the level of cobalt decreased both in plasma and in erythrocytes. The average decrease in plasma was 27 per cent and in red cells 11 per cent relative to the cobalt content of a normal adult person. No relationship was been found between the severity of anemia and the level of cobalt nor between the content of serum iron and the cobalt content.—E. K.

**SEVERE REACTION TO IRON DEXTRAN.** I. J. Forbes, Department of Medicine, University of Adelaide, South Australia. Med. J. Australia 2:500, 1960.

This is a case report describing clinical manifestations of toxicity to iron dextran (Imferon) given intramuscularly. The patient was a woman, aged 42 years, who developed urticaria after the sixth daily injection. Generalized lymphadenopathy, hepatomegaly and splenomegaly were found. Lymph node biopsy showed globose of unidentified substance in the cytoplasm of the sinusoidal macrophages, and liver biopsy revealed abnormal pigment granules in the parenchymal cells. The patient recovered completely.—G. C. de G.


The mean urinary excretion of radioactive 60CoB\textsubscript{12} in 30 Bantu women with megaloblastic
anemia following pregnancy was not significantly different from that found in a control group of 20 nonanemic women. All subjects in both groups were within one year of delivery, and all were still lactating. It is concluded that this form of megaloblastic anemia is not due to malabsorption of B₁₂.—R. M. H.


Of 77 patients with megaloblastic anemia of pregnancy characterised by a frankly megaloblastic marrow, 60 per cent were of blood group A, compared with an incidence of 42 per cent in 3500 routine antenatal cases in the same district. This difference is significant. The group A incidence of 98 pregnant women with minor grades of megaloblastic change in the marrow did not differ from the control group. It is suggested that the patients with advanced megaloblastic anemia of pregnancy constitute a special group in which there is an inherited predisposition to the disease.—R. M. H.


This paper gives the case report of a 48 year old epileptic woman who developed megaloblastic anemia and subacute combined degeneration of the spinal cord while on treatment with dilantin and phenobarbitone. The patient had folic acid deficiency combined with vitamin B₁₂ deficiency and latent iron deficiency. The authors state that their findings "confirm that more than one factor operating at the same time may be responsible for the development of megaloblastic anaemia in a patient receiving anticonvulsant drug therapy. Vitamin B₁₂ deficiency in such cases cannot be excluded unless the serum vitamin B₁₂ level is found to be normal."—C. C. de G.


This paper gives the case report of a 29 year old woman with infiltrative eosinophilia of the stomach and small bowel with associated macrocytic anemia which responded to the administration of vitamin B₁₂.—C. C. de G.


In primary cultures of human fetal lung and kidney the cells were capable of fixing blood group antibodies corresponding to the ABO group of the fetus. Antibody fixation was observed by the mixed agglutination technique. This capacity seemed to be lost after repeated subcultivation of the cells.—O. P. J.


A rewarding discussion of the theory and present status of the agglutination of erythrocytes by lactins in protein extracts.—R. E. R.


In an important contribution to the subject, the investigators separated saline and albumin Rh agglutinins from each other by (1) moving-boundary electrophoresis, (2) DEAE cellulose column chromatography and (3) mercaptoethanol destruction of macroglobulins. When saline agglutinins were recovered from a serum not revealing this activity prior to separation of γ₁- and γ₂-globulins, the γ₂-globulin fraction specifically blocked γ₁ saline agglutinins. Rabbit antibody to Waldenström macroglobulin, removed saline agglutinin activity but did not affect albumin agglutinins. On the other hand, anti-γ₂ (rabbit antibody to the heat eluates of Rh positive erythrocytes which had been sensitized with albumin agglutinins) removed albumin agglutinin activity but had no effect on Rh saline agglutinins. After removal of mercaptoethanol from 19S fractions that were obtained by DEAE column chromatography, saline agglutinin activity was not recovered; after papain digestion of γ₂ globulin fractions, albumin agglutinins could no longer be demonstrated. In both instances specific sensitization of erythrocytes for the antiglobulin test was retained, implying antigenic determinants near the specific combining sites of each antibody molecule. These results differed from those of Fudenberg (in Addendum), who studied the same human 19S agglutinins, and of Porter (Biochem. J. 73:119, 1959) who
ABSTRACTS

studied goat antibodies toward papain-digested rabbit Y_{2}-globulin antibodies.—R. E. R.


The hemoglobin content of three samples of blood was determined spectrophotometrically as cyanmethemoglobin, \(\alpha\)-hemoglobin and pyridine hemochromogen. The weighted means of the ratios between the values obtained with the three assay methods were: pyridine hemochromogen/cyanmethemoglobin = 0.986, and pyridine hemochromogen/\(\alpha\)-hemoglobin = 0.990. The pyridine hemochromogen determination is believed to give the correct value, but the differences between the methods are small enough to be negligible in routine work.—S. A. K.

MISCELLANEOUS


Two genetic polymorphic systems present in humans, haptoglobins and transferrins, have been studied in chimpanzees, monkeys, baboons, mice and fish. Haptoglobins were also studied in other species. Haptoglobin polymorphism was not found in primates other than humans. A polymorphism for transferrins exists in monkeys, but it includes proteins different from those found in human polymorphism. A thyroxin-binding protein in the prealbumin region occurred in three different positions in monkeys; this may also constitute a polymorphism.—H. H. F.


The transferrin groups have been studied in twenty-two monkeys using \(\text{Fe}^{59}\) and starch gel electrophoresis. Transferrin \(a\) was found two times, \(b\) three times, \(c\) seven times, \(d\) four times, \(e\) six times, \(f\) four times, \(g\) two times and \(h\) two times. Furthermore, the presence of a protein is noted with a mobility greater than that of “fast-\(a_2\)”. The transferrins showed a greater polymorphism in monkeys than that found in human beings. These findings may be of importance for the interpretation of phylogenetic data as well as for genetic and serological studies in monkeys.—G. M.


This paper gives an account of the investigation of the haptoglobin variants in a sample of 518 natives from the New Guinea Highlands, mostly from the eastern region. The \(\text{Hp}\) frequency is in the higher range of those so far reported for various populations, and the transferrin \(\text{TfD}_1\) frequencies are perhaps the highest known at present. No striking regional differences are apparent in this material, with the exception of a high frequency of haptoglobin-negative subjects in one area, which is discussed in relation to the effect of malaria on haptoglobin concentration.—G. C. de G.


This paper describes the localization of dehydrogenase activity in the cells of normal human blood and bone marrow by employing nitro BT as the tetrazolium salt, with or without the addition of a non-ionic surface active agent (Renex, Atlas Powder Co., Wilmington) to the incubating solution. The latter was employed because of the possibility that the tetrazolium salt was unable to pass through plasma and mitochondrial membranes. Endogenous dehydrogenase activity was observed in the mitochondria of most of the cells of the blood and bone marrow but the addition of succinate to the incubating solution definitely increased mitochondrial reactivity. Enzymatic inactivation by heat, iodoacetic acid, and ethyl maleimide inhibited the tetrazolium reaction. The localization of histochemically demonstrable dehydrogenase activity obtained in these studies conforms with biochemical evidence that has established the presence of Krebs cycle activity in human leukocytes.—O. P. J.


Although there is a relationship between RNA content and the uptake of amino acids into pro-
teins, the RNA content does not always reflect the degree of metabolic activity. Human bone marrow lends itself extremely well to a simultaneous investigation on the method of both RNA and protein because of the coexistence of different cellular classes at various stages of maturation and differentiation. Six normal bone marrows were studied by a high resolution autoradiographic technic utilizing labelled uridine, DL-Leucine and DL-B-phenylalanine as precursors. For the study of H3-uridine incorporation into RNA, H3-thymidine was also added to the sample. For all of the precursors, the incorporation increases linearly during the 3 hours of incubation, it occurs in all elements and progressively decreases with maturation and differentiation. Uridine incorporation appears to be the real index of RNA metabolism. Leucine and phenylalanine appeared to be incorporated both in the cytoplasm and in the cytoplasm at a rate of 5 to 1 respectively. The results indicate a strict interrelationship between RNA and protein metabolism.—O. P. J.


Certain aspects of hemato logical and immunological recovery of the irradiated, bone marrow-treated animal could be better studied if functional cells of a single type from the marrow or spleen could be isolated in reasonable quantity. A differential gradient centrifugation method, which makes use of differences in sedimentation rates in either bovine serum albumin or Kcl F-1 (fluorocarbon oil), layered bone marrow cells into 4 cell layers. Each layer was capable isologously of promoting survival of heavily irradiated mice; none was more effective than fresh whole bone marrow. Two mononuclear cell types along with many erythrocytes were concentrated in layer B. They were presumably capable of hemopoietic repopulation because they protected heavily irradiated mice.—O. P. J.


It has been possible to investigate the survival of cells frozen and thawed in various ways by injecting them into lethally irradiated mice, since recovery is caused by the proliferation of viable donor cells. Since non-electrolytes which can penetrate and are non-toxic are supposed to protect the cell at a concentration that is in direct proportion to the molecular weight, amino acids with their varied molecular structure and solubility in saline are suited for a study of the relation between structure and protective activity. Sixteen amino acids and one related compound were tested at 6 different concentrations in mice 12 to 16 weeks old. Correlation of survival with molecular weight and structure were found within groups of compounds having similar chemical structure. Compounds possessing a free-SH group, those basic in reaction and one with one amino and amide group did not protect. The difference between the results of mammalian red blood cells and for mouse bone marrow is probably only a reflection of the difference in the criteria of damage; namely, hemolysis versus the ability to reproduce.—O. P. J.


Electron-microscopy of the originally methyl-cholanthrene-induced MC1M tumor, when grown as an ascites tumor, showed same organelles as in normal cells plus a light perinuclear zone with fine paired lamellae.—F. G. R.


This profusely-illustrated paper reports a morphological study of white-cell concentrates, prepared by sedimentation in dextran and stained by May-Grunwald-Giemsa; the whole of the deposit was examined in every case (140 patients with malignant disease and 60 control subjects). The authors point out that many of the reports of malignant cells in blood smears in the past have been based on an inadequate appreciation of normal appearances, in particular some cellular elements found normally, especially megakaryocytic nuclei, may be confused with cancer cells by the inexperienced. Tumour cells were identified from the blood of only 7 out of 140 cases of malignant disease, and all these patients were in a terminal state. In patients with operable carcinoma, on the other hand, tumour cells could never be demonstrated—even if the blood sample was taken from the vein draining the neoplasm. The paper includes a full discussion, with illustrations, of the normal and abnormal appearances to be found in white-cell concentrates.—R. M. H.