ABSTRACTS OF SPECIAL INTEREST


The authors obtained rabbit antisera against whole normal, hemophilic, and von Willebrand plasma. The rabbit antihemophilic antiserum gave a double γ-globulin line with hemophilic plasma (except with circulating anticoagulant), usually with normal plasma, and never with von Willebrand plasma. They discuss this finding with respect to the so-called vascular factor of Niesson, and the precursor of A H G.-J. C.


Sarcomas were induced in non-isogenic rats by the subcutaneous implantation of a pellet of 3:4 benzpyrene. After these tumors reached a suitable size a small piece of the tumor was removed, irradiated with 10,000 r (a lethal dose) and then reimplanted into the same animal in the flank opposite the original tumor. The control series consisted of animals in which only a biopsy of the original tumor was performed. The original tumors were then given 2000 r. In the animals that received the tumor autografts a significantly greater number of tumors responded exceptionally well to the radiotherapy. The proposed mechanism is that tumor autografting augments the host immune response against these antigenic tumors and the tumor cells not destroyed by irradiation are destroyed by this immune response.—I. G.


A. Chromosome analysis of cells obtained from malignant effusions secondary to a vegetating cyst of the ovaries, an ovarian carcinoma and a breast adenocarcinoma, showed the following: 1) an abnormal chromosome, acrocentric, of the size of a 4-5 and called K1. This chromosome is present in all the cells from the ovarian vegetating cyst, most often in the disomic state, and in 30 per cent of the cells from the ovarian carcinoma; 2) an abnormal chromosome, subtelocentric, larger than a 1 and present in all the cells from the breast cancer. It was named K2; 3) two modes in the distribution of the chromosome numbers, one hypotetraploid at 88-90 and 52-62, respectively, in the two ovarian cancers. The hypotetraploid cells are rare in the breast cancer. B. Bone marrow cells and peripheral blood leukocytes obtained from individuals...
with a normal karyotype, were irradiated immediately after collection, at x-ray doses from 100 to 300 r. After 3 and 4 days in culture, chromosome analysis showed the following: 1) at equal doses, marrow cells exhibited less chromosome rearrangements than peripheral blood leukocytes. Frequencies of total aberrations varied in a linear fashion from 24 per cent and 28 per cent at 100 r. to 71 per cent and 80 per cent at 300 r.; 2) the observed chromosome rearrangements correspond to aberrations observed by others, namely polyploidy, dicentrics, ring chromosomes, and K1 and K2 types of rearrangements. C. These results are discussed with regard to the chromosome theory of carcinogenesis. Two hypotheses are considered: 1. chromosome rearrangements are an epiphenomenon of carcinogenesis, as well as an independent consequence of the known carcinogenic factors, i.e., certain virus infections, ionizing radiation, carcinogenic chemicals. 2. chromosome rearrangements are considered as the common pathway for these carcinogenic factors, and thus become the ultimate cause of carcinogenesis. The authors consider this second hypothesis as a more suitable one. —G. M.

LEUKOCYTES


These studies indicate that in the rat spleen, erythrocytes and particulate matter pass from the white pulp capillaries into a marginal sinus, formed by the anastomosed terminations of white pulp capillaries. From the sinus they pass through pores into the meshes of the marginal zone and then flow outward into the red pulp. Cells which were blackened by silver impregnation, similar but not identical to the reticoloendothelial system, represent a large reserve of potential phagocytes that may be mobilized quickly into active macrophages should the need arise. —O. P. J.


Electron microscopic studies revealed that the red pulp of the rabbit is composed of reticular cells, reticulum and elements of the blood circulating through it. The three types of reticular cells described followed no definite pattern as to types of spaces they lined and were not always adjacent to a basement membrane. The entire red pulp area was composed of intercommunicating pores and several spaces, and where no basement membrane existed, the cytoreticulum projections of the reticular cells formed a meshwork of spaces. This investigation supported the hypothesis that the red pulp of the spleen in the adult rabbit is a functionally dynamic area constantly changing its histologic structure, both with respect to cells and the state of vascular channels. —O. P. J.


Electron microscope studies were made of lymph nodes from mice for the purpose of observing possible pathways of differentiation of reticular cells, lymphocytes, plasma cells and phagocytes. Although one cannot be certain of developmental pathways in cell lines when a technique of comparing static cytologic images is employed, variations in cyttoplasmic appearance could be classified in such a way that their transitional pathways or patterns of cell differentiation could be postulated, beginning at the level of primitive reticular cells. This work supports the observation with the light microscope made by Reed ('02) and Downey and Weidenreich ('12) on heteroplastic formation of lymphocytes from reticular cells. It is possible that plasma cells may have a variable origin from two or more sources. —O. P. J.


Thymus glands from guinea pigs and C3H mice were fixed in 1 per cent osmium tetroxide, embedded in Araldite or Maraglas, and studied in the electron microscope. All of the thymic corpuscles displayed basically similar patterns of organization. One or more cells formed a central core. Flattened reticular cells were layered around the central elements. Signs of degeneration and deposits of intracellular fibrils were most
marked at the center of the corpuscle and were decreased toward the periphery. Extracellular connective tissue was absent. Occasionally concentric bodies were encountered which were almost entirely composed of degenerate fibrillar lamellae. Increased names and prominence of thymic corpuscles have been reported in some cases of lymphocytic depletion. A thymic corpuscle may have resulted from the collapse, rearrangement, and subsequent adhesion of elements of the cytoreticulum, perhaps provoked by local lymphocyte depletion with concomitant degeneration of the central cell or cells.—O. P. J.


Thymus glands from female guinea pigs were removed 20 to 25 days after stimulation by an injection of estradiol (Roussel). Tissue blocks were prepared for electron microscopic and histochemical studies. The Foa-Kurloff body is a dense intracytoplasmic inclusion with a unique membrane 40 to 50A thick. The core of this inclusion is homogeneous but its peripheral region contains some myelin figures. Its morphology excludes the possibility that it arose from the nucleus, parasites, rickettsia and phagocytes. The presence of a positive acid phosphatase reaction around and within the Foa-Kurloff relates their origin to lysosomes. Perhaps they have a secretory activity as an accessory sexual endocrine.—O. P. J.


This article deals with plasmocytopenia in sections of mediastinal lymph nodes obtained from 32, 10-week-old male normal albino rats. Sections were stained by the Dominici method, and a classification of plasmocytes was based on a measurement of nuclear diameters. The fate of the abundant large and medium plasmocytes originating from numerous mitoses is that these cells transform into smaller plasmocytes which leave the nodes by migrating along peculiar perivascular channels. It has been suggested that the cytoplasmic buds observed in sections might account for the release of antibodies in the larger plasmocytes.—O. P. J.


The widespread application of the short-term culture of human white blood cells to medical genetics and a more theoretical interest in the action of phytohemagglutinin (PHA) on leukocytes in vitro justified the need of a thorough investigation of the changes to which mature leukocytes are subject in the culture. The present article concerns the rate of DNA synthesis as a function of time in the population of human white cells cultured with the use of PHA.—O. P. J.


This study also shows, probably for the first time, that cells may be directly explanted in vitro in a chemically defined medium free of exogenous protein. These cells are being carried in vitro continuously in the chemically defined medium and have been through so many culture generations that any non-cellular protein material introduced initially with the explant must certainly no longer be present to influence the defined character of the medium. The removal of the fixed tissue cells from the populations in the first 48 hours, so that they were no longer present to overwhelm the lymphoma cells, certainly appeared as a valuable device for growing these P388 cells. Modifications and improvements in the concepts and techniques producing the present results may be desirable for establishing additional strains of circulating cells in vitro for metabolic and chemotherapeutic studies.—O. P. J.

This investigation was undertaken to assay and characterize mononuclear phagocyte (MN) enzymes that hydrolyze proteins, lipids, polysaccharides and nucleic acids, and similar enzymes in PMN and RBC were also evaluated. MN proteinases seem to be involved in many diverse biological phenomena, e.g., delayed hypersensitivity, the breakdown of antigens, the removal of effete red blood corpuscles, and the intracellular digestion of bacteria, viruses and protozoa. MN lipases are important because MN seem to be among the body’s main scavengers for many types of lipid material: immunological adjuvants, mycobacteria and necrotic tissue contain lipids that are ingested by MN. Perhaps the characterization of MN lipase may lead to a better understanding of how MN dispose of this material.

—O. P. J.


It has been observed that the normal rabbit alveolar macrophages contained more lysozyme than oil-induced peritoneal macrophages, based on analyses of aqueous extracts obtained from frozen and thawed cell suspensions. The present investigation has extended these observations. The results indicate that alveolar macrophages and oil-induced peritoneal macrophages are constitutively different in their enzymatic activities.

—O. P. J.


Lymph nodes from 76 germfree and 76 conventional mice were examined by histologic, histochemical, autoradiographic and immunocytochemical techniques, at intervals of 2 hours to 14 days after foot pad injection with killed E. coli organisms. These studies indicate that the lymphatic tissue of the germfree animal is capable of responding to antigenic stimulation. Previous experience with a microbial flora confers only minor advantages upon conventional animals, resulting in earlier and greater antibody production. In other respects the dormant lymphatic system of germfree animals reacts like its immunologically experienced conventional counterpart.—O. P. J.


In order to obtain information about protein and RNA metabolism in leukocytic nuclei, a system of isolated nuclei capable of incorporating labeled amino acids into protein and orotic acid into RNA was established. The results showed some similarity to protein synthesis in various isolated nuclei systems except that in the system investigated, some protein of high specific activity was released into the medium which may be concerned with leukocytic antibacterial activity.

—O. P. J.


Phytohemagglutinin (PHA), an extract of kidney bean, Phaseolus vulgaris, has been widely used to stimulate mitosis of human peripheral blood lymphocytes. No clear mechanism for the action of PHA has been established, but it has been suggested that it works as an antigen in stimulating cells to divide. The morphologic changes which have been observed, i.e., the change to plasma-like cells, seem to substantiate the suggestion that the small lymphocyte of the peripheral blood is indeed an immunologically competent cell.—O. P. J.


This monograph describes a new method to determine iso-antigens on leukocytes using sera with leukocyte agglutinins from pregnant women. After a review of the literature and a detailed chapter on methods, the author compares the results obtained with the leukocyte agglutination test using

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leukocytes from EDTA blood to those obtained when leukocytes from defibrinated blood are used. Optimal conditions regarding incubation time and temperature and, all important, number of leukocytes are outlined. The reproducibility of both methods was also studied; the method using leukocytes from EDTA blood proved to be the more reproducible, but also the least sensitive. Data are presented on the immunologic properties of these leukocyte agglutinins and their occurrence in the sera of more than 2500 pregnant women. A method is presented which allowed the recognition of a leukocyte group, group 4, with two alleles \( 4^a \) (with a gene frequency of 0.38) and \( 4^b \) (gene frequency 0.62). Essential features of the method were: a large number of observations which allowed the use of statistical methods, cross absorption to study the purity of the sera, and an insight into the shortcomings of the agglutination tests, especially concerning the occurrence of false negative reactions. Classical family studies showed that \( 4a \) and \( 4b \) are inherited as simple Mendelian autosomal codominant alleles. Part of this chapter that \( 4a \) and \( 4b \) are presented on the immunologic properties of these leukocyte agglutinins and their occurrence in the sera. These leukocyte agglutinins were found—C. M. E. A.


Variation in incidence of binucleated lymphocytes in the blood was studied in subjects who had not recently received any irradiation, so as eventually to serve as a basis for comparison in irradiated patients. Using a leukocyte concentration technic, 16 male and 16 female control subjects were examined in the males 0.94 binucleate lymphocytes per \( 10^4 \) lymphocytes; in the females, 1.62 binucleate lymphocytes per \( 10^4 \) lymphocytes were found.—C. M.


In previous experiments it was possible to cultivate cancerous tissue from mice and rats on embryonic chicken organs. This method was also employed for the tissue culture of human cancers. The problem arose as to whether small fragments of leukemic human bone marrow, such as one obtains from sternal puncture, would behave in a similar manner in organ culture. Normal human bone marrow retained its characteristic structure during the 3 weeks of its association with the chicken mesonephros in organ culture. Leukemic bone marrow did not manifest any invasive properties in regard to the mesonephros. Cell survival was markedly reduced in the case of the acute leukemia. On the other hand, explant survival and cellular proliferation were superior to those of normal marrows.—O. P. J.


Human peripheral blood lymphocytes were irradiated in vitro with 2 \( r \) and 5 \( r \) and then incubated at 37 C. for 5 days. Viability of lymphocytes was judged on the basis of morphology and motility of the cells. An easily detected decrease in survival of these irradiated lymphocytes was found.—I. C.

ERYTHROCYTES


These two articles describe a method for isolating nuclei from cell suspensions which is more rapid and less damaging than the usual methods which involve the mechanical description of the cell. It was found that one non-ionogenic surface active agent—Nonidet P 40 (NP 40) (Shell Ltd., Australia)—would lyse the plasma membrane quite differently than the nuclear membrane. One advantage of this and allied compounds is their solubility in both aqueous and non-aqueous media. —O. P. J.

A detailed description is given of a method for the quantitative fractionation of erythrocytes in a linear density gradient of bovine serum albumin. The cells segregated on the basis of buoyant density, and layer-wise fractionation of the tube contents provided material for further studies. The main factor responsible for distribution of buoyant density remains unknown, but it was suggested that it was not the distribution of lipid and probably not the water content. Erythrocytes in the densest fraction were less biconcave than lighter cells and reticulocytes formed a narrow distribution in the lightest quarter. Rabbit erythrocytes labeled with Fe59 in vivo, were followed in sequential analysis: labeled cells first appeared in lighter fractions, then became denser and the width of radioactivity distribution increased with time, indicating that the cells aged at different rates. Erythrocytes were shown to behave as perfect osmometers.—E. R. J.


A buffered, dextran-polyethylene glycol, aqueous two-phase system in an all-glass counter-current apparatus was employed to separate Fe59 in vivo-labeled erythrocytes of rats. Two populations of young cells were differentiated, associated with opposite ends of the distribution curve, and old erythrocytes were distinct from both young cell populations.—E. R. J.


This article describes the uptake of DNA and RNA precursors into polynucleotides in primitive erythrocytes of the chick embryo during development, as revealed by the technic of autoradiography. It was found that most of the erythrocytes from 5-day embryos were not labeled with isotopic thymidine, which indicates that these cells were not in a constant state of DNA synthesis. On the other hand, the RNA precursor, uridine, was taken up to quite a variable extent by most of these cells, indicating that RNA synthesis does not require a simultaneous synthesis of DNA. Mature primitive red blood cells do not synthesize DNA in the 7-day embryo but the early cells of the definitive cell line do take up the labeled precursor. In the 5-day-embryos' incubation there was a rapid synthesis of both RNA and of hemoglobin. As these early cells matured there was a loss in their RNA synthesizing capacity and ability to synthesize hemoproteins. It is possible to infer from these considerations that hemoglobin synthesis in these cells is closely associated with the synthesis of RNA and that the production of both types of these macro-molecules in the red blood cells of older embryos must occur while the erythrocytes are still within the hematopoietic tissue which produced them.—O. P. J.


When concentrations of Na and K are similar on both sides of the erythrocyte membrane (cat, dog), ATPase is not activated by Na and K, and it is not affected by ouabain. When there is a great difference in concentration between intracellular contents and plasma (human, rat), ATPase activity is stimulated 2 to 3 times by Na plus K, and the stimulation is abolished by ouabain. These findings are presented as further support for the hypothesis that the alkali-ion stimulated ATPase plays a role in the active transport of these ions.—E. R. J.


Ghosts of rabbit erythrocytes (high K) had about three times the Na- and K-stimulated ATPase activity of ghosts of cat erythrocytes (low K). Rabbit erythrocyte and cat marrow
cell ghosts had almost identical total ATPase activities, although the alkali-ion stimulated activity was higher in cat marrow cells. These studies indicated that there is sufficient Na- and K-stimulated ATPase in cat marrow to carry on Na and K transport.—E. R. J.


Rates of entry of neutral amino acids into washed human erythrocytes were shown to be directly related to the size of the hydrocarbon side chains, as long as no polar grouping was present. Entry was not due to lipid solubility, for uptake was subject to competition and saturation and showed stereospecificity and the phenomenon of countertransport. Similarities as well as differences between erythrocytes and Ehrlich ascites cells were noted. Three modes of uptake appeared to exist: 1) mediated transport, preferring long chain amino acids and easily saturated by them; 2) low-capacity uptake of alanine and glycine; 3) an apparently nonsaturable component limited to amino acids with large side chains.—E. R. J.


Phospholipids, extracted from human erythrocyte “ghosts” with hot ethanol and diethyl ether, formed complexes with dry glucose and other monosaccharides which were then soluble in the highly nonpolar solvent, hexane. Lecithins and lyssolecithins, but not phosphatidyl ethanolamine, neutral lipids or other ingredients of the extract, appeared to form such complexes. The authors suggest that these observations may be relevant to mediated sugar transport in erythrocytes, but they raise several unresolved questions.—E. R. J.


The increased rate of glucose utilization which resulted from the addition of inorganic phosphate (P$_1$) occurred without appreciable change in the cellular concentrations of ATP or G-6-P. Inhibition of erythrocyte hexokinase by G-6-P was overcome completely by P$_1$, and P$_1$ increased the apparent inhibition constant for G-6-P in intact erythrocytes. It was suggested that the effect of P$_1$ resulted from an exact coordination of its effects on the rates of hexokinase and phosphofructokinase activities. P$_1$, therefore, may affect the rate of ATP production via the Embden Meyerhof pathway without changing the rates of other metabolic processes.—E. R. J.


Evidence is presented to indicate that the rate at which glucose is utilized by intact human erythrocytes is inversely proportional to the amount of G-6-P in the cell and that this rate is strongly influenced by processes which determine the rate at which G-6-P is metabolized further. These latter processes appear to be, primarily, the G-6-P dehydrogenase and phosphofructokinase reactions, for evidence of phosphatase activity could not be obtained and the formation of glycogen in these cells remains an unanswered question. Addition of methylene blue enhanced glucose utilization only when G-6-P dehydrogenase activity was great enough to cause significant lowering in the concentration of G-6-P. The activity of hexokinase isolated from erythrocytes was inhibited by G-6-P, an inhibition which was only partly competitive with ATP. The inhibition of glucose utilization which occurred upon incubation of erythrocytes with inosine appeared to result from the accumulation of G-6-P.—E. R. J.


A 59-year-old white man with compensated nonspherocytic hemolytic anemia of 20 years’ duration and an unnamed neurologic disorder (optic atrophy, sluggish tendon reflexes) was found to have erythrocytes, leukocytes and platelets completely devoid of G-6-P dehydrogenase activity in
the standard assay system. Normal erythrocyte stroma failed to enhance activity of hemolysates of the patient’s erythrocytes, and stroma from his cells did not inhibit activity in normal hemolysates. Survival of his own Cr51-tagged erythrocytes was markedly shortened, whereas survival of erythrocytes of two female descendents with intermediate G-6-P dehydrogenase activities was normal. Administration of quinine sulfate was followed within hours by increased hemolysis and convulsions, and it was speculated that the neurologic disorder might also be causally related to the enzyme deficiency.—E. R. J.

**RED CELL GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY AND GLUTATHIONE DEFICIENCY. M. Oort. From the Central Laboratory of the Blood Transfusion Service of the Dutch Red Cross, Amsterdam, The Netherlands. Thesis, Amsterdam, 1964.**

Using the method of Kornberg and Horecker, the author found red cell G6PD deficiency in 17 patients: 13 males and 4 females. Only about half of them were of Dutch origin. Fifteen had episodes of acute hemolytic anemia caused by the Fava bean or drugs, particularly sulfonamides; of the 2 others one displayed icterus neonatorum followed by chronic non-spherocytic hemolytic anemia, and the other hepatosplenomegaly with transient icterus. One female patient, possibly homozygous, had been described earlier by Heeres and Zondag (Folia Med. Neerl. 4:121, 1961). On studying 225 relatives of these patients, 25 males with the deficiency were detected. Of the numerous deficient females, four were suspected to be homozygous, because of unusual low enzyme activity. In attempting to assess the gene frequency in the Dutch population the author detected among 100 healthy soldiers only one with a G6PD deficiency, whereas 3 of 100 healthy blood bank donors of Dutch origin, as well as 2 out of 100 Amboynese were grossly deficient.—E. A. L.


Extensive investigations of a boy with severe hemolytic anemia which improved somewhat after splenectomy demonstrated a severe deficiency in erythrocyte pyruvate kinase (PK) activity, abnormal autohemolysis-test results, which were not corrected by glucose or ATP, extremely short survival of Cr51-tagged cells in the patients’ own and in a normal circulation, and bizarre spiculated erythrocytes, macrocytes and balloon-shaped cells which could be seen in wet preparations examined within 5 seconds. Rouleaux formation, siderocytes, Pappenheimer and Heinz bodies were not observed. Although the morphologic abnormalities resembled those seen in acanthocytosis, none of the associated biochemical abnormalities was observed. Erythrocytes of both parents, 3 of 4 siblings, paternal grandmother, maternal aunt and uncle were found to have intermediate PK activities, findings compatible with a recessive, autosomal mode of inheritance. Although the patient’s erythrocytes contained increased levels of G-6-P dehydrogenase activity (reticulocytosis 34 to 94 per cent), erythrocytes from his mother, maternal aunt and maternal grandmother were found to
have intermediate activities, compatible with the carrier state. Survival of erythrocytes doubly heterozygous for PK and G-6-P dehydrogenase deficiency and heterozygous for G-6-P dehydrogenase deficiency was shortened alone in normal recipients, whereas heterozygous PK-deficient cells had a normal life span. The PK deficiency appeared to stem from Scotch and French-English ancestry; the G-6-P dehydrogenase deficiency appeared to have come from French-German ancestry.—E. R. J.

ASSAY FOR HEXOKINASE ACTIVITY IN INTACT RED CELLS AND ITS ALTERATION ON STORAGE. C. Bishop. From School of Medicine, State University of New York, Buffalo, N. Y. J. Biol. Chem. 239:1053–1056, 1964.

Hexokinase activity was equated with the oxygen uptake of intact erythrocytes incubated with glucose plus methylene blue. That G-6-P dehydrogenase activity and availability of TPN did not limit the reaction was concluded from the findings when inosine was used as substrate. Although the rate of the hexokinase reaction appeared to decrease in erythrocytes after storage in ACD solution under blood bank conditions, the decrease appeared to be due to a decrease in the supply of ATP, rather than to a decrease in hexokinase activity itself. Evidence for this conclusion was obtained with erythrocytes in which ATP levels were maintained by utilizing a modified preservative solution (heparin-glucose-phosphate-adenine) and with cells in which ATP levels were restored by preincubation with inosine and adenine.—E. R. J.


Whole blood from 4 patients in severe diabetic acidosis and from 10 control subjects was incubated with acetate-1-C14, and the fatty acids were isolated by gas liquid chromatography. Although there was no significant change in incorporation into fatty acids per unit volume of blood, a significant decrease in incorporation was noted when the results were expressed per number of leukocytes. Because of the possible effect of isotope dilution due to different pool sizes, determinations of percentage of recovered radioactivity in different classes of fatty acids were reported. A marked decrease of total radioactivity in lauric, myristic and palmitic acid, and a striking increase in stearic and oleic acids in blood from diabetics in acidosis was observed. Decrease in pH of normal blood to 6.6 did not result in an abnormal pattern, and addition of insulin in vitro did not correct the pattern of acidic blood, but normal patterns were observed with blood obtained 5 and 9 days after recovery from acidosis. The authors suggest as a possible explanation for these findings a decrease in the activity of the malonyl CoA pathway, due to decrease in TPNH, ATP, biotin etc., or reduction in activity of enzymes.—E. R. J.


No differences in the incorporation of oleic acid-1-C14 into diacyl phosphatides were noted upon incubation of hemolysates from the erythrocytes of 4 patients with spherocytosis and 3 control subjects. Acylating-enzyme activity, but not phospholipase-A activity, was demonstrated. In both types of cells. It was suggested that the protein portion of cellular lipoproteins may be important in determining the abnormality in spherocytic cells.—E. R. J.


Study of 18 of 189 known members of a kindred which could be traced to a French man who settled on the bank of Troublesome Creek in about 1800 revealed 4 individuals with methemoglobin concentrations greater than 1 Gm. per 100 ml. (7 to 12 per cent of total hemoglobin) whose erythrocytes were markedly deficient in DPNH-methemoglobin reductase activity. Methemoglobinemia was rapidly corrected by methylene blue intravenously, and spectrophotically and electrophonetically normal methemoglobin A was demonstrated. Eight acyanotic individuals had slightly
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Elevated methemoglobin concentrations (0.19 to 0.26 Gm. per 100 ml.) and their erythrocytes contained intermediate levels of DPNH-methemoglobin reductase activity. GSH concentrations and G-6-P dehydrogenase activities of the erythrocytes of homozygous subjects were normal. Ferrokinetic studies in one patient were normal, and the oxygen dissociation curve was shifted slightly to the left. Results of genetic studies were compatible with a recessive, autosomal mode of inheritance.—E. R. J.


Enzyme activity was determined by measuring the amount of ammonia released from serotonin or 5-methoxytryptamine by erythrocytes obtained from the blood of children (age 5–15) with rheumatic fever or rheumatic heart disease and from normal controls. Rate of ammonia production from both substrates was significantly lower in erythrocytes of rheumatic subjects, which were essentially unable to deaminate 5-methoxytryptamine. The authors suggested that either absence or inhibition of monoamine oxidase might account for the decreased ability to deaminate serotonin, although decreased, might be sufficient to account for apparently normal excretion of 5-hydroxyindoleacetic acid.—E. R. J.

BILIRUBIN: ACUTE EFFECTS IN NEWBORN RHESUS MONKEYS. R. E. Behrman and E. Hibbard.

Solutions of crystalline bilirubin were infused into newborn and infant monkeys to maintain serum concentrations between 30 and 45 mg./100 ml. Within 15 minutes, the concentration of oxygen molecules, measured by the polarographic technic with a platinum electrode implanted beneath loops of bowel, decreased and was stabilized after one hour at a level of 20 to 54 per cent of the initial value without a significant change in heart or respiratory rate or rectal temperature. Postmortem examinations revealed intravascular deposition of bilirubin crystals, but no bilirubin within cells. The authors postulated that high serum bilirubin concentrations may result in a decrease in the concentration of oxygen in tissues which may make the cells more susceptible to inward diffusion of bilirubin and that this effect may precede the intracellular action of bilirubin on oxygen uptake and phosphorylation.—E. R. J.


Plasma erythropoietic activity was assayed in 50 patients with primary or secondary erythemias. In 11 the activity was raised: In 1/9 patients with chronic respiratory insufficiency. In 2/6 patients with non-malignant renal lesions. In 3/13 patients with a primary erythremia and radiologically normal kidneys. In 4/18 primary cases in whom pyelography was not performed. In one patient with a malignant kidney tumor. The possible role of renal circulatory disturbances, organic or functional, in erythropoietin secretion is discussed. The difference in results between one case and another is thought to be due to the relative insensitivity of the method.—C. M.


The fate of non-hemoglobin iron in red-cell precursors was studied using human marrow cultures and radioiron labeling. A non-hemoglobin fraction (Fraction I) was separated from hemoglobin by a technic using chromatography; ferritin was precipitated from Fraction I with antiferritin serum. Studies showed an early uptake of radioiron by the non-hemoglobin iron-protein complex (Fraction I and ferritin) which acted as precursors to hemoglobin. When further labeling was stopped, some iron persisted in the non-hemoglobin complexes of the primitive cells without going to hemoglobin. However, none of the adult red cells contained ferritin. More iron was taken up from highly saturated transferrin solutions; the bulk of the extra iron was incorporated into ferritin; in iron deficiency no ferritin was found. Iron incorporated into ferritin leaves the red cells before they enter the circulation. This might explain, at least in part, ferrokinetic data, which indicate that some of the iron delivered to the
bone marrow enters a labile pool which may feed back into the plasma again.—R. O. W.


The first article shows that treatment of erythrocytes with proteolytic enzymes, such as trypsin or papain, lowered agglutinability and absorptive potency for species-specific hemagglutinins in anti-cell guinea pig serums. Treatment of erythrocytes with carbohydrate-reactive periodate was ineffective. These treatments produced opposite effects in control experiments with heterophile or blood-group A-specific hemagglutinins. The experimental results indicate that these species-specific proteins are antigenetically heterogeneous. In the second paper, it was shown that species-specific antigenecity as measured by inhibition of species-specific hemagglutinins are associated with particulate as well as soluble proteinaceous components throughout the cell including the nucleus. There was no indication for a particular, specially located cell constituent as a carrier of species-specificity. In contrast, blood group B-specific antigen was not found in the nuclear cell fraction of primary rhesus monkey kidney cells, but in the particulate as well as soluble cytoplasmic fractions tested.—O. P. J.

**HEMOSTASIS**

**ACTIVATION OF HAGEMAN FACTOR BY SOLUTIONS OF ELLAGIC ACID. O. D. Ratnoff and J. D. Crum. From Western Reserve University, School of Medicine, Cleveland, Ohio. J. Lab. & Clin. Med. 63:359, 1964.**

Aged solutions of tannic acid and of certain o-dihydroxyl compounds similar in structure to its gallic acid moiety were found to accelerate clotting. Ellagic acid was the most active compound tested, causing accelerated clotting at concentrations as low as 10^-8M. This compound did not clot fibrinogen directly, accelerate the thrombin fibrinogen reaction, or accelerate the clot-promoting effect of Russel viper venom and tissue thromboplastin. It did not correct the prolonged prothrombin time of plasma deficient in factors V, VII, or X. It had a minimal corrective effect upon the recalcification time of factor VIII and factor IX deficient plasma but none on Hageman deficient plasma. It did not activate crude preparations of PTA nor did it enhance the clot-accelerating effects of activated preparations of PTA. Evidence is presented which indicates that these compounds activate the Hageman factor and thus accelerate clotting. The chemical nature of the reaction was not elucidated but seemed to be related to the presence of o-dihydroxyl groups in the molecule.—R. G.


This is an excellent review. The areas discussed include: the molecular weight and amino acid composition of thrombin (bovine), the amino acid sequence of the active center of thrombin and comparison to that of trypsin and chymotrypsin, the bonds split by thrombin, the amino acid composition of fibrinogen, the peptide chain structure of fibrinogen, the conversion of fibrinogen to fibrin by thrombin, the amino-acid structure of the polypeptides A and B split from fibrinogen, the physiologic activity of these peptides, the polymerization phase of clot formation, the rate of the fibrin stabilizing factor (referred to as LIF), its activation by thrombin and its action on fibrin with the release of carbohydrate, and clot retraction.—R. G.

**THE EFFECT OF LANTHANIDES AND ACTINIDES ON BLOOD COAGULATION. 1. EVIDENCE FOR AND PROPERTIES OF A NEW SERUM THROMBOPLASTIC FACTOR. R. Colman and B. Alexander. Yamins Research Laboratory, Beth Israel Hospital and Department of Medicine, Harvard Medical School, Boston, Mass. J. Clin. Invest. 43:705, 1964.**

Thorotrast induces retarded coagulation in vivo, and in vitro causes a coagulation defect in both plasma and serum so that they do not support thromboplastin generation. The same abnormality is produced by thorium chloride and the related elements neodymium, lanthanum, cerium, and uranium. In plasma these compounds cause progressive inactivation of Factor V. When added to serum they cause a defect in thromboplastin formation which appears to involve an entity distinct
from other known clotting factors or intermediates. This is referred to as TVF (thorium vulnerable factor). It is adsorbable by BaSO₄, elutable by citrate, and it is present in the protein fraction precipitated at 50 to 60 per cent (NH₄)₂SO₄. It is relatively labile since it is markedly reduced in serum stored at 0 to 4 C. for 21 days, kept at 37 C. for two hours, or at 50 C. for 20 minutes. It has been separated from other known coagulation factors by starch-gel electrophoresis and column chromatography. Its activity can be increased 2 to 3 fold by trypsin. Replacing Inosithin by normal platelets in the TGT mixture corrects the thorium induced serum defect, but if the platelets are first treated with thorium, they become inert. Citrate and oxalate reverse the thorium induced defect, but EDTA does not. It is suggested by the authors that the lanthanides and actinides produce a defect in serum by forming a reversible complex with a serum protein which is necessary for thromboplastin generation. It is postulated that the biological function of the protein is compromised by chelation at specific sites on the protein essential for its clotting specificity. From the studies reported this clotting factor appears to be distinct from Factors I, II, V, VII, VIII, IX, X, XI, and XII. It resembles in some respects the coagulation activity first reported by Duckert et al. and designated at that time as Factor X and the labile serum fraction of Connor, Warner and Carter.

—R. G.

**ABSTRACTS**

**THE EFFECT OF LANTHANIDES AND ACTINIDES ON BLOOD COAGULATION. II. ASSAY OF A NEW SERUM THROMBOPLASTIC FACTOR VULNERABLE TO THESE ELEMENTS AND ITS VARIATIONS IN HEALTH AND DISEASE. R. Colman and B. Alexander.** Yamin's Research Laboratory, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass. J. Clin. Invest. 43:720, 1964.

An assay procedure for measuring the thorium vulnerable factor is described. It was found that: (1) the factor is markedly depressed in the newborn; (2) it is decreased in severe liver disease corresponding in degree to the depression of Factor V; (3) it is depressed by coumarin administration but not at the same rate or degree as Factors II, VII, or X, and; (4) it is not elevated in the gravid.—R. G.

**STUDIES ON THE SITE OF ACTION OF A CIRCULATING ANTICOAGULANT IN DISSEMINATED LUPUS ERYTHEMATOSUS. R. T. Breckenridge and O. D. Ratnoff.** From Western Reserve University School of Medicine, Cleveland, Ohio. Am. J. Med. 35:813, 1963.

The authors present evidence that the anticoagulant found in lupus erythematosus which is characterized by prolongation of the one-stage prothrombin time, behaves as an inhibitor to the formation of the "enzyme" (prothrombinase) which converts prothrombin to thrombin. This inhibitor appears to interfere with the interaction of activated Stuart Factor and Factor V. In each of the three cases studied the anticoagulant functioned after activation of Stuart Factor by Russell's viper venom but before the reaction between activated Stuart Factor and Factor V had been completed. The inhibition was maximal when the anticoagulant was present throughout the interaction of activated Stuart Factor and Factor V. The mechanism by which the inhibitor interferes in this interaction was not defined.—R. G.


Isolated liver of rats pretreated with vitamin K₁, perfused with red cells suspended in a nutrient medium devoid of coagulation factors, produced factors VII-X, factor V, and prothrombin. No production of plasminogen activators, proactivators, plasminogen and plasmin was noted, but antiplasmin release was observed.—R. G.

**ABNORMAL PLASMINOGEN-PLASMIN SYSTEM ACTIVITY (FIBRINOLYSIS) IN PATIENTS WITH HEPATIC CIRRHOSIS: ITS CAUSE AND CONSEQUENCES. A. P. Fletcher, O. Biederman, D. Moore, N. Alkaersig and S. Sherry.** From the Department of Internal Medicine, Washington University School of Medicine, and the Jewish Hospital, St. Louis, Mo. J. Clin. Invest. 43:681, 1964.

Nicotinic acid intravenously and electroshock which induce enhanced plasma thrombolytic activity of short duration in normal subjects produce an exaggerated response in patients with hepatic cirrhosis with both greatly enhanced peak activity and a more prolonged period of thrombolytic activity. With nicotinic acid injections of
100 mg, significant decreases in plasma-plasminogen and fibrinogen and prolongation of the thrombin time occurred in the cirrhotic but not in the normal. There was little if any difference of plasma inhibitors between the two groups. There was no difference in the in vitro decay rate of plasminogen activator between the normal and the cirrhotic, but in vivo plasma clearance of plasminogen activator was much more rapid in the normal. It is postulated that abnormal fibrinolysis in the cirrhotic may be due to failure of an hepatic clearance mechanism for plasminogen activator. —R. G.

**Effects of Epsilon Aminocaproic Acid on Coagulation and Fibrinolytic Mechanisms.**


This study evaluated the effect of daily oral administration of 10 Gm. of EACA for 1 week on the coagulative and fibrinolytic mechanisms in cirrhosis. Twenty-five patients were studied, 23 with Laennec's cirrhosis and 2 with post-hepatic cirrhosis. In these patients the most prominent coagulation deficiencies found were in platelet thromboplastin factor and plasma factors V, VII, X and prothrombin. Factor IX was occasionally reduced, and fibrinogen, factors VII and XII levels were normal. Prolonged plasma thrombin times were also found. These coagulation findings were not altered by the administration of EACA. In 16 of the 25 patients there was dissolution of the recalcified-plasma clot within 24 hours; of these 6 showed increased fibrinolytic activity. EACA prevented plasma-clot lysis, lowered the fibrinolytic activity, and suppressed urokinase excretion. No toxic manifestations attributed to EACA were encountered. —R. G.

**Effect of Plastic and Glass Surfaces on Clot Retraction and Serotonin Uptake of Platelet-Rich Plasma Stored at 4 C.**


The effect of plastic, glass, and siliconized glass surfaces on the number and function of blood platelets suspended in plasma and stored for various intervals at 4 C. was studied. Platelet function was tested by clot retraction and by the in vitro uptake of C14-labeled 5-hydroxytryptamine (serotonin). It was found that while there was no significant difference in the rate of decrease in platelet number between samples of platelet-rich plasma preserved in plastic and those preserved in siliconized and non-siliconized glass containers, the clot retraction and the serotonin-uptake properties of the platelets disappeared more rapidly when the platelet-rich plasma was preserved in plastic containers. Other findings indicated that the effect of the plastic surface on the clot retraction of preserved platelets was the result of a change in the platelets themselves and not of an alteration in the suspending plasma. When, however, whole blood was preserved instead of platelet-rich plasma, the unfavorable effect of the plastic surface could not be demonstrated. The serotonin uptake property of platelet-rich plasma obtained from EDTA blood was compared with that of ACD blood during storage at 4 C. It was found that in the presence of EDTA the platelets lost their serotonin-uptake property more rapidly than when ACD was used. —R. G.


There is good inverse correlation between the Ivy bleeding time and the number of “adhesive” platelets to the injured vessel wall. Both can be abnormal in thrombocytopenia, thrombathemia of Glanzmann with disturbance of platelet membrane metabolism, Von Willebrand disease due to a deficiency of a plasma factor, and tissue-collagen disorder. The Ivy has greater diagnostic value than the Duke bleeding time. Its correction by various therapeutic agents is much more difficult to obtain. —J. C.

**MISCELLANEOUS**

**Cytoc hemical Studies of Human Bone Marrow Fibroblast-Like Cells. II. Esterase and Acid Phosphatase.** *P. Farnes and B. E. Barker*. From Rhode Island Hospital, Providence, R. I. Am. J. Path. 44:481-489, 1964.

In a previous study it was shown that fibroblast-like cells cultivated from capillary endothelium have a demonstrable alkaline phosphatase activity in contrast to similar cells derived from peripheral blood “macrophages.” Because of histochemical characterization of morphologic cell
types appearing in newly explanted marrow and blood cells, cultures may contribute information about cell parentage—two enzymes which are prominent in histocytes. α-naphthyl acetate esterase activity was demonstrable in megakaryocytes, histiocytes and occasionally in plasma cells. Capillary endothelium and the remainder of hemic-cell types were not reactive for the enzyme. Acid phosphatase was prominent in stromal mononuclear cells of fresh marrow and plasma cells showed traces of activity. The distribution of acid phosphatase in cultured marrow was similar to that of nonspecific esterase and in marked contrast to that of alkaline phosphatase.—O. P. J.


Mammalian cells phagocytize DNA, but no convincing demonstration of transformation of these cells by the infested DNA has been presented. L-stain fibroblasts were allowed to infest DNA particles. The process was then followed by electron microscopy. The DNA was rapidly taken up into vacuoles, and the DNA lost its electron density. Cytocchemical reactions indicated the presence of esterases, acid phosphatases and nucleoside phosphatase in these vacuoles. The authors conclude that the injected DNA is hydrolyzed to nucleosides thus explaining the failure of this material to cause biological transformation.—I. G.


The effects of bacterial endotoxin on the formed elements of the blood were first described almost 75 years ago. The mechanism of the thrombocytopenic and leukopenic action of bacterial endotoxin is not clear. In order to obtain a cellular or subcellular localization of tagged endotoxins, a tritium-tagged endotoxin was developed for the radioautographic distribution of a tagged endotoxin with regard to the formed elements of the blood. Lymphocytes and red cells showed no association with silver grains. Approximately 4 to 8 per cent of granulocytes exhibited silver grains over cytoplasmic areas in smears made during the period of maximum labeling and the percentage of monocytes associated with graining was somewhat less and no consistent temporal pattern was established. A striking degree of platelet labeling was observed throughout the entire period. Apparently granulocytes, monocytes and platelets were associated with endotoxin.—O. P. J.


Anti-antibody in rabbits has been found to be a 19 S globulin with specificity for rabbit 7 S γ-globulin altered either by combination with antigen or by certain nonspecific methods. The anti-antibody combines with antigenic determinants on Fragments I and II, but not III, of the papain digested 7 S γ-globulin molecule.—H. F.


In vitro survival of Girardi Heart (clone 7 A6), an epithelial cell line decreases as rapid freezing rates are used during the freezing process. Cell survival falls from 4 per cent during slow freezing, to 0.2 per cent when fast freezing at 79 C is used; no cells survive when the cells are directly brought to −196 C. Cell survival increases when protective compounds are added to the medium; this increase is slight with mannitol and significant with glycerol. Results obtained from the tissue culture technic agree with those obtained from transplantation of isologous frozen bone marrow into lethally irradiated mice.—C. M.


The author reviews the effect of heparin, potassium oxalate and EDTA on the determination of CO₂-combining power. Only EDTA shows clear reduction in the expected level of CO₂ which is proportionate to the amount of anticoagulant. On the other hand, if potassium oxalate is dried above
180 C. a conspicuous increase results from the conversion of oxalate to carbonate at this temperature.—C. R. M.


Elevated urinary levels of 5-hydroxyindoleacetic acid, indole-3-acetic acid, and indican were found in untreated and treated celiac patients with steatorrhea, whereas normal levels of these metabolites were obtained in treated patients without steatorrhea. Tryptophan loading of the patients with steatorrhea showed significant increase in 5-hydroxyindoleacetic acid excretion, suggesting increased shunt of serotonin to 5-hydroxyindoleacetic acid. All patients with adult celiac disease failed to metabolize normally an oral tryptophan load, as manifested by increased urinary excretion of kynurenine, xanthurenic acid and kynurenic acid. It is suggested all patients with adult celiac disease be given supplementary vitamin B<sub>6</sub>—V. H.