MEGALOBLASTIC ANEMIAS

DISCUSSION: GASTRIC BIOPSY AND THE INVESTIGATION OF THE MEGALOBLASTIC ANEMIAS.


Dr. Doig has carried out gastric biopsy with a flexible tube on a thousand occasions and bleeding has been the sole complication, having occurred in 8 patients, 3 of whom needed transfusion. Abnormal mucosal appearances are divided into superficial gastritis, atrophic gastritis and gastric atrophy; a single sample is sufficiently representative of the whole body mucosa in the absence of a local ulcerative lesion of the stomach. Gastric atrophy is the characteristic lesion of the mucosa of the body of the stomach in pernicious anemia. In a study of 46 patients with pernicious anemia or subacute combined degeneration of the cord, only 3 were atypical in having atrophic gastritis. In two patients with gastric atrophy there were no anemia and no cord signs. The technic has no place in the direct diagnosis of gastric cancer. Dr. Turnbull has given vitamin B₁₂ labeled with Co⁶ on 23 instances to 10 control subjects and they excreted in the feces 14-47 per cent of the radioactivity in 0.5 μg. of vitamin B₁₂. In 39 observations on 22 pernicious anemia patients the feces contained 74 to 101 per cent of the radioactivity. When it was tried, the coincidental administration of a source of intrinsic factor reduced the fecal output of radioactivity. The findings in total gastrectomy cases were similar to those in pernicious anemia. In a few cases of steatorrhea, the recoveries were usually either normal or increased, but not affected by the giving of intrinsic factor. In one such patient, however, the fecal output of radioactivity was much increased and this was reduced by intrinsic factor. In megaloblastic anemia of pregnancy, recoveries of radioactivity were within normal limits.

Dr. Badenoch has attempted gastric biopsy in over 160 cases and obtained specimens in 130 of these. Bleeding was obvious in one. In four patients thought to have subacute combined degeneration of the cord there was gastric atrophy in two. In a third there was atrophic gastritis and normal absorption of radioactive vitamin B₁₂, and the neurological diagnosis was thought to be incorrect. The fourth boy, a strict vegetarian with neurological findings believed due to vitamin B₁₂ deficiency gave normal findings. In 24 patients with hypochromic anemia and achlorhydria, 6 had gross atrophy of the gastric mucosa, in one instance indistinguishable from that in pernicious anemia. In 3 of the 6 patients there was impaired absorption of radioactive vitamin B₁₂.

Two young women who had been receiving anticonvulsant drugs for epilepsy and whose diet had been unsatisfactory, developed megaloblastic anemia. There was normal gastric mucosa, a normal output of radioactivity from Co⁶ and normal serum B₁₂ levels. Fat absorption and gastro-intestinal X-rays were normal. One case responded to vitamin B₁₂ and the other to folic acid. It was thought that this might be a new form of megaloblastic anemia.
Drs. Molhin and Ross have, with the *Euglena* method, studied the serum vitamin B₁₂ levels of more than 280 patients with megaloblastic anemia. In 126 normal subjects the levels were 100 to 900 μg./ml. (mean 360 μg./ml.). In 190 pernicious anemia patients the levels were less than 100 μg./ml., and this was so even if the blood counts were relatively high. The administration of large oral doses of vitamin B₁₂, or of smaller doses with a source of intrinsic factor, maintained the serum vitamin B₁₂ level in the same way as did injected vitamin B₁₂.

The serum vitamin B₁₂ level may be low after total gastrectomy, partial gastrectomy, intestinal anastomoses, and in idiopathic steatorrhea, nutritional megaloblastic anemia and, (rarely) megaloblastic anemia of pregnancy. The great majority of patients with normal pre-treatment serum B₁₂ concentrations did not respond to vitamin B₁₂ therapy, but responded to folic or folinic acid. A few with severe megaloblastic anemia and low normal B₁₂ levels showed some response to large doses of vitamin B₁₂. Conversely, although most patients with low pre-treatment serum B₁₂ concentrations responded to vitamin B₁₂ therapy, a few responded incompletely unless folic or folinic acid was given.—R.H.G.


Twelve patients with pernicious anemia in relapse and three who had megaloblastic anemia after gastrectomy were treated. A single oral dose of vitamin B₁₂ of 1,000 to 9,000 μg. was given to seven fasting patients. In only two, given respectively 1,000 and 3,000 μg., the red cell gain did not satisfy the formula of Della Vida and Dyke. This was particularly the case with 1,000 μg. (a dosage given only to the one patient).

Daily doses of 50 μg. taken orally during fasting gave good clinical and hematological improvement in five patients and in one patient such maintenance therapy was satisfactory for more than a year. The surface agent “tween 20” did not appear to aid adsorption.—R. H. G.


In five patients with pernicious anemia and five controls, the urine was examined by paper partition chromatography, phenol and collidine being used as solvents. Before treatment of pernicious anemia, there was an increased excretion of taurine, lysine, cystine and leucine, and this was corrected by cyanocobalamin therapy. In a case considered to be one of subacute combined degeneration of the cord without anemia, there was an increased excretion of taurine, and it is suggested that this may be of assistance in diagnosis.—R. H. G.


Chicks fed underheated low quality soy bean oil meal have already been shown to become depleted of vitamin B₁₂, probably because of the antiproteolytic activity or soyin content of the underheated meal. Vitamin B₁₂ in the diet overcomes this effect. The present study concerns the effect of vitamin B₁₂ on the biological value of autoclaved soy bean meal.

To three groups of albino rats there was fed, respectively, basal diet containing autoclaved soy bean meal, the same with vitamin B₁₂ at 50 μg. per kilo of diet, and the same basic diet with 200 mg. aureomycin per kilo of diet. Growth was greater in the second and third groups.

In a second experiment three groups of rats were given 10 per cent casein in the basal diet in place of soy bean meal. Crude, active inhibitor preparations were made from soy bean meal, and the rats were given, respectively, casein plus inhibitor inactivated by heat, casein plus active inhibitor, and casein plus active inhibitor plus vitamin B₁₂. Vitamin B₁₂ just counteracted the effect of the inhibitor and showed no extra benefit.—R. H. G.
ABSTRACTS


The author has made this extensive study in order to elucidate the problem of why pernicious anemia patients have an excess mortality from gastric carcinoma. The occurrence of gastric carcinoma and cancer of other sites among 2,881 relatives of 234 patients with pernicious anemia was compared with the occurrence among 2,956 relatives of 226 controls of the same sex and age distribution. The mortality from gastric carcinoma proved significantly higher for the pernicious anemia relatives than for the controls, whereas the incidence of cancer of other sites did not differ significantly. It is concluded that the higher incidence of gastric cancer in patients with pernicious anemia and among their relatives, may depend on a common inherited predisposition to the two diseases. The same assumption has been made by previous authors on the basis of less extensive and less conclusive studies.—M.S.

MALIGNANT DEGENERATION IN ATROPHY OF THE STOMACH AND PERNICIOUS ANEMIA. D. Niemetz and F. B. Mead. University of Southern California, School of Medicine, Department of Medicine (Gastroenterology) and the Los Angeles County General Hospital, Los Angeles, California. Am. J. Gastroenterol., 22: 217-220, 1954.

In a survey of 110 patients with atrophic gastric mucosa, 85 had well established diagnoses of pernicious anemia. Specific causes for the atrophic gastritis in 25 were not found. Of the 110 patients, 3 were found to have gastrointestinal malignancies. One patient had plasma cell cytoma, one had leiomysosarcoma and although histologic evidence could not be obtained, the third had clinical evidence highly suggestive of gastric carcinoma.—W. N. J.


In the pig, intrinsic factor activity is greatest in mucosa from the antrum, cardia, and duodenum and absent or slight in that from the fundus. In man, activity is mainly found in the fundal mucosa. In the present experiments, juice from pyloric and duodenal fistulae in pigs was examined for intrinsic factor activity in man. The preparation under test was given by mouth to pernicious anemia patients together with vitamin B12 containing radioactive cobalt. It was found that the pyloric and duodenal secretions had intrinsic factor activity whereas the mucoid residue had none. In a single test, the dialysed filtrate from 50 ml. of duodenal juice appeared to possess intrinsic factor activity, although the total weight of solid in the sample was only 35 mg.—R. H. G.


Serial studies of the hepatic uptake following ingestion and parenteral administration of Co45 labeled B12 were made in 20 hematologically normal patients.

Of interest was the finding that a plateau of B12 concentration in the liver occurred 6-10 days after ingestion. The hepatic uptake, via intestinal absorption, decreased rapidly with increased amounts of B12. Whereas 0.45 µg. of an oral dose of 0.5 µg. of B12 was absorbed, a hundredfold increase in the dose administered (50 µg.) resulted in only a three fold increase (1.5 µg.) in absorption. In pernicious anemia, the negligible uptake of radioactive B12 is partially corrected by the simultaneous oral administration of normal human gastric juice, whereas in sprue, the normal gastric juice has no effect.

An interesting theory is proposed wherein the mechanism for absorption of B12 from the intestine is compared with the current concept of iron absorption.—W. N. J.
ABSTRACTS


When cyanocobalamin was added to certain crude liver extracts, assays carried out with Escherichia coli gave much lower results than were expected or than were given with Lactobacillus leichmannii. It seemed likely that such extracts contained a substance that interfered with the utilization of vitamin B₁₂ by E. coli. Other workers have shown that vitamin B₁₂ can be bound by the α-globulin fraction of human serum and by a protein in sow's milk.

Some of the vitamin B₁₂ in crude liver extracts cannot be assayed with the E. coli mutant, and cyanocobalamin can be bound by crude liver extracts. With E. coli the apparent vitamin B₁₂ content of the extract increases on boiling, but heating for 4 hours is optimal; when the crude boiled liver extract is allowed to stand for several days the apparent vitamin B₁₂ content falls to its original value. It is thought therefore that crude liver extracts contain a vitamin B₁₂ binding substance but that the binding is not due to the formation of a complex with a protein. The vitamin B₁₂ content of the extracts is not increased by treatment with cyanide; the bound vitamin B₁₂ can be dialyzed and the activity of the extracts is significantly increased by digestion with pancreatin, but not with papain or trypsin. The vitamin B₁₂ behaves differently in liver extracts than does cyanocobalamin in the presence of an intrinsic factor concentrate made from hog's stomach.

It is thought that in liver concentrates, cyanocobalamin usually accounts for only a small fraction of total cobalamin.—R.H.G.


Because of the long half life of Co⁵⁸ (five years) the total amount that can be given to one person to study vitamin B₁₂ absorption is small. The isotope Co⁵⁸ has a hard gamma radiation with a half life of seventy-two days. The disadvantage is the present difficulty in obtaining supplies. It has been obtained by irradiation of nickel, free from natural cobalt, in the Harwell pile. The cobalt was then added to culture medium and converted to vitamin B₁₂. There was an original specific activity of 0.57 microcuries per microgram of B₁₂, only 10 per cent of the activity being due to Co⁶⁰. The results of using Co⁵⁸ in pernicious anemia were similar to those with Co⁶⁰.—R.H.G.


A total of 86 patients not suffering from anemia was divided into four groups according to their age and type of illness, and the patients in each group were given, on different occasions, one or more injections of cyanocobalamin, of a refined liver extract, and of normal saline. The substances were so colored as to look alike. Standard questions were put to the patients including, "Do you feel better?", "Do you feel the same?", "Do you feel worse?". Following injections of cyanocobalamin and of refined liver extract, 49 per cent and 48 per cent, respectively, claimed improvement, but after a saline injection, 43 per cent had improvement. Improvement in the sense of well being and energy was great. Improvement in sleep and appetite was less. Women were more suggestible than men.—R.H.G.


One of the active principles in aqueous extracts of liver essential for the growth of Ochromonas malhamensis is vitamin B₁₂. Other chrysomonads isolated from brackish supra-littoral pools requiring cobalamin for growth are Monochrysis lutheri Droop (strain 60), Pyrannemium porrectum Carter (strain 65) and Syracosphaera elongata nom. prov. (strain 62).
ABSTRACTS

Maximum growth of all three strains was given by 30-100 mcg/ml. cobalamin/lit., compared with 100-200 mcg/ml. for Euglena. Concentration of essential nutrients in the pools is probably responsible for the blooms in them; cobalamin must attain a concentration of at least 30 mcg/ml. Preliminary assays with Syracosphaera suggest that coastal waters contained 5-10 mcg/ml. in February and March of this year. Cobalamin does not appear to be a limiting factor for growth in the early spring. —R.H.G.


There are given the details of seven patients, five of whom were considered to have subacute combined degeneration of the cord with insignificant peripheral blood changes and normoblastic bone marrows. The other two patients had peripheral neuropathy: with histamine fast achlorhydria and normal peripheral blood counts. In one the marrow had cells of the intermediate erythroblast type. Serum vitamin B₁₂ estimations were not done and investigations for steatorrhoea were not carried out.

It is suggested that the names "pernicious anemia" and "subacute combined degeneration of the cord" should be replaced by "vitamin B₁₂ deficiency" with appropriate qualifying terms.—R.H.G.

HEMOSTASIS


This is a study of coagulation factors in stored blood and desiccated plasma.

Stored blood. The blood was collected in citrate acid solution (100 or 200 cc.) and in ACD and preserved at 4 C. On the 7th and 21st day, in addition to the pH and proteins, the following coagulation factors were studied: prothrombin, proconvertin, proaccelerin, antihemophilic factors A and B, platelet factors, total thromboplastic activity, antithrombin and fibrinogen. Stored blood loses part of its antihemophilic activity and nearly all its accelerin, while the other factors remain but slightly modified. The activity of proconvertin and total thromboplastin may increase during preservation.

Desiccated plasma. The same factors were studied with diverse kinds of plasma: normal plasma from various sources and antihemophilic plasma tested before and after desiccation and at variable time intervals thereafter. Desiccated plasma prepared from fresh plasma loses the greater part of its accelerin and part of the antihemophilic factor B. The other factors remain nearly unchanged. The present work deals with results of these studies and with the inferences to be drawn from the therapeutical standpoint.—J.D.


The one-stage Quick’s test measures deficiency not only of prothrombin but also of factors V and VII. In liver disease a prolonged one-stage prothrombin time is usually due to moderate factor VII deficiency. In coumarin overdosage there is no strict correlation between the results of this test and the tendency to bleed.

In four patients under treatment with coumarol anticoagulants hemorrhage was associated with very low levels of prothrombin as estimated by a two-stage test while the "prothrombin times" by the one-stage tests were 40, 35, 29 and 38 seconds (control 12 seconds). In two cases of chronic liver disease the one-stage tests gave readings of 17 and 20 seconds respectively (controls 12 seconds) while prothrombin estimations by the two stage tests were, respectively, 10 to 20 per cent and 6 per cent.—R.H.G.
ABSTRACTS


A series of normal subjects took regular therapeutic doses of ethyl biscoumacetate or phenylindanedione. At set times a single oral dose of 100 mg. of vitamin K₁ was given in fruit juice. Prothrombin activity was measured by Quick's one-stage method. Vitamin K₁ significantly shortened the time taken for prothrombin activity to return to normal when ethyl biscoumacetate was stopped. If administration of the latter was continued, vitamin K₁ caused prothrombin activity to return to normal in 24 to 30 hours and to remain normal for one to three days. The response was slower when the anticoagulant was phenylindanedione.

In nine instances where ethyl biscoumacetate was stopped and no vitamin K₁ given, the average time taken for the prothrombin activity to return spontaneously to normal was 44.3 hours. In 18 instances, in which repeated testing was possible after individuals had received 100 mg. of vitamin K₁ at the time of taking their last dose of ethyl biscoumacetate, the average time for the prothrombin activity to return to normal was 10.3 hours. With phenylindanedione the figures were, respectively, 61.6 hours (6 subjects) and 12.6 hours (10 subjects). The danger of using 100 mg. of vitamin K₁ is that the patient is temporarily resistant to anticoagulants, and further thrombosis may occur. Doses of 10 to 15 mg. are advised.—R.H.G.


The thromboplastin generation test is invaluable for the investigation of bleeding disorders due to impaired generation of plasma thromboplastin. The most troublesome part of the test concerns the preparation of a suitable platelet suspension. The latter may be replaced by a brain extract. A standard human brain thromboplastin, prepared by acetone drying, is further washed with acetone until free of cholesterol. When 1 Gm. of the dried powder is extracted at room temperature with 50 ml. of chloroform, more than 300 mg. of active material is obtained by evaporation. The gummy residue is finely homogenized in 50 ml. of 0.85 per cent NaCl. An approximately 1:100 saline dilution of this concentrate is substituted for the platelet suspension.—R.H.G.


Hemorrhagic fever has been known for many years to the Russians and Chinese but has only recently become known to the Western physicians. It occurs particularly in the spring, and is rural in incidence. Isolated outbreaks have occurred in United Nations troops in Korea, and cross infection has not been a feature. The etiological agent is uncertain but is thought to be a virus. It is perhaps spread by the mite, and the rodent Apodemus agrarius may be the reservoir of the intermediate host.

The condition may be divided into invasive, toxic, and convalescent phases, and percutaneous and other hemorrhages are common. Shock and oliguria are frequent features. Serological studies have been negative. The mortality rate is about 5 per cent. Antibodies have no influence on the disease. Histologically there is generalized capillary dilatation, with necrosis. The marrow is hyperplastic. Clotting and prothrombin times are normal, but the bleeding time is prolonged. Tubular damage occurs in the kidneys.—R.H.G.