VITAMIN B-12 METABOLISM and PERNICIOUS ANEMIA

OBSERVATIONS ON THE BOUND FORM OF VITAMIN B₁₂ IN HUMAN SERUM. W. R. Pitney, M. F. Beard, and E. J. Van Loon. From the Department of Medicine, University of Louisville School of Medicine and the Medical Research Laboratory, Veterans Administration Hospital, Louisville, Ky. J. Biol. Chem. 207: 143-152, 1954.

The vitamin B₁₂ normally present in human serum is combined with protein primarily. It is split off, to a certain extent at least, when serum proteins are denatured by heat. A microbiological assay technic employing Euglena gracilis distinguishes between "free" and "bound" vitamin B₁₂. As parenteral injection of more than 80 micrograms of B₁₂ has been followed by the rapid urinary excretion of the major part of the dose, it has been suggested that there is a limit to the capacity of serum to bind the vitamin. Proteins of normal sera were fractionated by paper strip electrophoresis and the vitamin B₁₂ concentration measured by microbiological assay. Normally most, if not all of the B₁₂ was bound to the alpha globulins. The addition of crystalline B₁₂ to normal sera revealed a limited capacity to bind the vitamin. Consistently, however, the total amount of vitamin "recovered" by bioassay did not equal the amount added to the sera. This raises the question of whether some technical refinement is needed or whether there is in serum a substance that inhibits the activity of B₁₂ in this particular type of assay. It is possible megaloblastic anemia may result from a deficiency in vitamin B₁₂-binding protein. Further studies on correlation of vitamin B₁₂ and alpha globulin concentration in sera will be of interest.—P.F.W.


The authors demonstrate that H₂O₂ destroys the red acid of the Vitamin B₁₂ molecule by the action of peroxidase enzymes. Inorganic cobalt is liberated by this process from the molecule which is probably a cobalt porphyrin or similar compound. Bacteria producing hydrogen peroxide destroy vitamin B₁₂ in vitro only when their Redox-potential is high and when they produce peroxidase enzymes. In the stomach and the upper parts of the small intestines there are many bacteria producing hydrogen peroxide and also some peroxidases and katalases. Vitamin B₁₂ administered by mouth is largely destroyed in the gastrointestinal canal of patients with pernicious anemia. This process may be enhanced or inhibited by several factors. Bacteria in the alimentary tract are important in the pathogenesis of pernicious anemia.—C.M.

INFLUENCE OF FOLIC ACID AND VITAMIN B₁₂ ON THE IMPAIRMENT OF NUCLEIC ACID SYNTHESIS IN LACTOBACILLUS CASEI BY AUREOMYCIN. D. V. Rege and A. Sreenivasan. From
ABSTRACTS


A reversal of aureomycin action by folic acid and, to a lesser extent, vitamin B12, was observed with several micro-organisms. With *Lactobacillus casei* (A.T.C.C. 7469), cultures were allowed to grow for 48 hours and nucleic acid determinations were carried out, without aureomycin and with a concentration sufficient to produce half maximum growth inhibition in tube assays. Aureomycin is claimed to inhibit synthesis of both pentose nucleic acid and deoxypentose nucleic acid. Folic acid protects against this action, as does vitamin B12 in higher concentration.—R.H.G.


An enzyme preparation from pigeon liver was used together with various supplements, and its effects on the synthesis of (2-14C)-serine from (2-14C) glycine and formaldehyde, and of (3-14C) serine from unlabelled glycine and H14CHO were studied. Since the preparation also catalyses the breakdown of (2-14C) serine to (14C) glycine and formaldehyde, it was able to catalyse the formation of (2-14C) serine from unlabelled L-serine and (2-14C) glycine.

These and other results indicate that tetrahydro PGA, rather than leucovorin or N10 formyl PGA, plays the central part in one-carbon transfers, and suggest that tetrahydro PGA combines reversibly with formaldehyde in the presence of the enzyme, possibly forming the imidazoline derivative in which a methylene group links the N5 and N' positions of tetrahydro PGA. This intermediate might then react reversibly with glycine and water to form serine, and might transfer the methylene group derived from formaldehyde to homocysteine, forming methionine by a process in which vitamin B12 may be involved. In the incorporation of formate into serine, formate presumably reacts with tetrahydro PGA to form leucovorin, which is then reduced by a heat sensitive enzyme to the imidazolizin type of pteridine intermediate.—R.H.G.


From sow’s milk and desiccated pig stomach there have been isolated proteins which combine with cyanocobalamin and with vitamin B12-like substances with different nucleotide groups. Purification of the protein from sow’s milk whey was achieved by continuous paper electrophoresis and this was done more easily by saturating the impure concentrate with cyanocobalamin labeled with cobalt-60 and isolating the cyanocobalamin-protein complex.

The properties of the substance isolated from sow’s milk are given. Cyanocobalamin exists in the milk of the sow, woman, rat, goat and cow in bound forms; by an ultra filtration method it has been shown that all of these milks when raw and untreated can bind some added cyanocobalamin, but that compared with sow’s milk the amount bound by other milks is small. The electrophoretic mobility of the naturally occurring bound cyanocobalamin in all the milks and of the complexes formed by adding cyanocobalamin to these milks or to desiccated pig stomach was $-3.1 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ at pH 8.6, $\mu = 0.05$.

Investigations of the mode of linkage between cyanocobalamin and the protein suggest that the complex contains cyanocobalamin—rather than hydroxocobalamin. Free—SH groups were not necessary for the binding, but an—NH$_2$ or $>\text{NH}$ group may be involved.

For a quantitative microbiological estimation of the cyanocobalamin in the complex, preliminary digestion with papain or trypsin is necessary. It seems possible that the digest contains peptide conjugates of cyanocobalamin.—R.H.G.

Full therapeutic effect can be obtained from the oral administration of vitamin B₁₂ combined with far smaller doses of intrinsic factor than has hitherto been believed. The intrinsic factor was derived from dried pyloric mucosa of the pig’s stomach. A slight haemopoietic response occurred in pernicious anaemia from pyloric mucosa alone; this seemed to be more pronounced if the mucosal preparation was given at meal times. When 100 mg. of dried pyloric mucosa and 5 μg. of vitamin B₁₂ were given once to thrice daily, there was a marked response in eight out of ten instances. Satisfactory results were obtained with a commercial preparation containing 50 mg. of dried pyloric mucosa and 5 μg. of vitamin B₁₂, given thrice daily. For routine treatment, it is suggested that 300 mg. of dried pyloric mucosa and 30 μg. of vitamin B₁₂ should be given daily initially.—R.H.G.

BLOOD PRESERVATION


Red cells of group O type M or of Group A type N were stored at −20°C. in one of four mixtures. Solution I was trisodium citrate glycerol, II was tripotassium citrate glycerol, III was buffered tripotassium citrate glycerol, and IV was plasma citrate glycerol. Cells stored in solutions III and IV not only underwent very little haemolysis during three months, but retained their ability to survive after transfusion. With longer periods of storage, post-transfusion survival diminished progressively. In solutions I and II, red cells stored at −20°C. showed a progressive diminution in post-transfusion survival from the very beginning of the storage period. The damage observed in red cells stored in solutions I and II is probably due to alkalinity of these mixtures. The fact that chemical change occurs in solutions at −20°C. as shown by consumption of dextrose, suggests that preservation for an indefinite period cannot be expected at this temperature.

Red cells stored for a year at −20°C. in unbuffered citrate-glycerol mixtures react specifically with blood grouping sera, although their reactions may be weaker than those of fresh cells.—R.H.G.


These studies were undertaken to answer the following three questions: (1) What conditions of storage of artificially contaminated whole blood permit the abundant growth of gram-negative bacteria? (2) What therapeutic measures can be employed to reduce the mortality rate or extend the survival time of experimental animals receiving lethal amounts of contaminated blood? (3) Is the sudden death of experimental animals following injection of blood containing large numbers of ordinarily nonpathogenic gram-negative bacteria due primarily to an overwhelming invasive infection or to intoxication?

The two strains of gram-negative bacteria used were identified as E. intermedium and Paracolobacterium intermedium. White swiss mice were infected intraperitoneally with artificially contaminated blood and crude endotoxin.

A separate study was undertaken which showed that removal of the blood from 4°C. to room temperature for four hours did not greatly increase the rate of bacterial multiplication except with a very small inoculum. There was always an initial drop in the number of viable cells after inoculating whole blood, but regardless of the number of bacteria inoculated or destroyed within the first few hours, a few cells regularly persisted and eventually multiplied.
The injection of contaminated blood produced no deaths of mice until the viable bacterial population reached a certain critical concentration of $10^8$ to $10^9$ organisms per milliliter. Attempts to separate a soluble toxin-like material from the blood by Seitz filtration were unsuccessful. "With the exception of oxytetracycline against the coliform organisms, the antibiotics were capable of reducing the mortality rate significantly when given up to one hour after the injection of the contaminated blood. Combinations of streptomycin with chlortetracycline, oxytetracycline, or chloramphenicol, in dosages which singly were ineffective, protected all the animals. When the drugs were administered subcutaneously, however, much of the protective effects were lost, although the survival time was still prolonged." Cortisone produced no protective effects. Anti-serum against the coliform bacteria gave protection against the toxicity of the living cells but not against the crude endotoxins. Ten to 100 times as many killed as living cells produced the same effects. The authors conclude that this, coupled with the fact that anti-serum gave protection, indicates that endotoxins are primarily responsible for the acute reaction and death of the animals. -- T.R.T.

MISSED CONTAMINATIONS IN BIOLOGIC PRODUCTS: THE ROLE OF PSYCHROPHILIC BACTERIA.

The term psychrophilic is used to designate organisms which grow only at temperatures below the usual incubator temperature of 35 to 37 C.

"Performance of sterility tests on biologic products, under the standard conditions of incubation at 35 -37 C., has repeatedly failed to detect certain contaminants occurring in products prepared at the Institute of Laboratories and elsewhere."

"The routine performance of duplicate sterility tests at 20 -25 C. (room temperature) has repeatedly demonstrated the presence of contamination not detected at the higher temperature. Sixty-six, or 25 per cent of the contaminants detected in the years 1941 through 1951 at the Laboratories were found only by cultivation at 20-25 C. In some years the percentage reached above 50 per cent.

"Performance of sterility tests at 31-33 C. will detect most of the organisms preferring or requiring lower temperatures for growth. Not all such organisms, however, will be detected at this temperature.

"Psychrophilic contamination, in the study here reported, appears to have been largely associated with (a) processing of animal serums, (b) chemical fractionation or similar manipulation of biologic products, (c) processing and packaging without a preservative, or (d) a combination of any or all of these conditions. In general, the hazard has been most apparent clinically in the administration of human whole blood, probably because of the large volume injected, and the difficulty in setting up adequate sterility control procedures for whole blood.

"Psychrophilic contaminants represent not only a probable hazard of infection, but a well-established hazard from pyrogenic derivatives commonly liberated by such organisms. The detection of such contaminants is therefore an important component in the safety control of biologic products.

"In order to insure the maximum possible opportunity for detection of such psychrophilic organisms, it is recommended that duplicate sterility tests be carried out at both incubator and room temperatures, in the care of those biologic products in which the hazard of such contamination cannot be excluded. -- T.R.T.

ERYTHROCYTE PHYSIOLOGY

THE BLOOD FILAMENTS. A. Sole. From the Karolinen Pediatric Hospital, Vienna, Austria. Acta haemat. 11: 168-188. 1954.

Experimental investigations prove that red cells are the only source of the mobile structures in human blood, called filaments, which have often been described before in dark
field preparations. They can be obtained quickly and in large numbers. The exact fate of the filaments, their origin from the red cells, and the final filamentous destruction of the red cells are described. The filaments as products of the destruction of red cells seem to maintain certain properties of living matter, but they cannot be regarded as independent microscopic structures. These investigations show that the apparent finding of bacteria must be interpreted very cautiously when red cells are also present. The relationship between the filaments and agglutination and also the structure of red cells is discussed.—C.M.


The study was undertaken to evaluate the absorption, the distribution in the blood, and the excretion of diisopropylfluorophosphate (DFP) after the intramuscular injection of radioactive labelled substance (DFP²⁹) into two patients suffering from myasthenia gravis, one patient with cerebral arteriosclerosis and two normal persons. It was found that DFP could be demonstrated in the peripheral blood within a few minutes, and maximum radioactivity was reached in 90 minutes. In the course of these studies it was found that a portion of the injected PH was irreversibly bound to red cells and to plasma proteins. This portion disappeared from the blood at a rate corresponding to the normal replacement of these elements. Using this relationship, it is possible to calculate the rate of replacement of a plasma protein and the mean life span of red cells. In these studies, the half life of the plasma component labelled with PH was 12 to 14 days, while the mean life span of the red cells was reported as 116 and 129 days respectively. These figures compare closely with those obtained by other technics. The advantage of this radioisotope method is that there is no active metabolic pool that can be polluted with PH, and hence resynthesis of red cells and plasma protein with radioactive materials is not likely to interfere with turnover estimations. By extending this DFP²⁹ technic to in vitro labelling, it is also suggested by the authors that this may provide a good method for the determination of red cell and plasma volume, particularly in view of the irreversibility of the PH label.—R.B.C.


The results of an investigation on the hemolytic effect of organic mercury compounds are reported. Evidence is presented for the interpretation that this hemolytic effect is caused by the interaction of the organic mercurials with cellular—SH groups. It is concluded that—SH groups are essential for the maintenance of the intact erythrocyte structure, and observations from the literature which support this contention are cited and discussed.—C.E.R.


Erythrocytes from rabbits, pigs, horse, man and duck were extracted with methanol without addition of acids, and by continuous extraction in a Soxhlet apparatus, a dark red solution of haem was obtained. It was found best to use citrated cells washed twice with slightly hypertonic sodium chloride, to precipitate the protein with 10 vol. of acetone, to wash once with 5 vol. of methanol, to bring into a funnel and then to extract with the Soxhlet apparatus. It is suggested that methanol denatures the globin part of oxyhaemoglobin in such a way that the covalent linkage between the haem iron and the imidazole groups of globin is split, with release of oxygen and formation of denatured haemoglobin, which is then split into haem and denatured globin.—R.H.G.
ABSTRACTS


The laking of blood cells suspended in isotonic saline, by sonic oscillations, is explained as the result of rapid alterations of tension and compression produced in the surrounding medium by the vibrations. Washing and defibrination did not appear to injure the cells. However, hypertonic saline solution, saponin, preheating, alcohol, and ether decreased the fragility. Hypotonic saline, preheating to 50 C. for 3 minutes and hemolytic concentration of ether increased the fragility of red cells to 9000 cycle vibrations.—O.P.J.


The administration of ACTH and adrenal steroids induces a negative K balance both in man and in experimental animals. This loss of K involves skeletal muscle, but little information is available regarding the effects of these hormones on erythrocytes. In order to test this normal human beings were given intravenous infusions of ACTH or cortisone in 500 ml. 5 per cent dextrose solution over 8 hours. Heparinized blood samples were analyzed for absolute eosinophile count, mean corpuscular K concentration, and mean corpuscular hemoglobin concentration. The present results indicate that K is not lost by the erythrocytes and may well be gained following steroid administration or adrenocortical stimulation by ACTH. There is no data to explain this apparent difference between erythrocytes and the other body cells.—O.P.J.


The erythrocytes of various animal species, including the dog, differ from those of human beings by maintaining a high intracellular sodium and low potassium. The problem is to determine whether this difference in cellular ionic structure is associated with differences in the way these cells exchange potassium, but the present results do not make this possible.—O.P.J.


The radioactivity of normal human erythrocytes tagged in vitro with radioactive chromium describes a curve of recession within the circulation of normal individuals, which is comparable to the disappearance curve of nonagglutinable erythrocytes utilized in the Ashby differential agglutination method for the estimation of the survival of erythrocytes when both methods are applied at the same time. In order for the disappearance curve of Cr tagged erythrocytes to resemble the Ashby curve, the data derived from the former must be treated in a manner comparable to the treatment of the Ashby data.

Similar results have been obtained when the radioactive chromium tagging has been used with compatible heterotype erythrocytes used in the Ashby method (as type O given to a type A individual) and autogenous erythrocytes. The latter approach allows the estimation of the survival characteristics of erythrocytes within their own hemopoietic and circulatory systems.

The decay curve of radioactivity using heterotype or autogenous blood is slightly curvilinear.

Preliminary observations suggest the feasibility of the use of the radio-chromium technique herein described in the appraisal of hemolytic states.
The authors point out that unless data from different laboratories are similarly expressed, confusion of details may result. However, they seem not to have conformed to this rule entirely. The Cr⁶¹ labeled red cells in heterotransfusions can be shown from their own charts to have a half-survival time of about 35 to 38 days. The red cells in homotransfusions had an apparent half-survival time of about 27 to 32 days. Necheles et al. reported an apparent half-survival time of from 25 to 40 days. In spite of this, the authors state that it is only by using the same method of plotting data as that employed by Necheles et al. that an apparently “accelerated” destruction is obtained. They also neglected to point out that their Ashby data showed an apparent half-survival time of from 24 to 58 days. Finally they discuss the “extinction” of either Ashby or Cr⁶¹ labeled transfused cells, but they had not continued their observations to the time that this occurred. They also calculated percentage of cells destroyed per day by taking an arithmetic ratio of per cent cells destroyed in ninety days to that number of days, thus neglecting the fact that the Cr⁶¹ labeled cells are destroyed in a curvilinear fashion. Finally, they state that when plotted on semi-log paper their data do not form a straight line, whereas the one chart which they show seems highly suggestive of a straight line.

In general, the data presented in chart form appear to agree with those reported by other workers.—T.R.T.


Blood volume studies employing a new technic with radioactive chromium are reported. Although these observations indicate that the disappearance of radioactive chromium from the peripheral blood is related to the life span of the red cell, there is a discrepancy between these results and those obtained in studies by the Ashby isoagglutination method. An adequate explanation of this discrepancy will determine the actual value of this isotope in studies of anemia.—P.F.H.

LYMPHOMA and LEUKEMIA


Ten cases of primary leukemia in guinea pigs occurred in a series of 303 over a 10 year period. No significant difference was found in the incidence between irradiated animals and the control group, but the leukemia seemed to develop at an earlier age in the irradiated animals. The onset of leukemia was indicated by a sudden increase in leukocytes without any preleukemic alteration. The number of Kurloff cells was comparable in both groups. The chromatin was loose, lace-like and less dense than that of the normal small lymphocyte, but the leukemia cell closely resembled the normal lymphoblast. The latter may be a reticulo-lymphocyte according to Sundbery and Downey. Leukemia cells seemed to localize in the inflammatory areas of pulmonary tissue. Leukemic tissue of 5 of the guinea pigs with primary leukemia was transplanted to young inbred guinea pigs of the same strain with 100 per cent taken irrespective of the injection route. X-irradiation and gamma irradiation have not been established as leukogenic agents for the guinea pig.—O.P.J.


During the years 1945-1950, among 10,825 postmortems, 14 cases of lymphosarcoma of the digestive tract were seen; this number contrasts with 368 cases of carcinoma of the same organ. In 12 cases, the tumor was situated in the stomach, and in 2, in the intestine. The diagnosis with the naked eye is difficult. Actinotherapy, which is relatively effective with this category of tumors, is dependent upon correct biopsy diagnosis.—M.N.
AN OBSERVATION OF "DWARF CELL" LEUKAEMIA. J. Procházk. From the Medical Clinic, Military Medical Academy in Hradec Králové, Czechoslovakia. Cas. lék. čes. 92: 1289, 1953.

A case of atypical "dwarf cell" leukemia is reported. The clinical and hematologic picture is the same as in other forms of chronic aleukemic myelosis. The most characteristic feature is the discrepancy between the maturity of cytoplasm and the "immature" shape of the nucleus. There is predominance of round "dwarf" myelocytes. Polynuclears with more than two segments occur rarely. The nuclear arrangement is strikingly coarse. The resemblance and possible relation to Pelger Huet anomaly is discussed. This case is the fourth described in available literature.--M.N.


In three patients studied and in two of 29 suitably recorded cases in the same hospital, spontaneous regression of glands occurred; a histologic diagnosis of Hodgkin's disease was made in all. The duration of this regression was as long as a year in one instance. This did not appear to be due to elimination of any secondary infection.--R.H.G.


1. Five cases of Hodgkin's disease, 7 chronic myelosis, 7 chronic lymphadenosis, 1 of acute myeloid leukemia, 1 of lymphosarcoma and 2 of erythremia were treated with beta-naphthyl-di-2-chlorethylamine of Czechoslovak manufacture (TS 229).

2. The results are like those with other forms of nitrogen mustard, but the effect is weaker and the failure of treatment occurs more often.

3. The drug given by mouth 300-400 mg. per day and in usual dosage is not followed by nausea or vomiting.

4. Depression of bone marrow is rather rare, and of short duration.

5. The best results were noticed in Hodgkin's disease and in chronic myelosis. The failure in erythremia is explained by too small doses used.

6. The effect on the whole is rather inferior to other forms of treatment especially to x-ray therapy and to HN2, HN3. It does not seem to be a particular advantage in the chemotherapy of leukemia and malignant lymphomata, except in rare cases of generalized Hodgkin's disease, where intravenous route could not be utilized.—M.N.

COMBINED TREATMENT OF HEMOBLASTOSES WITH MICRODOSES OF NITROGEN MUSTARD AND X-RAY. S. Resín. From the Central Rtg. Department, State Hospital, Praha XVII and from the 3rd Medical Clinic, Charles University, Praha. Cas. lék. čes. 91: 1384, 1952.

The authors tried combined therapy of Hodgkin's disease, leukemic myelosis and lymphadenosis, using simultaneously x-rays and very small doses of nitrogen mustard (0.0075-0.03 mg/kg). No damage of hematopoiesis occurred.

Combined treatment seems to be very useful in advanced cases because small doses of nitrogen mustard apparently make the hematopoietic apparatus sensitive to x-rays. Thus the author is able to use much smaller doses of x-rays than usually and he no longer makes use of large doses of nitrogen mustard alone.—M.N.

ACTINOMYCIN in MALIGNANT HEMOPATHIES


Fifteen cases of Hodgkin's disease are treated with 200 to 400 gammas a day of actinomycin, with the following results: improvement of lymph nodes in only 2 cases, of fever
in only 2 cases, no improvement of splenomegaly and sedimentation rate. Only one case had a general improvement; 5 cases died during the treatment which was badly tolerated in most of the cases (anorexia, stomatitis in 5 cases, alopecia in 2 cases).—J.P.S.


Twenty-eight patients were treated with actinomycin C 300 to 400 gammas a day (16 Hodgkins, 6 lymphosarcomas, 4 reticulosarcomas, 1 Ewing sarcoma, 1 epithelioma). Only 8 cases of 22 had any improvement of the lymphnode enlargement. Eight instances of stomatitis were noted in 22 cases, diarrhea in 4 cases, alopecia in 3 cases.—J.P.S.


Six cases were treated with actinogen (3 Hodgkins, 1 carcinoma of the lung, 2 acute leukemias) without real improvement.—J.P.S.


Thirty patients were treated with 300 to 500 gammas a day of Actinomycine C. Of 21 cases of Hodgkin’s disease only 4 cases showed improvement.

Four cases of lymphosarcoma were not improved; 1 lymphoid leukemia and 4 carcinomas were modified.—J.P.S.


Fourteen patients were treated with an average daily dose of 200 gammas. Five cases of Hodgkin’s disease were treated without real advantage, 5 acute leukemias showed no improvement, and only one chronic lymphoid leukemia improved during the treatment. It was tolerated well.—J.P.S.


Twelve cases were treated, with only general improvement. Two patients died during the treatment.—J.P.S.


Eight cases of Hodgkin’s disease were treated with 200 gammas of Actinomycine C with no benefit, or very slight and very transient. Three cases of malignant reticulosis were not modified. One case of Brill-Symmers received 6000 gammas without untoward reactions.—J.P.S.


Eleven cases of carcinoma or Hodgkin’s disease were treated without striking results. The authors use the intravenous site of injection; some results were obtained with malignant disease of the reticulum but there were no beneficial effects in carcinomas.—J.P.S.
ABSTRACTS

Reticuloendothelial System Diseases


In 24 of the 29 patients, anemia, leukopenia, or thrombocytopenia was present. Anemia was most common. In 13 cases the leukocytes numbered less than 5,000 per cubic millimeter of blood. The lowest platelet count was 16,000 per cubic millimeter. In some instances the amount of indirect reacting serum bilirubin was elevated slightly.

Fifteen patients underwent splenectomy. Six of those patients were alive 11 to 20 years postoperatively. Of the 14 patients who did not undergo splenectomy, one was alive 12 years later and two were alive 14 years after their medical study.—P. F. W.


This describes Gaucher's disease in two sisters of a Greek family. The microscopic examination demonstrated in the Gaucher cells phagocytosis of blood platelets. Splenectomy had a very good clinical result. The surface of the spleen was completely irregular because of the proliferation of the reticulum cells. In two other members of the same family the authors found a bleeding tendency which could be identified as a disturbance of the capillary walls. Bone marrow puncture did not reveal any Gaucher cells in these cases.—C. M.


"This report presents a detailed clinical, anatomical, and chemical study of an unusual case of lipidosis. The patient was a 52 year old male who died 13 months after the onset of an illness, which was characterized by particularly severe involvement of the lungs. Histologic study before and after death suggested that this might be Niemann-Pick's disease in an adult. Chemical analysis of the lipid content of the lung, liver, and spleen indicated that the predominant lipid was sphingomyelin but that it was present in somewhat smaller amounts than previously reported in this condition. The tissue concentrations of certain other lipids were not only significantly higher than normal, but also higher than those previously reported in Niemann-Pick's disease in infants and adults. Accordingly, this case does not appear to fit precisely into any of the usual categories of lipidosis".—O. P. J.

Immunohematology

Genetic Linkage Between Ovalocytosis and the RH Blood Type. R. A. Marshall, R. M. Bird, H. K. Bailey, and E. Beckner. From the Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Okla. J. Clin. Investigation 33: 790-793, 1954.

This report summarizes genetic observations made of the family of a patient found to have ovalocytosis. Previous studies have established that this trait is inherited as a simple autosomal mendelian dominant. The present study was undertaken to determine if there is any linkage between the genes for ovalocytosis and those which determine the antigens in various blood groups. No close linkage was found between ovalocytosis and the ABO, MNS, Lewis, Kell, and Duffy systems. On the other hand it was found that the chromosomal distribution for the oval trait and the Rh pattern are identical. The authors postulate that the gene for ovalocytosis is on the same chromosome as the genes for the Rh blood groups. They raise the possibility that this associated linkage in a hereditary, human trait may provide a tool for the clinical investigation of the genetic predisposition to such familial diseases as idiopathic epilepsy and diabetes mellitus. It should also be pointed out that careful efforts were made to determine whether a hemolytic anemia may develop with ovalocytosis. Results established that this was not the situation.—R. B. C.
ABSTRACTS

A POTENT GROUP O CELL AGGLUTININ OF HUMAN ORIGIN WITH H-SPECIFIC CHARACTER.

Sera which agglutinate group O and group A cells preferentially can be divided into two groups on the results of inhibition tests with saliva; those whose power to agglutinate such cells is inhibited by saliva from secretors belonging to any of the groups within the ABO system are designated anti-H, and those whose power is not inhibited are provisionally grouped together and called anti-O. Not all anti-O sera are, however, of the same specificity. The differentiation into anti-H and anti-O antibodies cannot be distinguished merely by their reactivity with red cells.

A woman with carcinoma was found to have a powerful group O cell agglutinin in her serum, and this was found to be anti-H. The antibody reacted with authentic group A,A, cells more strongly than with a sample of known group A,O cells.

The results confirm that anti-H agglutinin does not detect a product of Bernstein's O gene, and that within the ABO system the homozygous (AA, BB) or heterozygous (AO, BO) nature of the cells cannot be determined by anti-H reagents.—R.H.G.

INACTIVATION OF THE D (Rh) FACTOR IN VITRO. B. Friedmann and J. Hoenigrohr. From the 1st Medical Clinic, Charles University, Praha. Čas. lék. čes. 83: 212, 1954.

The inactivation of the D (Rho) factor after incubation of red blood cells for one hour with nitrogen mustard at 100 mg. per cent and 50 mg. per cent concentration is described. Agglutinability was demonstrated with the agglutination method, the absorption method, and the indirect Coombs test. The agglutinability of the A, B, C (Rh'), M and N blood groups remained unchanged. The other blood groups were not examined.

The authors feel that the inactivation in vitro could be of great importance for the transfusion blood service. Diverse conditions which should be fulfilled during the process of inactivation are discussed.—M.N.


When group O blood is transfused into patients of group A, B or AB, anti-A or anti-B antibodies from the group O plasma may react with the recipients' A or B red cell antigens and give a hemolytic transfusion reaction. Samples of serum from 1960 group O donors taken at random were examined by a simple screening test within 24 hours of collecting the blood and 185 (9 per cent) were found to have strong anti-A or anti-B hemolysins. Whereas 10 per cent of group O sera containing strong anti-A hemolysins contained also immune anti-A agglutinins in a titer greater than 1 in 16, no immune agglutinins in this concentration were found in 772 group O sera lacking strong hemolysins. The simple screening test, which was compared with more complex technics, consisted of taking equal volumes of the serum under investigation and of 5 per cent suspensions in saline of red cells of groups A, B and O in separate tubes; these were allowed to stand at room temperature for an hour, then at 37 C. for an hour. The supernatant was examined by the naked eye for hemolysis. It was found that when the screening test for hemolysins could not be carried out within twenty-four hours of collecting the blood, fresh group O serum containing weak agglutinins but no hemolysins had to be added to the test serum to provide complement. It is recommended by the authors that all blood banks should adopt the following routine. After the examination for saline anti-A and anti-B agglutinins, the tubes containing the cell-serum mixture should be incubated for a further hour at 37 C. and the supernatant fluid examined for visible hemolysis. The bottle corresponding to any tube showing strong hemolysis should then be labeled to indicate that it should be transfused only to a group O recipient.—R.H.G.

Hemolytic disease of the newborn commonly occurs in Rh-negative women delivered of Rh-positive children, but may be seen in Rh-positive mothers; in the majority of such cases the maternal serum contains Rh antibodies, anti-E or anti-C. Antibodies to other blood group antigens, such as anti-Kell, may also be present in the maternal serum and give rise to the condition, and even rarer blood group antigens such as C* may be the cause. Family antigens may also be responsible; they appear to be of two kinds—those observed because they have given rise to hemolytic disease of the newborn and those found within a family but not associated with the occurrence of the disease.

In a third pregnancy, a patient gave birth to a child with hemolytic disease. The direct Coombs test omi the child’s cells was positive. The mother was A,R,R2(CDe/cDE). Detailed serological studies indicated that a rare Rh agglutinogen at the C-c locus, C*, was responsible. The maternal serum was tested with a total of 3,931 blood samples from healthy donors, Group O and A samples being tested with unabsorbed serum and Group B and AB with serum from which the anti-B agglutinins had been absorbed. With the cells of the father and the affected child and in only four other instances did the cells give positive results with the maternal serum; the albumin and Coombs technics and papainized-cell method were used. Tests with various anti-C sera indicated that these cells appeared to have a modified C antigen which gave characteristic reactions. Cells which gave positive results with the maternal serum all gave this characteristic pattern of reaction except where this was masked by the presence of a normal C antigen. Family studies suggested that the C* antigen was inherited as a mendelian dominant character.—R.H.G.


Premature delivery of babies with hemolytic disease of the newborn has been widely condemned but in individual cases it may be indicated. A 33 year old woman with a history of five pregnancies ending in still births became pregnant. Her blood group was A Rh-negative (cde/cde) and blocking antirhesus antibodies were present in the serum. The husband was O Rh-positive, the most likely genotype being CDe/cDE.

At the 33rd week the frequency and strength of foetal movements became less, and this had previously preceded intrauterine death. Caesarian section was carried out and a living male child obtained. It was group O Rh-positive, cDE/cde with a positive direct Coombs’ test and a hemoglobin level of 4.8 Gm. per 100 ml. Replacement transfusion was given. A further pregnancy occurred and again, when foetal movements diminished, a Caesarian section resulted in delivery of a living, anemic child.—R.H.G.

COAGULATION and PLATELETS


Doses of vitamin K1 varying from 5 to 50 mg. were given orally on approximately 96 different occasions to 70 patients receiving anticoagulant therapy, and in all but two instances it was effective. These two patients were desperately ill with myocardial infarction.

Where there is frank hemorrhage or a severe lowering of the blood prothrombin to a dangerous level, and anticoagulant therapy is stopped, 20-25 mg. of vitamin K1 will suffice with short-acting anticoagulants such as phenylindanedione and ethyl biscoumacetate but 20-50 mg. should be given if longer acting anticoagulants such as dicoumarol, phenylpropyldihydroxycoumarin or cyclocoumarol have been used.

If there is frank hemorrhage and it is desired to continue anticoagulants, 15 mg. may be required for the short acting anticoagulants and 15-25 mg. for the longer acting ones.
If there is severe reduction in the blood prothrombin without hemorrhagic manifestations and anticoagulant therapy is to be continued, 5-10 mg. of vitamin K, will suffice for those receiving short acting anticoagulants and 15 mg. for those receiving long acting ones. Response should be assessed by prothrombin estimations about eight hours after the K, is given.—R. H. G.

OUR EXPERIENCES WITH THE PROTHROMBIN CONSUMPTION TEST IN HEMORRHAGIC DISORDERS. F. Hérmanský, C. Šehla, and Z. Dienstbier. From the 1st Medical Clinic, Charles University, Praha. Čas. lék. čes. 92: 890, 1953.

1. The prothrombin consumption test was performed by the Soulier seconds method in 123 subjects, 144 times altogether. The values over 40" for the venous blood and over 3" for the capillary blood were considered as normal, when the activity of the thromboplastin used was 15".

2. This test was carried out 34 times in 27 hemophiliacs; normal prothrombin consumption was found only twice in the venous blood and on 6 occasions in the capillary blood. The remaining results detected, in most instances, a very marked decrease in the prothrombin consumption. A lower prothrombin consumption was observed also in 5 of the 6 examined mothers of hemophiliacs, although in lesser degree than in their sons.

3. In primary non-splenectomized thrombocytopenia, a faulty prothrombin consumption was found on 8 occasions in venous blood, while the examination of capillary blood gave, in the most cases, normal results. In one symptom-free patient with normal bleeding time, the consumption was normal in spite of a very low platelet count. Splenectomized subjects gave normal results, even when the platelet count remained low. In one case where splenectomy was without clinical benefit, faulty prothrombin consumption improved after ACTH administration, as the platelet count increased. In ten secondary thrombocytopenias, a distinct decrease in the prothrombin consumption was seen; however, in four myeloid leukemias with thrombocytopenia the findings were normal.

4. Three affected members of a family with Willebrand-Jürgens thrombopathy showed more or less defective prothrombin consumption. In two healthy members, the consumption was normal. In 6 cases of suspected thromboctoasthenias with normal platelet count (3 of them accompanying chronic myelosis), the prothrombin consumption was definitely decreased.

5. In anaphylactoid purpura, various liver diseases, and unclassifiable moderate hemorrhagic diatheses, the prothrombin consumption was in most instances normal.

The Soulier method is discussed in comparison with other technics, and their usefulness for detecting the disturbances in thromboplastin formation is stressed.—M. N.

INFLUENCE OF PENCILLIN ON BLOOD COAGULATION WITH REFERENCE TO THE PRINCIPLE OF "PROTECTED CLOT". Z. Dienstbier, F. Hérmanský, P. Málék and J. Koc. From the 1st Medical Clinic, Charles University, Praha, from the Institute for Experimental Surgery, Praha, and from the 2nd Surgical Clinic, Charles University, Praha. Čas. lék. čes. 92: 829, 1953.

1. The influence of penicillin on blood coagulation, when given by intravenous infusion during operations according to the principle of the "protected clot," was investigated. The following tests were used in order to detect the changes in the clotting process: the determination of the prothrombin time (one stage method), the recalcification time, and the heparin tolerance test in vitro.

2. The same tests were used when the changes in the clotting of oxalated plasma were investigated in vitro after the addition of various amounts of penicillin to this plasma.

3. In vivo no substantial changes in the clotting mechanism were detectable which could be attributed to the effect of penicillin.

4. In vitro a distinct lengthening of the prothrombin time occurred at a concentration of 10,000 U. per ml. of Czech crystalline penicillin as well as marked increase of the clotting times, determined by all three methods at the concentration of 50,000 U. per ml.

5. It can be concluded that penicillin applied according to the principles of the "pro-
tected blood clot” has no significant influence on blood coagulation. The results of the experiments in vitro require careful application of high concentrations of penicillin locally into the wound.—M.X.


In order to estimate the force of clot retraction, the author describes a new method. He measures the weight lifted during retraction. Normally this is 224-588 mg. In thrombocytopenia the weight is reduced to 28-56 mg. depending on the numbers of platelets. The importance of the force of clot retraction in the process of coagulation is discussed. When the readings obtained are normal, “physiological ligature” by occlusion of the vessel proceeds without disturbance. When readings are low, there is a tendency to bleeding as shown by prolonged bleeding time in thrombocytopenia. The phenomenon of the production of filaments of fibrin leading to retraction is described.—C.M.


Suspensions in normal saline of blood platelets from forty different bloods were found to be agglutinated by A, B or O-sera. Disodium sequestrene was used as an anticoagulant. Details of the method are given.—R.H.G.


A man, aged 49, was treated with gold for rheumatoid arthritis. Three days after the fifteenth injection (total 0.22 Gm.) purpura developed. The platelet count was 163,000 per cu. mm., but fell to 20,000 per cu. mm. despite blood transfusion. Epistaxis, hematuria, leg weakness, and third nerve paralysis occurred. Dimercaprol was given to a dosage of 1.3 Gm. over six days. Bleeding thereafter ceased but the platelet count remained low.

A 44 year old woman developed purpura and vaginal bleeding after 1 Gm. of gold. The platelet count was 20,100 per cu. mm. There was no response to ACTH and cortisone, (totals of 1.41 Gm. and 1.75 Gm. respectively). Dimercaprol was given in a dosage of 150 mg. every eight hours for 10 days, and there was an immediate and dramatic clinical and hematological response. Deterioration occurred when this treatment was withdrawn, and it had to be given again for 23 days, but was then withdrawn without relapse.—R.H.G.

THE MULTIPLEMENT OF PLASMIN INHIBITORS IN HUMAN SERUM, DEMONSTRATED BY THE EFFECT OF PRIMARY AMINO COMPOUNDS. O. Ratnoff, I. Lepow, and L. Pilleiner. From the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio. Bull. Johns Hopkins Hospital. 94: 169-179, 1954.

Human serum appears to contain at least three inhibitors of plasmin. One is destroyed by heating serum at 56 C. for 30 minutes. One of the heat-stable plasmin inhibitors can be inactivated by ammonia, hydrazine and certain primary amines, but secondary amines and polar-substituted primary amines are ineffective. The presence of a third component is indicated by the presence of plasmin-inhibitor activity in serum which has been successively treated by heat and hydrazine.—C.E.R.


For the past four years there has been much discussion as to whether or not corticotropin and cortisone therapy increased the incidence of thromboembolic complications. Cosgriff and others have presented reports indicating there was an increase. The present report is of observations on eighty-six patients with advanced cardiovascular and/or cerebrovascular
disease who received large dosages of cortisone in courses of therapy generally extending over a period of three weeks. No thromboembolic episodes were noted. Certainly there is no well-documented reason to use Dicumarol prophylactically in such cases. Those clinicians who do, should keep in mind that Dicumarol has been identified as one of the leading causes of death due to drugs.—P.F. W.

A NEW FORM OF HEREDITARY THROMBOPATHIA. W. Gerhard. From the 1st Medical Clinic Halle, Saale, Germany. Z. inn. Med. 8: 221–227. 1953.

This is a description of a new kind of hereditary thrombopathic bleeding disease. The bleeding time, the prothrombin consumption test, and the vascular fragility gave pathologic results. The formation of active thromboplastin was markedly delayed. Clot formation and platelet agglutination were normal. The author does not attribute the disease to the genuine thrombopenias, because the spleen was not enlarged. The heredity is of the irregular dominant type. Patients with the hematological defect only partly had other clinical manifestations.—C.M.


“...The correlation between capillary resistance and circulating eosinophils was studied in seven experimental conditions: following adrenalectomy in the normal rat and dog, during administration and withdrawal of cortisone in the adrenalectomized rat, during prolonged administration of cortisone in the adrenalectomized rat and dog, during prolonged administration of cortisone in the normal dog, after cold stress and forced muscular exercise in the normal rat, and during prolonged fasting in the normal dog.

A good inverse correlation was found in any situation presumably connected with a change in cortisone concentration of the body. (a) If cortisone concentration increases (cortisone administration to adrenalectomized or normal animals, first phase of stress response presumably associated with ACTH discharge), the capillary resistance rises and the eosinophils drop. (b) If cortisone level decreases (cortisone withdrawal in adrenalectomized or normal animals, adrenalectomy in normal rat, second phase of stress response presumably connected with cortical hypofunction), the capillary resistance precipitously drops and eosinophilia occurs.

A good inverse correlation (persistent high capillary resistance and persistent low eosinophil level) was found in any situation in which a high cortisone level was maintained during a long period of time by either exogenous (cortisone administration) or endogenous (protracted stress) method.

No persistent correlation was found in any situation connected with a permanently low cortisone level in the animals. After adrenalectomy or cortisone withdrawal there is at first a seesaw movement; the capillary resistance drops and eosinophilia sets in. This good correlation fades, however, when the cortisone level remains low, in that the high eosinophil count returns to normal average levels whereas the capillary resistance remains permanently low. In these conditions the eosinophils cease to reflect the cortisone level and only the capillary resistance remains a reliable indicator.”—T.R.T.
ABSTRACTS