
This is to certify that the

thesis entitled

The Relationship of Serum
Iron, Erythropoietin and
Erythrocyte Survival to
Anemia of Inflammatory Disease

presented by

Douglas J. Weiss

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pathology

Major professor

Date May 18, 1981

# THE RELATIONSHIP OF SERUM IRON, ERYTHROPOIETIN AND ERYTHROCYTE SURVIVAL TO ANEMIA OF INFLAMMATORY DISEASE

By

Douglas J. Weiss

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1981

ABSTRACT

THE RELATIONSHIP OF SERUM IRON, ERYTHROPOIETIN AND ERYTHROCYTE SURVIVAL TO ANEMIA OF INFLAMMATORY DISEASE

By

Douglas J. Weiss

Anemia frequently accompanies chronic infections and neoplastic diseases in humans and a variety of animal species. Induced sterile abscesses in cats provided an experimental model for the investigation of the complex pathogenesis of this disorder.

The role of serum iron, erythropoietin and erythrocyte survival in anemia of inflammatory disease was investigated. Maintenance of increased serum iron concentrations during the sterile abscess did not prevent but did allow the bone marrow to respond to the anemia. Daily cobalt injections in cats with abscesses produced a mild polycythemia, but the magnitude of the polycythemia was considerably less than in control cats. A significant reduction in erythrocyte survival was also observed in animals with sterile abscess.

The major conclusions of this study were: 1) the primary cause of anemia in the early stages of inflammatory disease is reduced erythrocyte lifespan; 2) low serum iron levels prevented the bone marrow from responding to the

anemia; and 3) serum iron concentrations, in addition to erythropoietin, were involved in the control of maximal erythropoiesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# INTRODUCTION

Anemia frequently accompanies chronic infections and neoplastic diseases in man and a variety of animal species. The term "anemia of chronic disorders" has been the most accepted name for this anemia. However, since inflammation has been found to be the common denominator in all cases, anemia of inflammatory disease would be a more precise and descriptive name.

Anemia of inflammatory disease has been clinically defined as a mild to moderate, poorly regenerative anemia with altered iron metabolism characterized by low serum iron and total iron binding capacity and normal to increased tissue iron.

The pathogenesis of the anemia has been found to be complex. At least 3 factors have been implicated: 1) impaired marrow response to the anemia, 2) impaired release of iron from mononuclear phagocytes, and 3) shortened erythrocyte survival. The relative importance of these factors and their underlying cause have not been established. The investigation of these problems will not only provide further knowledge of the pathogenesis of anemia of inflammatory disease but will help to define the interrelationship of the several mechanisms which control erythrocyte production and destruction.

Induced sterile abscesses have provided a simple experimental model for study of the pathogenesis of anemia of inflammatory diseases. This model will be used to explore 1) the mechanism of reduced erythropoiesis, 2) the mechanism of iron sequestration, and 3) the role of erythrocyte survival in anemia of inflammatory disease.

# LITERATURE REVIEW

This literature review will be divided into 4 parts: 1) iron metabolism--transferrin, ferritin, hemosiderin, lactoferrin and control of cellular uptake and release of iron; 2) erythropoietin--chemical properties, biosynthesis and control of secretion; 3) erythrocyte survival; and 4) anemia of inflammatory disease--clinical description, impaired marrow response to anemia, impaired iron release from mononuclear phagocytes and erythrocyte survival.

## Iron Metabolism

### Transferrin

Transferrin is the major plasma iron transport protein. The physical and chemical properties have been reviewed (Morgan, 1974; Harrison and Huehns, 1979). The molecule is a glycoprotein of approximately 76,000 molecular weight and 5.3% carbohydrate. Each of 2 lobes has a prolate ellipsoid shape with maximum overall dimensions of 10 x 50 x 40 Angstroms. A single iron binding site is present on each lobe. These 2 sites are identical in structure and have a very high affinity for iron. The interaction of ferric iron with the binding sites was found to involve 3 tyrosyl residues,

2 histidyl residues and the concurrent binding of 1 bicarbonate ion (Rogers, et al., 1977).

The relative availability of iron from the 2 binding sites to various tissues has been disputed. Fletcher and Huens, (1968) reported that iron from the 2 iron binding sites was not equally available for hemoglobin synthesis. They postulated that this functional dissimilarity regulated iron distribution within the body. Harrison and Aisen (1975), however, found no functional difference in the availability of iron from the 2 sites. In addition, they found that iron from diferric and monoferric transferrin was equally available to body tissues.

## Ferritin

Ferritin is known to be the major iron storage protein (Harrison, et al., 1974). The physical chemical properties of ferritin have been reviewed (Harrison, et al., 1974; Richter, 1978; Munro and Linder, 1928). Ferritin was found to be a water-soluble macromolecule consisting of subunits arranged in a octahedral fashion around a central core of iron. The subunits have been described as acidic proteins of approximately 18,500 daltons with 60% of the molecule in an alpha helical conformation (Harrison and Huehns, 1979). Harrison, et al., (1974) reported that the crystalline core of ferritin contained up to 4500 atoms of iron stored as a colloidal hydrous ferric oxide-phosphate complex.

Administration of iron to guinea pigs was found to cause rapid stimulation of apoferritin synthesis in the liver (Findberg and Greenberg, 1955). The first detectable product was an iron-poor apoferritin. The subunits of apoferritin were postulated to assemble spontaneously into a hollow shell which rapidly accumulated free iron (Harrison et al., 1974). Munro and Linder (1978) have postulated that apoferritin controls its own translation by preventing binding of messenger RNA to the endoplasmic reticulum. In their model increased cytosolic iron causes aggregation and re-distribution of apoferritin, thus removing the block for the binding of messenger RNA. Since ferritin messenger RNA is known to be very stable, this theory would provide a mechanism for rapid uptake and release of iron by ferritin. This function has been considered important to iron homeostasis in that free serum iron is toxic and cannot be rapidly excreted by the body.

Hemosiderin

Hemosiderin has been characterized by: 1) Prussian blue staining, 2) insolubility in water, and 3) molecular heterogeneity (Munro and Linder, 1978). Ultrastructural examination has suggested that hemosiderin was a degraded form of ferritin which resulted from partial loss of the protein shell (Munro and Linder, 1978). This transformation may occur in secondary lyososomes (Monro and Linder, 1978).

Munro and Linder (1978) conclude that hemosiderin as presently defined includes several intracellular forms of

iron. These forms include both loosely chelated iron and dense storage granules.

## Lactoferrin

Lactoferrin, an iron binding protein of 76,000 molecular weight, has been found in high concentrations in external body secretions and in the specific granules of neutrophilic granulocytes (Karle et al., 1979). Lactoferrin was found to share several biochemical properties with transferrin, including a closely related amino acid composition and similar iron binding sites (Karle et al., 1979). The molecules differed immunologically and in their affinity for iron (Morgan, 1974; Van Snick et al., 1974).

## Cellular Uptake and Release of Iron

Three cell types have been found to play unique roles in iron homeostasis: 1) mononuclear phagocytes, 2) reticulocytes, and 3) intestinal mucosa. Intestinal iron absorption has been reviewed and will not be further discussed (Turnbull, 1974).

Iron can be taken up by mononuclear phagocytes in several forms, including 1) uptake of intact erythrocytes, 2) uptake of iron from transferrin, 3) uptake of protein-bound hemoglobin and heme, and 4) uptake of serum ferritin (Munro and Linder, 1978).

Destruction of senescent erythrocytes by the mononuclear phagocyte system has been considered a normal physiologic mechanism for recycling iron and amino acids. The erythrocytes were found to be taken up by phagocytosis and

to be digested in secondary lysosomes (Munro and Linder, 1978).

Hepatocytes have been shown to have receptors for hemoglobin-haptoglobin complexes and for heme-hemopexin complexes. These complexes can be taken up by phagocytosis (Munro and Linder, 1978).

The mechanism by which transferrin interacts with cells has been primarily investigated using reticulocytes. Hemmaplardh and Morgan (1977) have shown that $^{125}$I-labeled transferrin first attached to a specific cell surface receptor and then entered the cell by endocytosis to become free in the cytoplasm. Fielder and Speyer (1974) suggested that transferrin did not enter the cell but gave up its iron at the cell surface. Whatever the mechanism, a large part of the transferrin was found to recirculate (Munro and Linder, 1978).

Factors affecting the rate of reticulocyte iron uptake have been reviewed by Morgan (1974). Increased transferrin saturation accelerated iron uptake. Chelating agents did not block uptake, which suggested that iron was not present in ionic form at any time during the absorption process. Anoxia and cell lysis were found to inhibit uptake, suggesting that uptake was an energy-requiring process dependent on cellular metabolism and cellular integrity.

Mazur et al. (1960) investigated the incorporation of transferrin-bound iron into ferritin in liver slices. They found that adenosine triphosphate (ATP) and ascorbic acid were essential to iron uptake. They concluded

that ascorbic acid was necessary for the reduction of ferric iron bound to transferrin. Egyed (1974) reported that ATP was directly involved in iron uptake by reticulocytes and proposed that ATP was bound to the cell membrane. Carver and Frieden (1972) reported that polyphosphate, adenosine diphosphate (ADP) and ATP accelerated iron release from transferrin by acting as intracellular chelators of iron. Graham and Bates (1974) proposed the following sequence of events in iron release from transferrin: 1) chelation of ferric iron with ATP or other cellular chelating agents, 2) reduction of ferric iron by reducing agents including ascorbic acid, 3) disruption of the bond between amino acids and the iron molecule, 4) allosteric alterations in the transferrin molecule, and 5) weakening of the anion thermodynamic linkage.

Munro and Linder (1978) proposed that iron from both transferrin and secondary lysosomes contributed to a chelatable iron pool in the cytosol. They postulated that this pool was in equilibrium with ferric iron in ferritin and that the size of the pool regulated synthesis of apoferritin through changes in the availability of messenger RNA for ferritin synthesis. The chelatable iron has also been shown to stabilize ferritin precursors and iron-poor ferritin (Drysdale and Munro, 1966).

Steps in the deposition of ferritin iron have been proposed by Crichton and Collet-Cossart (1977). These steps include: 1) ferrous iron binding to apoferritin, 2) formation of a dioxygen molecule with 2 ferrous ions,

3) reduction of ferrous iron to a peroxo-complex, and
4) hydrolysis of the peroxo-complex and migration of the ferric oxyhydroxide to the interior of the protein shell.

Possible mechanisms involved in the release of iron from ferritin have been reviewed by Harrison et al. (1974). Release may be regulated by the level of reducing agents and/or chelating agents within the cell. Ascorbic acid deficiency has been found to result in increased hepatic ferritin levels (Lipschitz et al., 1971).

Mazur, Carleton and Carlsen (1961) have provided evidence that the release of iron from ferritin was regulated by the state of oxidative metabolism within tissue. This theory was supported by the *in vitro* observation by Baker et al. (1977) that liver hypoxia resulted in release of iron from hepatocytes while hyperoxia caused decreased iron release. They proposed that decreased oxygen acted directly on an NADH-linked ferric reductase. Crichton and Collet-Cassart (1977) reported the existence of a ferro-reductase which required FMN as a coenzyme and NADH or NADPH as a source of electrons.

Mazur et al. (1958) provided both *in vitro* and *in vivo* evidence that xanthine oxidase reduces ferritin iron. Intravenous injection of substrates of xanthine oxidase into dogs, guinea pigs and rabbits resulted in prompt increase in serum iron. They proposed that iron mobilization from ferritin was regulated by the plasma level of xanthine oxidase substrates. Tissue hypoxia from anemia or other causes would increase the release of xanthine

oxidase substrates, thus stimulating release of ferritin iron. However, lowering of xanthine oxidase levels by feeding sodium tungstate and by treatment with allopurinol resulted in no accumulation of iron in the liver of rats (Kinney et al., 1961; Emmerson, 1966).

The formation of hemosiderin from ferritin has been reviewed by Matioli and Bates (1963). They proposed that ferritin initially undergoes oxidative denaturation, which causes the cell to sequester the denatured molecules in vacuoles. Conditions within the vacuoles favored aggregation of the denatured ferritin into granules. These granules, however, still maintained the ferritinic substructure. They further proposed that proteolytic degradation of this type of hemosiderin resulted in formation of hemosiderin in a nonferritinic form.

Banerjee and Chakrabarty (1965) reported that deposits of hemosiderin were greater than normal in liver of ascorbic acid deficient guinea pigs, while the soluble fraction was reduced. This led to the proposal that ascorbic acid prevented oxidation of ferritin.

Ferritin has been generally accepted as a readily mobilized iron storage pool, while hemosiderin was considered a more stable storage form (Richter, 1978). However, Shoden et al. (1963) reported that iron mobilized from tissue was derived from both hemosiderin and ferritin. They concluded that the 2 types of storage iron were functionally indistinguishable.

# Erythropoietin

## Chemical Properties

Sheep plasma erythropoietin and human urinary erythropoietin have been found to be acidic glycoproteins which were heterogeneous on isoelectric focusing (Wintrobe et al., 1974). The molecule was found to contain approximately 29% carbohydrate. The molecular weight has been estimated to be between 27,000 and 60,000 daltons (Goldwasser and Kung, 1971).

Goldwasser and Kung (1968) reported that removal of terminal sialic acid residues resulted in loss of *in vivo* biological activity but retention of *in vitro* biological activity. Lukowsky and Painter (1972) found that oxidation of asialo-erythropoietin with galactose oxidase restored biological activity *in vivo*. They concluded that, as with other proteins, exposure of the penultimate galactose residue resulted in rapid degradation of erythropoietin *in vivo*.

## Biosynthesis and Control of Secretion

The site of production of erythropoietin has been considered to be the kidney based on the association of nephrectomy and anemia (Wintrobe et al., 1974a). However, following bilateral nephrectomy in people erythropoietin could still be detected (Krantz and Jacobson, 1970).

Contrera and Gordon (1966) isolated a factor from the light mitochondrial fraction of rat kidneys which generated biologically active erythropoietin after

incubation with a plasma factor. It has been termed renal erythropoietic factor, or erythrogenin. Renal erythropoietic factor and plasma factor were both found to be immunochemically distinct from erythropoietin. Alternatively, Sherwood and Goldwasser (1978) found that extracts of rat, ox, dog and rabbit kidney contained biologically active erythropoietin and not a precursor hormone.

Rogers, Fisher and George (1974) reported that hypoxia and synthetic prostaglandin $E_1$ induced a renal protein kinase which activated renal erythropoietic factor by phosphorylation in the presence of cyclic AMP.

The effects of cobalt on erythropoiesis were first discovered by Waltner and Waltner (1926). They found that cobalt produced a polycythemia in experimental animals. Barron and Barron (1936) reported that daily subcutaneous injections of .01 gm of cobalt sulfate produced a polycythemia in rabbits within 6 or 7 days. The polycythemia was accompanied by reticulocytosis and nucleated erythrocytes in the peripheral blood and erythroid hyperplasia in the bone marrow. Based on *in vitro* culture of erythrocytes with cobalt, they concluded that cobalt acted directly by producing an anoxic condition within the bone marrow. However, Warren, Schubmehl and Wood (1944) were unable to reproduce these results.

Goldwasser et al. (1958) reported that cobalt caused an increase in plasma erythropoietin within 4 hours after injection. They postulated that cobalt acted by producing an anoxic state in the kidney.

## Erythrocyte Lifespan

Numerous methods have been used to study erythrocyte lifespan (Wintrobe et al., 1974b). These have been divided into cohort methods in which tracers are incorporated into newly formed cells in the marrow and random methods in which the tracers bind to erythrocytes in the circulation regardless of age.

Two methods for determination of *in vivo* erythrocyte survival were reviewed and standardized by the International Committee for Standardization in Hematology (1971). Tracers for these methods were $^{51}Cr$ sodium chromate and radioactive diisopropyl fluorophosphate.

Grey and Sterling (1950) first reported that erythrocytes mixed with radioactive sodium chromate rapidly took up chromium. Disappearance of $^{51}Cr$ from the circulation was studied by Ebaugh, Emmerson and Ross (1953). They found that $^{51}Cr$ eluted from erythrocytes and correction for this elution could be made.

Erythrocyte lifespan has been studied in cats by Kreier et al. (1970) and by Gillis and Mitchell (1974). Kreier et al. (1970) labeled erythrocytes concurrently with diisopropyl fluorophosphate and $^{51}Cr$ sodium chromate. They found that rapid elution of $^{51}Cr$ occurred at a rate of $5.88 \pm .90\%$ per day. Erythrocyte lifespan was found to be 70 days.

## Anemia of Inflammatory Disease

### Clinical Description

Anemia frequently accompanies chronic infectious and neoplastic diseases in man and a variety of animal species (Cartwright, 1966; Mahaffey and Smith, 1978). Finch (1978) suggested that inflammation was the common denominator among various causes of this anemia.

In humans, the anemia developed slowly over the first month of illness but thereafter plateaued (Cartwright, 1966). Similar anemias developed in dogs, cats and rats with experimental septic or nonseptic sterile abscesses (Lukens et al., 1967; Cartwright et al., 1946; Mahaffey and Smith, 1978). In rats and cats, the anemia was reported to develop more rapidly with significant decrease in hematocrit by 3 and 5 days, respectively (Mahaffey and Smith, 1978; Lukens et al., 1967).

Morphologically the anemia was usually normocytic, normochromic but normocytic, hypochromic or microcytic, hypochromic anemias were also observed (Cartwright, 1966). Reticulocyte counts were normal to decreased with minimal or no alterations in bone marrow cytology (Mahaffey and Smith, 1968; Cartwright, 1966).

Biochemically the anemia was characterized by: 1) decreased plasma iron, 2) decreased total iron binding capacity, 3) decreased saturation of transferrin, 4) decreased bone marrow sideroblasts, 5) normal or increased mononuclear phagocyte iron, 6) increased plasma copper, and 7) increased

free erythrocyte protoporphyrin (Cartwright, 1966). Cartwright and Lee (1971) stated that these changes were unique to anemia of inflammatory disease.

At least 3 factors have been implicated in the pathogenesis of the anemia: 1) impaired bone marrow response to the anemia, 2) impaired release of iron from mononuclear phagocytes, and 3) shortened erythrocyte survival. Each of these factors will be discussed separately.

## Impaired Bone Marrow Response to the Anemia

Cartwright and Lee (1971) implicated 2 factors which may have limited erythropoiesis: 1) inadequate iron supply to the marrow and 2) impaired erythropoietin release.

Prolonged iron deficiency has been clearly found to result in anemia (Wintrobe et al., 1974c). However, the role of iron supply in control of erythropoiesis in the non-iron-deficient state has been less well established. Hillman and Henderson (1969) investigated marrow production response of people to daily phlebotomies over a 3- to 4-week period. In response to sudden reduction of hematocrit to 32 to 37 volume %, marrow production increased over a 10-day period and reached a plateau at 1.8 to 3.5 times normal. When iron was provided as a single intravenous injection of iron dextran, marrow production increased 4.5 to 5.5 times normal. When iron was provided by infusion of nonviable erythrocytes, marrow production increased 6.6 to 7.8 times normal. They concluded that marrow iron supply played an important role in the control of erythropoiesis.

The role of erythropoietin in the pathogenesis of anemia of inflammatory disease has been disputed. This has been largely due to the relative insensitivity of the bioassay procedure at normal and low serum levels of erythropoietin. Zucker et al. (1974) reported that serum levels of erythropoietin were decreased relative to the degree of anemia. This conclusion was based on comparison of erythropoietin-hemoglobin ratios in people with chronic inflammatory disease and normal people. However, this relative decrease was not observed in cancer patients. Lukens (1973) determined erythropoietin levels in adjuvent-induced chronic inflammation in rats. Serum levels were only slightly lower than normal controls; however, erythropoietin concentrations following exposure to hypobaric conditions were markedly lower. These changes were interpreted as a relative failure in the production of biologically active erythropoietin. Douglas and Adamson (1975) studied anemia of inflammatory disease in people and reported that reduced erythropoietin concentrations were not a uniform finding. They concluded that marrow proliferation was primarily limited by the relative unavailability of iron. Ward et al. (1971) reported that erythropoietin levels in people with chronic infections and malignancies were significantly lower than in people with iron deficiency and hemolytic diseases.

Reissmann and Udupa (1978) reported that inflammation in mice caused a longlasting reduction in the number of erythroid colony forming units (CFU-E) in the bone marrow.

They attributed this effect to a blood-borne mediator which inhibited proliferation of CFU-E. However, they also found that CFU-E and hematopoietic stem cells (CFU-S) in the spleen were markedly increased.

Whitcomb et al. (1965) reported a serum inhibitor of erythropoiesis in anemia of inflammatory disease. Wallner et al. (1976) reported a similar inhibitor of erythropoiesis in anemia of chronic renal disease but were unable to demonstrate a serum inhibitor in anemia of inflammatory disease.

Rinehart et al. (1978) reported that peripheral blood monocytes and tissue macrophages could regulate erythropoiesis *in vitro*. They postulated that proliferation of monocytes during inflammation suppressed erythropoiesis.

Despite these conflicting reports, anemia of inflammatory disease has been shown to respond to the administration of purified erythropoietin, cobalt and hypoxia (Gutnesky and Van Dyke, 1963; Wintrobe et al., 1947). However, it was found that with all 3 stimuli the final hematocrits were not as great in rats with sterile abscesses as they were in the control groups. Thus, these stimuli did not completely abolish the defect in erythropoiesis produced by the inflammation (Cartwright, 1966). This discrepancy could be explained by: 1) decreased erythrocyte survival, 2) unavailability of iron for erythropoiesis, or 3) a serum inhibitor of erythropoietin or erythropoiesis.

## Impaired Iron Release from Mononuclear Phagocytes

Impaired release of iron from mononuclear phagocytes was first reported by Freireich et al. (1957) and has since been confirmed by others (Haurani et al., 1965; Quastel and Ross, 1966; Hershko et al., 1974). The impaired release has been demonstrated by infusion of nonviable erythrocytes which were labeled with radioiron. In anemia of inflammatory disease less than 40% of the radioiron was reutilized for hemoglobin synthesis as compared to 55 to 70% in normal subjects.

Several mechanisms for the iron sequestration have been proposed. They include: 1) increased ferritin synthesis (Konejn and Hershko, 1977), 2) oxidation of ferritin to hemosiderin (Feldman and Kaneko, 1978), 3) excessive lactoferrin release from neutrophils (Van Snick et al., 1974), and 4) increased oxidative metabolism in mononuclear phagocytes (Mazur et al., 1961).

Konijn and Hershko (1977) studied plasma iron turnover and ferritin synthesis in the liver and spleen of rats with sterile abscesses. They found that an increased rate of ferritin synthesis preceded, by several hours, changes in plasma iron turnover. They suggested that increased ferritin synthesis was responsible for the sequestration of iron in mononuclear phagocytes. They concluded by drawing an analogy between ferritin and "acute-phase" proteins and proposed that increased ferritin synthesis was part of a primary non-specific response to inflammation.

Kampschmidt and Upchurch (1969) reported that injection of leukocyte extracts resulted in a marked decrease in serum iron concentrations in rats. The extract has been termed leukocyte endogenous mediator and has been found to also have antimicrobial activity (Kamschmidt and Pulliam, 1975). A later report suggested that leukocyte endogenous mediator and leukocyte endogenous pyrogen were the same molecule (Merriman et al., 1977). Bornstein and Walsh (1978) injected endotoxin-free endogenous pyrogen into rabbits and found that it produced both an "acute-phase" reaction and hypoferremia.

Van Snick et al. (1974) reported that neutrophils released iron-free lactoferrin during phagocytosis *in vitro*. This lactoferrin was able to remove iron from transferrin at a pH of 7.0 or in the presence of a high concentration of citrate. Intravenous injection of human apolactoferrin into rats caused a marked decrease in plasma iron levels which could be retarded by mononuclear phagocyte blockade. Immunofluorescence studies indicated that lactoferrin was bound to and ingested by monocytes and that the iron was transferred to ferritin. They concluded that lactoferrin may mediate the hypoferremia associated with inflammation.

The turnover of radioiodine-labeled human lactoferrin in rabbits was studied by Karle et al. (1979). The half-life was found to be approximately 25 hours and most of the lactoferrin accumulated in the liver. The rate of synthesis in normal humans was estimated to be about 25 mg per day.

It was concluded that this form of iron transport was insignificant in the normal state. Although this pathway was increased in inflammatory disease, it also appeared to be insignificant when compared to toal iron turnover rate. However, this experiment did not take into account lactoferrin, which was metabolized by macrophages in the extravascular space and thus not entering the plasma pool.

Mazur et al. (1961) reported that increased oxidative metabolism in rat liver slices resulted in increased incorporation of transferrin-bound radioiron into tissue. They concluded that stimulation of ATP synthesis favored movement of serum iron into liver and spleen ferritin, whereas hypoxia favored iron release. They postulated that oxidative metabolism in mononuclear phagocytes during inflammation resulted in iron sequestration.

Feldman and Kaneko (1979) reported that hepatic superoxide dismutase activity was increased during anemia of inflammatory disease in dogs. They suggested that increased superoxide dismutase activity generated enough hydrogen peroxide to denature ferritin miscelles, producing deposits of less readily mobilized iron in the form of hemosiderin.

Erythrocyte Survival

Ferrokinetic studies have been performed on humans and experimental animals with anemia of inflammatory disease (Freireich et al., 1957; Quastel and Ross, 1966). In

general, the plasma iron turnover and erythrocyte iron turnover were normal to increased and the fraction of cells renewed daily was increased. These data suggested that the rate of erythropoiesis was normal or slightly increased and therefore that the rate of erythrocyte destruction must be increased. In addition, the rapidity with which the anemia developed in cats with turpentine abscesses could not be accounted for by decreased erythropoiesis alone (Mahaffey and Smith, 1978).

Erythrocyte survival has been determined in humans and experimental animals (Richmond et al., 1961; Rigby et al., 1962; Hyman, 1963; Karle, 1968a). In general, a modest shortening of erythrocyte survival has been demonstrated (Cartwright and Lee, 1971). However, Waggener et al. (1958) studied erythrocyte survival in patients with leukemia and lymphoma and found that erythrocyte survival was only 50% of normal in many patients.

Normal erythrocytes transfused into patients with anemia of inflammatory disease have been found to have a decreased erythrocyte survival, whereas erythrocytes from patients with anemia of inflammatory disease have a normal survival when transfused into normal patients. These studies have been interpreted as indicating an extracorpuscular hemolytic factor (Cartwright, 1966).

The mechanism of the decreased erythrocyte survival has not been defined. The 2 most commonly expressed theories include 1) a hemolytic factor elaborated from the site of the inflammation and 2) a hyperplastic mononuclear phagocyte

system which is overactive in destroying erythrocytes (Jacobs et al., 196; Cartwright, 1966). Decreased erythrocyte survival associated with splenic hyperplasia has been observed in patients with infectious and neoplastic diseases and in animals injected with particulate material (Ultmann, 1958; Jacobs et al., 1963). Conversely, splenic atrophy has been observed in animals following repeated bleeding (DeLangen, 1943). Jacobs et al. (1963) proposed that the size of the mononuclear phagocyte system in various organs is regulated by the total particulate workload presented to it. Ultmann (1958) demonstrated that splenic sequestration of erythrocytes was an important cause of anemia in patients with chronic lymphocytic leukemia and lymphosarcoma.

Evidence for a hemolytic factor in anemia of malignancy has been reviewed by Hyman (1963). Wasserman et al. (1955) proposed that non-specific antibodies may be produced by neoplastic tissue which cross react with erythrocyte antigens. Barry and Crosby (1957) reviewed 10 cases of hemolytic anemia related to ovarian cysts and teratomas. Many of these patients had a positive direct Coombs test. The anemia responded to removal of the cyst or tumor but not to splenectomy.

# MATERIALS AND METHODS

Eight major experiments will be described. These have been designated Experiments I through VIII (Table 2). Experiment V was divided into 5 subgroups designated Experiments VA through VE. The relationship of hematocrit, reticulocyte count, serum iron concentration, serum erythropoietin concentration and erythrocyte survival to experimental turpentine abscesses in cats was determined.

## Experimental Animals

Adult domestic cats were used in Experiments I through VIII. Adult Beagle dogs were used in Experiments VB, VC and VD. All animals were random source animals and were obtained from the Laboratory Animal Care Service at Michigan State University. The cats were preconditioned for 3 to 4 weeks by the Laboratory Animal Care Service. The conditioning program consisted of vaccinations for feline panleukopenia, calicivirus and herpesvirus, deworming and dusting for ectoparasites.

The cats and dogs were housed in the Veterinary Clinical Center in masonry cages with steel doors except for cats in Experiment VIII, which were housed in Laboratory Animal Care Service quarters. Cages were cleaned and animals fed twice daily.

## Experimental Design

### Experiment I

The objective of this experiment was to establish normal baseline values for tests performed in other experiments and to assess the effect of serial bleeding on hematologic parameters.

Five cats were used in the experiment. Daily, 1 ml of blood was aspirated from a cephalic vein into a sterile disposable syringe. Leaving the needle in the vein, an additional 0.5 ml of blood was aspirated into a second syringe containing 15 µl of ethylenediaminetetraacetate (EDTA). Tests performed were those listed in Table 1.

Table 1. Tests performed in Experiments I through VII

| | |
|---|---|
| Hemoglobin | Reticulocyte count |
| PCV | Plasma protein |
| RBC count | Serum iron |
| MCV | Total iron binding capacity |
| MCHC | Serum erythropoietin |
| Total leukocyte count | |

### Experiment II

The objective of this experiment was to assess the effects of a sterile abscess on the hematocrit, reticulocyte count, serum iron and serum erythropoietin. These results were used for comparison with later experiments in which

various treatments were employed to modify the effects of the sterile abscess.

Five cats were anesthetized with ketamine hydrochloride and .75 ml of filter-sterilized USP turpentine was injected subcutaneously in the gluteal region. Blood samples were drawn daily for the first 8 days and on alternate days for the next 6 days. The procedure for drawing the blood was that described in Experiment I, and the tests performed were those listed in Table 1.

Experiment III

The objective of this experiment was to assess the effects of cobalt injection on the hematocrit, reticulocyte count, serum iron and serum erythropoietin. These data were necessary to establish whether or not the effects of cobalt in cats were similar to those reported in other species.

Five cats received daily injections of 0.5 ml of cobalt chloride (50 mg/ml) in sterile water. Blood samples were drawn on alternate days for 2 weeks. The procedure for drawing blood samples was that described in Experiment I, and tests performed were those listed in Table 1.

Experiment IV

The objective of this experiment was to assess the capacity of cobalt to modify the hematologic effects of a sterile abscess. Five cats were anesthetized with ketamine hydrochloride and 0.75 ml of sterile USP turpentine was injected subcutaneously into the gluteal region. A cobalt

injection (0.5 ml of 50 mg/ml cobalt chloride) was given at the same time and repeated daily thereafter until the abscess opened. Blood samples were drawn daily for 8 days and on alternate days for the next 6 days. The procedure for drawing the blood samples was that described in Experiment I, and the tests performed were those listed in Table 1.

Experiment V

The objective of this experiment was to find a method for increasing serum iron concentration in cats and to assess the effects of elevated serum iron on normal cats. The most acceptable method would then be used to evaluate the hematologic effects of elevated serum iron during a sterile abscess.

In Experiment VA, inosine (50 mg/kg) in 3 divided doses was injected into 4 cats by jugular catheter. The injections were given at 15-minute intervals. Serum iron concentrations were determined at hourly or bi-hourly intervals.

Because serum iron concentrations failed to increase in cats, hypoxanthine (50 mg/kg) in 3 divided doses was injected into 5 dogs via a cephalic vein. Serum iron concentrations and uric acid concentrations were determined at hourly intervals for 3 hours.

In Experiment VC, the dogs were placed under barbituate anesthesia in order to reproduce conditions of previous studies. The experiment was otherwise similar to Experiment VB.

In Experiment VD, allopurinol (300 mg/day) was orally administered to 2 dogs for 7 consecutive days. Serum iron and uric acid concentrations were determined daily.

In Experiment VE, a solution containing ferric chloride was administered at a rate of 1 mg per hour by continuous intravenous drip to 2 cats over a 36-hour period. The solution was prepared by adding 25 mg ferric chloride and 250 mg of trisodium citrate to 30 ml of distilled water. The solution was filter-sterilized and added to 220 ml lactated Ringer's solution. The rate of administration was approximately 10 ml per hour. Blood samples were drawn twice daily. The procedure for drawing blood was that described in Experiment I, and tests performed were those listed in Table 1.

Experiment VI

The objective of this experiment was to assess the effects of continuous intravenous iron administration on the hematologic alterations associated with sterile abscesses. These data, when compared with Experiment II, provided a means of assessing the relative contribution of serum iron concentrations to the pathogenesis of anemia of inflammatory disease. Four cats were anesthetized and sterile abscesses were induced as described in Experiment II. An indwelling catheter was placed in a jugular or cephalic vein and an intravenous drip of the ferric chloride solution described in Experiment V was administered. Blood samples were drawn twice daily for the first 5 days, daily for the next 3 days, and every other day for the next 6 days. Tests performed were those listed in Table 1.

Experiment VII

The objective of this experiment was to assess the effects of combined intravenous iron and cobalt on the hematologic alterations associated with a sterile abscess. The procedure was similar to Experiment VI, except that daily cobalt injections (25 mg) were administered.

Experiment VIII

The objective of this experiment was to determine *in vivo* erythrocyte survival in control cats and in cats with sterile abscesses. Six cats were anesthetized and indwelling catheters were placed in a cephalic vein as previously described. Five milliliters of blood was withdrawn into a syringe containing 1 ml acid citrate-dextrose solution. The blood was placed in a sterile 10 ml tube. Fifty microcuries of sterile sodium chromate ($Na_2Cr^{51}O_4$) was added to each blood sample and the blood was incubated for 1 hour at 37 C. Ascorbic acid (30 mg) was added and the blood was promptly reinjected into the cats. Blood samples were drawn 1 hour following reinjection and daily thereafter for 17 days. Sterile abscesses were induced in 4 cats on day 10 (Table 2).

Table 2. Experimental design for study of anemia of inflammatory disease

| Experiment | Animals/Experiment | Treatments |
|---|---|---|
| I | 5 | Normal control |
| II | 5 | Abscess |
| III | 5 | Cobalt in normal |
| IV | 5 | Cobalt in abscess |
| V | 3 | Intravenous iron in normal |
| VI | 4 | Abscess + intravenous iron |
| VII | 4 | Abscess + cobalt + intravenous iron |
| VIII | 4 | RBC survival in normal and abscess |

## Procedures

### Complete Blood Count

The packed cell volume was determined by the microhematocrit method (McInroy, 1954). Hemoglobin, red cell count, MCV, MCHC and total leukocyte count were determined by standard methods with an electronic cell counter[a] (Schalm et al., 1975a).

[a]Coulter Counter, Model S, Coulter Electronics, Inc., Hialeah, Florida.

Plasma Protein

Total plasma proteins were determined by refractive index as measured by a refractometer.[b]

Reticulocyte Count

Reticulocytes were stained with new methylene blue according to the method of Brecher (1949). These cells were classified as Type I, II or III reticulocytes, as described by Schalm et al. (1975b). The relative number of each type was enumerated by counting 1000 red blood cells. Type I reticulocytes were those with isolated foci of reticulum. Type II reticulocytes were those with 1 to several filaments in addition to the isolated foci. Type III reticulocytes were those with an interwoven mass of reticulum which occupied a large portion of the cell.

Serum Iron and Total Iron Binding Capacity

Serum iron and total iron binding capacity were determined by a modification of Goodwin's method (Stookey, 1970). The reagents were purchased as a kit[c] and samples were analyzed on a centrifugal clinical chemistry analyzer.[d]

Preliminary evaluation of the kit revealed that color development was not stable. Color intensity continued to develop with time, giving significantly higher serum iron

---

[b]Goldberg Refractometer, American Optical Co., Buffalo, New York.

[c]Gemini Serum Iron and TIBC Kit, Electro-Nucleonics, Inc., Fairfield, New Jersey.

[d]Gemini Centrifugal Analyzer, Electro-Nucleonics, Inc., Fairfield, New Jersey.

concentrations. Good precision was obtained by allowing exactly 4 minutes between the end of the blank run and the beginning of the test run.

With each batch of samples a 500 µg/dl iron standard, normal feline control and commercial human control serum[e] were determined. Results were accepted only if all control sample values were within 2 standard deviations of their mean.

## Erythrocyte Survival

Radiolabeled whole blood (0.5 ml) was placed in a counting tube and lysed with distilled water (0.5 ml). Counting tubes were placed in a well-type scintillation counter. A minimum of 10,000 counts were accumulated on each sample by varying the counting time between 1 and 5 minutes. Raw counts were corrected for background and decay as described by Gillis and Mitchel (1974) and the percent activity remaining was plotted on semilog paper.

## Serum Erythropoietin

Serum erythropoietin concentrations were determined by Dr. R. D. Lange by an *in vitro* fetal mouse liver cell assay (Dunn, Lange and Jones, 1979). This assay has been shown to detect elevated erythropoietin concentrations in the serum of cats and dogs following exposure to hypobaric conditions and phlebotomy (Dunn, Lange and Jones, 1979;

---

[e]Monotrol I and II, Dade Division American Hospital Supply Corporation, Miami, Florida.

Dunn and Legendre, 1980). A purified human urinary standard and feline control serum were used. A highly significant correlation ( r=.6, p=.001) was found between the in vivo and the in vitro bioassay procedures. The index of precision for the procedure was .05-.15.

## Statistical Analysis

Means, standard deviations and standard errors of the mean were calculated for the data in each experiment. The significance of daily changes within gruops I through VII was analyzed by analysis of variance. The significance of day to day intergroup variation was analyzed either by analysis of variance (PCV) or by the nonparametric rank sum test (serum iron, reticulocytes-types I II and III and erythpopoietin). Bartlet's test was used to decide which of the two statistical tools was appropiate for each set of data.

In Experiment VIII, mean percent radioactivity remaining was plotted against time on semilog graph paper. A line of best fit was determined for days 1 through 9 and days 13 through 17 for both control and abscess groups. Lines of best fit were calculated by regression analysis following log transformation of the data. Slopes for each of the 4 regression lines were calculated and compaired by the Student's t-test.

# RESULTS

## Experiment I

Experiment I assessed hematologic alterations caused by the bleeding protocol. Total blood withdrawn over the 2-week period was approximately 16.5 ml. Results have been tabulated in Appendix Table A-1 and summarized in Table 3.

Significant changes in the tests were not observed except for type II reticulocytes, which increased during the experiment. The packed cell volume increased slightly, which indicated that the bleeding protocol by itself did not cause anemia.

Serum samples for erythropoietin determination were drawn on day 1 of the experiment. The mean serum erythropoietin concentration was found to be 22.5 $\pm$ 2.1 mU/ml (Table 4).

## Experiment II

Experiment II assessed the hematologic changes caused by a sterile abscess. Results have been listed in Table 5 and Appendix Table A-2.

The packed cell volumes were increased in all cats on day 2. Following day 2 the packed cell volume decreased

Table 3. Selected data from control cats (Experiment I)

| | Days | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 12 |
| PCV (Vol.%) | 34 ±2[a] | 35 ±2 | 35 ±2 | 35 ±2 | 37 ±2 | 36 ±2 |
| Serum iron (µg/dl) | 103 ±30 | 102 ±9 | 110 ±10 | 96 ±13 | 105 ±17 | 105 ±9 |
| Retic Type I ($x10^3/\mu l$) | 366 ±133 | 418 ±110 | 431 ±69 | 457 ±58 | 627 ±108 | 481 ±49 |
| Retic Type II ($x10^3/\mu l$) | 11 ±7 | 15 ±6 | 10 ±7 | 15 ±7 | 106[b] ±28 | 79 ±17 |
| Retic Type III ($x10^3/\mu l$) | 0 | 0 | 0 | 0 | 4.3 ±3 | 0 |

[a]Values represent mean ± standard error for 5 cats.

[b]Significantly different from day 1 ($p<.05$).

Table 4. Erythropoietin concentrations in control cats, cats with sterile abscesses, cats treated with cobalt and cats with abscesses treated with cobalt

| | Control | Abscess* | Cobalt* | Abscess and Cobalt* |
|---|---|---|---|---|
| | 19.6 | 32.1 | 16.2 | 28.5 |
| | 31.6 | 29.3 | 15.7 | 16.4 |
| | 19.0 | 29.3 | 38.2 | 16.3 |
| | 20.7 | 33.3 | 49.1 | 15.6 |
| | 22.7 | 31.2 | 31.0 | 31.6 |
| | 22.0 | | | |
| | 16.8 | | | |
| | 15.9 | | | |
| | 29.8 | | | |
| Mean | 22.5 | 31.0 | 30.0 | 21.7 |
| SD | 6.1 | 1.8 | 14.4 | 7.7 |
| SE | 2.1 | .8 | 6.4 | 3.5 |

*Samples drawn on day 5 after initiation of the abscess and/or cobalt.

Table 5. Selected data from cats with sterile abscesses (Experiment II)

| | Days | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 12 |
| PCV (Vol.%) | 32 ±2[a] | 39 ±1 | 31 ±2 | 24 ±2[b] | 28 ±2 | 29 ±2 |
| Serum iron (µg/dl) | 81 ±5 | 23 ±8[c] | 38 ±14[c] | 16 ±4[c] | 64 ±30 | 80 ±17 |
| Retic Type I ($x10^3/\mu l$) | 355 ±68 | 508 ±147 | 164 ±78 | 95 ±38[b] | 163 ±48 | 530 ±154 |
| Retic Type II ($x10^3/\mu l$) | 26 ±13 | 47 ±36 | 22 ±14 | 39 ±21 | 67 ±35 | 224 ±104[b] |
| Retic Type III ($x10^3/\mu l$) | .5 ±.5 | 3 ±3 | 0 | 0 | 7 ±4 | 27 ±12[b] |

[a]Values represent mean ± standard error for 5 cats.

[b]Significantly different from day 1 ($p<.05$).

[c]Significantly different from day 1 ($p<.01$).

progressively in all cats until the abscesses opened. This decrease was statistically significant ($p<.05$) by day 6 with an average decrease of 8 volume percent.

Serum iron concentrations were decreased in all cats by day 2 ($p<.01$). Iron concentrations remained low until the abscesses opened (days 6 to 8).

Type I reticulocytes decreased progressively between days 2 and 7. The decrease was statistically significant by day 5 ($p<.05$). Significant decreases in type II and type III reticulocytes were not observed.

Serum erythropoietin concentrations were determined on the fifth day after induction of the abscess. Mean serum erythropoietin concentrations were higher than the control group, but the change was not significant ($p=.1$).

## Experiment III

Experiment III assessed hematologic changes caused by cobalt administration. The data have been listed in Table 6 and Appendix Table A-3.

The packed cell volumes in all cats increased progressively during the experiment. An average increase of 13 volume percent occurred during the 2-week period of the experiment. A significant increase in type I and type II reticulocytes also occurred in all cats.

Mean serum erythropoietin concentrations (Table 4) varied considerably. Three of the cats had significantly increased concentrations, while 2 cats had values slightly lower than the normal group.

Table 6. Selected data from cats treated with cobalt (Experiment III)

| | Days | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 |
| PCV (Vol.%) | 36 ±2[a] | 39 ±2 | 44 ±2[c] | 45 ±2[c] | 47 ±3[c] |
| Serum iron (µg/dl) | 71 ±15 | 116 ±17[b] | --- | 137 ±24[b] | 105 ±15[b] |
| Retic Type I ($x10^3/\mu l$) | 876 ±85 | 1624 ±325[c] | 1396 ±352[c] | 1656 ±335[c] | 1708 ±227[c] |
| Retic Type II ($x10^3/\mu l$) | 15 ±6 | 46 ±32[b] | 101 ±77[c] | 173 ±117[c] | 211 ±115[c] |
| Retic Type III ($x10^3/\mu l$) | 1.5 ±1.5 | 21 ±11 | 67 ±44 | 69 ±31 | 47 ±24 |

[a]Values represent mean ± standard error for 5 cats.

[b]Significantly different from day 1 ($p<.05$).

[c]Significantly different from day 1 ($p<.01$).

## Experiment IV

Experiment IV assessed the effects of cobalt on the hematologic changes caused by a sterile abscess. The data have been presented in Table 7 and Appendix Table A-3.

The packed cell volumes of all cats incresaed significantly by day 2 ($p<.01$). The mean packed cell volume continued to increase until day 5 but thereafter decreased. The mean packed cell volume was significantly less than than in Experiment III by day 7 ($p<.01$).

Serum iron concentrations decreased slightly during the course of the abscess but remained significantly higher than in Experiment II ($p<.01$).

Types I, II and III reticulocytes increased significantly ($p<.01$) during the course of the abscess. By day 3 this change was significantly different from Experiment II, in which reticulocyte numbers decreased ($p<.01$). As in group III, erythropoietin concentrations varied considerably (Table 4). The mean concentration was essentially the same as in the control group.

## Experiment V

This group assessed the effects of elevated serum iron concentrations on control cats. Initially, attempts were made to increase serum iron by releasing storage iron. Inosine was injected into cats and hypoxanthine was injected into anesthetized and nonanesthetized dogs (Appendix Tables B-1 through B-4). All experiments were uniformly unsuccessful in increasing serum iron concentrations. However, uric acid levels were increased.

Table 7. The effects of cobalt on the hematologic alterations caused by a sterile abscess (Experiment IV)

| | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| PCV (Vol.%) | 32[a] ±1 | 39[b] ±1 | 42[c] ±2 | 41[b] ±3 | 38 ±3 | 36 ±4 | 35 ±2 |
| Serum iron (µg/dl) | 117 ±17 | 89 ±18 | 67[c] ±8 | 100 ±14 | 120 ±35 | 99 ±15 | 81 ±13 |
| Retic Type I ($x10^3/\mu l$) | 688 ±242 | 1241 ±493 | 1376[b] ±316 | 1100 ±188 | 771 ±107 | 890 ±88 | 961 ±57 |
| Retic Type II ($x10^3/\mu l$) | 5 ±5 | 11 ±7 | 86[b] 21 | 43 ±19 | 42 ±17 | 27 ±13 | 108 ±28 |
| Retic Type III ($x10^3/\mu l$) | 0 | 7 ±4 | 41[c] ±22 | 44[c] ±29 | 16 ±9 | 21 ±16 | 0 |

[a]Values represent mean ± standard error for 5 cats.

[b]Significantly different from day 1 ($p<.01$).

[c]Significantly different from day 1 ($p<.05$).

Alternatively, cats were given ferric chloride by slow intravenous drip at a rate of 1 mg/hr. It was technically difficult to maintain jugular and cephalic catheters in normal cats, as the cats could remove them by biting or scratching. Thus, the experiment was limited to 3 days. During this time serum iron levels were maintained between 400 and 550 µg/dl.

The mean packed cell volume increased from 33 to 37.5 (Tables 8 and Appendix Table A-5). Reticulocyte counts did not increase.

## Experiment VI

Experiment VI assessed the effects of intravenous iron on the hematologic alterations caused by a sterile abscess. Serum iron levels were maintained between 109 and 249 µg/dl.

As in Experiment II, the mean packed cell volume decreased significantly by day 6 ($p<.05$) and increased after the abscesses opened (Table 9 and Appendix Table A-6). The net decrease in PCV during the abscess was essentially the same as in Experiment II. Reticulocyte counts increased during the abscess rather than decreasing as they did in group II (Table 9 and Appendix Table A-6). Type I reticulocytes were significantly greater than in Experiment II by day 4 ($p<.05$) and types II and III reticulocytes were greater by day 6 ($p<.01$).

Table 8. Selected data from cats treated with intravenous iron (Experiment V)

| | Days | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| PCV (Vol.%) | 33 ±1[a] | 35 ±1 | 36 ±.5 |
| Serum iron (µg/dl) | 90 ±7 | 471 ±31 | 388 ±10 |
| Retic Type I ($x10^3/\mu l$) | 892 ±237 | 957 ±272 | 782 ±112 |
| Retic Type II ($x10^3/\mu l$) | 24 ±8 | 29 ±12 | 12 ±12 |
| Retic Type III ($x10^3/\mu l$) | 0 | 0 | 0 |

[a]Values represent mean ± standard error for 3 cats.

Table 9. The effects of intravenous iron on hematologic alterations caused by a sterile abscess (Experiment VI)

| | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| PCV (Vol.%) | 37[a] ±2 | 35 ±1 | 34 ±2 | 29[b] ±2 | 30 ±3 | 37 ±2 | 33 ±2 |
| Serum iron (µg/dl) | 131 ±19 | 236 ±34 | 223 ±58 | 195 ±35 | 125 ±49 | 108 ±56 | 112 ±56 |
| Retic Type I ($x10^3/\mu l$) | 737 ±192 | 485 ±239 | 718 ±195 | 628 ±276 | 537 ±147 | 583 ±72 | 985 ±200 |
| Retic Type II ($x10^3/\mu l$) | 34 ±23 | 8 ±8 | 91 ±23 | 168[b] ±32 | 368[c] ±61 | 415[c] ±74 | 262[c] ±54 |
| Retic Type III ($x10^3/\mu l$) | 0 | 0 | 8 ±5 | 30[b] ±8 | 77[c] ±24 | 57[b] ±33 | 58[b] ±34 |

[a]Values represent mean ± standard error for 4 cats.

[b]Significantly different from day 1 ($p<.05$).

[c]Significantly different from day 1 ($p<.01$).

## Experiment VII

Experiment VII assessed the effects of intravenous iron and cobalt on the hematologic alterations caused by a sterile abscess. Serum iron concentrations were maintained between 125 and 350 μg/dl, except for cat B, which removed its catheter on day 3. After 3 hours without iron supplementation, the serum iron had decreased to 51 μg/dl (Table 10 and Appendix Table A-7).

The changes in mean packed cell volume in this group generally paralleled Experiment IV, with an increase in the packed cell volume over the first several days followed by a drop after day 6 (Appendix Tables A-7 and A-8). In this experiment, however, the decrease was transient, followed by a progressive increase on days 8 and 10.

Reticulocyte counts increased markedly during the abscess. Both type I and type II reticulocytes were significantly increased by day 2 ($p<.01$ and $p<.05$). Type III reticulocytes were significantly increased by day 3 ($p<.05$). Type II and type III reticulocytes increased progressively during the abscess, while type I reticulocytes decreased on days 5 and 6. All 3 types of reticulocytes were significantly greater than reticulocytes in all other experiments ($p<.01$).

## Experiment VIII

Experiment VIII assessed changes in erythrocyte survival during a sterile abscess. In the control group the slope of days 1 through 9 was .037 ± .004, while the slope for days

Table 10. The effects of cobalt and intravenous iron on hematologic alterations caused by a sterile abscess (Experiment VII)

| | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| PCV (Vol.%) | 30 ±.5[a] | 37 ±2[b] | 38 ±3[b] | 40 ±5[c] | 39 ±4[b] | 45 ±6[c] | 44 ±4[c] |
| Serum iron (µg/dl) | 87 ±11 | 236 ±39 | 220 ±15 | 269 ±31 | 92 ±2 | 52 ±8 | 56 ±3 |
| Retic Type I ($x10^3/\mu l$) | 817 ±97 | 1426 ±137[c] | 1258 ±162[c] | 774 ±151 | 986 ±135 | 1295 ±60[c] | 2096 ±307[c] |
| Retic Type II ($x10^3/\mu l$) | 93 ±30 | 341 ±65[b] | 311 ±80[b] | 659 ±71[c] | 760 ±104[c] | 605 ±127[c] | 495 ±141[b] |
| Retic Type III ($x10^3/\mu l$) | 3 ±3 | 5 ±5 | 268 ±57[c] | 515 ±171[c] | 278 ±53[c] | 7 ±7 | 31 ±20 |

[a]Values represent mean ± standard error for 4 cats.

[b]Significantly different than day 1 ($p<.05$).

[c]Significantly different than day 1 ($p<.01$).

13 through 17 was .031 ± .007 (Table 11, Figure 7). In the sterile abscess group the slope for days 1 through 9 (pre-abscess) was .035 ± .001, while the slope during the abscess was .056 ± .005 (Figure 7). Pre-abscess and abscess slopes were significantly different ($p<.01$). These data indicate a significant decrease in erythrocyte survival during the abscess.

Table 11. Erythrocyte survival in normal cats and cats with abscesses induced on day 10

| Days | Control Group | Abscess Group |
|---|---|---|
| 2 | 10,660 ± 2,331[a] | 10,791 ± 281 |
| 3 | 8,694 ± 390 | 10,151 ± 367 |
| 4 | 8,110 ± 1,241 | 9,909 ± 543 |
| 5 | 7,465 ± 1,517 | 8,219 ± 485 |
| 6 | 7,345 ± 1,993 | 7,566 ± 444 |
| 7 | 6,542 ± 1,399 | 7,031 ± 388 |
| 8 | 5,865 ± 905 | 6,972 ± 418 |
| 9 | 5,653 ± 1,078 | 6,264 ± 279 |
| 10 | 5,880 ± 1,284 | 6,387 ± 463 |
| 11 | 6,332 ± 830 | 6,147 ± 290 |
| 12 | 4,838 ± 333 | 6,122 ± 339 |
| 13 | 4,240 ± 746 | 4,526 ± 424 |
| 14 | 4,104 ± 509 | 4,054 ± 469 |
| 15 | 3,827 ± 73 | 3,552 ± 316 |
| 16 | 3,684 ± 427 | 2,913 ± 263 |
| 17 | 3,524 ± 243 | 2,587 ± 293 |

[a]Values represent mean ± standard error for radioactivity in counts per minute/0.5 ml whole blood.

# DISCUSSION

The 8 experiments discussed herein investigated the pathogenesis of anemia of inflammatory disease. Although contemporary controls were not used, related experiments were conducted successively and all experiments were completed within 4 months. Environmental conditions within the kennel were carefully controlled throughout the study.

In the control group (Experiment I), significant changes were not observed except for type II reticulocytes which increased. The results indicated that the bleeding protocol by itself did not cause anemia. Erythropoietin concentrations (22.5 ± 2 mU/ml) were lower than normal values reported by the *in vivo* exhypoxic mouse assay (50 ± .03 mU/ml) and the *in vitro* fetal mouse liver cell assay (30 ± .01 mU/ml) (Dunn and Legendre, 1980). Although results of these 2 assays have been shown to correlate ($r=.6$, $p<.001$), the difference in mean normal values appeared to represent real differences in the amount of erythropoiesis-stimulating substances detected (Dunn, Lange and Jones, 1979). *In vitro* assays were shown to detect desilated erythropoietin, whereas *in vivo* assays did not (Dunn, Lange and Jones, 1979). Also, serum regulatory proteins, which modify the activity of erythropoietin, were reported to have more effect on *in vitro* assays

(Dunn, Lange and Jones, 1979). Thus, erythropoietin concentrations determined by present assay techniques should be considered only approximations of the true serum activity.

Cats with induced sterile abscesses (Experiment II) developed a hematologic disorder generally consistent with anemia of inflammatory disease as described in humans, dogs, rats and mice (Cartwright, 1966). A similar disorder described as "depression anemia" was reported in cats (Mahaffey and Smith, 1978). The anemia in all species in these reports was characterized by: 1) poorly regenerative, normocytic, normochromic or normocytic, hypochromic anemia, 2) low plasma iron, 3) decreased total iron binding capacity, 4) decreased saturation of transferrin, 5) increased storage iron, 6) decreased bone marrow sideroblasts, 7) increased plasma copper, 8) increased free erythrocyte protoporphyrin, and 9) shortened erythrocyte survival.

In these experiments detectable anemia developed by the fifth day after induction of the abscess in all cats (Figure 1). In rats with sterile abscesses, anemia was found to develop by the third day. However, in humans and dogs anemia developed only after 2 to 3 weeks (Lauritsen et al., 1946; Lukens et al., 1967; Cartwright and Lee, 1971). This species difference in onset time of anemia may relate to the normally shorter erythrocyte lifespans in cats and rats.

Serum iron concentrations decreased rapidly within the first 24 hours and remained low until the abscess opened (Figure 4). The rapid and consistent decrease in serum iron

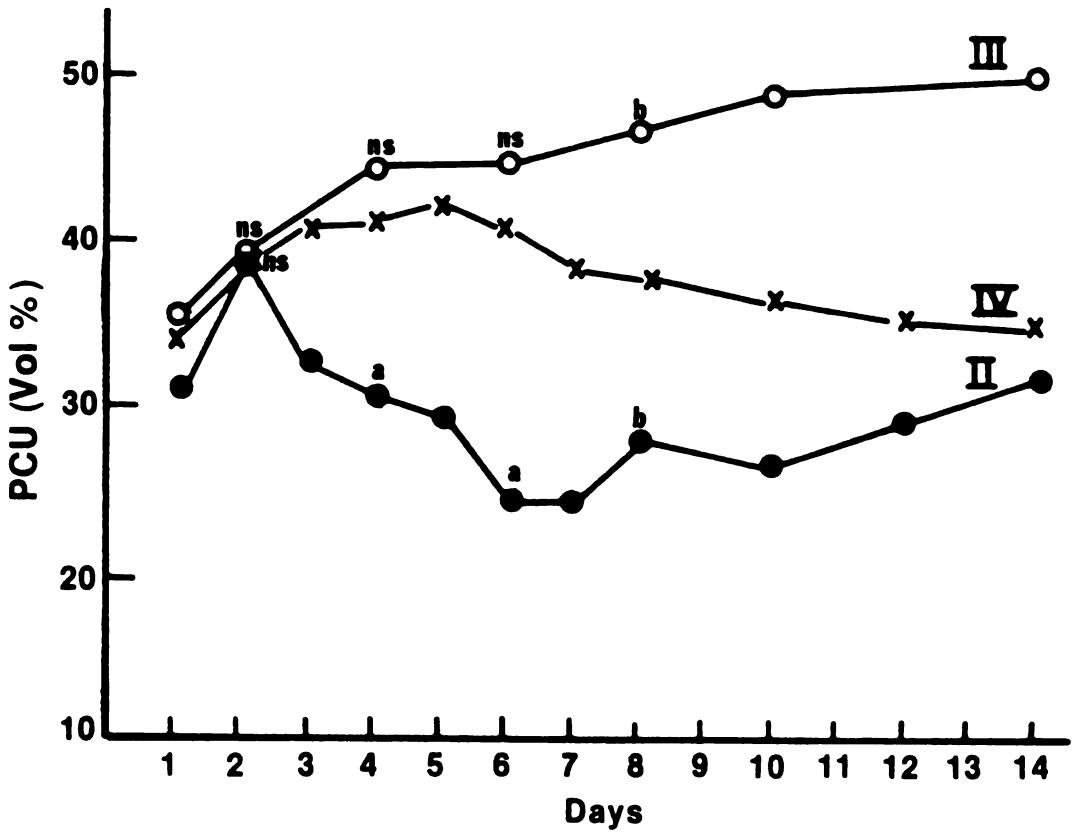


Figure 1. Sequential mean packed cell volumes in the sterile abscess group (Group II ●——●), cobalt treatment group (Group III ○——○), and combined abscess and cobalt treatment group (Group IV X——X ).

[a]Significantly different from the mean of group IV; p<.01; b-p<.05; ns - not significantly different.

has been considered a hallmark of anemia of inflammatory disease (Cartwright, 1966).

Type I reticulocytes decreased during the sterile abscess (Figure 5). This failure of the bone marrow to respond to the anemia was consistent with hypoproliferative nature of the anemia of inflammatory disease.

Administration of cobalt to normal cats (Experiment III) resulted in detectable polycythemia, reticulocytosis and hyperferremia by day 2. Normally a 3- to 5-day lag period is present between stimulation of the marrow and increased erythrocyte output (Schalm, 1975). Thus, other factors such as splenic contraction or subclinical hemoconcentration may have been involved in the early phase of the polycythemia (Mahaffey and Smith, 1978).

Erythropoietin concentrations in cobalt-treated cats varied (Table 4). Three cats had increased concentrations while 2 had concentrations in the low-normal range. These results are in contrast to those of Goldwasser et al. (1958). They reported that cobalt injection into rats caused a rapid increase in erythropoietin activity in serum. Erythropoietin concentrations in their study peaked about 10 hours after injection and then dropped rapidly over the next 10 hours. A maximum increase of 2-6 times baseline levels occurred at various doses of cobalt. The discrepancy in this study may have related to the time of sampling. Serum samples were collected 24 hours after the last cobalt injection. Thus, the erythropoietin peak may have been missed.

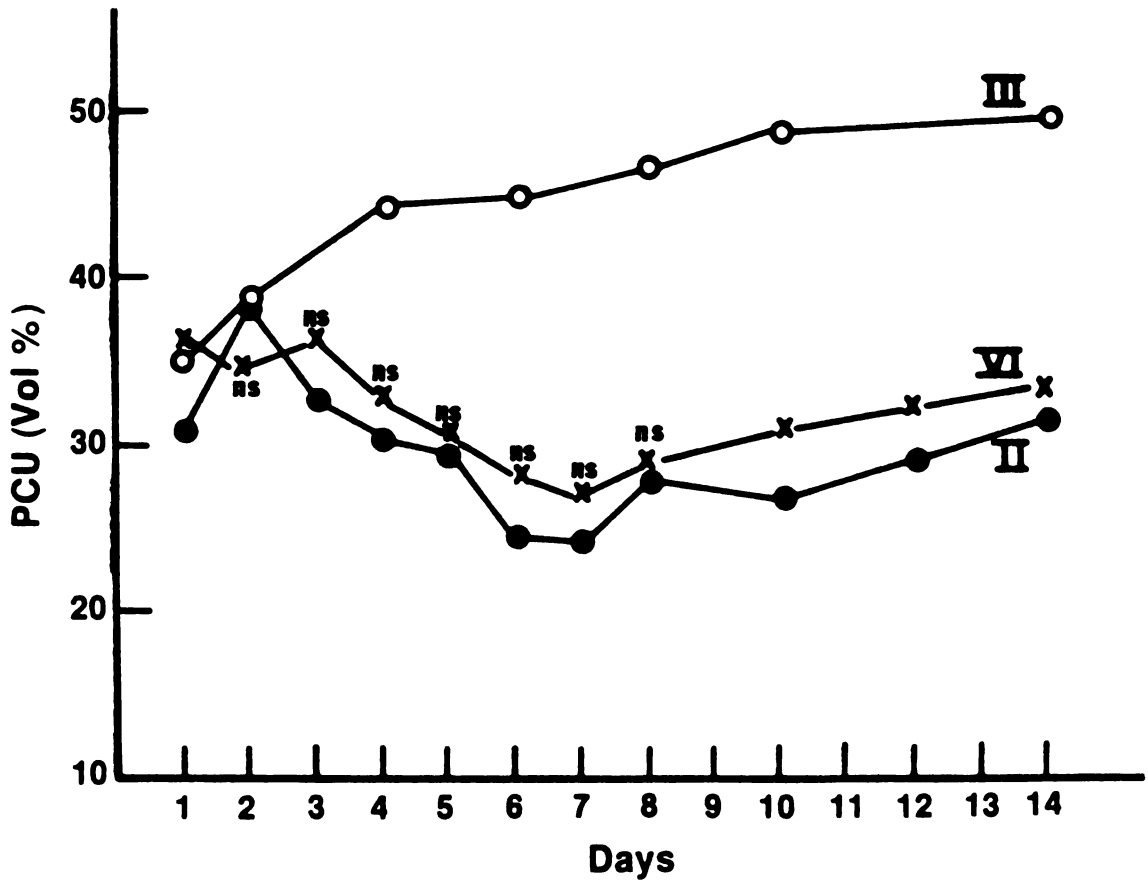


Figure 2. Sequential mean packed cell volumes in the sterile abscess group (Group II ●—● ), cobalt treatment group (Group III ○—○), and combined abscess and iron treatment group (Group VI X—X ).

ns - Not significantly different from the mean group II at the = 0.05 level.

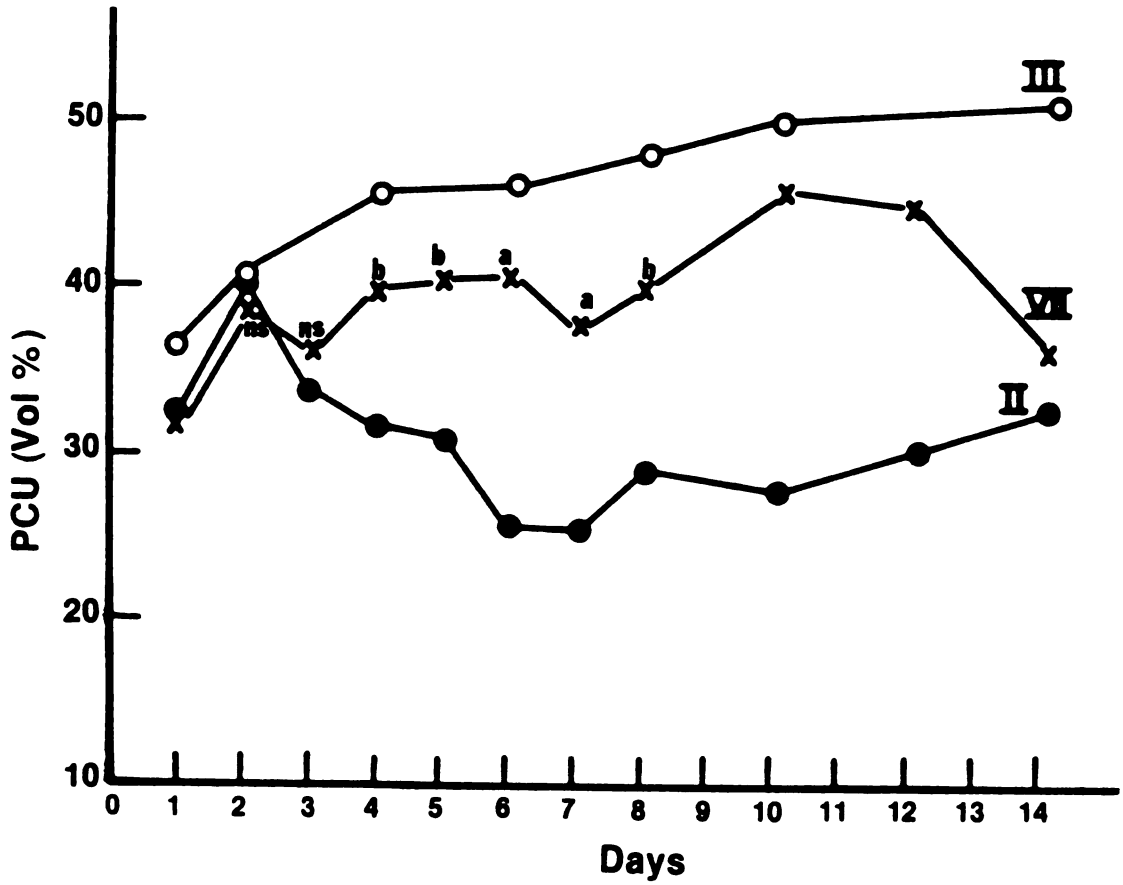


Figure 3. Sequential mean packed cell volumes in the sterile abscess group (Group II ●—● ), cobalt treatment group (Group III ○—○ ) and combined abscess, cobalt and iron treatment group (Group VII X—X ).

[a]Significantly different from the mean of group II; p<.01; b-p<.05; ns - not significantly different.

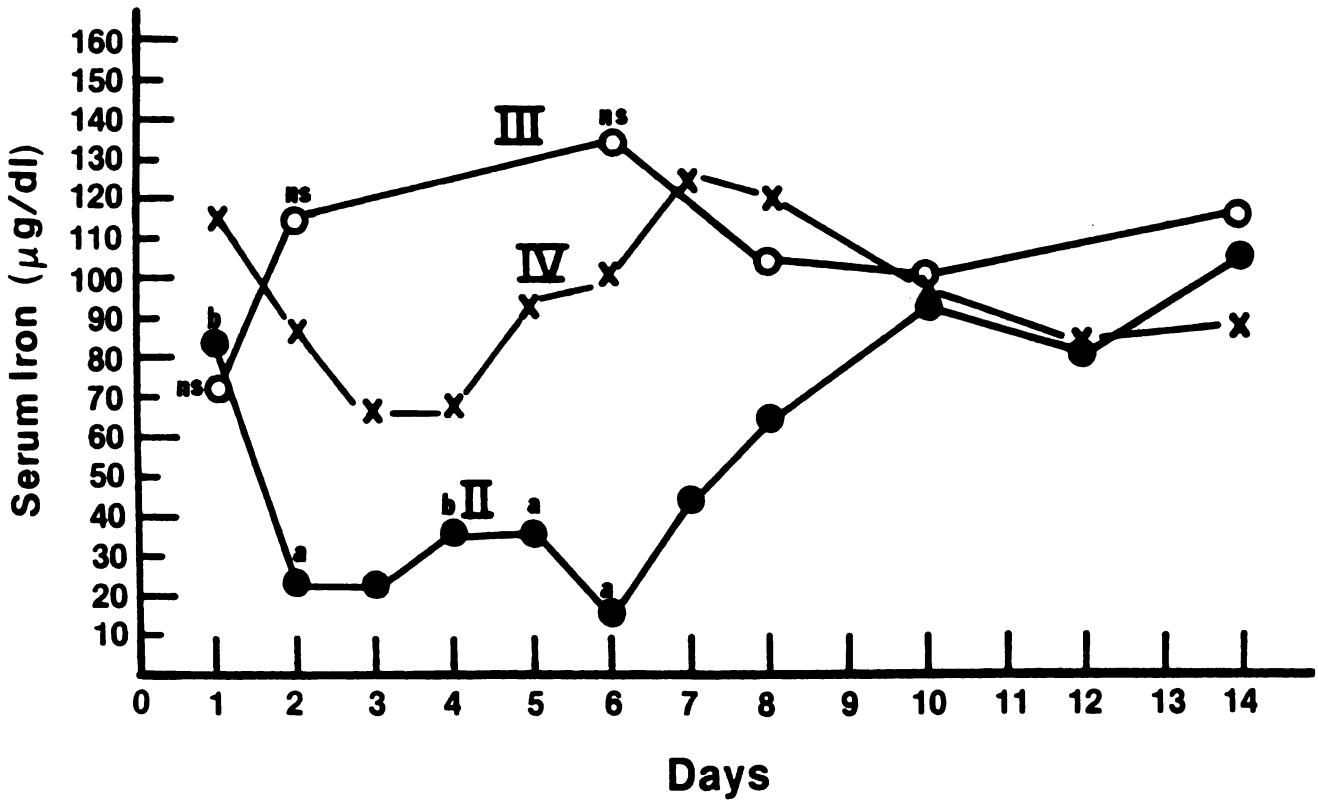


Figure 4. Sequential mean serum iron concentrations in the sterile abscess group (Group II ●—● ), cobalt treatment group (Group III ○—○ ) and combined abscess and cobalt treatment group (Group IV ✕—✕ ).

[a]Significantly different from the mean of group IV; p<.01; b-p<.05; ns - not significantly different.

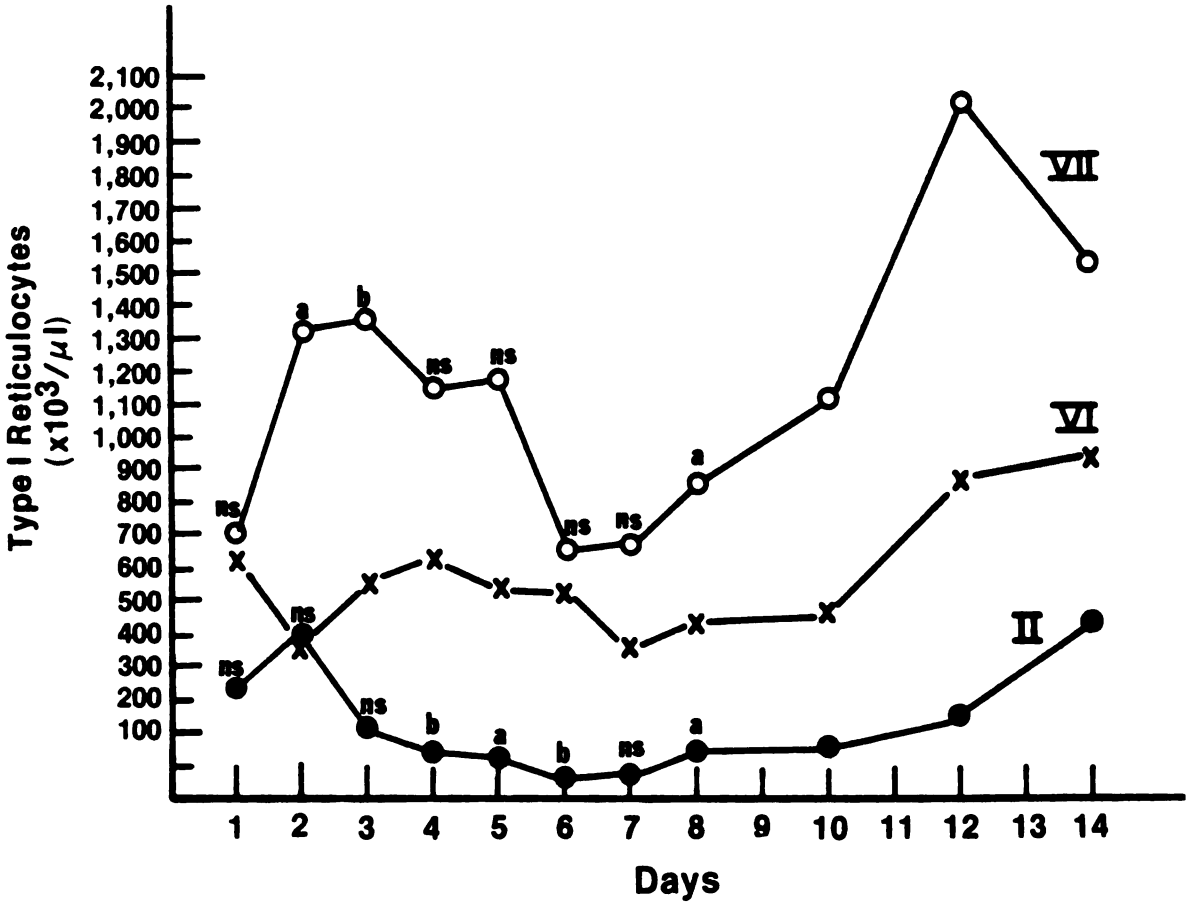


Figure 5. Sequential mean type I reticulocytes in the sterile abscess group (Group II ●—● ), combined abscess and iron treatment group (Group VI X—X ) and combined abscess, cobalt and iron treatment group (Group VII ○—○).

[a]Significantly different from the mean of group VI; p<.01; b-p<.05; ns - not significantly different.

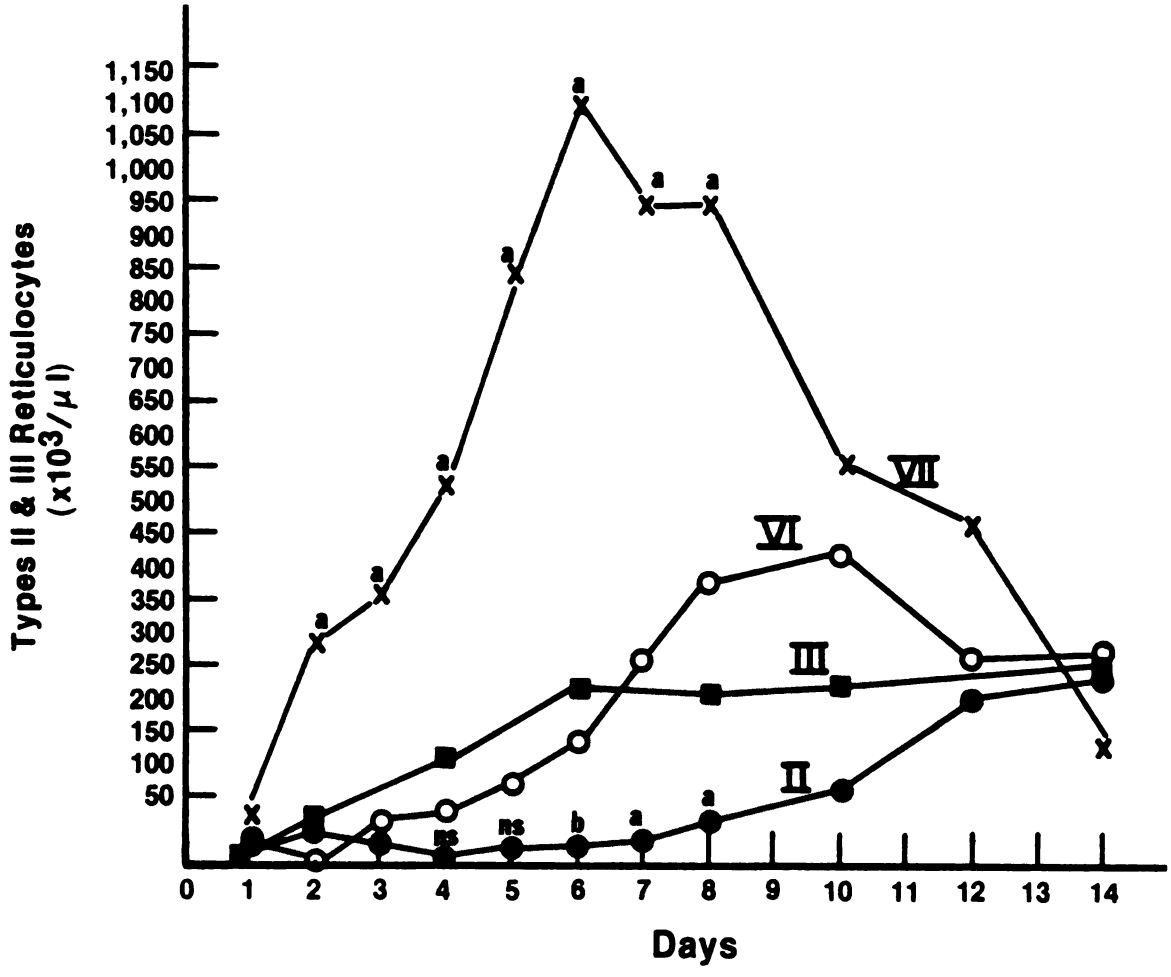


Figure 6. Sequential mean type II and type III reticulocytes in the sterile abscess group (Group II ●—●), cobalt treatment group (Group III ■—■), combined abscess and iron treatment group (Group VI ○—○) and combined abscess, cobalt and iron treatment group (Group VII ✕—✕).

[a]Significantly different from the mean of VI; p<.01; b-p<.05; ns - not significantly different.

Cobalt administration to cats with sterile abscesses (Experiment IV) prevented the development of the anemia and, instead, induced a polycythemia and reticulocytosis (Figures 1, 5 and 6). These findings were consistent with findings in rats and people and suggested that the bone marrow in anemia of inflammatory disease was capable of responding to erythropoietin (Shen et al., 1951; Wintrobe et al., 1947). However, the magnitude of the polycythemia was significantly less than in the non-abscess-cobalt treatment group (Figure 1).

Possible explanations of this decreased response to cobalt during an abscess include: 1) hypoferremia limiting erythropoiesis, 2) limited capacity to produce erythropoietin, 3) decreased erythrocyte lifespan, and 4) serum inhibitor of erythropoietin or erythropoiesis (Cartwright and Lee, 1971).

Intravenous infusion of iron into normal cats (Experiment V) resulted in increased mean packed cell volume from 33.0 to 37.5. These results suggested that iron supplementation alone stimulated erythropoiesis.

Intravenous iron infusion into cats with sterile abscesses (Experiment VI) did not prevent the development of anemia but did result in a reticulocytosis (Figures 2, 5 and 6). This was in contrast to Experiment II (sterile abscess alone), where reticulocyte counts decreased. These data were consistent with the conclusion that relative unavailability of iron prevented the bone marrow from responding to the anemia of inflammatory disease.

Few other reports of intravenous iron therapy in anemia of inflammatory disease have been published. Greenberg et al. (1947) infused iron ascorbate into people with chronic diseases for up to 72 hours. They found no improvement in the hemoglobin levels but did report a slight increase in reticulocyte counts. They concluded that hypoferremia was not the limiting factor in production of the anemia.

Richmond et al. (1958) reported the treatment of 26 rheumatoid arthritis patients with intravenous iron. The patients received daily injections of 200 mg saccharated iron oxide for 25 days. They found a 14% increase in hemoglobin 1 month after the last injection. However, iron deficiency anemia was reported to be present in at least 8 of the patients.

Combined administration of intravenous iron and cobalt to cats with sterile abscesses (Experiment VII) resulted in a marked reticulocytosis (Figures 5 and 6). The reticulocytosis was significantly greater than in Experiment III, where cobalt was given to non-abscessed cats. These data suggest that serum iron concentrations were involved in the stimulation of erythropoiesis. Thus, the regulation of serum iron in addition to erythropoietin concentrations may represent a dual control of erythropoiesis.

Cartwright and Lee (1971) proposed that mechanisms for the production of erythropoietin and for the release of storage iron were different. They concluded that the factors which control both mechanisms must be coupled to permit maximal erythropoiesis.

The release mechanism for storage iron has been poorly defined. In Experiment III, cobalt administration resulted in increased serum iron concentration (Figure 4). Thus, cobalt directly or indirectly resulted in release of storage iron. The failure of purine analogs to increase serum iron (Experiment V) suggests that xanthine oxidase is not a major regulation of iron release from cells.

The role of erythropoietin in the release of storage iron has been controversial. Cartwright and Lee (1971) proposed that erythropoietin was directly or indirectly responsible for release of storage iron. However, rats treated with large doses of erythropoietin had very low serum iron concentrations (Gutnesky and VanDyke, 1963).

Erythrocyte survival in cats with sterile abscesses (Experiment VIII) was found to be significantly reduced (Figure 7). The percentage loss of activity during the abscess was 4.28%/day, while the loss in the control group during the same period was 1.5%/day.

The abrupt flattening of the erythrocyte survival curve following induction of the abscess may have been due to subclinical hemoconcentration or splenic contraction. The concurrent increase in PCV and total plasma proteins in this experiment and in Experiment II would be consistent with this explanation.

Erythrocyte survival during sterile abscesses has been studied in dogs, rabbits and rats. Rigby et al. (1962) studied erythrocyte survival in dogs with turpentine abscesses. Two dogs which received 2 injections of

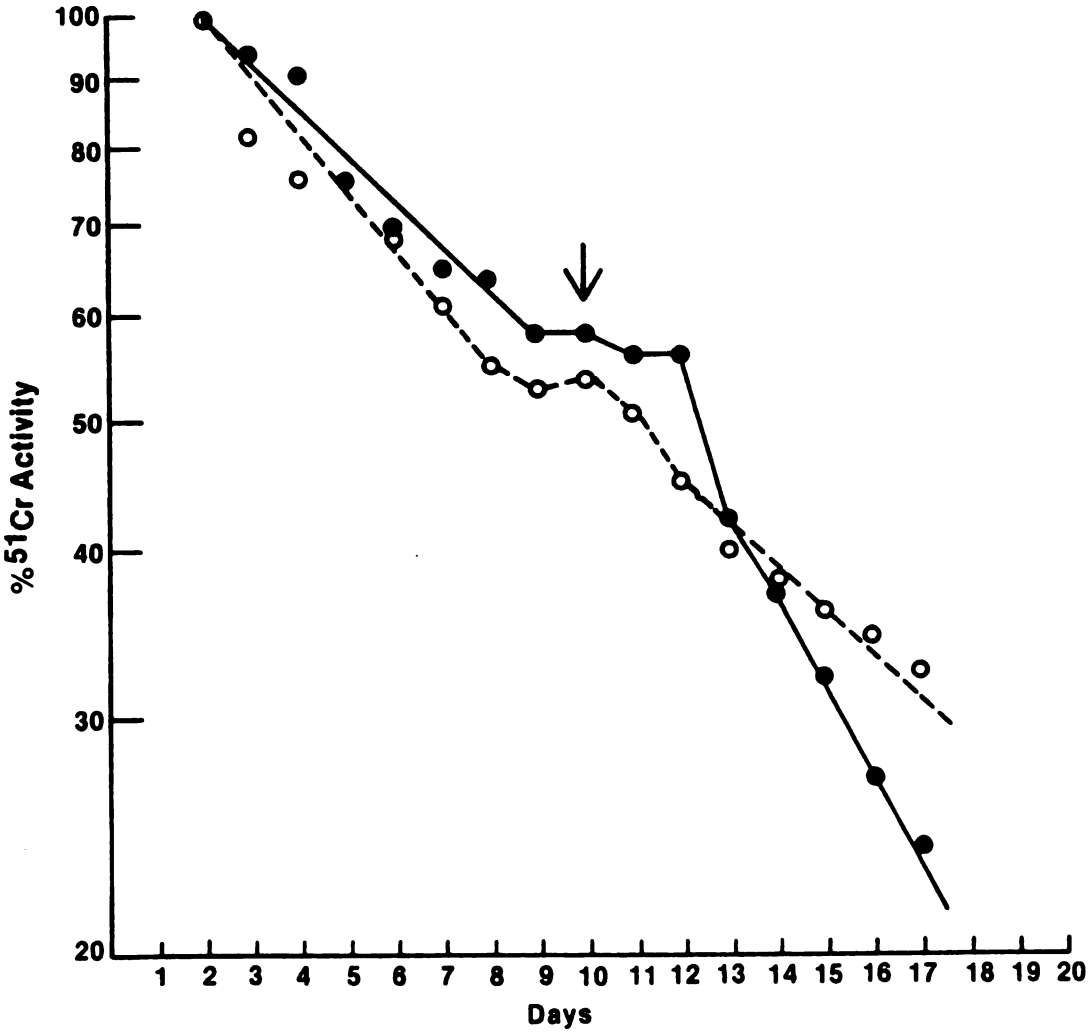


Figure 7. Mean $^{51}Cr$ erythrocyte survival in normal cats ( O- - -O) and cats with sterile abscesses induced on day 10 ( ●——● ).

turpentine had no detectable shortening of erythrocyte survival, while 1 dog which received 6 injections over a 28-day period had a mildly shortened erythrocyte lifespan. Mikolajew et al. (1969) reported a decreased erythrocyte lifespan in experimental adjuvant disease in rats. Karle (1969) studied the effects of fever induced by bacterial pyrogens, heated milk or external heating on erythrocyte survival in rabbits. A decreased erythrocyte survival occurred with all types of experimental hyperthermia. The maximum decrease occurred shortly after induction of the fever. Prolongation of the fever period did not result in continuation of the erythrocyte destruction. In another report, 2-generation-labeling of erythrocytes showed that fever-induced erythrocyte destruction was age-dependent (Karle, 1968a).

Two major questions remain concerning the pathogenesis of anemia of inflammatory disease. The first relates to the cause of the decreased erythrocyte survival and the second involves the mechanism of iron sequestration in the mononuclear phagocyte system.

Splenic size has been shown to increase during inflammation as a result of mononuclear phagocyte hyperplasia. This hyperplasia may result in increased splenic trapping and premature destruction of erythrocytes. Alternatively, splenic macrophages activated by inflammatory stimuli may more readily phagocytize erythrocytes. Future investigation could evaluate erythrocyte survival under conditions of mononuclear phagocyte blockade or mononuclear phagocyte

hyperplasia. These conditions can be produced by intravenous injection of particulate material at different dosage levels. These studies would serve to elucidate the role of mononuclear phagocytes in the decreased erythrocyte survival.

The second major factor remaining to be solved is the mechanism of iron sequestration. The most likely mediator of these changes is endogenous pyrogens (Bornstein and Walsh, 1978). Future studies in this area could be directed toward manipulation of isolated macrophages *in vitro*. These studies may include: 1) evaluating the effect of endogenous pyrogens on iron uptake by macrophages, 2) characterizing the effect of endogenous pyrogens on apoferritin synthesis, 3) determining the number of transferrin receptors on macrophages before and after activation, 4) determining the relative quantities of ferritin and hemosiderin in activated and non-activated macrophages, and 5) characterizing the effects of ATP and ascorbic acid on iron uptake.

Although the present study did not address these problems directly, it did document that iron sequestration and altered erythrocyte survival were the major factors in anemia of inflammatory disease. The further investigation of these problems should not only provide knowledge of the pathogenesis of anemia of inflammatory disease but help to define the complex mechanisms which control erythrocyte production and destruction.

# SUMMARY AND CONCLUSIONS

Cats with induced sterile abscesses developed a hematologic disorder consistent with anemia of inflammatory disease. The anemia was characterized by: 1) nonregenerative normocytic, normochromic anemia, 2) low plasma iron, 3) decreased total iron binding capacity, 4) decreased saturation of transferrin, and 5) shortened erythrocyte survival.

Some of the major findings of these experiments were: 1) cobalt administration to normal cats resulted in polycythemia and reticulocytosis; 2) cobalt administration to cats with sterile abscesses resulted in a lesser degree of polycythemia than in non-abscessed cats; 3) increasing serum iron concentrations by IV infusion enabled the bone marrow to respond to the anemia of inflammatory disease; 4) combined administration of iron and cobalt to cats with sterile abscesses resulted in a marked reticulocytosis, which suggseted that both erythropoietin and serum iron were involved in the control of erythropoiesis. The major cause of anemia due to a sterile abscess was a significantly reduced erythrocyte survival. Although the present study did not disclose the cause of the decreased erythrocyte survival and the iron sequestration, it does lay the groundwork for such investigations.

# REFERENCES

## REFERENCES


Barry, K. G., and Crosby, W. H.: Autoimmune hemolytic anemia arrested by removal of ovarian teratoma. Ann. Int. Med., 47, (1957): 1002.

Banerjee, S., and Chakrabarty, A. S.: Utilization of iron by scorbutic guinea pigs. Blood, 25, (1965): 839.

Baker, E., Vicary, F. R., and Huehn, E. R.: Iron mobilization from isolated rat hepatocytes. In *Proteins in Iron Metabolism*. Grune and Stratton, New York, (1977): 327-334.

Barron, A. G., and Barron, E. S.: Mechanisms of cobalt polycythemia. Effect of ascorbic acid. Proc. Soc. Exp. Biol. Med., 35, (1936): 407-409.

Bornstein, P. L., and Walsh, E. C.: Endogenous mediators of the acute-phase reaction 1. Rabbit granulocytic pyrogen and its chromatographic subfractions. J. Lab. Clin. Med., 91, (1978): 236-245.

Brecher, G.: New methylene blue as a reticulocyte stain. Am. J. Clin. Path., 19, (1949): 895-900.

Bush, J. A., Oshenbrucker, H., Cartwright, G. E., et al.: Anemia of infection. XX. The kinetics of iron metabolism in the anemia associated with chronic infection. J. Clin. Invest., 35, (1956): 89-97.

Cartwright, G. E.: The anemia of chronic disorders. Seminars in Hematology, 3, (1966): 351-375.

Cartwright, G. E., Lauritsen, M. A., Humphreys, S., et al.: The anemia of infection. II. The experimental production of hypoferremia and anemia in dogs. J. Clin. Invest., 25, (1946): 81-86.

Cartwright, G. E., and Lee, G. R.: The anemia of chronic disorders. Brit. J. Haematol., 21, (1971): 147-152.

Carver, F. J., and Frieden, E.: ATP-induced release of iron from transferrin. In *Proteins in Iron Metabolism*. Grune and Stratton, New York, (1977): 245-250.

Contrera, J. F., and Gordon, A. S.: Erythropoietin: production by a particulate fraction of rat kidney. Science, 152, (1966): 653-654.

Crichton, R. R., Collet-Cassart, D., Ponce-Ortez, Y., et al.: Ferritin: comparative structural studies, iron deposition and mobilization. In *Proteins in Iron Metabolism*. Grune and Stratton, New York, (1977): 13-22.

DeLongen, C. D.: Function of the spleen and blood. Acta Med. Scand., 115, (1943): 271.

De Monterice, B., Kaufman, N., and Kinney, T. D.: Liver iron and xanthine oxidase levels. Arch. Path., 72, (1961): 74-81.

Douglas, S. W., and Adamson, J. W.: The anemia of chronic disorders: studies of marrow regulation and iron metabolism. Blood, 45, (1975): 55-65.

Drysdale, J. W., and Munro, H. W.: Regulation of synthesis and turnover of ferritin in rat liver. J. Biol. Chem., 241, (1966): 3630-3637.

Dunn, C. D. R., Lange, R. D., and Jones, J. B.: Evidence that the fetal mouse liver cell assay detects erythroid regulatory factors. Exp. Heamtol., 7, (1979): 519-523.

Dunn, C. D. R., and Legendre, A.: Humoral regulation of erythropoiesis in cats: preliminary report. Am. J. Vet. Res., 41, (1980): 779-781.

Ebaugh, F. G., Emerson, C. P., and Ross, J. F.: The use of radioactive chromium 51 as an erythrocyte tagging agent for the determination of red cell survival *in vivo*. J. Clin. Invest., 32, (1953): 1260-1276.

Egyed, A.: Evidence of the direct involvement of ATP in the iron uptake by reticulocytes. In *Proteins in Iron Metabolism*. Grune and Stratton, New York, (1977): 237-244.

Emmerson, B. T.: Effects of allopurinol on iron metabolism in man. Ann. Rheum. Dis., 25, (1966): 700.

Feldman, B. F., and Kanako, J. J.: Hepatic superoxide dismutase activity, copper, zinc and non-heme iron levels in anemia of chronic inflammatory disease in the dog. Fed. Proc., 37, (1978): 710.

Fielding, J., and Speyer, B. E.: Ferritin as a cytosol transport protein: does transferrin enter reticulocytes? In *Proteins in Iron Metabolism.* Grune and Stratton, New York, (1977): 311-318.

Finch, C. A.: Anemia of chronic disease. Postgraduate Medicine, 64, (1978): 107-113.

Fineberg, R. A., and Greenberg, D. M.: Ferritin biosynthesis. II. Acceleration of synthesis by administration of iron. J. Biol. Chem., 214, (1955): 97-106.

Fletcher, J., and Huehns, E. R.: Function of transferrin. Nature, 218, (1968): 1211-1214.

Freireich, E. J., Miller, A., Emerson, C. P., et al.: The effect of inflammation on the utilization of erythrocyte and transferrin bound radioiron for red cell production. Blood, 12, (1957): 972-983.

Freireich, E. J., Ross, J. F., Bayles, T. B., et al.: Radioactive iron metabolism and erythrocyte survival studies of the mechanism of the anemia associated with rheumatoid arthritis. J. Clin. Invest., 36, (1957): 1043.

Gillis, D. B., and Mitchell, R. A.: Erythrokinetics in normal cats. Am. J. Vet. Res., 35, (1974): 31-33.

Goldwasser, E., Jacobson, L. O., Fried, W., and Plzak, L. F.: Studies on erythropoiesis. V. The effects of cobalt on the production of erythropoietin. Blood, 13, (1958): 550.

Goldwasser, E., and Kung, C. K. H.: Progress in the purification of erythropoietin. Ann. N.Y. Acad. Sci., 149, (1968): 49-53.

Goldwasser, E., and Kung, C. K. H.: Purification of erythropoietin. Proc. Nat. Acad. Sci. USA, 68, (1971): 697-698.

Graham, G. A., and Bates, G. W.: Factors influencing the rate of iron release from $Fe^{3+}$-transferrin-$CO_3^{2-}$. In *Proteins in Iron Metabolism.* Grune and Stratton, New York, (1977): 273-280.

Greenberg, G. R., Ashenbrucker, H., Lauritsen, M., et al.: The anemia of inflammation. IV. The lack of relationship between the diversion of iron from the plasma and the origin of the anemia. J. Clin. Invest., 26, (1947): 114-120.

Grey, S. J., and Sterling, K.: The tagging of red cells and plasma protein with radioactive chromium. J. Clin. Invest., 29, (1950): 1604.

Gutnesky, A., and Van Dyke, D.: Normal response to erythropoietin or hypoxia in rats made anemic with turpentine abscess. Proc. Soc. Exper. Biol. Med., 112, (1963): 75.

Harris, D. C., and Aisen, P.: Functional equivalence of the two iron-binding site of human transferrin. Nature, 257, (1975): 821-823.

Harrison, P., and Huehns, E. R.: Proteins of iron metabolism. Nature, 279, (1979): 476-477.

Haurani, F. I., Burke, W., and Martinez, E. J.: Defective reutilization of iron in the anemia of inflammation. J. Lab. Clin. Med., 65, (1965): 560-570.

Hemmaplardh, D., and Morgan, E. H.: The role of endocytosis in transferrin uptake by reticulocytes and bone marrow cells. Brit. J. Haematol., 36, (1977): 85-96.

Hershko, C., Cook, J. D., and Finch, C. A.: Storage iron kinetics. VI. The effects of inflammation on iron exchange in the rat. Brit. J. Haematol., 28, (1974): 67-75.

Hillman, R. S., and Henderson, P. A.: Control of marrow production by the level of iron supply. J. Clin. Invest., 48, (1969): 454-460.

Hyman, G. A.: Anemia in malignant neoplastic disease. J. Chronic Dis., 16, (1963): 645-666.

International Committee for Standardization in Hematology: Recommended methods for radioisotope red cell survival studies. Blood, 38, (1971): 378-386.

Jacob, H. S., MacDonald, R. A., and Jandl, J. H.: Regulation of spleen growth and sequestering function. J. Clin. Invest., 42, (1963): 1476-1490.

Jirtle, R. L., and Clifton, K. H.: Erythrokinetics in mice bearing tumours in either preirradiated or unirradiated tissue. Cell Tissue Kinetc., 11, (1978): 581-596.

Kampschmidt, R. F., and Pulliam, L. A.: Stimulation of antimicrobial activity in rat with leukocytic endogenous mediator. J. Reticuloendothelial Soc., 17, (1975): 162-169.

Kampschmidt, R. F., and Upchurch, H.: Lowering of plasma iron concentration in the rat with leukocytic extracts. Am. J. Physiol., 216, (1969): 1287-1291.

Karle, H.: Significance of red cell age to red cell destruction during experimental pyrexia. Brit. J. Haematol., 15, (1968a): 221.

Karle, H.: Elevated body temperature and the survival of red blood cells. Acta Med. Scand., 183, (1968b): 587-592.

Karle, H., Hansen, N. E., Malmquist, J., et al.: Turnover of human lactoferrin in the rabbit. Scand. J. Haematol., 23, (1979): 303-312.

Kinney, T. D., Kaufman, N., and Klavins, J. V.: Xanthine oxidase activity and iron storage iron in the liver. Proc. Soc. Exp. Biol. Med., 108, (1961): 22-27.

Kleinberg, W., Gordon, A. S., and Charipper, H.: Effect of cobalt on erythropoiesis in anemic rabbits. Proc. Soc. Exp. Biol. Med., 42, (1939): 119-120.

Konijn, A. M., and Hershko, C.: Ferritin synthesis in inflammation. I. Pathogenesis of impaired iron release. Brit. J. Haematol., 37, (1977): 7-16.

Krantz, S. B., and Jacobson, L. O.: *Erythropoietin and the Regulation of Erythropoiesis*. University of Chicago Press, Chicago, Illinois, (1970): 27.

Kreier, J. P., Swann, A. I., Taylor, W. M., and Wagner, W. M.: Erythrocyte life span and label elution in monkeys and cats determined with chromium-51 and diisopropyl fluorophosphate-32. Am. J. Vet. Res., 31, (1970): 1429-1435.

Lipschitz, D. A., Bothwell, T. H., Seftel, H. C., et al.: The role of ascorbic acid in the metabolism of storage iron. Brit. J. Haematol., 20, (1971): 155-163.

Lukens, J. N.: Control of erythropoiesis in rats with adjuvant-induced chronic inflammation. Blood, 41, (1973): 37-44.

Lukens, J. N.: Cartwright, G. E., and Wintrobe, M. M.: Anemia of adjuvant-induced inflammation in rats. Proc. Soc. Exp. Biol. Med., 126, (1967): 346-349.

Lukowski, W. A., and Painter, R. H.: Studies of the role of sialic acid in the physical and biological properties of erythropoietin. Can. J. Biochem., 50, (1972): 909-917.

Madewell, B. R., and Feldman, B. F.: Characterization of anemias associated with neoplasia in small animals. J. Am. Vet. Med. Assoc., 176, (1980): 419-425.

Matioli, G. T. and Baker, R. F.: Denaturation of ferritin and its relationship with hemosiderin. J. Ultrastructure Res., 8, (1963): 477-490.

Mazur, A., Carleton, A., and Carlsen, A.: Relation of oxidative metabolism to the incorporation of plasma iron into ferritin *in vivo*. J. Biol. Chem., 236, (1961): 1109-1116.

Mazur, A., Green, S., and Carleton, A.: Mechanism of plasma iron incorporation into hepatic ferritin. J. Biol. Chem., 235, (1960): 595-603.

Mazur, A., Green, S., Saha, A., and Carleton, A.: Mechanism of release of ferritin iron *in vivo* by xanthine oxidase. J. Clin. Invest., 37, (1958): 1809-1819.

McInroy, R. A.: A micro-haematocrit for determining the packed cell volume and haemoglobin concentration on capillary blood. J. Clin. Path., 7, (1954): 32.

Merriman, C. R., Pulliam, L. A., and Kampschmidt, R. F.: Comparison of leukocytic pyrogen and leukocytic endogenous mediator. Proc. Soc. Exp. Biol. Med., 154, (1977): 224-227.

Mikolajew, M., Kuratowska, Z., Kossakowska, M., et al.: Haematological changes in adjuvant disease in the rat. Ann. Rheum. Dis., 28, (1969): 172-178.

Morgan, E. H.: *Iron in Biochemistry and Medicine*, ed. by Jacobs and Worwood. Academic Press, New York, (1974): 29-71.

Munro, H. N., and Linder, M. C.: Ferritin: structure, biosynthesis, and role in iron metabolism. Physiol. Rev., 58, (1978): 318-396.

Quastel, M. R., and Ross, J. F.: The effect of acute inflammation on the utilization and distribution of transferrin-bound and erythrocyte radioiron. Blood, 28, (1966): 738-757.

Reissmann, K. R., and Udupa, K. B.: Effect of inflammation on erythroid precursors in bone marrow and spleen of mice. J. Lab. Clin. Med., 92, (1978): 22-29.

Richmond, J., Alexander, W. R. M., Potter, J. L., et al.: The nature of anemia in rheumatoid arthritis. V. Red cell survival measured by radioactive chromium. Ann. Rheum. Dis., 20, (1961): 133.

Richmond, J., Roy, L. M. H., Gardner, D. L., et al.: Nature of anemia in rheumatoid arthritis. IV. Effects of the intravenous administration of saccharated oxide of iron. Ann. Rheum. Dis., 17, (1958): 406-413.

Richter, G. W.: The iron-loaded cell--the cytopathology of iron storage. Am. J. Path., 91, (1978): 363-396.

Rigby, P. G., Strasser, H., Emmerson, C. P., et al.: Studies in the anemia of inflammatory states. I. Erythrocyte survival in dogs with acute and chronic turpentine abscesses. J. Lab. Clin. Med., 59, (1962): 244-248.

Rinehart, J. J., Zanjani, E. D., Nomdedeu, B., et al.: Cell-cell interaction in erythropoiesis. J. Clin. Invest., 62, (1978): 979-986.

Rodgers, G. M., Fisher, J. W., and George. W. J.: Activation of the renal erythropoietic factor by a cyclic AMP-dependent protein kinase. Proc. Soc. Exp. Biol., 145, (1974): 1207-1214.

Rogers, T. B., Gold, R. A., and Feiney, R. E.: Transferrins. In *Proteins in Iron Metabolism*. Grune and Stratton, New York, (1977): 161-168.

Schalm, O. W., Jain, N. C., and Carroll, E. J.: *Veterinary Hematology*. Lea & Febiger, Philadelphia, 3rd ed., (1975a): 15-81.

Schalm, O. W., Jain, N. C., and Carroll, E. J.: *Veterinary Hematology*. Lea & Febiger, Philadelphia, 3rd ed., (1975b): 113.

Shen, S. C., and Homburger, F.: The anemia of cancer patients and its relation to metastasis to the bone marrow. J. Lab. Clin. Med., 37, (1951): 182-198.

Sherwood, J. B., and Goldwasser, E.: Extraction of erythropoietin from normal kidneys. Endocrinology, 103, (1978): 866-870.

Shoden, A., Gabro, B. W., and Finch, C. A.: The relationship between ferritin and hemosiderin in rabbits and man. J. Biol. Chem., 204, (1953): 823-830.

Stanley, A. J., Hopps, H. C., and Hellbaum, A. A.: Observations on cobalt polycythemia. I. Studies on the peripheral blood of rats. Proc. Soc. Exp. Biol. Med., 61, (1946): 130-133.

Stookey, L. L.: Ferrozine - A new spectrophotometric reagent for iron. Anal. Chem., 42, (1970): 779-781.

Turnbull, A.: *Iron in Biochemistry and Medicine*, ed. by Jacobs and Warwood. Academic Press, New York, (1974): 370-403.

Ultmann, J. E.: The role of the spleen in the hemolytic anemia of cancer patients. Cancer Res., 18, (1958): 959.

Van Snick, J. L., Masson, P. L., and Heremans, J. F.: The involvement of lactoferrin in the hyposideremia of acute inflammation. J. Exp. Med., 140, (1974): 1068-1084.

Waggener, R. E., Pratt, P. T. and Hunt, H.: Erythrocyte survival in leukemia, Hodgkin's disease and malignant lymphoma as determined by radio-chromium. Am. J. Roentgenol., 79, (1958): 1045.

Wallner, S. F., Kurnick, J. E., Ward, H. P., et al.: The anemia of chronic renal failure and chronic disease. *In vitro* studies of erythropoiesis. Blood, 47, (1976): 561-569.

Waltner, K., and Waltner, K.: Kobalt und Blut. Klin. Wehnsehr, 8, (1929): 313.

Ward, H. P., Kurnick, J. E., and Pisarczyk, M.: Serum levels of erythropoietin in anemia associated with chronic infection, malignancy and primary hematopoietic disease. J. Clin. Invest., 50, (1971): 332-335.

Warren, C. O., Schubmehl, Q. D., and Wood, I. R.: Studies on the mechanism of cobalt polycythemia. Am. J. Physiol., 142, (1944): 173.

Wasserman, L.R., Stats, D., Schwartz, L., et al.: Symptomatic and hemopathic hemolytic anemia. Am. J. Med., 18, (1955): 961.

Whitcomb, W. H., Moore, M., Dille, R., et al.: Erythropoietin and erythropoiesis inhibitor activity in man. J. Clin. Invest., 44, (1965): 1110.

Wintrobe, M. M., Grinstein, M., Dubash, J. J., et al.: The anemia of infection. VI. The influence of cobalt on the anemia associated with inflammation. Blood, 2, (1947): 323-331.

Wintrobe, M. M., Lee, G. R., Boggs, D. R., et al.: *Clinical Hematology*. Lea & Febiger, Philadelphia, 7th ed., (1974a): 135-194.

Wintrobe, M. M., Lee, G. R., Boggs, D. R., et al.: *Clinical Hematology*. Lea & Febiger, Philadelphia, 7th ed., (1974b): 195-200.

Wintrobe, M. M., Lee, G. R., Boggs, D. R., et al.: *Clinical Hematology*. Lea & Febiger, Philadelphia, 7th ed., (1974c): 635-670.

Zucker, S., Friedman, S., and Lysik, R. M.: Bone marrow erythropoiesis in the anemia of infection, inflammation and malignancy. J. Clin. Invest., 53, (1974): 1132-1138.

# APPENDIX A

# INDIVIDUAL ANIMAL DATA FOR EXPERIMENTS I THROUGH VII

Table A-1. Individual animal data for Experiment I

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 34.0 | 35.0 | 38.0 | 36.0 | 35.0 | 36.0 | 35.0 | 41.0 | | 38.0 | |
| | Hemoglobin | g/dl | 12.5 | 12.5 | 13.5 | 12.7 | 12.6 | 12.8 | 12.7 | 14.3 | | 13.6 | |
| | Red blood cells | $X10^6$ /µl | 7.84 | 7.86 | 8.36 | 8.03 | 7.86 | 8.20 | 8.13 | 8.80 | | 8.38 | |
| | MCV | fl | 43.0 | 46.0 | 47.0 | 46.0 | 46.0 | 45.4 | 44.0 | 47.0 | | 47.0 | |
| | MCHC | g/dl | 36.7 | 34.5 | 34.3 | 35.0 | 35.0 | 35.3 | 35.5 | 34.9 | | 34.6 | |
| | Total leukocytes | µl | 9.7 | 10.5 | 11.6 | 11.6 | 9.4 | 12.0 | 13.0 | 16.4 | | 12.3 | |
| | Plasma protein | g/dl | 7.4 | 7.4 | 7.4 | 7.5 | 7.4 | 7.5 | 7.6 | 7.7 | | 7.6 | |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 94.1 | 123.1 | 167.2 | 189.2 | 204.4 | 187.2 | 195.1 | 448.8 | | 425.2 | |
| | Type I | $X10^3$ /µl | 94.1 | 123.1 | 167.2 | 189.2 | 204.4 | 187.2 | 195.1 | 369.6 | | 360.0 | |
| | Type II | $X10^3$ /µl | 0 | 0 | 0 | 0 | 7.86 | 4.38 | 0 | 70.4 | | 65.2 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| | Serum iron | µg/dl | 72.0 | 78.0 | 51.0 | 93.0 | 81.0 | 72.0 | 90.0 | 69.0 | | 88.0 | |
| | TIBC | µg/dl | | | | | 299.0 | | | | | | |
| | Transferrin saturation | % | | | | | 27 | | | | | | |

Table A-1 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 32.0 | 33.0 | 33.0 | 33.0 | 33.0 | 34.0 | 40.0 | 34.0 | | 34.0 | |
| | Hemoglobin | g/dl | 12.0 | 11.8 | 11.6 | 10.8 | 10.7 | 12.0 | 14.2 | 11.6 | | 12.1 | |
| | Red blood cells | $X10^6$ /µl | 8.22 | 7.93 | 7.88 | 7.40 | 7.34 | 8.2 | 9.63 | 7.93 | | 7.98 | |
| | MCV | fl | 42.0 | 43.0 | 43.0 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | | 42.0 | |
| | MCHC | g/dl | 35.0 | 34.6 | 34.3 | 34.8 | 35.0 | 34.8 | 34.9 | 34.8 | | 34.3 | |
| | Total leukocytes | µl | 12.9 | 11.2 | 12.5 | 10.8 | 10.3 | 12.9 | 19.7 | 9.1 | | 12.7 | |
| | Plasma protein | g/dl | 7.1 | 7.0 | 6.5 | 7.1 | 6.6 | 7.0 | 7.8 | 6.9 | | 7.1 | |
| | Reticulocytes; Total | $X10^3$ /µl | 98.6 | 300.2 | 701.3 | 456.0 | 447.7 | 770.7 | 982.2 | 396.5 | | 342.1 | |
| | Type I | $X10^3$ /µl | 98.6 | 278.2 | 622.5 | 447.7 | 374.3 | 759.3 | 972.6 | 253.8 | | 221.1 | |
| | Type II | $X10^3$ /µl | 0 | 22.0 | 78.8 | 8.4 | 73.4 | 11.4 | 9.6 | 142.7 | | 121.0 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | | 0 | |
| | Serum iron | µg/dl | | | 85.0 | 92.0 | 69.0 | 66.0 | | 72.0 | | | |
| | TIBC | µg/dl | | | 195.0 | | | | | | | | |
| | Transferrin saturation | % | | | 44 | | | | | | | | |

Table A-1 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 29.0 | 29.0 | 30.0 | 28.0 | 28.0 | 27.0 | 27.0 | 28.0 | | 28.0 | |
| | Hemoglobin | g/dl | 10.5 | 10.3 | 9.1 | 9.9 | 10.6 | 9.6 | 9.3 | 10.0 | | 10.2 | |
| | Red blood cells | $X10^6$ /µl | 6.95 | 6.85 | 6.21 | 6.57 | 7.01 | 6.32 | 6.14 | 6.54 | | 6.58 | |
| | MCV | fl | 41.0 | 42.0 | 43.0 | 42.6 | 42.0 | 43.0 | 43.0 | 44.0 | | 44.0 | |
| | MCHC | g/dl | 36.7 | 36.3 | 34.1 | 35.3 | 35.5 | 34.8 | 35.1 | 34.9 | | 35.0 | |
| | Total leukocytes | µl | 16.3 | 16.4 | 17.1 | 13.0 | 12.0 | 10.3 | 7.0 | 12.6 | | 12.5 | |
| | Plasma protein | g/dl | 7.0 | 6.8 | 6.3 | 6.78 | 6.9 | 6.8 | 6.8 | 6.9 | | 6.9 | |
| | Reticulocytes; Total | $X10^3$ /µl | 361.4 | 328.4 | 310.5 | | 378.5 | 315.5 | 356.1 | 654.0 | | 534.2 | |
| | Type I | $X10^3$ /µl | 361.4 | 328.4 | 310.5 | | 364.5 | 303.4 | 300.9 | 470.9 | | 434.0 | |
| | Type II | $X10^3$ /µl | 0 | 0 | 0 | | 14.0 | 12.1 | 55.3 | 170.0 | | 100.2 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | | 0 | 0 | 0 | 13.0 | | 0 | |
| | Serum iron | µg/dl | | 108.0 | 101.0 | 97.0 | 80.0 | 96.0 | 51.0 | 155.0 | | 128.0 | |
| | TIBC | µg/dl | | | | | | | | 336.0 | | | |
| | Transferrin saturation | % | | | | | | | | 46 | | | |

Table A-1 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 38.0 | 37.0 | 37.0 | 38.0 | 37.0 | 35.0 | 35.0 | 42.0 | | 39.0 | |
| | Hemoglobin | g/dl | 13.1 | 13.0 | 13.3 | 13.0 | 12.0 | 12.5 | 12.6 | 13.8 | | 13.4 | |
| | Red blood cells | $X10^6$ /µl | 7.63 | 7.64 | 7.64 | 7.63 | 7.48 | 7.38 | 7.37 | 8.09 | | 7.92 | |
| | MCV | fl | 51.0 | 51.2 | 52.0 | 51.0 | 52.0 | 50.0 | 50.0 | 51.0 | | 51.0 | |
| | MCHC | g/dl | 34.1 | 33.9 | 33.7 | 33.9 | 33.5 | 34.6 | 34.6 | 33.4 | | 34.0 | |
| | Total leukocytes | µl | 13.5 | 11.9 | 9.0 | 11.2 | 15.8 | 13.1 | 13.9 | 7.1 | | 8.3 | |
| | Plasma protein | g/dl | 7.0 | 6.9 | 7.0 | 7.0 | 7.0 | 6.9 | 6.8 | 6.9 | | 6.9 | |
| | Reticulocytes; Total | $X10^3$ /µl | 640.9 | 632.8 | 702.9 | 621.3 | 643.3 | 683.3 | 722.3 | 1011.3 | | 883.2 | |
| | Type I | $X10^3$ /µl | 610.4 | 604.3 | 657.0 | 589.9 | 568.5 | 640.1 | 707.5 | 873.7 | | 796.9 | |
| | Type II | $X10^3$ /µl | 30.5 | 28.5 | 30.6 | 22.4 | 59.8 | 43.2 | 14.7 | 129.4 | | 86.3 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 15.3 | 0 | 14.9 | 0 | 0 | 8.1 | | 0 | |
| | Serum iron | µg/dl | | 107.0 | 144.0 | 138.0 | 71.0 | 105.0 | | | | 124.0 | |
| | TIBC | µg/dl | | | 357.0 | | 348.0 | | | | | | |
| | Transferrin saturation | % | | | 40 | | 20 | | | | | | |

Table A-1 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | PCV | vol% | 39.0 | 39.0 | 38.0 | 39.0 | 40.0 | 40.0 | 39.0 | 39.0 | | 39.0 | |
| | Hemoglobin | g/dl | 13.6 | 13.4 | 13.2 | 13.8 | 14.0 | 13.8 | 13.5 | 13.6 | | 13.6 | |
| | Red blood cells | $X10^6$ /µl | 8.43 | 8.22 | 8.17 | 8.62 | 8.80 | 8.73 | 8.56 | 8.52 | | 8.48 | |
| | MCV | fl | 48.0 | 48.0 | 48.0 | 48.0 | 49.0 | 49.0 | 49.0 | 49.0 | | 49.0 | |
| | MCHC | g/dl | 34.1 | 34.0 | 33.9 | 34.2 | 34.4 | 34.3 | 33.9 | 34.2 | | 33.8 | |
| | Total leukocytes | µl | 6.3 | 6.1 | 5.4 | 6.2 | 6.4 | 8.1 | 7.3 | 6.1 | | 8.4 | |
| | Plasma protein | g/dl | 7.4 | 7.2 | 7.2 | 7.3 | 7.7 | 7.4 | 7.3 | 7.4 | | 7.1 | |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 733.4 | 710.0 | 555.6 | 521.7 | 545.6 | 558.3 | 707.8 | 622.0 | | 554.6 | |
| | Type I | $X10^3$ /µl | 708.1 | 687.3 | 539.2 | 521.7 | 545.6 | 556.0 | 599.2 | 604.9 | | 533.2 | |
| | Type II | $X10^3$ /µl | 25.3 | 22.7 | 16.3 | 0 | 0 | 2.3 | 8.6 | 17.0 | | 21.4 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| | Serum iron | µg/dl | 133.0 | 132.0 | 125.0 | 128.0 | 139.0 | 139.0 | 128.0 | 127.0 | | 97.0 | |
| | TIBC | µg/dl | 339.0 | | | | | | | | | | |
| | Transferrin saturation | % | 39 | | | | | | | | | | |

Table A-2. Individual animal data for Experiment II

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 32.0 | 40.0 | 33.3 | 35.0 | 33.0 | 21.0 | 26.0 | 25.0 | 25.0 | 27.0 | 29.0 |
| | Hemoglobin | g/dl | 11.5 | 14.2 | 12.2 | 13.1 | 13.6 | 10.2 | 11.7 | 10.6 | 11.1 | 11.3 | 11.4 |
| | Red blood cells | $X10^6$ /µl | 7.01 | 8.29 | 7.36 | 8.09 | 8.01 | 6.20 | 7.42 | 6.59 | 6.84 | 7.03 | 6.95 |
| | MCV | fl | 48.0 | 50.0 | 49.0 | 48.0 | 48.0 | 46.0 | 47.0 | 46.0 | 46.0 | 46.0 | 48.0 |
| | MCHC | g/dl | 34.5 | 34.1 | 33.9 | 33.7 | 35.2 | 35.3 | 33.0 | 34.9 | 35.6 | 35.3 | 34.3 |
| | Total leukocytes | µl | 8.6 | 25.9 | 18.0 | 12.5 | 11.4 | 12.9 | 17.6 | 12.6 | 12.4 | 7.2 | 4.9 |
| | Plasma protein | g/dl | 6.5 | 6.9 | 6.8 | 7.8 | 7.8 | 6.9 | 6.8 | 7.1 | 6.2 | 7.0 | 7.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 252.4 | 232.1 | 176.6 | 80.9 | 32.0 | 12.4 | 29.7 | 0 | 13.7 | 140.6 | 500.4 |
| | Type I | $X10^3$ /µl | 252.4 | 232.1 | 176.6 | 80.9 | 32.0 | 12.4 | 29.7 | 0 | 13.7 | 140.6 | 458.7 |
| | Type II | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 41.7 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Serum iron | µg/dl | 84.0 | 29.0 | 34.0 | 89.0 | 65.0 | 10.0 | 14.0 | 123.0 | 94.0 | 117.0 | 96.0 |
| | TIBC | µg/dl | 190.0 | 202.0 | 156.0 | 185.0 | 185.0 | | 350.0 | 206.0 | | 240.0 | |
| | Transferrin saturation | % | 44 | 14 | 32 | 48 | 35 | | | 60 | | 39 | |

Table A-2 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 28.0 | 35.0 | 29.0 | 30.0 | 25.0 | 20.0 | 18.0 | 27.0 | 24.0 | 25.0 | 32.0 |
| | Hemoglobin | g/dl | 10.1 | 12.5 | 11.3 | 11.3 | 9.9 | 9.3 | | 10.7 | 10.6 | 10.4 | 12.2 |
| | Red blood cells | $X10^6$ /µl | 6.74 | 8.37 | 7.52 | 7.54 | 6.45 | 6.32 | | 7.03 | 7.08 | 7.08 | 8.29 |
| | MCV | fl | 44.0 | 43.0 | 43.0 | 44.0 | 45.0 | 41.0 | | 43.0 | 42.0 | 42.0 | 44.0 |
| | MCHC | g/dl | 34.1 | 34.7 | 34.8 | 33.9 | 34.3 | 36.3 | | 34.0 | 35.8 | 35.0 | 33.5 |
| | Total leukocytes | µl | 11.8 | 28.0 | 23.8 | 17.9 | 12.7 | 12.7 | | 16.7 | 19.8 | 15.2 | 14.7 |
| | Plasma protein | g/dl | 6.4 | 6.3 | 6.1 | 6.5 | 6.2 | 6.3 | 6.9 | 6.4 | 6.3 | 6.2 | 6.5 |
| | Reticulocytes; Total | $X10^3$ /µl | 572.9 | 502.2 | 90.2 | 45.2 | 77.4 | 12.6 | 0 | 309.3 | 28.3 | 0 | 1011.0 |
| | Type I | $X10^3$ /µl | 572.9 | 502.2 | 90.2 | 45.2 | 77.4 | 12.6 | 0 | 295.3 | 28.3 | 0 | 994.8 |
| | Type II | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14.1 | 0 | 0 | 16.6 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Serum iron | µg/dl | 85.0 | 50.0 | 14.0 | 24.0 | 36.0 | 10.0 | 34.0 | 44.0 | | | 84.0 |
| | TIBC | µg/dl | 184.0 | 176.0 | 164.0 | 167.0 | 161.0 | | 122.0 | 192.0 | | | |
| | Transferrin saturation | % | 46 | 29 | 9 | 14 | 22 | | 28 | 23 | | | |

Table A-2 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 36.0 | 41.0 | 31.0 | 25.0 | 29.0 | 30.0 | 29.0 | 35.0 | 34.0 | 33.0 | 34.0 |
| | Hemoglobin | g/dl | 12.8 | 14.3 | 11.3 | 8.8 | 11.2 | 11.1 | 10.7 | 12.8 | 11.9 | 11.2 | 12.2 |
| | Red blood cells | $X10^6$ /µl | 8.20 | 9.1 | 7.5 | 5.75 | 7.30 | 7.30 | 6.98 | 8.14 | 7.67 | 7.32 | 7.82 |
| | MCV | fl | 45.4 | 47.0 | 46.0 | 45.0 | 45.0 | 44.4 | 44.0 | 45.0 | 47.0 | 46.0 | 46.0 |
| | MCHC | g/dl | 35.3 | 33.5 | 33.3 | 34.0 | 34.3 | 34.3 | 35.1 | 34.6 | 32.7 | 33.3 | 34.0 |
| | Total leukocytes | µl | 12.0 | 18.6 | 18.2 | 10.1 | 15.9 | 19.9 | 18.1 | 13.3 | 12.7 | 11.9 | 18.6 |
| | Plasma protein | g/dl | 7.5 | 7.8 | 7.3 | 7.0 | 7.8 | 8.4 | 8.1 | 8.3 | 7.2 | 7.6 | 7.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 221.4 | 227.5 | 75.0 | 120.8 | 233.6 | 131.4 | 125.6 | 350.0 | 406.5 | 409.9 | 500.5 |
| | Type I | $X10^3$ /µl | 205.0 | 218.4 | 75.0 | 109.3 | 131.4 | 87.6 | 55.8 | 146.5 | 191.8 | 131.8 | 211.1 |
| | Type II | $X10^3$ /µl | 16.4 | 9.1 | 0 | 11.5 | 87.6 | 43.8 | 69.8 | 195.4 | 199.4 | 205.0 | 250.2 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 14.6 | 0 | 0 | 8.1 | 15.3 | 73.2 | 39.1 |
| | Serum iron | µg/dl | 73.0 | 10.0 | 53.0 | 20.0 | | 22.0 | 80.0 | | 114.0 | 92.0 | |
| | TIBC | µg/dl | | 164.0 | 171.0 | 148.0 | | 212.0 | 229.0 | | 227.0 | 267.0 | |
| | Transferrin saturation | % | | 6 | 32 | 14 | | 10 | 35 | | 41 | 34 | |

Table A-2 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 35.0 | 40.0 | 34.0 | 32.0 | 29.0 | 29.0 | 29.0 | 31.0 | 31.0 | 35.0 | 37.0 |
| | Hemoglobin | g/dl | 12.0 | 13.8 | 12.3 | 11.4 | 10.5 | 10.5 | 10.7 | 10.9 | 10.2 | 12.7 | 12.4 |
| | Red blood cells | $X10^6$ /µl | 8.2 | 9.67 | 8.32 | 7.94 | 7.25 | 7.22 | 7.35 | 7.2 | 7.18 | 8.55 | 8.46 |
| | MCV | fl | 42.0 | 42.0 | 43.0 | 42.0 | 43.0 | 42.0 | 43.0 | 43.0 | 43.0 | 42.0 | 43.0 |
| | MCHC | g/dl | 34.8 | 34.0 | 34.8 | 34.8 | 33.6 | 34.8 | 34.4 | 33.9 | 32.8 | 35.0 | 34.0 |
| | Total leukocytes | µl | 12.9 | 13.8 | 15.2 | 13.5 | 19.6 | 18.5 | 15.7 | 16.8 | 15.4 | 6.7 | 21.2 |
| | Plasma protein | g/dl | 7.0 | 7.6 | 7.1 | 7.2 | 7.7 | 7.5 | 6.9 | 6.8 | 7.0 | 7.0 | 6.8 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 516.6 | 580.2 | 199.6 | 134.9 | 290.0 | 317.6 | 441.0 | 266.4 | 674.9 | 1299.6 | 1455.1 |
| | Type I | $X10^3$ /µl | 451.0 | 541.5 | 158.0 | 111.1 | 261.0 | 202.1 | 338.1 | 180.0 | 402.0 | 718.2 | 964.4 |
| | Type II | $X10^3$ /µl | 65.6 | 38.6 | 58.2 | 23.8 | 29.0 | 115.5 | 102.9 | 79.2 | 272.8 | 513.0 | 439.9 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7.2 | 0 | 68.4 | 50.7 |
| | Serum iron | µg/dl | 66.0 | 9.0 | 10.0 | 11.0 | 10.0 | 10.0 | 58.0 | | | 76.0 | |
| | TIBC | µg/dl | | | | 191.0 | | 128.0 | 197.0 | | | 273.0 | |
| | Transferrin saturation | % | | | | 6 | | 8 | 29 | | | 28 | |

Table A-2 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | PCV | vol% | 28.0 | 38.0 | 39.0 | 32.0 | 32.0 | 25.0 | 22.0 | 22.0 | 18.5 | 25.0 | 27.0 |
| | Hemoglobin | g/dl | 9.9 | 12.9 | 13.7 | 11.0 | 11.4 | 9.1 | 7.8 | 8.0 | 6.9 | 8.7 | 8.5 |
| | Red blood cells | $X10^6$ /µl | 6.57 | 8.59 | 9.14 | 7.41 | 7.63 | 6.13 | 5.23 | 5.49 | 4.84 | 6.11 | 5.52 |
| | MCV | fl | 43.0 | 44.0 | 46.0 | 46.0 | 44.0 | 45.0 | 45.0 | 47.0 | 46.0 | 49.0 | 48.0 |
| | MCHC | g/dl | 35.3 | 34.0 | 32.0 | 32.1 | 33.9 | 33.1 | 32.8 | 30.9 | 31.1 | 29.3 | 32.0 |
| | Total leukocytes | µl | 13.0 | 31.3 | 25.4 | 33.8 | 24.3 | 38.0 | 27.4 | 39.0 | 34.0 | 42.0 | 46.7 |
| | Plasma protein | g/dl | 6.8 | 7.7 | 7.4 | 6.5 | 6.4 | 5.8 | 5.8 | 6.3 | 6.5 | 7.3 | 7.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 354.7 | 1219.7 | 804.3 | 548.3 | 228.9 | 196.1 | 115.0 | 263.5 | 242.0 | 769.8 | 792.8 |
| | Type I | $X10^3$ /µl | 293.0 | 1030.8 | 639.8 | 474.2 | 183.1 | 159.3 | 52.3 | 197.6 | 125.8 | 268.8 | 220.8 |
| | Type II | $X10^3$ /µl | 48.6 | 188.9 | 146.2 | 74.1 | 45.1 | 36.7 | 41.8 | 43.9 | 96.8 | 403.2 | 518.8 |
| | Type III | $X10^3$ /µl | 2.6 | 17.1 | 18.2 | 0 | 0 | 0 | 20.9 | 21.9 | 1.93 | 97.7 | 55.2 |
| | Serum iron | µg/dl | 97.0 | 19.0 | 12.0 | 46.0 | | 29.0 | 34.0 | 24.0 | 67.0 | 36.0 | |
| | TIBC | µg/dl | | 336.0 | 358.0 | 247.0 | | 302.0 | 242.0 | 221.0 | 274.0 | 279.0 | |
| | Transferrin saturation | % | | 6 | 3 | 19 | | 10 | 14 | 11 | 24 | 13 | |

Table A-3. Individual animal data for Experiment III

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 37.0 | 43.0 | | 42.0 | | 41.0 | | 44.0 | 45.0 | | 46.0 |
| | Hemoglobin | g/dl | 13.0 | 15.1 | | 13.6 | | 13.9 | | 14.4 | 14.9 | | 15.1 |
| | Red blood cells | $X10^6$ /µl | 7.64 | 8.96 | | 7.92 | | 8.19 | | 8.55 | 8.71 | | 8.92 |
| | MCV | fl | 51.0 | 50.0 | | 50.0 | | 51.0 | | 52.0 | 50.0 | | 51.0 |
| | MCHC | g/dl | 33.9 | 34.0 | | 34.7 | | 33.4 | | 32.2 | 35.0 | | 35.0 |
| | Total leukocytes | µl | 11.9 | 9.70 | | 12.3 | | 13.3 | | 12.4 | 13.8 | | 12.8 |
| | Plasma protein | g/dl | 6.9 | 7.3 | | 6.7 | | 6.7 | | 7.3 | 7.0 | | 7.0 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 741.0 | 1084.1 | | 918.7 | | 1466.0 | | 1692.9 | 1681.0 | | 1680.0 |
| | Type I | $X10^3$ /µl | 679.9 | 913.9 | | 586.0 | | 769.8 | | 889.2 | 722.9 | | 721.0 |
| | Type II | $X10^3$ /µl | 30.5 | 170.2 | | 332.6 | | 638.8 | | 666.9 | 818.7 | | 818.8 |
| | Type III | $X10^3$ /µl | 7.6 | 44.8 | | 0 | | 57.3 | | 136.8 | 139.3 | | 139.4 |
| | Serum iron | µg/dl | 107.0 | 82.0 | | 185.0 | | 184.0 | | 139.0 | 96.0 | | 122.0 |
| | TIBC | µg/dl | | | | | | | | | | | |
| | Transferrin saturation | % | | | | | | | | | | | |

Table A-3 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 39.0 | 48.0 | | 49.0 | | 52.0 | | 58.0 | 59.0 | | 59.0 |
| | Hemoglobin | g/dl | 13.5 | 17.4 | | 16.5 | | 17.2 | | 18.2 | 22.6 | | 22.3 |
| | Red blood cells | $X10^6$ /µl | 8.68 | 11.3 | | 11.2 | | 11.8 | | 13.6 | 14.2 | | 14.4 |
| | MCV | fl | 47.0 | 47.0 | | 44.0 | | 46.0 | | 46.0 | 45.0 | | 45.0 |
| | MCHC | g/dl | 33.3 | 32.4 | | 34.0 | | 32.0 | | 29.0 | 34.4 | | 34.3 |
| | Total leukocytes | µl | 15.4 | 21.6 | | 15.4 | | 18.1 | | 18.2 | 9.2 | | 12.3 |
| | Plasma protein | g/dl | 7.5 | 8.2 | | 7.3 | | 7.6 | | 9.6 | 8.9 | | 8.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 1111.0 | 1649.8 | | 1859.2 | | 1770.0 | | 2339.2 | 2016.4 | | 2318.4 |
| | Type I | $X10^3$ /µl | 1111.0 | 1649.8 | | 1814.4 | | 1699.2 | | 2257.6 | 2016.4 | | 2304.0 |
| | Type II | $X10^3$ /µl | 0 | 0 | | 2.2 | | 47.2 | | 54.4 | 0 | | 144.0 |
| | Type III | $X10^3$ /µl | 0 | 0 | | 2.2 | | 23.6 | | 27.2 | 0 | | 0 |
| | Serum iron | µg/dl | 75.0 | 140.0 | | | | 170.0 | | 126.0 | 114.0 | | 123.0 |
| | TIBC | µg/dl | | | | | | | | | | | |
| | Transferrin saturation | % | | | | | | | | | | | |

Table A-3 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 34.0 | 42.0 | | 47.0 | | 47.0 | | 46.0 | 50.0 | | 50.0 |
| | Hemoglobin | g/dl | 12.2 | 14.2 | | 15.1 | | 14.9 | | 14.9 | | | 16.8 |
| | Red blood cells | X10$^6$ /µl | 8.66 | 10.3 | | 11.7 | | 11.7 | | 11.6 | | | 11.0 |
| | MCV | fl | 43.0 | 44.0 | | 42.0 | | 43.0 | | 41.0 | | | 43.0 |
| | MCHC | g/dl | 34.4 | 31.7 | | 31.0 | | 30.0 | | 31.0 | | | 32.0 |
| | Total leukocytes | µl | 12.8 | 23.0 | | 19.8 | | 23.0 | | 14.9 | | | 13.2 |
| | Plasma protein | g/dl | 7.6 | 7.7 | | 7.7 | | 7.5 | | 7.1 | 6.8 | | 7.1 |
| | Reticulocytes; Total | X10$^3$ /µl | 1039.2 | 2286.6 | | 2363.4 | | 2550.6 | | 1948.8 | | | 1948.8 |
| | Type I | X10$^3$ /µl | 1021.8 | 2266.0 | | 2129.4 | | 2328.3 | | 1879.2 | | | 1829.2 |
| | Type II | X10$^3$ /µl | 17.3 | 10.3 | | 46.8 | | 70.2 | | 69.6 | | | 69.6 |
| | Type III | X10$^3$ /µl | 0 | 10.3 | | 187.2 | | 187.2 | | 0 | | | 0 |
| | Serum iron | µg/dl | 40.0 | | | | | 111.0 | | 60.0 | 69.0 | | 89.0 |
| | TIBC | µg/dl | | | | | | | | | | | |
| | Transferrin saturation | % | | | | | | | | | | | |

Table A-3 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 30.0 | 37.0 | | 43.0 | | 42.0 | | 42.0 | 43.0 | | 44.0 |
| | Hemoglobin | g/dl | 10.0 | 11.9 | | | | 13.1 | | 12.9 | 13.7 | | 14.2 |
| | Red blood cells | $X10^6$ /µl | 6.87 | 8.01 | | | | 9.06 | | 9.05 | 9.78 | | 9.75 |
| | MCV | fl | 47.0 | 45.0 | | | | 44.0 | | 45.0 | 43.0 | | 44.0 |
| | MCHC | g/dl | 31.3 | 33.2 | | | | 34.0 | | 32.1 | 33.1 | | 33.2 |
| | Total leukocytes | µl | 28.3 | 33.7 | | | | 23.6 | | 20.7 | 16.8 | | 16.9 |
| | Plasma protein | g/dl | 7.3 | 7.8 | | 8.2 | | 7.6 | | 7.4 | 6.8 | | 6.9 |
| | Reticulocytes; Total | $X10^3$ /µl | 858.7 | 2483.1 | | | | 2500.5 | | 2353.0 | 1858.2 | | 2086.5 |
| | Type I | $X10^3$ /µl | 858.7 | 2419.0 | | | | 2446.2 | | 2226.3 | 1779.9 | | 1891.5 |
| | Type II | $X10^3$ /µl | 0 | 16.0 | | | | 36.2 | | 108.6 | 78.2 | | 195.0 |
| | Type III | $X10^3$ /µl | 0 | 48.0 | | | | 18.1 | | 18.1 | 0 | | 0 |
| | Serum iron | µg/dl | 102 | | | | | 81.0 | | 80.0 | 109.0 | | 111.0 |
| | TIBC | µg/dl | | | | | | | | | | | |
| | Transferrin saturation | % | | | | | | | | | | | |

Table A-3 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | PCV | vol% | 39.0 | 41.0 | | 40.0 | | 41.0 | | 44.0 | 45.0 | | 45.0 |
| | Hemoglobin | g/dl | 13.6 | 13.9 | | 13.0 | | 12.1 | | 14.4 | 14.8 | | 14.9 |
| | Red blood cells | $X10^6$ /µl | 8.43 | 8.39 | | 7.81 | | 7.41 | | 8.80 | 9.34 | | 9.52 |
| | MCV | fl | 48.0 | 48.0 | | 48.0 | | 50.0 | | 48.0 | 48.0 | | 48.0 |
| | MCHC | g/dl | 34.1 | 34.8 | | 34.9 | | 33.0 | | 34.0 | 33.0 | | 33.8 |
| | Total leukocytes | µl | 6.3 | 11.1 | | 13.9 | | 11.0 | | 13.6 | 13.4 | | 13.9 |
| | Plasma protein | g/dl | 7.4 | 7.2 | | 7.5 | | 7.4 | | 7.8 | 7.8 | | 7.8 |
| | Reticulocytes; Total | $X10^3$ /µl | 773.4 | 906.1 | | 1155.8 | | 1170.7 | | 1584.0 | 1419.6 | | 1542.2 |
| | Type I | $X10^3$ /µl | 708.1 | 872.5 | | 1054.3 | | 1037.4 | | 1372.8 | 1307.6 | | 1437.5 |
| | Type II | $X10^3$ /µl | 25.2 | 33.5 | | 23.4 | | 74.1 | | 158.4 | 74.7 | | 95.2 |
| | Type III | $X10^3$ /µl | 0 | 0 | | 78.1 | | 59.2 | | 52.8 | 37.3 | | 9.5 |
| | Serum iron | µg/dl | 133.0 | 125.0 | | | | | | 120.0 | 111.0 | | 118.0 |
| | TIBC | µg/dl | 339.0 | | | | | | | | | | |
| | Transferrin saturation | % | 43 | | | | | | | | | | |

Table A-4. Individual animal data for Experiment IV

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 31.0 | 35.0 | 41.0 | 37.0 | 40.0 | 38.0 | 35.0 | 34.0 | 28.0 | 30.0 | 28.0 |
| | Hemoglobin | g/dl | 10.2 | 11.2 | 12.4 | 11.9 | 12.1 | 12.0 | 10.7 | 10.5 | 9.2 | 9.4 | 8.9 |
| | Red blood cells | $X10^6$ /µl | 7.46 | 8.26 | 8.65 | 8.80 | 8.90 | 8.63 | 7.79 | 7.74 | 6.94 | 7.10 | 6.75 |
| | MCV | fl | 42.0 | 43.0 | 45.0 | 43.0 | 43.0 | 45.0 | 44.0 | 43.0 | 43.0 | 43.0 | 43.0 |
| | MCHC | g/dl | 33.3 | 31.8 | 32.5 | 31.7 | 32.0 | 31.2 | 31.7 | 31.9 | 31.4 | 31.4 | 30.8 |
| | Total leukocytes | µl | 16.3 | 41.5 | 43.7 | 47.1 | 33.5 | 40.3 | 40.5 | 38.7 | 28.9 | 37.2 | 38.5 |
| | Plasma protein | g/dl | 5.4 | 5.4 | 5.8 | 6.0 | 5.8 | 6.7 | 6.6 | 6.3 | 6.3 | 6.7 | 7.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 104.4 | 247.8 | 553.6 | 756.8 | 498.4 | 949.3 | 747.8 | 928.8 | 846.6 | 1050.8 | 1215.0 |
| | Type I | $X10^3$ /µl | 104.4 | 231.2 | 50.17 | 475.2 | 409.4 | 690.4 | 669.9 | 758.5 | 777.2 | 1036.6 | 1174.5 |
| | Type II | $X10^3$ /µl | 0 | 16.5 | 34.6 | 158.4 | 35.6 | 103.5 | 63.3 | 46.4 | 69.4 | 14.2 | 40.5 |
| | Type III | $X10^3$ /µl | 0 | 0 | 17.3 | 123.2 | 53.4 | 155.3 | 15.5 | 46.4 | 0 | 0 | 0 |
| | Serum iron | µg/dl | 55.0 | 67.0 | 55.0 | 47.0 | | 86.0 | 58.0 | 30.0 | 49.0 | 69.0 | 79.0 |
| | TIBC | µg/dl | 501.0 | 550.0 | 511.0 | 230.0 | | 149.0 | 145.0 | 144.0 | 198.0 | 143.0 | 181.0 |
| | Transferrin saturation | % | 11 | 12 | 11 | 43 | | 58 | 40 | 21 | 25 | 48 | 44 |

Table A-4 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 35.0 | 43.0 | 44.0 | 46.0 | 45.0 | 43.0 | 42.0 | 40.0 | 37.0 | 35.0 | 33.0 |
| | Hemoglobin | g/dl | 11.8 | 14.7 | 14.6 | 15.4 | 14.8 | 14.1 | 14.1 | 14.1 | 12.8 | 11.7 | 11.3 |
| | Red blood cells | $X10^6$ /µl | 7.31 | 9.04 | 9.17 | 9.64 | 9.01 | 8.87 | 8.78 | 8.84 | 8.04 | 7.51 | 7.28 |
| | MCV | fl | 49.0 | 49.0 | 49.0 | 48.0 | 50.0 | 50.0 | 49.0 | 49.0 | 49.0 | 50.0 | 49.0 |
| | MCHC | g/dl | 33.7 | 33.7 | 33.3 | 33.9 | 33.2 | 32.4 | 33.9 | 33.2 | 33.0 | 31.6 | 32.6 |
| | Total leukocytes | µl | 13.7 | 27.6 | 21.3 | 17.6 | 17.5 | 21.9 | 16.1 | 12.1 | 19.1 | 20.4 | 12.5 |
| | Plasma protein | g/dl | 7.2 | 7.9 | 8.0 | 7.8 | 7.3 | 7.2 | 7.6 | 7.3 | 6.5 | 7.0 | 7.0 |
| | Reticulocytes; Total | $X10^3$ /µl | 1462.0 | 2712.0 | 1632.2 | 1985.8 | 1639.8 | 1383.7 | 1299.4 | 830.9 | 611.0 | 976.3 | 1208.4 |
| | Type I | $X10^3$ /µl | 1462.0 | 2621.6 | 1613.9 | 1908.7 | 1621.8 | 1365.9 | 1246.7 | 830.9 | 611.0 | 961.2 | 1106.5 |
| | Type II | $X10^3$ /µl | 0 | 36.1 | 9.1 | 38.5 | 18.0 | 17.7 | 52.6 | 0 | 0 | 75.1 | 101.9 |
| | Type III | $X10^3$ /µl | 0 | 0 | 9.1 | 38.5 | 18.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Serum iron | µg/dl | 149.0 | 56.0 | 79.0 | 90.0 | | 108.0 | 142.0 | 155.0 | 134.0 | 65.0 | 70.0 |
| | TIBC | µg/dl | 407.0 | 290.0 | 316.0 | 364.0 | | 361.0 | 395.0 | 350.0 | 374.0 | 339.0 | 256.0 |
| | Transferrin saturation | % | 37 | 19 | 25 | 25 | | 30 | 36 | 44 | 36 | 19 | 27 |

Table A-4 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 31.0 | 37.0 | 42.0 | 44.0 | 49.0 | 48.0 | 42.0 | 47.0 | 47.0 | 37.0 | 40.0 |
| | Hemoglobin | g/dl | 10.3 | 13.2 | 14.3 | 15.7 | 16.8 | 16.0 | 15.1 | 16.4 | 15.9 | 13.3 | 13.1 |
| | Red blood cells | $X10^6$ /µl | 6.17 | 7.65 | 7.90 | 9.13 | 9.74 | 9.25 | 8.83 | 9.58 | 9.31 | 7.75 | 7.76 |
| | MCV | fl | 51.0 | 52.0 | 51.0 | 52.0 | 53.0 | 53.0 | 52.0 | 53.0 | 53.0 | 53.0 | 52.0 |
| | MCHC | g/dl | 33.2 | 33.6 | 36.0 | 33.7 | 32.9 | 32.9 | 33.7 | 33.1 | 32.6 | 32.5 | 33.0 |
| | Total leukocytes | µl | 13.1 | 18.9 | 12.4 | 16.7 | 16.6 | 21.1 | 10.8 | 17.4 | 42.1 | 21.9 | 18.0 |
| | Plasma protein | g/dl | 6.4 | 6.9 | 7.2 | 7.0 | 7.7 | 7.1 | 6.8 | 6.8 | 6.8 | 6.3 | 7.0 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 999.5 | 2126.7 | 1611.6 | 2227.7 | 1967.4 | 1591.0 | 1130.2 | 997.1 | 1117.2 | 1240.0 | 771.1 |
| | Type I | $X10^3$ /µl | 974.8 | 2111.4 | 1595.8 | 2118.1 | 1753.2 | 1498.5 | 971.3 | 881.3 | 1079.9 | 1069.5 | 586.7 |
| | Type II | $X10^3$ /µl | 24.6 | 0 | 15.8 | 91.3 | 779.0 | 74.0 | 158.9 | 95.8 | 37.2 | 120.5 | 154.4 |
| | Type III | $X10^3$ /µl | 0 | 15.3 | 0 | 18.2 | 136.3 | 18.5 | 0 | 0 | 0 | 0 | 0 |
| | Serum iron | µg/dl | 107.0 | 158.0 | 74.0 | 89.0 | 119.0 | 119.0 | 195.0 | 228.0 | 80.0 | 91.0 | 102.0 |
| | TIBC | µg/dl | 409.0 | 491.0 | 290.0 | 226.0 | | 341.0 | 344.0 | 433.0 | 397.0 | 298.0 | 309.0 |
| | Transferrin saturation | % | 27 | 32 | 26 | 33 | | 35 | 57 | 53 | 20 | 31 | 33 |

Table A-4 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 37.0 | 40.0 | 45.0 | 44.0 | 45.0 | 43.0 | 42.0 | 40.0 | 40.0 | 41.0 | 41.0 |
| | Hemoglobin | g/dl | 13.3 | 14.3 | 16.2 | 16.4 | 16.2 | 15.8 | 14.7 | 13.5 | 13.4 | 14.1 | 14.0 |
| | Red blood cells | $X10^6$ /µl | 8.91 | 9.49 | 11.22 | 11.3 | 11.2 | 11.01 | 9.80 | 8.91 | 9.08 | 9.39 | 9.32 |
| | MCV | fl | 43.0 | 45.0 | 43.0 | 44.0 | 46.0 | 44.0 | 45.0 | 45.0 | 45.0 | 47.0 | 46.0 |
| | MCHC | g/dl | 35.3 | 34.0 | 34.5 | 33.4 | 33.6 | 32.6 | 34.4 | 34.1 | 33.2 | 32.8 | 33.3 |
| | Total leukocytes | µl | 5.9 | 10.6 | 10.5 | 10.2 | 6.3 | 9.0 | 10.7 | 19.9 | 19.0 | 16.0 | 14.4 |
| | Plasma protein | g/dl | 6.5 | 7.3 | 7.4 | 7.3 | 7.7 | 7.7 | 8.2 | 8.1 | 8.3 | 7.9 | 7.0 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 392.0 | 436.5 | 896.0 | 1243.0 | 963.2 | 1078.9 | 725.2 | 748.4 | 1035.1 | 1051.6 | 1360.7 |
| | Type I | $X10^3$ /µl | 392.0 | 436.5 | 873.6 | 1130.0 | 963.2 | 1012.9 | 725.2 | 677.1 | 1016.9 | 920.2 | 1080.0 |
| | Type II | $X10^3$ /µl | 0 | 0 | 0 | 90.4 | 0 | 22.0 | 0 | 53.4 | 0 | 131.4 | 40.8 |
| | Type III | $X10^3$ /µl | 0 | 0 | 22.4 | 22.6 | 0 | 44.0 | 0 | 17.8 | 18.1 | 0 | 0 |
| | Serum iron | µg/dl | 137.0 | 85.0 | 86.0 | 77.0 | 113.0 | 135.0 | 180.0 | 130.0 | 125.0 | 129.0 | 105.0 |
| | TIBC | µg/dl | 466.0 | 343.0 | 332.0 | 312.0 | | | 413.0 | 429.0 | 438.0 | 251.0 | 259.0 |
| | Transferrin saturation | % | 30 | 25 | 27 | 25 | | | 44 | 30 | 29 | 51 | 41 |

Table A-4 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | PCV | vol% | 31.0 | 38.0 | 36.0 | 37.0 | 32.0 | 32.0 | 30.0 | 29.0 | 28.0 | 28.0 | 32.0 |
| | Hemoglobin | g/dl | 10.7 | 12.9 | 12.6 | 12.3 | 10.3 | 11.5 | 10.3 | 9.9 | 9.6 | 10.1 | 11.2 |
| | Red blood cells | $X10^6$ /µl | 7.53 | 9.12 | 8.96 | 8.80 | 7.30 | 8.03 | 7.50 | 7.19 | 7.04 | 7.43 | 8.12 |
| | MCV | fl | 44.0 | 43.0 | 42.0 | 44.0 | 42.0 | 44.0 | 42.0 | 42.0 | 43.0 | 44.0 | 43.0 |
| | MCHC | g/dl | 33.2 | 33.5 | 34.1 | 32.4 | 33.7 | 33.0 | 33.0 | 33.0 | 32.3 | 31.3 | 32.6 |
| | Total leukocytes | µl | 6.7 | 7.1 | 6.4 | 7.6 | 6.2 | 15.4 | 13.9 | 10.6 | 9.6 | 8.0 | 10.2 |
| | Plasma protein | g/dl | 7.3 | 8.0 | 8.0 | 8.2 | 8.3 | 8.3 | 8.6 | 8.3 | 8.1 | 8.0 | 8.4 |
| | Reticulocytes; Total | $X10^3$ /µl | 481.9 | 656.6 | 734.7 | 668.8 | 394.2 | 497.8 | 465.0 | 373.8 | 884.8 | 832.1 | 1445.3 |
| | Type I | $X10^3$ /µl | 481.9 | 638.4 | 645.1 | 616.0 | 379.6 | 497.8 | 465.0 | 345.1 | 732.1 | 683.5 | 1250.4 |
| | Type II | $X10^3$ /µl | 0 | 0 | 35.8 | 52.8 | 0 | 0 | 0 | 14.3 | 28.1 | 148.6 | 194.8 |
| | Type III | $X10^3$ /µl | 0 | 18.2 | 53.7 | 0 | 14.6 | 0 | 0 | 14.3 | 84.4 | 0 | 0 |
| | Serum iron | µg/dl | 139.0 | 81.0 | 42.0 | 46.0 | 52.0 | 52.0 | 59.0 | 58.0 | 105.0 | 53.0 | 94.0 |
| | TIBC | µg/dl | 379.0 | 234.0 | 253.0 | 315.0 | 332.0 | 358.0 | 363.0 | 356.0 | 449.0 | 268.0 | 453.0 |
| | Transferrin saturation | % | 36 | 31 | 16 | 15 | 16 | 15 | 16 | 16 | 23 | 20 | 21 |

Table A-5. Individual animal data for Experiment V

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 32-34 | 36-35 | 36-37 | | | | | | | | |
| | Hemoglobin | g/dl | 11.1 | 11.5 | 11.1 | | | | | | | | |
| | Red blood cells | $X10^6$ /µl | 8.12 | 8.30 | 7.98 | | | | | | | | |
| | MCV | fl | 43.0 | 42.0 | 43.0 | | | | | | | | |
| | MCHC | g/dl | 33.4 | 33.3 | 33.2 | | | | | | | | |
| | Total leukocytes | µl | 29.2 | 28.1 | 37.0 | | | | | | | | |
| | Plasma protein | g/dl | 6.0 | 6.0 | 6.3 | | | | | | | | |
| | Reticulocytes; Total | $X10^3$ /µl | 1161.1 | 1245.0 | 893.7 | | | | | | | | |
| | Type I | $X10^3$ /µl | 1128.6 | 1228.4 | 893.7 | | | | | | | | |
| | Type II | $X10^3$ /µl | 32.4 | 16.6 | 0 | | | | | | | | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | | | | | | | | |
| | Serum iron | µg/dl | 83-484 | 440-507 | 379-408 | | | | | | | | |
| | TIBC | µg/dl | 414 | 456-475 | 389-425 | | | | | | | | |
| | Transferrin saturation | % | 20 | 96-100 | 97-96 | | | | | | | | |

Table A-5 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 34-35 | 34-35 | 35-38 | | | | | | | | |
| | Hemoglobin | g/dl | 11.2 | 11.1 | 11.3 | | | | | | | | |
| | Red blood cells | $X10^6$ /µl | 8.18 | 8.16 | 8.18 | | | | | | | | |
| | MCV | fl | 43.0 | 43.0 | 43.0 | | | | | | | | |
| | MCHC | g/dl | 34.0 | 33.0 | 33.0 | | | | | | | | |
| | Total leukocytes | µl | 14.5 | 16.2 | 17.3 | | | | | | | | |
| | Plasma protein | g/dl | 6.7 | 6.7 | 6.8 | | | | | | | | |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 670.7 | 726.2 | 695.3 | | | | | | | | |
| | Type I | $X10^3$ /µl | 654.4 | 685.4 | 670.7 | | | | | | | | |
| | Type II | $X10^3$ /µl | 16.3 | 40.8 | 24.5 | | | | | | | | |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | | | | | | | | |
| | Serum iron | µg/dl | 97-480 | 507-497 | 398-432 | | | | | | | | |
| | TIBC | µg/dl | | | | | | | | | | | |
| | Transferrin saturation | % | | | | | | | | | | | |

Table A-6. Individual animal data for Experiment VI

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 32.0 | 36.0 | 40.0 | 40.0 | 34.0 | 27.0 | 29.0 | 31.0 | 34.0 | 35.0 | 37.0 |
| | Hemoglobin | g/dl | 10.6 | 12.5 | 14.0 | 14.2 | 12.0 | 9.6 | 10.8 | 11.1 | 11.3 | 11.8 | 12.2 |
| | Red blood cells | $X10^6$ /µl | 6.87 | 7.88 | 8.98 | 8.94 | 7.48 | 6.00 | 6.94 | 6.92 | 6.83 | 7.02 | 7.23 |
| | MCV | fl | 48.0 | 47.0 | 49.0 | 50.0 | 52.0 | 50.0 | 51.0 | 51.0 | 50.0 | 49.0 | 49.0 |
| | MCHC | g/dl | 32.9 | 34.1 | 32.4 | 32.4 | 31.4 | 32.2 | 30.9 | 32.1 | 33.2 | 34.0 | 33.7 |
| | Total leukocytes | µl | 8.8 | 9.7 | 6.1 | 6.3 | 8.4 | 4.0 | 5.2 | 8.9 | 18.4 | 15.6 | 17.6 |
| | Plasma protein | g/dl | 6.0 | 5.8 | 6.0 | 5.6 | 5.5 | 5.4 | 5.4 | 5.0 | 5.5 | 5.6 | 5.7 |
| | Reticulocytes; Total | $X10^3$ /µl | 467.1 | 189.1 | 682.4 | 661.5 | 658.2 | 564.0 | 596.8 | 1107.2 | 983.5 | 1165.3 | 1446.0 |
| | Type I | $X10^3$ /µl | 425.9 | 189.1 | 520.8 | 518.5 | 568.4 | 432.0 | 416.4 | 594.1 | 450.7 | 603.7 | 1185.7 |
| | Type II | $X10^3$ /µl | 41.2 | 0 | 71.8 | 35.7 | 89.7 | 120.0 | 124.9 | 373.6 | 382.4 | 407.1 | 260.2 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 17.8 | 0 | 12.0 | 55.5 | 138.4 | 150.2 | 154.4 | 0 |
| | Serum iron | µg/dl | 114-256 | 309 | 374 | 389-345 | 208-224 | 280 | 317 | 267 | 273 | 279 | 310 |
| | TIBC | µg/dl | 296 | 332 | 411 | 421 | 259 | 280 | 372 | 309 | 337 | 342 | 368 |
| | Transferrin saturation | % | 39 | 93 | 91 | 92 | 80 | 100 | 85 | 86 | 81 | 82 | 84 |

Table A-6 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 38.0 | 33.0 | 32.0 | 30.0 | 32.0 | 33.0 | 29.0 | 37.0 | 35.0 | 34.0 | 35.0 |
| | Hemoglobin | g/dl | 13.4 | 12.2 | 11.4 | 10.4 | 11.9 | 13.6 | 10.8 | 13.0 | 12.2 | 10.7 | 11.5 |
| | Red blood cells | $X10^6$ /µl | 8.04 | 7.56 | 6.99 | 6.39 | 7.27 | 7.98 | 6.66 | 7.56 | 7.27 | 6.46 | 6.72 |
| | MCV | fl | 49.0 | 48.0 | 51.0 | 50.0 | 49.0 | 51.0 | 50.0 | 51.0 | 50.0 | 50.0 | 49.0 |
| | MCHC | g/dl | 34.3 | 34.1 | 32.7 | 33.0 | 33.0 | 34.3 | 32.7 | 33.7 | 33.3 | 32.5 | 34.7 |
| | Total leukocytes | µl | 15.7 | 9.9 | 9.2 | 5.5 | 8.1 | 6.8 | 9.1 | 8.6 | 7.2 | 9.3 | 18.7 |
| | Plasma protein | g/dl | 7.8 | 7.1 | 6.5 | 7.2 | 7.9 | 8.3 | 7.2 | 7.4 | 7.6 | 7.2 | 7.4 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 1382.8 | 1224.7 | 1202.2 | 1380.2 | 1599.4 | 1739.6 | 1198.0 | 1406.1 | 770.6 | 994.8 | 1223.0 |
| | Type I | $X10^3$ /µl | 1286.4 | 1194.4 | 1188.3 | 1290.7 | 1323.1 | 1452.3 | 905.7 | 922.3 | 465.2 | 826.8 | 1008.0 |
| | Type II | $X10^3$ /µl | 96.4 | 30.2 | 13.9 | 89.4 | 276.2 | 255.3 | 319.6 | 423.3 | 305.2 | 167.9 | 188.1 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 0 | 31.9 | 39.9 | 60.4 | 0 | 0 | 26.8 |
| | Serum iron | µg/dl | 124-332 | 278 | 221 | 216-214 | 257-235 | 156 | 117 | 108 | 78 | 68 | 60 |
| | TIBC | µg/dl | 368 | 292 | 263 | 252 | 306 | | 312 | 313 | 299 | 291 | 300 |
| | Transferrin saturation | % | 34 | 95 | 84 | 86 | 84 | | 38 | 35 | 26 | 23 | 20 |

Table A-6 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 41.0 | 37.0 | 37.0 | 30.0 | 27.0 | 26.0 | 21.0 | 25.0 | 25.0 | 26.0 | 26.0 |
| | Hemoglobin | g/dl | 14.0 | 12.9 | 12.8 | 10.9 | 9.5 | 8.7 | 7.4 | 8.5 | 8.3 | 8.6 | 8.3 |
| | Red blood cells | $X10^6$ /µl | 8.57 | 7.82 | 7.65 | 6.33 | 5.84 | 5.15 | 4.51 | 4.77 | 4.78 | 4.99 | 4.99 |
| | MCV | fl | 51.0 | 50.0 | 51.0 | 51.0 | 50.0 | 52.0 | 52.0 | 57.0 | 56.0 | 54.0 | 52.0 |
| | MCHC | g/dl | 32.5 | 33.3 | 33.6 | 32.8 | 32.6 | 32.7 | 32.0 | 31.0 | 30.6 | 31.3 | 31.6 |
| | Total leukocytes | µl | 13.3 | 9.2 | 6.9 | 6.5 | 7.6 | 7.9 | 10.9 | 13.8 | 13.2 | 8.9 | 8.5 |
| | Plasma protein | g/dl | 5.8 | 5.2 | 5.5 | 5.8 | 6.1 | 6.5 | 5.3 | 6.3 | 6.4 | 6.3 | 6.5 |
| | Reticulocytes; Total | $X10^3$ /µl | 702.7 | 344.0 | 673.2 | 721.6 | 408.8 | 525.3 | 532.1 | 954.0 | 1357.5 | 1856.2 | 1916.1 |
| | Type I | $X10^3$ /µl | 702.7 | 344.0 | 627.3 | 633.0 | 292.0 | 298.7 | 279.6 | 391.1 | 707.4 | 1546.9 | 1586.8 |
| | Type II | $X10^3$ /µl | 0 | 0 | 45.9 | 88.6 | 81.2 | 175.1 | 207.4 | 477.0 | 630.9 | 279.4 | 289.4 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 0 | 35.0 | 51.5 | 45.1 | 85.8 | 19.1 | 29.9 | 39.9 |
| | Serum iron | µg/dl | 186-234 | 181 | 165-164 | 153-155 | 217-235 | 220 | 111 | 50 | 32 | 49 | 44 |
| | TIBC | µg/dl | 244 | 201 | 181 | 190 | 248 | 250 | 200 | 263 | 226 | 232 | 211 |
| | Transferrin saturation | % | 69 | 90 | 91 | 81 | 88 | 88 | 56 | 19 | 14 | 21 | 21 |

Table A-6 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 38.0 | 32.0 | 40.0 | 34.0 | 31.0 | 28.0 | 32.0 | 26.0 | 33.0 | 35.0 | 35.0 |
| | Hemoglobin | g/dl | 13.5 | 11.4 | 13.9 | 12.0 | 11.4 | 9.9 | 11.7 | 8.9 | | 11.2 | 11.0 |
| | Red blood cells | $X10^6$ /µl | 8.05 | 7.08 | 8.34 | 7.42 | 7.09 | 6.11 | 7.01 | 5.48 | | 6.01 | 6.65 |
| | MCV | fl | 51.0 | 49.0 | 51.0 | 51.0 | 50.0 | 50.0 | 52.0 | 51.0 | | 52.0 | 50.0 |
| | MCHC | g/dl | 33.2 | 33.1 | 33.2 | 32.5 | 32.8 | 32.0 | 32.8 | 31.9 | | 32.3 | 32.5 |
| | Total leukocytes | µl | 10.3 | 9.6 | 10.4 | 6.8 | 7.3 | 7.1 | 11.3 | 8.6 | | 12.7 | 7.5 |
| | Plasma protein | g/dl | 5.5 | 5.2 | 6.0 | 6.3 | 6.5 | 6.4 | 6.8 | 6.8 | 6.8 | 6.8 | 7.2 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 531.3 | 212.4 | 450.3 | 593.6 | 510.4 | 476.5 | 869.2 | 460.3 | 1136.5 | 1153.9 | 1502.9 |
| | Type I | $X10^3$ /µl | 531.3 | 212.4 | 300.2 | 430.3 | 439.5 | 329.9 | 332.4 | 241.1 | 708.4 | 913.5 | 984.2 |
| | Type II | $X10^3$ /µl | 0 | 0 | 150.1 | 148.4 | 56.7 | 122.2 | 364.5 | 197.2 | 339.4 | 192.3 | 425.6 |
| | Type III | $X10^3$ /µl | 0 | 0 | 0 | 14.8 | 14.1 | 24.4 | 182.2 | 21.9 | 59.0 | 48.0 | 93.1 |
| | Serum iron | µg/dl | 99-249 | 176 | 156-140 | 135-148 | 184-207 | 124-220 | 86 | 75 | 48 | 50 | 73 |
| | TIBC | µg/dl | 272 | 205 | 194 | 170 | 241 | 179 | | 223 | 226 | 237 | 266 |
| | Transferrin saturation | % | 36 | 86 | 77 | 79 | 76 | 69 | | 34 | 21 | 21 | 27 |

Table A-7. Individual animal data for Experiment VII

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PCV | vol% | 31.0 | 44.0 | 37.0 | 45.0 | 46.0 | 52.0 | 42.0 | 45.0 | 56.0 | 53.0 | |
| | Hemoglobin | g/dl | 10.2 | 15.1 | 12.7 | 15.6 | 15.9 | 15.3 | 14.5 | 15.8 | 18.2 | 19.8 | |
| | Red blood cells | $X10^6$ /µl | 6.78 | 9.55 | 8.06 | 9.64 | 9.68 | 9.04 | 7.94 | 8.92 | 11.20 | 12.06 | |
| | MCV | fl | 47.0 | 49.0 | 50.0 | 51.0 | 53.0 | 53.0 | 58.0 | 56.0 | 55.0 | 47.0 | |
| | MCHC | g/dl | 31.8 | 31.8 | 31.3 | 31.6 | 32.6 | 31.5 | 31.5 | 32.8 | 29.5 | 34.2 | |
| | Total leukocytes | µl | 11.6 | 13.1 | 38.1 | 34.2 | 17.3 | 13.9 | 14.0 | 20.8 | 26.2 | 22.8 | |
| | Plasma protein | g/dl | 7.4 | 8.1 | 6.9 | 7.4 | 7.5 | 7.3 | 6.4 | 6.7 | 6.2 | 5.0 | |
| | Reticulocytes; Total | $X10^3$ /µl | 908.5 | 2158.3 | 1547.5 | 2159.3 | 2884.6 | 2386.5 | 1857.9 | 2301.3 | 2086.8 | 3087.3 | |
| | Type I | $X10^3$ /µl | 895.0 | 1795.4 | 1305.7 | 1368.8 | 1742.4 | 1102.8 | 698.7 | 1213.1 | 1354.2 | 2701.4 | |
| | Type II | $X10^3$ /µl | 13.6 | 343.8 | 241.8 | 424.1 | 658.2 | 524.3 | 651.0 | 767.1 | 732.6 | 385.9 | |
| | Type III | $X10^3$ /µl | 0 | 19.1 | 0 | 366.3 | 484.0 | 759.3 | 508.1 | 321.1 | 0 | 0 | |
| | Serum iron | µg/dl | 60-225 | 238-226 | 109-213 | 227 | 219 | 296 | 242 | 90 | 60 | | |
| | TIBC | µg/dl | 268 | 289 | 155 | 275 | 266 | 336 | 269 | | | | |
| | Transferrin saturation | % | 22 | 82 | 70 | 83 | 82 | 88 | 90 | | | | |

Table A-7 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | PCV | vol% | 30.0 | 35.0 | 34.0 | 40.0 | 45.0 | 42.0 | 42.0 | 45.0 | | 45.0 | |
| | Hemoglobin | g/dl | 10.7 | 13.8 | 13.2 | 15.0 | 15.8 | 15.2 | 15.1 | 16.1 | | 16.0 | |
| | Red blood cells | $X10^6$ /µl | 6.27 | 7.86 | 7.82 | 8.41 | 8.62 | 8.52 | 8.51 | 8.99 | | 8.55 | |
| | MCV | fl | 49.0 | 52.0 | 52.0 | 53.0 | 54.0 | 55.0 | 55.0 | 59.0 | | 54.0 | |
| | MCHC | g/dl | 34.2 | 33.3 | 31.9 | 33.5 | 33.3 | 32.9 | 32.9 | 33.2 | | 34.2 | |
| | Total leukocytes | µl | 17.4 | 25.2 | 33.9 | 33.4 | 28.3 | 25.5 | 24.8 | 30.2 | | 23.8 | |
| | Plasma protein | g/dl | 7.0 | 7.5 | 7.0 | 7.2 | 7.3 | 6.1 | 5.8 | 6.3 | | 4.5 | |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 1141.1 | 1650.6 | 1970.6 | 2455.7 | 2465.3 | 2556.0 | 2416.8 | 2553.1 | | 2274.3 | |
| | Type I | $X10^3$ /µl | 1015.7 | 1336.2 | 1595.2 | 1665.1 | 1413.6 | 1022.4 | 953.1 | 1150.7 | | 1881.0 | |
| | Type II | $X10^3$ /µl | 125.4 | 314.4 | 328.4 | 454.1 | 672.3 | 698.6 | 987.1 | 1042.8 | | 324.9 | |
| | Type III | $X10^3$ /µl | 0 | 0 | 46.9 | 336.4 | 379.2 | 834.9 | 476.5 | 354.6 | | 68.4 | |
| | Serum iron | µg/dl | 96-222 | 203-205 | 51-208 | 188 | 211 | 229 | 194 | | | 53 | |
| | TIBC | µg/dl | 267 | 243 | 233 | 267 | 254 | 260 | 221 | | | 187 | |
| | Transferrin saturation | % | 36 | 84 | 22 | 70 | 83 | 88 | 88 | | | 28 | |

Table A-7 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | PCV | vol% | 31.0 | 34.0 | 35.0 | 38.0 | 37.0 | 37.0 | 35.0 | 37.0 | 46.0 | 44.0 | 35.0 |
| | Hemoglobin | g/dl | 10.3 | 11.4 | 13.2 | 14.1 | 12.8 | 12.7 | 12.6 | 12.8 | | | 11.4 |
| | Red blood cells | $X10^6$ /µl | 6.82 | 7.52 | 8.55 | 8.96 | 8.13 | 8.09 | 7.92 | 8.21 | | | 6.97 |
| | MCV | fl | 45.0 | 47.0 | 45.0 | 46.0 | 48.0 | 48.0 | 48.0 | 48.0 | | | 47.0 |
| | MCHC | g/dl | 33.7 | 32.4 | 33.7 | 33.9 | 32.9 | 32.8 | 32.8 | 32.8 | | | 34.6 |
| | Total leukocytes | µl | 10.0 | 11.5 | 16.1 | 17.7 | 17.2 | 15.9 | 14.9 | 12.9 | | | 12.0 |
| | Plasma protein | g/dl | 6.4 | 7.0 | 7.2 | 7.5 | 7.2 | 7.4 | 6.0 | 7.5 | 6.5 | 6.4 | 6.4 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 709.3 | 1338.6 | 2103.3 | 1505.2 | 2048.7 | 1747.4 | 1742.4 | 1953.9 | | | 1449.7 |
| | Type I | $X10^3$ /µl | 559.2 | 1143.0 | 1556.1 | 1003.5 | 1219.5 | 550.1 | 887.0 | 968.7 | | | 1268.5 |
| | Type II | $X10^3$ /µl | 150.0 | 195.5 | 513.0 | 250.8 | 569.1 | 841.3 | 601.9 | 673.2 | | | 167.2 |
| | Type III | $X10^3$ /µl | 0 | 0 | 34.2 | 250.8 | 260.1 | 355.9 | 253.4 | 311.9 | | | 13.9 |
| | Serum iron | µg/dl | 80-167 | 162-183 | 178-176 | 205 | 199 | 198 | | | | | 53 |
| | TIBC | µg/dl | 201 | 222 | 218 | | | | | | | | |
| | Transferrin saturation | % | 40 | 73 | 82 | | | | | | | | |

Table A-7 (continued)

| Animal | Determination | Units | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | PCV | vol% | 29.0 | 36.0 | 32.0 | 30.0 | 28.0 | 28.0 | 26.0 | 30.0 | 34.0 | 34.0 | 34.0 |
| | Hemoglobin | g/dl | 9.5 | 12.0 | 11.4 | 10.1 | 9.5 | 9.1 | 8.8 | 10.6 | 10.3 | 10.0 | 11.9 |
| | Red blood cells | $X10^6$ /µl | 6.77 | 8.50 | 7.81 | 7.09 | 6.59 | 6.20 | 5.80 | 6.79 | 6.64 | 6.46 | 7.69 |
| | MCV | fl | 43.0 | 45.0 | 45.0 | 43.0 | 45.0 | 45.0 | 47.0 | 48.0 | 49.0 | 47.0 | 46.0 |
| | MCHC | g/dl | 32.6 | 31.1 | 32.4 | 32.8 | 31.6 | 32.1 | 32.1 | 32.0 | 31.8 | 32.7 | 34.0 |
| | Total leukocytes | µl | 9.7 | 7.3 | 15.6 | 7.9 | 8.7 | 7.3 | 7.4 | 9.8 | 11.4 | 10.6 | 13.6 |
| | Plasma protein | g/dl | 7.8 | 8.4 | 8.0 | 7.8 | 7.4 | 7.9 | 7.6 | 8.3 | 8.3 | 8.4 | 9.0 |
| | Reticulocytes; | $X10^3$ | | | | | | | | | | | |
| | Total | /µl | 893.6 | 1938.0 | 1936.8 | 1219.4 | 1344.3 | 1103.6 | 1183.2 | 1290.1 | 1726.4 | 1808.8 | 2137.8 |
| | Type I | $X10^3$ /µl | 798.6 | 1428.0 | 1437.0 | 992.6 | 803.9 | 421.6 | 638.0 | 611.1 | 1235.0 | 1705.4 | 1968.6 |
| | Type II | $X10^3$ /µl | 81.2 | 510.0 | 437.3 | 113.4 | 408.5 | 570.4 | 440.8 | 556.7 | 478.0 | 775.0 | 169.2 |
| | Type III | $X10^3$ /µl | 13.5 | 0 | 62.4 | 113.4 | 131.8 | 111.6 | 104.4 | 122.2 | 13.2 | 25.8 | 0 |
| | Serum iron | µg/dl | 111-338 | 342-261 | 267-247 | 258 | 228 | 244 | 181 | 93 | 44 | 58 | 119 |
| | TIBC | µg/dl | 395 | 389 | 316 | 261 | 258 | 259 | | | 301 | 323 | 338 |
| | Transferrin saturation | % | 28 | 88 | 84 | 99 | 88 | 94 | | | 15 | 18 | 35 |

# APPENDIX B

# EFFECTS OF INOSINE, HYPOXANTHINE AND ALLOPURINOL ON SERUM IRON AND URIC ACID CONCENTRATIONS

Table B-1. The effect of inosine injection on serum iron concentrations (μg/dl) in cats

| Animal | Hours | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 6 |
| A | 77 | 78 | 78 | 78 | 121 |
| B | 138 | 140 | 138 | 145 | 129 |
| C | 150 | | 138 | 129 | 140 |
| D | 111 | | 108 | 100 | 84 |

Table B-2. The effects of hypoxanthine injection on serum iron (μg/dl) and serum uric acid (mg/dl) concentrations in dogs

| Animal | Determination | Hours | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| A | iron | 75.0 | 67.0 | 69.0 | 69.0 |
| | uric acid | .4 | 2.3 | .82 | |
| B | iron | 138.0 | 142.0 | 146.0 | 139.0 |
| | uric acid | .5 | .8 | .5 | |
| C | iron | 51.0 | 34.0 | 56.0 | 74.0 |
| | uric acid | .4 | .8 | | |
| D | iron | 151.0 | 158.0 | 128.0 | 135.0 |
| | uric acid | .6 | 2.1 | .6 | .7 |
| E | iron | 165.0 | 164.0 | 167.0 | 165.0 |
| | uric acid | .5 | | .8 | .5 |

Table B-3. The effects of hypoxanthine injection on serum iron (μg/dl) and serum uric acid (mg/dl) concentrations in anesthetized dogs

| Animal | Determination | Hours | | | |
|---|---|---|---|---|---|
| | | 0 | .5 | 1 | 2 |
| A | iron | 111.0 | 112.0 | 118.0 | 117.0 |
| | uric acid | 1.0 | 2.3 | 2.7 | 1.7 |
| B | iron | 151.0 | 145.0 | 142.0 | 149.0 |
| | uric acid | 1.2 | 3.0 | 2.6 | 1.8 |
| C | iron | 122.0 | 172.0 | 124.0 | 170.0 |
| | uric acid | 1.0 | 3.1 | 2.8 | 1.5 |

Table B-4. The effects of allopurinol injection on serum iron (μg/dl) and serum uric acid (mg/dl) concentrations in dogs

| Animal | Determination | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| A | iron | 109.0 | | 92.0 | 130.0 | | 130.0 | 159.0 | 121.0 |
| | uric acid | .9 | | .7 | .7 | | .4 | .3 | .4 |
| B | iron | 156.0 | 218.0 | 163.0 | 127.0 | 144.0 | 184.0 | 202.0 | 184.0 |
| | uric acid | .9 | .6 | .7 | .6 | .4 | .6 | .3 | .4 |