ABSTRACTS

THEODORE H. SPAET, M.D., Editor

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ABSTRACTS OF SPECIAL INTEREST


Experiments were done with 1746 splenectomized female white mice. Two weeks after splenectomy 690 r of whole body irradiation (LD90) were administered. Surprisingly, splenectomy resulted in a reduction of the morbidity from 85 per cent (controls) to 32.9 per cent. The best results were obtained when splenectomy was performed one hour after irradiation. The presence of an irradiated spleen seems to be detrimental to the organism. Consequently, whole body irradiation is not a contraindication for splenectomy: on the contrary, it might be possible that splenectomy offers new prophylactic and therapeutic approach to x-ray damage.—H. M.


Under the action of light, bilirubin pigment denatures rapidly. This property was originally applied by Perryman and Richards in 1937 as treatment for neonatal jaundice. The author used the method successfully in the treatment of severe hyperbilirubinemia without feto-maternal incompatibility as well as in cases of ABO and Rh incompatibility. Striking and rapid reduction of bilirubin was obtained.—M. J.


The authors describe a fast moving Hb component in a patient with a severe iron deficiency anemia. This component disappeared after the patient’s recovery. This fast moving component is most probably related to the fast moving fraction of normal adult hemoglobin as described by Kunkel. Further studies of this component in severe iron deficiency anemias would be of interest.—B. R.


The authors studied fractions of free amino acids in the serum and urine of patients with
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Received Politamin, an amino acid complex, or hydroxyproline, and they showed some tendency to recover from the anemic state with improvement of the general condition. Sera from the patients generally contained an extremely low level of free amino acids, especially glycine and alanine which were markedly decreased to absent. In addition, some unknown fractions were found. Increased glycine was found in the urine. Although administration of adrenocortical hormones improved the anemia and general condition, no change of amino acid fractions occurred. The free amino acid patterns in the patients with myeloma resembled those in patients with idiopathic hypoplastic anemia.—K. F.


The enzymatic conversion of fibrinogen to fibrin by thrombin is now known to involve the release of several peptides, designated as cofibrins. These cofibrins are released at different rates. The authors have shown that two cofibrins, A and B, are liberated presumably by action of rabbit thrombin when rabbit plasma is clotted with brain thromboplastin and calcium. Cofibrin A is liberated at a much greater initial rate than B. When the reaction was stopped before completion, it was noted that about half of the cofibrin A was liberated before B was detectable. The liberation of B was concurrent with visible increases in turbidity. The same findings were noted during the reaction of purified fibrinogen with bovine thrombin. A cold-precipitable, thrombin-cougalublable protein, designated as cryoprofibrin, was separated from plasma of rabbits treated with E. coli endotoxin. Cryoprofibrin was shown to be a fibrin intermediate, consisting of fibrinogen that had lost only a portion of the peptides liberated during the conversion of fibrinogen to fibrin. On reaction with thrombin, fibrinogen and cryoprofibrin yield the same amount of cofibrin B; however cryoprofibrin yields 30 per cent less cofibrin A than did fibrinogen. This is felt to be evidence of limited action of thrombin in vivo in these endotoxin-treated rabbits. Evidence is given to show that the cryoprofibrin was not formed in the plasma after bleeding. An alternative approach to measurement of thrombin activity involving recovery of cofibrin A from urine proved inapplicable, because no cofibrin A was found to be excreted in the urine after intravenous injection of cofibrin A. The demonstration of increased thrombin activity by this method after endotoxin treatment, the authors feel, implicates thrombin in the mechanism of fibrinoid deposition. It is suggested that the study of fibrin intermediates may be useful for discriminating between a generalized or localized origin of fibrin deposited in lesions.—R. G.

RADIATION EFFECTS


Human homografting, facilitated by whole-body irradiation, is discussed from the alternate approaches (1) of high doses intended to ablate the host bone marrow with the intention of establishing a foreign system permanently, and (2) more moderate doses calculated to depress the host immune system during establishment of foreign cell colonies, with the hope that subsequently regenerating host cells may develop the "fetal" tolerance to the newcomers. Either way, if successful, would mean subsequent transplantation of other donor tissues to the host should be feasible. Two humans, following nephrectomy of single kidneys for severe trauma, received high doses of whole body irradiation followed by bone marrow transplants. One also received a transplanted kidney, the other patient being too near death to warrant this procedure. Four patients in the terminal stages of chronic and subacute glomerulonephritis received more moderate doses of whole body irradiation with subsequent kidney transplants (except one patient). Of the total, three died from marrow failure at 22-32 days, and two from uremia at 18-19 days post irradiation. The survivor is presented in a separate current paper.—J. L. B.


Nine successful kidney transplants between identical twins have been made since 1955, after testing each time for immunological compatibility by reciprocal skin grafts. This paper presents the case of a man of 24, in terminal uremia due to chronic glomerulonephritis, who received a transplanted kidney in January 1959, from his nonidentical male twin. Blood grouping eventually revealed identity in 25 groups. Preliminary reciprocal skin grafts resulted in rejection at 22 days by the healthy twin, and accelerated loss of
a second graft in the same route, but maintenance of the graft to the sick twin. Whole body 250 kvp X-irradiation of 250 r was done, followed by 200 r one week later. Kidney transplantation was accomplished the following day. Thereafter the patient improved and has continued to do so. The authors discuss the apparent take in the face of dissimilar tissue antigens, and follow up by sections from the initial donor skin graft to the patient, and subsequent treatment by radiation and steroids.—J. L. B.


A twin Co60 (bilateral) irradiation facility was operated at 0.48-0.56 rads minute (30-40 r/hour) to irradiate dogs and humans. The low dose rate necessitated exposure up to 45 hours to obtain 1,500 r. Feasibility of autologous and homologous bone marrow and kidney transplants in dog was then studied, with autologous marrow grafts found generally more successful than homologous transplants, only one male dog with female leucocytes eight months post treatment being cited. A male canine recipient of a female kidney died of pneumonia 5 days post treatment, a few weeks after removal of his own kidneys. Several successful isologous marrow transplants from normal to leukemic identical twins (post irradiation of up to 1,600 r) were achieved. The authors conclude that they have obtained no permanent homologous marrow "takes" in humans, and that many of the transplantation difficulties are common to man and dog. (Abstractor's note: Considering the very low dose rate to dogs and humans in this group and the benign post irradiation courses, marked dose rate effect is suggested; LD50 data from the author's control animals would have been helpful.)—J. L. B.


The authors present a historical survey of the major research leading to the present understanding that radiation protection by donor spleen and hemopoietic tissue is due to population of the host by donor cells rather than by action of a humoral or other non-cellular mechanism. A brief discussion is presented of technics of producing radiation chimeras.—J. L. B.


The authors studied preserved dog blood after X-ray irradiation (200 kV, 15 mA, filter 0.5 mm Cu + 1 mm Al, OK 30 and 23 cm) with 2,500, 9,000, 18,000 and 35,000 r. Changes in the number of leucocytes and thrombocytes, the concentration of sodium and potassium in plasma and erythrocytes, the amount of free hemoglobin in plasma and the osmotic resistance of red blood cells were followed. The blood was kept in a refrigerator at +4 C and the estimations were made after weekly intervals, usually for a period of five weeks. Perceptible changes were found only with doses over 18,000 r and consisted in a slight rise of free plasma hemoglobin and a slight increase of Rbc osmotic fragility. The decrease of leucocytes and thrombocytes was small as compared with controls and hardly significant. The ratio of electrolytes in plasma and red blood cells did not undergo any substantial changes. Simultaneously transfusions of large volumes of preserved dog blood irradiated with 20,000 r were made. The blood was kept after withdrawal and irradiation for 10 days in the refrigerator. The animals tolerated the transfusion well and no post-transfusion reactions developed. The experiments provided evidence of a considerable resistance of preserved blood to the action of ionizing radiation.—L. D.


The authors studied peripheral changes of leucocytes, neutrophiles, lymphocytes, reticulocytes and thrombocytes after a single injection of P32 in doses 0.031, 0.125, 0.5, 1.25 and 1.6 uc/g body weight in white rats. The absorbed doses of radiation for bones and the rest of the body were calculated by means of an exponential model of elimination and are summarized in tabular form. The dynamics of changes of white blood cells were similar to those after external radiation except for a progressive fall of leucocytes and especially lymphocytes in the 3rd and 4th week with the smallest doses. In contrast to external radiation the authors observed a reticulocytosis lasting from the first day to the 4th week. Thrombocytopenia, usual after external radiation, did not occur.—L. D.

STUDIES ON SUSCEPTIBILITY TO INFECTION FOLLOWING IOnIZING RADIATION. C. P. Miller, C.
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Groups of 10 week old CF-1 female mice were exposed to 250 KVP X-irradiation at dose levels of 300, 400, and 500 r. Mice drawn at intervals from each dose group were then inoculated intraperitoneally with graded inocula of a Pseudomonas aeruginosa culture. Mortality data then yielded LD_{50} values for each dose level at each interval. The 300 r group showed little increase in susceptibility, but the 400 r group had increased susceptibility to infection on the 3rd day post-irradiation, recovering by the 17th day. Five hundred r caused marked susceptibility to infection lasting from the 3rd to 11th day. Resistance to infection recovered more quickly than did the white blood cell count in the last group.—J. L. B.


Eighty-eight Sprague-Dawley female rats 40 days old were divided into three groups and treated as follows: (1) controls, (2) 200 r of Co^{60} whole body irradiation at 40 r/min, and (3) 400 r. Animals were closely followed to 15 months post-exposure, with tumors excised and animals returned to the study. After 15 months, animals were followed to death, or sacrificed if tumor appeared. Incidence of four detected types of breast tumors was plotted together and separately against time. Non-breast tumors showed little increase in incidence in irradiated versus non-irradiated animals. For breast, incidence of (1) adenocarcinoma, (2) fibrosarcoma, and (3) fibroadenoma was almost uniformly twice as great at 400 r as at 200. The adenofibromas did not show this proportionality. Final cumulative incidence of rats with breast tumors (one or more) was (1) Controls—40 per cent, (2) 200 r—64 per cent and (3) 400 r—90 per cent.—J. L. B.


The Atomic Bomb Casualty Commission accumulates and evaluates data on human injury attributed to the atomic bombings of Hiroshima and Nagasaki. Correlation with radiation dose is a primary aim, and replicates of Japanese houses have been employed to obtain better estimates of exposure doses. The proportion of dose due to neutrons (compared to gamma) was less at Nagasaki due to bomb construction. Examination has revealed no increased incidence of stillbirths, infant mortality, or congenital abnormalities in 27,000 offspring born from 1948 to 1954 of heavily irradiated survivors. Evidence, however, for production of a sex linked lethal mutant was obtained in this group, by finding an altered sex ratio in the newborn. Bomb survivors were examined in 1949 for overt cataracts, 9 being found in Hiroshima, and 1 in Nagasaki. In 1951–3, examination of survivors with a history of major epilation after the bombings revealed an 84 per cent incidence of lenticular opacities compared with 10 per cent in a comparable control group. Proportionality of opacification to degree of post-bombing epilation was noted. Leukemia, particularly of the chronic granulocytic type, was found to pass through a peak incidence (for survivors) in the years 1950–52 with frequency appearing to be a linear function of proximity to the Hiroshima hypocenter. Calculation shows in survivors, the leukemia incidence to be over three times that expected during the entire anticipated life span of a comparable non-irradiated group, refuting the thesis of an “advanced biological clock” mechanism. Carcinomas of lung, breast, stomach, ovary, and cervix appear increased in heavily irradiated bomb blast survivors, but further data are needed. Fifteen microcephalic and mentally defective children were born to women pregnant less than four months at the bombing, and sixteen microcephalic children of normal mentality were similarly recorded. Incidence appeared related to dose, with eight of the first group appearing in mothers less than 1200 meters from the hypocenter.—J. L. B.


Previous studies by the authors had indicated the ability of 50 r X-irradiation of pregnant mice to produce exencephaly (brain herniation) in up to 6 per cent of fetuses, as found on sacrifice of the mother at 17.5 days gestation (almost full term). Up to 42 per cent resorption also occurred, but is seen from 5–10 per cent in normals, whereas exencephalies are not. Hence the interest in the latter. Using a timed mating technique to produce 387 pregnancies, paired 184 Kv X-irradia-
totaling 50 r were effected at various spacings through pregnancy. Exencephalies were most frequent with initial exposure on the 1.5 day, and least with this on the 4.5 day. A slight decrease of effect was felt to result from fractionation, particularly for the longer intervals.—J. L. B.


Data and conclusions presented by M. P. Finkel in 1958 relating to life shortening and bone tumor induction in mice by injection of Strontium-90 are reviewed by the authors. The data are felt to be comprehensive and accurate, but plotting of response against initial dose injected is objected to as not allowing for differences in lifespan and consequently, accumulated irradiation dose. Archer and Carroll present revised graphs of the original data, revealing linearity of dose-response, and extrapolation to normal incidence of tumors at zero dose. They find no threshold dose for either criterion—J. L. B.


A survey was undertaken of 1,644 children who had been irradiated to the head, neck, and chest between 1932 and 1950, with over 99 percent of the family histories obtained through personal interview by trained assistants. The figure "1,644" represents over 73 percent successful followup of a total of 2,230 theoretically available from hospital records; 3,777 non-irradiated siblings were evaluated in the same way. Incidence of thyroid cancer was zero in the controls, but 11 in the irradiated group, about one-hundred times that normally expected. Additional findings were (1) increased disease generally in the patients, and (2) a greater death rate in the siblings.—J. L. B.


The authors queried 36 collaborators by mail, and visited some personally, obtaining information on 71,582 patients treated with radium for cancer of the cervix. A total of 22 cases of leukemia was obtained, with six of these being disallowed as coexistent. The net incidence in 191,750 patient years at risk, came to 115 cases per million. Removal of the lymphatic types resulted in 62 cases per million. These results are considered to coincide well with normal incidence of 58 per million for Great Britain to 90 per million for the United States. The authors conclude that radium treatment for cancer of cervix does not induce an increase in the incidence of leukemia among the survivors.—J. L. B.

HEMOSTASIS


The authors observed marked thrombocytopoiesis in animals treated with pantetine (a derivative of pantothenic acid), and after the injection of small amounts of serum of pantetine treated animals. The peak of thrombocytopoiesis took place 20 to 40 minutes after the injection of serum. The existence of a thrombopoietic factor in serum is postulated on the basis of these results.—P. d. N.


Platelet-rich plasma was lyophilized and platelet count and morphology were studied after reconstitution. No significant modifications of platelet count and morphology were observed but there was a marked tendency for agglutination to occur.—P. d. N.


In lyophilized, platelet-rich plasma the aldolasic activity of platelets is not significantly modified after reconstitution.—P. d. N.

RESEARCH ON THE ADSORPTION OF COAGULATION FACTORS ON THE SURFACE OF SILICA AND BENTONITE. CONNECTIONS WITH THE PATHOGENESIS OF
The adsorption of coagulation and fibrinolytic factors was studied by means of bentonite, quartz and barium sulfate, using the following determinations: prothrombin time, prothrombin, factor V, factor VII, factor IX, factor X, fibrinolysis (fibrin plate method, plasma, euglobulin fractions with and without activator). Strong adsorbing activity of bentonite was demonstrated for factors with and without activator. Strong adsorbing brin plate method, plasma, euglobulin fractions P. d. N.

![Image]


Fibrinolytic activity is greatly reduced during pregnancy from about the 15th week. After delivery there is a rapid increase to normal levels within 24 hours. Both the investigations reported in these papers aim to show whether the changes during pregnancy are due to an increase of antifibrinolytic activity or to alterations in the fibrinolytic system itself. Biezenski, estimating the titers of serum antiplasmin, finds that they do not differ from those in non-pregnant women. He concludes that the marked decrease in spontaneous fibrinolytic activity in late pregnancy and labor is due to an actual reduction in the circulating active lytic enzyme and is not associated with a change in antifibrinolytic activity. Naidoo, Hathorn and Gillman, however, find a significant decrease in antifibrinolytic activity within 24 hours after delivery in eight out of nine African women, as well as a decreased euglobulin lysis time. They therefore suggest that the parturum increase in fibrinolytic activity to non-pregnant levels is due to changes in circulating antifibrinolytin as well as to alterations in the fibrinolytic system.—R. M. H.


A fatal bleeding disorder occurring during labor and affecting both mother and fetus is described. All stages of the coagulation mechanism apart from the fibrinogen-fibrin reaction appeared to be normal. The defect could be corrected in vitro by the addition of protamine sulphate or toluidine blue to the patient's plasma but there was no other evidence of hyperheparinemia. The authors show no direct evidence for the presence of a circulating anticoagulant and the cause of the defect in fibrinogen conversion remains unexplained.—R. M. H.

Erythrocytes


This paper reports the results of studies with Fe59 on the kinetics of iron uptake and heme synthesis in rat, pigeon and human reticulocytes in vitro and on the effect of certain metabolic inhibitors on the uptake pattern. It is shown that the uptake of radioiron by mammalian reticulocytes is a rapid process and heme is synthesized from the iron almost immediately. The process is slower with avian erythrocytes and this is possibly related to a slower but more prolonged synthesis of protoporphyrin by the nucleated cells. This synthesis must account for all the Fe59 hemoglobin formed in vitro under the experimental conditions. Incubation of both reticulocytes and nucleated cells, before addition of iron, reduces both uptake of iron and synthesis of heme. It is postulated that there are two stages in the process of intracellular heme synthesis. The first is the entry of iron into the cell and this is inhibited by mercuric chloride but not by other enzyme inhibitors. The second phase, heme synthesis, is inhibited by potassium cyanide, and is therefore apparently dependent on oxidative respiration.—G. C. de G.


Erythrocyte neuraminic acid was found to be 19 mg./100 ml. of erythrocytes in normal children (mean values of 15 determinations), with a range between 18 and 23.—P. d. N.

This article further emphasizes the statement that "the fetal liver is a superlative tissue for a number of cytological problems." Material used for these studies was obtained from rabbit fetuses of various ages from 12 to 30 days gestation and it was fixed in Dalton's osmium-dichromate fixative. Electron microscop observations were made of erythroblasts, heterophile granulocytes, megakaryocytes, macrophages, and hepatic cells. The latter were readily distinguished from hematopoietic cells by their size, amount of cytoplasm, lipid inclusions and characteristic mitochondria. This is indeed a timely observation in view of the conclusion of Thomas, Russell, and Yoffey (Nature 197:876-877, 1960) that hepatic hemocytoblasts are derived not from the mesenchyme but from endothelium, namely, the as yet undifferentiated liver cells. There was no evidence in the livers of rabbit fetuses that mitochondria give rise to the specific granules of the granulocyte, megakaryocyte, and platelet. The appearance of small granules in the Golgi zone suggest this region as the site of granule development. The transmission of ferritin by a process likened to pinocytosis, but called ruphhecytosis, was observed between hepatic cells and erythroblasts. This suggests that intercellular transfer of material is a more widely spread process than has previously been described. At times a small finger-like process appeared to extend from a hepatic cell into an erythroblast causing an invagination in the erythroblastic plasma membrane.—O. P. J.


The earthworm blood circulatory system has a respiratory pigment, erythrocruorin, which is in solution in the plasma. The erythrocruorin particle is about 36 times as large a hemoglobin. All of the vessels are lined with a well defined internal basement membrane which intervenes between the suspended erythrocruorin particles and the inner surfaces of the endothelium. Surrounding this basement membrane in all of the vessels is a continuous complete layer of endothelial cells, more or less specialized. The basement membrane inside the capillaries forms a barrier which prevents the blood pigment from leaving the vessels or from making contact with the endothelial cells. In this respect the basement membrane appears to have a function analogous to the red blood cell surface membrane.—O. P. J.


It has been shown that albumin is one of the components of serum that inhibit hemolysis induced by lysins such as saponin. It also prevents the prehemolytic sphere of erythrocytes placed in saline between slide and coverslip. However, very little is known about the effect of albumin on hypotonic hemolysis. In the present article, the effect of albumin on hypotonic hemolysis was studied by placing erythrocytes in hypotonic salt solutions containing albumin. It was demonstrated that albumin is an effective inhibitor of osmotic hemolysis. The erythrocyte in hypotonic albumin solutions becomes permeable to cations. If the osmotic pressure of the albumin in the solution balances that of the hemoglobin in the cell, no hemolysis occurs. If the osmotic pressure of the albumin is less than that of the hemoglobin, partial hemolysis ensues until the osmotic pressures of the albumin plus hemoglobin outside the cell balance that of the remaining hemoglobin in the cell. This is in terms of the colloid-osmotic theory of hemolysis.—O. P. J.


Iron metabolism of 25 premature infants (14 males and 11 females) weighing 1,300 to 2,480 Gm. was studied. Increased serum iron values were found during the first few days after birth. Return to normal values was observed after the tenth day. Unsaturated transferrin was markedly increased but only during the first two days after birth. Erythrocytic free protoporphyrin was increased in the first four days after birth. No strict correlation was found between serum iron and transferrin. A functional deficiency of iron metabolism in premature infants was postulated on the basis of these findings.—P. d. N.

This paper describes a method of estimating the plasma iron concentration and the total iron-binding capacity. The method is adapted from previously described methods and has been found suitable for routine use in a hospital laboratory. The iron is released from plasma iron-binding protein by the action of dilute hydrochloric acid and thioglycolic acid, the plasma proteins are precipitated by trichloracetic acid without heating and the iron concentration is determined colorimetrically using the water-soluble reagent, sulfonated 4:7 diphenyl 1:10 phenanthrolmine. Total iron-binding capacity is estimated by first adding iron to the plasma in excess of the iron-binding capacity, removing the excess iron with powdered magnesium carbonate, and estimating the iron remaining in the same manner as for plasma iron.—G. C. de G.


The folic acid activity of blood was measured by streptococcus fecalis assay before and after specific therapy in 10 patients with Addisonian pernicious anemia, 8 with non-Addisonian megaloblastic anemia and 15 with iron-deficiency anemia, and in 7 patients recovering from acute blood loss. A temporary increase in folic acid activity was demonstrated during the most active period of blood regeneration in all these groups of subjects, usually after the maximum reticulocyte count. This increase was clearly not due to any specific therapy, and the authors conclude that it is chiefly associated with hemoglobin regeneration. Full details of the assay method are given, together with normal ranges and limits of variation.—R. M. H.


The authors modified a clinically valid assay of folic acid deficiency in man, and then demonstrated their modification was clinically useless. It is possible that their failure was due to use of too high autoclave pressure. The successful report they attempted to reproduce (Clin. Chem. 5:275, 1959) erroneously recorded autoclave pressure as 10 p.s.i. At that pressure, unfortunately for the present authors, serum folic acid activity is destroyed in a steam environment (V. Herbert, J. Clin. Invest., January, 1961).—V. H.


A comparison was made between Ochromonas Malhamensis and E.Coli 113–3 as test organisms for the determination of Vitamin B12. The vitamin was obtained from the growth of Propionibacterium shermanii under anaerobic conditions. The values obtained with Ochromonas were lower or identical but never higher than those obtained with E.Coli. Where quantities detected with E.Coli were higher, two or three paper chromatographic spots could be recognized, but only one spot in the other twain instances. The results point to a high specificity of the Ochromonas Malhamensis method for the true Vitamin B12 assay. Included is a detailed description of the Ochromonas assay, preparation of media, conditions of culture and assessment of growth.—J. J. B.

Distribution of Radioactivity in Man After the Oral Ingestion of Small Test Doses of Radiolabeled Cyanocobalamin. A. Doscherholm, P. R. Finlay and P. S. Hagen. From the Veterans Administration Hospital, Minneapolis, Minn. J. Lab. & Clin. Med. 56:547, 1960.

Doses of 0.56 μg Co60B12 plus hog intrinsic factor concentrate was administered orally to 11 patients who died 2 hours to 113 days later. Liver, spleen, and kidneys had similar concentrations of radioactivity during the first two days after administration, with the concentration subsequently rising in the liver and falling in spleen and kidney. The two patients who died 2 and 7 hours after Co60B12 had most body radioactivity in the jejunum; the patient who died 4 hours after Co60B12 had most body radioactivity in the ileum. Those who died 1 to 13 days after Co60B12 had most small intestine activity in the ileum. These findings add to evidence favoring the ileum as the major site of B12 absorption, but indicate that the jejunum cannot be excluded as an area of absorption.—V. H.


Urine amino acid chromatograms were studied in 10 patients having Addisonian anemia, and a further 5 in whom Vitamin B12 deficiency was secondary to gastrectomy or intestinal disease.
In 5 out of the 10 cases of Addisonian anemia, an increase in taurine excretion was found: of the non-Addisonian group, none had an increase in taurine excretion, but in two patients taurine excretion relative to glycine was increased. An increase in taurine excretion was also found in 5 patients with adult celiac disease who had megaloblastic anemia without Vitamin B₁₂ deficiency, and in one out of 5 cases of iron-deficiency anemia, but it was not detected in three cases of megaloblastic anemia of pregnancy. In almost all patients, the abnormal amino acids began to disappear when the appropriate specific therapy was started, whether this was Vitamin B₁₂, folic acid, or iron. The authors conclude that urine amino acid chromatography does not offer any assistance in the differential diagnosis of anemia.—R. M. H.


The authors present a case of congential syphilis in a man aged 19 who had experienced episodes of "hematuria" on exposure to cold over the previous five years. On admission during an episode of hemolysis, hemoglobin was found in the urine, but there were no red blood cells; methemalbumin was present in the plasma, the cold agglutinin titer was 1 in 8, and the Wasserman, Kahn and Price reactions were strongly positive. The Donath-Landsteiner and Coombs tests were studied in detail, and the Donath-Landsteiner antibody was found to behave in the Coombs test like the cold antibodies occurring in other conditions. Thus, agglutination was due to interaction of the antiglobulin serum with complement adsorbed onto the test cells; the antiglobulin serum had to be used in a higher concentration than in rhesus testing, and the positive results persisted after the addition of γ-globulin, unless a very high concentration was reached.—R. M. H.


This paper gives an account of the rate of in vivo survival of rabbit red cells as determined by the Cr³¹ method, after the cells had been treated in vitro with papain, trypsin or receptor destroying enzyme (RDE). The papain-treated cells were destroyed at random at a fairly constant rate. Trypsin produced irreversible changes in the cells and the rate of destruction was greatest shortly after injection. RDE-treated cells were removed for the first four or five days but thereafter survived normally. The changes produced by the enzyme were apparently reversible. RDE solution was injected intravenously and produced panagglutinable changes in the red cells. These altered cells were removed from the circulation by the anti-T agglutinin in the serum and the rate of removal was dependent on the amount of anti-T present.—G. C. de G.


Two cases of autoimmune hemolytic anemia are described; one presented as acute hemolysis shortly after childbirth in a woman aged 27, the other was found during routine blood investigations and remained subacute. In both cases, the direct Coombs test was positive both at room temperature and at 37°C, and at 4°C there was a marked autoagglutination, which was enhanced by Coombs serum. The patients' blood groups were A₁ Rh positive (CCDee) and A₁ Rh positive (CCDe) respectively. By using anti-I serum and I cells, it could be shown that both patients' cells were I-positive and that their sera contained an anti-I antibody. Their sera also agglutinated enzyme-treated I cells to a slight extent, and in this they differed from "naturally" occurring anti-I. The authors suggest that if test cells and sera were available, many of the antibodies of the acquired hemolytic anemias would show blood-group specificity.—R. M. H.


An allele bearing Hu and He as well as N and S antigens (NSHuHe) has been observed. In the same family no untoward reactions were encountered in typing for the various antigens in the MNSs⁹, Hu, He system. The occurrence of this MNS allele bearing both the Hunter and Henshaw antigens further indicates the considerable complexity of this blood group system.—H. F.

**Problems in Rh Typing as Revealed by a Single Negro Family.** R. E. Rosenfield, G. V. Haber,
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Routine Rh typing of a Negro family revealed a mother of type Rh Rh (ccDEE) with three children of phenotype Rh Rh (ccDee). Further testing revealed an Rh allele, termed R* by the author, which gave only trace reactions with anti-rh' (anti-C) and anti-rh" (anti-e), as defined by some reagents. Because of such trace reactions, this allele differed from the R* (-D-) allele present in Caucasians. Further, excess Rh Rh (D) was not produced. Such an allele introduces a significant source of error into serologic tests for disputed paternity involving Negroes. This family also had the "Negro" r' allele (r'). This finding too introduces a source of error into Rh-Hr serologic tests in Negroes. It is becoming increasingly apparent that the various Rh-Hr alleles in Negroes are genetically and serologically different from the superficially identical Rh-Hr alleles in whites, with the reactions encountered depending upon source of the antisera used in testing.—H. F.


The ABO distributions of Rh-nonsensitized and Rh-sensitized Rh-incompatibly mated population samples deviate significantly from one another; they also deviate in opposite directions from Hardy-Weinberg expectancies. The authors explain the simultaneous deficiency of ABO-incompatibles in the Rh sensitized series and the excess of ABO-incompatibles in the Rh-nonsensitized series on the basis that ABO incompatibility tends to inhibit Rh-immunization, and possibly modifies its consequences as well. The possible biological mechanisms underlying such ABO-Rh interaction are discussed, as well as the possible impact on allele frequencies in future generations.—H. F.


Total lipids, cholesterol, phosphorus and phospholipids were studied in the erythrocytes of five children with thalassemia major, seven of their relatives and four newborn infants with Rh erythroblastosis. A definite increase of the values was observed in all cases. The ratio of cholesterol/total lipids and the ratio P/cholesterol are decreased in the cases of thalassemia major. A relative diminution of the cholesterol and of P is demonstrable in the same cases. In the relatives the ratios P/total lipids, cholesterol/total lipids and P/cholesterol are identical with those of normal adults. In hemolytic disease of the newborn the ratio P/total lipids is normal, whereas the ratios cholesterol/total lipids and P/cholesterol are modified, thus indicating a slight increase of cholesterol, both relative and absolute.—P. d. N.


This is the first report of the occurrence of Hb-O outside Indonesia. Four children of a Hb-O trait carrier father and a sickle cell trait carrier mother were examined: one possessed hemoglobins A and O, one hemoglobins A and S, and two possessed both the abnormal hemoglobins as well as Hb-A. As Hb-S is a β-chain mutant, it might be expected from its combination with hemoglobins A and O in the same individual that Hb-O is an α-chain mutant; the authors state, however, that they believe that this is not the case. No further explanation of this apparent paradox is given in the present paper.—R. M. H.

LEUKOCYTES


When homologous adult chicken spleen cells are implanted on, or injected into, the chorio-allantoic membrane of the chick embryo, splenomegaly eventually occurs in the host embryo. The immunological reaction of the donor cells with the host antigen is accompanied by cellular proliferation and as these donor cells have colonized the host's spleen, the reaction is manifest as splenomegaly in the host chick embryo. In a recent series of experiments, spleen implants from white Leghorn chick embryos 10-18 days of incubation and from juvenile chicks from hatching to seven weeks post hatching were used as the donor tissues. No significant increase in the mean weight of the spleens or livers of the hosts occurred when donor spleens from embryos or from chicks up to four days post hatching were used. Immunological competence (as measured by the extent of splenomegaly in the host) was found with every donor within the age
range five days to seven weeks post hatching. The increase in net weight of these spleens was due to cellular proliferation as the RNA, DNA and protein contents also increased to the same extent. Most of the enlarged spleens were found in the female embryo hosts.—O. P. J.


Mast cells in the mouse do not react uniformly to a combined Alcian blue-safran stain and such a variability may have physiologic implications possibly indicating functional depletion of sulfated mucopolysaccharides. Furthermore, the Alcian blue-staining mast cells were commonly found in older mice in close proximity to large phagocytes filled with granules, yellow-brown pigment indicative of hemosiderin or lipofuscin. Mouse tissues were therefore examined for these three components in young and aged mice. The results showed that in many tissues, the numbers of mast cells increase concurrently with siderophages and lipophages. The histochemical characteristics of the mast cells accompanied by abundant siderophages and lipophages indicate depletion of their sulfated mucopolysaccharides.—O. P. J.


The authors suggest the following diluent for both visual and electronic counting of leucocytes: cetrimide 0.5 per cent, glacial acetic acid 0.5 per cent, formalin 1 per cent, in distilled water. This solution was found to be an excellent stromalysin: leucocytes could be counted within a few minutes, and remained unaffected for 24 hours at room temperature. When usual and electronic counts were performed in parallel on large numbers of cells, very close correlation was achieved. On comparison with other recommended diluents, these were all found to suffer from disadvantages such as turbidity or the necessity for immediate counting.—R. M. H.


Mouse leukemias and lymphomas grew even in hamsters not conditioned with cortisone, but retained specificity for mouse strain of origin. Human tumors could be maintained in hamsters, but could generally not be transferred serially.—P. G. R.


Case report of a young woman with refractory anemia, thrombocytopenia and a hypercellular bone marrow for 10 months before a diagnosis of leukemia could be made. Abstractors note: Many reports of prolonged preleukemic states in myeloblastic leukemia are appearing. They may resemble megaloblastic ( Nord.Med. 63:221, 1960) or aplastic anemia.—P. G. R.


In 25 patients with leukemia the survival of red blood cells labeled with radiochromium Cr51 was investigated. The direct Combs test was negative in all patients. In "compensated" patients the biological half-life was normal or slightly reduced. In other patients who were compensated at the time of the clinical and laboratory examinations, but, had a reduced half-life, decomposition developed within a few weeks. In uncompensated patients the half-life was reduced to a different degree. The results confirm the participation of the hyperhemolytic mechanism in the pathogenesis of anemia in leukemia. No correlation was evident between the size of the spleen and the survival of erythrocytes. The number of reticulocytes was not proportional to the hyperhemolysis and an important role must thus be ascribed to deficient erythropoiesis.—L. D.


The authors investigated 33 patients with hemoblastosis (8 chronic lymphadenoses, 5 chronic myeloses, 11 acute and or subacute myeloses, 8 malignant lymphogranulomas and 1 malignant reticulosis). In 25 cases they found, by the method of differential agglutination, shortened survival of red blood cells of varying degree. These results show that hemolysis is often present with anemia in these diseases. In the majority of cases they found a direct relationship between the degree
RBC destruction and the stage of disease. In most cases the life span of erythrocytes was shorter with a more malignant course and a graver general condition of the patient. Of the usual clinical signs of increased hemolysis an inconstant increase of reticulocytes was most frequently encountered. It was found in more than 50 per cent of cases. On the other hand, no correlation was found between serum bilirubin level and erythrocyte survival. Likewise, findings in sternal punctates in most cases did not furnish convincing evidence of increased hemolysis. No correlation was found between splenomegaly and shortened erythrocyte survival. Likewise, findings in all relation was found between serum bilirubin.

It was found usual clinical signs of increased hemolysis clear-cut in the remainder ere hyperhemolysis. The authors was found. no direct relation of these two factors disturbance of renal function s'as investigated in 35 patients with multiple myeloma. Disturbance of renal function was found in 74 per cent, elevation of nonprotein nitrogen in 54 per cent. In patients with Bence-Jones protein in the urine and with high plasma calcium the disturbance of renal function was more frequent and more serious but no direct relation of these two factors was found. The authors came to the same conclusion in investigating the relation of renal function to paraproteinemia. The laboratory findings were established by autopsy in 17 patients. In 88 per cent of them the histological picture was typical. In 11 (65 per cent) uremia was the cause of the death. In one case renal failure occurred acutely following intravenous pyelography.

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Lithium was investigated in 35 patients with multiple myeloma. Disturbance of renal function was found in 74 per cent, elevation of nonprotein nitrogen in 54 per cent. In patients with Bence-Jones protein in the urine and with high plasma calcium the disturbance of renal function was more frequent and more serious but no direct relation of these two factors was found. The authors came to the same conclusion in investigating the relation of renal function to paraproteinemia. The laboratory findings were established by autopsy in 17 patients. In 88 per cent of them the histological picture was typical. In 11 (65 per cent) uremia was the cause of the death. In one case renal failure occurred acutely following intravenous pyelography.


Energy for phagocytic activity of leukocytes is obtained partly from glycolysis and partly from the processes of oxidative phosphorylation. Addition of monoiodoacetate interfered with glycolysis and reduced the phagocytic activity from the normal by about 40 per cent to 25 per cent. Inclusion of 2,4-dinitrophenol (DNP), which interferes with phosphorylation, reduced the activity to 8 per cent. Addition of ATP to the suspension of leukocytes after DNP did not alter phagocytosis, irrespective of the ATP concentration. The results seem to indicate stronger dependence of phagocytosis upon the oxidative phosphorylation processes than upon glycolysis.


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The authors study biochemical processes in terms of electron transfers from donor to acceptor
occupied molecular orbital (empty molecular orbital) is the lowest values of numerical energies of various molecules. The donor or acceptor properties of effective as anti-tumor agents, six ineffective purine-analogs of nucleic purine analogs, and the purine bases DP N as compared to DPN. The elevation of both HOMO and LEMO in 6 AN-AN-DPN to function as anti-tumor agents, six ineffective purine analogs, and the purine bases of nucleic acids. A correlation was found between the electronic structure of the purine skeleton ring nitrogens of the antimetabolites and their anti-tumor activity. The formal positive charge of the N9 in the purine skeleton is 0.431 -0.399e in active antimetabolites, 0.437-9.441e in inactive ones and 0.400-0.419e in reference purines. Thus, in active antimetabolites it is of the order of magnitude of the normal purines, whereas inactive antimetabolites have a greater charge. N9 is the place of ribosilation, and the ribosides are unstable if N9-charge is high. In addition, the remaining ring nitrogens bear a negative charge which is often greater in the active than in the inactive compounds. Thus, the basicity of active antimetabolites is closer to that of natural bases than the basicity of inactive compounds.—P. G. R.


Rabbits fed for 105 days on a cholesterol-rich diet were divided into three groups: (1) control, killed at once; (2) kept for a further 112 days on normal diet, (3) kept for the same period of time on normal diet plus 50 μg Vitamin B12 subcutaneously daily. After the preliminary high cholesterol feeding serum levels of cholesterol and phospholipids were very much increased, with a reduced phospholipid: cholesterol ratio. In groups two and three, those levels fell considerably, particularly in the third group but not until the end of the experimental period. Marked aortic atherosclerotic lesions were evident in the second group, less so in the first group and hardly any in the group receiving the vitamin. Histological examinations of the aortae showed virtual absence of fatty deposits in the intima of the third group, with an increase, however, of fine collagen fibers apparently in lieu of the atheromatous plaques.

It seems that B12 might in some way induce a regression of the artificially produced aortic atheroma in rabbits though obviously not by lowering of serum cholesterol. The histological examination points to actual resorption of the atheromatous lesions followed by healing.—J. J. B.


Repeated examination of plasma levels of iron, copper and zinc (in several cases also of magnesium) in 26 patients with myocardial infarction revealed decrease of plasma iron and zinc and increase of copper; these changes were most obvious during the first week following infarction and returned to normal gradually. Follow-up study of zinc metalloenzymes (lactic and malonic acid dehydrogenase) in the course of the disease has shown an increase of their activity, but the correlation with metal levels was poor. The fluctuations of levels of tracer elements in the serum were not diagnostic of myocardial infarction.

They were also seen in other serious cardiovascular conditions such as pulmonary infarction and dissecting aneurysm.—L. D.


Multiple subcutaneous injections of casein cause amyloidosis in a reproducible manner in the spleen, kidney and liver of rabbits. The organ in which the lesion was first observed was invariably the spleen. The amyloid appeared to be deposited in close relationship to the connective tissue beneath the splenic sinuses. Electron microscopic studies were undertaken in order to study (a) the alterations in the reticuloendothelial system during the inception of the disorder; (b) the fine structure of the amyloid substance; and (c) the relationship of amyloid to the basement membrane of splenic sinuses. The results indicate that, prior to the development of clear-cut amyloidosis, there is a thickening of the subendothelial basement membrane. Amyloid accumulated progressively, forming large nodules separated by cytoplasmic processes. Endothelial cells stretched over the masses of amyloid and separated it from direct contact with the blood stream. Intracellular organelles were unaltered. Amyloid itself had a granular and fine filamentous appearance, and contained many cytoplasmic projections.—O. P. J.