ABSTRACTS OF SPECIAL INTEREST

MEGALOBLASTIC ANEMIA IN A 3 YEAR OLD CHILD. C. J. Sievers. From the Copenhagen County Hospital, Gentofte, Denmark. Ugesk. laeger 125: 1744, 1963.

Case report of a girl, 3, with megaloblastic anemia. After vitamin B12 the marrow became normoblastic and the hemoglobin rose to normal. pH of gastric juice: 4. Two Schilling tests performed 1 year apart, both with and without intrinsic factor, showed no absorption of vitamin B12. Suppression of the intestinal flora with neomycin and bacitracin failed to increase vitamin B12 absorption. Gastric juice from the patient normalized intestinal B12 absorption in a patient with proved pernicious anemia. Extensive liver function tests and intestinal absorption tests (iron absorption, glucose tolerance, xylose absorption, fat balance study) were normal. No gross abnormalities were noted in a GI series. Trace proteinuria was constantly present. Electrophoresis of urine proteins showed that the slight proteinuria was predominantly due to albumin (69 per cent) and α-globulin (21 per cent). Serum cholesterol was normal. Urinary amino acids were within normal limits. The urinary sediment and an IVP were normal. The parents and three brothers of the patient are healthy. The parents are cousins. [Abstracters comment: This interesting syndrome of apparently specific intestinal malabsorption of vitamin B12 and proteinuria has now been encountered in Finland (Gräsbeck et al.: Acta med. Scandinav. 167:289, 1960), Norway (Imerslund: Acta paediat. suppl. 119, 1960) and Denmark. Is this another Scandinavian specialty? — S. A. K.]


The authors describe a 20-month-old girl with megaloblastic anemia, permanent proteinuria, and pulmonary, pleural, and dermal changes. Vitamin B12 therapy given 2½ years after the onset of symptoms resulted in complete normalization of hemopoiesis. Biopsy material obtained from the skin and kidney revealed considerable changes in the structure of tubular epithelium with defects noted in the absorptive portion of epithelial cells. The disease is designated congenital ectomesodermal dysplasia and is considered by the authors as an essential epitheliopathy with megaloblastic anemia. — J. K.

ERYTHROCYTES

TRANSFER OF BILIRUBIN-C14 ACROSS MONKEY PLACENTA. R. Lester, R. E. Behrman and J. F.
Two successful experiments in which nearly physiologic amounts of unconjugated bilirubin C14 were injected into a fetal limb vein of a Rhesus monkey in utero were described. Total placental transfer of the fetal dose equaled 19 per cent in 6 hours and 3 per cent in 2 1/2 hours; most of the radioactivity was recovered in crystallized bilirubin. Major transplacental hemorrhage was excluded. Limited fetal hepatic bilirubin excretion appeared to have occurred in utero, compatible with low fetal glucuronyl transferase activity. It was suggested that the placenta is a major site of fetal bilirubin transfer in primates. —E. R. J.

A COMPARATIVE STUDY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN MAMMALS. B. Ramot and S. Bauminger. From Tel-HaShomer Government Hospital, Tel-Aviv, Israel. Biochim. et biophys. acta 73:186-192, 1963.

Antisera against G-6-PD purified from human erythrocytes were prepared after injection into rabbits, alone or with an adjuvant. Inhibition of G-6-PD activity was observed upon incubating the crude antibodies with enzyme isolated from human, monkey and pig erythrocytes, but not from rabbit cells. TPN did not reactivate inhibited enzyme nor did TPN prevent inhibition. Enzyme prepared from erythrocytes of subjects with G-6-PD deficiency was capable of removing the inhibitory antibody, whereas enzyme prepared from cells of normal subjects and diluted to the same low level of activity failed to remove the inhibitory activity of the antiserum. These findings were interpreted as indicating that an inactive form of G-6-PD with normal immunologic reactivity existed in the erythrocytes of deficient subjects. Variability in the behavior of antiserum prepared by the two methods, and heterogeneity of the antigens employed were described.—E. R. J.

ABSTRACTS

A 43,500-fold purification of G-6-PD from human erythrocytes obtained from outdated blood bank blood was attained with a yield of 7.5 per cent after repeated fractionation with DEAE-cellulose, ammonium sulfate, calcium phosphate gel and carboxymethyl cellulose with added TPN. The enzyme preparation, about 80 per cent pure, had a sedimentation constant of 6.5–7.1S, a diffusion constant of 3.4 x 10^{-7} cm.² per second, a molecular weight of 190,000, and it appeared to be a highly asymmetrical molecule with an axial ratio of 11. A component with a sedimentation constant of 3.9S was also observed, but was almost completely removed by further purification. A minimal value of 1 mole each of N-terminus alanine and tyrosine per 200,000 Gm. of protein was determined with C¹⁴-fluorodinitrobenzene, and it was suggested that there are at least two peptide chains per molecule of enzyme. Although the specific activity of 113 units per mg. was considerably less than that of the crystallized enzyme from yeast, the present purification is the highest yet reported for the enzyme from mammalian tissues. Each mole of enzyme contained 2 moles of tightly associated TPN that could be reduced by the enzyme. Apoenzyme, produced by treatment with acid ammonium sulfate, was very unstable, but its activity could be restored by incubation with TPN or TPNH. Removal of TPN resulted in a change in the sedimentation constant of the major protein component from 7S to 4S and recombination with TPN resulted in a 7S kinetic unit. It was suggested that the apoenzyme dissociates into two catalytically inactive subunits, each of approximately one-half of the molecular weight of the native enzyme and consisting of nonidentical peptide chains. DPN was not able to replace TPN in protecting the enzyme. A model was proposed in which a segment of the polypeptide chain of each subunit in the native enzyme is maintained by interaction with TPN in a specific configuration which allows both interaction with TPN and association of the two subunits into a catalytically active structure.

—E. R. J.


Ghosts of human erythrocytes, prepared by water hemolysis, metabolized the pentose moiety of inosine rapidly to xylulose-5-phosphate which eventually equilibrated with ribose-5-phosphate and then decreased to a low level during 6 hours of incubation. Glucose-6-phosphate equilibrated with fructose-6-phosphate and hexosemonophosphate accounted for 45 per cent of the inosine metabolized in 4 hours, whereas total recovery of ester carbon in all of the esters measured accounted for 75 per cent of the pentose metabolized. Triosephosphate, mostly dihydroxyacetonephosphate, accumulated and reached a maximum in 4 hours. The activity of glyceraldehyde-3-phosphate dehydrogenase was presumed to be a rate-limiting enzyme in ghosts. The concentrations of inosine and inorganic phosphate were found to influence the formation of hexose- and triosephosphates. These studies provided further evidence for the presence of the enzymes of the pentose phosphate pathway in human erythrocyte ghosts.

—E. R. J.


G-6-PD and acetylcholinesterase (AChE) activities of erythrocytes from normal full-term infants at age 0 to 3 days, 3 to 9 weeks and 10 to 17 weeks were compared with previously reported studies of Cr⁵¹-tagged erythrocyte half-life of other infants during the same age periods. The initially elevated G-6-PD level was decreased to nearly adult levels at 3–9 weeks and then appeared to increase again in the third age group. AChE activity, initially lower than that of erythrocytes of adults, declined further in the second age group and began to approach adult levels at 10–17 weeks. It was postulated that the similar alterations in erythrocyte survival and erythrocyte enzyme activities reflected reduced erythropoiesis after birth that resulted in an older erythrocyte population.

—E. R. J.


Differential centrifugation of blood from normal human subjects and from rabbits with reticulocytosis induced by bleeding was used to prepare layers of relatively young (top) and relatively
old (bottom) erythrocytes. Previously reported findings of the author and others were confirmed: the bottom layer of cells had a higher density and dry weight and contained less water, K, Cl, CO₂, PO₄, and total organic anion than the top layer, but similar amounts of Na and Mg, regardless of whether the results were expressed on a dry weight or per unit cell basis. Even greater differences were noted between layers of rabbit erythrocytes in which the top layer contained about 42 per cent reticulocytes; slightly increased corpuscular hemoglobin content and considerably more non-hemoglobin material were present in the reticulocyte-rich layer. Reticulocyte-rich top layers of rabbit blood had a higher K and lower Na flux and a higher uptake of Cr⁵¹ and triiodothyronine than did reticulocyte-poor and lower layers. Bottom layers of human blood had a higher influx of K⁴², a similar Na²⁺ influx, a lower Cr⁵¹ uptake and a similar triiodothyronine uptake when compared with top layers. Although significant differences in the concentrations of leukocytes between the layers were reported, the contributions of these cells to the biochemical differences remain unknown. It was postulated that maturation of mammalian erythrocytes is associated with alterations in the cell membrane.—E. R. J.


Erythrocytes of normal subjects and of splenectomized and nonsplenectomized patients with HS were incubated in plasma-phosphate buffer alone or with added methylene blue or iodoacetate for 2½ hours. Glucose utilization, pyruvate and lactate formation, pentose, 2,3-diphosphoglycerate, ketose, triose and ATP accumulation were measured. Glycolytic rates were nearly identical in normal and HS erythrocytes. Bound (nucleoside and nucleotide) pentose failed to accumulate in HS cells in the presence of methylene blue, less ketose phosphate accumulated in spherocytes and the sensitivity of glycolysis to inhibition by iodoacetate appeared to be greater in HS cells. The concentration of ATP was maintained as effectively in HS erythrocytes as in normal cells, and the hexosemonophosphate pathway was intact. No complete explanations for the observed differences were presented, but it was again suggested that alterations in the membrane and in its permeability might be the primary defect.—E. R. J.


Clinical and laboratory studies of three patients and five relatives confirmed previously reported observations of the abnormalities in erythrocytes deficient in pyruvic kinase activity. Column chromatography of extracts of whole blood before and after 8 hours of incubation demonstrated a 20 per cent decrease in ATP content of normal blood, a decrease of 48 and 35 per cent in the blood of two homozygous subjects and a 32 per cent decrease in the blood of one heterozygous individual. An abnormally low initial ATP content was noted in the blood of one patient, while a normal value was found in the blood of the other patient, despite elevated reticulocyte percentages in both bloods. A marked fall in DPN content during incubation of blood from two pyruvic kinase-deficient subjects that did not occur in the blood of normals was also noted. The data presented were compatible with an autosomal recessive mode of inheritance and the variability...
of the clinical manifestations of the disease was discussed.—E. R. J.


A progressive decrease in MCHC, associated with a progressive increase in free erythrocyte protoporphyrin (FEP), occurred during hematologic remissions in patients successfully treated with testosterone and corticosteroids, but was not associated with a progressive increase in free erythrocyte protoporphyrin (FEP). These changes disappeared after withdrawal of therapy. Alterations in MCHC and FEP were not observed in the erythrocytes of patients with congenital hypoplastic (pure red cell) anemia treated with cortisone. It was suggested that testosterone stimulated cellular maturation without affecting the final stage of hemoglobin synthesis.—E. R. J.


Although this paper does not have anything to do with blood directly, it should be brought before the attention of hematologists because in every respect electron microscope images of phytoferritin, derived from pea embryos, appeared to be identical with images of animal ferritin secured by the same procedure. The localization of phytoferritin in plastids makes it distinct from animal ferritin. Its presence in both plants and animals suggests that ferritin is an ancient protein from the evolutionary point of view.—O. P. J.


This article summarizes certain properties of ferritin, e.g., it is a protein shell of apoferritin, having a diameter of 120 Å, with a core of inorganic iron, having a diameter of 55 Å; its iron content varies from 0–35 per cent as shown by analytical ultracentrifugation; its electrophoretic mobility is the same as that of apoferritin; reducing agents remove its iron. Apoferritin is composed of 20 spheroidal subunits. The author was able to induce synthesis of apoferritin in HeLa cells under the influence of iron, and iron that acts as a stimulus becomes part of the final product, ferritin.—R. O. W.


Iron absorption and utilization were measured in 14 healthy prematures, 1 to 10 weeks of age, using radioiron incorporated in the infants’ formulae. Absorption ranged from 7 to 74 per cent (mean 32 per cent). Iron utilization between the 7th and 14th days ranged from 8 to 103 per cent (mean 52 per cent). Both absorption and utilization varied directly with the growth rate. Premature infants absorbed and utilized iron to a greater degree than did older infants.—R. O. W.


Four examples of Donath-Landsteiner antibodies failed to react with red cells of type TJ(a–). This is further evidence that auto-agglutinins have blood group specificity.—R. E. R.


The data reported indicate that a stromal glycoprotein, homogeneous by several criteria, is an active inhibitor of viral hemagglutination and also has M-N blood group specificity. The results obtained when the protein is removed from solution by mixing with influenza virus and centrifuging the mixture, or when it is reacted with anti-M-N serum indicate that a single protein possesses both the properties.—H. H. F.

An Hereditary Anomaly of the Erythrocytic Membrane with a Positive Coombs Test and Modification of the N Antigen. M. Jeannet,

A positive Coombs' test of the $\gamma_{1M}$ type, combined with modification of the group antigen N, was found in 11 members of a family from Berne, Switzerland. None showed signs of hemolytic anemia. The biochemical nature of the anomaly of the erythrocyte membrane is unknown, but it seems likely that an alteration in erythrocyte sialic acid metabolism is involved. The defect was inherited as an autosomal dominant.—H. H. F.


The RNA derivatives, cytidine sulfate and cytidylic acid, weakly inhibit Rh agglutination. A number of other compounds structurally related by virtue of containing rigid ring systems and phosphate ester groups were more potent inhibitors. $\alpha$-tocopherol phosphate gave the strongest inhibition at the lowest concentration. The inhibition is probably not specific as is indicated by (1) the lack of effect on Coomb's reactions, and (2) by the inhibition of blood group systems unrelated to Rh.—H. H. F.


After treatment with preparations containing both $\beta$ galactosidase and $\beta$ glucosaminidase, human erythrocytes displayed decreased reactivity with human anti-I and with horse anti-Pn XIV sera. Type A, cells were altered more rapidly than type O. Stroma treated similarly failed to absorb anti-I and released galactose and N-acetylglucosamine as monosaccharides.—R. E. R.

LEUKOCYTES


In 20 macrosomic newborn infants (over 4000 Gm. weight), a statistically significant increase of the cytoplasmatic lipid granule leukocytes was found.—P. d. N.


There is accumulating evidence that the lymphocyte is involved in delayed hypersensitivity and in the homograft rejection phenomenon. Therefore, this is an exceedingly timely article, for little is known about the cells that circulate in the human thoracic duct because of the obvious difficulty of obtaining suitable specimens. The thoracic ducts of seven patients were cannulated, and in none was there any indication of disease affecting the lymphoid organs or the immune mechanism. Although the formed elements of the thoracic duct fluid consisted almost entirely of lymphocytes, the heterogeneity of the cell population was very striking. Plasma cells were rarely encountered, with an incidence of less than 0.5 per cent. The absence of neutrophils, basophils and thrombocytes was noted. Eosinophils, on the other hand, were always present. The count ranged from 0.2 to 2.0 per cent. The number of erythrocytes varied from 4 to 10 per 100 leukocytes. Many lymphocytes revealed profiles of rough endoplasmic reticulum, which suggests that some lymphocytes are metabolically more active than others. The cytoplasm of some lymphocytes revealed bundles of fibrils which have been described only in malignant cells and peritoneal macrophages. Perhaps the occurrence of fibrils in typical lymphocytes is somehow related to the functions of freely circulating cells or their ability to migrate through tissues.—O. P. J.


When cells from the peripheral blood or thoracic duct lymph were cultured for 3 days in a medium containing the mitogen, phytohaemaglutinin, there was a 10-fold increase in the proportion of large lymphocytes. The transformed cells did not resemble the earlier cells of the lymphocyte series but they did have many of the ultrastructural characteristics of a cancer cell. Of particular interest was the occurrence of the crystal-like structure in the cytoplasm of cells derived from the blood.—O. P. J.
ABSTRACTS


Spleenic tissue was obtained from rats, guinea pigs, squirrels, monkeys and 2-week-old pups. The red pulp was composed of a system of sinuses and the pulp cords of Billroth. In the 2-week-old dog, evidence of hemopoiesis was found within the pulp cords. Some of the immature hemapoietic cells had plasma membranes that interdigitated with each other. This may partially explain why megakaryocytes and their precursors infrequently get into the circulation, while the nucleated erythrocytes which are not interlocked with littoral cells readily migrate from the pulp cord into the open sinus.—O. P. J.


Lymphoid cells labeled with H3-thymidine were infused into isologous recipients 29-30 hours partial hepatectomy. The recipient mice were killed 28-30 hours later, and the liver, intestine, Peyer’s patch and spleen were examined. Heat killed H3-thymidine-labeled isologous lymphocytes were also employed. Seventy-two to 78 per cent hepatic cell nuclei, 30-35 per cent of liver RE cell nuclei, and 90-95 per cent of intestinal crypt nuclei were labeled. Only a few intensely labeled lymphocytes and a somewhat larger number of weakly labeled lymphocytes were present in recipient animals. When labeled dead lymphocytes were infused, the results were similar except that intensely labeled lymphocytes were absent. These results suggest that reutilized DNA can be derived from dead cells. The reutilized DNA is probably in the form of nucleosides and nucleotides. DNA reutilization occurs in lymphoid organs, but to a lesser degree than in intestine and liver. The author speculates that reutilization of DNA may account for the preservation of immunologic memory, and also that dead cells may act as “trephocytes” by supplying DNA to proliferating cells. What the impact of these findings will have on the studies using H3-thymidine, which had assumed little or no reutilization of this material, is not mentioned by the author.—I. G.


The incidence of leukemia in a long-lived group of homologous radiation chimeras, C57H± LAF1, was 9 per cent. All leukemias were of the lymphocytic type. In a similar group of long-lived isologous radiation chimeras, LAF1 LAF1, there were no leukemic animals found. The incidence of other types of non-hematopoietic lesions were the same in both groups. No attempt was made to identify the genotype of the leukemic cell. The authors speculate that one possible cause of the higher incidence of leukemias present in the homologous radiation chimera is the intense isoantigenic stimulation received by the lymphocyte cell populations in this group of mice. (Abstracter’s note: This report lends support to the antigen deletion, immunoselection concepts of carcinogenesis.)—I. G.


The author reasons that if the increased incidence of leukemia among radiologists is because of more accurate diagnosis and not because of x-ray exposure, then the incidence of chronic lymphatic leukemia should also be increased. A survey of causes of 425 deaths between 1948-1961 in American radiologists between the ages of 35-74 was made. There was a statistically increased number of deaths from acute leukemia, aplastic anemia and multiple myeloma. There was no increased incidence of chronic lymphatic leukemia. The author points out the unusual association between radiation exposure and multiple myeloma which previously had not been noted. The question posed by the author, “Do excessive numbers of deaths from leukemia continue to occur in American radiologists?” is answered in the affirmative.—I. G.


Sera were obtained from 12 children with leukemia and from their first degree relatives. The
HEMOSTASIS


Arteriovenous shunts of polyethylene tubing were made between normal dogs and dogs made prothrombin-deficient by dicumarol; the shunts were maintained for 15 minutes, 1 hour, or 4 hours. The plasma prothrombin activity of each dog was determined at intervals during the period of cross-circulation and for 3 to 4 days after stopping the cross-circulation. The immediate effect of the procedure was simple dilution of the plasma prothrombin of the normal dog with that of the prothrombin-deficient plasma of the dicumarol-treated dog. After cessation of cross-circulation the prothrombin of the dicumarol-treated animals fell to previous levels within 12 hours whether the cross circulation time was 15 minutes or 4 hours. The prothrombin in the normal dog returned slowly towards normal over the course of many hours. The studies are interpreted as indicating that prothrombin is not present in extravascular sites in amounts sufficient to influence plasma prothrombin activity. The rapid loss of the acquired plasma prothrombin in the dicumarol-treated dogs did not appear to be primarily due to equilibration of plasma prothrombin with extravascular prothrombin.—R. G.


The metabolism of warfarin in man was studied by determination of unchanged warfarin in biologic fluids with a spectrophotometric method described in the paper. A standard dose of warfarin (1.5 mg. per Kg. body weight) was administered orally to 14 normal subjects. Maximal concentration occurred in the plasma in 2 to 12 hours. During the phase of elimination the half-time of warfarin's disappearance from plasma varied from 15 to 58 hours with a mean of 42 hours. Maximal decrease in prothrombin complex activity occurred between 36 to 72 hours. The subjects with the least depression of prothrombin complex had the most rapid elimination of warfarin and those with the greatest depressions had the slowest elimination. The absorption of warfarin from the gastrointestinal tract appeared to be complete, as no warfarin was found in the stools even after massive oral doses. Warfarin levels in plasma and prothrombin complex responses were virtually identical with oral and intravenous administration. Warfarin was found not to enter red cells but, as previously reported, in plasma almost all is bound to protein (albumin). The warfarin space was found to be the same size as the albumin space (2 to 6 times the plasma volume). Virtually no unchanged warfarin was excreted in the urine, but significant quantities of a metabolite were. Oral administration of vitamin K₁ did not influence the rates of absorption or chemical transformation of warfarin.—R. G.

Action of Carbon Disulphide on Fibrinolysis.


Carbon disulphide inhibits in vitro fibrinolysis, probably through the inhibition of activator. Streptokinase reduces the antifibrinolytic action of carbon disulphide. In workers, employed in viscose rayon factories, before and after 8 hours of work, a decrease of the fibrinolytic activity was found to be proportional to the concentration of carbon disulphide in the air; the effects were transient.
ABSTRACTS

An inhibition of SH groups is postulated for interpreting the action of carbon disulphide.—P. d. N.


Factor VIII was found to be absorbed to and to be removed from citrated plasma by aluminum hydroxide when polybrene was added to the plasma prior to the adsorption; little if any Factor VIII was adsorbed in the absence of polybrene. It was also found that significant amounts of polybrene were removed from the plasma by the aluminum hydroxide as measured by a heparin-neutralizing technic. The authors feel that the findings suggest that there is a direct interaction between factor VIII and polybrene. They speculate that since factor IX has been reported to react with heparin, a highly negatively charged compound, and factor VIII appears to react with polybrene, a positively charged compound, it is possible that this affinity for oppositely charged compounds is a clue to the mechanism by which factor VIII and IX interact.—R. G.


Previously reported molecular weights of polybrene were found to be in error because of the presence of fluorescent material which made the determination of molecular weight by the light scattering method inaccurate. Determination of molecular weight by ultracentrifuge methods revealed that they were considerably less than previously reported. A sample of polybrene was separated into three fractions by passage through a column of Sephadex G 50. For each fraction the molecular weight was determined from sedimentation equilibrium, and its toxicity and anti-heparin activity measured. There was a direct relationship between molecular weight and toxicity and an inverse relationship between molecular weight and anti-heparin activity. These studies revealed that a polymer of four monomer units (M. W. 1800) would be the preferred one for clinical use.—R. G.

MISCELLANEOUS

Studies in Antigenic Overloading with Massive Skin Homografts in Rats. J. M. Converse,
The authors tested the concept that very large skin grafts survive longer than small skin grafts. Nine by 6 cm. transplants were exchanged between two different strains of rats. At the same time, small skin grafts 1.3 x 1.3 cm. of a third strain, and also a small skin homograft of the same type as the large grafts, were grafted. The results indicate that large skin homografts survive twice as long as small control homografts. The specificity of the prolongation of the survival time of large grafts is shown by the fact that the small graft from the third strain was rejected in a normal time, whereas the survival of the small skin homografts present in the animal with the same type of large skin homograft was prolonged to the same degree as the large skin homograft. The data confirm the previous work of Zotikov, and lend support to the concept that antigen dosage may be one of the critical factors in producing tolerance to "self" or artificially administered antigens.—I. C.


Sera from 98 patients with myasthenia gravis were examined. Antibodies against muscle were present in 38 cases and against thymus in 2. Thirty-six out of 111 sera had antibodies against thyroid, and 10 sera also had anti-nuclear factor.—I. C.


The molecular properties of albumin isolated from macroglobulinemic human serum were compared with those of normal serum albumin. The effect of salts on the sedimentation rates indicated that there were small differences between the two albums.—H. H. F.


This is a study of the chromosome abnormalities found in peripheral blood of persons exposed to (a) occupational chronic irradiation (including a group of "distinguished" radiologists), (b) acute high-level irradiation, and (c) a control group having no occupational exposure. In group (a) the incidence of pseudo-diploid cells, dicentrics and other chromosome aberrations was considerably higher than in the control group. This occurred even when the calculated exposure (by film badge, etc.) was well within the presently prescribed safety limits. In group (b), 60-70 per cent of metaphases were pseudo-diploid 2 weeks after irradiation. This latter group was not followed long enough to calculate the rate of elimination of radiation-induced abnormalities. (Abstracter's comment: This report again raises the question as to whether any dose of irradiation is entirely safe?)—I. C.