HEMATOPOIETIC TISSUES

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Owing to the increasing number of medical research problems which require experimentation on dogs, it is very fitting that a well controlled study of dogs’ blood be presented at this time. The results reported in this article were obtained from studies on 18 normal mongrel dogs ranging in age from 1 day old to adult. The red blood cell count, hemoglobin values, and hematocrit readings decreased steadily during the first 1 to 3 weeks after birth, then they gradually increased until most dogs were 6 months old—when the hematopoietic system was stabilized in the adult form. The blood specific gravity readings followed the general trend of the red blood cell changes. Mean corpuscular volume and mean corpuscular hemoglobin values decreased after the first 3 days of life to approach the adult figure at about 4 weeks of age. Only minor changes in the mean corpuscular hemoglobin concentration were noted with age.


Although the present article is of great importance in veterinary medicine, there are certain general principles which might very well obtain in the human being. In order to study the fate of intracutaneous injections in the ox and the lymphatic drainage of the skin, either India ink or a 1 per cent aqueous solution of trypan blue was injected in amounts ranging from 0.1 to 10.0 cc. The cattle were killed at intervals varying from a few seconds to 24 hours after injection and the prescapular lymph node removed. Four skin areas of the ox were associated with drainage to particular portions of the prescapular lymph node. These skin areas were the cranial third of neck, the scapular region, the ventral aspect of the neck and brisket, and the forelimb from distal humerus to the carpus. Lymph drainage from each of these four skin areas was confined to a particular portion of the lymph node. This knowledge is of importance in veterinary medicine because the prescapular lymph node, easily palpable in the living and accessible in the dead animal, receives afferent vessels from that part of the body most commonly used for intracutaneous and subcutaneous injections.


For a number of years it has been known that amoebae move as a result of reversible sol-gel changes. Leukocytes have been observed to have similar changes when they migrate, and in addition, Lewis (1942) reported the presence of ‘constriction rings’ which remain stationary as the cell moves. De Bruyn reports the results of studying these changes in tissue cultures of rabbit lymph nodes and bone marrow. The leukocyte movements were recorded with time-lapse photographs on reversible film at the rate of 60 per minute. Constriction rings were observed in cells regardless of whether they had the ‘handmirror’ or ‘wormlike’ types of motion but are much more frequent in the latter. If a given cell had a constriction ring at a certain place in the culture, other cells passing this point would also have a constriction ring as they too passed this place. This suggested that these rings were caused by physical obstacles within the culture medium. Similar results were obtained when leukocytes moved on the flat surface of the cover glass. From these observations it was concluded that constriction rings do not play an essential role in the movement of cells. Lateral protuberances on the body of the cells were observed to be more or less stationary rather than ‘waves.’ These lateral protuberances were formed when the anterior pseudopodial area became immobilized, and they disappeared when the tail gradually approached this area. These prot-
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Tuberances are probably due to a superficial plasma-gel formation which may contract and drive the cell forward. The possible mechanisms concerned with plasma-sol and plasma-gel changes are discussed.

BLOOD TRANSFUSIONS AND ERYTHROCYTE SURVIVAL

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The authors studied the fate of transfused normal red cells administered to six patients with congenital hemolytic jaundice manifested by chronic anemia, hyperbilirubinemia, reticulocytosis, spherocytosis, and increased osmotic erythrocyte fragility. The rate of destruction of the donor cells following transfusion was determined by means of serial red counts, employing the selective agglutination technic of Ashby, when group O cells were received by individuals of other blood groups, or the method of Wiener, utilizing differences in the M and N grouping. In similar fashion the survival of red cells obtained from one of these patients before and after splenectomy was determined after injection of the blood into individuals with mild secondary anemia. The purpose of the investigation was to discover whether the excessive blood destruction associated with congenital hemolytic jaundice is explainable on the basis of an abnormal mechanism of blood destruction, in which a circulating red-cell antibody or pathologic splenic function might be operative, or whether the red cells are inherently defective and consequently short-lived.

It was found that normal erythrocytes, injected into five patients with congenital hemolytic jaundice, survived as long as in recipients without hemolytic disease, the survival periods totaling from 100 to 130 days. In one patient complete destruction of donor cells occurred within 60 days; in this instance, however, an immunologic hemolytic mechanism could be implicated, namely, the stimulation of anti-Rh iso-antibodies in response to the transfusion of Rh positive blood.

The reverse experiment, in which one of these patients served as a blood donor for a patient with iron deficiency anemia, demonstrated a marked susceptibility to destruction on the part of congenital hemolytic jaundice red cells. Blood samples obtained prior to, and one year following, splenectomy were destroyed at comparable rates, disappearing completely from the recipients' blood within 14 and 19 days, respectively.

Similar studies were carried out in a patient with paroxysmal nocturnal hemoglobinuria, in whom transfused red cells survived in normal fashion, providing confirmatory evidence that the mechanism of excessive blood destruction in this disease, as in congenital hemolytic jaundice, is related to a pathologic property of the red cell.


Patients with various types of anemia were transfused with red cells from normal group O donors. The survival of the injected cells in these recipients, whose blood groups were other than group O, was thereafter estimated by the Ashby technic.

The rate of donor cell destruction in six patients with anemia due to chronic iron deficiency was relatively constant, the total duration of survival approximating 100 days, the average life span, 50 days. Three patients with clinical evidences of acquired hemolytic disease eliminated the donor cells much more rapidly, the average life of the transfused erythrocytes in these cases ranging from 7 to 13 days. The rate of donor cell destruction moreover did not follow a linear curve, as in patients with iron deficiency, but an exponential type of curve, the rapidity of disappearance apparently being a function of the concentration of donor cells in the recipient's blood. A disappearance curve of this type is interpreted as evidence of an abnormal hemolytic process or iso-antibody operating indiscriminately against all red cells, irrespective of their age or origin.

Similar results were obtained in cases with anemia complicating acute infections, in a patient with pernicious anemia of pregnancy and one with multiple myeloma. On the other hand the destruction of donor cells in patients with anemia attributed to chronic infection and chronic nephritis, and in a patient...
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with pernicious anemia receiving liver therapy, occurred at a normal rate, implying the absence, in these cases, of a hemolytic disorder.


Blood transfusion has been applied by the authors in studying the hemolytic mechanisms responsible for blackwater fever. Two patients exhibiting this syndrome in the course of chronic malaria and quinine therapy were transfused with normal group O blood. The destruction of the donor cells, measured by the Ashby technic, was excessively rapid, as rapid as was the destruction of the recipients' own red cells, when transfusions were given during hemolytic crises. During convalescent periods more prolonged erythrocyte survivals were observed, the life span of injected cells eventually becoming normal. Red cells obtained from a patient with blackwater fever during a hemolytic crisis, and a second specimen withdrawn 10 days following the episode (5 days prior to another crisis), survived for abnormally brief periods when injected into recipients in whom there were no evidences of hemolytic disease.

The authors conclude that the syndrome of blackwater fever is produced by some unidentified extracellular agent, or factor, capable of destroying more normal donor cells as well as the patient's erythrocytes. Red cells that escape destruction during the acute crisis are nevertheless irreversibly damaged by the hemolytic mechanism, and, whether they continue to reside in the patient's circulation or are transferred to a normal environment, their survival is consequently brief. A direct attempt to demonstrate a circulating hemolysin by injecting 500 cc. of plasma, obtained from a patient during a crisis, into a recipient with active P. falciparum malaria receiving quinine, failed to provoked a hemolytic reaction. This failure, as the authors point out, may signify merely that complete exhaustion of the hypothetical circulating hemolysin had already occurred, possibly by cellular adsorption.


An unusually direct and ingenious approach to the problem of reticulocyte maturation is described by the authors, whose methods of investigation included experimental blood transfusion followed by selective agglutination studies of the recipient's blood. A child with aplastic anemia, blood group A, received blood from a patient with an atypical hemolytic anemia, blood group O. Three hundred and fifty cc. of red cells were injected, 73 per cent of which were reticulocytes. The fate of the mature and of the reticulated donor cells was determined by means of Ashby counts in combination with reticulocyte counts. The data obtained indicated that maturation of the transfused reticulocytes occurred in the course of approximately 140 hours; almost identical findings, with respect to maturation rate, were obtained on in vitro incubation of the donor blood in Simmel's solution. Destruction of the donor cells was abnormally rapid, none being detected in the recipient's circulation after 8 days. Nevertheless, the injected reticulocytes appeared to be immune to destruction until they had attained maturity.

It is concluded that, if the average maturation time of reticulocytes is as prolonged as that found in this case, the reticulocyte count must be considered unreliable as a quantitative index of the rate of hemopoiesis, and reticulocyte experiments inadequate as a basis for computing the life span of red cells. Thus, if all reticulocytes mature after an average period of 140 hours in the peripheral blood, and if the average percentage of reticulocytes in the normal blood is 0.7, it follows that the average life span of the normal red cells is 833 days, more than 8 times greater than indicated by transfusion studies or measurements of pigment excretion. The authors regard this discrepancy as evidence that many erythrocytes must leave the bone marrow as mature cells, excepting when erythropoiesis is excessively rapid. An alternate explanation might also be hypothesized, namely, that reticulocytes may enter the peripheral circulation at various stages of maturity, depending on the rate of blood production, and that completion of their maturation in the peripheral blood may normally require much less than the 140 hour period observed in this case of severe hemolytic disease.
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