ABSTRACTS

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VITAMIN B12, FOLIC ACID, and ERYTHROCYTE METABOLISM


The preparation by Lester Smith of radioactive vitamin B12 of high specific activity (420 μc./mg.) enabled the investigators to give a standard dose of 0.5 μg. of radioactive vitamin B12 containing about 0.2 μc. of radioactivity to thirteen pernicious anemia patients, mostly in remission, and to ten controls. In the latter, a mean of 31.0 per cent (standard deviation 6.9 per cent) of the radioactivity in the test dose was recovered from the feces. In the former, a mean of 88.7 per cent (standard deviation 6.8 per cent) was recovered.

When sources of intrinsic factor were given with radioactive vitamin B12 to pernicious anemia patients, the amount of radioactivity recovered from the feces was diminished, although the various intrinsic factor preparations appeared to differ in activity.—R.H.G.

Intrinsic Factor Studies. II. The Effect of Gastric Juice on the Urinary Excretion of Radioactivity after the Oral Administration of Radioactive Vitamin B12. H. E. Schilling. From the Department of Medicine, University of Wisconsin Medical School, Madison, Wis. J. Lab. & Clin. Med. 42: 800-806, 1953.

It has been reported by Heine and co-workers that the fecal excretion of radioactivity in four pernicious anemia patients receiving radioactive Vitamin B12 (B12Co48) by mouth was definitely greater than in normal subjects. They also reported that the simultaneous oral administration of an intrinsic factor preparation and B12Co48 was followed by a definite reduction in fecal radioactivity.

This paper reports observations on the effect of orally administered normal human gastric juice upon the quantity of radioactivity in the urine of pernicious anemia patients who were given a small oral dose of B12Co48. Two hours after the oral dose of B12Co48 each patient was given a subcutaneous injection of 1000 μg. of nonradioactive B12. Total urine volumes were collected at 2, 4, 6, 8, 12, and 24 hours.

Six normal people all excreted definite amounts of radioactive vitamin B12 when 2 μg. of B12Co48 were administered orally in 1000 ml. of water and 1000 μg. of vitamin B12 were given subcutaneously as described above.

Six patients with pernicious anemia in remission excreted no detectable radioactivity in their urine when B12Co48 was administered by mouth in the same way, but if active gastric juice was added each patient excreted easily detectable amounts beginning at about 4 to 6 hours after the oral dose. When the 1000 μg. of subcutaneous B12 was omitted neither group excreted detectable B12Co48.

This test has every appearance of being "the test we have been waiting for" in order to diagnose those cases of pernicious anemia which have been made obscure by previous therapy, and provides a new method for the study of intrinsic factor activity because observations can be made on pernicious anemia patients in hematologic remission.—T.R.T.
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STUDIES OF VITAMIN B₁₂ IN SERUM AND URINE FOLLOWING ORAL AND PARENTERAL ADMINISTRATION. W. G. Unglaub, H. L. Rosenthal, and G. A. Goldsmith. From the Division of Nutrition and Metabolism, Department of Medicine, and the Department of Biochemistry, the Tulane University School of Medicine, and the Charity Hospital of Louisiana, New Orleans, La., J. Lab. & Clin. Med. 43: 143-156, 1954.

These studies are based upon a microbiologic assay technique for measuring the presence and amount of vitamin B₁₂ in serum or urine.

Total serum vitamin B₁₂ activity was found to be significantly lower in patients with macrocytic anemia than in normal subjects. Vitamin B₁₂ activity in urine was similar in the two groups.

Following intramuscular injection of 10, 25, 50, and 100 μg. of vitamin B₁₂ in normal subjects, total serum vitamin B₁₂ activity increased rapidly and in proportion to the size of the dose injected. Urinary excretion of the vitamin increased roughly in proportion to the amount injected but variation was wide. Findings in two patients with pernicious anemia were similar to those in normal subjects.

Oral administration of vitamin B₁₂ to normal subjects was followed by little or no change in total serum B₁₂ activity after 500 μg., a slight increase after 1000 μg., and a definite rise after 3000 μg., which was comparable to that observed after intramuscular injection of 10 to 25 μg. of the vitamin. Oral administration to patients with macrocytic anemia in relapse was followed by a slight increase in serum vitamin B₁₂ activity, within the normal range, in two of three subjects after 500 μg. and by a definite increase in one subject after 1000 μg. When 3000 μg. was administered, a moderate to marked rise in serum vitamin B₁₂ activity was observed in five of six patients. A hematopoietic response followed oral administration of the vitamin in each instance but was suboptimal with doses of less than 3000 μg.

Urine vitamin B₁₂ activity following oral doses of 500 to 1000 μg. differed little from that prior to administration of the vitamin. Following doses of 3000 μg., an increase in urine vitamin B₁₂ activity equivalent to that which followed intramuscular injection of 10 μg. of the vitamin was noted in most instances. No definite relationship between maximum total serum vitamin B₁₂ activity and urinary excretion of the vitamin was apparent. Maximum levels of total serum vitamin B₁₂ activity and urinary excretion tended to increase with repeated oral doses of the vitamin. No correlation was observed between maximum total serum vitamin B₁₂ activity and hematopoietic response.—T.R.T.


These authors continue their series of papers by classifying megaloblastic anemia among Africans into three groups.

Type 1 responds to penicillin, to oral and intramuscular cyanocobalamin, to pteroylglutamic acid, and to liver.

Type 2 does not respond to penicillin or to oral cyanocobalamin, but still responds to intramuscular cyanocobalamin and to liver.

Type 3 responds only to pteroylglutamic acid or to crude liver.

Altogether twenty-four cases of megaloblastic anemia have been treated with penicillin (dosage and route not specified). Of these, sixteen gave responses; four did not respond to penicillin or oral cyanocobalamin but did respond to injections of the latter: four responded only to pteroylglutamic acid.

The authors consider that penicillin affects the synthesis, metabolism, or absorption of vitamin B₁₂ but not of folic acid and that it acts by influencing the biosynthesis and utilization of intestinal vitamin B₁₂.—R.H.G.

In a child with congenital hypoplastic anemia (erythrogenesis imperfecta) who was studied for four years, a bluish fluorescent substance was noted in the urine. This was identified as anthranilic acid. Oral administration of 1.6 Gm. of L-tryptophan led to increased urinary excretion of anthranilic acid as well as to the excretion of other intermediary metabolites of tryptophan. There appeared to be a decrease in the amount of anthranilic acid excreted when riboflavin was administered.

In eight additional such cases anthranilic acid was found in the urine in every instance.—

R.H.G.


Wheat grain was dissected manually under the low powered microscope and the grain divided into the principal anatomic regions, namely pericarp + testa, aleurone, embryo, scutellum, and endosperm. In each fraction microbiologic assays of the content of riboflavin, pantothenic acid, nicotinic acid, aneurin, biotin, folic acid, and pyridoxin were carried out, but accurate information about the first four alone has so far been obtained. The distribution of the various vitamins in the fractions of grain varies, but there was a high content of all four in the aleurone layer.

Various flours were analyzed for their content of the seven vitamins referred to above, and it was considered that the distribution of folic acid and pyridoxin was similar to that of riboflavin. Thirty-seven per cent of the riboflavin is in the aleurone fraction and 32 per cent in the endosperm layer, with 12 per cent in the embryo and 14 per cent in the scutellum.

—R.H.G.

IRON METABOLISM


Previous work by a number of investigators has shown that the nucleated erythrocytes of the duck as well as human reticulocytes are capable of assimilating iron in vitro, and that an appreciable fraction of the radioiron added in vitro is recoverable in the heme fraction. The authors considered it possible that measurement of iron uptake by red cells might be used as a means of assaying the degree of heme synthesis in vitro under various experimental conditions. It was therefore decided to study factors influencing the movement of iron into duck erythrocytes and its distribution in these cells.

It was found that the quantity of iron taken up by a given number of avian nucleated red cells depends upon the amount of iron available in the plasma, the temperature at which the incubation is carried out, and the proportion of cells which contain reticulum. Mature nonreticulated cells are able to synthesize heme only to a limited extent. Desoxypyridoxine markedly inhibited iron uptake but failed to modify incorporation into the nonheme fraction. Pyridoxine added simultaneously did not modify this inhibition. 4-Aminopteroylglutamic acid and pantoyltaurine (a pantothenic acid antagonist) had no influence on the uptake of iron by erythrocytes. Potassium cyanide inhibits heme synthesis.

The iron uptake of human reticulocytes was about one-fifth that of duck blood containing a similar number of reticulocytes.

Duck erythrocytes incubated at 37 C. take up iron when suspended in plasma or 0.15 M. sodium chloride. In such a system approximately 85 per cent of the iron which enters the erythrocytes becomes irreversibly incorporated into heme and 15 per cent is held within the cell in a form which has been termed the "nonheme RBC iron pool ."

In order to make the amount of heme into which the radio-iron was incorporated, more protoporphyrin is required than normally exists in the free state in duck erythrocytes. It is postulated that new porphyrin synthesis must have occurred during these experiments in vitro.
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It was also found that human fetal cells take up more iron than do normal adult cells.—T.R.T.


The methods commonly in use for measurement of the iron binding capacity of serum have been based on Schade's demonstration that stepwise additions of ionic iron to the serum in vitro produced progressive increases in color of the iron protein complex, until the protein became saturated, when no further color developed. The disadvantages of this method are said to be the difficulty of selecting an optimum wave length, the inability to use lipemic or icteric serum, or serum older than 24 hours. In addition, the method is limited in accuracy to 50 μg. per 100 ml.

The authors therefore investigated the use of radioiron for this determination. The method involves the addition of radioiron of known specific activity in an amount greater than that required to saturate the protein, allowing the iron to combine with the protein, separating the iron-saturated protein from the solution, measuring the excess of unbound iron, and thereby calculating the quantity of iron bound. Ammonium sulfate (saturated) was used to precipitate to protein.

The data indicate that this is a reliable method. This reviewer is impressed with the fact that it is considerably more elaborate than the older methods, and would suggest that the older methods are usually adequate for diagnostic purposes.—T.R.T.

ERYTHROCYTE PRESERVATION and SURVIVAL

SURVIVAL OF TRANSFUSED RED CELLS IN SCURVY. C. Merskey. From the Department of Medicine, University of Cape Town, S. Africa. Brit. M. J. 2: 1353-1356, 1953.

In fourteen patients with scurvy and anemia and two with scurvy but no anemia the survival time of transfused red cells was estimated. The patients (fifteen Bantus and one Indian) were at rest in bed on a vitamin C free diet. In fourteen instances the red cell survival time was abnormally short, and in some of these cases it was shown that the abnormality persisted for some days after the commencement of ascorbic acid therapy. It seemed possible that blood transfusion materially increased metabolic demands for vitamin C, but red cell survival was shown to be abnormal even when a replacement transfusion was substituted for simple transfusion.—R.H.G.


Study of the oxygen and carbon dioxide dissociation curves of blood stored in an acid-citrate-dextrose medium at 4 C. showed that the oxygen dissociation curve was shifted to the left and the amount of carbon dioxide released for each volume per cent of hemoglobin saturation with oxygen was reduced. The investigations which were carried out on over one hundred samples of blood stored for up to ninety days indicated that the changes were progressive with storage.

Transfusion studies on ten patients, all anemic, receiving fourteen transfusions given at a speed of a pint in 1 or 2 hours, showed that the oxygen dissociation curve of patients after transfusion with citrated blood stored seven days or more was substantially shifted to the left immediately after transfusion. This effect lasted several hours. The magnitude and duration of the shift were proportional to the volume and length of time of storage of the transfused blood. As a result of the shift, an anemic recipient's blood may be unable, for a few hours after transfusion, to release as much oxygen as it did before transfusion.

For the full development of these abnormalities, storage, acid reaction, and citrate are necessary. They are not due to abnormal pigments or to alteration of the pH of the recipient's plasma.—R.H.G.
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IMMUNOHEMATOLOGY


A case is presented in which the occurrence of a single and specific intragroup hemolytic antibody was noted. This antibody was responsible for hemolytic transfusion reactions. It was active against red blood cells of 64 per cent of fifty donors examined. The patient had apparently been sensitized by previous transfusions. An antibody to the Duffy factor, and certain unidentified antibodies demonstrable by the conglutinin technic were also found. These were distinct from the isohemolysin.—T.R.T.


A donor, believed to be group O, was found to have blood that was incompatible with the serum of a nullipara without transfusion history. It was thought that the serum of the recipient contained a unique antibody and that the cells of the donor contained a unique antigen. When Group O serum was used for testing it was found that the unique antigen was in fact A, the occurrence of which among British people is about 1 in 30,000. It is recommended that all so-called family or private blood groups be tested with group O serum, that group O serum is an essential reagent in ABO grouping, and that the racial origin of both the donor of a family antigen and the corresponding antibody be considered.—R.H.G.

ABO BLOOD GROUPS AND HUMAN FERTILITY. T. M. Allan.


This is an analysis of the collected material of Waterhouse and Hogben (Brit. J. Soc. Med. 1: 1, 1947) in terms of relative fertility by mating class. In a composite sample of 1239 families with 4139 children, these authors had found a highly significant shortage of group A offspring in the mating class father A × mother O as compared with father O × mother A, and an appreciable shortage of group B offspring in the mating class father O × mother B as compared with father B × mother O.

It is now suggested that the first of these findings is part of a shortage of offspring of A fathers generally and that the second is part of a general shortage of offspring of group B mothers.—R.H.G.

DIRECT OBSERVATIONS OF INTRAVASCULAR AGGLUTINATION OF RED CELLS IN ACQUIRED AUTOIMMUNE HEMOLYTIC ANEMIA. C. Wasastjerna, W. Dameshek, and Z. D. Komninos.

From the Ziskind Laboratory (Blood Research Laboratory) of the New England Center Hospital, and the Department of Medicine, Tufts College Medical School, Boston, Mass. J. Lab. & Clin. Med. 43: 98-106, 1954.

'The appearance presented by the blood stream in the superficial vessels of the conjunctival sclerae of 15 healthy persons and of 53 patients with hematologic disease was studied by means of the slit lamp microscope. Intravascular red-cell agglutination was noted in all 14 patients having acquired autoimmune hemolytic anemia. The agglutination was slight to moderate in those patients who were in good clinical remission, and very marked in manifest hemolytic anemia. In cases of hemolytic anemia associated with an intracorpuscular defect, the Coombs test being negative, there was no agglutination or at most fine granularity of the streaming blood, even with severe anemia. All patients suffering from leukemia or other malignant blood diseases revealed a varying degree of intravascular agglutination of red cells. Although the agglutination of red cells coated with antibodies appears to be of pathogenetic importance in the hemolysis of immunohemolytic anemia, it is probable that other mechanisms also contribute to the destruction of red cells in these disorders. It is not clear from these studies as to the relationship of the agglutina-
tion phenomenon observed here and the 'sludging' phenomenon of Knisely. We have the impression, however, that 'sludging,' as noted in many conditions, e.g., infections, is more closely related to rouleau formation than to the intravascular agglutination seen in many hemolytic processes."—T.R.T.


Among the seventy-eight patients treated in Leeds with these drugs were three children with acquired hemolytic anemia. One responded after transfusion and ACTH: a second responded to ACTH both before and after splenectomy, but required to be maintained on cortisone: the third relapsed when ACTH was withdrawn after splenectomy, but later it was found possible to cease hormone therapy. Two cases of aplastic anemia did not benefit from ACTH. A patient who developed agranulocytosis while taking thiouracil improved rapidly with ACTH, but it is not stated whether the thiouracil treatment was stopped. In two other granulocytopenic patients there was no benefit. In two patients with thrombocytopenia there was no hematologic response although bleeding stopped, and three patients with myeloid leukemia did not improve with ACTH therapy.—R.H.G.


This second report deals with sixty-five patients, of whom thirty-seven received cortisone, twenty-three ACTH, and five both. Higher doses of the hormones were given than in previous investigations.

Hemolytic anemia. Seven idiopathic acquired and three symptomatic cases were treated. The latter all responded well, and only two of the former showed no response. It was thought that inadequate dosage did not account for failure in response.

Purpura. Twenty-two patients were treated; of these fifteen had idiopathic thrombocytopenic purpura and ten responded favorably. There was no obvious relationship between duration of illness and response to hormones in these fifteen, and no suggestion that responses were significantly influenced by variations in dosage or duration of treatment. Splenectomy had favorable results both in those who had responded to hormones and in those who had not.

Aplastic anemia, agranulocytosis, etc. Of seven patients treated there was a good response in one case of agranulocytosis and a partial response in one case of aplastic anemia and one of pancytopenia.

Leukemia, etc. Twenty-four cases treated. Only five responded at all and in only 2 of these was the temporary response satisfactory.

Follow-up studies of cases reported previously. Only six patients out of the eighty-eight previously reported appear to have improved and remained well after a single course of ACTH or cortisone.—R.H.G.


In one hundred seventy-five samples of normal and anemic blood, examination for fetal hemoglobin was carried out by three methods.

The 70 to 80 per cent of fetal hemoglobin present at birth is rapidly replaced by adult hemoglobin in the first four months of life. Not more than a trace of fetal hemoglobin was present in the blood from cases of acute leukemia, erythroleukemia, myelosclerosis, paroxysmal nocturnal hemoglobinuria, aplastic, hypochromic, and megaloblastic anemia, target cell anemia in liver disease, or from a wide variety of cases of familial hemolytic anemia associated with spherocytosis or ovalocytosis or in atypical forms of the condition.

In Mediterranean and sickle cell anemias, fetal hemoglobin was encountered more frequently.—R.H.G.