VITAMIN B₁₂ and FOLIC ACID


This is an interesting report of several cases treated by different methods with vitamin B₁₂. The authors conclude that when intrinsic factor of Castle is in combination with cobalamin it is relatively heat stable and resistant to trypsin digestion. They conclude there is a parallelism between intrinsic factor activity of several preparations and their ability to combine with cobalamin thereby rendering the vitamin incapable of stimulating growth of certain micro-organisms. The significance of any such conclusion, if it continues to be apparent in further work, is debatable.—P.F.W.


The recent discovery of 5,6-dimethylbenzimidazole among products resulting from and hydrolysis of vitamin B₁₂ indicated to the authors that benzimidazole and its derivatives are substances of considerable biological interest.

The authors developed a colorimetric and fluorometric determination of 5,6-dimethylbenzimidazole based on reactions of 4,5-dimethyl-o-phenylenediamine, obtained from the benzimidazole by benzylation and subsequent cleavage of the 4,5-dimethylbenzyl-o-phenylenediamine with concentrated sulfuric acid. Under standard conditions of hydrolysis one mole of 5,6-dimethylbenzimidazole mole B₁₂ is regularly obtained and can be determined by the colorimetric procedure.

Although this report is preliminary, it appears that an accurate chemical assay method for B₁₂ is at hand.—C.E.R.


The response of a group of patients comprising 4 with pernicious anemia, 4 with tropical sprue and 2 with nutritional macrocytic anemia to the substance or substances known as folinic acid or citrovorum factor was studied. Six patients were arbitrarily given the folinic acid preparation in low dosages (200 units intramuscularly daily for ten days). Only 2 of these patients showed even a slight response to this dosage. All 4 patients, however, who
received the preparation of citrovorum factor, 20 million units intramuscularly daily for
ten days (or approximately 10,000 times the dosage of the folinic acid preparation given to
the first group of patients), exhibited a satisfactory clinical and hematologic response. Data
is presented on 4 of the 10 patients.

Further study is needed to establish the minimum effective dose of this substance and
its clinical activity as compared to folic acid. One would expect that those dangers attend-
ing the prolonged use of folic acid in pernicious anemia would also be present when citro-
vorum factor was used in maintenance therapy.—H.W.B.

ANEMIA

THE ANEMIA OF THERMAL INJURY. I STUDIES OF PIGMENT EXCRETION. G. W. James, III,
O. J. Purnell and E. I. Evans. From the Laboratory for Clinical Investigation, Department
of Medicine, and the Surgical Research Laboratory, Department of Surgery, Medical

Previous observations have demonstrated that anemia may develop rapidly in the first
4 days after thermal injury and that moderate anemia may develop during convalescence
from a small burn. The present study was undertaken to determine the role that hemolysis
may play in the rapid development of anemia and to study the anemia of the convalescent
stage. By means of quantitative urine and fecal urobilinogen studies, hemograms and the
determination of the hemolytic index, the authors have studied the course of 17 burn pa-
tients. The data so obtained showed that hemolysis occurs in burns of all degrees. Such
hemolysis is very great in third degree burns of more than 20 per cent. There is also a con-
stant increase in the urinary urobilinogen noted on the third day after injury. The authors
feel that this probably denotes early hepatic dysfunction and inability to properly catab-
olize an increased amount of urobilinogen. Observations during the period of convalescence
showed that the ratio of the fecal urobilinogen to the total circulating hemoglobin (hemoly-
ic index) was usually low. This would seem to point to decreased hemolytic activity,
which may result from decreased hemoglobin synthesis. Decreased hemoglobin synthesis
may be due to infection of the burned area. Other data obtained showed that oral aure-
omycin reduced the fecal and urinary urobilinogen to very small amounts.—R.B.C.

THE ANEMIA OF THERMAL INJURY. II STUDIES OF LIVER FUNCTION. G. W. James, III, O.
J. Purnell and E. I. Evans. From the Laboratory for Clinical Investigation, Department
of Medicine, and the Surgical Research Laboratory, Department of Surgery, Medical

In a previously reported study the authors demonstrated that increased excretion of
urinary urobilinogen was an early and constant finding after thermal injury. They inter-
preted this finding to be a manifestation of hepatic injury. In the present investigation,
liver function studies revealed impaired function followed mild to extensive third degree
burns. The more severe the burn, the greater the change in liver function. Autopsy studies
on 5 fatal burn cases showed no constant histopathologic changes but there was evidence of
fatty infiltration, cloudy swelling, increased pigments in the reticulo-endothelial cells,
focal necrosis and congestion. The mechanism of these pathologic changes was not evident.
The authors emphasize that preventive therapy against liver damage should be initiated
immediately. Preliminary efforts using dietary measures, lipotropic agents and oral aure-
omycin were ineffective in so far as changes in liver function were concerned.—R.B.C.

THE ANEMIA OF INFECTION. XIII. STUDIES ON EXPERIMENTALLY PRODUCED ACUTE HYPOR
FERREMIA IN DOGS AND THE RELATIONSHIP OF ADRENAL CORTEX TO HYPOFERREMIA. G.
E. Carterwright, L. D. Hamilton, C. J. Gubler, N. M. Fellows, H. Ashenbrucker and M. M.
Wintrobe. From the Departments of Medicine and Surgery, College of Medicine, Uni-

Hypoferremia is one of the most consistent features of the anemia associated with chronic
infection. The mechanism by which inflammation produced hypoferremia has been the
subject of many studies by this group of investigators. In the present study, serial observations of the plasma iron levels of a large number of intact and adrenalectomized dogs were made under a series of conditions of stress.

A variety of agents other than bacterial and sterile turpentine abscesses produced acute hypoferremia in dogs. These included histamine, epinephrine, fracture, anaphylactic shock and mild stress. Adrenocortical extract and ACTH produced a similar hypoferremia. Adrenalectomy abolished the hypoferremia produced by mild stress and by ACTH, and reduced that produced by epinephrine. Intravenous saccharated oxide of iron disappeared rapidly from the plasma of intact dogs given ACTH and more slowly from the plasma of adrenalectomized dogs. Intravenous Di-benamine did not block the hypoferremia effect of epinephrine and itself produced a hyperferremia in 2 hours, followed by hypoferremia with a maximum at 24 hours.

The authors speculate that stress produces hypoferremia through an increase in the uptake of iron by the reticulo-endothelial system as a result of stimulation by cortical hormones. It should be pointed out, however, that epinephrine and turpentine can produce some hypoferremia even in adrenalectomized dogs. They also emphasize that these observations were made on dogs. Although a similar mechanism may account for the hypoferremia of chronic infection in man, no evidence is presented to support this possibility.—R.B.C.

**Biochemical and Haematological Changes in Type 1 and Type 2 Nephritis. M H. Roscoe. From the Department of Medicine Manchester University, England. Quart. J. Med. 19: 161-186, 1950.**

Sixty-one cases of nephritis were studied over a three year period. In type 1 nephritis, anemia develops early in the acute attack, due to blood dilution, but if the attack is prolonged a true normochromic anemia develops.

In type 2 nephritis, the patient rarely has anemia when the edema develops and it is concluded that the plasma volume is normal. The hemoglobin usually remains normal until late in the course when uremia develops.

No studies were done to explain the basic mechanisms involved. Actual plasma volume determination with T 1824 was done in only one case. It is unfortunate that the careful clinical observations in this large group of cases were not correlated with more complete hematologic studies.—C.E.R.


Three patients suffering from Raynaud’s phenomena and chronic hemolytic anemia are reported. Hemoglobinuria on exposure to cold occurred in 2. The sera in all 3 contained cold agglutinins, cold hemolysins and incomplete cold antibodies in considerable titer. The etiology of the disorder was not discovered.

An analysis of 9 similar cases from the literature is included.—C.E.R.

**The Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria. I. The Role of Complement and Its Components in the Donath-Landsteiner Reaction. W. S. Jordan, Jr., L. Pillemer and J. H. Dingle. From the Departments of Preventive Medicine and of Medicine, and the Institute of Pathology, School of Medicine, Western Reserve University, and the University Hospitals, Cleveland, Ohio. J. Clin. Investigation 30: 11-21, 1951.**

Previous investigation by Donath and Landsteiner has demonstrated that the abnormal factor responsible for hemolysis in paroxysmal cold hemoglobinuria is in the patient’s serum. Inactivation of the serum by heat prevented hemolysis, but the addition of fresh serum restored the activity. The hemolytic reaction in vitro occurred in two phases: (1) a
ABSTRACTS

cold phase during which the antibody (hemolysin) is adsorbed on the erythrocytes; (2) a warm phase during which the sensitized cells are lysed in the presence of complement (fresh serum).

In the present study it was found that large amounts of complement may be necessary for hemolysis in the Donath-Landsteiner reaction. It was also demonstrated that complement is essential in both the cold and warm phases of this reaction. The erythrocyte—PCH antibody system is unique in that complement is necessary for antibody fixation as well as for subsequent hemolysis. In addition, utilizing the four components of complement (C'1, C'2, C'3, and C'4) the authors demonstrated that only two components (C'2 and C'4) of complement need be present. If C'4 is missing in the cold phase or C'2 is missing in the warm phase, hemolysis will not occur. Since only two components of complement are necessary for hemolysis in paroxysmal cold hemoglobinuria, titration of complement in serum by the sheep cell-anticoceptor system, which requires all four components, may not measure the capacity of serum to produce hemolysis with PCH antibody. Finally, the antibody level in PCH may be determined by the agglutination by antilglobulin serum of erythrocytes which have been sensitized in dilutions of paroxysmal cold hemoglobinuria serum.—R.B.C.

THE MECHANISM OF HEMOLYSIS IN PAROXYSMAL COLD HEMOGLOBINURIA. II. OBSERVATIONS ON THE BEHAVIOR AND NATURE OF THE ANTIBODY. W. S. Jordan, Jr., L. Pillener and J. H. Dingle. From the Departments of Preventive Medicine and of Medicine, and the Institute of Pathology, School of Medicine, Western Reserve University, and the University Hospitals, Cleveland, Ohio. J. Clin. Investigation 30: 22-30, 1951.

In paroxysmal cold hemoglobinuria an abnormal factor is present in the serum as a result of infection with the spirochaete of syphilis. Under proper conditions this serum antibody will hemolyze both the patient's own erythrocytes and those of other individuals. The hemolysin is activated in an antigens-antibody-complement system. In the presence of complement (component C'4) antibody fixation occurs when erythrocytes and PCH serum are chilled. When sensitized red cells are warmed in the presence of complement (component C'2), hemolysis occurs. This reaction is unique in requiring complement for antibody fixation in the cold phase and in requiring only two of the four components of complement. The abnormal antibody in PCH may also be identified and measured by using antilglobulin serum (Coombs reaction).

In the present study, the authors report further on the behavior of this abnormal antibody. Erythrocytes from a patient with paroxysmal cold hemoglobinuria were found to be agglutinable in antiglobulin serum (direct Coombs test). Such in vivo "sensitized" cells were not hemolyzed when warmed with complement. They were hemolyzed, however, in vivo if the patient was chilled and in vitro if they were chilled and warmed in PCH serum. The patients' erythrocytes did not give a direct Coombs test six hours after in vivo hemolysis had occurred, nor was any concomitant change noted in either serum antibody or complement level.

Utilizing the indirect Coombs test and the Donath-Landsteiner reaction, the behavior of the abnormal antibody present in the serum of patients with PCH was studied. These studies indicated that the PCH serum factor which reacts with antilglobulin serum was identified as the PCH hemolysin. This PCH antibody is a water soluble (pseudoglobulin) gamma globulin.—R.B.C.


This is a discussion of hemolytic anemias associated with circulating red blood cell antibodies. The causes for false-negative and false-positive reactions in the Coombs test are mentioned. The rather unpredictable clinical course such cases may have is stressed.
Caution in interpreting the effect of therapy in any individual case is therefore necessary. However, ACTH and cortisone apparently may have a beneficial effect in such patients. —P.F.W.


Utilizing the differential transfusion technic of Ashby, the authors have studied 4 patients with lymphatic leukemia to determine whether or not excessive hemolysis is an important factor in the production of the anemia of lymphatic leukemia even when the usual signs of excessive hemolysis are absent, and whether in any cases there is evidence of the presence of a hemolytic process not seen in normal subjects. In 3 of the 4 cases, transfused erythrocytes were destroyed at a rate faster than normal. In 2 of the cases, the hemolytic mechanism appeared exponential instead of linear. This exponential hemolytic mechanism is considered to be abnormal by the authors. The authors conclude that in at least some cases of lymphatic leukemia, the anemia is contributed to by increased hemolysis and that in some cases this hemolysis may be of an abnormal type (exponential instead of linear). They emphasize the fact that hemolysis may be demonstrated when not evident by the usual indexes of hemolysis and suggest that hemolysis may be an important factor in anemia more often than is now believed.—R.B.C.

LEUKOCYTES

EXPERIMENTAL LEUKOPENIA IN RATS AND THEIR THERAPEUTIC RESPONSE TO VITAMINS, METALS AND OTHER SUBSTANCES. R. Jürgens and A. Studer. From the Research Laboratory of the Hoffmann-La Roche Company, Basle, Switzerland. Acta haematologica 5: 47-64, 1951.

Sucinylsulfothiazol was fed to 1,000 rats in order to produce a striking granulocytopenia. This was used as a test for the examination of the leukopoetic effect of various substances. Especially effective was folic acid, even in experiments with additional damage of the bone marrow by nitrogen mustard. Vitamins A and C and iron had a similar leukopoetic effect. No response was seen using the vitamins B1, B6, B12, E, the nucleic acid derivatives hypoxanthinedesoxiriboside, guanidinesoxiriboside thymin, thymidin, uracil, furthermore choline, testosteronepropionate, methionine and thyrosine.—C.M.


The author reviews the different Pelger families described in the literature and discusses the connection with other anomalies of the leukocytes in human beings and animals. Of all similar nuclear deformities in animals only the Pelger anomaly in rabbits can be recognized as related to the human defect. In a second part the results of investigations with homozygote rabbits are described and an exact anatomic picture is presented. The skeletal system of those rabbits is related to Chondro dystrophy. A large bibliography is added.—C.M.

BLOOD VOLUME


Simultaneous direct measurements of plasma volume and whole blood volume using Evans blue dye and radioactive phosphorus were made on 10 patients with pulmonic stenosis immediately before and three and one-half to thirteen months after operation. The results indicated that after an adequate pulmonary blood flow has been established by opera-
ABSTRACTS 383

tion there is in addition to a great reduction in red cell volume a rise in plasma volume with the result that the whole blood volume, although somewhat reduced, remains moderately elevated.—C.E.R.

HEMORRHAGIC DISEASE and BLOOD COAGULATION


Seven generations of a hemophilic family are reported with 4 hemophiliac females in the fifth generation. The affected females resulted from the marriage of a hemophiliac male to his first cousin in the fourth generation. Two of the 4 hemophiliac females were personally studied by the author who found the clinical histories and laboratory findings to be typical of hemophilia except that the prolongation of clotting time was slight and both patients had positive tourniquet tests.—C.E.R.

THE ROLE OF SURFACE AND OF CALCIUM IN THE COAGULATION OF GLOBULIN FRACTION OF PLATELET-DEFICIENT PLASMA. O. D. Ratnoff and C. L. Conley. From the Department of Medicine, The Western Reserve University and the Mount Sinai Hospital, Cleveland, Ohio, and the Department of Medicine, the Johns Hopkins University and Hospital, Baltimore, Md. Bull. Johns Hopkins Hosp. 89: 245-254, 1951.

A globulin fraction was prepared from natural plasma after separation of the formed elements by high speed centrifugation in silicon treated equipment. The platelet free globulin fraction clotted in glass tubes without the addition of ionized calcium but did not clot when contact with glass was prevented. This suggests that the clot promoting effect of glass is due to its action on the precursor of the plasma thromboplastic factor which is deficient in hemophilia. When normal or hemophilic globulin was mixed with dilute thromboplastin alone in glass tubes, clotting was accelerated. In these experiments, normal and hemophilic globulins behaved in similar fashion. These studies provided evidence that the defect in hemophilia is an inability to form thromboplastins in shed blood.

The data presented in this report are offered as further evidence that platelets are not needed for the initiation of clotting and that plasma itself contains those factors necessary for coagulation.—C.E.R.


This study reports a series of observations demonstrating the role of a plasma and serum component, "labile factor," in the coagulation of blood, with special emphasis on its effect on the rate of conversion of prothrombin to thrombin. By virtue of its influence on the velocity of prothrombin conversion to thrombin in serum, the amount of labile factor present influences the prothrombin determination by the one-stage procedure. Under such circumstances, the computation of prothrombin consumption from differences between plasma and serum prothrombin activities may be erroneous.

It is the opinion of the authors that labile factor is identical to plasma A2-globulin and to Factor V of Owen. It is necessary for the rapid conversion of prothrombin to thrombin in the presence of calcium. As coagulation occurs, labile factor disappears promptly from the blood. That which is not consumed represents the labile factor of serum. The addition of thromboplastin to normal blood leads to more consumption of labile factor and hence less serum labile factor remains. On the other hand, dicumarol reduces the amount of prothrombin available for conversion and, in turn, less labile factor is consumed. More serum labile factor remains and dicumarol serum has a strong restorative effect on aged plasma (in which the labile factor has deteriorated). Thromboplastin added to dicumarol blood did not result in decreased serum labile factor, as occurred when it was added to normal blood.
Hemophilic serum has more restorative effect on aged plasma than normal serum because more serum labile factor is present. Supplementing hemophilic blood with thromboplastin, which accelerates coagulation, yields serum with far less labile factor activity. In thrombocytopenia and thrombosthenia, two disorders in which prothrombin consumption is poor, serum labile factor activity is markedly elevated.

In the opinion of the authors, serum labile factor, i.e., plasma labile factor (plasma Ac-globulin) not consumed during the conversion of prothrombin to thrombin, represents one part of “serum” Ac-globulin. The other constituent of “serum” Ac-globulin is SPCA, serum prothrombin conversion accelerator. This latter substance is evolved during coagulation and in the presence of labile factor accelerates prothrombin conversion. SPCA exerts the major effect in accelerating prothrombin conversion and in restoring prothrombin conversion of aged plasma. However, SPCA in serum may be removed by adsorption with BaSO4. The restorative activity then remaining is dependent on serum labile factor. This technic serves as a means of measuring serum labile factor activity. This article is worthy of careful review by all individuals interested in the coagulation mechanism.—R.B.C.


The authors have performed further investigations into the role that proteolytic enzymes may play in the mechanism of blood coagulation. They have demonstrated that crystalline trypsins and “purified” serum fibrinolysin accelerate clotting of ordinary recalcified plasma and platelet-free plasma obtained with silicone technic. These enzymes accelerate and increase thrombin yield from heat-delipirinated plasma or other crude prothrombins. On the other hand, they have no significant effect on the thrombin-fibrinogen reaction nor upon the activation of purified prothrombin with Ca or accelerator globulin but negligible thromboplastin. Both substances markedly potentiate thromboplastic actions. Antilysic agents completely abolish the platelet thromboplastin potentiating effects of trypsins but do not abolish those of fibrinolysin. It is suggested that the data supports the clot-aiding role of “thromboplastic enzymes” as far as trypsins is concerned. However, it appears that fibrinolysin may not have such a function in the coagulation process.—R.B.C.

EFFECT OF PLATELET EXTRACT ON HEMOPHILIC BLOOD. E. Mond and K. Singer. From the Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill. J. Clin. Investigation 30: 77-83, 1951.

In a previous report the authors noted that a concentrated homogenate of platelets shortened the coagulation time of hemophilic blood. These homogenates had definite thromboplastic activity. The present study describes the action of platelet homogenates and dilutions of tissue thromboplastin on the clotting time and prothrombin consumption time of a group of 8 patients with hemophilia. The clotting time of hemophilic blood was consistently reduced to normal by these homogenates, and saline thromboplastin dilutions of 1:500 and 1:800 had the same effect. The prothrombin consumption time was increased regularly but not always significantly by these homogenates. By a process of exclusion, it is the opinion of the authors that these effects may be explained most satisfactorily by attributing them to the thromboplastin activity of the platelet preparation. Comparative studies with dilutions of tissue thromboplastin demonstrate that very small amounts of thromboplastin are fully capable of producing all the effects noticed with platelet homogenates. This is cited as supporting indirect evidence of the suggested mechanism of action of platelet homogenates. Fluctuations in the clotting status of hemophilies may reflect variations in the patient’s ability to produce thromboplastin.—R.B.C.

HEMATOLOGIC BIOCHEMISTRY

SOME OBSERVATIONS WITH THE PHASE-CONTRAST MICROSCOPE UPON INCINERATED HUMAN BLOOD CELLS. J. Krużyński. From the Histological Laboratory, Department of Physiology and Histology, University of Liverpool, Liverpool, England. J. Physiol. 111: 89-95, 1950.
The use of micro-incineration to determine the mineral constitution of erythrocytes has been reported previously. This present work uses the above technic plus a phase contrast microscope, and uses microchemical tests to identify the metallic ions. Na, K, and Fe were identified in erythrocytes, with wide variations in Na and K content in normal cells. The ash of lymphocytes and monocytes consists mainly of Ca. —R.C.C.

METHODS

A Benzidine-Thionine Method for the Demonstration of Hemoglobin in Formaldehyde-Fixed, Paraffin-Embedded Tissue. F. A. Patl. From Department of Pathology, Yale University School of Medicine, New Haven, Conn. Arch. Path. 52: 293-294, 1951.

Hemoglobin with this technic is stained olive green or greenish brown and fading of the stain is not appreciable after one year. —O.P.J.


After the incubation of blood at 37 C. for 12 to 32 hours, corpuscles may hemolyze in isotonic saline or even in serum. In this study dog blood was diluted 1:100 in various concentrations of buffered saline solution. The degree of hemolysis was determined for each saline diluted blood sample after incubation at 37, 42 and 45 C. It was observed that the resulting hemolysis varied directly although not proportionately with the osmotic tonicity of the saline. It was also observed that 95 per cent or more of the expected hemolysis resulting from incubation in 0.70 per cent saline at 37 C. could be prevented by the addition of 32 mg. of dextrose to 100 ml of the 1:100 saline dilution of blood. It is suggested that the increases in erythrocytic fragility during incubation may be due to an increase in the metabolic processes producing an increase in the osmotically active constituents of the erythrocytes. —R.B.C.

ERRATA

In the article entitled "A Second Example of Anti-Cellano (Anti-k)," by Philip Levine, A. B. Kuhmichel and Milton Wigod (Blood 7: 251-254, 1952), the word "Anti-k" should have been spelled with a small k (the small k signifying Cellano, the capital K signifying Kell).

In the article entitled "Analytical Review: The Interpretation of Red Cell Survival Curves," by A. C. Dornhorst (Blood 6: 1284-1292, 1951), an error in Equation 2, page 1285 occurred. Equation 2 should read:

\[ N_t = N_0 \int_{t_1}^{t_f} \varphi(a) \, da \]