HEMOSTASIS


Sera were obtained from 120 women whose pregnancy varied from four weeks to full term; from 41 women whose delivery had been from one to twelve weeks previously; from cord blood at 30 births; and from 15 babies aged 1 to 14 days. All pregnancies were normal. Antithrombin content was measured in each case. In all the pregnant patients the antithrombin level was lower than in 794 of 800 ‘normal’ sera. In the post-partum cases the titer was found to be low for at least four weeks after delivery, returning to normal some time between five and twelve weeks after delivery. The test may provide a simple and reliable aid to the diagnosis of pregnancy in difficult cases.

The sera from cord blood gave abnormally low results, and this was true also of the sera of babies 1 to 14 days old. A normal result was obtained in 2-months-old babies.—R.H.G.


A 56 year old man with multiple myelomatosis had papilledema with retinal hemorrhage. When blood was withdrawn, the serum became a firm gel. By electrophoresis this was shown to be connected with a marked increase in the y-globulin. At autopsy were found multiple petechial hemorrhages in the brain due to occlusion of small vessels by masses of eosinophilic protein.—R.H.G.


By absorbing anti-A and anti-B sera with highly concentrated suspensions of platelets, it was possible to show in these elements the presence of the A and B agglutinogens in persons belonging to the A, B or AB groups. These antigens are absent from platelets of the O type. Such results were only obtained with platelet suspensions containing twenty million elements per cu. mm., after crushing these elements. Control studies showed that the absorption of antibody was not due to the presence of antigens dissolved in the serum or plasma, or to the presence of red cells persisting in the platelet suspensions. The antigen seemed to be located inside the protoplasm of the platelets.—R.H.G.


The condition is one of acute hemolytic anemia, thrombocytopenic purpura, fever, and fluctuating neurological disturbances. Microscopically there is widespread patchy thrombosis of minute blood vessels.
A 38 year old man with a six months' history of leg ulcers developed abdominal pain, vomiting and red urine. Treatment with sulphadimidine was begun, but the above features developed (without abnormal neurological signs). The patient was group O Rhesus positive, and there were no atypical antibodies. The Coombs test was negative. Penicillin was given and treatment with cortisone and ACTH begun, but death ensued. The necropsy findings were those of thrombotic microangiopathy.—R.H.G.


A hospital matron aged 46 developed jaundice, followed by petechial hemorrhages. There was anemia and thrombocytopenia. The urine contained bile rather than urobilinogen but hemolytic anemia was considered to be present. The bleeding tendency increased, fever developed, and there was evidence of an upper motor neurone lesion followed by death. At autopsy the spleen contained large quantities of hemosiderin. The principal thrombotic changes were in the venules of the renal cortex. A diagnosis of thrombotic thrombocytopenic purpura was made in retrospect.—R.H.G.


Using serum from coumarin-treated patients as a source of factor VII and Christmas factor in the blood thromboplastin generation test, it is found that thromboplastin generation may be impaired when the one stage Quick’s test which measures principally factor VII is normal or nearly so. This occurs early in treatment before the one stage test has lengthened, and on cessation of treatment; it has also been found, after a single dose of a coumarin drug, to be insufficient to make any change in the one stage test. The sera from these patients gave normal blood thromboplastin generation in mixtures of platelets and plasma treated with aluminium hydroxide when they were not under the influence of the drug.

The thromboplastin generation test has been carried out on more than 200 samples of coumarinized serum in mixtures of normal platelets and plasma treated with aluminium hydroxide, and there is no apparent correlation between the results and moderate factor VII deficiency as indicated by the Quick test. A similar phenomenon has been found in patients with hepatocellular disease, but not in obstructive jaundice.

This evidence is considered to support Koller’s suggestion that there is an additional factor present in serum, deficient in the blood of patients receiving coumarin anticoagulants. Further investigations of Christmas factor under these conditions is required.—R.H.G.


The thromboplastin generation test, which can be modified to allow measurement of antihaemophilic globulin (AHG), enables blood levels of the latter to be estimated. For reasonable hemostatic efficacy, 30–50 per cent of the normal amount of AHG is needed in the blood. Usual estimates of the therapeutic level are much lower than this, because in most hemophilic patients 2–5 per cent of the normal AHG in the blood is probably sufficient to give a normal clotting time. For optimum therapy relatively large amounts of blood or of AHG are required. Accordingly AHG has been prepared from ox blood, which has an average AHG activity sixteen times that of human blood. The best material was prepared by salt fractionation, and sterilization was by centrifuging at high speed and exposing to UV light.

Three hemophilic patients were given the material intravenously successfully but one developed thrombocytopenia without purpura. The bovine material would agglutinate human platelets and produced doubtfully positive skin tests and precipitin reactions.—R.H.G.

A 40 year old woman who had had nine previous normal confinements had some vaginal bleeding during pregnancy and in the 30th week had an eclamptic fit followed by delivery of a dead-born fetus. Bleeding was severe despite blood transfusion and packing of the uterus. The coagulation time was prolonged.

The patient was then given 2 Gm. of fibrinogen intravenously and bleeding stopped within fifteen minutes. The coagulation time was now normal and recovery was complicated only by a femoral thrombosis.

In a year at the Rotunda Hospital 64 cases of accidental hemorrhage occurred. Of these 18 were severe; the blood was incoagulable only in the present case and one other.

The incoagulability of the blood is considered to follow the accidental hemorrhage, not to precede it. The coagulation defect is transient. Blood transfusion alone is not a satisfactory method of treatment.—R.H.G.


Several workers have suggested that postpartum hemorrhage may sometimes be due to a lack of circulating fibrinogen. The present authors support the view that thromboplastins are liberated from the uterus or its contents and gain access to the maternal circulation; there they cause blood coagulation to occur with extensive intravascular clotting and depletion of circulating fibrinogen so that a hemorrhagic diathesis occurs. A fibrinolysin is produced in the maternal blood, and this makes matters worse.

Two cases are described in whom there was an absence of fibrinogen bands in micro electrophoresis patterns. An hourly observation of the coagulation time is recommended to give warning of developing fibrinogen deficiency, and treatment with pure human fibrinogen is strongly advised.—R.H.G.


During administration of ACTH and cortisone there was noted to be a higher incidence of thrombo-embolic complications. When treatment with tromexan (ethyl biscoumacetate) was given, it was found that a larger dosage was often required in patients undergoing hormonal therapy for the maintenance of an optimal plasma 'prothrombin' level. Two patients, one with idiopathic acquired hemolytic anemia and one with acute thrombophlebitis, were studied, and this dosage phenomenon was found. The mechanism is not clear, but the phenomenon should be remembered and unless absolutely indicated for their known specific effects, ACTH and cortisone should be withheld in the presence of thrombo-embolic complications.—R.H.G.


A normal clotting time in the one stage "prothrombin test" depends upon at least factors V and VII in addition to prothrombin, and when coumarin anticoagulants are employed, it is factor VII that is reduced. This affects coagulation by impairment of intrinsic blood thromboplastin generation, but in patients given coumarin drugs, the generation of blood thromboplastin may be impaired at a time when the one stage test is normal. This has been noted both before the one stage test has been prolonged and on cessation of treatment after it has returned to normal.

Impaired blood thromboplastin generation has also been found in patients under treatment whose one stage clotting times had temporarily fallen below 20 seconds and in patients in whom the conventional therapeutic level was not achieved. This phenomenon is not due
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to lack of factor VII unless it is assumed that the thromboplastin generation test is a much more sensitive measure of factor VII deficiency than the one stage test. It may equally be Christmas factor which cannot be measured by the one stage test.

There is, beyond certain limits, no hard and fast correlation between the results of the Quick test in therapeutics and the tendency to bleed: the hemorrhage may not be due to factor VII deficiency. It is possible that most patients given coumarin substances do not bleed because their prothrombin concentration is adequate. Three patients have bled with very low prothrombin levels but with one stage times within the accepted therapeutic level. For research purposes a period of more intensive study of these different factors is now indicated to determine the safest and most effective method of using coumarin drugs.—R.H.G.

BLOOD COAGULATION


The coagulation activity of normal serum on normal and pelentanized (tromexanized) plasma has been investigated. At the same time the coagulation activity of pelentanized serum on normal and pelentanized plasma has been determined. The examinations were performed serially between the first and second hour after the blood was obtained with native sera (without thromboplastin or calcium). The examinations were carried out in order to find out if the clotting times obtained by mixing normal plasma with normal serum compared with the clotting times obtained by mixing pelentanized plasma with pelentanized serum could be used for a control of Pelentan treatment as proposed by Petrášek.

Our conclusions are as follows:

1. The coagulation activity of pelentanized serum on normal plasma decreases much more slowly in comparison with the coagulation activity of normal serum on normal plasma. This is due to the greater amount of the factor VI in pelentanized serum caused by the decreased utilization of factor V during the clotting process of pelentanized blood. The thrombin activity of pelentanized serum (tested on fibrinogen) is very low and decreases very rapidly.

2. The coagulation activity of serum, especially of the pelentanized one, is caused largely by activation of factor V to factor VI. This suggestion is stressed since examinations with aged plasma showed considerable lengthening of clotting times. At the same time, the difference between the activities of normal and pelentanized serum was not more detectable.

The long lasting activity of pelentanized serum is mostly concealed or overcome by the decrease of prothrombin and factor VII when the activity is tested on pelentanized plasma. The lengthening of the coagulation times and the faster decrease of activity in this case do not run parallel with Quick’s test. These changes are sometimes not impressive and many values are of the same order as those of normal individuals, where these values are also very variable.

4. The values are very much dependent on the temperature of the surroundings. They become considerably longer at higher temperatures.

5. The method proposed by Petrášek is unsuitable in the present form for the control of Pelentan treatment and cannot replace the test of Quick.—M. N.

ERYTHROCYTES AND ERYTHROCYTIC DISEASE


Blood from a male West African was submitted to paper electrophoresis at pH 8.6. The hemoglobin separated into the normal adult variety (A) and another component differing slightly in its speed of migration from sickle cell hemoglobin (S). The red cells of this individual did not sickle on chemical reduction. The unidentified hemoglobin differed from fetal hemoglobin (F) and from the sickle cell form. The name hemoglobin G is suggested for this new variety which also appears to differ from hemoglobins C, D and E.—R.H.G.

A 35 year old female of the Ga tribe of West Africa had anemia. By paper electrophoresis this was found to be associated with the presence of sickle cell hemoglobin and hemoglobin C. In a preliminary survey of 200 out-patients, hemoglobin C was found in 12 per cent. Two further individuals with sickle cell hemoglobin and hemoglobin C were found and also one patient who was homozygous for hemoglobin C. Of nine anemic patients, one had sickle cell hemoglobin and hemoglobin C, and another was a hemoglobin C homozygote. — R. H. G.


Exposure of newborn rats to hypoxia (6 hours daily for 14 days, altitude 15,000 feet) failed to produce evidence of increased erythropoiesis as judged by increased hematocrit, hemoglobin and red cell volume. If rats 18 days and older were exposed to a similar degree of hypoxia, the expected increased erythropoiesis occurred. Authors interpret these findings as evidence that hypoxia is unlikely to be the cause for the high erythroid values found at birth. They conclude that termination of hypoxia at birth, therefore, does not explain postnatal anemia. — A. G. M.


This article is based on extensive studies of reticulocytes in blood and bone marrow. The author has shown that all red cells normally leave the blood-forming organs as reticulocytes, the last part of the maturation to fully mature, non-reticulated erythrocytes taking place in the peripheral blood. The reticulocyte content of the normal bone marrow tissue is estimated at 25 to 30 per cent of the total cell population of the red bone marrow. When this is taken into consideration one will find that precursors of red cells are approximately as numerous in the bone marrow as precursors of the white cells.

The maturation of reticulocytes has been studied by incubation of heparinized blood in vitro at 37 C. (using siliconized test tubes). By interpretation of the reticulocyte maturation curves it is possible to get a fairly accurate estimate of the rate of formation of red cells, and, under certain conditions, of the red cell survival.

The rate of erythrocyte formation is normally regulated within certain limits by the oxygen supply to the organism. The author presents new evidence in favor of the theory that the oxygen tension in a blood-regulating center in the basal part of the brain is the important factor in this connection. The presence in some animals of a humoral erythropoiesis-regulating mechanism seems to be established by well controlled experiments by different investigators. Evidence is presented by the author indicating that a similar regulating mechanism is instrumental in human beings as well. — M. S.


Measurement was made by a photoelectric oxyhemoglobin method of the hemoglobin levels of 337 men and 116 women of different races living in Singapore. Venous blood was used. Europeans, Chinese, Indians and other Asians were included. The mean hemoglobin concentration of men was 16.12 Gm. per 100 ml., and of women was 13.88 Gm. per 100 ml. There was no racial difference. — R. H. G.

Oxygen dissociation curves were studied in 29 cases of anemia. The curves were significantly shifted to the right in 10 out of 11 cases of megaloblastic anemia in relapse, and in 9 out of 10 patients with hypochromic and normochromic anemias. No shift was observed in one out of two cases of aplastic anemia and in all three cases of hemolytic anemia studied. Oxygen dissociation curves were derived from study of whole blood. When oxyhemoglobin solutions were examined, results remained the same. Displacement of the oxygen dissociation curve persisted during the period of recovery in the cases of megaloblastic anemia but was not present in therapeutic remission. Reticulocytosis did not influence the position of the curve. A major part of displacement appeared to be due to a slightly decreased cell pH. However, authors believe that pH changes did not entirely explain the observed shift to the right. Displacement of the oxygen dissociation curve to the right increases the amount of oxygen released to the tissues, and thus may represent a compensatory adjustment for anemic anoxia.

It has recently been shown that the oxygen dissociation curve of hemoglobin solutions from three patients with sickle cell anemia did not differ from the curves obtained with normal hemoglobin (Rev. d'hémat. 9: 155, 1954). The oxygen dissociation curve in one case of homozygous hemoglobin C also has been reported as normal (New England J. Med. 3: 251: 365, 1954).—A.G.M.


Previous studies by the Army research unit at the University of Chicago demonstrated development of acute hemolytic anemia in a number of Negroes when the antimalarial drug primaquine was given in daily doses of 30 mg. base. Complete hematologic recovery followed although drug administration was continued. Hematologic observations and studies with Cr<sup>51</sup>-labeled erythrocytes demonstrated an abrupt decrease in rate of hemolysis after approximately one week of drug administration. The primaquine-sensitive tagged red cells, when transfused into nonsensitive recipients pre-fed with primaquine, remained sensitive and were hemolyzed. Similarly such sensitive red cells given to recipients in the recovery phase of primaquine hemolysis were destroyed. On the other hand red cells obtained from donors in the recovery phase of primaquine hemolysis showed normal red cell survival. Authors conclude that self-limitation of hemolysis was due to a change in the reactivity of the red cell population.—A.G.M.


The erythrocytes of primaquine-sensitive and -nonsensitive individuals were compared in the following respects:
A. Morphology.
B. Antigenic characteristics.
C. Sensitivity to acid hemolysis.
D. Hemoglobin types and methemoglobin.
E. Mechanical osmotic and chemical fragility.
F. Fragility to the in vitro hemolytic action of primaquine and similar compounds.
G. Susceptibility to the accelerating of naphthalene and primaquine on taurocholate or saponin hemolysis.

No differences could be detected. Similarly, morphology, Coombs test, hemoglobin types and osmotic and mechanical fragility of red cells did not alter during the various phases of the primaquine-induced hemolytic reactions. However, Heinz bodies appeared in erythrocytes of sensitive men just before and at the beginning of hemolysis and disappeared rapidly.
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as hemolysis progressed. These findings were interpreted by the hypothesis that primaquine inflicts injury on sensitive cells which are then destroyed by in vivo mechanism.—A.G.M.

THE HEMOLYTIC EFFECT OF PRIMAQUINE. IV. RELATIONSHIPS OF CELL AGE TO HEMOLYSIS.

Isotopic iron Fe59 was administered to a primaquine-sensitive individual to produce a tagged red cell population of relatively uniform age. Administration of primaquine when labeled red cells were 8 to 21 days old failed to destroy the young tagged erythrocytes. A second 6-day course of primaquine when the tagged cells varied between 63 and 70 days produced significant destruction of the labeled cells. Authors conclude that older red cells are particularly sensitive to the action of primaquine. This explains the self-limited nature of the hemolytic process. It is suggested that a deficiency in a red cell enzyme system (not catalase) accounts for the abnormal sensitivity. Certain similarities to hemolysis by aniline compounds are pointed out.—A.G.M.

SICKLE CELL TRAIT AND SPLENIC INFARCTION ASSOCIATED WITH HIGH ALTITUDE FLYING.

Conn reports on two soldiers with electrophoretically proven sickle trait who developed signs and symptoms of splenic infarction during high-altitude flight. Splenic enlargement was absent. The patients were treated conservatively. Harvey described splenic infarction during flight in a Negro soldier with a positive sickling test, splenomegaly and nucleated red cells in the peripheral blood. Electrophoretic studies of the patient’s hemoglobin were not done. Splenectomy was performed in Harvey’s case.

In view of the enlarged spleen and the presence of nucleated red cells it is extremely likely that Harvey’s patient was affected with sickling-hemoglobin C disease. During the past year Cooley et al. reported on six cases of splenic infarction occurring during flight in soldiers with a positive sickling test. It is significant that the syndrome of splenic infarction may occur in patients with a sickling trait as well as those with sickling-hemoglobin C disease and associated splenomegaly. Although hypoxia presumably plays an important role in the development of the syndrome both Harvey and Conn point out that the exact factors determining the production of splenic infarcts in some patients and not in others remains unknown. Conn feels that conservative management rather than splenectomy is the treatment of choice.—A.G.M.


The electrophoretic examination of hemoglobin samples from Africans with sickle cell trait showed that the mobilities were not constant and did not agree with values reported by other observers. Samples taken from one individual varied from time to time. The variations occurred both with the Tiselius apparatus and with paper electrophoresis. Normal hemoglobin can be resolved into fractions with different physical properties and the difference of electrophoretic mobility may be a reflection of such heterogeneity.

Usually the proportions of normal and sickle hemoglobin components were reversed in the ascending and descending columns. This might indicate the formation of a complex between hemoglobin and some other constituent of the red cells; sickle cell hemoglobin might differ from hemoglobin in being such a complex with some unidentified macromolecule. Normal hemoglobin was mixed with nucleic acid and the mixture dialyzed against phosphate buffer and submitted to electrophoresis. The mobility of the original hemoglobin was altered and a new peak, presumably corresponding to a hemoglobin-nucleic acid complex, appeared.

Abnormal hemoglobins that have been described may merely be complexes of hemoglobin
with some other macromolecule which is constantly present and genetically inherited, and which, in some instances, may alter the solubility properties of the hemoglobin to give rise to the 'sickle' phenomenon.—R.H.G.


The term T-transformation applies to the production of the specific receptor-T in erythrocytes through the activity of an enzyme formed by various bacterial species. It has proved practicable to produce hemolytic anemia in guinea pigs by T-transformation of their erythrocytes in vivo. This work was carried out on four guinea pigs: two had a high anti-T titer and their erythrocytes were T-transformed by intravenous injection of concentrated enzyme prepared from pneumococcus 19. These developed hemolytic anemia. The other two animals had a low anti-T content of the serum and served as controls. They were given intravenous injections of saline, and anemia did not develop.—R.H.G.


In eleven patients with the hepato-splenoegalic form of Manson's schistosomiasis the blood volume was determined simultaneously for the plasma volume with T-1824 dye (Evans blue) and for the red cell volume with Pbs. There was observed a regular increase of the whole blood volume due to a plasma volume increase. The reduction of the red cell volume was out of proportion to the degree of anemia. The authors considered this anemia as being due in great part to dilution by the expanded plasma volume. The red cell volume, expressed in ml. per Kg. of body weight, was not reduced; it was slightly reduced when the absolute values were compared with the accepted normal standards.—M.A.J.


The first case concerns a 51 year old man who was first seen in 1952 after a history of anemia of two years duration. The hemoglobin was 30-50 per cent; the direct and indirect Coombs test was positive. Treatment with Cortisone, X-ray of the enlarged spleen, and blood transfusions were completely ineffective. So, the spleen was removed (weight 720 Gm.). After a remission of 6 months, a new relapse of the anemia occurred and the patient died.

The other patient was 57 years old and was observed in January 1953; he had been anemic for 6 months. Hemoglobin was 31 per cent. Splenectomy (weight 320 Gm.) was performed after two months of internal treatment. Afterwards, the hemoglobin rose to 75 per cent. Ten months later the general condition was still very good. The direct Coombs test was positive, whereas the titer of the indirect test was decreasing.

The authors, in opposition to the general concept, have the opinion that splenectomy should be done early in these cases.—C.M.


A nine year accumulation of data relative to 469 cases of erythroblastosis fetalis is analyzed with particular attention to the problem of stillbirth. The natural history of the erythroblastotic stillbirth (23 per cent for this period) is closely related to the past maternal history and titer. When the titer was 1:256 or higher and a previous baby had died, 60 per cent were stillborn, and another 14 per cent were hydropic. The primary cause of intra-
uterine death appears to be anemia and the prevention of this anemia is therefore of primary consider-
ableness. It is emphasized that each case be considered individually in the light of previous history, maternal titer, Rh-genotype of the husband, and the availability of exchange transfusion therapy. Delivery before term of these selected cases (when it is considered as a safe procedure for the mother) does reduce the frequency of erythroblastotic stillbirth.—J.H.A.

Blood Club Meeting

The Eighth Annual meeting of the Blood Club will be held on Sunday, May 1, 1955, at 7:45 p.m., in the Vernon Room, Haddon Hall Hotel, Atlantic City, New Jersey. The program will be as follows:

THE METABOLISM OF THE RED BLOOD CELLS

1. Adenosine and Red Cell Metabolism
   Dr. Clement A. Finch, Department of Medicine, Seattle, Washington

2. Carbohydrate and Phosphorous Metabolism of the Normal Red Cells
   Dr. Grant Bartlett, Scripps Metabolic Clinic, La Jolla, California

3. Abnormalities of Carbohydrate Metabolism in Spherocytes
   Dr. Kurt Altman and Dr. Lawrence Young, Department of Medicine, University of Rochester, New York

4. The Erythropoietic Factor
   Dr. Allan J. Erslev, Murphy Army Hospital, Waltham, Massachusetts

5. Evidence for an Erythropoietic Factor in the Pituitary
   Dr. A. N. Contopolous, Institute of Experimental Biology, The Donner Laboratory, Berkeley, California

It is hoped to have the discussion opened by Dr. J. V. Dacie, Department of Pathology, Post Graduate School, London, England, and Dr. William B. Castle, Thorndike Memorial Laboratory, Boston, Mass.

At the conclusion of the meeting, refreshments will be served in an adjacent room to facilitate social and scientific discourse.

Committee on Arrangements:

Chairman: Charles A. Doan, M.D., The Ohio State University
Secretary: George E. Cartwright, M.D., University of Utah
Robert F. Schilling, University of Wisconsin