HEMATOPOIETIC TISSUES AND CYTOLOGY
OF BLOOD CELLS


In the past many purely morphologic studies of the first circulating blood cells in mammalian yolk sacs have been undertaken to determine their nature. The present paper is unique in that it brings a new and interesting technic into this controversial field, the results of which now shed new light on this problem. Pieces of rat yolk sac from 11 to 17 day embryos were grafted into the anterior chamber of adult rat eyes. These were permitted to remain there for periods ranging from 1 to 30 days after transplantation. Grafts were vascularized by the third or fourth day and the debris incident to transplantation was cleared away. One of the first and most spectacular changes was the rapid and complete disappearance of primitive erythroblasts. The mechanism for this is not clearly understood. While this process was going on, large numbers of definitive erythroblasts, megakaryocytes, and myelocytes developed from mesenchymal cells. This finding is most striking since these cells normally form an inconsequential percentage of the yolk sac cells. From a theoretical point of view this is extremely important because it demonstrates that cells which develop primarily into primitive erythroblasts can unfold other potentialities by changing their environment. In addition to these experiments, Bleck also attempted unsuccessfully to influence embryonic erythropoiesis by administering various substances to the mother.

O. P. J.


For a number of years Wislocki and his associates have been investigating various tissues by means of physical and histochemical technics. The results of the present article should come as a breath of fresh air to some morphologic hematologists because Wislocki and Dempsey have investigated the lipoids, glycogen, calcium, iron, mucoproteins, nucleoproteins, and various enzymes present in several hematopoietic tissues from rhesus monkeys. Lipid material was demonstrated in neutrophilic and eosinophilic granules. The latter, quite unlike those reported by Baililif and Kimbrough (Anat. Rec. 96 [Suppl.]: 67, 1946), were described and pictured as solid spheres. Minute sudanophilic bodies were also found in megakaryocytic cytoplasm and in blood platelets of the circulating blood. Glycogen was demonstrated only in neutrophilic leukocytes. From a histologic point of view, it is important to learn that nerve fibers actually accompany the arterial vessels in the marrow. While others have shown that the basophilia of lymphocytes is due to the presence of ribonucleoprotein, the present authors have demonstrated that this also obtains for erythroblasts, myeloblasts, and myelocytes. Tissue mast cells of the rat and human being were studied more extensively than other cells, and the results indicated that there is a
species difference with respect to the presence of lipoidal material. The basophilic granules were not digested by either ribonuclease or hyaluronidase.

O. P. J.


Blood spots in chicken eggs are of historical importance since they were first described by Aristotle. At present they should attract the attention of investigators working on leukemias since this may be the way in which avian leukemia is transmitted. Lucas studied blood spots in eggs present in the oviduct as well as those held for varying periods after laying. Because of Bloom’s criticism of the dry smear method, Lucas relied chiefly on wet fixed smears, which in some instances were followed by the Feulgen reaction. Differential blood counts were made of the blood spots and blood of the hens. Lymphocytes and monocytes seemed more susceptible to damage than eosinophils and basophils. The latter were most resistant to degeneration. Macrophages in blood spots had their origin from fibroblasts of parent tissue rather than from vascular lymphocytes.

O. P. J.


Mast cells and their granules have been the subject of many morphologic and experimental studies. The present authors have attempted to determine their chemical nature and physiologic significance by using various histochemical techniques. The preputial gland and mesentery of the rat were the tissues selected for study because of the relatively high incidence of mast cells. Various techniques were used to demonstrate the following: alkaline and acid phosphatase, cytochrome C, cytochrome oxidase, lipase, glycogen, peroxidase, free iron, lipoids, and aldehydes. The results showed that alkaline phosphatase was localized in the majority of mast cell granules. Acid phosphatase activity was present in only a few. The cytochrome C. cytochrome oxidase system was also present. Glycogen and lipoid substances were absent.

O. P. J.


The present article confirms the observations made by Sheehan that leukocytic granules contain lipoid (J. Path. & Bact. 45: 580, 1935). Discombe was unable to demonstrate by means of Baker’s formol-calcium Sudan black technic a Golgi apparatus in the pale-staining clear area around which specific granules first appear. The difference in the appearance of neutrophilic granules stained with Leishman’s stain and those colored with Sudan black appears to be due to the absorption of one of the azures on the surface of the lipoid granule. The size, appearance, and distribution of these granules were indistinguishable from those seen in oxidase preparations.

O. P. J.


In previous articles Andrew and his associates have shown that lymphocytes migrate through epithelial cells of the duodenum in black mice of strain C 57. During this intracellular passage lymphocytes undergo certain changes. The present article reports the results of studying a different strain of mice in an attempt to confirm the previous findings and make additional cytologic observations. Twenty-three white mice ranging from 2 days of age to adults were studied. The youngest animals had fewer migrating lymphocytes in the villi than in the crypts of Lieberkühn. Activity in both of these locations increases with age. Some intracellular lymphocytes undergo mitosis, which may be aberrant. Lymphocytes on the apical side of epithelial nuclei are frequently twice the size of those on the basal side. The Golgi apparatus (Cajal technic) of lymphocytes in the apical region also becomes modified. At first it hypertrophies and later regresses by golgiorrhexis and golgiolysis.

O. P. J.

Kelsall has observed that small lymphocytes contain more desoxyribonucleoprotein (thymonucleic acid), as indicated by the Feulgen reaction, than the nuclei of several other somatic tissues. In view of the fact that Andrew and his associates have recently studied the occurrence of intracellular lymphocytes in intestinal epithelium, Kelsall considered it necessary to determine whether or not there were differences in the chemical composition of these lymphocytes and also those present in animals with a rapidly growing neoplasm. Tissues from 5 control hamsters and 5 with a subpanniculary implanted mixed-cell sarcoma were studied. The Peyer's patch nearest the ileocecal junction was removed in each case with an adjacent piece of ileum. The vast majority (96 per cent) of the small lymphocytes within the columnar epithelial cells were located between the nucleus and the basement membrane. Neither the presence of a neoplasm nor the intracellular position of small lymphocytes altered the density of lymphocytic chromatin as compared with similar cells in Peyer's patches. Extracellular particles of desoxyribonucleoprotein were very scarce in the cytoplasm of epithelial cells. This has been interpreted to indicate that small lymphocytes in this location neither disintegrate nor act as trophocytes. Plasma cells were found in the stroma of villi and a few were also present in the basal cytoplasm of columnar epithelial cells. The author has suggested that perhaps small lymphocytes within the epithelial cells may synthesize basophilic cytoplasm and then migrate to the stroma of the villi. Many of the findings reported in this article are contrary to those of Andrew and Collings (Anat. Rec. 96: 445, 1946) and they may be explained on the basis of species difference as well as differences in the intestinal tract from which the material was obtained.

O. P. J.


Duodena obtained from 10 autopsied human subjects were studied to determine whether or not lymphocytes entered epithelial cells and transformed there in a manner similar to that described previously for the mouse (Anat. Rec. 93: 252, 1945). The present authors reported that lymphocytes near the basal ends of epithelial cells are intercellular while those in the apical ends are intracellular. Lymphocytes in the latter position appeared to be undergoing degeneration changes as indicated by pyknosis and fragmentation. In spite of their intracellular position, lymphocytes may undergo mitosis. These findings, contrary to those made by Kelsall on the hamster, may be due to species differences.

O. P. J.


Siderocytes, or red cells containing blue-green granules after staining with αα'-dipyridyl and potassium thiocyanate in dilute hydrochloric acid, were demonstrated in the blood of cat, dog, and man (and of other mammals by other observers). Siderocytes appeared most rapidly and in largest numbers in blood exposed to unfavorable conditions of storage and to chemical agents such as acetyl phenylhydrazine. Once the siderotic granules have been extruded, the erythrocyte appears to be normal morphologically but is probably more susceptible of phagocytosis. The siderocyte is probably the source of urinary siderotic granules described by Rous. The author considers it likely that the siderotic change can take place only once in any given cell, and that only a definite (and probably small) fraction of hemoglobin can be degraded by this mechanism. It is thought that the stainable, nonhematin iron of the siderotic granules is closely associated, if not identical, with the "easily split" blood iron described by other observers, and that this iron should be regarded as catabolic.

There is an obvious need for further studies on siderocytes, particularly in mammals with anemia and with various forms of jaundice.

L. E. Y.
The in vitro culture of bone marrow cells is potentially a powerful tool for the study of the development of marrow cells, but to date technical difficulties have discouraged widespread use of this procedure. At best, such culture can be successfully maintained for only a matter of hours; during this time, however, studies of various types can be most instructive.

Warren employed the Warburg microrespirometer to study the metabolic requirements of the cells of the bone marrow. His studies led to several conclusions:

1. Erythroid cells showed a predominance of respiratory (oxygen-utilizing) over glycolytic (lactic-acid-forming) activity. Myeloid cells, on the contrary, showed a predominantly glycolytic metabolism.
2. The normal metabolism of the myeloid cells was virtually identical with that of tumor cells.
3. Potassium arsenite was found to depress the (oxygen) respiration of normal and leukemic myeloid cells. Thio-uracil in high concentrations, but of the same magnitude as attained in the bone marrow of patients receiving the drug, also depressed myeloid respiration. It was postulated that this might be the mode of production of neutropenia by these drugs. It is of especial interest that pyridoxine was of no protective value in this regard in vitro.
4. When serum instead of saline was used as the substrate, both respiration and glycolysis were increased to twice their rates.

Details and further results with this and similar technics may be expected to provide fundamental explanations of certain problems of bone marrow cells, and of growth in general.

S. E.

ANEMIA


The anemia which develops in most patients after full thickness burns involving 20 per cent or more of the body surface has been intensively studied by the authors. Bone marrow activity was estimated by reticulocyte counts and by measuring the rate of utilization of radioiron. Transfusions of red cells containing radioiron were used in estimating total red cell mass of the burned patients. Following such transfusions the concentration of radioactivity in the peripheral blood remained constant under normal conditions, decreased if the donated cells were rapidly destroyed or if the patient’s marrow became active, and increased if the patient’s own cells were preferentially destroyed. Operative blood losses were computed from the concentration of acid hematin or of the radioactivity in the washings of sponges and drapes.

Three stages of the anemia of burns are described and it is explained that only one or two of the stages may be encountered in any one case. The first stage, lasting only a few days, is due to destruction of cells and is associated with a transient increase in erythrocyte fragility, the exact cause of which was not investigated by the authors. Depression of erythropoiesis is considered a likely cause of the second stage of anemia, observed during the first week or ten days following severe burns. The third stage, which may be separated from the second by a brief period of positive red cell balance, appears in the third or fourth week and is attributed to multiple causes including hemorrhage from the wound. The amounts of blood lost during excision of wounds and in changing dressings and the number of transfusions required to maintain a normal total red cell mass were surprisingly large. The roles of infection, iron deviation, and the “alarm reaction” are discussed, but no mention is made of the part that may be played by intravascular sludge formation. The need for early replacement of red cells is stressed and it is concluded that in controlling therapy there is no adequate substitute for serial measurements of total red cell volume.
All physicians who may be charged with the responsibility of treating severely burned patients would do well to read this impressive paper.

L. E. Y.


The authors analyze 10 cases having flame burns of from 30 to 80 per cent of their body surfaces. None of the 7 patients who survived the initial period of shock received adequate amounts of whole blood during the first week of illness to prevent the development of anemia. The data presented, however, serve to emphasize the urgent need for intensive, controlled whole blood transfusion therapy during the shock period. Whole blood is advocated in preference to blood substitutes because (1) it restores viscosity if plasma proteins have been depleted, (2) hemoglobin is a good source of protein, (3) the transfused red cell mass has a sparing action on body proteins by displacing plasma volume, (4) assimilation of protein given by mouth is improved after correction of anemia, and (5) because there may be a physiologic increase in blood volume when a large granulating surface is forming. It is admitted that the last-named consideration requires further study.

In addition to transfusions of whole blood, the authors recommend oral administration of sodium chloride solution in combination with lactate or bicarbonate in preference to sodium lactate solution alone.

L. E. Y.


These two reports describe, for the first time, the occurrence of aplasia of the marrow following the use of tridione for convulsive disease. In the first case a 16 year old girl with grand mal was maintained on tridione and methyl-phenyl-ethyl hydantoin (a compound related to dilantin) for six months, when she rapidly developed aplastic anemia and died despite therapy. The second case was that of a 24 year old woman who had had petit mal since the age of 5. After receiving tridione and phenobarbital for a period of ten months, with good symptomatic response and no untoward effects, she suddenly developed marrow aplasia and died seventeen days later despite various attempts at therapy.

It is a fair assumption that in both instances the offending chemical was tridione (3, 5, 5-trimethyl-oxazolidine-2, 4-dione). The cautious use of this material, as recommended by the authors, is certainly indicated.

S. E.


The advent of radioactive iron promises to provide a powerful tool for hematologic investigations. The present report demonstrates an ingenious mode of its application to the labeling of red cells.

It is known that radioactive iron given intravenously is incorporated into hemoglobin and begins to appear in the blood stream within twenty-four hours. That is, radioactive iron is taken up by erythrocytes only during the period of their formation. Hence, the radioactivity of a blood sample after a given dose of radioactive iron is a measure of the rate of addition of new erythrocytes to the blood stream. A patient who was inoculated with P. vivax was given radioactive iron intravenously, and blood was subsequently taken for erythrocyte counts, parasite counts, and parasite concentration. It was found that the radioactivity of the concentrates greatly exceeded the radioactivity of whole blood. Since radioactivity is limited to red cells formed after the injection of the radioactive iron, the concentrate
therefore contained a large number of young cells. The method of concentration, however, was for the malarial parasite; i.e., the concentrate contained largely parasitized cells. Hence, it could be concluded that the parasite preferentially invades the young red cell.

Of even greater importance than this result is the ingenuity of the method, which serves to demonstrate the tremendous potentialities of the use of radioactivity in fundamental hematologic investigations.

S. E.

CONGENITAL HEMOLYTIC ANEMIA—A CASE REQUIRING EARLY SPLENECTOMY. E. Conrad and R. E. Schmidt.

From the Department of Pediatrics, Duke University School of Medicine and Duke Hospital, Durham, N. C. Am. J. Dis. Child. 72: 731–733, 1946.

A case is reported of a white boy who developed classical evidence of congenital hemolytic anemia at the age of 17 days. Pertinent findings were anemia, jaundice, splenomegaly, hepatomegaly, microspherocytosis, reticulocytosis, nucleated red cells in the peripheral blood, and increased fragility of erythrocytes in hypotonic saline. The mother's blood also showed spheroctysis and increased osmotic fragility. Although detailed studies on the survival of donated cells were not made it is stated that the child’s hemoglobin fell rapidly following transfusion. Splenectomy was performed at the age of 10 weeks with good response. It is implied that this is the earliest age at which symptoms and signs of congenital hemolytic anemia have been reported. Sections of the spleen are described as showing an increase of iron pigment and fibrosis in the sinusoidal tissues. No mention is made of the degree to which the splenic pulp was filled with red cells.

L. E. Y.

MORPHOLOGICAL CHANGES IN THE RED CELLS IN RELATION TO SEVERE BURNS. A. Brown.


It was reported previously that when burns involve more than 15 per cent of the body surface, they are frequently accompanied by a moderately severe anemia. The present article extends observations along this line to include variations in the mean corpuscular volume, mean corpuscular diameter, and mean corpuscular average thickness in relation to osmotic fragility. In cases with the less severe burns microspherocytosis was present in the stained films, while cases with the more severe burns showed fragmentation and degeneration of red cells within a few hours after burning. Brown concluded that these morphologic changes were due to the direct action of heat on the cells.

O. P. J.

POLYCYTHEMIA AND THE LIFE SPAN OF THE ERYTHROCYTE

The Increase in Hypoxia Tolerance of Normal Men Accompanying the Polycythemia Induced by Transfusion of Erythrocytes. N. Pace, E. L. Locner, W. V. Consolazio, G. C. Pitts, and L. J. Pecora.

From the Naval Medical Research Institute, Bethesda, Maryland. Am. J. Physiol. 145: 151–163, 1946.

These studies were undertaken in an attempt to determine the effect of artificially produced polycythemia on tolerance to high altitudes. One thousand cc. of red blood cells were transfused into each of 5 normal subjects during a period of four days. The mean hematocrit was increased from 46.1 to 58.3 per cent, an increase greater than had been expected, and believed to have been accounted for by hemococoncentration. No adverse symptoms attributable to the polycythemia were encountered, and there was definite increase in the tolerance of the subjects to hypoxia, as judged by the pulse rate during exercise under conditions of lowered oxygen tension. The exercise pulse rate dropped sharply following the transfusion of red cells and gradually increased during the following fifty days as the hematocrit decreased toward normal. It was estimated that the transfused subjects at an altitude of 15,500 feet had the same tolerance to exercise that the untransfused controls had at 10,300 feet.

The artificially induced polycythemia persisted for approximately fifty days, about half the time estimated for the maximum life of transfused red blood cells. This would indicate that either there had
been an increased rate of erythrocyte destruction or a decreased rate of new erythrocyte formation. The latter seemed more probable, since there was no increase in urinary pigment excretion, but a definite decrease in reticulocytes followed the transfusions. This suggested that normal erythropoiesis was inhibited to some degree by the presence of the injected erythrocytes.

J. F. R.


Glycine labeled with N\textsuperscript{15} was fed to a man, resulting in the formation within the erythrocyte of heme containing a comparatively high concentration of the isotope. The level of N\textsuperscript{15} was followed in the circulating blood. The value rose rapidly to a high level, remained constant for about three months, and then fell. From this the average erythrocyte life span was calculated to be 127 days.

It was apparent that the heme pigment was a fixed molecule, not involved in the dynamic metabolic exchange shown to exist in most tissues. Its final disappearance coincided with the destruction of the erythrocyte. The figure of red cell life span by this method corresponds well to measurements by other reliable technics.

C. A. F.

**Blood Grouping and the Rh Factor**


Cerebral spinal fluid of normal chloride and protein content was found to have an inhibitory action on the anti-Rh\textsubscript{0} agglutinating antibody. Agglutination of Rh\textsubscript{1} cells was completely inhibited when cerebrospinal fluid was used in place of saline as the diluent for the sera and the suspension media of the cells in three out of six sera, and the inhibition was partial but incomplete with three sera. This inhibitory effect was partially counteracted by the addition of human serum and was completely overcome by the addition of albumin. Fluids containing the inorganic and some of the organic constituents of cerebrospinal fluid failed to produce inhibition. Five of the fifteen samples of cerebrospinal fluid used were Rh negative individuals, so the inhibition of agglutination was not due to the presence of the specific substance and was probably dependent on a variable factor in the serum. Cerebrospinal fluids from other species exerted an inhibitory effect, as did abnormal human fluids of increased protein content. Since the inhibitory effect of cerebrospinal fluid is overcome by agents known to aid agglutination of cells by the blocking antibody, it is suggested that inhibition may be related to the conversion of the agglutinating antibody to a blocking antibody. It is not clear whether the presence of cerebrospinal fluid prevents the sensitization of cells by the anti-Rh antibody or fails to provide the necessary media for agglutination. Observations designed to detect sensitization of cells exposed to the cerebrospinal fluid anti-Rh serum mixture should settle this point.

R. S. E.

**Hemoglobinemia and Bilirubinemia**


This is the first report calling attention to the hazard of intravascular hemolysis in transurethral
prostatectomy. The authors report a case of fatal anurea and postulate that the distilled water used as irrigating fluid during the operative procedure caused hemolysis by gaining access to the blood stream. Similar observations have been made at least two other clinics and provide ample evidence of the potential hazard of this operation when distilled water is used.

C. A. F.


By the fifth day of life, infants generally show an increase in the serum bilirubin. It has been shown that the level of free fatty acids and soaps in the serum of animals and human beings increases after the ingestion of fat; and, according to the authors, are enough to account for hemolysis sufficient to cause a significant bilirubinemia. Furthermore, the diet of the newborn infant is stated to have a higher fat content than that of the fetus.

On the basis of these postulates, Freeman and his co-workers obtained umbilical cord blood from 50 newborn children and then divided them into three groups according to the (controlled) fat contents of their respective formulae. Their results were as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum bilirubin at birth</th>
<th>Serum bilirubin on 5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (3.6% fat)</td>
<td>1.36 mg. %</td>
<td>5.41 mg. %</td>
</tr>
<tr>
<td>Group I (1.8% fat)</td>
<td>1.36 mg. %</td>
<td>4.21 mg. %</td>
</tr>
<tr>
<td>Group II (0.05% fat)</td>
<td>1.36 mg. %</td>
<td>3.09 mg. %</td>
</tr>
</tbody>
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The conclusion drawn from these results is that the amount of fat ingested plays some role in the production of neonatal icterus, by affecting the amount of hemolysis. This conclusion seems justified, although it would be desirable to have erythrocyte levels to judge the relationship between the occurrence of bilirubinemia and destruction of red cells. There are various causes for jaundice in the newborn; and even so-called ‘physiologic’ jaundice is probably a heterogeneous group, due perhaps in some cases to the mechanism here postulated.

S. E.

**AGRANULOCYTOSIS AND LEUKOPENIA**

**SPHINGOMYELINS: THEIR ACTION ON BLOOD CELLS, PARTICULARLY LYMPHOCYTES; THEIR SHARE IN THE NUCLEINATE-LIKE ACTION OF THE ETHER-INSOLUBLE FRACTION OF BRAIN LIPIDS.** E. H. Tompkins.

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn., and the Laboratory of Applied Physiology, Yale University, New Haven, Conn. Bull. Johns Hopkins Hosp. 63: 57-77, 1946.

The author has presented evidence previously to show that ether insoluble fractions of lipoids (''protagon'') produced a leukopenia followed by a lymphopenia and granulocytosis, then a lymphocytosis following injection into rabbits. Sphingomyelin obtained by further fractionation of the above lipoids produced a reaction characterized by a lymphopenia and a moderate granulocytosis. The circulating lymphocytes began to decrease in numbers shortly after injection and reached the lowest in 5 to 7 hours later when the number varied from 55 to 80 per cent of the control value. The granulocytes reached a peak of increase 3 to 12 hours after injection, showing a 25 to 108 per cent increase over the control values. The number of monocytes varied in relation to the lymphocytes and the numbers of all three elements were back to the control level 12 to 15 hours after injection. Control observations with 5 per cent glucose failed to show similar reactions although egg lecithin produced results somewhat similar but lesser in degree. The author calls attention to the differences and similarities in results obtained with sphingomyelin and the parent material ''protagon'' and suggests that sphingomyelin is responsible for the lymphopenia produced by ''protagon.''' The similarity of the reaction to sphingomyelins to that following injections of pituitary adrenotropic and adrenal cortical hormone is pointed out and the theory of reciprocal relationship between the number of circulating lymphocytes and granulocytes is discussed.
The importance of observations dealing with the effect of organic substances on the number and type of leukocytes in the circulation cannot be overemphasized. Further studies on the changes in the blood picture following the injections of phospholipids in other species should follow.

R. S. E.


The author describes the development of severe granulocytopenia (18 to 50 neutrophils per cu. mm.) in a woman who had received 10 grams of thiouracil in a period of seven weeks. On administration of large amounts of vitamin B6 (pyridoxine), and discontinuance of thiouracil, a prompt increase of the number of circulating neutrophils occurred, as well as their appearance in the bone marrow; and the patient was discharged well.

The relationship between the hematologic response and the use of pyridoxine is difficult to establish. In in vitro studies of bone marrow cells, Warren found that the depression of respiration of myeloid cells by thiouracil could not be prevented by pyridoxine (Tr. N. Y. Acad. Sc. 8: 111-127, 1946). It may be expected that the problem of agranulocytosis in the thiouracil therapy of hyperthyroidism will be eliminated by the use of propylthiouracil. In granulocytopenia due to other causes, especially drugs, the use of so-called ‘bone marrow stimulants’ (liver, folic acid, pyridoxine) will continue on the rationale of possible benefit and no harmful effects, but it is doubtful if they are of any value except in those conditions in which neutropenia is a result of their lack.

S. E.

Agranulocytosis from Thiouracil Occurring during Prophylactic Treatment with Folic Acid.

E. V. Newman and B. F. Jones. From the Johns Hopkins University School of Medicine, Baltimore, Md. J. A. M. A. 132: 77-78, 1946.

Because of the reports of neutropenia developing during thiouracil therapy, and the possible value of folic acid in such neutropenias, the authors attempted to prevent the development of neutropenia by the prophylactic administration of folic acid to a patient receiving thiouracil. The patient was a 35 year old woman with hyperthyroidism, who was given a daily total of 0.050 grams of folic acid, 1.5 cc. of Lugol’s iodine, and 0.6 grams of thiouracil. On the twentieth day of such treatment, she was found to have a severe neutropenia, from which she recovered after two days of cessation of thiouracil together with the administration of penicillin and more folic acid (0.060 grams daily).

It is probable that folic acid plays no role in the elimination of neutropenias which are not due to a specific lack of folic acid or some related compound. The search for an over-all, nonspecific ‘bone marrow stimulant’ is yet unrewarded: pentnucleotide, liver extract, yellow bone marrow, pyridoxine, and folic acid do not seem to qualify.

S. E.

The Effect of Liver Extract and Methyl Acetamide with Para-Chloro-Xylenol on Artificially Induced Leukopenia in Rats. W. R. Platt, S. G. Bluestein, and R. G. Sisson. From the Department of Pathology, Yale University School of Medicine, New Haven, Conn. Yale J. Biol. & Med. 18: 275-279, 1946.

Concise data are presented to show that oral liver extract corrects the leukopenia produced in white rats by feeding insoluble sulfonamides. Control observations showed that CXM (0.5 cc. of methyl acetamide in 15 per cent para chloroxylenol), which ordinarily produces a marked leukocytosis, had no effect on the leukopenia. Liver extract failed to affect the leukopenia produced by benzene although there was a response in this instance to CXM. The active principle in liver extract is thought to be folic acid, which is reduced in amount in the intestinal tract when a purified diet with succinyl-sulfathiazole is given. The reduction in folic acid content may be due to the suppression of bacterial flora responsible for its synthesis. Since liver extract was used instead of a pure form of folic acid the hematologic response cannot be said to be due to folic acid alone in this instance.

R. S. E.
ON THE QUANTITATIVE RELATIONSHIP BETWEEN CALCIUM AND PROTHROMBIN. A. J. Quick. From the Department of Biochemistry, Marquette University School of Medicine, Milwaukee. Am. J. Physiol. 148: 211-221, 1946.

The minimum concentration of calcium chloride required for maximum prothrombin activity of human plasma was found to be approximately 0.0011 M and for dog plasma 0.0004 M. Higher concentrations had no further effect on prothrombin time except that a depressing action began when the calcium exceeded 0.02 M. The optimum or critical concentrations for both human and dog plasma are below the level of the free or ionized calcium.

Quick conjectures that calcium reacts with thromboplastin through an intermediary plasma factor termed a "calcium co-factor." When prothrombin is decreased by feeding dicumarol the minimum calcium concentration required to obtain the shortest prothrombin time is definitely increased (in a cited example, it is increased by a factor of 2). Thus when the prothrombin time is over 10 seconds the amount of ionized calcium present in the blood is no longer adequate for optimum prothrombin activity. This suggests that the increase in coagulation time in marked hypoprothrombinemia from dicumarol is probably due to the combined effect of reduced prothrombin and insufficient calcium. If this is established it will be the first recognized condition in which an insufficiency of calcium is a factor influencing coagulation of the blood.

J. F. R.

THE ROLE OF THE ENDOCRINE GLANDS IN HEMATOPOIESIS


The object of this paper was to determine whether the hypophysectomized rat would respond to a reduced oxygen tension with a bone marrow hyperplasia, a reticulocytosis, an increase in total erythrocyte count, and an increase in hemoglobin that is typical of normal animals under these conditions.

The pressure was reduced in all experiments to 412 mm. Hg., equivalent oxygen tension, 12 per cent of the atmosphere. This corresponds to an altitude of 16,000 feet.

The control rats, in the chamber for periods of 3 to 12 days, showed a 2.1 Gm. increase in hemoglobin, a percentage increase of 5 in reticulocytes, and a 700,000 increase in the total erythrocyte count. The bone marrow was hyperplastic.

Seven rats were hypophysectomized. Eight to 9 days after surgery they were placed in the chamber. They showed changes typical of the normal rats.

Another 7 rats were hypophysectomized. Twenty-five to 30 days after surgery they were placed in the chamber. These animals showed no response to the reduced oxygen tension.

The hypophysis would seem to be necessary for the rat to respond to a decreased oxygen tension with bone marrow hyperplasia, reticulocytosis, and an increase in erythrocyte and hemoglobin levels.

R. C. C.


This paper is concerned with three groups of animals: (1) control adult rats upon which control surgery was performed, the exact technic for hypophysectomy being followed except for the removal of the gland, (2) hypophysectomized animals, and (3) normal control rats maintained on a decreased intake of food comparable to the decrease seen in hypophysectomized animals.
ABSTRACTS

Blood was obtained from the tail and studied at weekly intervals. Six weeks after hypophysectomy the total erythrocyte count had decreased from 8.35 million cells per cu. mm. to between 5.0 and 6.0 million cells; the hemoglobin from 104 per cent to 70 per cent. The control operated rats showed no change from normal. The rats on the starvation diet showed an increase in the total erythrocyte count and the hemoglobin level.

Reticulocyte counts, though variable, showed a decrease following hypophysectomy but remained normal in the other two groups of animals.

There were no changes found in the total white cell count or in the differential white cell count. The bone marrows after hypophysectomy were hypoplastic. No differential counts were made on the bone marrows.

Hypophysectomy in adult rats produced a decrease in total erythrocyte count, a decrease in hemoglobin, a decrease in reticulocyte percentage, and a hypoplasia of the bone marrow. These results were not due to the surgery or the decreased food intake. There were no changes in the white cell counts.

R. C. C.

The Hypophysis and Hemopoiesis. O. O. Meyer, E. W. Thelis, and H. P. Rusch. From the Department of Medicine, University of Wisconsin Medical School, Madison, Wis. Endocrinology 27: 932, 1940.

This paper is involved with attempts to prevent the development of the anemia which is induced by hypophysectomy with injections of various hormones.

Growth hormone from the anterior lobe of the pituitary increased the number of reticulocytes, but this increase was not accompanied by an increase in the total erythrocyte count or the hemoglobin level.

Injections of 0.4 mg. of thyroxine every second day into hypophysectomized rats produced a reticulocytosis. The total erythrocyte count and the hemoglobin showed a gradual decline, but this anemia was not so severe as after hypophysectomy and no treatment. Thyrotropic hormone, from the anterior lobe of the pituitary, showed similar results.

Adrenotropic hormone also produced a reticulocytosis but the total erythrocyte count and the hemoglobin decreased just as if no treatment had been given.

Following thyrotophine hormone and thyroxine treatment, the anemia of hypophysectomized animals was not so severe. It would seem that the atrophy of the thyroid gland, known to occur following hypophysectomy, might be one factor in the cause of this anemia.

R. C. C.

The Effects of Endocrines on the Formed Elements of the Blood. Part I: The Effects of Hypophysectomy, Thyroidectomy, and Adrenalectomy on the Blood of the Adult Female Rat. R. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. Endocrinology 29: 596, 1941.

The object of this paper was to compare the effects of hypophysectomy, thyroidectomy, and adrenalectomy on hemopoiesis in the adult female rat in an attempt to determine whether the effects produced by hypophysectomy were primarily due to the removal of that gland or secondarily due to the decreased activity in the thyroid and adrenal cortex known to occur following hypophysectomy.

Hypophysectomy produced, in 40 days, a decrease in the total erythrocyte count from the normal level of 8.20 million cells per cu. mm. to between 5.0 and 6.0 million cells. The hemoglobin dropped from 100 per cent to 70 per cent. Reticulocyte percentage dropped from 1.7 per cent to below 1.0 per cent in all cases. The total white cell count, although quite variable, showed a significant rise from an average of 8.8 thousand cells per cu. mm. to 15.8 thousand cells. There was no change in the differential white cell count.

Thyroidectomy produced the following results. The erythrocyte count decreased to 6.47 million cells in 90 days. The anemia was much more gradual in its development than that following hypophysectomy. The hemoglobin decreased slightly. The total white cell count remained normal but the differential cell count showed a lymphocytopenia and a neutrophilia.

Adrenalectomy produced the following results. (All animals were maintained on 1 per cent NaCl.) The erythrocyte count and hemoglobin both showed temporary decreases but then returned to normal levels. All other features remained normal.

The bone marrows were not studied.
These data indicate that the thyroid may play a role in the anemia which is induced by hypophysectomy but that the adrenal cortex does not. The possible roles of water balance, inanition, and posterior lobe removal are discussed.

R. C. C.


Testosterone propionate injections raised the erythrocyte count of hypophysectomized rats from the typical anemic levels to normal. The hemoglobin did not return to normal levels but did increase. Pregnant mare serum injections, known to stimulate the release of androgens from the testes, had similar effects. In both groups of rats a reticulocytosis and a bone marrow hyperplasia occurred.

Estradiol benzoate, on the other hand, had opposite effects. The anemia of hypophysectomized animals was increased. Injections of pregnant mare serum in the hypophysectomized female rats had no effect. Reticulocyte counts were low and the bone marrow exhibited a hypoplasia.

Pregnancy urine extracts produced no changes and progesterone injections gave inconsistent results.

These data indicate that androgens can be classed as erythropoietic agents while estrogens cannot.

R. C. C.


Adult male rats were hypophysectomized and the subsequent anemia allowed to develop. Attempts were made to eliminate this anemia with injections of (1) adrenal cortical extract, (2) desoxycorticosterone acetate, (3) prolactin, (4) crystalline thyroxine, and (5) thyroid powder.

Adrenal cortical extract: 5 rats were given 1 to 2 cc. of this extract daily for 5 weeks. No beneficial effects were obtained.

Desoxycorticosterone acetate: 6 rats were given daily injections of 1.0 mg. of this material for 40 days. A slight increase in erythrocyte count occurred. There was no change in reticulocyte counts or hemoglobin levels.

Thyroid powder plus desoxycorticosterone acetate: 7 rats were given 1.0 mg. of DOCA plus a ration containing 8.3 mg. of thyroid powder per Gm. each day. The latter was given orally. The erythrocyte count returned to normal levels. The hemoglobin increased “approximately to normal.” The reticulocytes increased initially and then decreased. The bone marrow showed considerable repair.

Crystalline thyroxine: 8 rats were injected with daily doses of 0.01 to 0.05 mg. of thyroxine for 5 weeks. The erythrocyte count increased from 6.40 million cells per cu. mm. to 8.12 million cells. The hemoglobin also increased but the percentage increase was only half as great as obtained in the erythrocyte count. The reticulocyte response varied from no response to a slight response. The bone marrows gave the appearance of complete repair.

Prolactin: 4 rats were given daily doses of 0.5 to 1.5 mg. of this material for 40 days. These injections produced moderate gains in erythrocyte and hemoglobin levels and a fair repair of the bone marrow.

Of these hormones, thyroxine seems to have produced the most beneficial effects.

R. C. C.


Twelve men were treated with testosterone propionate, methyl testosterone, or both combined. Eight were eunuchoids, 2 had hypogonadism, and 2 were sexually mature men. Studies were made on erythrocytes, hemoglobin, hematocrits, and leukocytes over periods varying from 1 month to 8 years.

With therapy, 7 of the 8 eunuchoids exhibited a rise in hemoglobin, erythrocyte, and hematocrit levels. These were decreased upon withdrawal of therapy.

Those patients showing a rise in the blood count also showed a rise in the basal metabolic rate.

These data showed that there was an increase in the BMR when androgen injections were given.
to the human beings, indicating that androgens possibly stimulate hemopoiesis through increasing the metabolic rate.

R. C. C.

The Effects of Iron, Copper, and Thyroxine on the Anemia Induced by Hypophysectomy in the Adult Female Rat. R. C. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. Am. J. Anat. 79: 167, 1946.

This report deals with findings obtained in an attempt to determine why a severe anemia occurs after hypophysectomy.

This work revealed that the anemia induced by hypophysectomy was of the microcytic hypochromic type, which was accompanied by a hypoplasia of the bone marrow. Hypophysectomized rats were, accordingly, treated with ferrous sulfate, ferrous sulfate plus cupric sulfate, thyroxine, and ferrous sulfate plus cupric sulfate plus thyroxine.

Daily injections of 0.01 mg. of thyroxine, 0.5 mg. of ferrous sulfate, and 0.025 mg. of cupric sulfate for 30 days, followed by daily injections of 0.01 mg. of thyroxine, 2.0 mg. of ferrous sulfate, and 0.1 mg. of cupric sulfate for 20 days produced the best results. The combination of these three materials, injected into hypophysectomized adult female rats, maintained a normal erythrocyte count, completely prevented the microcytosis, practically prevented the hypochromia, not only prevented the hypoplasia of the bone marrow but produced a hyperplasia, and increased the number of erythroid elements in the bone marrow from the mean normal control level of 40.2 per cent to 49.3 per cent of marrow cells.

The author has summarized the literature and pointed out that the materials which have been most beneficial in the hypophysectomized rat have been hormones which would stimulate the basal metabolic rate, i.e., crude pituitary preparations, thyroxine, androgens, and iron-copper-thyroxine combinations. Differential counts were made on the bone marrows, and photomicrographs of erythrocytes and bone marrows are included.

R. C. C.

Effects of Hypophysectomy, Castration, and Testosterone Propionate on Hemopoiesis in the Adult Male Rat. R. C. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. Endocrinology 39: 401, 1946.

Adult male rats were hypophysectomized, hypophysectomized and treated with 2.0 mg. of testosterone propionate, castrated, and castrated and treated with 2.0 mg. of testosterone propionate. Treatment was started immediately after surgery. Injections were given daily.

Hypophysectomy caused a severe microcytic hypochromic anemia, marked decrease in hemoglobin, hypoplasia of the bone marrow, a decrease in percentage of erythroid elements in the bone marrow, and an increase in the leukocyte count.

Androgen therapy in hypophysectomized adult male rats prevented the decrease in erythrocyte count, increase in total white cell count, and hypoplasia of the bone marrow; and partially prevented the hypochromia, the microcytosis, and decrease in the total number of erythroid elements.

Castration produced a slight decrease in erythrocyte and hemoglobin levels. These changes did not compare in severity with those following hypophysectomy.

Androgen therapy in castrated animals produced a hyperplasia of the bone marrow and increased the number of erythroid elements. Other factors were not studied in this group.

These data indicate that androgens are erythropoietic agents but that the loss of androgen via hypophysectomy is not the cause of the anemia in hypophysectomized animals.

R. C.C.

Comparative Studies on the Effects of Estradiol and stilbestrol upon the Blood, Liver, and Bone Marrow. D. Catrodale, O. Bierbaum, E. B. Helvig, and C. M. MacBryde. From the Department of Medicine, Washington University School of Medicine, St. Louis, Mo. Endocrinology 29: 365, 1941.

Adult male and female dogs were treated with doses of stilbestrol ranging from 1.0 mg. to 100 mg. daily or doses of estradiol benzoate ranging from 1.66 mg. to 3.32 mg. Studies were made on the blood, liver, and bone marrow. The results obtained were as follows:

Blood: A sharp increase in the total white cell count occurred which was followed by a marked leu-
kopenia. In one case the leukocyte count went up as high as 133,000 cells per cu. mm. Differential counts showed that the neutrophils were the cells responding. The other cells remained at normal levels. A gradual decrease in erythrocyte and hemoglobin levels accompanied this rise in total white count. The blood platelets decreased in number to such an extent that thrombocytopenia followed.

Bone marrow: The bone marrow became hyperplastic, the myeloid elements being responsible. This was followed by areas of hypoplasia. No differential counts were made on the bone marrow.

Liver: The results were inconstant. Four dogs showed some fatty degeneration, moderate central necrosis, and 2 showed no changes.

Compared by estrogenic potency, estradiol produced more rapid and more profound changes in the bone marrow and the blood than did stilbestrol.

R. C. C.


Adult female dogs were treated with daily injections of either estradiol benzoate or stilbestrol. The severity of the response varied from animal to animal. A typical response was as follows:

An adult female dog was given injections of 5 mg. of stilbestrol for 27 days followed by 10.0 mg. for 7 days. This resulted in a sharp rise in the total white cell count to a peak of 543 thousand cells per cu. mm. in 13 days. This was followed by a rapid drop to 100 cells per cu. mm. by the 34th day, the day of death. Differential counts showed the neutrophils to be responsible for this rise. The erythrocyte count gradually decreased from 6.4 million cells to 4.0 million. Thrombocytopenic hemorrhagic purpura started on day 25.

The other dogs reacted in a similar manner except that they withstood the injections for longer periods, one lasting 112 days before it died.

This same treatment, with 100 mg. of stilbestrol, was given to adult female rhesus monkeys. This daily dose of estrogen, a fourfold dose per body weight, had no effect upon hemopoiesis in the monkey. With partial liver damage, imposed by oral administration of CCl4, estrogens would produce an anemia in the monkey. At no time, however, was there a leukocyte response such as observed in the dogs.

This paper goes into detail on the normal hematologic figures for the monkey, comparing results obtained with those reported in the literature. The first blood sample obtained from a monkey, being an intractable beast, was very misleading. This first count showed a high white cell count with lymphocytes predominating. After a few weeks of training, the figures for the monkey were observed to be similar to those for the human being.

These data indicate that any estrogen in large doses is toxic to the bone marrow in dogs. Bone marrow studies were not included in this report.

R. C. C.


The object of this paper was to present evidence that the pituitary adrenotropic hormone is the factor which regulates the number of blood lymphocytes.

Single injections of adrenotropic hormone in rats, rabbits, and mice produced an absolute lymphopenia. This reaction occurred within a few hours after the injection. This response could not be elicited in adrenalectomized animals or in intact animals treated with pure protein. The authors thus claim that the lymphocyte response is specific to the hormone injection.

The total erythrocyte count and the hemoglobin levels showed a slight decrease.

These same responses could be elicited with adrenal cortical extract, adrenal cortical steroids in oil, corticoserone, or compound F of Wintersteiner.

These same authors, in other publications, have found a correlation between this decrease in lymphocyte number and an increase in serum protein, indicating the release of the protein from the broken-down lymphocytes.

R. C. C.
LEUKEMIA AND LYMPHOMA


These three reports summarize extensive investigations of the effects of the nitrogen mustards on neoplastic diseases, especially disorders of the hematopoietic system. A total of some 160 patients were treated. The official statement of Rhoads summarizes the results in a series of general statements:

1. The toxic effects of the methyl-bis compound are (a) local inflammation, if the material escapes from the vein; (b) nausea, vomiting, weakness, anorexia, and headaches, in a matter of hours; and (c) lymphopenia, neutropenia, anemia, and (rarely) hemorrhagic tendencies, often with thrombocytopenia.

2. The limits of therapy are as follows: (a) the nitrogen mustards do not cure; (b) the tumor regressions are transient, rarely extending beyond several months; (c) the chief toxicologic effect is hematopoietic damage, which at times may exceed damage to the tumor treated.

3. The effects of therapy are as follows: (a) In Hodgkin’s disease, there may be dramatic dissolution of lymph nodes, with marked systemic improvement. The effect lasts from two weeks to eight months; subsequent relapses respond less and less to repeated therapy. In some cases, nitrogen mustards seemed to make radioresistant tumor masses more radiosensitive. (b) In lymphosarcoma, giant follicle lymphoma, chronic lymphatic leukemia, and chronic myelogenous leukemia, the methyl-bis compound seems to be approximately as effective as x-ray therapy. Goodman et al. reported occasional good results in lymphosarcoma. (c) There was no effect in the acute leukemias. (d) Rhoads et al., found encouraging results in anaplastic carcinoma of the lung. (e) Jacobson et al. found good results in polycythemia vera.

S. E.


The authors review the symptoms and findings in 83 patients proven by marrow biopsy and/or autopsy to have had multiple myeloma. Treatment was uniformly of no value. Death occurred in from 1 month to 84 months after the onset of the disease, with an average of some 19 months. Tabulation of the principal abnormalities was as follows:

- Pain: 86%
- Multiple bone lesions: 78%
- Elevated serum protein: 73%
- (In all but three cases, the ratio of albumin to globulin was reversed.)
- Renal dysfunction: 67%
- Marked rouleau formation: 59%
- Anemia: 31%
- Bence-Jones proteinuria: 33%
- Negative x-rays: 19%
- Myeloma cells in peripheral blood: 10%

There was no prognostic value to the presence or absence of Bence-Jones proteinuria, or to the level of the serum proteins. It is of interest that, during the course of this study, Bence-Jones protein was found in 4 patients who did not have myeloma (1 with chronic lymphatic leukemia; 1 with carcinoma of the stomach; 1 with carcinoma of the kidney; and 1 with pulmonary tuberculosis and prostatitis).

S. E.

Multiple myeloma has, in common with the otherwise unrelated disease kala-azar, an almost constant rise in the level of serum globulin. Because stilbamidine (diamidino stilbene; there is no antimony in this compound) is curative in kala-azar, Snapper decided to test its effect in myeloma, with remarkable results.

The effect of stilbamidine on the myeloma cells was to produce large cytoplasmic granules, which Snapper believes to consist of ribose nucleic acid. No such granules appeared in the other cells of the bone marrow. The effect of stilbamidine on the patient was to cause a disappearance of bone pain. The author believes that the drug arrests the proliferation of the myeloma cells for a period of time, and thereby eliminates the mechanism of bone pain in the disorder. Objective x-ray bone changes, in the direction of healing, occurred in some patients. There was no effect on the level of the serum globulin or on the excretion of Bence-Jones protein in the urine. The drug was ineffective unless the patient received, at the same time, a diet poor in animal protein—presumably because stilbamidine and arginine, which resemble each other chemically, may compete for the same chemical group in the myeloma cell. Caution is recommended in patients with renal disease. In a few cases a related compound, pentamidine, was also found useful.

The value of this discovery cannot be overemphasized. It may allow insight into the nature of the myeloma cell, with which stilbamidine apparently combines specifically; and if indeed the myeloma cell is the source of the abnormal serum globulins and the Bence-Jones protein found in the disease, further study of these abnormalities may prove profitable. It might also be of value to test the effect of stilbamidine on bone pain due to other disorders (presumably such pains would not be affected) and the effect of arginine (animal protein) on myeloma, in support of the author's mechanism of the action of stilbamidine. To the patient, stilbamidine and pentamidine may be symptomatic boons.

S. E.


Like so many other articles on the subject of monocytic leukemia, the present one commences by stating that the first case was reported by Reschad and Schilling-Torgau in 1913. Downey (Handbook of Hematology) has thoroughly reviewed this subject and has pointed out why the so-called first case should be excluded from this group. Downey has also shown that monocytes in the leukemias may arise from the reticulo-endothelial system or from the myeloblast. In the present article histopathologic changes in 8 cases of leukemia have been studied, reported, and illustrated. Although Herbut and Miller were unable to demonstrate in all cases a definite hyperplasia of the reticulo-endothelial system resulting in a transformation of these cells into monocytes, they believe the reticulum acts as the precursor of monocytes.

O. P. J.

MONOCYTIC LEUKEMOID REACTION ASSOCIATED WITH TUBERCULOSIS AND A MEDIASTINAL TERATOMA. A. Gibson. From the Central Pathological Laboratory of Sector 8, E. M. S., St. Thomas's Hospital, Godalming. J. Path. & Bact. 58: 469-476, 1946.

Although much has been published about the various monocytic leukemias, the origin of monocytes is still open for discussion and little has been published about monocytic leukemoid reaction. The present case report focuses attention on the latter. Autopsy revealed that the bone marrow and splenic sinuses were filled with monocytes, whereas the alveolar exudate, stroma of the teratoma, and many smaller blood vessels contained numerous monocytes. No real leukemic infiltrations were found in the lungs, liver, kidneys, or suprarenals. Monocytes were present in relation to tuberculosis foci in the lung and liver.

O. P. J.