ANEMIA


Since 1936, xanthopterin has been reported as curative of certain (nutritional) anemias and leukopenias in rats and fish. In 1940, xanthopterin was isolated from liver extract used in treating pernicious anemia. The present report concerns the effect of xanthopterin on erythropoiesis and leukopoiesis in bone marrow cultures in vitro.

The authors suspended bone marrow (rabbit, beef, sheep, rat, cat) in Tyrode’s solution, added specified supplements (Tyrode’s solution, folic acid, xanthopterin, normal human serum), and counted the various blood cells after three and six hours of incubation of the resultant mixture. They found that xanthopterin caused an increase in the rate of cellular proliferation of white cells, reticulocytes, and red cells; and that such an effect was optimal when the concentration of xanthopterin was about five micrograms per milliliter of suspension. The results obtained with xanthopterin and with normal human serum were of the same order of magnitude and were prompt. Folic acid, on the other hand, had no enhancing effect on proliferation of cells in these suspensions; suggesting, as the authors subsequently point out, that folic acid as such is unavailable to the marrow cells for growth. In a subsequent report dealing with nutritional anemia in rats, folic acid similarly was found to have an effect only after a lag period of several days; whereas the hematopoietic effect of xanthopterin was immediate (Am. J. Physiol. 152: 182, 1948).

S.E.


Anemia was produced in rats by feeding a purified diet augmented with 1 per cent sulfathiazole. The rats were then divided into three groups (with corresponding controls), and treated with xanthopterin or pteroylglutamic (folic) acid. The effect on the blood values was studied.

Group 1: These rats received 100 micrograms of xanthopterin daily for five days. There was an immediate and marked rise in red cells, hemoglobin, and hematocrit, which did not occur in the controls. A peak was reached, and then the blood values began to fall rapidly to their pretreatment ranges.

Group 2: These rats received single injections of xanthopterin (15, 50, or 100 micrograms) or synthetic pteroylglutamic acid (100 micrograms). The control group, which received no injections, died in seven days. Xanthopterin-injected rats showed a prompt rise in red cells, reticulocytes, white cells, and hematocrit, the rise being greater the greater the dose of xanthopterin. Rats receiving pteroylglutamic acid also showed a rapid rise in all blood elements, but there was a delay period of three to five days before this rise began.

Group 3: These rats received, by a single injection, either 100 micrograms of xanthopterin, or 300 micrograms of pteroylglutamic acid (this amount contains the same weight of pteridin as 100 micrograms of xanthopterin). Again, there was a rise in all blood values; but whereas the rise with xanthopterin was immediate, that with pteroylglutamic acid followed a lag period of one to three days.

It is suggested, as a result of these experiments and similar results in vitro with bone marrow culture...
ABSTRACTS

(Am. J. Physiol. 152: 175-178, 1948), that xanthopterin can be utilized directly by the bone marrow in hematopoiesis; whereas pteroylglycine acid cannot be utilized as such, but must be altered in form before it can affect hematopoiesis. It is strange that, in contrast, fragmentary experiences with xanthopterin in pernicious anemia suggested that it was of no value in the dosages employed (South. M. J. 40: 46-35, 1947), although, as is well known, pteroylglycine acid is highly effective.


Crystalline vitamin B12 was administered by single intramuscular injection to two patients with pernicious anemia, two patients with nutritional macrocytic anemia, and one patient with nontropical sprue. Five of the patients received six micrograms of the material; the sixth, 1 microgram. Within three to five days after the injection, the patients felt better and stronger, the soreness and burning of the tongue disappeared, and there was objective clinical and hematological improvement. Reticulocytosis (twelve to twenty per cent) occurred by the fifth to ninth day; and a rise in red count and hemoglobin followed. Although bone marrow examinations were done, no details are given in this brief report. There was also no extended followup.

This report confirms similar experiences in three patients with pernicious anemia following single injection of three to 150 micrograms of vitamin B12 (West, Science 107: 398, 1948). It is possible that this material isolated from potent liver extracts, may be the essential substance required for pernicious anemia.


In two patients with typical tropical sprue, the administration of eight micrograms of crystalline vitamin B12 intramuscularly was followed within three days by marked subjective and objective improvement (mouth and tongue symptoms relieved; strength increased); as well as by a rise in the various blood elements, with reticulocyte peak in each case on the eighth day. No data beyond the eighth day of treatment are given.

SE. HIGH SERUM ACETYLCHOLINE CONCENTRATIONS IN PERNICIOUS ANEMIA AND THEIR REDUCTION BY EFFECTIVE THERAPY. J. E. Davis. From the Departments of Physiology and Pharmacology, University of Arkansas School of Medicine. Am. J. Dig. Dis. 15: 53-55, 1948.

In dogs, the continued injection of acetylcholine produces a hyperchromic anemia which responds to antipernicious-anemia therapy; and the injection of derivatives of acetylcholine in dogs may produce central nervous system changes resembling those found in human pernicious anemia. The author, having developed a bio-assay method for the determination of the amount of acetylcholine in serum, measured the serum acetylcholine in five patients with relapsed pernicious anemia, two patients with remitted pernicious anemia, six normal individuals, and six individuals with "secondary" anemias (lymphosarcoma, leukemia, sickle-cell disease). The following results were obtained: It was found that the normal level of acetylcholine in serum ranged from 6 to 8.3 micrograms per 100 cc. of serum; the level in "secondary" anemias was the same, but in relapsed pernicious anemia, levels of 15 to 33 micrograms per 100 cc. were obtained. When specific treatment of these latter patients was instituted (liver extract, stomach extract, or pteroylglycine acid), there was a marked reduction in the serum acetylcholine to normal levels within four to seven days.

It is speculative, whether the aberration in serum acetylcholine is fundamentally related to the occurrence of pernicious anemia. The author suggests the possibility that something produces an excess of acetylcholine in the serum, which in turn depresses or arrests erythropoiesis within the marrow, resulting in the disease. Actually, he points out, the serum cholinesterase in pernicious anemia is normal or only
slightly decreased, and in remitting pernicious anemia the cholinesterase may actually fall even further; whereas in normal and leukemic individuals, pteroylglutamic acid causes a rise in serum cholinesterase. It is possible, therefore, that perhaps the effect of antiperi- nicious-anemia medicaments is to raise the level of cholinesterase in the blood cells rather than the serum (for this there is some evidence); that a fall in acetylcholine results from this; and that, with the fall, erythropoiesis reverts toward normal. These speculations are still tentative and, of course, do not suggest the basic cause for the alterations in the serum levels of acetylcholine or cholinesterase.

S.E.


A diminution in the blood serum cholinesterase (ChE) has recently been reported in pernicious anemia; and it has been found that the administration of liver extract or pteroylglutamic acid in such cases was followed by a rise in the serum ChE, concomitant with the clinical and hematological improvement following such treatment. It has been suggested, therefore, that the role of liver extract or pteroylglutamic acid may be to cause an increase in serum ChE, which in turn is followed by an improvement in the anemia. The purpose of the present article was to test whether these materials actually cause a rise in serum ChE in vitro or in vivo.

Several experiments were performed.

1. Various amounts of pteroylglutamic acid and liver extract were added to normal human and dog plasma in vitro, and the mixture incubated at body temperature. The ChE levels of the plasma remained unchanged.

2. Dog plasma depleted of ChE by the use of diisopropyl-fluorophosphate (DFP), which irreversibly inactivates ChE, was similarly incubated in vitro with folic acid and liver extract. There was no rise in the ChE level.

3. An attempt was made to produce macrocytic anemia (with the accompanying decrease in ChE) in dogs by administration of acetylsalicylic and physostigmine. No anemia could be so produced; and, furthermore, there were no deviations of serum ChE levels in these dogs during this regimen, or after the addition of liver extract or pteroylglutamic acid.

4. Four dogs were given DFP, so that their plasma ChE level was much reduced. Two were followed without further therapy; two, mg. of pteroylglutamic acid were administered daily; and to the fourth, 2 units of liver extract daily. The recovery of plasma ChE to normal values was identical in all four dogs.

These data negate the suggestion that it is possible to cause a increase in the amount of serum ChE by the use of the antiperi- nicious-anemia drugs, under the conditions of the experiments. They do not support the hypothesis that an acetylcholine-cholinesterase mechanism is concerned in erythropoiesis. Previous work had already shown that plasma ChE rises (in states of depletion) at a rate similar to the rate of regeneration of other plasma proteins. It seems probable, therefore, as the authors suggest, that the rise of plasma ChE in the successful treatment of pernicious anemia is the result, rather than the cause, of recovery.

S.E.


Streptomycin, according to the authors, has been used in some 900 patients in the literature. Of these patients, eight developed mild, self-limiting leukopenia; and one developed "agranulocytosis," the outcome of which was unstated. Of 400 additional patients who received streptomycin in the authors' hospital, two developed aplastic anemia on the 79th and 95th days of treatment, respectively. In the absence of other etiologic agents for marrow involvement (other drugs, disseminated tuberculosis), the aplasia had to be attributed to the streptomycin.

The first patient, a 46 year old man with moderately advanced tuberculosis, received two grams of streptomycin daily for 79 days, when his blood, previously normal, showed anemia, leukopenia, and neutropenia. Although the drug was discontinued, and various supportive measures used, the pancy-
topenia progressed, and the patient died 19 days later. Prior to death the white cell count was 800, with two per cent neutrophils (16 per cu.mm.); and red cells and platelets were much reduced. The marrow at autopsy was hypoplastic.

The second patient, a 27 year old man with moderately advanced tuberculosis, also received 2 grams of streptomycin daily; on the 95th day, pancytopenia was noted and progressed despite discontinuation of the medication. Marrow punctures showed hypocellularity of the marrow. The patient was still living at the time of the report.

SE.

IDIOPATHIC HYPOPLASTIC ANAEMIA WITH BONE MARROW HYPERPLASIA. E. S. Mills. From the Departments of Medicine, McGill University and the Montreal General Hospital, Montreal, Quebec. Canad. M. A. J. 57: 227-231, 1947.

Three fatal cases of idiopathic anemia are described and autopsy findings in two of the cases are included. All three patients were males and each presented variants from the generally accepted pattern of so-called aplastic or hypoplastic anemia. One patient, who received 196 transfusions of 500 cc. each, developed generalized hemosiderosis and brownish pigmentation of the skin. Remission occurred in this case after 11 years of illness, but death occurred soon thereafter as a result of pneumococcal infection.

Erythroid hyperplasia of the bone marrow in two of the cases is stressed and it is suggested that there is a maturation arrest in this disease due to deficiency of a factor other than that which is lacking in pernicious anemia.

In the author’s interesting discussion of these cases is included a statement that the life span of a red cell is probably less than forty days. This statement should be challenged in view of the conclusive evidence from many laboratories that the life span of normal human erythrocytes is about 120 days.

L.E.Y.


The authors have summarized the variety of information to be gained from sternal puncture. The methods of marrow aspiration in infancy and childhood are discussed, as well as normal and pathological cytologic findings. Attention is called to certain bacterial and protozoal infections and metabolic disorders in which diagnosis may be made by sternal aspiration.

C.A.F.


The response of sixty-three patients with severe anemia to therapy were studied by the authors. Of these, forty-three were macrocytic, twelve normocytic and two microcytic. Improvement following administration of iron and liver extracts was variable and not related to the red cell size. Crude liver extracts were more effective than highly concentrated liver, similar to the cases reported by Watson and Castle (Am. J. M. Sc. 227: 514). The incidence of hookworm infestation (eighty per cent) was higher among these patients than in the general hospital admissions (forty-six per cent). The authors felt that tropical anemia differs from pernicious anemia in not showing a true megaloblastic anemia and in its response to liver therapy. They believe it represents several deficiencies rather than deficiency of the intrinsic factor.

C.A.F.


Previous surveys of the nutritional status of Indian troops indicated that there was more of a correlation between hemoglobin and serum protein levels than had been reported in similar studies in Europe and North America. Although several methods were used for determining serum protein, preference was given to the copper sulphate gravity method of Phillips et al. In these cases the hemoglobin determina-
tion seemed to be a more sensitive test for malnutrition than the determination of serum protein. In macrocytic anemias the dietary deficiency produces low serum protein levels. But in hypochromic or normochromic anemias due to a dietary deficiency in iron, the serum protein is not necessarily lowered.

O.P.J.


Blood was removed from four young women and two male medical students who belonged to group A and were Rh-positive. It was replaced by equal volumes of group O blood. By differential agglutination it was determined that the survival time was about 90-100 days in women and 110-120 days in men. The curve of decay of transfused cells was linear for men and slightly curved for women. Apparently blood destruction is greater in women. The estimated figure of 400 cc. per month is much larger than that lost by menstruation alone.

O.P.J.

HEMOLYTIC ANEMIA


This paper suggests that the fat diet of the newborn infant plays a role in the rapid destruction of erythrocytes which gives rise to physiologic icterus of the newborn. The authors compared the serum bilirubin in cord blood at birth, with that in venous blood on the fifth day of life, in three groups of newborn infants: those on a routine diet (3.6 per cent fat), those on a low-fat diet (1.8 per cent), and those on a fat-free diet (0.03 per cent). (An attempt to evaluate a fourth group, on a 3.5 per cent fat diet, was unsuccessful because of refusal of feedings.) The average serum bilirubin at birth was 1.36 mg. per cent. On the fifth day of life, high-fat babies showed 5.4 mg. per cent; low-fat babies showed 5.4 mg. per cent; and fat-free, 3.09 mg. per cent.

Although these results are striking, they must be considered suggestive rather than conclusive, chiefly because of the small number of cases. Thus, there were ten children in the first group (3.6 per cent fat); although six showed a rise in serum bilirubin, four showed either no change or an actual fall. Of twelve children on the low-fat diet (1.8 per cent fat), eight showed the rise, but four showed no rise or a fall, and of nine children on the fat-free diet, six showed a rise, with the other three showing a definite fall. The procedure of averaging such small groups, as the authors themselves point out, may lead to erroneous results. There is, however, a trend, and it is not unlikely that sudden exposure of the newborn to a relatively high fat diet (fat is thought to be barred by the placenta) plays some role in physiologic icterus. That it is not the only factor is obvious.

S.E.


The author reports eight patients in two families with hemolytic anemia. The anemia was macrocytic, normochromic, accompanied by reticulocytosis, bilirubinemia, and splenomegaly. Fragility tests were normal and no spherocytes were seen. In two patients of one family, hemoglobinemia and hemolysis of erythrocytes on incubation was observed, but the Donath Landsteiner and acid hemolysis tests were negative. In the second family, stippled cells were consistently observed. The two groups show significant differences and neither fit into conventionally recognized types of hemolytic anemia. The author suggests that these are instances of congenital structural defects of the erythrocytes.

C.A.F.


The authors report the occurrence of jaundice in an eighteen year old boy, otherwise in perfect health.
This jaundice had existed since he was three years old. It consisted of a frank chronic mucocutaneous coloration, with occasional slight remissions. There had been no similar case in the family. The blood count was normal. Hemolysis of the red cells started in 0.48 per cent saline and was complete at 0.4 per cent. Rouleaux formation was normal. The average red cell diameter was 7.5 μ and there was no spherocytosis. The blood group was O, Rh positive. The myelogram was normal (13 per cent erythroblasts). There were no bile pigments or bile salts in the urine. Urinary urobilin was 0.3 mg. per litre, and 0.73 over twenty-four hours. Fecal urobilinogen (Watson method), 23 mg. Indirect blood bilirubin, 18.75 units; i.e., 98.4 mg., and two days later, 24.60 units, i.e., 98.4 mg. Cholalemia was 10 mg. per litre of serum. Finally, the urinary porphyrins were 40 per 1000. All liver function tests gave normal results. The authors discuss the nosological significance of this very definite observation and compare it with that of Dameshek and Singer (Arch. Int. Med. 67: 359, 1941) and of the hereditary jaundice of the rat studied by Mallay and Lowenstein. They think it is a nonhemolytic jaundice caused by an inborn error of the metabolism of the bile pigments with a kind of regurgitation of the indirect bilirubin.

J.P.S.


Sickling of susceptible red cells takes place only if the hemoglobin within the red cell is in the form of reduced hemoglobin. Oxygenating the reduced hemoglobin causes a reversal of the sickled cell to a normal biconcave disc. The various tests for sickling depend, correspondingly, upon reduction of the contained hemoglobin; and their speed and efficiency may be expected to vary directly (other things being equal) with their ability to cause this reduction.

The present authors utilize a culture of nonpathogenic bacteria (B. subtilis) to eliminate the oxygen in a blood sample, and thereby produce favorable and necessary conditions for sickling to occur. A drop of blood is placed upon a coverslip, a drop of the bacterial culture added to it, and the coverslip is placed upon a glass slide and ringed with paraffin. The preparation is then incubated at 37°C for five minutes and examined; if negative, it is incubated for an additional ten minutes and re-examined. Most positive tests appear within five minutes, and all within fifteen minutes; so that, according to the authors, those that are negative after fifteen minutes of incubation may be considered to be truly negative. The test does not discriminate between the severe sickle-cell anemia and the milder sickle-cell trait, since in both conditions the same abnormality of the red cell, a tendency to sickle under conditions of anoxia, is present.

This is the simplest of the reliable tests for the detection of sickling yet reported. The maintenance of an active culture of B. subtilis is discussed by the authors: once started from agar slants, such a culture is carried with ease by day-to-day reincoclusion of media; but refreshment from agar cultures at intervals is required. The speed of the results is of course a great asset.

S.E.


It has been known that some red corpuscles from patients with severe hemolytic anemia following splenectomy contain basophilic iron positive granules. The present authors have shown that not all of the basophilic Romanowsky-positive bodies contain iron. This may be due to the fact that the iron content is too small for detection. Negative reactions for iron of basophilic stippling and Howell-Jolly bodies were obtained. In marrow from these patients, siderotic granules were found in nucleated red cells and in some instances they showed a perinuclear arrangement. It has been suggested that their appearance parallels that of hemoglobin because siderotic granules were absent from the more primitive nonhemoglobinized red cell precursor.

O.P.J.


The authors present the coincidence, in a patient with familial spherocytic hemolytic anemia,
superimposed chronic iron deficiency (lip fissures, atrophic glossitis, koilonychia, hypochromic anemia) which included dysphagia and, on x-ray, spasm of the esophagus. The hypochromia responded to iron therapy, but there were no changes in the glossitis, nail changes, or esophageal picture; and reticulocytosis and increase in hypotonic fragility of the red cells persisted after the blood picture had lost its hypochromia.

The occurrence of both disorders in one patient must be very rare.

S.E.


Kernicterus, the staining of certain parts of the brain by bilirubin in congenital hemolytic anemia, may be followed by permanent neurologic and mental changes in the surviving child. The author of this review presents the current concepts of erythroblastosis fetalis, and then discusses the possible relation of Rh incompatibility between child and mother and subsequent mental difficulties in general. Three forms of erythroblastosis are recognized: hydrops fetalis (with the child usually stillborn), icterus gravis neonatorum, and congenital hemolytic anemia. Kernicterus was noted only in those children who survived beyond the first few days of life; thus, in the stillborn with hydrops fetalis, kernicterus has not yet had time to occur; but if the newborn child with hydrops or severe icterus dies several days later, kernicterus is present. Kernicterus was not found in mild hemolytic anemias without jaundice, but only if jaundice was present. It was not possible to correlate the occurrence of kernicterus with the type of antibody found, the abnormality being found no matter which antibody predominated (agglutinins, blocking antibodies). In all infants surviving icterus gravis, evidence of nervous damage in later life was present in some 17 per cent of the cases.

The author does not agree that agglutination thrombi of red cells are responsible for the neurologic abnormalities (death and staining of nerve cells), and cannot suggest the reason for selective involvement of the cortex and basal ganglia.

With respect to the general question of the possible relation of mental deficiency in general to icterus neonatorum, the author reports on 100 mental defectives. He was unable to find a mother-child Rh incompatibility in various types of nonspecific mental defects as compared to cases of Mongolism, and as compared to normal children. This contradicts the work of Yannet and Liebenman (J.A.M.A. 138: 335, 1946), who found that, in a group of Rh positive mental defectives, there were twice as many Rh negative mothers as would be expected on a random basis; but Cappell points out how their figures were inadvertently weighted. It is generally agreed that there is a relationship between the occurrence of kernicterus and the severity of blood destruction (V. C. Vaughn, J. Ped. 20: 462, 1946), and that mental defects occur only if kernicterus has occurred (M. Creak, Arch. Dis. Childhood 22: 180, 1947).

S.E.


An unusual case of kala azar refractory to a variety of agents and several courses of therapy is described, occurring in a twenty-three year old Negro soldier. In addition, a severe anemia with reticulocytosis and hyperbilirubinemia was present along with a leukopenia. Fifty-one liters of blood were given over a period of twenty-two months. Following removal of the spleen weighing 3500 gms., the patient made a recovery from the anemia and leukopenia and had no further symptoms of leishmaniasis. The organisms were recovered from the spleen at the time of the operation by hamster injection. The authors are anxious not to give the impression that splenectomy is indicated or necessary in most cases of kala azar, although in this instance of refractory disease complicated by hemolytic anemia it was particularly effective.

R.S.E.

BLOOD COAGULATION AND HEMORRHAGIC DISEASE

Amerlite IR-100, a phenol formaldehyde resin, is capable of removing calcium from blood serum without otherwise producing any changes in the blood. The authors utilized this material to study the effects of various concentrations of calcium, strontium, magnesium, and barium on blood coagulation. Blood was obtained from man, dogs, and rabbits; decalcified with the Amerlite; and stored on ice. The coagulation time of the decalcified blood was determined after addition of specified concentrations and amounts of the various ions. Thromboplastin was reduced to a minimum by the use of silicon-coated needles, syringes, and glassware.

It was found that coagulation did not occur until the concentration of calcium chloride in the blood was of the order of 0.0015 M. This is the actual level of calcium in the blood in the human body. Once this level of calcium was reached, there was a relatively wide range of concentrations in which coagulation occurred normally. A delay in coagulation did not occur until the concentration of calcium was reduced below one-half of normal. Above a calcium level of 0.004 M., the coagulation time of the blood became more and more prolonged.

Strontium behaved similarly to calcium in its ability to cause coagulation of decalcified blood; but was much weaker. Magnesium was found incapable of causing coagulation, except in the presence of thromboplastin; and then was weak in its action. Barium was incapable of producing coagulation even in the presence of thromboplastin. It was found, in this connection, that the presence of thromboplastin allowed coagulation at lower levels of calcium and strontium than when it was absent; and the more the thromboplastin, the lower the requisite concentration of the calcium or strontium ion. Changes in calcium level in physiologic and pathologic states are never within the extreme ranges of alteration in these experiments; and are probably never sufficient to alter the speed of coagulation in the human patient.

Hemorrhagic Diathesis in Hiroshima, Nagasaki and at Bikini Atomic Bomb Test. A. L. Copley. From the Laboratory of Cellular Physiology, Department of Biology, New York University, N.Y.C. J.A. M.A. 137: 145, 1948.

It is known that petechial hemorrhages and thrombocytopenia occurred after the atomic bomb explosions; and that, in addition to thrombocytopenia, hyperheparinemia occurs after exposure of dogs to X-rays, and is in part responsible for the resultant bleeding tendency. The present author seeks to correlate the occurrence of hyperheparinemia and thrombocytopenia, with resultant hemorrhage, by pointing out that (1) heparin induces clumping of platelets, thereby producing platelet-agglutination in vitro, and thrombocytopenia in vivo; and (2) the injection of heparin in various animals induces not only petechiae but also "white emboli" (platelet-clump plus white blood cells), which also result in vascular damage. He suggests, therefore, that ionizing radiation may produce its hemorrhagic effect by inducing hyperheparinemia (mechanism unknown), the heparin then clumping platelets (thrombocytopenia plus petechiae) and causing "white" embolization (petechiae).

The observations upon which these suggestions are based—i.e., the effect of heparin on platelets, etc.—are as yet unconfirmed.

Leukemia and Lymphoma


Na$^{24}$ (half-life 14.8 hours) was given orally in single doses of about 20 millicuries. The amount of radiosodium in urine was less than 10 per cent of administered dose. Cases treated included chronic myelogenous leukemia (7), subacute and acute leukemias (4), chronic lymphatic leukemia (4), acute lymphatic leukemia (2), polycythemia vera (1), and malignant lymphomas (1). Case reports are provided on many of the patients. There was no irradiation sickness. In chronic leukemia changes in the peripheral blood were similar to those following other types of irradiation. However, the therapeutic results are not superior to those with P$^{32}$ or X-ray and the short half life of the isotope would make its general usage impractical.

C.A.F.
ABSTRACTS


This is a largely clinical discussion on recent attempts to influence malignant disorders by various chemical means. Included are short discourses on estrogens in carcinoma of the prostate, chlorocthylamines (nitrogen mustards) in Hodgkin's disease, and urethane in leukemias.

S.E.


Experiences are reported with the use of nitrogen mustard in the treatment of lymphomatous disorders. The results duplicate those found in other centers: there was no effect in two patients with melanocarcinoma and two patients with lymphosarcoma; a fair response in a patient with giant follicle lymphosarcoma; and excellent responses in five of six patients with Hodgkin's disease. Relapse in the latter cases occurred within four months in most instances, although one patient was still in remission 11 months after treatment. It was noted that the lymphocytes in the blood typically became pale, granulated, deformed, and otherwise changed after nitrogen mustard, showing changes of noxious nature.

S.E.


This short clinical report, admittedly preliminary and inconclusive, seeks to suggest that the use of nitrogen mustards may accelerate remissions in widespread sarcoidosis. Four cases are presented, in all of whom the diagnosis was established ultimately by node biopsy, and in all of whom there was involvement of eyes, lungs, nodes, and sometimes skin. Methyl-bis (beta chloroethyl) amine hydrochloride was administered in two courses one month apart, and the patients followed for signs of change. Marked retrogression of enlarged nodes, lung infiltrations, and ocular and other abnormalities occurred in the first three cases, beginning from two weeks to three months after initiation of treatment; the fourth case remained stationary.

The natural course of the disease makes evaluation of this and any similar short-term report most difficult, as the author himself points out. A complicating factor is the presence of tuberculosis in case 2, as demonstrated by sputum inoculation of a guinea pig; and it is at best questionable whether nitrogen mustards should be used in tuberculosis. It is true that many of the changes to normal were remarkable; but whether chloroethyl amine played a role in these changes cannot be deduced from the evidence presented.

S.E.


This is a review of thirteen children with sarcoidosis, aged from 9 to 14 years, in whom observation was made for periods as long as seven years. The authors point out that there are only twenty-one previously reported cases in the literature, and that the disease, although uncommon, is not rare in the young age groups.

S.E.

HEMATOPOIETIC TISSUES


Erythrocytes of the salmon, carp and fowl have been used as a source for obtaining isolated chromosomes. The procedure for the isolation of chromosomes was the first part of a method used by the authors to prepare a deoxyribose nucleoprotein complex-chromatin. The isolated threads showed a definite organization along their axis, a tightly coiled helix and at least two chromonemata. Since few of the
cells were in mitosis, the isolated chromosomes were liberated from interphase nuclei. This is additional evidence that chromosomes retain their individuality during interphase, or the so-called resting stage.

O.P.J.


Lymphocytes of the calf thymus are a convenient source for obtaining isolated chromosomes. The method of isolation was somewhat similar to that used for fish and fowl blood. By fractionation of the isolated chromosomes it was shown that 90 to 97 per cent of the mass was largely desoxyribose nucleohistone. The insoluble residue (or residual chromosome) contained a nonhistone, tryptophane-containing protein, 12 to 14 per cent ribose nucleic acid, and about 3 per cent desoxyribose nucleic acid. For years hematologists and cytologists have described basicchromatin and oxychromatin. It now appears that these two chromatin differ chemically.

O.P.J.


The use of cryptorchid animals made it possible to study the influence of the secretion of the interstitial tissue of the testis upon the thymus and mediastinal lymph nodes. Unlike the results obtained in castrated albino rats, there was no striking enlargement of these organs.

O.P.J.


There has been an increasing amount of evidence to substantiate the concept that cytoplasmic basophilia and a high ribonucleic acid content are characteristic of young and actively growing cells. White has contributed to this by applying Brachet's cytochemical test to bone marrow aspirations from six normal individuals and nineteen with various pathologic conditions. Ribonuclease abolished the basophilia of the cytoplasm and nucleoli in proerythroblasts and myeloblasts. In such cells a fine ring of nucleolus-associated chromatin remained which was more deeply staining in the former. After nucleoli have disappeared in the more mature cells, distinct and deeply staining chromatin persists at their site. In pernicious anemia, hemocytoblasts, young normoblasts and young megaloblasts had a high content of cytoplasmic ribonucleic acid. The granular nature of basophilic cytoplasm was shown by the use of acid fuchsin to be due to the presence of numerous mitochondria.

O.P.J.


Bone marrows from several rhesus monkeys and six guinea pigs were studied after applying various cytologic and histochemical technics. Both the basophilia and metachromasia of megakaryocytic cytoplasm were abolished after digestion in a solution of ribonuclease. Although this was true for the rhesus monkey, it was not entirely so for guinea pig megakaryocytes, because they retained some metachromasia after exposure to the enzyme. The distribution of mitochondria seemed to be identical with that of the cytoplasmic objects with an affinity for sudan black. Blood platelets exhibited a faint basophilia and metachromasia. The presence in blood platelets of lipoidal particles, mitochondria and bodies tinged supravitally with neutral red similar to those found in the cytoplasm of megakaryocytes reaffirms Wright's theory for their origin. Further studies were also made on tissue eosinophils and basophils.

O.P.J.

Metachromasia is of interest to the hematologist because certain blood cells possess that property of inducing chemical and chromatic changes when stained with suitable dyes. Wislocki and his associates have investigated this in tissue sections of material from rhesus monkeys fixed in four per cent basic lead acetate and stained with one-half per cent toluidin blue. Some metachromasia is due to the presence of mucopolysaccharides, but in other instances it may be referable to the presence of other nucleoproteins and substances of unknown composition. Tissue mast cell granules have a strong affinity for both toluidin and methylene blue. The metachromatic reaction was unaltered by exposure to hyaluronidase. These granules did not give the Bauer reaction after digestion with saliva. These authors were unable to abolish the metachromasia present in megakaryocyte cytoplasm after exposure to ribonuclease.

O.P.J.


The authors have developed a new method for using sudan black in differentiating various granules as seen in dry smears of normal blood. The results have been very consistent and better than those obtained previously. Such things as fixation in formaldehyde vapor and the use of a buffered solution containing phenol have improved the technic. Sheehan and Storey attribute a mordanting action to the phenol. In this connection it must be pointed out that another interpretation of the action of phenol is possible; namely, that of lipophanerosis. The authors made the interesting comment that in certain blood diseases neutrophilic (polymorph) leukocytes do not contain sudanophilic granules. This will be described in a subsequent paper.

O.P.J.


The effects of solutions containing 1 part lead salt to 40,000 parts water on the hematopoietic systems of larval bullfrogs was studied in 40 control and 143 test animals. Although both lead acetate and lead nitrate produced similar results, the action of the latter was more rapid. Among the first changes observed were those in the mature erythrocyte which became fragile and subject to fragmentation. There was a subsequent increase of immature erythrocytes and hemoblasts. Differentiation seemed to occur in the peripheral blood with the eventual formation of some spheroidal erythroplastids. The liver, which is not normally a hematopoietic organ in the tadpole, developed lymphogranulopoietic areas in the periportal connective tissue. In advanced stages of lead poisoning degenerating erythrocytes were present in the sinuses of the spleen. In this situation they remained unphagocytized because of toxic alterations in the histiocytes. Normally the intertubular mesonephric connective tissue of the larval frog is the chief hematopoietic center. This became rapidly depleted of its cellular elements and, for some unknown reason, eosinophilic myelocytes actually increased there.

O.P.J.


This review of the basic anatomic physiology of cellular membranes—specifically the capillary wall—discusses fundamental rather than clinical interpretations of the problems of capillary tonus, permeability, and fragility. From the point of view of structure, three types of membrane may be discriminated: (1) one in which the cell itself determines the permeability of the membrane (e.g., the proximal tubule of the kidney); (2) one in which both the cell and an intercellular material determine the permeability (e.g., intestinal mucosa); and (3) one in which permeability of the membrane is determined exclusively or largely by the intercellular material. Into this last group the authors place the capillary membrane.

The capillary wall consists of endothelial cells plus an interendothelial cement elaborated by these cells; an endocapillary lining which seems to be an adsorbed layer of a blood protein; and a pericapillary sheath of connective tissue. With regard to the interchange of material between blood and tissue (i.e., permeability of the capillary), the interendothelial cement substance serves as the basic framework. It acts as an ultrafilter the selective properties of which depend directly on variations in its porosity.
Its porosity (and therefore the permeability of the membrane) is high, as compared with the permeability of the endothelial cells themselves, which is low. The porosity of the intercellular material may be affected by mechanical stretching, by chemical and pH changes of the surrounding medium, etc. This cement substance is thought to be produced by the endothelial cells, and to be in a constant state of usage and replacement.

The endocapillary lining seems to be an adsorbed layer of blood protein, probably albumin, which penetrates the porous interstices of the cement and helps reduce the pore size of the cement filter. In this way, it helps to reduce capillary permeability. The pericapillary sheath is a condensation of connective tissue which serves mechanically to support the blood capillary.

Changes may occur in any of the three components of the capillary and result in increased permeability. It is of interest that the variations in capillary permeability can be explained by changes in the nonliving components of the capillary wall: the intercellular cement, the endocapillary protein, and the pericapillary connective tissue sheath. The endothelial cell itself apparently often merely produces the cement substance. It is apparent, correspondingly, that it may be difficult to explain a given change in permeability on a single basis, and that it is unreasonable to assume a single permeability factor to account for fluid balance in the body.

This article discusses further the topography of the capillary bed; the roles of precapillary vasomotion and of hydrostatic and colloid osmotic pressures in permeability, the nervous control of capillary permeability, and the significance of various agents affecting capillary permeability. The manner of action of histamine, adrenal cortex, and various agents affecting capillary permeability, are discussed.

S.E.


An ingenious technic is presented for mounting flat segments of vein walls so that the inner coats of the wall can be stained and studied. Benzidine, which selectively stains red blood cells, was used to outline the vascular plexus in vein walls, and silver nitrate was employed in staining and studying the inner coats of vein walls.

Increasing endothelial desquamation was found in vein segments which were dissected free from all blood, lymphatic and nerve supply to the walls, but which were left unobstructed in situ. Despite the presence of raw, muscular surfaces, however, intravascular clotting seldom occurred, presumably because the velocity of blood flow was maintained. When blood flow was reduced by eighty to ninety per cent following the application of small metal clamps without isolating the vein from its bed, there was less resultant endothelial damage but slightly greater tendency toward thrombosis. When isolation and partial obstruction of vein segments were combined, there was a high incidence of intravascular clotting, but it is acknowledged that further experience with this procedure will be required before firm conclusions can be drawn regarding the mechanisms involved.

L.E.Y.


The Italian authors have assembled in a book the results of twelve years experience concerning bone marrow.

They studied in succession normal marrow, pernicious anemia, leukemia, and erythroblastosis.

In vitro, normal marrow loses its organic characteristics; immature granulocytes develop in a slow and restricted manner. During the course of this development, one may see a new generation of granuloblastic cells which are derived either directly from hemocytoblasts or possibly from preexisting granuloblasts. Erythroblasts develop much more rapidly and completely than granulocytes to the orthochromatic stage or even, after a reticular formation, arrive at the red anuclear globular stage. Very few immature erythroblasts develop to replace the elements which have evolved.

When after fifteen to twenty days the parenchymal elements have gradually disappeared the histoid cells develop and are soon transformed into a pure culture of fibroblastic elements.

In myeloid leukemia, one meets a similar evolution; the degree of development is no different, and the time of survival of the parenchymal cells is analogous.
ABSTRACTS

In lymphoid leukemia, the same time of survival and development of the fibroblastic cells is observed. One may note, in addition, morphologic modifications of the lymphocytes (the chromatin less thick, the protoplasm more diffused), and this would not be a matter of actual return to the most immature forms, but the mere adaptation of the cells to a new environment. It is important to note that the histiomyocytic cells observed do not ever result in lymphocytic elements; the matter concerns two separate developments, and the differences can only make themselves plain successively in the culture: the lymphocytes disappear when the histiocytes are transformed into the fibroblasts, which alone survive.

In acute leukemia, a new fact is obtained: the possible survival of parenchymal elements after a month of culture. Different in structure, the root cells of acute leukemia do not give rise to macrophages or to true monocytes, nor to fibroblasts. The extremely polymorphic elements which can take shape, although of histoid morphology, are of hemocyto-myeloblastic appearance, but do not appear to form any cells of a normal grouping. The cells of acute leukemia are of the 'preparenchymal' type, which doubtless explains their particular faculty for survival, though they do not evolve toward the fibroblastic stage.

Thus chronic leukemia and acute leukemia appear to possess very different histogenesis.

The authors expect shortly to issue a classification of the leukemias. We limit ourselves here to a brief resume of the results of medullary culture in the leukemias, which displays at the same time the interesting aspects and the actual limits of the method of study to which Fieschi and Astaldi have devoted themselves.

J.P.S.

THE EFFECTS OF EXPERIMENTAL HYPERPYREXIA AND RESTRAINT ON THE BLOOD AND HEMOPOIETIC ORGANS OF THE ALBINO RAT. J. S. Latta and W. W. Nelson. From the Department of Anatomy, College of Medicine, University of Nebraska, Omaha, Nebraska. Am. J. Anat. 82: 321-351.

The authors studied the effects of sustained fever on the blood values of white rats, and in a preliminary study they used as controls two animals which were untreated except for handling and one which was restrained but untreated. Quite unexpectedly the restrained animal showed marked changes. Hence, the problem of determining what effects on the blood and blood-forming organs were attributable to hyperpyrexia alone arose.

Albino rats were placed in a Kettering hypertherm and after rectal temperatures reached 103-104°F., they were maintained at this temperature for five hours. Blood samples were obtained from the tail. Both sustained fever and restraint produced a marked lymphopenia after the first hour. Neutrophils first increased then decreased moderately and finally increased again during the fourth and fifth hours of hyperpyrexia. In the case of restraint, neutrophils increased steadily to twice the absolute value in five to six hours. This increase was not sufficient to offset the lymphopenia in heat-treated animals. Consequently, a leukocytosis did not occur. Tissue studies indicated a relative marrow depression in hyperpyrexia and no evidence of a neutrophilic infiltration in various organs. The marked lymphopenia was accounted for in part by a failure of delivery and to a greater extent by degeneration and destruction of lymphocytes.

O.P.J.


Most hematologists are so engrossed with problems related to clinical hematology that not infrequently they forget about the vast and relatively unexplored field of comparative hematology. In the human, some disturbances of the hematopoietic organs may be recognized and classified according to the type, amount and distribution of hematopoietic tissue. Biologists have put somewhat similar information to an entirely different use. Knowledge that hematopoietic loci may be variously distributed in the organs of amphibia, and that they may be either lymphoerythropoietic or lymphogranulopoietic, has made it possible to determine the phylogenetic position of certain genera.

O.P.J.

In view of the work of Selye with the alarm reaction, and of White and Dougherty with an interrelationship between adrenal cortex and lymph nodes, it was thought of value to see whether other agents known to affect the adrenal glands might lead to similar effects in the lymph nodes. Specifically, since insulin is known to stimulate the adrenal medulla by way of the sympathetic nervous system, would the same results upon lymphoid tissue occur following this endogenous release of epinephrine, as occur, for example, following an alarm reaction, or the injection of (exogenous) adrenalin?

The author injected one group of rats with epinephrine daily for three to seven days; and another group of rats with ascending doses of insulin daily for three to seven days. Other rats served as controls. The rats were then killed and their tissues weighed. With few exceptions, it was found that adrenal tissue enlarged after insulin or epinephrine; and that lymphatic tissue shrank after insulin or epinephrine.

The author postulates that various stimuli (e.g., injury; insulin) which cause discharge of adrenal medulla secretion, in so doing also cause slower but more sustained cortical activity (after injury, for example, the cortex enlarges, loses its lipids, and secretes its hormones). Hence, perhaps, the adrenal medulla is a link in the pituitary-cortex interrelationship; and, also, stimuli which affect this relationship may therefrom affect the lymph nodes.

Although no blood counts were done, mention is made of a previous observation that insulin produces lymphocytopenia and dissolution of cells in lymph nodes (Latt, J. S., and Henderson, J. W.; Folia haematol. 2: 1106, 1937); i.e., a reaction similar to that found by Selye following an alarm reaction, and by White and Dougherty following the injection of adrenal cortex.

**SE.**

**AN EXPERIMENTAL STUDY OF LYMPH NODE REGENERATION IN RABBITS.** 

Although many reports have been made on the regeneration of lymph nodes, they have not been too careful in reporting the age of the animals, method of node removal and length of postoperative period. The results in the present article were obtained after a study of 270 specimens of popliteal nodes obtained by unilateral and bilateral excisions of 152 rabbits (three days to three and one-half years in age). Popliteal lymph nodes prior to fixation measured 8 X 12 mm. Supernumerary nodes were very rare. Regeneration, progressive differentiation of undifferentiated tissue, was optimum in incidence and quality in rabbits about one and one-half months old, thirty days postoperatively. This occurred from lymphoreticular tissue in perinodal areolar tissue. One of the most outstanding findings was that senile nodes may be activated by typhoid vaccine so that there is perhaps no complete loss of structure and function.

O.P.J.

**AGE CHANGES IN LYMPH NODES.** 

Over three hundred lymph nodes from deep cervical, inguinal, bronchial, mesenteric and axillary groups were obtained from 150 autopsies. Three groups of patients were equally divided among those derived from (a) accident cases, (b) acute medical and surgical cases, and (c) chronic disease cases. With respect to structural differences and response to aging, lymph nodes may be divided into two groups—superficial and deep. There is a difference between cortical and medullary reticulum (argyrophil fibers) in both. In the cortex the meshwork is larger, more open and polyhedral in shape than in the medulla. Superficial nodes undergo regression at puberty. This is characterized by a shrinkage of lymphoid tissue on the capsule rather than the hilum which results in a cup-like structure filled with connective tissue. Germinal centers of the superficial nodes are fewer in number, smaller in size and they possess smaller pale centers than those of deep nodes. Although there is a diminution in size of germinal centers of deep lymph nodes from puberty onward, they can still be found at the age of eighty. Regressive changes in deep lymph nodes are unlike those in the superficial group in that there is a gradual return to the fetal type of node.

O.P.J.
AGE CHANGES IN THE DEEP CERVICAL LYMPH NODES OF 100 WISTAR INSTITUTE RATS. W. Andrew and N. V. Andreu. From the Department of Anatomy, George Washington University School of Medicine, Washington, D. C. Am. J. Anat. 82: 105-165, 1948.

The present article is part of a general study of age changes in lymphoid tissue. Since it has been shown that lymph nodes vary histologically in different regions of the same individual, only deep cervical lymph nodes from 100 Wistar rats were studied. The range in age was from 21 to 1170 days. Weights of nodes from young adults were about five times greater than those from younger animals. This significant weight increase was usually maintained in the senile group. There was a reversal of this relationship with age, so that senile animals had nodes with practically no cortex. Reaction or germinal centers were present in about forty per cent of the younger animals; they increased in the young adults and then gradually decreased in the senile ones. Clusters of epithelial cells which were found in younger animals tended to disappear with age. The origin and significance of these cell masses are unexplained. Lymph nodes from senile rats contained large cavities lined by reticulo-endothelial cells, usually located in the medulla, but some involved the cortex. These cavities contained coagulated fluid, lymphocytes, neutrophil and a few macrophages. Some lymphocytes seemed to be undergoing a pycnotic degeneration. Mast cells and eosinophils were much more frequent in senile rats than in younger ones. In general the capsule was thicker in the senile group.

O.P.J.


Andrew and his associates previously studied and reported the behavior of lymphocytes in intestinal epithelium. The present investigation on tracheal and bronchial epithelium was along similar lines. Lymphocytes which were the predominant cell type were usually found in a basal position. There was no evidence for the intercellular transformation of lymphocytes into neutrophils. Pycnosis of lymphocytic nuclei was more pronounced after the cells had reached an apical position.

O.P.J.


Murray was able to maintain growing cultures of pure thymus epithelium because in making subcultures he left a bit of epithelium in place and removed the remainder. This procedure apparently reduced the amount of trauma sufficiently so that the cultures would resume their growth after a quiescent period. Thymocytes behaved in a manner similar to true lymphocytes. Macrophages derived from both sources were indistinguishable. One of the most important observations was the finding on two occasions of mitoses resulting in two different daughter cells. One cell resembled pure thymic epithelium and the other a lymphocyte. This is of interest because most hematologists have agreed with Maximow's concept of differentiating mitoses. Sabin, however, considered that endothelial cells might give rise to daughter endothelial cells or megaloblasts depending upon the orientation of the spindle. If it can be proved that the present findings were not due to a diminished oxygen supply, then not only would Sabin's views be supported but also those regarding the derivation of thymocytes from epithelium.

O.P.J.

THE SPLEEN


Since Barcroft's demonstrations in 1923-1925 that the spleen of dogs is capable of storing large quantities of blood for use in emergencies (exercise, administration of epinephrine), the reservoir function of the human spleen has been considered correspondingly well established. Little experimental verification of this thesis, however, has been offered.

In the present report, Nylin tested whether severe muscular work could be shown to result in splenic
contraction and emptying of the postulated stored blood in human adults. He injected blood containing labeled red blood cells (labeled with radioactive phosphorus according to a technic of the author) into 5 healthy men, and took blood samples in 10 and again in 15 minutes after the injection. The subject was then made to do severe muscular work, and two further blood samples taken; the first at 25-32 minutes, the second at 27-39 minutes. All samples were subjected to radioactivity determinations in a Geiger-Müller counter. It had previously been shown by the author that the radioactivity of the blood remains constant for at least 60 minutes after such an injection; hence, the volume of the circulating red cells could be measured by the radioactivity of the blood. Presumably, if any reservoir of blood was present which responded to the severe muscular exercise, discharge of red cells from this reservoir would change the radioactivity of the circulating blood.

Nylin found, actually, that there was no significant change in the specific radioactivity of the blood after exercise within the time studied. For all the patients, the mean circulating cell volume before work was 1,408 ml., as compared with 1,471 ml. after work; and the mean circulating total blood volume before work was 4,934 ml., as compared with 4,855 ml. after work. Since the amount of the red cells was unchanged, it was concluded that there was no reservoir which empties red cells into the circulation after work. This result is in contrast with the work of Bancroft (in dogs), and with the commonly held opinions that epinephrine contracts the spleen and thereby increases the numbers of circulating red cells. If verified, these conclusions would be of great theoretic importance.

S. E.