LEUKOCYTE MORPHOLOGY and PHYSIOLOGY


When nontoxic doses of compounds belonging to the diaminoacridine group are administered, either parenterally or orally, to rabbits, guinea pigs, rats, or mice and tissues of the various organs fixed by the technic of Altman-Gersh, the localization of these compounds can be determined by the presence of fluorescence excited by near ultraviolet light. The fluorescence in nuclei stained in vivo with any of these compounds is discrete rather than diffuse and it resembles the chromatin pattern after staining with ordinary dyes. In lymphatic tissue, bone marrow and spleen, the nuclei of reticular cells fluoresce brighter than those of the free blood cells. Tissue cultures of cells so treated did not behave in any way differently from cultures of blood cells of noninjected animals. Membrane permeability and nucleoprotein concentration may play a role in the variations of fluorescence intensity of different nuclei. The fate of transfused leukocytes has also been studied by this technic (Farr, R. S.: Anat. Rec. 106: 194 (Abst.) 1950).—O. P. J.


The ubiquitous lymphocyte can seemingly leave lymphatic tissue, circulate in the blood stream, leave it and, either re-enter the lymphatic tissue or enter the bone marrow and intestinal wall. It may remain morphologically the same or, because of its pleuripotentiality, may transform into another cell type. Obviously, in order to follow lymphocytes, some kind of labeling must be used. To this end, one of the diaminoacridines, a nontoxic vital nuclear stain which fluoresces in near ultra violet light, has been used to label lymphocytes collected from popliteal lymph nodes of rabbits. Suspensions of lymph node material in tyrode solution, containing about 4,000 cells per cu. mm., were transfused autogenously into the ear vein.

Blood and tissues were examined for the presence of labeled lymphocytes in the near ultra violet light with conventional optics and then with lenses for phase microscopy in order to identify the cell in white light. Most of the labeled lymphocytes disappeared from the circulation during the first ninety minutes. Two hours after injection, labeled lymphocytes were found in the bone marrow, thymus, mesenteric lymph nodes, appendix, Peyer's patches and white pulp of the spleen. After twelve hours, labeled lymphocytes were still present in lymphatic tissue, but those in the bone marrow had differentiated into myelocytes. Also at this time some labeled lymphocytes were present in the lamina propria and submucosa. The implications derived from these experiments are manifold. We now have another method for following and studying the fate of transfused leukocytes, which may shed some light on their sequestration in such organs as the lungs.—O. P. J.

The fate of transfused blood cells and transplanted tissues in the recipient have been studied by various means, viz., differential agglutination, "tagging" with radioactive isotopes and staining with various vital dyes. In the recent experiments by Farr ('46 and '50) acriflavine hydrochloride, which fluoresces in ultraviolet light, was used to label leukocytes. In the present experiments, the fate of injecting suspensions of finely divided lymph nodes was determined by studying the distribution of large lymphocytes and lymphoblasts.

With the exception of the newly introduced cells being in a medium of blood, the procedure is actually one of transplantation of hematopoietic tissue. The suspensions of lymph node material were obtained by collecting mesenteric lymph nodes from 3 to 5 healthy rabbits about 6 months of age. These were finely divided, suspended in saline, centrifuged and strained thru gauze. About 30 cc. of this material was injected into either the ear vein or mesenteric vein of the recipient. In the latter case, one-half the amount injected into the ear vein produced similar results. Control suspensions of killed cells were made by freezing and thawing or by heating at 50 C. for thirty minutes. The suspensions contained about 150,000 lymphocytes per cc. Tissue sections of the lung, liver, spleen, bone marrow, lymph nodes and small and large intestine were studied. During the first twenty-four hours the major portion of lymphocytes were arrested in the lung capillaries. Between twenty-four and forty-eight hours, the lymphocytes gradually decreased in the lung and accumulated in the liver and spleen. The collections of lymphocytes were found in the interlobular portal veins and perportal tissue spaces.

The mechanism whereby lymphocytes change from an intravascular to extravascular position is as follows: lymphocytes attached to one side of the vein become covered with a thin layer of fibrins, endothelial cells proliferate and spread along these fibrin threads, eventually the lymphocytes are no longer in direct contact with the blood stream, and finally the original superficial endothelial cells atrophy and disintegrate. These perportal accumulations of lymphocytes resemble the metaplasia of lymphocytes found in lymphatic leukemia. There is a tendency to form lymphatic nodules without germinal centers and mitotic activity is evident. Injected lymphocytes also collected in the perifollicular regions of the spleen and showed signs of degenerating there. The bone marrow did not show any increase in lymphocytes and there was no sign of extensive elimination of injected lymphocytes by the small and large intestine. In the control experiments, killed cells did not accumulate in the lung, liver and spleen, but apparently disintegrated rapidly in the blood or tissues of the recipient.—O.P.J.


When electronegative colloidal substances are injected into the peritoneal cavity, they are removed and transferred to the tissues over three routes: through the diaphragm to the parasternal and mediastinal lymphatic channels, through the intestinal lymphatics and directly into the subperitoneal tissues. Experiments were planned to study the effects of such colloids on the structure and activities of lymph nodes.

Ninety-two albino rats, about 5 months old, were given either daily subtoxic doses or larger doses which usually caused the toxic syndrome to appear during the second or third week. The colloids used were Bierbrich's scarlet, chlorazol black E, and colloidal mercuric sulphide. The reaction of the reticulo-endothelium occurred in three overlapping phases. These have been called the activation period, the active plateau period and the period of regression. It was found that a period of at least several days is needed before a normal node can be converted into one capable of widespread phagocytosis. If the dosage remains at a subtoxic level, it is possible to secure some degree of equilibrium between phagocytosis and phagocytogenesis on one hand, and lymphocytogenesis on the other. However, this
cannot be maintained indefinitely and the node will eventually undergo regression. Gradually the creation of new macrophages decreases, lymphocytopenia is increased and the organ parenchyma reestablished. The presence of foreign particulate matter in lymph nodes has a depressing effect on mitotic activity.—O.P.J.


Fowl leukemia accounts for a high proportion of deaths in domestic fowl. Therefore, the importance of an accurate but simple method for enumerating white and red cells in the blood of fowls is obvious. The author has developed a diluting fluid, utilizing Giemsa stain, which enables the simultaneous counting of red cells, lymphocytes, monocytes and granulocytes. Since thrombocytes frequently clump, they may be difficult to count.—O.P.J.

**PITUITARY GLAND AND BLOOD LYMPHOCYTES. H. F. Colfer, J. deGroot, and G. W. Harris.**


This paper shows that the usual decrease in lymphocytes follows conditions of stress in the rabbit as well as other animals. The decrease was maximum at three hours after the stress. Emotional stress in the hypophysectomized rabbit, however, produced no lymphopenia. The hypophyseal-adrenal cortex mechanism in the hypophysectomized rabbit is still functional, for injections of ACTH in the hypophysectomized animal produced the usual lymphopenia. Denervation of the adrenal glands had no effect on the mechanism and injections of adrenaline produced no lymphopenia. The author claims that this lymphopenic response is the quickest indicator of anterior pituitary activity known at present.—R.C.C.

**THE IN VITRO REACTION OF CELLS TO ADRENAL CORtical STEROIDS WITH SPECIAL REFERENCE TO LYMPHOCYTES. J. D. Feldman.**

From Yale University School of Medicine, New Haven, Conn. Endocrinology 46: 552–563, 1950.

The object of this experiment was to settle the problem of whether lymphocytes are destroyed by adrenal cortical steroids under in vitro conditions. The literature on this subject is quite contradictory. Tissues of rats, rabbits, guinea pigs and human beings were studied. These experiments show that Lipo Adrenal Cortex (Upjohn) is a general cytotoxic agent which rapidly and completely injures lymphocytes, marrow cells, testicular cells, pseudoeosinophils and peripheral white blood cells in vitro. Compound A (dehydrocorticosterone acetate, Merck) acted similarly but less rapidly. Aqueous adrenal cortical extract (Wilson and Upjohn) was less damaging. This extract required a higher dose and a longer interval of time to produce comparable results. Agitation and increased temperature hastened the damaging effects. Compound E, desoxycorticosterone, testosterone propionate, growth hormone, ACTH and insulin were not damaging.—R.C.C.

**THE MECHANISM OF INSULIN-INDUCED EOSINOPENIA IN RATS. A. Dury.**


Both injections of insulin and epinephrine are known to produce an eosinopenia in rats. This experiment showed that epinephrine would produce an eosinopenia in intact and in denudated rats, but that insulin produced the effect only in intact rats. It is therefore concluded that the effect of insulin on the eosinophils is not a direct action but due to the release of epinephrine after the insulin injection.—R.C.C.

**THE LEVEL OF THE CIRCULATING EOSINOPHILS FOLLOWING TRAUMA. J. L. Gabriolave.**


Daily eosinophil counts were made on fasting patients after surgery, coronary occlusion, administration of adrenocorticotropic, 17-hydroxy-11-dehydrocorticosterone and testo-
sterone. All above treatments produced a decrease in eosinophil count except the testosterone therapy. The eosinophil level decrease was accompanied by an increase in urinary corticoids.—R.C.C.


Using a “window technic” which is described in detail, the authors produced the L.E. phenomenon experimentally in the skin of two normal human volunteers. After abrading the skin, preparing the “window” (coverslips applied over the abraded area), and adding diphtheria toxoid, plasma from a patient with acute disseminated lupus erythematosus was added. At intervals the coverslip was removed with its adherent cellular exudate. This removed coverslip was stained and examined microscopically. Another coverslip was placed over the lesion. This procedure could be repeated as desired at intervals and the progression of the cellular changes occurring in vivo could be thus observed. Under the conditions of the experiment, the neutrophilic L.E. cell usually developed as a result of the ingestion of other neutrophilic nuclear lobes or nuclei which had undergone a previous partial lysis. Less commonly the L.E. cell may represent the originally affected neutrophilic leukocyte in which lobar lysis is out of step in the individual nuclear lobes. The authors advise against repetition of these experiments in man until such a time as it is possible to utilize active necrotizing L.E. factor plasma known to be free of any contamination with the virus of homologous serum hepatitis.—R.B.C.

**LEUKOCYTIC DISEASES**

**PULMONARY INFILTRATION AND BLOOD EOSINOPHILIA IN CHILDREN (LOEFFLER’S SYNDROME). A REVIEW WITH REPORT OF EIGHT CASES. R. L. Nemir, A. Heyman, J. D. Gorvoy and E. N. Ervin.** From the Department of Pediatrics, College of Medicine, New York University, Bellevue Medical Center, and the Children’s Division, Sea View Hospital for Tuberculosis, New York, N. Y. J. Pediat. 37: 819–843, 1950.

The authors review a considerable portion of the literature relating to Loeffler’s syndrome with special emphasis on its possible etiologies, its pathology and its pathogenesis. The close relationship to allergy and particularly to parasitic infestations is discussed in detail. Eosinophilia of a considerable degree is a feature along with shifting pulmonary infiltrations. Although it is a benign condition, the course may sometimes be prolonged for a matter of months. Weight loss and impairment of liver function may occur. Eight patients with pulmonary infiltrations and eosinophilia are described. Five of the cases were mild. Three cases were chronic, and the condition persisted for months. Three patients were for a time mistakenly diagnosed as miliary tuberculosis. The importance of differentiating Loeffler’s syndrome in patients with a positive tuberculin reaction is emphasized. In 7 of the 8 patients intestinal parasitism was found.—R.B.C.


It has been known for thirty years that the cerebroside-keratin is present in lipid extracts of spleen in Gaucher’s disease. However, there are certain observations which make it doubtful that keratin is per se the sole abnormal chemical feature of the Gaucher cell and that it most likely is bound to some normal cellular constituent. This article describes the isolation and properties of a lipoprotein faction which was isolated from two spleens removed from patients with Gaucher’s disease. The protein moiety constituted 38 per cent of the lipoprotein faction and it did not present any significant feature in its amino acid composition, except for the absence of valine. Apparently keratin is embedded in a mesh of peptide coils, which will have to be considered in future concepts regarding the pathogenesis of Gaucher’s disease.—O.P.J.

Since the first description by Oberling and Wöringer in 1927 of Gaucher’s disease in infants, several cases have been described, but the histologic studies of the central nervous system very seldom has been done. In the case here reported the symptomatology was unusual of neurologic involvement, and discrete splenomegaly involvement. The death occurred in the first year of the life. In this case, in contrast to several published accounts, very numerous foam cells were found (as shown by a microphotography) in the nervous tissues. In the knowledge of the authors, Gaucher cells are not found in neurologic sections even when neurologic disturbances are present, except in one adult studied by Gunnar Tellum (1944), and in the case here presented.—J.P.S.


The authors review briefly the literature pertaining to central nervous system complications of infectious mononucleosis. Serous meningitis, manifested by nuchal rigidity, headache and pleocytosis of the spinal fluid occurs moderately frequently. Encephalitis and encephalomyelitis may also develop not uncommonly. Papilledema, probably due to primary optic neuritis, with or without serous meningitis or encephalomyelitis may also occur. The present report describes such a case with bilateral papilledema, serous meningitis and encephalomyelitis in a young man of 21. It is again emphasized that infectious mononucleosis is not always the benign disease it formerly was considered to be.—R.B.C.


A review has been made of consecutive cases of leukemia in children under 15 years of age who were admitted to the Pediatric Service of the University of California Hospital from 1939 to 1948. There were 90 cases of lymphogenous leukemia under 14 years of age, of which 76 were analyzed relative to duration of survival. The average duration of survival of 18 cases of untreated lymphogenous leukemia was 5.6 months. The average survival of 17 cases treated with irradiation plus blood transfusions and antibodies was 5.8 months. The survival of 24 patients who received transfusions but no antibiotics averaged 6.0 months. The survival of 17 patients who received blood transfusions and antibiotics averaged 8.9 months, a significantly prolonged survival time in comparison with the first three groups. The authors emphasize that in evaluating the effect of chemotherapeutic agents on survival in leukemia, it is essential that a statistical comparison be made with a comparable group that has not received the chemotherapeutic agent.—R.B.C.


Blood urethane levels were determined in patients receiving enteric coated urethane tablets for leukemia or multiple myeloma. Blood levels ranging from 0 to 27 mg. per 100 cc. of blood were obtained. The correlation between the dosage of the drug, the blood level, and the clinical response was poor. Hence, such a determination does not appear of value in the regulation of oral urethane therapy.—R.B.C.
ABSTRACTS

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Twenty-five patients with various blood diseases were treated with urethane. In 8 cases the authors observed psychic (catatonic) and neurologic disturbances, which partially required discontinuing the treatment. In 3 cases the autopsy showed degenerated ganglion cells in the brain and in the medulla with glial proliferations in the neighborhood.—C.M.

LEUKEMIA and MALIGNANT LYMPHOMA

THE SKELETAL LESIONS OF LEUKEMIC CHILDREN TREATED WITH AMINOPTERIN. F. E. Karpinski, Jr. and J. F. Martin. From the Department of Pediatrics and the Department of Radiology of Western Reserve University School of Medicine, and of Babies and Childrens Hospital, Cleveland, Ohio. J. Pediat. 37: 208-223, 1950.

This paper reports the roentgen observations made of the skeletal lesions in a group of 35 children with acute leukemia. Lesions of the skull, osteolytic in character and unresponsive to aminopterin therapy, were observed in 6 cases. Widening of the suture lines, presumably due to increased intracranial pressure, was observed in 4 patients. The long bones revealed evidence of abnormality in 81 per cent of the patients. Osteolysis was identified in 12 cases, superosteal new bone formation in 11, transverse bands of diminished density at the ends of the shafts of the long bones in 22 and transverse bands of increased density in 5 cases.

This last finding occurred in 1 patient who did not receive aminopterin, in 1 patient before aminopterin and in 3 patients after aminopterin therapy. In a single patient a true destructive bone lesion disappeared after aminopterin. However, in general it appeared that progression of bone lesions occurred despite therapy with aminopterin and there is no conclusive evidence that aminopterin will prevent the formation of new bone lesions. The authors conclude that the specific action of aminopterin on the skeletal lesions cannot be stated with certainty.—R.B.C.

ACUTE MYELOGENOUS LEUKEMIA WITH GIANT CELL CARCINOMA OF THYROID. P. Daron and P. Pizzolato. From Department of Pathology, Louisiana State University School of Medicine, New Orleans, La. Arch. Path. 51: 72-75, 1951.

This is the case report of a 54 year old Negro farmer who was admitted to the hospital after six weeks' illness with a sudden onset, characterized by a daily elevation of temperature, a migratory polyarthritis, dyspnea, weakness and a cough that produced white sputum.

The initial laboratory work revealed 1,770,000 red blood cells and 104,000 white cells per cc. There were 49 per cent myeloblasts. A sternal marrow aspiration performed four hours post mortem showed a hyperplasia of very immature cells—chiefly myeloblasts. At autopsy a firm, yellowish white nodule, well circumscribed, measuring 2 cm. in diameter was found in the left lobe of the thyroid. Microscopic examination of this nodule showed that it was composed of large, pleomorphic, single-nucleated and multi-nucleated giant cells with a variable amount of reticulated pink cytoplasm. This carcinoma had invaded skeletal muscle and metastasized to a hilar lymph node. The authors point out the extreme malignancy of both diseases and the infrequency of the combination.—O.P.J.


A brief case history, without discussion, of a young female patient with acute monocytic leukemia who presented ulcerative vaginitis as one of the manifestations of her disease.—R.B.C.

From the Department of Pathology, University of California, School of Medicine, San Francisco, Calif. California Med. 74: 111–114, 1951.

The author presents an excellent brief review of the subject with 28 references and 3 tables. Table I: supposed “etiologic organisms” in Hodgkin’s disease. Table II: Hodgkin’s disease treatment with biological preparations. Table III: Animals inoculated with lymph node mash derived from subjects with Hodgkin’s disease.

The author’s work showing that Seitz-filtered, sterile Hodgkin’s disease lymph node extract can be consistently passed serially in fertile chicken eggs and that the amniotic fluid from these eggs possesses the capacity to interfere with the growth of influenza virus in eggs is mentioned. The author feels that these interference phenomena offer for the first time a practical tool by which Hodgkin’s disease can be extensively studied from a promising etiologic standpoint.—C.E.R.


In the European literature a few cases of myeloma are described in which plasma cells are very large with a condensed chromatin in the eccentric nucleus. A widespread cytoplasm, also large, and numerous vacuoles give the cytoplasm its characteristic aspect.

In the case here reported, besides the typical vacuolated aspect of the cells, azurophil granulations were seen. The story was one of typical plasmocytoma with bone localizations. A very high peak of gamma globulin was found in the serum. (Five good photographs and 12 references.)—J.P.S.


This case report describes the clinical course of a primary reticulum cell sarcoma of the stomach occurring in a 21 year old woman. Death occurred three and one-half years after the onset of symptoms. The features of this lesion are briefly discussed. The incidence of primary gastric sarcoma is stated as ranging from 0.3 to 2.0 per cent. There is no pathognomonic clinical syndrome. It is emphasized that this lesion may mimic a benign gastric ulcer or hypertrophic gastritis.—R.B.C.


This interesting case report deals with an unusual case characterized by recurrent abdominal symptoms over a period of twelve years. There was an associated eosinophilia ranging as high as 63 per cent with a mild leukocytosis. Pyloric obstruction finally developed leading to a subtotal gastric resection. Pathologic examination revealed marked thickening of the wall of the stomach and upper duodenum with a diffuse infiltration of the muscularis propria with adult eosinophils. Previous sternal marrow examination had revealed similar infiltration with mature eosinophils. Extensive studies, including repeated stool examinations for parasites, failed to demonstrate any underlying causal factor or factors.—R.B.C.

RADIATION


This report relates in detail a study on the capacity of the rabbit to form antibodies after the whole body, except for the spleen or appendix, has been exposed to 800 r x-radiation. Such a dose is followed by death of approximately one-half of the animals. Such an
exposure without shielding of the spleen or appendix suppressed antibody formation. If the spleen or appendix of the rabbit is protected by lead shielding during total body irradiation, the capacity to produce antibodies to an injected particulate antigen is retained to a marked degree even though lymphatic tissue elsewhere in the body is temporarily destroyed.

Similarly, lead protection of the spleen obviated the development of anemia and significantly lessened the severity and duration of the leukopenia and thrombocytopenia. Moreover, the regeneration of hematopoietic tissue elsewhere in the body is markedly hastened if the spleen is shielded during the exposure to x-radiation. The authors also observed that the removal of the lead shielded spleen three to four days after whole body radiation (two or three days after antigen injection) or the removal of the lead shielded appendix five days after irradiation (four days after antigen injection) will not prevent antibody formation. —R.B.C.


Four renal bile fistula dogs were exposed to total body radiation in single doses of 150 r (1 dog) and 250 r (3 dogs). The excretion of bilirubin in the urine by each of the 4 dogs increased significantly during the first or second week following exposure. Possible sources of the increments in bilirubin output are discussed and it is considered that accelerated destruction of mature erythrocytes could be neither established nor disproved by these experiments. It is considered likely that some bilirubin is derived from destruction of partly hemoglobinized normoblasts in the marrow. It is also suggested that somewhat more than the normal portion of the total bilirubin excretion might stem from hemoglobin precursors if such molecules should be less successfully incorporated into erythroid cells after radiation.

Ten ml. aliquots of heparinized blood from a normal human donor were exposed in vitro to x-radiation. The osmotic and mechanical fragility of the red cells was normal when tested immediately after radiation and again after incubation at 37 C. for twenty-four hours following exposure, except in the sample treated with 20,000 r which showed slightly greater than normal increase in osmotic fragility after incubation. Rates or autohemolysis and glycolysis during incubation in vitro were neither accelerated nor decelerated by radiation of the blood prior to incubation.—G.E.C.


When frogs were given single exposures in dosages ranging from 600 to 2000 r, the death rate was highest between two to five weeks after exposure. The 50 per cent lethal dose (at six weeks after exposure) was about 700 r. Peripheral blood changes were studied following 600 r and 900 r. Lymphocytes were the most sensitive of the blood cells, and in both dosage groups the mean count reached a minimum at seven days. Mean heterophil counts reached a minimum at two and twenty-eight days after exposure in both dosage groups. No significant effect was seen on the number of thrombocytes, erythrocytes or the hemoglobin concentration.—O.P.J.

Porphyrens and Porphyria

This is the third of a series of articles dealing with the incorporation of $^{15}$N into the coproporphyrin, uroporphyrin, stercobilin and hippuric acid of a patient suffering from congenital porphyria who had been fed isotopically labelled glycine. The present paper deals with the $^{15}$N content of the heme and the glycine content of the globin, and attempts to define the metabolic abnormalities in congenital porphyria.

The studies are well conceived and reported and are recommended to students of this aspect of hematology.—T.R.T.Jr.


Evidence is presented which indicates that porphobilinogen, as excreted in cases of acute porphyria, is a mixture of either dipyrrylmethenes or of monopyrrolic compounds. This is in contrast to the belief of Waldenström that porphobilinogen is a mixture of two dipyrrylmethenes. There is a further discussion of an ether soluble precursor of coproporphyrin which, it is suggested, may be a metal complex of coproporphyrin.—T.R.T.Jr.


Protoporphyrin in combination with iron and proteins forms hemoglobin, myoglobin, cytochrome, peroxidase and catalase. It also occurs in apparently uncombined or free form in erythrocytes. The origin, function and fate of this free erythrocyte protoporphyrin is uncertain. It may serve as an intermediary substance in the synthesis of hemoglobin. An increase of free erythrocyte protoporphyrin is thought to occur if synthesis of hemoglobin is interrupted. This study deals with the amount of this protoporphyrin in the red cells of normals, patients with liver disease and miscellaneous other conditions. The method of determination is given in detail and showed an error that did not exceed 10 per cent. Normal values for free erythrocyte protoporphyrin ranged from 13 to 139 micrograms per 100 ml of erythrocyte with a mean of 43 µg. In 26 cases of obstructive jaundice, the mean value was 47 µg, and the free erythrocyte protoporphyrin in 28 cases of parenchymatous liver disease ranged from 20 to 249 µg. with 24 of these giving a mean of 46 µg. Patients with pernicious anemia under treatment with vitamin $B_12$ showed increased protoporphyrin during reticulocytosis. In a miscellaneous group the value of free protoporphyrin was generally normal. In 3 of 4 cases of nontropical sprue the values of free erythrocyte protoporphyrin were greatly in excess of the normal mean, although only one value exceeded the highest value found in a normal subject. Repeated determinations of the free erythrocyte protoporphyrin of the same subject over a period of six weeks showed little variation.—R.B.C.


Four cases diagnosed as acute porphyria are presented. At the time of writing the patients were still living. The authors ascribe clinical remissions partially to the administration of large doses of parenteral liver and vitamins.—P.F.W.

**BLOOD VOLUME**


This is an excellent comparative study and critical review of these two methods of determining blood volume.
The authors conclude that the 15 per cent difference between body and venous hematocrit is due to a plasma occlusion factor as well as an inherent difference between venous blood and the blood in the entire circulation. They believe that the radiophosphorus red cell method for total blood volume is simple, accurate and avoids the error of plasma occlusion and other centrifugation errors.—T.R.T.Jr.

**The Tagging of Red Cells and Plasma Proteins with Radioactive Chromium.** S. J. Gray and K. Sterling. From the Biophysical Laboratory and the Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass. J. Clin. Investigation 29: 1604-1613, 1950.

A new biological tracer, radioactive chromium (Cr⁴¹) with a half-life of 26.5 days has been found to tag both red cells and plasma proteins. The anionic hexavalent form labeled the red cells and the cationic trivalent form labeled plasma proteins. In vitro addition of Na₂Cr⁴¹O₄ to a suspension of red cells resulted in an uptake of 80 to 90 per cent of the radioactivity in two hours. Rapid tagging of red cells also occurred after intravenous administration of Na₂Cr⁴¹O₄ and tagged cells injected intravenously into dogs retained their activity without loss to plasma for twenty-four hours. The globin portion of hemoglobin is apparently tagged and trivalent Cr⁴¹ is bound more readily than hexavalent Cr⁴¹. However, cationic trivalent chromium is not taken up by the red cell and it is suggested that anionic hexavalent chromium enters the red cell by diffusion. Presumably, within the red cell it is reduced to cationic trivalent chromium and firmly bound to hemoglobin.—R.B.C.

**Determination of the Circulating Red Cell Volume in Man by Radioactive Chromium.** K. Sterling and S. J. Gray. From the Biophysical Laboratory and the Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass. J. Clin. Investigation 29: 1614-1619, 1950.

A new biological tracer, radioactive chromium (Cr⁴¹), with a half-life of 26.5 days, has been employed for the determination of circulating red cell volume. Red cells are tagged in vitro by Na₂Cr⁴¹O₄. The tagged red cells retained their radioactivity without significant loss to the plasma for periods of one day or more after intravenous injection into experimental animals. Using Cr⁴¹ tagged red cells the red cell volumes of 25 normal males were determined. The mean circulating red cell volume in these subjects was 2,351 cc. as determined by the radioactive chromium method compared to 2,208 cc. using radioactive iron. The accuracy of the method was verified by a second Cr⁴¹ determination of the red cell volume after the transfusion or hemorrhage of a known volume of red cells. The results agreed with the calculated values within 3 per cent. The mean red cell volume was 31.8 cc. per Kg. of body weight and 1.21 L. per square meter surface area. Calculations were based on the isotope dilution principle. The technic is outlined in detail. The low radioactivity dosage (total dose of 0.1 rep or less) permits repeated use of the Cr⁴¹ method on the same individual. The authors state that the method combines the accuracy of the radioiron technic with the simplicity of the radiophosphorus method.—R.B.C.


Studies on the influence of the hypophysis on hemopoiesis have shown that a 30 per cent reduction occurs in erythrocytes, hematocrit and hemoglobin upon removal of the hypophysis in rats. The hypophysectomized animal has been known to be in a dehydrated condition which would make the above anemia appear less severe than actually. The present experiment shows that to be true. Using P⁹¹ labeled erythrocytes to determine the actual total circulating red cell volume, these authors have found that the actual decrease in erythrocytes is a 45 per cent decrease rather than a 30 per cent decrease.—R.C.C.
ANEMIA

ANEMIA


The clinical features and course of 25 cases of aplastic or "refractory" anemia are reviewed. In 16 cases the peripheral blood picture was macrocytic, in the other 9, normocytic; the sternal marrow was hyperplastic in 5 cases, hypoplastic in 9 and completely aplastic in 9.

Six of the patients recovered completely (3 with the symptomatic and 3 with the idiopathic disease) and another patient was on the way to recovery at the time of this report.

The author stresses the fact that every patient with refractory anemia has a chance of recovery.—C.E.R.


The findings in 27 cases of aplastic anemia are described. In contrast to previous published series, only 3 patients had been exposed to potential marrow poisons. The marrow was aplastic or hypoplastic in all except 1 case where there was hyperplasia. In the peripheral blood, macrocytosis without much anisocytosis was a feature. Liver function tests were normal. The prognosis was poor, more than half the patients dying within two years of onset.—S.T.C.

Cooley's Erythoblastic Anemia. Some Skeletal Findings in Adolescents and Young Adults. J. Caffey. From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University and Babies Hospital, New York, N. Y. Am. J. Roentgenol. 68: 547-560, 1951.

Observations on 4 patients demonstrated regression with age of radiologically demonstrable lesions in the bones of the extremities while the changes in the central segments of bone persisted. Pneumatization of cranial air sinuses was retarded in all patients.—P.F.W.


In the oxygen unsaturated state the abnormal sickle cell hemoglobin molecules undergo orderly orientation forming long chains of hemoglobin elements. Parallel alignment of these elements produce so-called birefringent tactoids. The birefringent sickle cell is probably a membrane-covered hemoglobin tactoid. These abnormal hemoglobin molecules produce increased viscosity of hemoglobin solution. The viscosity of whole blood is increased and the mechanical fragility of sickled cells are significantly increased at lowered oxygen tensions. The increase in viscosity may explain the multiple venous thrombosis and the increase in mechanical fragility may explain the hemolytic anemia phenomenon. Sickling and increases in viscosity and in mechanical fragility occur within the range of oxygen saturation of venous blood. Sickle cell anemia erythrocytes when transfused into normal recipients do not long survive. In the trait, only 25 to 44 per cent. of the hemoglobin is abnormal and the condition is without clinical manifestations. Sickling, increases in viscosity and mechanical fragility can be produced only by oxygen saturations well below those usual for venous blood.—R.B.C.


This author has found that blood of normal individuals will show the sickling phenomenon when mixed with thick gelatin "solutions" such as Lepage's glue. The author states that "in sicklemia there is apparently an intrinsic change in the red blood cells, whereas with glue the change is brought about by extrinsic factors."—R.C.C.
THE HEMOLYTIC FACTOR IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA. W. H. Crosby.

It has been established that the hemolytic process in paroxysmal nocturnal hemoglobinuria involves the destruction of defective red cells. Ham and Dingle have demonstrated that hemolysis of these abnormal red cells was mediated by a normal heat labile plasma factor that had many of the characteristics of complement. The present investigation was undertaken when it was noted that serum became increasingly hemolytic against the red cells of PNH when tested serially after coagulation had occurred. This intensification of hemolysis after clotting appears to implicate the clotting mechanism. In a series of experiments the author observed the effect of the various factors involved in the clotting mechanism. It became apparent that there was a similarity between the behavior of the hemolytic factor and the coagulation accelerator, Ac-globulin, and the author believes that the evidence strongly suggests that the coagulation accelerator was the hemolytic factor.

With this relationship in mind, dicumarol was administered to a patient with PNH with the idea of modifying the coagulation mechanism and, in turn, the hemolytic process. There occurred a drop in his plasma hemoglobin and hemoglobinuria ceased. Simultaneously, his serum accelerator activity dropped. The response was not permanent and even under therapy, hemolytic crises continued to occur and there was a sharp hemolytic reaction when dicumarol was discontinued. The inert precursor of the hemolytic factor did not diminish during dicumarol therapy. The reaction to dicumarol appeared to be due to the lack of prothrombin, hence the lack of thrombin, the activator of the hemolytic factor. An explanation is also offered as to how this hemolytic factor related to blood coagulation exerts its effect in the circulation where coagulation does not occur. This explanation is simply that the clotting mechanism in the circulation is in a state of dynamic equilibrium so that Ac-globulin may be continually activated in vivo and so function as a hemolytic factor. Finally, it is emphasized that the ultimate understanding and control of paroxysmal nocturnal hemoglobinuria must await the definition of the red cell defect.—R.B.C.

INFLUENCE OF HYPOPHYSECTOMY ON THE HEMOPOIETIC RESPONSE OF RATS TO LOWERED BAROMETRIC PRESSURES. W. M. Feigin and A. S. Gordon. From the Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, N. Y. Endocrinology 47: 364–369, 1950.

An important finding which linked the hypophysis and hemopoiesis was that of Stewart, Greep and Meyer (Proc. Soc. Exper. Biol. & Med. 35: 112, 1935) which showed that hypophysectomy prevented the usual rise in erythrocyte number and hemoglobin level which accompanies exposure to lowered barometric pressures. The present work was performed to repeat the above work and to determine whether decreasing the barometric pressure even lower would produce the usual effects. Subjection of hypophysectomized rats to reduced pressures of 411 mm. Hg (16,000 ft.) for periods up to fourteen days produced no significant effects on peripheral blood. Unoperated control rats did respond under these conditions. These results are similar to those of Stewart, Greep and Meyer. Pressures of 321 mm. Hg (22,000 ft.), however, evoked an erythropoietic response in the hypophysectomized rats of approximately the same magnitude as shown in the normal rats.—R.C.C.


One hundred ninety-three patients with advanced cancer were studied by means of sternal marrow and peripheral blood examinations in addition to accepted radiographic and clinical observations. Of these, 116 had hemoglobin levels below 80 per cent (15.6 Gm. per 100 cc. of blood = 100 per cent). It was determined by these studies that 28.5 per cent were due to blood loss, 2.6 per cent to hemolysis, 56 per cent were of the myelopathic type and 12.9 per cent of the latter were complicated by blood loss. The definition of myelopathy was "either metastases to bone, demonstrable by marrow aspiration or by x-ray, or else had no characteristics which would permit classification in any of the preceding categories."
ABSTRACTS

Cobaltous chloride 60 to 240 mg. daily was administered to 16 anemic patients (8 with and 8 without osseous metastases). In 6 patients with bone lesions who received an adequate trial on cobalt a reticuloocyte response was elicited. Two of these 6 died before a rise in hemoglobin could be expected; 1 failed to show such a rise; and in the other 3 patients the hemoglobin rose to normal levels. Of the 4 without bone metastases who tolerated cobalt for an adequate period, 3 had a rise in reticuloocytes followed by an increase in hemoglobin.

Of the 193 patients 25.9 per cent had evidence of osseous metastases; 48 per cent of these were anemic. Of the 116 patients with anemia 20.7 per cent had some evidence of bony metastases, whereas in the 77 patients without anemia 33.8 per cent had osseous metastases. In the 50 patients with metastases 24 (48 per cent) had anemia and 92 (64 per cent) of the 143 patients without metastases had anemia. On this evidence they conclude the incidence of anemia is not directly related to presence or absence of bony metastases.—T.R.T. Jr.

STUDIES OF HEMAGGLUTININS IN HEREDITARY SPHEROCYTOSIS AND IN ACQUIRED HEMOLYTIC ANEMIA; THEIR RELATIONSHIP TO THE HYPERSPLENIC MECHANISM. C. S. Wright, M. C. Dodd, B. A. Bouroncle, C. A. Doan and R. M. Zollinger. From the Departments of Medicine, Bacteriology and Surgery, The Ohio State University, Columbus, Ohio. J. Lab. & Clin. Med. 37: 165-181, 1951.

The authors present the results of their studies of incomplete antibodies, by means of the Coombs (developing) test and the use of enzyme (trypsin) treated red cells, in patients with hereditary spherocytosis and acquired hemolytic anemia.

The trypsinated red blood cell and developing technics were employed 1,716 times in 465 persons; 185 of these were patients with some type of hematologic abnormality, of whom 133 had some degree of hypersplenic cytopenia. The remaining 280 individuals were either normal or had some nonhematologic disease. The Coombs test was positive in 371 determinations on sera of patients from the first group; the trypsinated red blood cell test was positive in 406 determinations. Sera from the remaining group were all negative.

Twenty-five cases of hereditary spherocytosis were studied. One or both tests were positive in 8 cases. The Coombs test was positive in 4, the trypsinated red blood cell test was positive in 6 and both were positive in 2. There was no consistent response of titer to splenectomy. There was no relationship between the presence or titer of antibodies and the activity of the hemolytic process.

Fifty-eight cases of acquired hemolytic anemia were studied. The Coombs test was positive in 42; the trypsinated red blood cell test was positive in 43; and both were positive in 37. Both tests were negative in 10 cases. There was no definite or reliable correlation between antibody titer and intensity of the hemolytic process.

Six of 9 cases of thalassemia showed a positive trypsinated red blood cell test; 3 of these 6 showed a positive Coombs test; and both tests were negative in 3 cases.

Twenty-two cases of essential thrombocytopenic purpura were studied. In 6 of these both tests were positive; and the remaining cases were negative. There was no evidence of a hemolytic process in any of these cases.

Of 6 cases of multiple myeloma there were three in which both tests were positive and one case in which only the trypsinated red blood cell test was positive. No evidence of hemolysis or hypersplenism was found in this group.

In a separate phase of these studies, splenic residual blood generally exhibited higher titers than circulating peripheral blood. Of the 24 individuals with demonstrable antibody in the splenic residual blood, only 19 had antibodies in the circulating peripheral blood.

The finding that 8 of 25 cases of hereditary spherocytosis exhibited incomplete antibodies indicates that the Coombs and trypsinated red blood cell tests are not reliable as a sole means of differentiating acquired from hereditary hemolytic anemia. Furthermore, there was no direct relationship between the presence or titer of antibodies and the hemolytic process. Seventy-seven patients of the 185 had one or both tests positive; of these, 68 had active hemolysis or history thereof, or an inherited trait of defective erythrocytes.

The authors state that, "The newer data revealed with the recently devised techniques are requiring a revision of our concepts and thus our investigative approach to many hematologic problems." The relationship of the incomplete antibodies to erythrocytes, granulocytes and thrombocytes is discussed.—T.R.T., Jr.