Hematopoiesis and Comparative Hematology

Direct Action of Vitamin B₁₂ upon Human Bone Marrow. The Effect of Instillation of Vitamin B₁₂ and Folic Acid into the Bone Marrow as Studied by Nucleic Acid Staining Techniques. D. Horrigan, T. Jarrole and R. H. Filler. From the Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio. J. Clin. Investigation 30: 31-36, 1951.

In this study vitamin B₁₂ in 1 mg. doses and folic acid in 1 or 2 mg. doses were instilled directly into the marrow cavity of one iliac crest. Forty-eight hours later, bone marrow was aspirated from the exact site of previous instillation, from the opposite iliac crest, and in one instance from sites 4 and 8 cm. distant from the site of instillation of the vitamin B₁₂. Coverslip preparations of the marrow were stained with Giemsa stain and with nucleic acid staining techniques. Vitamin B₁₂ was utilized locally by the marrow cells, stimulating erythroid maturation and correcting a qualitative abnormality in cellular ribonucleic acid in persons with pernicious anemia in relapse. Vitamin B₁₂ need not be altered by stomach or liver extract to exert this effect. On the other hand, folic acid was not utilized locally by bone marrow cells within 48 hours of instillation. If folic acid is administered orally or parenterally, it has the same cytologic and cytochemical effects as vitamin B₁₂. It would appear that folic acid must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body.—R.B.C.


This is the third of a series of articles dealing with the establishment of extramedullary hematopoiesis. Evidence presented in the previous articles indicated that when a large quantity of cellular elements from bone marrow or lymph nodes was injected, they accumulated metastatically in organs and tissues according to differences in cell characters. (See abstracts in Blood 6: 575 and 757, 1951.) In order to further test this concept, experiments were devised in which the animal’s own immature myeloid cells were vigorously liberated into the circulation by the action of a single sublethal dose of saponin. Immature myeloid cells were found in the lung capillaries in greater numbers than in the peripheral blood, and up to 24 hours the percentage there was greater than that for the liver, spleen, or bone marrow. However, by 72 hours there was a striking accumulation of immature myeloid elements in the sinusoids of the liver and red pulp of the spleen. The marrow parenchyma reflected this outpouring of cells by appearing hypocellular and flooded with blood. Saponin is apparently less injurious to the myeloid cells than the trauma inflicted by transplantation of cells from one animal to another. In general, the characteristics of the manner in which immature myeloid cells accumulate in foreign organs were the same as described previously.—O.P.J.
ABSTRACTS


These two papers report results of studies upon factors that condition the susceptibility of tissues to destruction by irradiation. Bullfrog tadpoles were selected for these experiments because they can live at temperature levels from 0 to 33.5°C and their hematopoietic tissue is highly susceptible to irradiation. The standard dosage used in these experiments was 500 r requiring about 6 minutes. Tadpoles were placed in water during irradiation at a depth of 7.0 mm, leaving the dorsal half above the surface. The index of rate of division in tadpole hematopoietic tissue (which fills the spaces between renal tubules) was determined by using the mitotic inhibitor colchicine. Data show that post-irradiation temperature controls the rate of destruction of the hematopoietic tissue. Irradiation produces injuries that may remain invisible for some time, awaiting reactions or changes in the cells. Cell destruction in a given period is directly correlated with the rate of cell division during the same period.—O.P.J.


There are several differences between the adult and fetal hemoglobin of various animals, and between invertebrate and vertebrate hemoglobins. Some of these have been studied in the bullfrog, eel and lamprey. In the case of tadpole and adult bullfrog hemoglobin, the molecular weight is not significantly different. There is no effect of pH upon oxygen equilibrium in the tadpole, whose oxygen affinity is about 7 times as great as that of the adult. Oxygen equilibrium studies of eel blood indicate that its hemoglobin behaves like that of almost all vertebrates. The lamprey, a primitive vertebrate, has a blood hemoglobin which is characteristic partly for invertebrates and partly for vertebrates.—O.P.J.


It has been known that the blood urea concentration of elasmobranch fishes exerted about one-third of the total measured osmotic pressure of such blood and that the erythrocytes of these animals are relatively permeable to urea. The present experiments show that the urea exerts no measurable influence on the equilibrium volume of the red cells but that this volume is determined by the salt concentration.—O.P.J.

HEMATOLOGIC BIOCHEMISTRY


Nitrogen mustards produce cytotoxic effects that result in the inhibition of mitosis,
nuclear fragmentation, chromosomal aberrations and even mutations. In the present study, actively proliferating tissue from the bone marrow, spleen and thymus of rabbits was analyzed for its phosphorus content under various conditions. The hydrochloride of methyl bis-amine was administered in physiological saline intravenously in a dose of 2.0 mg per Kg. The rabbits were sacrificed 1 to 5 days after administration of the mustard. Homogenates of bone marrow, spleen and thymus were made using 3 volumes of water for the marrow and 5 volumes each for the spleen and thymus. Analyses were carried out in duplicate on 1 cc. aliquots of these homogenates. The nucleic acid content of these organs was depressed 12 to 24 hours following administration and it returned to normal in the bone marrow and spleen by the fourth day, whereas the value for the thymus remained depressed. The utilization of $^{32}P$ for desoxyribonucleic acid synthesis in mustard treated animals was retarded at first but it rose to values higher than normal on the second to fourth days.—O.P.J.

**Effect of Intravenous Folic Acid on the Cholinesterase Activity of the Blood and the Folic Acid Levels.** H. H. Scudamore, G. J. Gabuzda and L. J. Vorhaus, II. From the Army Medical Nutrition Laboratory and the Department of Medicine, University of Illinois, College of Medicine, Chicago, Ill. J. Lab. & Clin. Med. 38: 183-187, 1951.

Folic acid was injected intravenously into 10 individuals, in amounts of either 3.75 or 15.0 mg. The individuals studied included: 3 healthy males, 3 males with hepatic cirrhosis, 1 male with essential hypertension and 3 males with pernicious anemia (1 during remission and 2 after a reticulocyte response to liver therapy). Plasma and erythrocyte cholinesterase activities and folic acid blood levels were determined before and at intervals after the administration of folic acid.

There was no significant alteration of cholinesterase activity in any of the subjects. It is suggested that the increases in erythrocyte and cholinesterase activity of patients ill with pernicious anemia treated with folic acid or liver extract are brought about by erythropoiesis and recovery and not by direct stimulation of cholinesterase activity.—T.R.T., Jr.

**The Action of Some Hemolysis Accelerators upon Lipid and Protein Monolayers.** H. L. Chen and H. B. Collier. From Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada. J. Gen. Physiol. 35: 17-22, 1951.

The action of hemolytic agents upon artificial monolayers parallels hemolytic activity which may be due to the penetration of the lipoprotein membrane of the mammalian erythrocyte. Certain agents (acetylphenylhydrazine, 9-aminoacridine and phenothiazine), which accelerate hemolysis, have been studied for their effect on monolayers. They apparently enhance hemolytic activity by affecting the protein component of the erythrocyte membrane.—O.P.J.

**Immunohematology**


A specific hemagglutination reaction has been described between the sera of tuberculous experimental animals or human beings and sheep erythrocytes treated with extracts or products of the culture filtrates of tubercle bacilli. The author has found that homologous human erythrocytes may be sensitized and substituted for the sheep erythrocytes in this reaction. In the process of studying this phase of the reaction it was observed that the addition of complement to the reaction system transformed the reaction from one of hemagglutination to one of hemolysis. The specific technic of performing the hemagglutination test using sensitized homologous red cells or sheep cells is described. The hemolytic modification of this test is also described. Comparative studies of the sera of tuberculous patients
have shown that the hemagglutination test and its hemolytic modification do not measure the same serologic properties and presumably not the same antibodies.—R.B.C.


The titer of Rh agglutinins was determined in 36 patients at different stages of pregnancy, employing frozen serum so that the titers of a given patient could be performed simultaneously. A rise or fall in titer was nearly always associated with an Rh-positive fetus which suffered from hemolytic disease of the newborn, but the majority of the infants produced no change in the maternal agglutinins.

The authors suggest the hypothesis that the fetus absorbs the maternal agglutinins during pregnancy. On this basis a rise in titer would be observed when maternal production exceeded fetal absorption, but when the reverse occurred, a fall in titer would be found. It was noted that a rise in titer is usually seen postpartum when fetal absorption is no longer possible and in one case there was a pronounced increase in titer after the death in utero and before delivery of the fetus.

The authors conclude that although examinations for the detection of agglutinins are most important, titration of any agglutinins so found is of limited value as a prognostic aid.—C.E.R.


The authors immunized rabbits by means of known quantities of sheep red cells, and studied the ability of the immunized rabbits to develop anti-sheep-red-cell antibodies (hemolysins). Studies were made of the relative abilities of intact and splenectomized rabbits to form these hemolysins. The details of the method, and the attempts to arrive at quantitative results, are given in detail.

It was found that, in intact animals, there was an initial period, lasting some four days after a single injection of sheep red cell suspension, during which there was a rapid rise in the antibody titer. This was followed by a second, slower rise of titer; and then by a third period, during which the titer remained level. When splenectomy was performed in the rabbits on the day of immunization, or within 4 days thereafter, there was a significant depression of antibody formation; but there was no effect if splenectomy was done after the fifth day following injection of the antigen.

The conclusion was reached that, during the period of the initial rise in antibody, it was the spleen that formed most of the antibody. Then, about the fourth day after the single injection of antigen, the spleen abruptly stopped forming antibody; following this time, remaining new antibody was formed from nonsplenic sources, while already formed antibody decayed at a given, calculable rate. It is of interest, as the authors point out, that previous work had shown that hemolysin production could be almost completely suppressed in rabbits receiving total body irradiation of 800 r, but largely retained in such rabbits if the spleen was shielded with lead during such irradiation (L. O. Jacobson et al., Argonne National Laboratory, Biological and Medical Divisions. Quart. Rep., May-July 1949, pp. 27–36).—S.E.


This study was carried out to evaluate under controlled conditions the effect of sodium salicylate in guinea pigs on the antibodies formed in response to the antigens in R + type O human red blood cells. Antibody titers produced in guinea pigs receiving Rh + type O human red blood cells intraperitoneally were compared each week after immunization with those produced in similarly treated guinea pigs which also received varying doses of sodium salicylate. It is concluded that the natural antihuman hemoagglutinins cannot be depressed.
by sodium salicylate. The depressant action of sodium salicylate on the Rh + type O human antigen-antibody reaction in guinea pigs was limited by the toxicity of salicylates. Rh + and Rh − titers between 1:136 and 1:819 were depressed by nontoxic plasma levels of sodium salicylates (15 to 40 mg. per cent); whereas Rh + and Rh − titers of 1:1500 were not depressed even by toxic plasma levels (approximately 50 mg. per cent).—R.B.C.


Antibody production and splenomegaly in mammals are not as great in young animals as in older ones. Different results have also been obtained after the injection of soluble and particulate antigens. Somewhat similar results have been reported for chickens. In the present experiments hybrid chickens of both sexes, ranging in age from 1 day to 12 weeks, were used. The antigens, either crystalline beef albumin or undiluted beef serum, were injected in the same amount for the same age groups. Spleen weights tended to increase with the amount of antigen injected, and two injections were more effective than the same amount given as a single injection. The spleens of chickens younger than three weeks responded irregularly, and a measurable titer appeared more slowly and disappeared more rapidly than in older chickens. Increase in spleen weight was not due to edema but due to increased secondary follicles in the swollen capsule and white pulp. In general a more rapid development of antibody titer was observed in the older age groups. Results on adult spleens indicated the increase in spleen size is maintained for longer periods in hyperimmunized chickens. The differences in results that may arise from using animals with different periods of immunization were emphasized.—O. P. J.


A low blood serum complement is often found in patients suffering widespread disease of the bones. This study was carried out to investigate the complement activity of serous fluid expressed from the coagulate obtained from sternal bone marrow. In many cases the complement activity of sternal fluid compared with that of blood serum. In a small number of marrow fluids no complement activity was demonstrable while the sera showed normal complement activity. Moreover complement components from normal sera failed to reactivate these marrow fluids and, in turn, these same marrow fluids inhibited the complement activity of normal sera. This anticomplementary factor was heat stable. Splenomegaly was present in 60 per cent of the patients whose sternal fluid complement levels were less than 20 per cent of their corresponding blood sera values. Splenomegaly occurred in only 20 per cent of those cases in whom the sternal marrow complement values do not deviate more than 20 per cent from their corresponding blood value.—R.B.C.


The icterus index, red cell count, hemoglobin, A, B, O and Rh-Hr blood types were studied in 21 randomly selected newborn infants. No relationship was found between the blood groups investigated and the physiologic icterus of the newborn. The possible relationships of cold antibodies to the syndrome of physiologic icterus is discussed.—S.T.C.


Following the line of investigation of Kahirer and Miller (J. Obst. 54: 1. 1947), the authors propose to introduce hapten-like substances into erythroblasticotis mothers in order
to absorb the rhesus antibodies and to prevent them from entering the placental circuit. In vitro experiments allowed the authors to extract from the urine of rhesus positive individuals these substances which show phosphatid-like structure. Further investigation will be carried out, trying to synthetize such haptens in order to use them therapeutically in humans. —C.M.


This paper summarizes previous work on the occurrence of the agglutinin anti-S and describes the finding of anti-S in a further case. In this instance, as in one of the nine previous examples, the antibody appeared to be naturally occurring. An addendum mentions an eleventh example from a fatal transfusion reaction.—S.T.C.


A 30 year old Negro patient suffering from an undefined collagen disease required multiple transfusions. Evidence of a hemolytic reaction was noticed five days after the third transfusion. Six weeks later, difficulty was encountered in cross matching more blood. On investigation the serum was shown to contain anti-A, -M, -S, -C, -E and anti Fy, the patient's own blood group being B, N, cDe/cde or cDe/cDe. Subsequent transfusions were uneventful.—S.T.C.


It has been shown previously that the only method for detecting the Duffy, or Fy, antibody is by means of the indirect antiglobulin test. Data are presented which show that after treatment of Duffy positive, Fy, cells, with trypsin, papain or ficin they can no longer be coated with their specific antibody as evidenced by a negative indirect antiglobulin test. This action is quite independent of the action of enzyme on the Rh factor. Inasmuch as several post-transfusion hemolytic reactions due to the Fy factor have been reported, these findings are of clinical importance.—T.R.T., Jr.

BLOOD COAGULATION and HEMORRHAGIC DISEASES

ON THE COAGULATION ACCELERATING EFFECT OF AMNIOTIC FLUID. F. D. Rendelstein, H. Frischauf and E. Deutsch. From the first Medical Clinic of the University of Vienna, Austria. Acta haemat. 6: 18-31, 1951.

Amniotic fluid contains a thermolabile, and a thermostable thrombokinase and calcium. It shortens the recalcification time of normal, dicoumarolized, and stored plasma. The prothrombin time of plasma is only little reduced, whereas that of dicoumarolized and stored plasma reduces the coagulation time of hemophilic plasma to normal. The i.v. injection of amniotic fluid in high dosage can lead to intravascular fibrin formation and fibrinopenia.—C.M.

HYPERCOAGULABILITY OF THE BLOOD ASSOCIATED WITH ACTH AND CORTISONE THERAPY. S. W. Cosgriff, A. F. Diefenbach and W. Vogt, Jr. From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y. Am. J. Med. 8: 752-756, 1950.

The observation of numerous thromboembolic episodes in patients receiving ACTH or cortisone initiated a study of the blood coagulation mechanism in a group of these patients.
The changes which were noted in coagulation time and heparin-retarded coagulation time strongly suggested that ACTH and cortisone were in many instances responsible for a state of hypercoagulability of the blood. The mechanism of this alteration in blood coagulation is unknown and was not further clarified by the supplementary determinations of prothrombin time, fibrinogen B and protamine titration. Further studies are now in progress.

These observations are of interest in view of the probably large number of patients undergoing treatment with ACTH or cortisone who have other factors predisposing to thromboembolism. It is suggested that prophylactic anticoagulant therapy be considered in some of these patients.—H.W.B.

THE THROMBIN-INHIBITORY EFFECT OF CERTAIN THROMBOPLASTIN PREPARATIONS. R. C. Hartmann. From the Division of Clinical Microscopy, Department of Medicine, the Johns Hopkins University and Hospital, Baltimore, Md. Am. J. M. Sc. 222: 279-284, 1951.

Thromboplastin from a variety of sources was found to have variable thrombin-inhibitory activity. The substance is nondialyzable, heat stable, not extractable with ether and not filterable through a Seitz filter. It is suggested that this may explain the discrepancies noted in the prothrombin estimations using different thromboplastins.—T.R.T., Jr.


The authors used a desiccated thromboplastin extract containing ionizable calcium and sodium chloride. This was reconstituted before use by the addition of distilled water. It is concluded that this leads to greater accuracy and simplicity in the performance of the one stage test for prothrombin time.—T.R.T., Jr.


A patient treated by tromexan (dicumarol derivative) with a prothrombin level of 11 per cent, was for the twentieth time treated by a sympathetic block. A short time after injection a deep located pain increased in the right side of the abdomen, the day after a mass was palpated. Red cells fell to 2,650,000. Anticoagulant treatment was stopped and 24 hours later the prothrombin level was 48 p. 100. Hematoma did not increase. But the following day embolism recurred and the patient died, 15 days after the onset of the hematoma. Postmortem findings showed the hematoma to be in the psoas muscle. The authors conclude that the use of anesthetic block during anticoagulant therapy is contraindicated.—J.S.


Serial prothrombin determinations were done in 21 infants with acute gastroenteritis of varying severity and etiology. In general the prothrombin levels tended to reflect the course of the diarrhea. With one exception all initial prothrombin values were lowered (average 52 per cent). As recovery occurred these rose to normal but in the 2 fatal cases the levels fell progressively. Thirteen of the infants were considered to be chronically malnourished and in 4 of these low prothrombin levels persisted following recovery from the acute diarrhea.

The various factors contributing to the hypoprothrombinemia are discussed. It would appear, however, that in patients of this age group an interference with the intestinal synthesis of vitamin K was of prime importance. Of particular interest was the observation that the prothrombin level was not appreciably affected by the administration of sulfaguanidine, succinylsulfathiazole or oral streptomycin.
ABSTRACTS

While the hypoprothrombinemia was not alarming in most of these cases, several very low prothrombin levels (lowest 12.5 per cent) and one instance of severe hemorrhage were encountered. Proper management of even mild cases of diarrhea in infants should, therefore, include an awareness of the possibility of a hypoprothrombinemia of significance.—H.W.B.


A case of thrombocytopenic purpura is reported in which a hypersensitivity to quinidine was proved. The patient was followed carefully with platelet counts and bone marrow studies throughout the hospital course which included a spontaneous recovery and an intentionally produced recurrence with subsequent recovery. Evidence is presented which suggests that allergic thrombocytopenic purpura may be due in part to a destruction of circulating platelets as well as to an inhibition of platelet production. Serum prothrombin times were performed also and prothrombin consumption curves plotted. In general the slope of the prothrombin consumption curves increased as the platelet count rose, although these curves did not provide an accurate measure of the platelet count. The difficulties in interpretation of these results are discussed.

In view of the similarities between allergic and idiopathic thrombocytopenic purpura, the authors stress the value of careful search for evidence of an allergic reaction in cases which appear to be of the idiopathic type.—H.W.B.

METHODS


These two methods of determining the erythrocyte sedimentation rate were used on each of 2,700 blood samples. In general, there was excellent correlation between the two methods. A chart is presented which shows the relationship of the results obtained by the two different methods.—T.R.T., Jr.


The study revealed that the amount of blood which must be ingested to give positive reactions by normal subjects on a meat free and fish free diet for the different reagents tested were as follows: orthotolidin 1 ml., benzidine 3.5 ml., phenolphthalein 3.5 ml. and guaiac 20 ml. Normal subjects on an unrestricted diet showed faintly positive reactions to guaiac after the ingestion of 2 to 3 ml. of blood.

The ingestion of ferrous sulfate did not produce false-positive reactions with these reagents with the possible exception of orthotolidin.

The detection of occult blood in the urine with these reagents is not recommended, since the urine itself inhibits the reaction between blood and the indicators.—T.R.T., Jr.