ABSTRACTS OF SPECIAL INTEREST


Neonatally thymectomized mice were implanted intraperitoneally at 7 days of age with cell-tight Millipore diffusion chambers containing either embryonic or neonatal thymus tissue. Suitable control studies were performed. In contrast to the controls most mice implanted with thymus-filled chambers gained weight satisfactorily, did not develop a wasting syndrome, showed a good serum antibody response to sheep erythrocytes, and were able to reject skin homografts. At autopsy the implanted mice had much more nearly normal amounts of lymphoid tissue present than did the control mice. The tissue recovered after 1-2 months from the diffusion chambers showed only epithelial-reticular cells but no lymphoid cells. It is suggested that a humoral factor produced by the thymus epithelial-reticular complex may be responsible for stimulating lymphopoiesis and also for endowing lymphoid cells with immunological competence.—T. E. B.


An association between phenylbutazone therapy and subsequent leukemia has been reported. This paper reports another 5 cases of acute leukemia, 4 myeloblastic and 1 lymphoblastic in adults following such therapy.—I. C.


A case of myelomonocytic leukemia in a 44-year-old female following periodic phenylbutazone therapy over 4 years.—I. C.


Many forms of immune reaction and a heterogeneity of antibodies may relate to structural differences among the γ-globulins and Bence Jones proteins. For structural studies, 63 samples of myeloma globulins, pathologic macroglobulins, Bence Jones proteins, and normal γ-globulins were collected and purified by salting out fractionation and then DEAE-cellulose column chromatography. Fragments of these globulins obtained by papain digestion or cleavage with mercaptoethanol were compared with each other or with Bence Jones proteins. The antisera were specific for Bence Jones protein since antisera against type I Bence Jones protein reacted with only type I and
antiseria against type II with only type II proteins. Slow moving fragments of papain digested \( \gamma \)-myeloma globulin were antigenically similar to mixed Bence Jones proteins corresponding to the same type. A myeloma globulin in the serum and a Bence Jones protein in the urine were the same type, I or II, if both of the samples were obtained from a single patient. An antigenic map of proteins belonging to the \( \gamma \)-globulin system was presented. According to the map, hyper-gammaglobulinemia due to increased monoclonal \( \gamma \)-globulin can be classified into: \( \gamma \)-type myeloma I or II, \( \gamma \),-type myeloma I or II, Waldenström macroglobulinemia I or II, micromolecular type myeloma I or II, and H\(\gamma\)\(\beta\) disease (Franklin). Analysis of these protein structures by a peptide map of tryptic digest coincided completely with the results from the antigenic map. Bence Jones protein type I and II were also distinguished by the measurement of optical rotatory dispersion or ultraviolet spectra. In order to classify Bence Jones protein type, a simple test by means of ultraviolet spectra was presented for clinical use.—K. F.

**ERYTHROCYTES**


An 18,000-fold purification of glutathione reductase (GSSG-R) from hemolysate prepared from washed erythrocytes obtained from outdated blood bank blood was attained with ammonium sulfate and calcium phosphate gel fractionation. The enzyme appeared to contain flavin which resembled FAD, was active with TPNH, DPNH and deamino-DPNH, but not with other DPNH analogues, had only a little diaphorase and no met-hemoglobin reductase activity, had inherent dihydrolipoic dehydrogenase activity, had no pyridine nucleotide transhydrogenase activity, had an equilibrium constant which favored formation of GSH, and was inhibited by excess substrates and by reaction products. Although the rate of reduction of GSSG by DPNH was only 13 per cent of that found with TPNH, a single enzyme was thought to be responsible for TPNH-GSSG-R, DPNH-GSSG-R and dihydrolipoic dehydrogenase activities. However, pH optima, temperature dependence, effect of ionic strength and binding of substrates appeared to differ for the three reactions. The kinetics of the GSSG-R reaction were postulated to involve the formation and decomposition of successive binary complexes of the two substrates with the enzyme.—E. R. J.


Carbonic anhydrase I (CA-I) was isolated from hemolysates of erythrocytes of man, chimpanzee, baboon and rhesus monkey by chloroform-ethanol extraction with partial purification by passage through DEAE and carboxymethyl cellulose columns. The order of carboxylic ester hydrolase activity, estimated by formation of \( \beta \)-naphthol from \( \beta \)-naphthyl acetate, was: rhesus, baboon, chimp, man. The order of enzymatic hydration of \( \text{CO}_2 \) (hydrolase activity) was: chimp, man, rhesus, baboon. Erythrocyte carbonic anhydrase may also hydrolyze carboxylic ester linkages and further studies on inter- and intraspecies variation and on genetically determined variants are in progress. —E. R. J.


Insoluble human erythrocyte membrane ATPase was fractionated by treating with 0.6M NaI and then with 2 M NaI. ATPase activity of 0.6M fraction was insensitive to ouabain, with or without Na\(^+\) and K\(^+\), was activated slightly by Na\(^+\) or K\(^+\), and had a pH optimum of 7.2. Activity of 2M fraction was extremely sensitive to ouabain, was not activated by either Na\(^+\) or K\(^+\), but was activated by both ions added together, and had a pH optimum of 7.7. The authors concluded that at least two different ATPases exist in human erythrocytes.—E. R. J.


NADase and NADPase are contained in erythrocytes, and their respiratory activities were de-
Retardation of Sodium Exchange in Dog


Evidence was obtained that aldosterone, in physiologic or pathologic concentrations, could influence the exchange of sodium between erythrocytes and plasma of dogs. The sodium-rich erythrocytes were even more sensitive to the effect of aldosterone after the dogs underwent bilateral adrenalectomy. It was suggested that the effect resulted from the inhibition of sodium influx. —E. R. J.


Comparison of turnip greens obtained from plants grown without shade with basic fertilization, unshaded with high nitrate fertilization, and shaded with high nitrate fertilization revealed: 1) significant increase in nitrate content with increased fertilization and shading; 2) nitrate was not a significant factor in loss of ascorbic acid during cooking. Ascorbic acid added to oxalated human blood failed to protect against methemoglobin formation by nitrite. Similarly, in vivo studies with guinea pigs failed to demonstrate a protective effect from ascorbic acid. Thus, accidental or climatic shading of highly fertilized turnip greens introduces no health hazard, even though the greens have a low ascorbic acid and high nitrate content.—E. R. J.


Phenolics (phenol, methyl and/or chlorine substituted), alcohols (complex, chlorinated), mercapturals, cationic surfactants, parabens and sodium formaldehyde sulfoxylate were prepared in 0.9 per cent sodium chloride solution. All of the preservatives studied, except for parabens, sodium formaldehyde sulfoxylate and thimerosal, caused complete hemolysis of rabbit and human erythrocytes in concentrations normally used in parenteral solutions. It was suggested that the antibacterial mechanism of action of a preservative may be similar to that which causes hemolysis. This hemolytic effect must be taken into consideration in evaluating the results of investigations in which materials containing preservatives are added to blood.—E. R. J.


Incubation of human erythrocytes or hemolysates with more than 5 μg of chromate ion (expressed as elemental chromium) per ml. of cells, but not with trivalent chronic ion, resulted in up to 80 per cent inhibition of glutathione reductase (GSSG-R) activity. Activities of other enzymes studied (Embden-Meyerhof and hexose monophosphate shunt pathways, acetylcholinesterase, glyoxalase) were not inhibited. Chromate-treated erythrocytes had an increased tendency to form Heinz bodies and their GSH content decreased more than did that of control cells upon subsequent incubation with acetylphenylhydrazine. Although the authors emphasized that the concentration of chromate required to obtain GSSG-R inhibition was much greater than the amount currently employed in clinical studies, the findings in older investigations must be re-evaluated in the light of these results. (Abstractor’s comment: Could less readily detectable effects of chromate on GSSG-R activity have anything to do with the observed short T ½ of G51-tagged cells which has been attributed, in part, to elution of G51 from the cells?)—E. R. J.

A detailed description and statistical analysis of a study of hemolysates prepared from finger-prick blood from 343 American Negroes and subjected to vertical starch gel electrophoresis and staining for G-6-P dehydrogenase activity. Type A mothers had type A sons, type AB mothers had either type A or type B sons, and type B mothers had type B sons; the only exceptions were sons whose erythrocytes were deficient in G-6-P dehydrogenase activity with a slightly faster band (A−) which made identification difficult. The data are compatible with a sex-linked mode of inheritance of the different enzymes. The findings are compared with those previously reported by Boyer et al. and it is suggested that differences in procedure may explain the contrasting observations. Caution is advised in designating the mode of inheritance and further investigations are suggested by the authors.—E. R. J.


Glyoxalase activity of intact erythrocytes and of hemolysates was determined from the amount of CO₂ formed from methyglyoxal. This activity is dependent upon GSH. Glyoxalase activity of intact G-6-P dehydrogenase deficient erythrocytes did not differ from that of normal cells, but hemolysates of such deficient cells, assayed with added GSH, had statistically slightly higher glyoxalase activity than did normal erythrocyte hemolysates. Glyoxalase activities of relatively old and relatively young erythrocytes, separated by differential centrifugation, were almost identical. During primaquine-induced hemolysis in sensitive subjects, glyoxalase activity in intact cells decreased coincident with the fall in GSH, rose above the baseline after drug was stopped, but lagged behind the reticulocyte increase. Activity in hemolysates also decreased during hemolysis, rose with reticulocytosis and remained markedly elevated after subsidence of reticulocytosis. Changes in cell and hemolysate glyoxalase activity during anemia induced by acute phlebotomy were small or equivocal. It was concluded that the increased glyoxalase activity in hemolysates of G-6-P dehydrogenase deficient cells was not due to their younger than normal average age, that it might be a compensatory mechanism for the lowered concentration of GSH and that the reticulocytes produced as a result of primaquine in sensitive subjects are different from those produced by normal or sensitive subjects after blood loss. Although the role of methyglyoxal in metabolism is still uncertain, it was pointed out that methyglyoxal is toxic and can inhibit several enzymes. The importance of evaluating enzyme activities in intact cells, rather than in hemolysates alone, was emphasized.—E. R. J.


A careful study of 110 members of a Japanese kindred in which 3 acatalasic individuals were noted appeared to establish the fact that there are at least two forms of acatalasia. In addition, a previous study suggested the possibility of a third variant with completely recessive inheritance. In the present study, rather than observing the usual trimodal distribution of erythrocyte catalase activities which will differentiate homozygous, heterozygous and normal subjects, a broad continuum, exclusive of zero values, was noted. The apparent variability in expression of the carrier state and the overlap of carrier values with normals observed in this kindred resembled the findings reported in Swiss kindreds. Two hypotheses were advanced: 1) two different, but allelic, genes for acatalasia, one new and one previously described; 2) presence of a new gene which may or may not be allelic to that previously described and
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which is more sensitive to modifying influences than the other mutant. No satisfactory explanation, however, was advanced to account for the higher mean catalase values in the presumed heterozygotes in this kindred.—E. R. J.


Hb H and Hb Bart's was present in two children of mixed Chinese, Siamese, Indonesian and European descent. Both were thought to have α-thalassaemia. When a modified starch-gel electrophoretic technic was used (reduction in thickness of starch-gel to 2–3 mm.) a fraction with the same mobility as Hb H was present in all other members of the family. It is suggested that both parents have α-thalassaemia trait and the two more severely affected children are homozygotes.—I. C.


Small human embryos (C-R length less than 8.5 cm.) contain 2 slow moving components named Hb-Gower 1 and Hb-Gower 2. In the smallest embryos Hb-Gower 1 constituted up to 20 per cent of the Hb present and Hb-Gower 2 10 to 16 per cent. Thereafter they were present in only small amounts and none was present when the C-R length exceeded 10 cm. Hb-Gower 2 has also been found in 1 out of 300 normal cord bloods and in the red cells of all of 6 neonates with the D1 trisomy syndrome. Peptide analysis of Gower 2 showed 2 normal α chains and what appear to be 2 hitherto undescribed polypeptides termed ε chains. Thus Hb-Gower 2 is α2ε2. Less information is available about Hb-Gower 1. It does not appear to contain α chains and it may consist solely of 4 ε-chains.—I. C.


In 14 subjects with thalassemia major, requirements varied for transfusion both before and after splenectomy in different patients. The need for blood transfusion was not closely correlated with hereditary aspects, erythrocyte morphology, reticulocyte levels or the survival and sites of destruction of either autologous or donor erythrocytes. In subjects with thalassemia major, no single parameter alone may be relied upon to determine management.—H. H. F.


A high frequency of thalassemia was found among Kurdish and Indian Jews. Most of the patients had β-thalassemia. Hb A2 level determinations using the paper electrophoretic technic of Lehmann and Ager was found to be reliable and suitable as a screening method.—B. R.


The red cell antigen i is maximally developed at birth and decreases in strength during the first year of life. Thus fetal cells react best with anti-i. The authors tested the red cells from 17 patients with thalassemia major with an anti-i serum and these reacted almost as well as fetal cells. A similar phenomenon was noted with red cells in aplastic anemia and acute leukemia. Cells from thalassemia minor are more variable.—I. C.


The effect of the Forssman antibody and guinea pig complement in producing lysis of sheep red cells is accompanied by the production of "holes" in the cell membrane seen on electron microscopy. It had been predicted that production of a single lesion in the red cell membrane will lead to lysis, and under experimental conditions the number of lesions can be predicted. The authors attempted to correlate the predicted number of lesions with
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the number actually observed on the red cell surface, and on the whole a fair degree of correlation was found. This is held to support the "one-hole" theory of complement lysis.—I. C.


The red blood cells of the patient reported and of his mother gave positive direct antiglobulin (Coomb's) reactions. Increased levels of γ globulins and macroglobulins, rheumatoid factors, antinuclear factors and antibodies to cardiac and thyroid tissue were present in various combinations in the serum of the patient, his mother and other close relatives. A sibling had died of polyarteritis nodosa. The evidence indicates that a genetic predisposition is a necessary but not sufficient condition for the development of diseases associated with immunologic hyperreactivity.—H. F.

ULCERATIVE COLITIS WITH ANTI-ERYTHROCYTE ANTIBODIES. Susie Fong, H. Fudenberg, and P. Perlmman. From the University of California School of Medicine, San Francisco, Calif. Vox Sang. 8:668, 1963.

Antibodies to both red cell and colon antigens were detected in the serum of a patient with ulcerative colitis and Coomb's positive hemolytic anemia. The erythrocyte coating material proved to be typical 7 S. No cross reactivity between the erythrocyte and colon antibodies could be demonstrated in absorption studies. The possibility of an underlying genetic predisposition to aberrant immunologic response in patients with ulcerative colitis is discussed.—H. H. F.


The univalent fragments produced by papain digestion of human bivalent 7 S antibody γ-globulin and subsequent chromatographic separation are termed A and C. The data presented show that any individual molecule of 7 S γ-globulin has two A and no C or two C and no A fragment, depending on net charge of the molecule. All molecules have one fragment devoid of antibody combining sites. Univalent fragments of incomplete anti-Rh antibodies do not agglutinate cells either untreated or enzyme treated, but do combine with the cells to produce positive Coomb's reaction. Hence, truly univalent antibody differs in serologic behavior from "incomplete" antibody: the serologic behavior of "incomplete" antibodies such as anti-Rh depends on properties other than "univalence." —H. H. F.


These studies were carried out with a purified 131I-labeled anti-D obtained from a single donor. The effect of the pH of the suspending medium on the equilibrium constant between anti-D and the D antigen was studied. The highest values of the equilibrium constant were found in the pH range 5.5 to 7.0. The values were considerably reduced at pH values below 6.0 and above 8.0. Study of the rate of dissociation of antibody from the red cells at pH values below 6.0 revealed considerably heterogeneity of the antibody with respect to its rate of dissociation. For instance, a small percentage of antibody dissociated in the pH range 5.0–6.0, and this antibody was also found to dissociate rapidly at neutral pH. On the other hand, antibody which dissociated at pH 2.0–3.5 was found to dissociate relatively slowly at neutral pH. The effect of ionic strength on the rate of association was investigated and it was found that a reduction from 0.175 I to 0.03 I brought about a 1000-fold increase in the rate of association.—I. C.


Five cases are reported in which increased amounts of specific blood-group substance were present in the serum and caused difficulty or errors in blood grouping. The importance of checking the results of blood groupings in adults by a study for anti-A and anti-B, and in newborn infants by repeating the determination on washed blood cells is emphasized. Only weak blood-cell suspensions or washed blood cells should be used for compatibility tests.—H. H. F.
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Indirect reticulocyte counts after staining with 0.05 per cent and 1 per cent brilliant cresyl blue were compared. With the latter staining procedure, reticulocyte counts were about 50 per cent higher than with the former. Differential counts of reticulocytes according to their state of maturation showed that about 50 per cent of the most mature reticulocytes are missed with the 0.05 per cent stain. The frequency of less mature reticulocytes did not vary with the concentration of the stain. The indirect counts were also compared with the direct reticulocyte count method of Björkman. With the direct method, reticulocyte counts were about 3% of the indirect counts after staining with 1 per cent brilliant cresyl blue.

—S.-A. K.


In rat liver, synthesis of coenzyme B₁₂ from Co⁶⁰ labeled 5,6-dimethylbenzimidazolylcobamide cyanide was demonstrated.—E. K.


Evidence is presented that a higher value for the estimation of L. casei (folate) activity is obtained if the dilution of serum in the preparation of the extract for assay is increased from 1:10 to 1:100. This procedure results in a better differentiation between the values in normal subjects as opposed to those with folic-acid deficiency. The value of ascorbic acid as a preservative for the L. casei activity of stored serum is confirmed.

—I. C.


By conjugase treatment and chromatography, varying quantities of N¹⁰ formyl pteroylglutamic acid, folic acid, and pteroylglutamic acid were extracted from 12 foodstuffs. While this study represents a further advance in the study of food folates, the reader must bear in mind that extraction of a given form of folate from a given food does not mean that the food itself contains that folate in that particular form prior to the extraction procedure.—V. H.


A steady decline in the serum B₁₂ level was noted in Rhesus monkeys in captivity on vegetarian diets. Low B₁₂ levels were found in pregnant monkeys but if the monkeys were given parenteral B₁₂, high B₁₂ levels were found in the baby. It is suggested that the relative infertility of captive animals may be related to these low B₁₂ levels.

—I. C.


The total daily excretion of vitamin B₁₂ by all routes was calculated to be 0.66 to 2.1 μg. (mean 1.3 μg.).—V. H.


Seventy-five patients infested with dibotriocephalus latus were studied. Gastric suction biopsy was normal in 28 and showed varying degrees of gastritis in 47 (superficial gastritis in 19, slight, moderate, and severe atrophic gastritis in 14, 8 and 6, respectively). Hydrochloric acid and pepsin secretion, low serum B₁₂ concentration, and low Schilling test values correlated well with the histologic findings. Sixteen patients had manifest B₁₂ deficiency; among these, 13 had atrophic gastritis of varying degree and 2 had superficial gastritis. Follow-up studies were done in 32 patients with gastritis about 6 months after expulsion of the worm. In 8 cases some improvement of the gastric mucosa was observed, in 22 patients there was no change, and in 2 progression was noted. From the data it cannot be decided whether gastritis was a cause or an effect of vitamin B₁₂ deficiency.—S.-A. K.

There is a decline in the L. casei (Folate) activity of serum and of the serum vitamin B₁₂ level throughout pregnancy. Nevertheless patients with megaloblastic anemia in pregnancy could not be distinguished from other pregnant subjects by using these tests. Serum appropriately diluted was heated in the presence of ascorbic acid and in its absence, and thereafter was assayed with L. casei. The difference between these values is referred to as labile folic acid activity of serum. Measurement of the labile folic acid activity resulted in a better distinction between megaloblastic and normoblastic pregnancy.—I. C.


Three consecutive cases with chronic myelofibrosis are described who presented with anemia and with megaloblasts in either peripheral blood or marrow. Two had a good response to folic acid therapy and the third a rather doubtful response. Megaloblastic anemia due to folic acid deficiency is an important but unfortunately not generally recognized cause of marrow failure in megaloblastic anemia.—I. C.


Although pyridoxine-responsive anemia has usually been considered characterized by normoblastic erythropoiesis, hypochromic, microcytic adult erythrocytes and evidence of iron overload, the patients described have indications that other morphologic expressions occur in appreciable numbers and indicate that a clinical trial of pyridoxine should be assayed in obscure anemias accompanied by iron overload or familial involvement.—V. H.


Using rat liver slices an increased uptake of Fe⁵⁹ was demonstrated when the diets of these rats were supplemented with ethionine for 9–10 days.—I. C.


Although iron absorption is claimed to be increased in chronic pancreatitis, iron absorption using Fe⁵⁹-labeled hemoglobin was decreased in a group of 15 children with cystic fibrosis. These children had received pancreatin therapy, but iron absorption remained low after its withdrawal for 5 days.—I. C.


The incorporation of tritiated thymidine has been demonstrated to be a simple, sensitive method for evaluating the mitotic activity of tissues. By this method it has been shown again that erythropoietin stimulates erythropoiesis in the rat spleen. This erythropoiesis appears not to be achieved by an increase in mitotic activity within the stem cell compartment, but rather the results are consistent with the hypothesis that erythropoietin causes differentiation of reticuloendothelial cells into erythrocytic elements.—H. H. F.


Erythropoietin is not precipitated by polyphosphoric acid. Erythropoietin can be concentrated from polyphosphoric acid-precipitated plasma or tissue extract by adsorption to DEAE-Sephadex. —S.-A. K.

The Precipitation of Erythropoietin from Urine by Means of Tannic Acid. Use in Analysis and Production of Erythropoietin-Rich Preparation. P. Hansen. From the
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Protein precipitated from urine with tannic acid contains the majority of erythropoietin activity. The protein containing the erythropoietin can be released from the tannic acid precipitate by means of caffeine. The method can be used for concentration of erythropoietin.—S.-A. K.


After hypertransfusion, normal C57B1/6 and anemic C57B1/6 mice show the same increase in red cell volume, decrease in reticulocytes, and temporary cessation of blood formation. The hematocrit value at which new erythrocytes are again released into the circulation depends upon the genotype of functioning blood-forming tissue. Injections of erythropoietin stimulate red cell formation in normal C57B1/6 mice hypertransfused to polycthemic levels, but have much less effect upon genetically anemic C57B1/6 mice hypertransfused to similar levels.—H. H. F.


Among 32 rabbits subjected to constriction of one renal artery, one animal developed erythrocythemia. Total red cell mass was about 2.5 times greater in the controls. The marrow was hyperplastic. Cardiopulmonary disease which might have explained the erythrocythemia could not be demonstrated at autopsy. Juxtaglomerular granules were numerous in the clamped kidney but scarce in the intact kidney. Erythropoietin could be demonstrated in rabbit kidney extracts after clamping the afferent artery. No activity was demonstrable in the contralateral, unclamped kidneys. It remains unexplained why only one animal developed erythrocythemia.—S.-A. K.

LEUKOCYTES


Phagocytic activity was measured by the ability of leukocytes to ingest heat-killed organisms in a standardized suspension at 37°C. The phagocytic activity of neutrophils is increased in whole blood in cases of iron-deficiency anemia. A similar increase in activity was produced by an artificial reduction of the volume of red cells in normal blood.—I. C.


Glycogen phosphorylase activity varies widely in leukocytes from different donors, but mean activity is about the same in chronic granulocytic and lymphocytic leukemic leukocytes as it is in leukocytes from normal donors. Most leukocyte glycogen phosphorylase seems to exist in the a form.—T. E. B.


Chronic myelocytic leukemic leukocytes have been shown to contain low levels of glycogen when compared to normal leukocytes. The authors incubated leukocytes for 3 hours in the presence of a glucose-containing medium. They found that chronic myelocytic leukemic leukocytes were able to accumulate glycogen three times as rapidly as were normal leukocytes. However, when normal leukocytes were first incubated for 1.5 hours in the absence of glucose in order to deplete them of their glycogen stores, they were then found to accumulate glycogen at the same rate as did the leukemic cells. It is suggested that intracellular mechanisms for glycogen synthesis are not impaired in the leukemic leukocyte, and that some other mechanism is responsible for the low glycogen levels found in chronic myelocytic leukemic leukocytes.—T. E. B.

Data are presented suggesting that thymidine phosphorylase activity is present in leukocyte homogenates and in preparations of intact leukocytes. Normal leukocytes contain significantly higher levels of thymidine phosphorylase activity than do leukemic leukocytes. Low thymidine phosphorylase activity may be a function of cellular immaturity and capacity for DNA synthesis rather than a characteristic of leukemia per se. The different levels of thymidine phosphorylase activity in different cell populations may profoundly affect their uptake of tritiated thymidine and should be taken into account in the interpretation of experiments using tritiated thymidine as a DNA label.—T. E. B.


The formation of 19 S and 7 S antibody to polio virus in rabbits differed in (a) antigen dose requirements for induction and maintained synthesis; (b) kinetics; (c) retention of memory; and (d) sensitivity to prior x-irradiation. These differences are best explained on the assumption that the two antibodies are produced by different cells.—H. H. F.

**Fluorometric Analysis of Amidase and Alkaline Phosphatase in Neonatal Rat Lymphocytes: Peak Activities at Two Days of Age.**


The activity of four amidases and alkaline phosphatase was tested in thymic cell suspensions from neonatal rats. These enzymes were most active on the second day and their activity declined by day 3 and 4. It was thought that this activity was a feature of the large thymocyte.—I. C.

**Histocompatibility and Immunologic Competence in Renal Homotransplantation.**


Circulating human lymphocytes grown in tissue culture are probably stimulated to form large cells and to undergo mitosis when antigenic material or phytohemagglutinin is present. Cultures of lymphocytes may perhaps be used to assay histocompatibility. The lymphocytes from potential kidney recipients are cultured with those from potential donors, and the percentage of large cells and mitoses stimulated is determined. Also in kidney recipients receiving immunosuppressive therapy the effect of therapy can be evaluated by determining the responsiveness to phytohemagglutinin of lymphocytes cultured from recipients.—T. E. B.

**Studies of Immune Destruction of Lymphoid Tissue. I. Lymphocytotoxic Effect of Rabbit-Anti-Rat-Lymphocyte Antiserum.**


A destructive agent selective for lymphoid tissue is being sought in order to allow a more thorough study of the physiology of the lymphoid system and to aid in achieving successful homotransplantation. The authors describe production of a rabbit antiserum which destroys rat lymphocytes in vitro, produces a marked lymphopenia in vivo, and has surprisingly little effect on peripheral blood neutrophil counts of the recipient rats. In contrast to previous studies, daily intraperitoneal injections of the antiserum for 21 days produced a sustained lymphopenia, perhaps because recipient lymphoid tissue was damaged enough to prevent inactivation of the administered antiserum by newly formed rat antibodies.—T. E. B.

**The Lymphocyte Content of the Bone Marrow in Thymectomized-Lymphadenectomized Rats.**


Eleven young rats, mean weight 75 Gm. were thymectomized. At the same time about 70 per cent of their total lymph nodes were removed. Seventeen control animals were sham-operated. Two months later quantitative bone marrow studies were performed. No significant differences between the two groups were found with respect to total cells, erythroid cells, myeloid cells, and lymphocytes in the marrow. Mitotic index counts were done in Peyer’s patches and thoracic lymph nodes. No difference between groups was found.


The case is reported of a girl who succumbed at 15 days to overwhelming bacterial infection. At post-mortem she was found to have alymphoplasia of the thymus and a marked poverty of lymphocytes in all other lymphoid tissues. Her peripheral blood had shown lymphopenia. An additional finding of great interest was the total absence of granulocytes in her tissues, blood, and bone marrow at post-mortem, though the marrow contained abundant megakaryocytes and some nucleated red blood cells.—T. E. B.


A 10-year-old girl with chronic neutropenia unimproved after splenectomy is described. Her bone marrow showed abundant granulocytes at all levels of differentiation with marrow segmented neutrophils actually being relatively increased. The segmented neutrophils looked abnormal because of frequent cytoplasmic vacuolation, excessive length and thinness of the filaments connecting the lobes, and a tendency to hypersegmentation. By several different methods it was shown that the patient's mature marrow granulocytes had decreased viability and functional activity. It is suggested that an increased rate of intramedullary cell death and retention, in the marrow, of cells damaged were the mechanisms responsible for the peripheral granulocytopenia.—T. E. B.


A case of fatal chlorpropamide agranulocytosis is presented. Leukoagglutinins were present in the patient's serum and were accentuated by the addition of chlorpropamide in vitro. Platelet agglutinins activated by chlorpropamide were also detected.—T. E. B.


Nine examples of this syndrome were encountered in the course of 173 open-heart perfusions (5 per cent). Pyrexia appeared after 3 weeks and was associated with splenomegaly and generalized lymphadenopathy. A transient maculopapular rash was seen in four patients. The total white count was normal in seven and 10,000 and 14,000 in the two other patients respectively. Atypical lymphocytes were noted in eight of the nine cases at the time of diagnosis and in the 9th case these cells appeared later. The Paul-Bunnell was positive in two cases.—I. C.


Three cases of hyperthyroidism associated with lymphatic hyperplasia have been presented, with emphasis on the diagnostic difficulties which arise when the hyperthyroidism is not obvious. One of the patients showed marked hilar adenopathy on chest X-ray; this disappeared after propyl-thioracil therapy. The others had splenomegaly, and even Pal-Ebstein-like fever. In any lymphoma-like state, long standing hyperthyroidism should be considered.—B. R.


Bone marrow from patients with either acute or chronic myeloid leukemia was inoculated into human embryo tissue cultures. A virus was isolated from each of 10 cases, and became cytopathic for the cells in the course of serial passage. Comparable cultures inoculated with bone-marrow from control cases showed no such changes. The viruses produced no pathologic changes after inoculation into newborn mice, hamsters and ferrets. The viruses were weakly neutralized by sera from patients with leukemia. The author concludes that the virus or a group of antigenically related viruses, is frequently associated with human leukemia.—I. C.

It was unexpectedly found that the addition of phytohemagglutinin to purified suspensions of lymphocytes from normal human blood resulted in an apparent striking protection of the lymphocyte suspension against the cytotoxic effect of irradiation. Morphologic criteria were used for determining whether or not the irradiated lymphocytes had been seriously damaged. Some radioprotection of lymphocytes was observed even when phytohemagglutinin was added to the suspensions 2 days after irradiation had been given.—T. E. B.


The Philadelphia chromosome (Ph1) was demonstrated at a very early stage of the disease in six cases. One of these had polycythemia vera and two others had total white cell counts of 24,000 and 28,000 per cu. mm.—I. C.


The Bence Jones protein forms an insoluble aggregate between 50 C. and 70 C., which dissolves on heating to 100 C. owing to deaggregation and degradation to polypeptide chains. On cooling, the various polypeptide chains recombine to form a product resembling the starting material, which may reaggregate at temperatures ranging between 70 C. and 50 C.—H. H. F.


Thirty-seven cases with myelomatosis treated with melphalan were observed for more than 1 year. The total treated was 70. A daily dose of 5 mg. was given for 16, 20 or 25 days and after 1 to 2 weeks treatment was continued with a maintenance dose of 2 mg. a day. Significant leukopenia was the usual consequence of therapy. Thrombocytopenia occasionally occurred. Severe anemia was regarded as a contraindication to therapy. A high proportion of patients showed some response to this therapy both clinically and biochemically.—I. C.

Thrombocytopenia occasionally occurred. Severe anemia was regarded as a contraindication to therapy. A high proportion of patients showed some response to this therapy both clinically and biochemically.—I. C.

Hemostasis


It could be shown that erythrocytes contain a factor which stabilizes fibrin clots. The factor is activated by thrombin in the presence of Ca ions, and is inactivated by antithrombin II. A new technic for titration of stabilizing factor is described. It could also be shown, that globin enhances the titer of the stabilizing activity.—C. K.


This paper is devoted to a detailed account of the methodology of the assay of the components of the fibrinolytic system.—I. C.


Changes in the electrophoretic pattern of plasma proteins were induced by prolonged fibrinolysis induced by stasis of venous blood or by nicotinic acid. The changes consisted in increase of the $\alpha_2$-fraction and decrease of the $\beta$-globulins fraction and were transient.—E. K.


The formation of FDP was followed in the course of plasmin-induced proteolysis and the inhibitory effect of these products on the fibrin-
HEMORRHAGE IN HEMOPHILIA, WITH A SPECIAL
REFERENCE TO FIBRINOLOGY. O. Katsum. From
Nagoya University School of Medicine, Nagoya,

In 45 patients with hemophilia, there was good
correlation between fibrinolytic activity and sever-
ity of hemorrhage. Thus, in a group with severe
bleeding, decreased antiplasmin was observed; with
moderate hemorrhage there were increased plas-
min and normal antiplasmin; with mild hemor-
rhage, fibrinolytic activity was found to be en-
tirely normal. Twenty cases of hemophilia with se-
vere bleeding were given oral e-aminocaproic acid
(e-ACA) 100 to 200 mg./Kg. daily. Eighteen
of twenty cases showed marked improvement of
bleeding. In the effective group, inhibition of
fibrinolytic activity and a decrease in inhibitor of
thromboplastin formation were observed. Both in
vivo and in vitro experiments revealed the close
relation between the inhibitor and fibrinolysis.
The concentration of e-ACA in the patient’s blood
was quantitated by means of paper chromatography. In
seven healthy subjects given similar doses of e-
ACA maximum blood levels were 70 to 110 y/ml.,
and e-ACA disappeared from the blood within 4
hours after administration. In hemophiliacs, espe-
cially with severe hemorrhage, the maximum con-
centration was only 25 to 52 y/ml., and e-ACA
was no longer demonstrable in the blood 1 hour
after administration. In the patients without bleed-
ing, both the maximum concentration and dura-
tion in the blood showed no difference from those
of the normal subjects.—K. F.

THROMBOCTOPHENIC SERUM: AN ARTIFICIAL FAC-
TOR VIII-DEFICIENT REAGENT. E. Davidson and
S. Tomlin. From University of Cambridge. J.

It is suggested that such a serum provides a
satisfactory source of factor V in the assay of
factor VIII in the thromboplastin generation test.
This was prepared by collecting whole blood into
siliconized tubes and removing both red cells and
platelets by rapid centrifugation. The plasma was
allowed to clot and the resultant serum stored.
—I. C.

EFFECT OF ADENOSINE DIPHOSPHATE ON CIRCULAT-
ING PLATELETS IN MAN. M. G. Davey and H.
Lander. From University of Adelaide, South
1964.

Adenosine diphosphate (ADP) not only aggre-
gates platelets in vitro, but infused into human
volunteers produced very transient falls in the
circulating platelet count. It is suggested that this
was due to their transient concentration within
either the splanchnic or pulmonary circulations.
—I. C.

BLOOD CLOTTING FACTORS IN RENAL DISEASE. K.
Satake. From Nippon Medical School, Tokyo.

The association of an hemorrhagic tendency
with renal diseases, especially with uremia, has
been recognized for many years. This paper re-
ports selected clotting factors in 113 patients with
renal disease without regard to hemorrhage. Re-
duced activities of factors II, V, VII, IX and X,
were observed in chronic renal insufficiency. Elev-
ated activities of various clotting factors, espe-
cially of factors V, VII and X were found in the ne-
phrotic syndrome. Elevation of plasma fibrinogen
was common to almost all renal diseases. Signifi-
cant correlations were demonstrated between
various clotting activities and renal function tests
(NPN, GFR and RBF). However, histologic study
of the liver in autopsied cases of chronic renal in-
sufficiency revealed considerable damage. There-
fore, hepatorenal dysfunction is considered to be
responsible for the changes in clotting factors in
chronic renal insufficiency. This view was partly
supported by animal experiments. Significant posi-
tive correlations of factors V and VII complex with
serum cholesterol level were observed.—K. F.

HOMOCYSTINURIA, THROMBOSIS AND THE BLOOD-
PLATELETS. L. McDonald, C. Bray, C. Field, F.
Love and B. Davies. From The London Hospi-

Homocystinuria is an inborn error of metabo-
libism. Clinically spontaneous arterial and venous
thrombosis is a feature. All these children showed
an increase in platelet stickiness. When homocys-
teine was added to normal blood there was a
similar enhancement of platelet stickiness. There
was no abnormal tendency to platelet aggregation.
—I. C.

Cells thought to be circulating megakaryocytes were about 40 times more common in the antecubital-vein blood of patients with pelvic or head and neck cancer than in persons without cancer. Such cells can readily be confused with embolic cancer cells in the blood. Interestingly the megakaryocytes were about 10 times more common in blood from veins draining the site of the cancer than in antecubital-vein blood. There was no obvious relationship between numbers of circulating megakaryocytes and either extent of the cancer or prognosis.—T. E. B.

MISCELLANEOUS


Hybrid, bivalent antibody molecules bearing specific combining sites for both ovalbumin and bovine γ-globulin were produced by reoxidation of a mixture of the 3.5 S fragments of the two specifically purified antibodies. The dual specificity and combining properties of the hybrid antibody were demonstrated by mixed agglutination and two-step agglutination experiments, and by test systems utilizing inhibition of agglutination or dispersal of agglutinates followed by antoglobulin reactions. Visual demonstration of the dual specificity of the hybrid was shown by coupling one homologous antigen to oval nucleated chicken cells and the other to round mammalian erythrocytes.—H. H. F.


Low molecular weight γ-globulins in normal human urine are antigenically related to the S fragment of papain-digested γ-globulin. There are at least two distinct populations of γ-u, designated γ-u A and γ-u B, which are respectively related to Korngold and Lipari Type A Bence Jones proteins. The low molecular weight γ-u-globulins are increased in patients with various diseases associated with hypergammaglobulinemia and diminished in patients with hypogammaglobulinemia.—H. H. F.


The agglutination by rheumatoid serum of red cells sensitized by anti-D serum (Moore) was inhibited by γ-globulins of some normal serums. The inhibitor, tentatively named Gm(p), is associated only with 7 S γ-globulins and it differs from other previously defined serum groups. It is much more common in Caucasians than in Negroes, and probably is determined by a simple dominant gene.—H. H. F.


The authors checked a new disposable Wintrobe tube against the standard reusable tube, and showed that there was no significant difference in the result obtained for hematocrit or sedimentation rate.—C. R. M.