ABSTRACTS OF SPECIAL INTEREST


On starch block electrophoresis, rabbit hemoglobin fractionates into a main fraction $L_1$ and a minor fraction $L_2$, incompletely separated. The use of valine-$\text{C}^{14}$ and arginine-$\text{C}^{14}$ as labels for hemoglobin has shown that the specific activity of the main fraction varies with time: a rapidly ascending curve up to the 5th day, then a plateau until the 50th day, followed by a decrease. The specific activity of the minor fraction, however, shows no plateau. It increases until the 40th day and then exceeds the main fraction. The only possible interpretation is that the minor fraction represents an aged fraction of hemoglobin, for simple metabolic exchanges between the major and minor fractions could only lead to an equalization of their specific activities. During aging of the red blood corpuscle, hemoglobin itself undergoes modifications: the nature of this aging process is discussed.—G. M.


One of the pathways of histidine catabolism is to urocanic acid, formimino-glutamic acid and glutamic acid. The last step requires a competent folic acid co-enzyme to act as acceptor of the formimono-group. Attention has been focused on the urinary excretion of formimino-glutamic acid as an index of folic acid function in man. The authors find that the excretion of formimino-glutamic acid in the urine is usually accompanied by the excretion of urocanic acid and frequently urocanic acid is the predominant histidine derivative appearing in urine.—I. C.


EDTA appeared to have a colchicine-like effect on chromosomes. Thus, leukocyte concentrates of blood or marrow taken into EDTA as the anticoagulant gave adequate chromosome preparations without the need for preliminary culture. Chromosomal abnormalities in leukemia were confirmed by this method.—I. C.

COMPARISON OF EFFECTS OF PROTAMINE AND POLYBRENE, WITH SPECIAL EMPHASIS ON THE FACTOR VIII (ANTI-HEMOPHILIC GLOBULIN)
ABSTRACTS

From the Memorial Blood Bank of the San Francisco Medical Society, San Francisco, Calif.

Studies on dogs revealed that when protamine or Polybrene in a dose of 3 or 5 mg. per kilo was rapidly administered intravenously, profound hypotension was induced which lasted for several minutes and was not influenced by the presence or absence of heparin. There was also a brief tachycardia followed by a protracted bradycardia, and a pronounced slowing of respiration. Both drugs induced marked thrombocytopenia with a slow return to normal levels. Polybrene neutralized more heparin than a comparable dose of protamine, while the cephalin time were found to be abnormal and that found when comparable amounts of these agents were added to blood in vitro. In addition to clotting times, serum prothrombin consumption and cephalin time were found to be abnormal even after Polybrene or protamine activity could no longer be demonstrated in the blood (one hour after administration). It was found that factor V was elevated and factor VIII was markedly depressed. The mechanism for the decrease in factor VIII could not be determined, but it was found that slow administration of the drugs resulted in less of an effect upon the factor VIII level, and that administration of a second dose did not further depress the factor VIII activity. When heparin was injected prior to polybrene there was no change in factor VIII from the control value, but when heparin was injected 10 minutes after polybrene, it had no effect on the course of the low factor VIII levels. It is suggested that similar factor VIII deficiencies may be induced in humans by the use of excessive doses of heparin antagonists following open heart surgery.—R. G.

ERYTHROCYTES


Histochemical methods were used to study the presence of the enzymes, succinate dehydrogenase and cytochrome oxidase, in the buccal mucosa of patients with iron-deficiency anemia. Only cytochrome oxidase was absent in some cases of iron deficiency anemia, and in these it was restored within 72 hours of parenteral iron therapy.—I. C.

STUDIES SUGGESTING THE PRESENCE OF INTRINSIC FACTOR IN BILE. V. Herbert and M. E. Kaplan.

Human and to a lesser extent rat bile enhances CoB12 uptake by rat liver homogenate. Some rat bile specimens enhance CoB12 uptake by everted sacs of rat small intestine. Some rat bile specimens appear to substitute for rat intrinsic factor in a gastrectomized rat. In Ouchterlony agar double diffusion analysis, a reaction of identity was formed by human bile and intrinsic factor concentrates from hog, rat, and human sources to antibody induced by injection of purified hog intrinsic factor concentrate. If these findings in fact indicate that bile contains intrinsic factor, it is possible that an enterohepatic circulation of intrinsic factor exists as a further phase of its activity with relation to vitamin B12.—V. H.


Radioactive vitamin B12 was injected into the rat and the products obtained from a cannula in the bile duct were analyzed. Seventy five per cent of the radioactivity in the bile was B12 and the remaining 25 per cent analogues of B12. It is suggested that, whereas the B12 is reabsorbed from the gut, the analogue will be excreted. If this proves correct in man it would cast doubt on the fecal excretion method as a test of B12 absorption.—I. C.


Schilling tests were carried out on eight cases with megaloblastic anemia due to anticonvulsant drugs before therapy with folic acid and again after treatment. In six cases the subsequent tests showed an increased urinary excretion of radioactivity as compared to the first test. Free acid was usually present in the gastric juice, and it was concluded that the improved B12 absorption was due to an improvement in gut function following folic acid therapy. The results are difficult to interpret. Carbachol (a stimulant of intrinsic factor
secretion) was not used; nor is any information available about renal function and serum B₁₂ levels. Paired Schilling tests performed in seven nonanemic control subjects (and not having pernicious anemia?) showed that half the tests gave abnormal results. The authors’ hypothesis remains unproved.—I. C.


Intestinal absorption of radioactive vitamin B₁₂ was poor and serum vitamin B₁₂ levels were low in both untreated and folic acid-treated patients with tropical sprue. There was usually a gradual rise in serum vitamin B₁₂ with prolonged folic acid therapy. Ten patients treated with 30 μg vitamin B₁₂ weekly had excellent clinical and hematologic responses, equaling the response usually observed with folic acid. The authors summarize that “vitamin B₁₂ was found therapeutically as effective as folic acid in the treatment and management of patients with tropical sprue.” However, it is possible that 30 μg of vitamin B₁₂ weekly is a sufficiently large dose to partially compensate for a continued deficiency of folic acid. It is also interesting that neurologic lesions were not observed in any of the patients studied, although paresthesias occurred in 18 per cent of almost 100 patients with malabsorption syndrome, many of them from Puerto Rico, studied in New York (J. Mt. Sinai Hosp. 24:296, 1957).—V. H.


A patient with clinical scourvy, macrocytic anemia, a low serum folic acid activity and a slightly elevated urinary formiminoglutamic acid excretion manifested a megaloblastic bone marrow and a marked increase in formiminoglutamic acid (FIGLU) excretion within two weeks on a vitamin-free diet. Intravenous injection of large daily doses of synthetic ascorbic acid failed to prevent progression of this process. Subsequently, small doses of synthetic pteroylglutamic (folic) acid (50 μg. intramuscularly a day) initiated hematologic improvement, which may or may not have been augmented by subsequent therapy with other components of the vitamin B complex. The findings in this patient suggest that in patients with borderline folic acid deficiency, overt megaloblastic bone-marrow changes may develop within two weeks when they are totally deprived of dietary folic acid, that ascorbic acid per se has no hematopoietic effect in the presence of such a deficiency and that a total daily dose of 50 μg. of pteroylglutamic acid, which may induce a suboptimal hematologic response in the subject with megaloblastic anemia who is deficient in folic acid, is therefore probably in the range of the minimal daily adult requirement for this form of folic acid. In therapeutic trials, daily doses of 50 to 100 μg. of pteroylglutamic acid given parenterally may be preferable to larger doses.—V. H.


Urinary excretion of folic acid was estimated using the Streptococcus fecalis assay of Capps et al. Nonanemic tapeworm carriers showed lower excretion than normal controls but not as low as carriers with megaloblastic anemia. It is suggested that the tapeworm may compete with the host for folic acid as well as for vitamin B₁₂.—S. A. K.


Red cells from fetuses with hydrops and erythroblastosis fetalis whose hemoglobin contained an electrophoretically fast moving component (Hb Bart’s) showed sickling phenomenon resembling the holly leaf formation in sickle cell trait. The phenomenon was not dependent on de-oxygenation.—I. C.


The resistance of fetal hemoglobin in red cells to elution by acid phosphate buffer was used to scan maternal blood for cells of fetal origin. This was found in 18 per cent of antepartum women and 17 per cent of postpartum mothers. The highest incidence followed forceps delivery.—I. C.
ABSTRACTS


1. Immuno-electrophoretic analysis showed the presence of 12 antigens, only two of which corresponded to hemoglobin fractions. Catalase constituted a third antigen. Four other antigens, although possessing no enzyme activity demonstrable by immuno-electrophoresis, seemed to be alies-terases: they had in gel electrophoresis bands identical to those of four esterase fractions demonstrated in normal human hemolysates. Enzyme techniques showed AChE in red cell stroma. On the other hand, no soluble fraction seemed to be AChE. 2. A protease was extracted from red cell stroma with butyl alcohol. Its activity was demonstrated by incubation with Hb denatured by HCl. Electrophoretic study of this fraction showed its localization in the area corresponding to serum albumin. Immuno-electrophoretic analysis showed a line of precipitation at the level of this protease, which could not be proved as the responsible antigen.—H. F.


The surface of the normal erythrocyte by electron microscopy appears as a series of plaques 200–500 Å in diameter. After sensitization with anti-D the diameter of the plaques increased to 500–800 Å. The red cells from patients with cancer often showed the larger type of plaque. This suggested that the cells had an abnormal protein on the surface. This could be correlated with a weakly positive antiglobulin test in these patients.—I. C.


In a series of 122 matings which resulted in abortion, ABO incompatibility was present in 45 per cent (control series 30 per cent). Hemolysins against A, B and O cells were present in 59 per cent of the mothers (control series 9 per cent).—I. C.

LEUKOCYTES


Cytoplasmic granules of the polymorphonuclear leukocyte contain the antimicrobial agent phagocytin and a number of digestive enzymes. Degranulation and thus a release of these agents into the cytoplasm or within the phagocytic vacuole accompanies phagocytosis. The present paper reports the use of guinea pig peritoneal exudate cells and the insoluble starch granules of Amaranthus cruentus as a test particle. In these studies, the use of inert starch particles eliminated the possibility of degranulation due to any substrate, or carrier-induced lysis of granules.—O. P. J.


Cellular physiologists have shown considerable interest in tension at the surface of living cells with particular reference to cellular morphology and to ameboid and cytokinetic movements. The morphology of mast cells in rat peritoneal fluid undergoes a progressive change with aging of the animal. Mast cell populations in rats of different ages showed that deformed cells of older rats are slower to regain sphericity than their counterparts from young animals. The results indicate that nonspherical mast cells normally encountered in peritoneal fluid of older rats can become spherical in vitro just as centrifugally deformed cells do, so that the experimental design appears to resemble closely the physiologic situation. Mast cells of young rats behaved as if invested with an elastic envelope.—O. P. J.


In previous reports it was shown that oxygen uptake of polymuclear leukocytes incubated in glucose containing media is (a) dependent on the presence of a suitable CO₂ tension, and (b) cannot be completely accounted for by the cytochrome chain activity. The present paper deals
with experiments showing that respiration of leukocytes in bicarbonate-CO₂ medium is inhibited by amylal and is unaffected by antimycin. This would be in line with the hypothesis that malic enzyme and malic-dehydrogenase, coupled with extra-mitochondrial TPN-dependent reactions, may represent one of the main systems of reoxidation of external reduced pyridine-nucleotides.

—O. P. J.


A comparative study was made of the reductase activity of leukocytes of mice, guinea pigs, rabbits, and of human blood following incubation at room temperature in a pipette with tetrazole INT. It was noted that leukocytes of mice showed the greatest amount of reductase activity, that leukocytes of rabbits and human donors showed an intermediate amount of activity, and that leukocytes of guinea pigs showed the least amount of activity. Reductase activity was observed to vary between different species, between leukocytes, and between different human donors.—O. P. J.


The glycogen content of leukocytes from 25 non-diabetic fasting and resting subjects averaged 148 ± 9 mg. per 10¹⁰ leukocytes. The leukocytes of 14 diabetic patients in poor control without insulin contained 111 ± 10 mg. of glycogen per 10¹⁰ leukocytes.—S. A. K.


Using an antiglobin consumption method, the authors were able to demonstrate that sera from patients with D.L.E. possessed antibodies not only against leukocyte nuclear material but against cytoplasmic protein as well.—I. C.

CHROMOSOME DAMAGE DURING NITROGEN MUSTARD THERAPY. P. E. Conen and G. S. Lansky.


Chromosomal abnormalities were demonstrated by leukocyte culture in a man with bronchogenic carcinoma after he had been treated with nitrogen mustard.—I. C.


A family is described in whom three sibs had died, two from acute leukemia and one from bronchopneumonia. The surviving sib was shown to have a sex-chromosome abnormality (XY/XXY type). The parents were clinically normal and had an apparently normal chromosome complement.—I. C.


Serial immuno-electrophoretic studies of sera from myeloma patients have shown that, as the myelomatosis progresses, immune proteins disappear, and clinical symptoms develop corresponding to an antibody deficiency state. Furthermore, in patients with γ-type myeloma, an increase of the α-globulin subfraction, especially the ceruloplasmic fraction, and of the prefraction was observed in every determination.—H. H. F.

HEMOSTASIS


Autoprothrombin II activity developed when prothrombin preparations of human origin were activated with purified thrombin at pH 8.2. Early during the activation an inhibitor seemed to form. Concentrates of the autoprothrombin II could replace serum in the thromboplastin generation test, provided platelets were used, but not if a soy bean phosphatide was used. Dogs were given Coumadin in doses that lowered their own autoprothrombin II concentrations practically to zero. Then, while continuing the anticoagulant, purified
AUTOPROTHROMBIN was infused intravenously. This was tolerated well and the autoprothrombin II concentration stayed at normal levels for 7 hours; in 24 hours none remained. The infusion was followed by a shortening of the whole blood clotting time, although the prothrombin time was still long. Autoprothrombin II concentrates had a procoagulant effect on blood and plasma from persons with hemophilia A as well as hemophilia B. — R. G.


Studies were carried out to determine the effect of Polybrene on various stages of blood coagulation. In concentrations comparable to dosages administered clinically, Polybrene prolonged whole blood clotting, probably by interfering with the production of "thromboplastic" activity, thus hindering conversion of prothrombin to thrombin. The degree of inhibition of coagulation was found to be inversely related to the number of platelets present. In the thromboplastin generation test, polybrene was found to be inhibitory. Its inhibitory power was counteracted by heparin, platelets, and serum factors, particularly prothrombin, factor IX, and to a lesser extent, platelet-like activity of serum, but not by crude cephalin or adsorbed plasma. Polybrene was found to be fibrinoplastic in the thrombin-fibrinogen reaction, but did not itself precipitate fibrinogen from plasma. In relatively higher concentrations it delayed the one-stage prothrombin time and agglutinated red cells. — R. G.


Iodinated dog fibrinogen was infused into normal dogs, and the fibrinogen 1131 of each was measured as the amount of radioactivity clottable by thrombin or precipitated at 56°C. The half-life was 2.4 days. Fibrinogen was rapidly distributed between intra- and extravascular compartments, being demonstrable in the lymph by 30 minutes after infusion. Equilibration, as determined by the change in slope of the disappearance curve, was reached in approximately 24 hours; at this point 66 per cent of the infused fibrinogen 1131 remained in the plasma. Antiocoagulants (heparin or Coumadin) did not effect fibrinogen survival time. A fibrinolytic agent caused marked fibrinogenolysis. Infusion of dog brain thromboplastin resulted in the appearance of an anticoagulant which interfered with clot formation on addition of thrombin. — R. G.


This method is based upon the photoelectric measurement of the turbidity produced when fibrinogen is polymerized by the action of thrombin. Plasma is diluted 12-fold in a barbitone-saline buffer, a calcium-thrombin solution is added, and after 20 minutes the optical density of the solution is read at 470 μm wave length. The fibrinogen can then be calculated from the formula given. The method was accurate to within approximately 5 per cent over a wide range of fibrinogen concentration when compared to chemical methods. — R. G.

SEDIMENTATION OF PLASMA ANTHEMOPHILIC FACTOR. G. M. Theilin and R. H. Wagner. From the University of North Carolina School of Medicine, Chapel Hill, N. C. Arch. Biochem. 95:70, 1961.

Purified bovine plasma antihemophilic factor preparations and canine plasma were sedimented in a swinging bucket type rotor of a preparative ultracentrifuge. A technic is described for sampling contents of the tubes for analysis after sedimentation. Sedimentation diagrams and S20,w values are presented for AHF and fibrinogen at 40.21°C. It was shown that AHF in canine plasma probably exists in an associated form, either as a polymer or associated with fibrinogen. Different degrees of association seemed to exist in bovine fractions, with the degree of association depending upon the ionic strength of the solution. The S20,w value of 4.3S probably represents the sedimentation coefficient of the AHF monomer. — R. G.


New method with fairly good reproducibility (coefficients of variation 10, 8, and 5 per cent
for antihemophilic factor A, B, and C respectively).
—S. A. K.

THE ASSAY OF HEPARIN IN THROMBIN SYSTEMS.

The anticoagulant effect of heparin, as measured by the prolongation of thrombin time, is sensitive to variations in pH, temperature, ionic strength, calcium ions, and various proteins. The protein effect may influence the results of the heparin tolerance test: a plasma which contained large amounts of macroglobulins showed increased heparin tolerance but was normalized in this respect when the pathologic protein was removed. None of the factors mentioned influence the titration of heparin with Polybrene, a synthetic antiheparin.—S. A. K.


These studies confirm earlier studies that factor VII activity is increased during the last trimester of pregnancy. In addition, this study demonstrated that factor X is also frequently elevated. In some cases neither was specifically elevated, yet the orthodox Owren assay procedure, which measures both, revealed elevated activities, suggesting another, as yet undetermined, coagulation factor concerned with prothrombin activation by thromboplastin. In contrast, plasma prothrombin was rarely, if ever, found to be increased. The elevated levels of factor VII and X seemed not to reflect "activated" material, but more probably, increased levels of the relatively inert precursors which can be profoundly activated by thromboplastin.—R. G.


Results obtained in guinea pig, dog, and man show that pretreatment with barbiturates may antagonize the hypoprothrombinemic effect of coumarin anticoagulants. This effect has been correlated with lower plasma levels of the anticoagulant. In man, in contrast to what was found in guinea pig and dog, barbiturates did not have an inhibitory effect upon parenterally administered coumarin anticoagulants, whereas they did result in lower plasma levels of the anticoagulants and a decrease in hypoprothrombinemics effect when the coumarin was given orally.—R. G.


PTA levels were found to be normal in patients receiving Dicumarol, Coumadin, or phenylindanedione and depressed in patients with Laennec's cirrhosis. The degree of depression in this latter group paralleled the prothrombin time value.—R. G.


Of 736 patients treated for acute myocardial infarction between July 1946 and Dec. 1957, 583 were studied over a two-year period or until death. The patients were divided into three groups: Group I were treated with long-term anticoagulants, group II served as a control, while group III were treated for about half of this period and served as control for the remainder of the time. The two-year mortality was 13 per cent for group I and 26 per cent for group II. It was further observed that a significant reduction in mortality was also manifest for the males or females alone, patients with or without previous myocardial infarction, and those with hypertension. While patients with previous infarction showed a more dramatic benefit from long-term anticoagulant therapy, a significant benefit occurred even after the first infarction. The patients treated with anticoagulants during only part of the two-year study had a markedly lower incidence of recurrent infarction, and death while on therapy than while off therapy. When all patients were included, the treated patients had a mortality rate of 5.6 per cent compared to 14.4 per cent per year respectively. There was some evidence to suggest that there is an increased danger of recurrent thromboembolic episodes during the first month after cessation of anticoagulant therapy. In the small group of diabetics studied, anticoagulant therapy appeared not to be of any benefit.—R. G.
ABSTRACTS


Changes in platelet levels of patients undergoing cardiac surgery with extracorporeal circulation were studied. The lowest platelet counts (average of 40 per cent of preoperative levels) were reached on the second post-operative day and recovery to preoperative levels took seven to eight days. The heparinized blood used could be stored for 24 to 48 hours without altering the patients' platelet responses. A few times, blood less than four hours old was used and a similar fall in platelet levels was found. The authors feel the data suggest that both donor and patient platelets were damaged by passage through the pump oxygenator. In patients perfused with one-day-old heparinized blood, there was no relation between postoperative transfusion requirements and the postperfusion in vitro coagulation tests done. With the use of the transfusion requirement as an index of possible bleeding tendencies, the perfusion of two-day-old blood resulted in no greater incidence of clinical bleeding than perfusion of one-day-old blood.

—R. G.


The case is described of a woman with idiopathic thrombocytopenic purpura who had had four pregnancies; in three the infants showed purpura and thrombocytopenia. Using an antigenlobulin consumption test, antibodies were demonstrated in the serum of both mother and child. The antibodies in the infant were no longer detectable after the 14th week.—I. C.

MISCELLANEOUS


Attempts to culture bone marrow by a variety of technics have resulted in the development of fibroblast-like cells (F.L.C.). Recently the enzyme collagenase, derived from Clostridium histolyticum, has been utilized by tissue culture workers to produce disaggregation of fibrous tissue. The present work was undertaken to determine whether collagenase can inhibit these cells, allowing cultivation of hematopoietic cells without fibroblastic overgrowth. The results indicate that collagenase in concentration of 0.2 mg./ml. of medium did not appear detrimental to the hemic cells, but it did inhibit the formation of F.L.C. in marrow cultures. —O. F. J.


After the addition of colchicine to cultures of Chinese hamster "fibroblasts," the medium was decanted and replaced with hypotonic saline. Within three or four minutes, cells arrested in metaphase became dislodged from the glass. Several hundred cells in about 0.5 ml. of medium were drawn into the tip of a braking pipette and dipped into a reservoir of glacial acetic acid, and an equal volume was drawn up. After 5 to 10 seconds, the entire contents were expelled onto a clean microscope slide over which a drop of 3:1 (alcohol:acetic acid) fixative had been allowed to spread a few seconds earlier.—O. P. J.


The hematologic evolution of 13 isologous radiochimeras and nine heterologous radiochimeras have been studied in the mouse. The isologous radiochimeras recover their normal hematologic values after 30 days. The heterologous radiochimeras show: (a) anemia with compensating erythropoietic activity due to increased peripheral destruction followed, on the 30th day, by relative medullary insufficiency; (b) polymorphonuclear leukocytosis and a marked lymphopenia; (c) moderate thrombocytopenia. These results are similar to those observed in human allogenic radiochimeras.—H. H. F.

The author noticed that there were apparent differences in the blood color and content of *Amblystoma tigrinum* taken from three different localities. No correlations between the altitudes of ponds from which they were obtained nor the average body weight were found. The problem of fragmentation of the cytoplasm of red blood cells had to be considered in the study of hematocrit determinations and cell numbers. The most consistent uniformity in readings or counts was the differential blood picture. The average counts were lymphocytes 92 per cent; neutrophils, 4 per cent; monocytes, 3 per cent, and eosinophils, 2 per cent. —O. P. J.


Intestinal absorption of fat after exchange transfusion in a child with congenital absence of β-lipoproteins has been studied. Absorption of fats is not modified by the increased level of β-lipoproteins. Deficiency in fat absorption therefore is not a consequence of the lack of β-lipoproteins in the plasma. This experiment permitted the study of the life span of injected lipoproteins. The space of diffusion of β-lipoproteins equals that of the blood volume while the diffusion space of α-lipoproteins is much less. The half-life of α and β-lipoproteins given by exchange transfusion is identical and is about 30 hours. —H. H. F.


A pathologic macroglobulin was isolated from a serum and then dissolved in 0.15 M NaCl at pH 6.5. Several physicochemical measurements have been made in order to get the morphologic features of this protein. It appears spherical with a mean radius 100 Å and a molecular weight average of 750,000. However, some differences exist between this protein and the classical globular proteins as to internal hydration and polydispersity. The weight of bound water is twice that of the dry protein, in good agreement with qualitative x-ray analysis. From sedimentation measurements the distribution of hydrodynamic radii was determined and comparison was made with direct photography obtained by electron microscopy. From these results a schematic picture of the macroglobulin is given. —H. H. F.
ABSTRACTS