IRON AND SELENIUM SUPPLEMENTATION OF SHEEP

by

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ABSTRACT

Iron deficiency and a possible interaction between iron and selenium were investigated in lambs raised under an intensive management system. Trial 1 compared 2 levels of iron dextran treatment, 0 and 500 mg Fe, using 35 lambs injected once at birth. Trial 2 involved 66 lambs and 3 levels of iron: 0, 250, and 500 mg. A third trial was replicated 3 times, using a total of 121 lambs, in order to determine if the iron treatment response was limited by the marginal Se status of the lambs. Treatments were control, +1.5 mg Se, +500 mg Fe, and 1.5 mg Se + 500 mg Fe.

The parameters measured included hemoglobin, hematocrit, weight, plasma iron, and a plasma profile (Ca, P_i, glucose, BUN, total protein, albumin, AP, LDH and AT). Additionally, plasma Se, plasma protein fractions and disease resistance were measured in Trial 3.

Injection of 500 mg Fe significantly ($P<0.05$) increased hemoglobin from 2 to 11 weeks of age in Trial 1, and from 1 to 8 weeks in Trial 3. While iron dosages of either 250 or 500 mg prevented the depression of hemoglobin from birth to 30 days, plasma iron and hemoglobin ($P<0.05$) were significantly higher at 4 weeks in lambs receiving 500 mg Fe. In all studies, a significant proportion of control lambs were anemic at 3-4 weeks of age.

Preliminary information was provided on the effect of iron deficiency and other factors (breed, sex, rearing, birth weight and growth rate) on the lamb plasma profile at 4 weeks. The data indicate that iron deficiency affects plasma metabolites similarly in lambs and humans. P_i, glucose, cholesterol, total protein, alkaline phosphatase and aspartate

transaminase responded linearly to iron dosage. Many parameters were also significantly correlated with plasma iron.

The interaction of iron with selenium was significant ($P<0.05$) only for plasma selenium levels. Plasma selenium at 4 weeks was increased in lambs injected with selenium and not injected with iron. Means were 0.085 ppm (control), 0.086 ppm (+Fe), 0.107 ppm (+Se) and 0.088 ppm Se(+Fe+Se), with 18 to 20 lambs per treatment.

Disease resistance was assessed by susceptibility of lambs to soremouth; hemagglutination titer to a chicken RBC antigen; and gamma globulin levels from 2 to 6 weeks. Selenium but not iron treatment influenced susceptibility of lambs to soremouth. The response of lambs to antigenic challenge from chicken RBC's was also increased ($P<0.05$) by Se treatment at birth, even though by the time of initial challenge at 4 weeks, plasma Se was only slightly higher in the Se-injected lambs (0.098 ppm vs. 0.086 ppm). Iron had little effect on titer, except in selenium-treated lambs. Although iron treatment enhanced gamma globulin production at 6 weeks of age, iron may be more crucial to cellular rather than humoral immunity.

This study consistently demonstrated a dramatic response of blood hemoglobin to iron treatment, but also indicated that other aspects of iron deficiency may be more important than anemia. Marginal deficiencies of both iron and selenium may affect lamb health, and thus have an economic impact on intensive sheep production systems.

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INTRODUCTION

Iron deficiency is not considered to be of practical importance in ruminants (Ammerman and Goodrich, 1983). This assumption should be reevaluated as iron deficiency may be a more serious problem under intensive management systems of sheep production. Research is lacking on the effects of iron deficiency in livestock, other than on hemoglobin production, which may be the most visible but least important aspect of iron deficiency.

Various studies have documented the development of iron deficiency anemia in suckling lambs (Carlson et al., 1961; Holman and Dew, 1966; Holz et al., 1961; Hibbs et al., 1963; Ricketts et al., 1965; Ullrey et al., 1965) dairy calves (Matrone et al., 1957; Mollerberg, 1975; Mollerberg et al., 1975), and even in beef calves on range (Raleigh and Wallace, 1962). Iron deficiency anemia of pre-natal origin has also been demonstrated in as many as 30% of dairy calves at birth, even though the dams have normal hemoglobin levels (Hibbs et al., 1963; Tennant et al., 1975b). Most of these studies looked only at changes in hemoglobin and red cell indices in blood, reflecting the prevalent attitude of physicians and veterinarians:

> "that iron deficiency had no symptomatology, no morbidity, and no mortality; that it was for all practical purposes clinically irrelevant, except to the extent that a little bit of anemia was good for most people" (Fielding, 1975).

Recently, interest in the prevalence of iron deficiency in livestock and possible effects on health and mortality has been stimulated by current findings on the role of iron in disease resistance, tissue enzymes, tissue morphology, and nutrient absorption. Most research on these subjects concerns humans, as iron deficiency is the leading worldwide nutritional

problem. Iron deficiency has occurred in humans for thousands of years, particularly in peoples with low-meat diets. While the early Greeks believed that iron was able to impart strength and force to persons suffering from weakness (Moore and Dubach, 1960), the connection between dietary iron and iron-deficiency anemia was only appreciated since 1895 (Witts, 1969, p. 4).

The history of iron deficiency in livestock is much more recent, and less well understood. Iron deficiency in livestock was first identified in the 1920's in piglets farrowed and raised in confinement (Witts, 1969, p. 4). Since then iron injection of pigs has become a standard practice. Iron deficiency was subsequently identified in milk fed veal calves, but is otherwise believed to be unusual in ruminants. Nonetheless, iron deficiency may develop in the rapidly growing young of almost any mammalian species, including rabbits, monkeys, and elephants (Morgan, 1980; Klos and Lang, 1982).

Lambs may be susceptible to iron deficiency for three reasons: low placental iron transfer (Hoskins and Hansard, 1964), low milk iron content (Underwood, 1966) and high growth rate. Iron deficiency is primarily a production disease, as high growth rate is the most significant variable in the etiology of the disease. Thus conditions conducive to maximum growth are most likely to be associated with anemia (Silverman et al., 1970). The increasing trend towards confinement housing of sheep, and improved management practices such as accelerated lambing schedules, may intensify iron deficiency problems in lambs.

This study was undertaken to investigate iron deficiency in lambs raised under an intensive management system. In the first two trials,

control lambs were compared with lambs injected with iron at birth. Hemoglobin, hematocrit, and weight were measured at birth and weekly until weaning, and plasma iron was measured at 3-4 weeks. The impact of iron deficiency on overall metabolism of the lamb was assessed by measuring a profile of plasma metabolites. No information was previously available on the effect of iron status on the plasma profile. These experiments also investigated on the influence of factors such as growth rate, sex, breed and rearing.

The first trial compared treatment effects for 2 levels of iron, 0 and 500 mg, using 35 lambs. The second trial involved 66 lambs, which were divided into 3 treatment groups: 0, 250, and 500 mg Fe. A third trial, which was replicated three times, investigated the effects of injecting lambs with 500 mg iron, with or without 1.5 mg supplementary selenium.

As the experimental flock was marginal in selenium status, as shown by the chronic but low incidence of white muscle disease (WMD), the validity of the iron treatment results was questioned for several reasons. Firstly, selenium and/or vitamin E may be required for erythropoiesis, or may affect hemolysis and consequently red cell turnover. Secondly, the incidence of WMD appeared to be increased by iron supplementation in the first two experiments. Since iron injection may cause toxicity in marginally Se-deficient pigs, the question arose - was iron injection causing muscle damage similar to and mistaken for WMD? Finally, the response to iron may have been limited by a deficiency in selenium, and therefore an additive or synergistic response to both iron and selenium might be expected. In order to answer these questions, the third trial tested the effects of iron and selenium, separately and combined, on a variety of

parameters: hemoglobin, PCV, plasma iron, selenium and metabolites, plasma proteins, and disease resistance as measured by the hemagglutination test and observations on soremouth.

LITERATURE REVIEW

IRON

FUNCTIONS OF IRON

Iron proteins evolved out of the necessity to utilize oxygen effectively, and to control its toxicity. Iron is essential to the oxidation of organic substances by molecular oxygen, and consequently, to the energy metabolism of all living cells (Frieden, 1974).

The iron proteins are a diverse group, structurally and functionally. For convenience, they are usually classified as heme and non-heme iron compounds (Harrison, 1969). Quantitatively, the heme group of iron proteins dominates iron metabolism. The heme molecule contains an iron atom in the center of a porphyrin ring, chelated to the pyrolle nitrogen atoms. Heme can be synthesized by all aerobic mammalian cells except normal RBC's (Harris and Kellermeyer, 1970, p.3).

Hemoglobin and myoglobin function as oxygen carrying proteins. Hemoglobin increases the oxygen carrying capacity of the blood about seventy times that carried by diffusion (Harrison, 1969). Myoglobin is similar structurally to hemoglobin, but has only one heme group instead of four, and is located in the sarcoplasm of skeletal and heart muscle. As myoglobin has a higher affinity for oxygen than does hemoglobin, it accepts oxygen released from hemoglobin and acts as a tissue reservoir (Moore and Dubach, 1960).

Heme proteins include the cytochromes. These redox enzymes catalyze electron transfer reactions through the ability of the heme iron to undergo reversible oxidation. They transport hydrogen to molecular oxygen as part of the cellular electron transport system (Malstrom, 1970; Wrigglesworth

and Baum, 1980). Cytochromes a (cytochrome oxidase), b, and c, are located in the mitochondria cristae. Cytochrome P_{450} is located in microsomal membranes, and functions in the oxidative degradation of drugs and other metabolites, especially in the liver (NRC, 1979, p. 120).

The catalases and peroxidases are important heme enzymes which destroy toxic oxygen derivatives such as peroxides. They are found in the cytoplasm of most animal cells, but catalase is also found in organelles such as peroxisomes. Peroxidases are involved in biological defence mechanisms. For example, myeloperoxidase in blood cells and intestinal mucosa is important in disease resistance, and lactoperoxidase in milk and saliva has antibacterial activity (Wrigglesworth and Baum, 1980).

The non-heme iron enzymes catalyze a wide variety of metabolic reactions. The metalloflavoproteins include succinate dehydrogenase, α-glycerophosphate dehydrogenase, and NADA-dehydrogenase, all located in mitochondria, and monoamine oxidase, xanthine oxidase, and aldehyde dehydrogenase in the cytoplasm (NRC, 1979, p. 120). Most of these are iron-sulphur proteins.

Among the major iron-dependent, non-heme iron enzymes which do not contain sulphur are the prolyl and lysyl hydroxylases (collagen synthesis), phenylalanine hydroxylase (catabolism of phenylalanine), tyrosine hydroxylase (melanin and epinephrine synthesis), and tryptophan hydroxylase (serotonin synthesis). Ribonucleotide reductase catalyzes an essential, controlled step of DNA synthesis and thus may affect the activity of non-heme enzyme systems (Wrigglesworth and Baum, 1980).

IRON METABOLISM

Iron homeostasis is achieved through control of iron absorption as iron excretion is limited. Very little iron is excreted through the usual routes of urine, skin, hair and endogenous secretions into the gastrointestinal tract, as iron is tenaciously conserved and reutilized. Quantitatively, the daily intake of iron represents only a fraction of the amount of iron circulated through the plasma daily and used in synthesis of iron compounds.

Maximal absorption of iron occurs when the iron requirement is high, as in young and gestating animals. Percentage absorption generally rises with decreasing iron content of the feed, but total absorption decreases.

Many factors in addition to age and diet affect iron absorption in the ruminant. They include iron status; health; gastrointestinal conditions such as motility, pH, mucosal turnover and parasitism; hypoxia; and blood loss. Any conditions stimulating erythropoiesis normally increase iron absorption (Kolb, 1963; Underwood, 1971). Conversely, in many disease states, the release of a leukocyte endogenous mediator from white blood cells limits absorption regardless of iron status (Kampschmidt et al., 1973)

The most important site of iron absorption is the duodenum and jejunum, in both man and animals. Some iron can also be absorbed through the stomach, ileum, and colon (Bothwell et al., 1979, p. 269). The proximal intestine, where most iron absorption occurs, contains specific, glycoprotein, receptor sites in the brush border. More receptor sites are produced during iron deficiency, after a lag phase related to turnover of mucosal cells.

The process of iron absorption can be divided into two steps: uptake from the intestinal lumen into the mucosal cell, and transfer from the serosal surface of the cell to the plasma.

The uptake step of iron absorption involves either an energy-dependent active transport process, as reviewed by Linder and Munro (1977), or a passive diffusion process as described by May and Williams (1980).

According to the active transport theory, ionic divalent iron absorbed by receptors in the brush borders is moved into the cell in a process requiring energy and intact protein synthesis (Linder and Munro, 1977). Consequently, cycloheximide, tetracycline, and other antibiotics impair iron absorption by inhibition of protein synthesis (Forth, 1974). While the active transport mechanism has been the subject of numerous reports, it has not been unequivocally established. Much of the research may be better explained in terms of simple, passive diffusion of chelated iron in equilibrium with various interacting pools of iron (May and Williams, 1980).

The passive diffusion theory proposes that iron is absorbed as chelated complexes. The importance of chelation to iron absorption has been gradually recognized (Thomas, 1970). Low molecular weight, lipophilic complexes of iron in either oxidation state are equally well absorbed. Contrary to earlier work, Fe^{+3} is absorbed as well as Fe^{+2} (Christopher et al., 1974). The more lipophilic the iron complex, the greater the amount of iron transferred through the membrane (May and Williams, 1980).

Probably the many factors which influence iron absorption are not mediated by a single rate-determining step (May and Williams, 1980). In the mucosal cell, equilibrium is established between a rapidly exchangeable

iron pool, the slowly exchangeable ferritin iron pool, and the transferrin in plasma. The rapidly exchangeable iron pool, possibly a protein or polypeptide, has a major influence on iron metabolism. The size and degree of saturation of this pool regulate how incoming iron is proportioned between cell storage and plasma transferrin.

When the labile iron pool is close to saturation, most iron is diverted to ferritin synthesis. As this pool is only slowly exchangeable, excess iron is sequestered in this form and lost during normal cell exfoliation, preventing iron overload. In iron deficiency, labile binding to this pool directs most incoming iron immediately to the circulation.

A carrier protein may be involved in the transport of iron across the mucosal cell, a distance 10,000 to 20,000 times the diameter of the iron atom (Linder and Munro, 1977). The carrier may be a transferrin-like protein. The concentration of this protein is increased in mucosal cells of iron deficient mice, but not in iron deficient sla mice, which are genetically incapable of transferring adequate iron. Sla is the gene involved (Bothwell et al., 1979, p. 274).

The release of iron from the mucosal cell to the plasma is the final step of iron absorption. During the initial, rapid phase of iron release 60 to 80% of the eventual total may be transferred within 30 minutes. Iron released in this phase originates from the rapidly exchangeable iron pool. The slow phase lasts 12-24 hours, as iron is gradually released from ferritin (Bothwell et al., 1979, p. 272).

The importance of individual dietary factors to iron absorption is uncertain, even for monogastrics. Anions forming insoluble or only weakly soluble salts with iron limit its absorption, as iron must be in a soluble

complex. Chelating agents can improve or depress iron absorption. As previously mentioned, weaker chelating agents are necessary for iron absorption. They may improve iron availability by preventing the formation of insoluble iron phosphates and hydroxides, and also by maintaining the iron in a soluble, absorbable state (Conrad, 1970). Organic acids and reducing agents such as ascorbate, citrate, lactate, pyruvate, succinate, cysteine, histidine, lysine, and some sugars thus facilitate absorption (Thomas, 1970). HCl is of major importance as a complexing agent, in adult humans, infants, and pigs (Beutler and Fairbanks, 1980; Bothwell et al., 1979, p. 267; and Hannan, 1971).

Strong intraluminal chelating agents depress iron absorption by competing with the cellular acceptor site for iron. Phytates, endotoxins, alkalinizing agents, pancreatic secretions, phosphates, oxalates, and other endogenous and exogenous chelating agents depress iron absorption in this way (Thomas, 1970).

Iron is transported by transferrin between sites of absorption, storage, utilization, and excretion, and in both plasma and extravascular spaces (Aisen, 1980). Transferrin minimizes the loss of iron from the body, by depositing surplus iron in tissues adapted for iron storage. Transferrin is also important in distributing iron in proportion to need (Bothwell et al., 1979, p. 293).

Transferrin, or siderophilin, is a β-globulin containing a carbohydrate fraction. The liver is the major site of synthesis. Transferrin has two metal binding sites which are capable of binding a wide variety of bivalent and trivalent metals, but have the highest affinity for Fe^{+3} (Brown, 1977).

Normally, transferrin is one-third saturated with iron. The delivery of iron is affected by the degree of saturation of transferrin, as uptake of iron is highest from diferric transferrin for all tissues (Bothwell et al., 1979, p. 293). The two iron-binding sites of transferrin appear functionally similar. However, one site of differric transferrin may preferentially donate iron to developing red blood cells and the placenta (Jacobs, 1977a).

Najean et al. (1970) suggested a labile iron pool may be instrumental in maintaining a desirable, stable level of plasma iron. The transit pool may be a cysteine-containing non-heme protein (Najean et al, 1970), or a low molecular weight complex (Jacobs, 1977b). Evidence for an intracellular transit iron pool has been obtained for reticuloendothelial cells, red cell precursors, cultured Chang cells and liver. Iron may enter the transit pool from endogenous heme breakdown, mobilization of ferritin, and exchange with transferrin (Jacobs, 1977b).

The main storage forms of iron are ferritin and hemosiderin. Ferritin stores trivalent iron in a soluble form which can be mobilized as required, whereas hemosiderin iron is less available (Crichton, 1975). Ferritin has a large capacity for iron, eg. 4500 Fe atoms per molecule, but usually maintains a reserve capacity of one third of this (Harrison et al., 1980). Ferritin contains on average 21% iron, stored as a ferric-hydroxide-phosphate core inside of a spherical protein shell. Iron passes freely in and out of the shell through six channels; the protein shell is thought to have enzyme activity. Ferritin is formed in all cells in response to enlargement of the labile protein pool. The major site of synthesis is the liver, and also spleen and bone marrow (Kaneko, 1980).

Hemosiderin is insoluble, contains 25-33% Fe^{+3} in addition to Fe^{+2}, and is much larger than ferritin (Kaneko, 1980). Hemosiderin is considered to be a breakdown product of ferritin (Harrison et al., 1980).

IRON INTERACTIONS

Most trace minerals, including iron, are metals of the first transition series on the periodic table. Many examples of iron interactions with other minerals involve substitution or antagonism between minerals of similar size and electron structure. Interactions can occur in the lumen of the gut, at absorptive sites, and at various metabolic levels.

The iron requirement is increased by high dietary levels of zinc, cadmium, copper and manganese. These minerals can compete with iron for the iron binding sites in the intestinal mucosa (Underwood, 1971). Conversely, high levels of iron can induce Co, Cu, Zn, Mn and Se deficiencies (Puls, 1981). Iron deficiency results in increased absorption of heavy metals, mainly Fe, Mn, Zn and Ni, but not Cu (Bothwell et al., 1979, p. 273). Possibly the site of interaction in this case is not the iron-binding site, but transferrin, which is responsible for iron uptake from the mucosa. The fact that the electron structure of Mn^{+2} and Fe^{+3} are functionally identical (Thomas, 1970) and that transferrin is an important protein for the transport of Zn (Brown, 1977) supports this interpretation. Furthermore, Zn may reduce iron absorption by interfering with iron incorporation into, or release from, ferritin (Underwood, 1971).

Biological substitution of iron with other minerals is most likely to occur between Mn^{+2} and Fe^{+3}, and Fe^{+2} and Co^{+3}. These ion pairs share similar electron structures. When dietary manganese is high, or

during anemia, the degree of biological substitution of manganese for iron is greatly increased. Mn is incorporated into the heme molecule, with the same rate of synthesis and turnover as the iron porphyrin (Thomas, 1970).

The availability of iron at the metabolic level is dependent on copper enzymes (Frieden, 1974). Nearly all metabolic processes involving iron depend on the interconversion of ferrous and ferric iron. Two copper containing enzymes, or ferroxidases, are known to catalyze the oxidation of ferrous to ferric iron. One of these is ceruloplasmin. Different ferroxidases occur in various species. Iron accumulates in the liver very rapidly in response to even a mild copper deficiency, as a shortage of ceruloplasmin impedes its mobilization (Grassman and Kirchgessner, 1974). On the other hand, the copper content of the liver increases greatly as a result of iron deficiency, indicating that iron is required for copper utilization.

Iron interactions with phosphorus are important at high dietary levels of iron. Too much iron in the diet interferes with phosphorus absorpton by forming an insoluble phosphate. Rickets may then result on an otherwise adequate diet with a good phosphorus content. Studies with piglets fed different levels of iron as ferrous sulfate indicate that the amont of iron required to produce a toxicity depends on the amount and source of phosphorus in the diet. In one experiment, feeding 5000 ppm iron reduced growth rate, serum inorganic phosphorus, and femur ash within 5 weeks, (O'Donovan et al., 1962). However, ruminants appear to be sensitive to much lower levels of iron than are piglets, and 500 ppm should be regarded as the maximum level tolerable by ruminants (ARC, 1980, p. 242).

DIETARY IRON

Most feeds contain generous amounts of iron. There is usually more than 20 tonnes of iron per acre in the top 15 cm of soil, so soil contamination can greatly influence the iron content of feeds (Wretlind, 1968). In the Fraser Valley of British Columbia, mean and ranges of iron in common feeds were: grass hay, 540 ppm (130-1370); alfalfa hay, 580 ppm (40-2185); corn silage, 384 ppm (40-1490); grass silage 1373 ppm (40-5550); and pasture 1076 ppm (40-5370), (Cathcart et al., 1980). Cereal grains are poor sources of iron, containing 30-60 ppm iron; oilseed meals frequently contain 100-200 ppm iron (Underwood, 1981).

Solubility of inorganic iron sources appears to be a primary, but not exclusive, determinant of their availability for ruminants. Ammerman et al. (1967) tested the availability of four inorganic iron sources for ruminants. On the basis of tissue Fe^{59} retention, ferrous sulphate, ferrous carbonate and ferric chloride were ranked in decreasing order of availability, but differences were not significant. Iron in ferric chloride was 3 to 4 times as available as that in ferric oxide (Fe_2O_3) to iron-depleted calves. Ferrous sulphate and ferric chloride are very soluble, and ferrous carbonate and ferric oxide are slightly or non-soluble.

The absorption coefficient of soluble iron as ferric chloride was 0.60 for young calves when the diet provided 30 mg iron daily, and 0.30 when the diet provided 60 mg iron daily (Matrone et al., 1957). Hemoglobin synthesis was used as the criterion of availability in this study.

Iron in plant products appears to be less available than iron in soluble iron salts. Most of the iron in plants is in the ferric (Fe^{+3})

form in organic complexes (NRC, 1980, p. 243). Iron in grass was 48 to 63% as effective, and iron in legumes 47 to 57% as effective, compared to ferric chloride for improvement of hemoglobin (Raven and Thompson, 1959; Thompson and Raven, 1959).

Hoskins and Hansard (1964) estimated that the true availability of iron from a diet of maize, soybean meal, and cottonseed hulls was 0.29 for pregnant ewes. The diet provided 19 ppm iron.

Milk iron availability was found to be 26% for calves and 30% for piglets (ARC, 1972). A similar value should prevail for the pre-ruminant lamb. These values reflect the low iron content of milk and the large demand for iron.

IRON REQUIREMENTS

The iron requirements of sheep and cattle are not well defined. Few experiments comparing levels of iron have been conducted on a long-term basis; the requirement for maintenance and/or deposition of iron stores is generally ignored; and the source of iron is typically a soluble iron salt more available than feed iron.

Work with calves indicated that the average daily gain during growth, rather than body weight of the animal, is the major factor determining the amount of iron required. The requirement for maintenance is low compared to that for growth (Matrone et al., 1957). Mollerberg et al. (1975a) calculated that the iron requirement for 1 kg growth in calves was 40-45 mg. Assuming 25% maximum dietary iron retention, the actual requirement for 1 kg growth would be at least 160-180 mg iron (Mollerberg et al., 1975a).

Subsequently, Suttle (1979) found that the iron concentrations of lamb and calf carcasses are similar. Iron concentration ranged from 52.6 - 75.1 mg/kg fresh carcass weight for lambs weighing 18 - 69 kg, decreasing slightly with age at slaughter. The value of 55 mg iron/kg carcass gain was taken to represent the approximate net growth requirement, exclusive of iron storage. The value for calves was similar. Suttle (1979) concluded that the total net requirement of ruminants for iron should be defined in terms of dietary intake rather than concentration because of the relatively large and constant contribution of the growth component.

Most studies set the iron requirement as the minimal level for hemoglobin maintenance, and thus may underestimate the total iron requirement. Demands on dietary iron for hemoglobin synthesis supersede demands for myoglobin maintenance in the calf. For example, significant increases in myoglobin occurred when dietary iron fed to dairy calves was increased from 24 mg/kg to 44 and 104 mg/kg diet (Bremner et al., 1976). Other studies with dairy calves indicate a minimum iron requirement as high as 100 mg/kg dry matter (ARC, 1980, p. 236). This figure is in line with a possible requirement of 100-125 ppm iron for milk-fed baby pigs (Hitchcock et al., 1974; Ullrey et al., 1960), but much higher than the generally accepted requirement of 30-60 ppm based on earlier work (Matrone et al., 1957).

The iron requirements of the milk-fed lamb have not been studied experimentally. In view of the work with cattle (Matrone et al., 1957; Mollerberg et al., 1975) and sheep and cattle (Suttle, 1979), it is likely that the iron requirement of the suckling lamb is higher on a dry matter basis than that of the older, weaned lamb.

Two experiments on the iron requirement of weaned lambs were carried out by Lawlor et al. (1965). A dietary level of 70 ppm iron was adequate for growing-finishing lambs fed a semi-purified diet. In the second trial, 40 ppm iron met the requirements of weaned lambs, but feed conversion efficiency was poor compared to the diet with 70 ppm iron.

The iron requirement of adult sheep is stated to be 30-50 ppm, or mg/kg, of diet dry matter (NRC, 1975, p. 47). Iron deficiency is not believed to be a common problem of mature ruminants. However, the lower range of iron levels in feeds were marginal for two Canadian surveys (Peterson and Waldern, 1977; Cathcart et al., 1980). While iron deficiency is improbable, ruminant reproduction could be adversely affected by low iron availability in some roughages, as a high correlation is observed between iron in body fluids and fertility (Hidiroglou, 1979).

METHODS OF IRON SUPPLEMENTATION

Oral, parenteral and intravenous methods of iron therapy are used. Intravenous iron therapy has little advantage in rate and magnitude of response compared to parenteral injection, and cannot be justified even in acutely deficient human patients, especially as more risk is involved. This leaves the oral and parenteral routes of iron administration.

Orally, ferrous fumarate, sulphate, succinate and gluconate are among the iron salts used successfully to treat iron deficiency in man and animals. The tolerable dose is limited, as large amounts of iron salts cause diarrhea and damage the intestinal mucosa. The treatment must be repeated at short intervals. Iron supplementation of the feed of suckling animals is ineffective, due to low consumption of solid feed for the first weeks of life.

Parenteral iron administration has become the method of choice for both commercial and experimental iron supplementation of livestock. It is convenient and dependable. Piglets are routinely injected with iron dextran or iron dextrin within the first few days of life. The iron is used efficiently for Hb synthesis: about 94% of the dose is found in the red blood cells two weeks later (Thoren-Tolling, 175, p. 44). Due to the high molecular weight of iron dextran complexes circulating n the plasma, renal clearance is minimal.

Iron dextran, or Imferon, is the most widely used of the parenteral iron products. It has extensive use world-wide in human and animal iron therapy. Others are iron sorbitex and dextriferron (McCurdy, 1970).

The effects of iron dextran injection have been investigated (Kolb, 1963; Beresford et al., 1957; Martin et al., 1955). An acute inflammatory reaction develops at the injection site. Lymphatic absorption of iron is rapid, and the lymph nodes may act as temporary iron stores (Thoren-Tolling, 1975). Most iron is removed through the local inflammatory reaction; but iron which diffuses away from the site is taken up and retained by tissue macrophages.

Most of the iron is absorbed within three days, accompanied by a sharp rise in the plasma iron content. This greatly exceeds the iron-binding capacity, but has no toxic effects due to the high stability of the iron dextran complex. Iron dextran must be processed through the reticulo-endothelial system to split the iron from the dextran (Szilagyi and Erslev, 1970). As a result, iron dextran takes three days to measurably increase blood Hb, compared to less than a day for iron sorbitex (McCurdy, 1970).

Side effects of iron dextran injection are only well known in humans. Reactions such as fever, dealyed arthralgia, local discomfort, and skin staining bear little relationship to dosage. Rarely, both fatal and non-fatal anaphylactic reactions are caused by a repeated dose later than 5 days after the initial dose in humans (McCurdy, 1970) and in beef cattle (Perry et al., 1967).

STAGES OF IRON DEFICIENCY

Iron deficiency progresses through several stages between iron depletion and actual iron deficiency anemia. Initially, a negative iron balance is counteracted by several mechanisms. Iron is mobilized from the body stores of liver, muscle, spleen, and marrow in order to maintain Hb production, while iron absorption is increased. Normal serum iron, and serum iron saturation percentage, are maintained (Bothwell et al., 1979, pp. 44-45). Iron depletion is sometimes considered a pathological condition, as the tissue concentration of some iron-containing enzymes is diminished (Verloop et al., 1970). For example, liver enzyme activity in the pentose phosphate shunt is affected at an unexpectedly early stage in iron deficient rats (Jacobs, 1977a).

In latent iron deficiency, the increased iron absorption still helps maintain normal hemoglobin levels. The serum iron saturation percentage is decreased, as serum iron is reduced and/or transferrin production is increased. Iron stores are absent. At this stage, growth rate, general well-being or disease resistance may respond to iron supplementation.

Iron deficiency anemia, the final stage of deficency, results when the normal hemoglobin level cannot be maintained. An equilibrium can be

reached at any level of Hb below the norm, or Hb may continue to decline until death occurs. Serum iron may fall below 35 μg/dℓ in anemic humans, and values near zero are not uncommon. The erythrocyte protoporphyrin content is elevated, denoting defective heme synthesis (Harris and Kellermeyer, 1970, pp. 116-117).

Hematologically, a well-defined iron deficiency anemia is characterized by a large proportion of red blood cells which are small in size (microcytic) and poorly filled with hemoglobin (hypochromic). As new cell formation is restricted, the reticulocyte count (immature red blood cells) tends to be low. Initially, the level of blood hemoglobin may be depressed more than either the red blood cell count or hematocrit (Harris and Kellermeyer, 1970, p. 115). Bone marrow examination also demonstrates characteristic changes during iron deficiency, including normoblastic hyperplasia (Beutler and Fairbanks, 1980).

MEASUREMENTS OF IRON STATUS

Different techniques are used to assess iron status depending on whether or not anemia is present. When a microcytic, hypochromic anemia can be demonstrated, the few possible causes other than iron lack can be readily eliminated (ie. inflammation, infection, copper deficiency, lead poisoning) or are extremely rare. Thus, iron deficiency anemia can usually be easily identified by use of hemoglobin or hematocrit tests, though it is desirable to confirm the iron deficiency by other measurements. The ultimate proof is a specific, orderly response of blood hemoglobin to iron therapy (Harris and Kellermeyer, 1970, p. 120).

In iron deficiency anemia, the plasma iron concentration is low, total iron binding capacity (transferrin) is high, and percentage saturation of transferrin is very low. These measurements can be used to confirm the presence of iron-deficiency anemia.

The plasma iron concentration is affected by a wide range of conditions, and thus is not very specific for iron status. Plasma iron is increased in association with decreased erythropoiesis, increased hemolysis, or increased release of iron from body stores, while reduced levels of iron are consistently seen during acute and chronic infections, even if body stores are high. The decrease in plasma iron level is a late development in iron deficiency and may occur only after the mobile iron reserves are completely exhausted (Fielding, 1980).

The percentage iron saturation of transferrin best measures the supply of iron to the erythroid marrow. A saturation of less than 16% depresses basal erythropoiesis. Normally, transferrin is about one-third saturated. In conditions associated with impaired protein production, the transferrin level is decreased. Consequently, the quantity of Tr, also known as the total iron-binding capacity (TIBC) should be measured as well as the percentage saturation (Bothwell et al., 1979, pp. 50-56; Crosby, 1975; Finch, 1970).

Hemoglobin and hematocrit tests are the most rapid, accurate and convenient tests, but cannot assess the full range of iron status except for obvious anemias. The lower values of normal hemoglobin and hematocrit values also overlap the upper range of anemic values (Garby and Killander, 1968). Transferrin measurements may also be ineffective in diagnosing borderline cases of iron overload or deficiency (Beutler et al., 1954; Crosby, 1975).

In the pre-anemic stages of iron deficiency, the best tests for iron status assess the level of iron stores. Marrow aspiration or the level of absorption of a test dose of iron have been the best methods of evaluating iron depletion. Marrow aspiration is used to determine the presence of hemosiderin, a storage form of iron, in the reticuloendothelial cells (Beutler et al., 1954). This test is laborious and unsuited for screening purposes and nutritional trials. The iron absorption test is also not appropriate for experiments with growing animals, especially as it is not valid in cases of increased erythropoiesis. The level of urinary iron excretion after a test dose of desferrioxamine is supposed to be correlated with the level of body stores, but is also inappropriate for most animal research purposes. Consequently, the degree of iron depletion is difficult to assess (Bothwell et al., 1979, pp. 88-104; Harris and Kellermeyer, 1970, p. 118).

A new technique that will receive widespread clinical application is a rapid, 2-site radioimmune assay for serum ferritin. Serum ferritin is highly correlated with body iron stores in all states from iron deficiency to iron overload (Jacobs, 1977c; Powell et al., 1975). The role of serum ferritin in iron transport and metabolism is uncertain (Worwood et al., 1975; Worwood, 1980). Should the serum ferritin test prove feasible for nutritional studies in animals, the measurement of both serum ferritin and either hemoglobin or hematocrit would provide the optimum picture of iron status.

EFFECTS OF IRON DEFICIENCY

There is increasing evidence that symptoms of ill-health, reduced growth rate, and possibly decreased disease resistance can occur even in

mild cases of iron deficiency. The irreversible or long-term effects of iron deficiency on iron enzymes and cell structure and function may be more important than the readily reversible anemia.

The susceptibility of cells to iron deficiency is determined by the rate of cell turnover, and the rate of turnover of the iron-containing compounds within the cell (Fielding, 1975). Rapidly proliferating tissues, such as the gastrointestinal mucosa, respond most rapidly to changes in available iron. On the other hand, the higher the rate of turnover of the iron-containing enzymes and proteins within the cell, the more reversible the deficiency condition. The cytochrome oxidase activity in the intestinal mucosa reaches normal 48 hours after iron treatment, whereas skeletal muscle cytochrome c activity recovers very slowly (Dallman, 1971).

Iron deficiency causes a variety of enzymatic and morphological defects in solid tissues. Many cytochromes and other enzymes are significantly decreased by iron deficiency. The enzymes are differentially affected within the cell and between tissues (Jacobs, 1975; Jacobs, 1977a; Buetler, 1963; Beutler and Fairbanks, 1980).

The role of iron in DNA synthesis may be responsible for the highly significant reduction of various non-iron enzymes during iron deficiency. For example, the pentose phosphate shunt enzymes (phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase) suffer a major reduction early in iron deficiency. The disaccharidase activity in the brush border of the intestinal mucosa is also affected (Jacobs, 1975), as is glutathione peroxidase activity in the erythrocyte (Macdougall, 1972).

Defects are often observed in iron-deficient mitochondria. Many workers have found evidence of increased mitochondrial fragility,

swelling, vacuolation, and membrane breakdown in lymphocytes, intestine and marrow cells. Abnormal mitochondrial morphology in iron-deficient rats was reversible within 5 days of iron treatment, much more rapidly than the mitochondrial cytochrome deficiency (Dallman, 1971; Jacobs, 1975; Jacobs, 1977a).

Epithelial lesions are widespread in iron deficiency. All proliferating tissues are affected, especially the mucous membranes. Enzyme defects have been found in the buccal mucosa, stomach and small intestine but as yet a cause and effect relationship between the decreased iron enzymes and the epithelial lesions cannot be inferred (Verloop et al., 1970).

Specific lesions of iron deficiency have been well documented in humans. Most studies have revealed a high frequency of atrophic gastric mucosal changes, ie. 85% (Beutler and Fairbanks, 1980). Gastric atrophy is of major interest, as it leads to hypochlorhydria which further depresses iron absorption, especially in the young (Witts, 1966; Witts, 1969, pp. 39-44). Sores at the corners of the mouth, atrophy of the papillae of the tongue, esophageal ulceration, poor hair growth, and dry, fissured skin are other typical lesions (Heilmeyer and Harwerth, 1970; Harris and Kellermeyer, 1970, p. 114). Similar lesions, especially of the gastrointestinal mucosa, have been observed in swine and other monogastrics but have not been studied in ruminants.

In general, iron deficiency causes widespread metabolic changes, as might be expected through the many functions of iron enzymes. Serum triglyceride levels are elevated, folic acid levels depressed, and basal metabolic rate is depressed (Bothwell et al., 1979, p. 31). A reduction in the mitochondrial enzyme α-glycerophosphate dehydrogenase in muscle

tissue leads to excessive production of lactic acid, impairing work capacity (Saltman et al., 1982). This can be demonstrated in iron deficient animals even in the absence of anemia (Bothwell et al., 1979, p. 31). Iron deficient animals voluntarily restrict physical activity, and appetite is reduced in the early stages of anemia, at least in calves (Bremner et al., 1976). Consequently, it is difficult to directly compare the efficiency of feed utilization in normal and iron-deficient animals.

IRON AND DISEASE RESISTANCE

Disease resistance is depressed during iron deficiency in all species studied. Enzymatic and metabolic defects are found in many components of the immune system (Pearson and Robinson, 1976). The number of leukocytes and lymphocytes are often significantly reduced by iron deficiency, resulting in the impairment of both phagocytic function and cell-mediated immunity, respectively. The effect of iron status on tissue morphology, resident bacterial populations (Fletcher et al., 1975) and inactivation of bacterial exotoxins and endotoxins (Weinberg, 1971; Janoff and Zweifach, 1960) may also be important.

Mortality in farm livestock is increased by iron deficiency. In clinically normal but anemic dairy calves, mortality was 22%, mainly from septicemic and enteric infections (Tennant et al., 1975a). The incidence of enteritis was significantly less ($P<0.001$) in iron-injected calves than in anemic calves fed a commercial veal calf milk replacer containing 19 mg Fe/kg diet (Mollerberg et al., 1975a). Comparable data is not available for lambs, but Holz et al. (1961) noted that mortality was 30.5%, 38.6%, and 13.5% for 72 lambs injected with 0, 150 and 300 mg iron at

birth. Iron treatment of piglets resulted in a significant reduction of infectious diseases, particularly diseases associated with E. coli organisms, such as scours. Mortality was 13% in the iron-treated piglets compared to 17% in the controls, out of a total of 494 piglets (Hopson and Ashmead, 1976).

The susceptibility of iron-deficient animals to gastrointestinal infections may involve a failure to produce adequate numbers of myeloperoxidase-containing cells. Neutrophils, the most important phagocytic cells, contain myeloperoxidase, an iron-containing enzyme which affects bactericidal capacity. Iron deficient rats had fewer MPO-cells in the lamina propria and submucosa, and were much more susceptible to challenge with Salmonella typhimurium (Baggs and Miller, 1973). In another study, the iron deficient rats responded very slowly compared to controls in production of intestinal MPO, which was correlated with survival and retention of S. typhimurium in the gut (Baggs and Miller, 1974). Studies with humans indicated that phagocytosis by neutrophils was unaffected in iron deficiency, but intracellular bacterial killing was significantly ($P<0.001$) less in iron deficient patients (Higashi et al., 1967; Chandra, 1973).

Iron deficiency significantly affects the size, structure and function of lymphoid tissues. Various studies document effects such as reduction of antibody-forming spleen cells; reduction in thymus weight and in thymus mononuclear cells (Chandra et al., 1977). Iron deficiency may be most important during development of these tissues. Studies with rats indicated that iron deprivation during gestation and lactation was more serious than after weaning, as the subsequent disease resistance was

reduced, even after a period of nutritional rehabilitation (Baggs and Miller, 1973).

While iron supplementation of young animals would appear to be beneficial in improving disease resistance, Knight et al. (1983) have recently cautioned against oversupplementation, stating that:

> "Although confinement-reared pigs required Fe supplementation to prevent anemia, the data presented here and the large amount of previously reported evidence from other species indicate potential detrimental effects from over- as well as undersupplementation. It appears prudent that the effects of susceptibility to infection be included in determining the optimum Fe supplementation levels."

These concerns were based partly on in vitro studies on the growth of two E. coli strains, in serum taken from iron dextran injected piglets at various intervals after injection. The iron treatment significantly enhanced bacterial growth in vitro in some cases, but not consistently, and not later than day 3 post-injection (Knight et al., 1983).

As iron is a crucial trace mineral for microbial growth, an increase in plasma iron (hyperferremia) in humans may be associated with susceptibility to bacterial and fungal pathogens (Weinberg, 1974). It should be recognized, though, that the hyperferremia is extreme and/or long-term, and is typically caused by disease conditions such as viral hepatitis, sickle cell anemia, malaria and others which could tax the immune system regardless of iron level. Thus, a comparison between hyperferremia-associated diseases in humans and the risks of therapeutic iron supplementation in livestock is not completely valid.

Whether or not iron supplementation increases disease susceptibility depends on several factors. In order to neutralize the microbiostatic action of serum, enough iron must be provided to saturate at least 60-80%

of the serum transferrin (Weinberg, 1974). Iron dextran is a particularly safe iron supplement as no more than 1-3% of the iron can be directly transferred to transferrin (Szilagyi and Erslev, 1970). Iron administration during experimental infections of rats has been shown to dramatically lower the LD_{50} dose for a variety of bacteria, provided the iron is in a form able to diffuse to the site of bacterial replication, and is administered by iv, ip or im routes. Consequently, iron dextran and iron dextrin are inactive in enhancing microbial growth, whereas ferric ammonium citrate, iron sorbital citrate and hemoglobin are highly active (Weinberg, 1971).

Accordingly, iron supplementation of iron-deficient animals appears to confer little risk, especially when iron is provided as iron dextran, while the improvement in iron status allows optimal functioning of many aspects of the immune system.

SELENIUM

FUNCTIONS OF SELENIUM

Biological functions of selenium include the maintenance of muscle and membrane (erythrocyte, vascular endothelium, and cell organelle) integrity; stimulation of antibody and ubiquinone synthesis; maintenance of essential enzyme systems, pancreatic function, and vigor and mobility of sperm (Calvin et al., 1981; Combs and Bunk, 1981; Ganther et al., 1976; Hidiroglou et al., 1968; Spallholz et al., 1975). Many of these functions involve glutathione peroxidase.

The selenoenzyme, glutathione peroxidase (GSH-Px, EC 1.11.1.9) is part of a complex mechanism, including the superoxide dismutases, catalase, and vitamin E, which defends the cell against cytotoxic oxygen derivatives. Due to the impossibility of isolating these compounds in vivo, the models proposed for the action of GSH-Px are still tentative (Flohe et al., 1979).

GSH-Px protects the cell from damage caused by peroxides and free radicals. GSH-Px probably catalyzes the reduction of many hydroperoxides to their corresponding alcohols, or H_2O, in the case of H_2O_2. H_2O_2 is a natural by-product of many enzyme reactions, including those of xanthine oxidase and d-amino acid oxidase (Rotruck, 1981). Free radicals may be produced by mitochondrial respiration, autooxidation, irradiation damage or environmental pollutants (Csallany et al., 1981) and by interaction between H_2O_2 and metal ions or O_2^- (Rotruck, 1981). O_2^- is generated in large amounts by many electron transport steps in mitochondrial enzyme systems, and catalyzes the peroxidation of membrane polyunsaturated fatty acids (Diplock, 1981).

The intimate relationship between vitamin E and selenium can partly be explained by their related functions. GSH-Px, located both in the cytosol and mitochondria in a 70:30 ratio, reduces lipid peroxides to nontoxic hydroxy fatty acids. Vitamin E, located within membranes, prevents or decreases the formation of lipid peroxides. Hoekstra (1974) has discussed the nutritional implications of this scheme, but emphasized that GSH-Px should not be considered the only biochemical function of Se.

While more than 9 different selenium-containing proteins have been found in lamb tissues one of these was present only in heart and muscle of normal lambs and not in lambs affected with White Muscle Disease. Significantly, heart and muscle tissue are the target sites during Se/vitamin E deficiency in sheep. Furthermore, a link between oxygen generation or energy utilization and WMD is indicated by the nature of this protein. It is a cytochrome containing a heme group identical to cytochrome c, but has an amino acid composition and weight similar to cytochrome B_5 (Whanger et al., 1974).

It has been hypothesized that a primary role of selenium is as an oxidant-labile selenide in a class of non-heme iron proteins present in the mitochondria and smooth endoplasmic reticulum, and protected from oxidation by vitamin E (Diplock and Lucy, 1973; Diplock, 1974; Caygill and Diplock, 1973; Giasuddin et al., 1975). Deficiency of vitamin E would lead to a replacement of the selenide with a more stable, but less catalytically active, sulphur group, as the selenide in modified non-heme Fe proteins is much more vulnerable to oxidation than the sulphur group (Diplock, 1970).

Increasing evidence suggests a major function of selenium may be in electron transport, possibly involving the same protein investigated by

Diplock (1970) and Whanger et al. (1974). Levander et al. (1974) were the first to present evidence of a role for Se in catalyzing electron transfer. They point out that with adequate dietary Se, the Se could "short-circuit" the respiratory chain, avoiding the H_2O_2 generating step, by directly transferring electrons from GSH to cytochrome c.

SELENIUM METABOLISM

The dietary selenium sources of ruminants are mainly organic compounds of Se in feedstuffs and selenite or selenate salt supplements. Ruminants absorb significantly less of both inorganic and organic sources of selenium than do monogastrics. Based on the insoluble nature of fecal Se and the known ability of the anaerobic, highly reducing rumen environment to reduce less susceptible sulphur compounds, it is likely that rumen microbes reduce Se compounds to unavailable forms such as selenide or elemental selenium. At least 50% of fecal selenium is in such insoluble forms (Cousins and Cairney, 1961). Conversely, rumen microbes can increase the availability of dietary Se. They concentrate Se at 2 to 78 times the dietary concentration. These organic forms, including selenoamino acids incorporated into microbial protein, are more easily absorbed (Whanger et al., 1978a). The spontaneous recovery of WMD-affected lambs which survive to 6 weeks of age may be explained by the increased availability of Se incorporated into rumen microorganisms (Whanger et al., 1970).

The proportion of selenium absorbed increases with decreased dietary selenium and/or deficient Se status, and is not affected by vitamin E level. Similarly, Se retention of injected Se is also inversely proportional to dietary selenium (Ku et al., 1972; Kincaid et al., 1977; Van

Fleet, 1975). Sodium selenite is more effective than selenomethionine in increasing tissue and blood levels of Se and GSH-Px, when supplemented at 0.1 ppm Se. However, at a dietary level of 1.0 ppm, selenomethionine is more effective than sodium selenite (Moknes and Norheim, 1983).

The main site of Se absorption is the duodenum. Selenium is not absorbed from the rumen or abomasum in sheep. Selenite and selenocystine are absorbed by passive transport, while selenomethionine is transported against a concentration gradient. As methionine and selenomethionine share the same transport system, methionine can inhibit selenomethionine uptake. This may partly explain the protective effect of high protein diets against selenosis. Methionine may be effective only in combination with adequate levels of vitamin E (Levander, 1976; White and Somers, 1977).

Selenium is transported by plasma proteins (Porter et al., 1979). It is gradually taken up from the blood by the liver and kidney cortex, and less readily by the spleen, muscle, heart and lungs. Organic forms of selenium tend to be retained more tenaciously by tissues compared to inorganic forms (Martin and Gerlach, 1972).

Wright and Bell (1964) suggest that the tissues with highest Se content after dosing have the greatest ability to synthesize organoselenium compounds from inorganic selenium. Subsequently, these compounds are redistributed, especially to the muscle which is vulnerable to Se deficiency in sheep. Surprisingly, Se^{75} studies by Wright and Bell (1964) and others, indicate that 48 hours after dosing, Se^{75} uptake in muscle from Se-deficient sheep is lower than in muscle from Se-supplemented sheep.

Selenium is excreted by fecal, urinary and respiratory routes. Fecal excretion is more important to ruminants than monogastrics, due to lower rates of absorption. At high levels of dietary Se, selenium detoxification by GSH-dependent methylation becomes important. Dimethyl selenide and trimethylselenonium ion are the major pulmonary and urinary Se metabolites, respectively (Levander, 1976).

Selenium is found in many proteins, including heme proteins (hemoglobin, cytochrome c, myoglobin), enzymes (myosin, aldolase, urokinase, fibrinase) and nucleoproteins. Previously, Se was thought to be a contaminant mistaken for S due to its close chemical similarity. While selenomethionine is "accidentally" incorporated into proteins in place of methionine (McConnell et al., 1970), selenocysteine is now known to form the active center of various selenoproteins which have been investigated (Wilhelmsen et al., 1981).

Selenium metabolism, while sharing some pathways with sulphur, is othewise unique (Levander, 1976). Animals readily reduce such forms of inorganic Se as selenate or selenite, but cannot reduce even sulfate or sulfite within body cells. Reduction is crucial to selenite metabolism, as selenium must be reduced from the +4 oxidation state to the -2 oxidation state. Ganther and Hsieh (1974) have described a probable biochemical mechanism.

SELENIUM INTERACTIONS

Selenium interactions with a variety of nutrients have been documented, most commonly with arsenic, cadmium, coper, lead, mercury, silver, tellurium, zinc and sulphate. Selenium metabolism is influenced by

selenium's tendency to complex with heavy metals. These minerals reduce the toxicity of high levels of dietary selenium, and also high levels can induce a deficiency when dietary selenium is marginal (Lee and Jones, 1976). Selenium also interacts, directly and indirectly, with vitamin E, protein, certain carbohydrate fractions, and other factors which remain to be identified.

Dietary sulphur at levels found naturally in feeds affects selenium absorption and retention significantly. High levels of sulphate increase the Se requirement in sheep (Ekermans and Schneider, 1982). Major changes in blood Se occur between 0.05 and 0.10% dietary S, but little change occurs at higher levels. Increasing dietary sulphur increases urinary Se secretion. On a high sulphur diet, the increased population of Desulphovibrio bacteria in the rumen may reduce more selenium to H_2Se through sulphate reduction pathways. H_2Se is thought to dissociate to more stable but less soluble forms, either elemental selenium or highly insoluble metal selenides (Pope et al., 1979).

The selenium content of rumen microorganisms, and potentially the availability of Se, is reduced by sulphur deficiency (Whanger et al., 1978a). Sulphur deficiency also increases selenosis at high Se levels, as sulphur in glutathione and glutathione reductase is needed for the formation of excretory products such as dimethyl selenide and trimethyl selenonium ion (Pope et al., 1979).

Conflicting evidence of the effect of sulphur on increasing the incidence of white muscle disease may be resolved by the observation that sulphur competes with selenium only when present as the selenium analogue. Thus, "inorganic sulphur may alter the metabolism of inorganic selenium more than organic selenium" (Whanger et al., 1969a).

Protein source may be a major influence on the absorption of selenium. Studies with beef cattle indicated that dietary selenium levels may be more critical on diets marginal or deficient in protein. However, a response in weight gain to additional inorganic selenium was apparent in growing, but not in finishing, cattle. The degree of protein deposition may be a factor (Trevis, 1979). Feeding a high protein diet, on the other hand, has been recommended in cases of Se toxicity (Ekermans and Schneider, 1982).

Linseed oil meal has long been known to have a protective effect against chronic selenosis. Two cyanogenic glycosides in linseed oil meal release CN- which interacts with metabolic selenium to form SeCN-. This interaction could be detrimental in animals marginally deficient in selenium (Palmer, 1981).

Dystrophogenic factors in feeds may reduce the availability of selenium or vitamin E. The vitamin E in alfalfa may not be completely available for calves or chicks. A compound extracted in ethanol from alfalfa increased excretion of α-tocopherol from both alfalfa and added sources (Pudelkiewicz and Matterson, 1960). A succinoxidase inhibitor antagonized by α-tocopherol may occur in high levels in dystrophogenic feeds (Diplock, 1970).

DIETARY SELENIUM AND TOCOPHEROL

Selenium deficiency occurs naturally in large parts of the world, including the Pacific Northwest; Midwest, Southeast and Northeast North America. In Canada, low selenium soils are prevalent in central B.C., west-central Alberta, Northern Ontario, the Atlantic provinces and parts of

Quebec (Ullrey, 1981). Forages sampled in the Fraser Valley of B.C. were also consistently inadequate in selenium (Cathcart et al., 1980). The availability of Se to plants is increased in well-aerated, alkaline soils and decreased in acid, water-logged soils. Studies in the Kootenays in B.C. demonstrated striking differences between Se availability in adjacent soil types, which were more highly correlated with soil pH and moisture regime than soil Se (Van Ryswyk et al., 1976).

Selenium concentration in forage can be markedly and suddenly decreased by changing cultural practises, as shown by a dramatic increase in WMD in New Zealand after 1957. Increasing yields by fertilization, seeding of more productive species, irrigation (and associated leaching) seems to dilute the amount of selenium in the feed, and heavy cropping may remove more selenium than is recycled. Intensive stocking may also decrease the availability of Se to plants, as much fecal selenium is tied up in elemental and other insoluble forms of selenium (Counsins and Cairney, 1961).

Tocopherol content of forages does not seem to be affected by selenium level. However, it is sensitive to stage of maturity, drying losses in hay, rain, processing and storage losses (Kivimae and Carpena, 1973). Tocopherol in concentrates is destroyed by grinding, mixing with minerals or fat, and pelleting (MacDonald et al., 1976).

SELENIUM REQUIREMENT

The minimum selenium requirement of sheep is approximately 0.1 ppm (NRC, 1975, p. 47). The form of dietary selenium, the criteria used to assess adequacy, and the diet composition including vitamin E, will

influence the requirement. Levels of 0.11 to 0.12 ppm Se were required in order to maintain tissue PSH-Px levels in sheep fed a practical-type ration (Oh et al., 1974). Selenium-responsive disorders in ruminants become increasingly prevalent as dietary Se falls below 0.08 mg /kg D.M. Dietary selenium levels between 0.03 and 0.05 ppm are marginal (ARC, 1980, p. 243). Under B.C. conditions, 0.2 ppm is considered adequate, and <0.2 ppm may be marginal (Puls, 1981, pp. 76-87).

The dietary vitamin E may be important only at "marginal" intakes of Se (ARC, 1980, p. 244-251). It was suggested that the dietary Se requirement for all species is 0.1 - 0.15 ppm when vitamin E is sufficient, but may be as high as 0.5 - 1.0 ppm when vitamin E is low (Hoffman and La Roche, 1971).

The selenium requirement may be markedly increased when dystrophogenic feeds such as cull kidney beans are included in the ration. The addition of 0.17 ppm Se to a kidney bean and hay ration during lactation did not significantly reduce clinical NMD in lambs; the authors suggested that a level of 1.0 ppm added Se may not be completely effective under these circumstances (Hintz and Hogue, 1964).

SELENIUM DEFICIENCY AND WHITE MUSCLE DISEASE

Numerous diseases have been identified as responsive to selenium and vitamin E. Some respond to only vitamin E or only selenium, while others such as white muscle disease (WMD) may respond to either depending on the deficient nutrient and other nutritional stresses. By far the most common worldwide Se/Vitamin E deficiency in ruminants is white muscle disease, also known as nutritional muscular dystrophy or stiff lamb disease.

Recently, retained placenta in dairy cows, lameness in breeding stock, "sawdust liver" in feedlot steers, and illthrift, peridontal disease, reduced fertility and poor wool production in sheep have also been associated with selenium deficiency (Julien et al., 1976; MacDonald et al., 1976; Scales, 1976; Ullrey et al., 1977; Ammerman and Miller, 1975).

WMD most commonly affects young calves and lambs between birth and weaning, or sometimes just after weaning or other stresses. Morbidity may be 65% or greater, although immediate Se/Vitamin E treatment usually helps prevent severe losses. Seasonally, WMD is most common in spring, and frequently, lambs and ewes are on lush pasture, presumably rich in α-tocopherol.

Symptoms of WMD are similar for lambs and calves. Mortality from uncomplicated WMD invariably results from cardiac failure, and may not be properly diagnosed when clinical signs do not precede death. In pigs the disease is called mulberry heart disease because extensive cardiac hemorrhage results in a reddish-purple appearance. WMD affects heart function even before histopathological lesions develop. Deficient calves display a marked decrease in heart rate at the same time as initial signs of muscular dystrophy. Differences in ECG tracings were observed between calves on normal and dystrophogenic diets (Safford et al., 1954). Similar abnormalities have been seen in vitamin E deficient lambs as well (Bacigalupo et al., 1953). More information on the role of Se in heart function may come from current studies on Keshan disease, a cardiomyopathy affecting thousands of people in China but now controlled by large scale Se supplementation (Chen et al., 1981; Shamberger, 1981).

The first sign of stiff lamb disease is reluctance to walk, and then a definite stiffness, especially of the back legs, and the characteristic arched back stance. The muscles of the front and hind legs are affected first and most severely, then those of the shoulder, rump, loin and neck may be involved. In extreme cases lesions may affect the diaphragm, intercostal muscles and tongue (Culik et al., 1951). However, loss of appetite is rare.

Pathologically, WMD is characterized by degeneration of striated muscle, either skeletal or cardiac muscle, or both. Degeneration seems closely related to the degree of muscle stress (Young and Keeler, 1962). White or grey spots and streaks in muscle tissue indicate localized damage and calcium precipitation.

Two types of lesions - fiber and vascular - are found in WMD, and one or both may occur in other Se/vitamin E responsive diseases, such as exudative diathesis in chicks.

Fiber lesions are very similar in swine and in ruminants. Van Fleet et al. (1977a) studied ultrastructural changes in fibers of Se-vitamin E deficient swine, and found evidence of concurrent myofibrillar and mitochondrial changes, both probably initiated by cellular peroxidation. Similarly, Godwin et al. (1974) observed an increase in membrane lability in organelles in Se-vitamin E deficient tissues. The membrane damage appears to affect intracellular fluid balance and energy production. Damaged fibers eventually become mineralized, and the healed lesions persist as patches of stromal condensation and fibrosis. Regeneration may occur in sheep skeletal muscle, but not in cardiac muscle, at least in swine (Van Fleet et al., 1977a).

Vascular damage is frequently found in the hearts and other tissues of selenium or vitamin E deficient animals. Microvascular lesions and hemorrhages occur in the kidney, intestine, liver, skeletal muscle, stomach and skin as well as the heart in swine (Van Fleet et al., 1977b), developing independently of fiber lesions. The heart of lambs and calves with WMD is typically edematous.

The vascular lesions apparently arise from lipoperoxidation damage to the endothelial cells lining arterioles and capillaries. The thin, tightly joined endothelial cells lining the lumen of normal vessels give way to loosely attached, thickened but intact endothelium. The increased permeability allows leakage of blood proteins into the vessel wall, causing accumulation of fibrinoid, and perivascular edema. Sudden massive hemorrhaging may develop in cases of spontaneous WMD.

SELENIUM AND DISEASE RESISTANCE

Since 1972 numerous reports have been published on the effects of selenium and vitamin E on both humoral and cellular immunity. Most studies have been conducted using sodium selenite, however, organic Se compounds appear less effective than equivalent amounts of Se as selenite or selenide (Spallholz, 1981). Deficiencies of selenium or vitamin E are associated with impaired immunity and increased susceptibility to experimental bacterial and fungal infections, but supplementation of "normal diets" leads to a further increase in antibody production.

Antibody production in response to various vaccines or SRBC-antigen is enhanced in cattle, dogs, mice, rabbits, and chicks simultaneously injected with Se (Spallholz, 1981). Generally, dietary Se levels above 0.1

ppm are also effective. The number of plaque-forming cells in spleen of mice were increased proportionately as dietary selenite was increased from 0 to 1.25 ppm (Spallholz et al., 1973). (Plaque-forming cells produce antibody.) Diets containing 1 to 3 ppm selenite, levels greatly in excess of the normal requirement, potentiated the synthesis of IgM and IgG immunoglobulins in mice (Spallholz et al., 1974). However, when given intraperitoneally, the amount of Se required to enhance the primary immune response in mice was not much greater than the estimated daily Se requirement (Spallholz et al., 1975).

Some effects of selenium and vitamin E on the immune system may be interchangeable. The 2-week-old chick requires both Se and vitamin E for optimal imune function, but by 3 weeks, Se alone can facilitate optimal immune function (Baumgartner, 1979). Selenium apparently can duplicate some of the effects of vitamin E on the immune system, which may involve a role in ubiquinone and prostaglandin synthesis (Heinzerling et al., 1974; Tengerdy and Nockels, 1975; Tengerdy et al., 1978). Alternatively, some of the immunostimulatory effects of Se and vitamin E may involve provision of the proper biochemical environment for cellular interactions, and may be replaceable by synthetic antioxidants. Some of the nonspecific mitogenic factors of macrophages are antioxidants, and it is suggested that antioxidants such as Se and vitamin E have a similar adjuvant effect, ie. they attract macrophages (Baumgartner, 1979).

Defective microbicidal activity is typically observed in neutrophils of selenium deficient animals. The viability of neutrophils and ingestion of bacterial or fungal cells is not impaired, but the ability to kill these cells is greatly impaired for both rats and cattle, among others (Serfass

and Ganther, 1975; Boyne and Arthur, 1978). The selenium deficiency reduces neutrophil GSH-Px, and consequently the ability to metabolize H_2O_2. The H_2O_2 accumulation results in destruction of the O_2^--generating system, which is not altered by vitamin E status, at least in rats (Baker and Cohen, 1983). In contrast, Bass et al. (1977) compared bactericidal activity of neutrophils from species varying greatly in normal GSH-Px content, and concluded that post-phagocytic oxidative responces and bacterial killing "were not compromised by complete absence of GSH-Px, even in species with the highest natural levels of this enzyme".

Sex differences in antibody response to Se dosage have been observed by several groups working with chicks, but have not been investigated in other species. At dietary levels in slight excess of that required to prevent deficiency diseases, antibody titer is significantly depressed in male but not female chicks (Marsh et al., 1981; Baumgartner, 1979).

IRON AND SELENIUM INTERACTIONS

Three possible areas of metabolic interaction between selenium and iron have been documented. Selenium deficiency may reduce red cell life-span, indirectly increasing iron turnover, and in severe cases causing hemolytic anemia. Selenium deficiency may be directly involved in the utilization of iron for heme synthesis. Finally, iron status influences levels of the selenoenzyme, GSH-Px in red blood cells.

SELENIUM DEFICIENCY AND ANEMIA

Selenium and/or vitamin E deficiencies are associated with hemolytic anemia in swine, monkeys and other animals. Both vitamin E and selenium are important in maintaining RBC membrane integrity, but only selenium, as GSH-Px, is effective against Hb oxidation, due to the membrane localization of vitamin E (Hoekstra, 1974). Cells containing oxidized Hb, or Heinz bodies, break down prematurely. Similarly, an inherited deficiency of reduced glutathione (GSH), the substrate for GSH-Px, also reduces the potential lifespan of RBC's in sheep and in man (Tucker, 1974).

SELENIUM AND HEME SYNTHESIS

Tissue Enzymes

Recent research on Se in heme synthesis and catabolism suggests a role for selenium, distinct from GSH-Px, in the utilization of iron. A marked increase in both heme synthesis and catabolism was found in liver but not in spleen of Se-deficient rats (Correia and Burk, 1976). Further investigations revealed that selenium is essential for normal utilization of heme in rat liver, and selenium deficiency leads to "wasted" heme; that

the effect of selenium is not mediated by GSH-Px, nor is an antioxidant effect of the element involved; and the nutritional selenium requirement of the rat is lower for maintenance of hepatic heme utilization than for maintenance of hepatic GSH-Px levels (Burk and Correia, 1981).

Whanger et al. (1977) reported the first data on the effects of selenium on ovine hepatic microsomal heme proteins. Hepatic microsomal cytochrome P_{450} and total heme content were significantly lower in WMD lambs, but cytochrome b_5 content was not affected. Hepatic mitochondrial heme protein content and levels of cytochromes a, b, and c + c_1 did not differ between normal and WMD lambs. Thus microsomal but not mitochondrial heme proteins were affected in this case. Whanger et al. (1977) felt that a deficiency of both vitamin E and Se was necessary to alter microsomal heme compounds in the ovine liver.

The role of vitamin E in the regulation of heme synthesis has been investigated by Nair et al. (1972), while a detailed study on the effect of vitamin E deficiency on cellular membranes and membrane-bound enzymes has also been reported (Hulstaert et al., 1975). The relationship between selenium and vitamin E and heme metabolism is not yet fully understood.

SELENIUM AND HEME SYNTHESIS

Erythropoiesis

Selenium and/or vitamin E deficiency may also interfere with erythropoiesis. An indirect effect of selenium in stabilizing heme enzyme systems may occur, as was observed in liver (Whanger et al., 1977).

Bone marrow abnormalities - erythrocyte hyperplasia and multinucleated precursor cells - were found in Se-E deficient swine (Niyo et al.,

1980). Multinucleated erythrocyte precursor cells are associated with delayed erythrocyte maturation, which could eventually be manifested by low blood hemoglobin levels. Hemoglobin gradually decreased in vitamin E deficient lambs (Culik et al., 1951).

Baustad and Nafstad (1972) observed changes in swine hematology consistent with impairment of hematopoiesis during vitamin E deficiency. The reticulocyte count in vitamin E-treated piglets was significantly higher than in untreated littermates at 2 weeks of age (6.95 vs. 3.26% of erythrocytes). Total Hb, PCV, and RBC count were also higher in vitamin E-treated piglets. Additionally, bone marrow abnormalities typical of those described by Nafstad (1973) were found in vitamin E deficient piglets of any age from newborn to 5 weeks.

Fontaine et al. (1977a, 1977b) demonstrated that selenium, but not vitamin E, may have a specific role in erythropoiesis. Much work remains to be done in this area. So far, the limited information on Se and erythropoiesis in sheep is confusing, as two papers reported that selenium treatment depressed Hb levels.

Buchanan-Smith et al. (1969) observed a depression in PCV, and more slowly in Hb, in 4 month old lambs supplemented with selenium compared to selenium-deficient controls. Horton et al. (1978) compared four methods of Se/vitamin E supplementation, finding the greatest depression in red cell count and Hb with the most effective methods of Se supplementation.

IRON AND SELENIUM UTILIZATION

Iron status affects the concentration of GSH-Px in red blood cells, indicating an unanticipated role for iron in selenium metabolism. Studies

in humans demonstrate that red blood cell GSH-Px is significantly decreased in iron deficiency anemia (Cellerino et al., 1976; Macdougall, 1972).

The effect was not dependent on anemia per se, as GSH-Px was either unaffected or increased in other types of anemia (Cellerino et al., 1976). Furthermore, GSH-Px was significantly correlated with serum iron levels. Red cell GSH-Px was also reduced in iron deficient calves (Horber et al., 1980), and iron-deficient rabbits (Rodvien et al., 1974). The decrease in GSH-Px observed with iron deficiency may result from an inability to utilize dietary Se for GSH-Px synthesis (Ganther et al., 1976). However, this theory would not explain why GSH-Px activity in Se-deficient animals might be influenced by high levels of iron.

Red blood cell GSH-Px levels may be affected by iron when selenium is deficient. A single study was done using rats (Lee et al., 1981). High levels of dietary iron failed to influence RBC GSH-Px when Se was adequate. However, GSH-Px was higher in Se- and E-deficient rats fed 1255 ppm iron than those fed 305 ppm iron. Unfortunately, the effect of deficient levels of iron was not considered.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

In all trials, lambs were born over a 3-6 week lambing period, and randomly allocated at birth to one of the injection treatments. The number of treatments varied according to the trial.

Iron Supplementation (Trial 1)

Trial 1 investigated the effects of iron supplementation using 17 control and 18 iron treated lambs. The iron dosages were 0 and 500 mg of iron, administered within 3 days of birth. As breed (Finn or Dorset sired), sex and rearing (as single or twin), may markedly influence weight gains and possibly other parameters, these factors were included in the experimental model. Consequently, the trial was set up as a completely randomized 2X2X2X2 design.

Weight, hemoglobin and PCV were measured weekly from birth to 11 weeks of age. Plasma samples taken at 24-25 days of age were analyzed for a blood profile including calcium, inorganic phosphate, glucose, blood urea nitrogen, total protein, albumin, alkaline phosphatase, lactate dehydrogenase, aspartate transaminase and plasma iron. This profile was selected to assess the effect of iron treatment and/or anemia on other physiological parameters. For example, glucose and cholesterol are affected by iron deficiency anemia in humans.

The following least squares model was used to analyze all the Trial 1 data:

$$Y_{ijkl} = u + T_i + B_j + S_k + R_l + T_iB_j + T_iS_k + T_iR_l + B_jS_k + B_jR_l + S_kR_l + W_{ijkl} + E_{ijkl}.$$

where Y_{ijkl} = the dependent variable hemoglobin, PCV, etc.

u = the overall mean common to all samples

T_i = the effect of the i'th treatment

B_j = the effect of the j'th breed

S_k = the effect of the k'th sex

R_l = the effect of the l'th rearing

T_iB_j = the interaction of the i'th treatment with the j'th breed

T_iS_k = the interaction of the i'th treatment with the k'th sex

T_iR_l = the interaction of the i'th treatment with the l'th rearing

B_jS_k = the interaction of the j'th breed with the k'th sex

B_jR_l = the interaction of the j'th breed with the l'th rearing

S_kR_l = the interaction of the k'th sex with the l'th rearing

W_{ijkl} = the covariable birth weight

E_{ijkl} = the unexplained residual error associated with each sample

The above model was altered for analysis of the plasma profile data by the addition of a hemolysis covariable for Trials 1 and 2. Hemolysis was ranked on the scale of 0 (no hemolysis) to 4 (severe hemolysis) as some hemolysis was unavoidable.

Level of Iron Supplementation (Trial 3)

Trial 2 compared the effects of 3 levels of iron treatment: 0, 250 mg, and 500 mg iron. As before, breed, sex and rearing were other sources of variation, resulting in a 3X2X2X2 design. Sixty-six lambs were used. The same least squares model was used as in Trial 1.

Iron and/or Selenium Supplementation (Trial 3)

In Trial 3, iron and selenium treatments were combined into 4 treatment combinations. These were control, 1.5 mg Se only, 500 mg Fe only, and 1.5 mg Se + 500 mg Fe. The experiment was replicated 3 times, with a total of 121 lambs divided between the 3 lambing periods. Hb, PCV, weight and plasma profile data were collected as before. However, plasma selenium and plasma protein data were obtained from replicates 2 and 3 only, and hemagglutination and soremouth data from replicate 3 only.

The following linear model was used to measure the effects of replicate, treatment, breed, sex and rearing on Trial 3 Hb, PCV, weight and plasma profile data:

$$Y_{ijklm} = u + P_i + T_j + B_k + S_l + R_m + P_iT_j + P_iB_k + T_jB_k + T_jS_l + T_jR_m + B_kR_m + E_{ijklm}$$

where Y_{ijklm} = the dependent variable Hb, PCV, etc.

u = the overall mean common to all samples

P_i = the effect of the i'th replicate

T_j = the effect of the j'th treatment

B_k = the effect of the k'th breed

S_l = the effect o the l'th sex

R_m = the effect of the m'th rearing

P_iT_j = the interaction of the i'th replicate with the j'th treatment

P_iB_k = the interaction of the i'th replicate with the k'th breed

T_jB_k = the interaction of the j'th treatment with the k'th breed

T_jS_l = the interaction of the j'th treatment with the l'th sex

T_jR_m = the interaction of the j'th treatment with the m'th rearing

B_kR_m = the interaction of the j'th breed with the m'th rearing

W_{ijklm} = the birthweight covariable

E_{ijklm} = the unexplained residual error associated with each sample

Plasma proteins were analyzed using a simplified linear model, which included total protein as a covariable:

$$Y_{ijklm} = u + T_i + B_j + S_k + R_l + P_{ijkl} + E_{ijkl}$$

where Y_{ijkl} = the dependent variable albumin, beta-globulin, etc.

u = the overall mean common to all samples

T_i = the effect of the i'th treatment

B_j = the effect of the j'th breed

S_k = the effect of the k'th sex

R_l = the effect of the l'th rearing

P_{ijkl} = the total protein covariable

E_{ijkl} = the unexplained residual error associated with each sample

Plasma selenium data were analyzed using this model:

$$Y_{ijklm} = u + I_i + X_j + B_k + S_l + R_m + I_iX_j + X_jS_l + E_{ijklm}$$

where Y_{ijklm} = the dependent variable selenium

u = the overall mean common to all samples

I_i = the effect of the i'th iron treatment

X_j = the effect of the j'th selenium treatment

B_k = the effect of the k'th breed

S_l = the effect of the l'th sex

R_m = the effect of the m'th rearing

I_iX_j = the interaction of the i'th iron treatment with the j'th selenium treatment

X_jS_l = the interaction of the j'th selenium treatment with the l'th sex

E_{ijklm} = the unexplained residual error associated with each sample

The model for analysis of the hemagglutination data was the same, with the addition of the interactions of selenium with rearing, iron with sex, and iron with rearing.

STATISTICAL ANALYSIS

Analysis of variance was done using UBC BMD:10V (1975), a General Linear Hypothesis packaged program. A major advantage of this program was its ability to manipulate unbalanced cells and missing dtaa, although not missing cells. Analysis of covariance was used instead of ANOVA when a concomitant variable, which could be measured but not controlled, affected a dependent variable. For example, despite random allocation of lambs to treatments, mean birth weight tended to be higher for some treatments. As

birth weight is related to weight gain, it was very useful to be able to adjust means for the effect of birth weight on weight.

ANOVA with BMD:10V also enabled the testing of single degrees of freedom contrasts. A priori, orthogonal hypotheses were:

for all iron level data, Trial 2:

1. Control lambs do not differ from iron treated lambs.
2. High level of iron injection does not differ from low level.

similarly, for data in Trial 3:

1. Iron treated lambs do not differ from non-iron treated lambs.
2. Selenium treated lambs do not differ from non-selenium treated lambs.
3. Iron and selenium do not interact.

The BMD:10V program was run for all sets of data collected, changing the model as warranted. When insignificant interactions were obtained, the SS' and d.f.'s were added into the experimental error to increase the precision, then the F's were recalculated by the program. Consequently, three-way and four-way interactions were normally eliminated, and many biologically meaningless two-way interactions. The first model given was adjusted in this manner, while the remaining models represent the final versions of the complete initial models. This frequently resulted in highly significant main effects.

Correlations between Trial 2 plasma metabolites and plasma iron, Hb, PCV, weight gain, and birth weight were investigated using UBC TRP (1978), a triangular regression package. They were of interest as covariance analysis was not appropriate for looking at correlations, yet the existence

of certain correlations had a major impact on the data. TRP used a forward stepwise regression technique to derive regression equations.

UBC TRP (1978) was also used to do plots. Throughout the trials, it was tedious to measure both Hb and PCV, when they might be of equal value in assessing iron deficiency. Regression analysis derived predictive equations for Hb from PCV at different ages, and was used to assess the closeness of the relationship between the two variables. TRP also plotted scattergrams of Hb versus PCV, with and without a severe level of outlier rejection of 5% of the data. Simple regression equations were calculated for all plots.

All means are given with variation expressed as the standard error, and not as the standard deviation.

ANIMAL MANAGEMENT

Lambs were housed with their dams in sawdust-bedded group pens within an open-eaved unheated building on the University of British Columbia research farm. Dams were Dorset and FinnXDorset breeding. Dorset, Finn and Suffolk rams were used.

Lambs were docked at 3-7 days of age. Males were not castrated. Lambs were weaned at an average age of eight weeks.

Water and cobalt-iodized salt were available ad lib. Ewes were fed alfalfa cubes and a barley-based grain mixture twice daily. Starting at ten days of age, the lambs began eating small amounts of a creep-feed ration having a calculated iron content of 95 ppm (Appendix 1). By six weeks of age, lambs were consuming about 0.5 kg of creepfeed per head per day.

Health problems were never severe. Contagious pustular dermatitis (soremouth) was endemic, appearing in each lamb crop several weeks after the start of lambing. Isolated cases of both E. coli scours and Corynebacterium ovis joint abscesses occurred; the last rarely affected growth rate. White muscle disease affected some lambs at various ages between birth and 4 weeks of age in Trials 1 and 2 only. The affected animals usually responded to a combined selenium/vitamin E injection. Overall mortality was low and varied from 0 to 7%; main causes were premature/difficult births, trampling and pneumonia.

Lambs were treated with iron and/or selenium depending on the experiment. Both iron and selenium were administered by intramuscular injection between 0 and 3 days of age. The products used were Haemalift and Dystosel, both from the Rogar/STB division of BTI Products, Inc., London, Ontario. Haemalift provided 100 mg actual iron per ml as a ferric hydroxide complex with dextran. Dystosel contained 3 mg selenium per ml as sodium selenite, and 163 IU d-alpha tocopheryl acetate.

ANALYTICAL PROCEDURES

Weight

Lambs under 15 kg were weighed in a pail with a spring balance (accuracy ± 0.1 kg). When lambs reached 15 kg, a large beam balance with an accuracy of ± 0.5 kg was used.

Blood Samples

All samples were obtained by jugular venipuncture using 20 gauge needles. Five ml tubes containing a small amount of sodium heparin were

used to collect small (2 ml) initial samples for hemoglobin and packed cell volume determinations. Subsequently, 10 ml heparinized vacutainer tubes were used to collect blood for Hb, PCV, plasma profile, and mineral analyses. Vacutainer tubes containing potassium oxalate instead of heparin were used to collect samples for plasma protein electrophoresis. Plain or silicon-coated vacutainers were used when serum was required.

Hemoglobin

Blood hemoglobin levels were measured by the cyanmethemoglobin technique (Schoen and Solomon, 1962; Eilers, 1967). It is a colorimetric technique which measures all hemoglobin derivatives using cyanide reagents. Duplicate analyses were done for each sample within 24 hours of collection; however, hemoglobin is stable for over 7 days at 4°C, or a month and more at -20°C. The equipment involved consisted of Vanlab 20 mm^3 ($\pm$1%) disposable micropipettes, Hycel No. 117 Cyanmethemoglobin Certified Standard, and a Gilford Stasar II spectrophotometer.

Packed Cell Volume

Duplicate microhematocrits were done on each blood sample, generally on the day of collection. Dade and Canlab heparinized microhematocrit capillary tubes were two-thirds filled with blood, capped with Critoseal, and centrifuged for 15 minutes in a Canlab International Microcapillary Centrifuge with Reader (Models MB and CR).

Plasma Profile

Plasma was extracted from 10 ml whole blood samples, frozen and later analyzed by a commercial laboratory (B.C. Biomedical Laboratories Ltd.,

7845 Edmonds, Burnaby, B.C.). Eleven plasma constituents were measured - calcium (Ca), inorganic phosphate (P_i), glucose, blood urea nitrogen (BUN), cholesterol, total protein, albumin, alkaline phosphatase (AP), lactate dehydrogenase (LDH), aspartate transaminase (AT), and iron. References to analytical procedures for each metabolite are given in Appendix 2.

Plasma Protein Electrophoresis and Total Protein

Plasma preparation. Plasma was separated from whole blood collected in vacutainer tubes containing potassium oxalate. Heparin is a unsuitable anticoagulant as it may interfere with various protein bands in electrophoresis.

After thawing, samples were centrifuged and decanted as necessary to remove turbidity caused by lipids and denatured proteins. Hemolysis was evident in some samples but was not judged severe enough to necessitate analysis of Hb and use of a correction factor. (The cyanmethemoglobin technique is not appropriate for Hb concentrations less that 4 g/dℓ.) However, hemolysis could well be a source of error in both total protein (TP) and plasma protein electrophoresis.

Total protein. The biuret method (Gornall et al., 1949; Cannon et al., 1974) was chosen for total protein determination, as it produces a stable colour that obeys Beer's Law and is unaffected by the ratio of albumin to globulin.

Protein fractions. Plasma protein fractions were separated by electrophoresis on prepared agarose gel in the Corning Cassette Electorphoresis Cell System. The Corning procedure was followed ("Determination

of serum proteins (Amido Black 10B)", Corning Medical, Corning Glass Works, Medfield, Massachusetts). In order to maximize the resolution of the protein fractions, various buffers were tested at varying pH's and ionic strengths (Cannon et al., 1974). The optimum combination seemed to be sodium barbital buffer, ionic strength μ=0.05, pH=8.6, in preference to Corning Universal Barbital Buffer containing EDTA. The electrophoretograms were scanned in the Transidyne General 2980 Scanning Densitometer. Protein fractions were calculated as percentages of total protein by calculating the relative surface area under each peak. Actual values of the 5 protein fractions were then calculated from the percentages using total protein values obtained by the biuret technique.

Production of Anti-erythrocyte Serum

Aseptic collection of chicken blood. Laying hens from the University of British Columbia Department of Poultry Science supplied the erythrocytes for both antiserum production and testing. Using different birds each time, ten or more birds were bled three times weekly. Ten mls of blood were withdrawn from a medial vein on the underside of the wing (Garvey et al., 1977, p. 31). The equipment included ethanol, cotton gauze, 21 guage needles rinsed with concentrated sodium heparin solution, 12 ml syringes containing 2-3 mls of Alsever's solution plus sodium heparin, and collection flasks containing Alsever's solution. Heparin was necessary to prevent coagulation, especially in the needle and syringe. All equipment and solutions were sterile.

Standardization of chicken erythrocytes. Blood samples were combined in a large volume of Alsever's solution. Cells were washed by mixing with

sterile citrate/saline solution (Garvey et al., 1977, p. 524), centrifuged in sterile 40 ml tubes in a refrigerated centrifuge, then the supernatant was decanted. These steps were repeated 4-6 times to remove plasma proteins and lipids.

The erythrocyte concentration was standardized by adapting a method for the photometric standardization of sheep erythrocytes (Garvey et al., 1977, pp. 140-143). Two mls of a 2.5-3.0% cell suspension were added to 10 mls of distilled water. The lysate was read against a distilled water blank at a wavelength of 520 nm, and adjusted to give an optical density of 0.500. The original suspension was then adjusted accordingly by the addition of cells or buffer solution, resulting in a 2.5% cell suspension containing approximately 4 X 10^8 cells per ml.

Antiserum production. Sheep on all four iron and selenium treatments in Replicate 3 of Trial 3 were injected intraperitoneally with 1 ml of the freshly-prepared cell suspension at weekly intervals from 4 to 9 weeks of age. This was done three times weekly at the usual sampling times to minimize age variability within the sampling periods. Simultaneously, blood samples were taken for serum. As every effort was made to ensure aseptic conditions, no deleterious side effects resulted from the erythrocyte injections.

The sheep blood samples were allowed to clot at room temperature, and centrifuged. Serum was decanted and initially, refrigerated at 4°C for immediate antisera testng using a passive hemagglutination method. Because of time restraints, most samples were frozen and tested 4-5 months later. The initial serum samples were also frozen and retested.

Hemagglutination Test

The passive hemagglutination test was performed on the thawed, heat-treated antiserum samples following the method described by Garvey et al. (1977, pp. 356-360). As the technique specified tannic acid treated sheep rbc's coated with bovine serum albumin (BSA) as antigen, and anti-BSA from rabbits, some modifications were necessary: 1) the antigen was chicken rbc's, the antiserum was sheep anti-chicken-rbc; 2) the antiserum required at most 1:2 and 1:10 dilution, rather than 1:10,000 dilution; 3) erythrocytes rather than BSA were the antigen, therefore tannic acid coating onto rbc's was unnecessary; and 4) control erythrocytes could not be used for the same reason as 3). Titres were obtained as dilution units, and converted to natural log units for statistical analysis.

RESULTS AND DISCUSSION

TRIAL 1 IRON SUPPLEMENTATION

Effect of Iron on Hemoglobin, PCV, and Plasma Iron

Iron supplementation of lambs substantially affected the pattern of hemoglobin and hematocrit changes during the first 11 weeks of life. The rapid decline in hemoglobin and PCV between birth and three weeks of age in control lambs was prevented by iron dextran injection. The single injection of 500 mg iron at birth significantly increased Hb, PCV and plasma iron from 2 to 11 weeks of age ($P<0.05$). Although few control lambs became anemic, Hb, PCV, and plasma iron values indicated that erythropoiesis was restricted by iron deficiency in control lambs.

The initial blood samples were taken at various times between birth and 3 days of age. Mean Hb was 14.12±0.30 g/dℓ, which is similar to the mean birth hemoglobin value of 14.17 g/dℓ reported by Holz et al. (1961). Mean initial values of both Hb and PCV were slightly higher for the iron-treated group than the control group, with means of 14.5±0.4 vs. 13.7±0.6 g/dℓ Hb, and 40.2±1.2 vs. 37.8±1.5% PCV. This difference was not significant ($P<0.05$) due to the high variability of Hb and PCV at this age. Contributing factors include polycythemia in some lambs at birth, and a rapid drop in total red cells during the first 12 hours of life, followed by a slower decrease in Hb and PCV (Blunt, 1975). Consequently, Hb and PCV values change rapidly during the first three days of life.

The Hb levels of both control and iron-treated lambs continued to fall until 1 week of age (12.3±0.5 vs. 12.9±0.2 g/dℓ), but were not significantly different ($P>0.5$). However, at 2 weeks of age the Hb was further

depressed to 10.9±0.4 g/dℓ in the control group, but had increased to 13.3±0.2 g/dℓ in the iron treated group (P<0.001).

The Hb level in the control group fell to 10.7±0.4 g/dℓ at 3 weeks, then increased slowly to 12.0±0.2 g/dℓ at 7 weeks and stabilized at that level until the end of the trial at 11 weeks (Appendix 3). In comparison, the Hb level in the iron-treated group increased steadily from a low of 12.9±0.2 g/dℓ at 1 week to 13.7±0.3 g/dℓ at 4 weeks, and remained near that level until 7 weeks. As shown in Figure 1, the Hb level declined slightly from 8 to 11 weeks but remained higher than in the controls. The treatment difference was still significant (P<0.001) at 7 weeks, and remained significant at 11 weeks (P<0.05).

Changes in hematocrit values in the two treatment groups paralleled changes in hemoglobin almost exactly, as shown in Figure 2. Treatment effect was significant at 2 weeks (31.2±1.1% vs. 38.8±0.8% PCV, P<0.001). Subsequently, PCV declined in the control group to a low of 30.9±1.3% at 3 weeks of age, and increased steadily from 3 to 11 weeks, reaching a mean value of 36.0±0.7%. PCV levels in the iron-treated group increased from a low of 35.8±0.8% at 1 week to a high of 39.5±0.7% at 5 weeks. The treatment effect was significant (P<0.001) from 2 to 7 weeks of age, and remained significant (P<0.05) at 11 weeks. Data is given in Appendix 3.

While actual Hb and PCV values may vary from study to study, the same pattern of both minimum levels in controls and maximum response to iron at 3 weeks has been consistently observed (Holz et al., 1961; Ricketts et al., 1965; Ullrey et al., 1965; Tait and Dubeski, 1979). Ullrey et al. (1965) measured average Hb and PCV values of 6.2 g/dℓ Hb and 20.5% PCV in 3 week old control lambs, compared to an iron-treated group with 10.8 g/dℓ Hb and

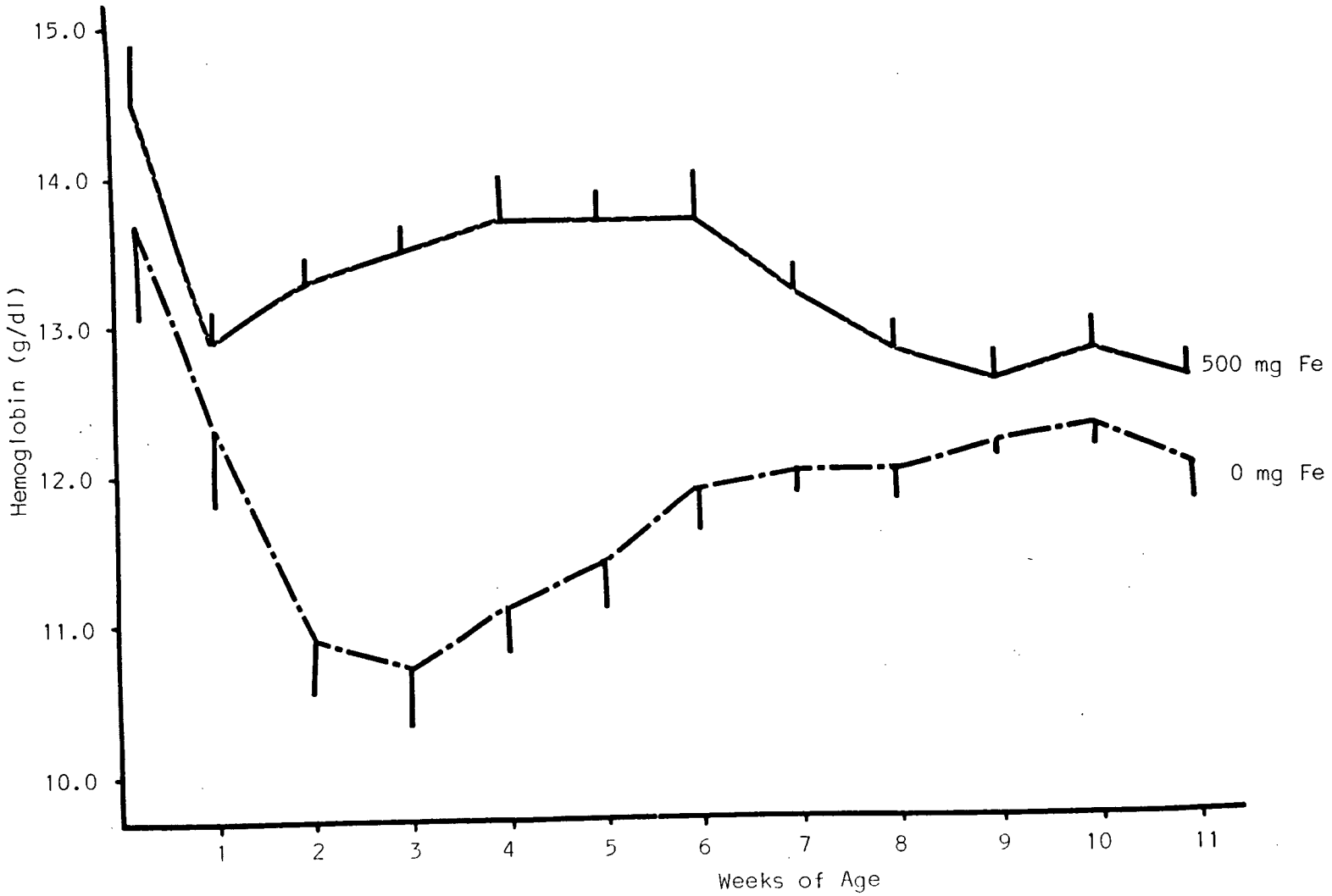

Figure 1. Effect of iron supplementation on hemoglobin (Trial 1).

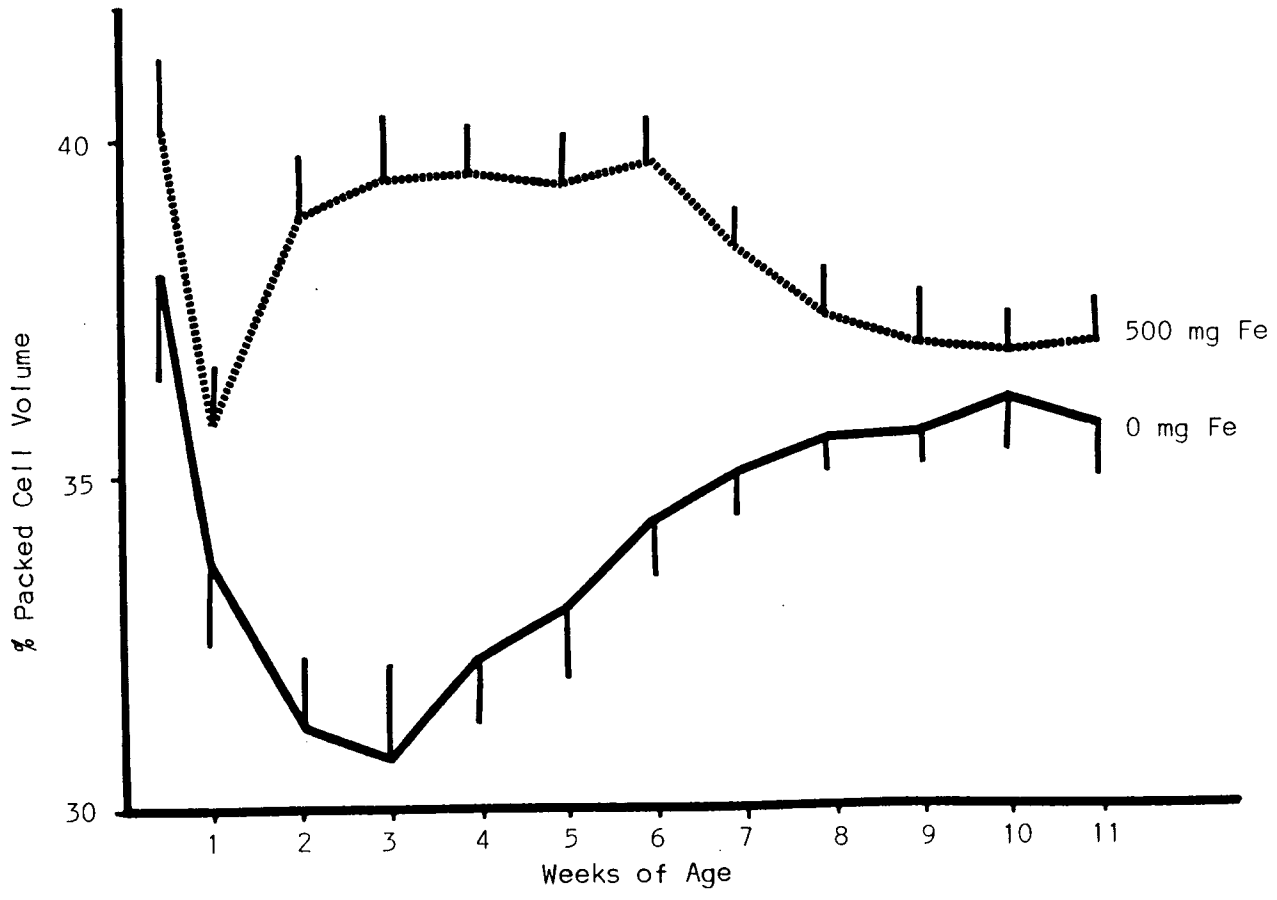

Figure 2. Effect of iron supplementation on packed cell volume (Trial 1).

33.6% PCV. In another study, the minimum Hb level averaged 8.37 g/dℓ in 3 week old lambs (Holz et al., 1961). More recently, Wohlt (1982) found levels of 23% PCV and 9.4 g/dℓ Hb in 42 14-day old lambs that had not been docked. In the present study, the minimum Hb and PCV levels in controls were much higher, at 10.7 g/dℓ Hb and 30.9% PCV, yet iron treatment still resulted in a dramatic hematological response.

Rearing consistently affected Hb and PCV, but the effect was significant only at 2 and 3 weeks of age ($P<0.05$). Both parameters were slightly higher in singles compared to twins at birth (14.25±vs. 13.99 g/dℓ Hb, 39.24 vs. 38.86% PCV). By 2 and 3 weeks of age singletons had significantly higher PCV and Hb levels than twins, within each treatment group. This indicates that higher body reserves of iron present in the single lamb at birth may significantly affect hematology in early life.

Work with other species provides evidence that twinning leads to reduced storage of iron in each fetus. A study of newborn dairy calves revealed a significant incidence of severe neonatal anemia with 15.8% of single calves, and 37.5% of twin calves having <20% PCV at birth (Tennant et al., 1975b). Possibly blood transfusion between fetuses resulted from unequal functioning of the two circulatory systems, as occurs in human twins. Twinning, low birthweight and prematurity predispose iron-lack anemia in human infants (Betke, 1970). The type and degree of transplacental iron transfer would determine whether twinning or low birthweight predispose anemia in sheep. So far the limited information on the subject is inconclusive (Hidiroglou, 1980; Hoskins and Hansard, 1964).

Mean plasma iron at 4 weeks was 185 μg/dℓ. Iron treatment significantly ($P<0.005$) elevated plasma iron from a mean of 141±17 μg/dℓ in the

controls to 223± 15 μg/dℓ in the iron-treated lambs. Breed, sex and rearing did not affect plasma iron. However, control single males had the highest growth rate, and tended to have extremely low iron levels. Thus, even though single lambs are presumed to have greater tissue iron stores at birth, they may be most benefitted by supplementary iron due to their high growth rate.

Normal levels of plasma iron were reported to be 159 μg/dℓ in 2 week old lambs, and 228 μg/dℓ in 6 week old lambs (Hidiroglou & Jenkins, 1971). In older lambs normal values were between 200 and 300 μg/dℓ, which is much higher than the normal level of 115 μg/dℓ in humans (Bothwell et al., 1979, p. 297). It is not known if the plasma iron threshold for iron-deficient erythropoiesis is the same for all species.

Plasma Profile

Variability was high for most metabolites. In spite of minimizing age variation, and accounting for breed, sex and rearing effects, the high overall variability tended to obscure subtle treatment differences, if any. Alkaline phosphatase was the only metabolite significantly affected by iron treatment (Table I). The applicability of the plasma profile test to nutritional studies has been questioned (Rowlands, 1980). Large numbers of animals per treatment may be required to compensate for high innate variability in the data.

Calcium

The mean plasma calcium value at 24-25 days of age was 11.56±0.12 mg/dℓ, within the normal range of 9-12 mg/dℓ (Simesen, 1980). Similarly, Mitruka and Rawnsley (1977) gave a range of 10.4-14.0 mg/dℓ from the

TABLE I.

Effect of iron treatment on plasma profile at 24-25 days (Trial 1)

	CONTROL	IRON	S.E.M.
n	17	18	
Calcium (mg/dℓ)	11.7	11.4	0.1
Inorganic Phosphate (mg/dℓ)	10.3	10.0	0.3
Glucose (mg/dℓ)	95.3	93.9	2.8
Blood Urea Nitrogen (mg/dℓ)	16.4	17.4	0.8
Cholesterol (mg/dℓ)	142	132	7
Total Protein (g/dℓ)	6.04	5.92	0.09
Albumin (g/dℓ)	3.28	3.20	0.06
Alkaline Phosphatase (IU/ℓ)	755_a	627_b	30
Lactate Dehydrogenase (IU/ℓ)	569	587	31
Aspartate Transaminase (IU/ℓ)	104.6	113.1	2.8
Plasma Iron (μg/ℓ)	141_a	223_b	13

a-b Denotes statistical differences between treatment means in the same row, $P<0.05$.

literature, with a mean of 11.4 mg/dℓ. Plasma calcium and phosphorus have been observed to vary with age in lambs (Long et al., 1965; Moodie, 1975) as well as in calves (Simesen, 1970, p. 320). A comprehensive study of lamb hematology reported 2 week serum calcium levels of 12.2±0.19 mg/dℓ, falling to 11.5±0.14 mg/dℓ at 4 weeks (Long et al., 1965).

Iron treatment did not significantly affect plasma calcium ($P>0.05$). Calcium values were 11.73±0.15 mg/dℓ and 11.41±0.17 mg/dℓ for the control and iron-treated groups, respectively.

Phosphorus

The mean plasma inorganic phosphate (P_i) was 10.14±0.29 mg/dℓ. Values in the literature show great variability because of age and dietary effects. Long et al. (1965) obtained very similar results to the present study. The mean P_i from 2-4 weeks of age was 11.0 mg/dℓ. Weight gain and feed intake are correlated with P_i, resulting in high variability even among animals fo the same age and diet (Moodie, 1975; Little et al., 1977).

Plasma P_i is more sensitive than plasma Ca to dietary factors. P_i varies markedly with dietary phosphorus, and also intimately linked with carbohydrate metabolism (Simesen, 1970). Iron treatment did not significantly ($P>0.05$) affect P_i. Plasma contained 10.33±0.46 mg/dℓ P_i in the control group compared to 9.97±0.37 mg/dℓ P_i in the iron-treated group.

Glucose

Blood glucose levels in young ruminants and monogastrics are comparable, but are significantly lower in the adult ruminant. Blood

glucose has been positively correlated with feed intake and weight gain in calves (Little et al., 1977) and in one year old sheep (Bensadoun et al., 1962).

Literature values of ovine plasma glucose range from 55.0 to 131 mg/dℓ (Mitruka and Rawnsley, 1977). Hackett et al. (1957) reported a mean of 53.5±2.3 mg/dℓ, with no difference between ewes and lambs under range conditions, but lamb age was not specified. Lindsay and Leat (1975) listed mean glucose levels of 103.4 mg/dℓ and 96.9 mg/dℓ for 17-24 day old and 25-32 day old lambs, respectively. These means are similar to the mean in Trial 1 of 94.6±2.8 mg/dℓ.

The blood glucose falls steadily with advancing age in the growing lamb, reaching adult levels at 6-9 weeks (Reid, 1953). In spite of the metabolic shift in emphasis from glucose to volatile fatty acids, the change in blood glucose does not have a close relationship with rumen development. Various workers have attributed the blood glucose changes to a shift in erythrocyte metabolism, associated with the replacement of fetal erythrocytes with adult-type cells (Reid, 1953; Kappy, 1982). Adult erythrocytes differ in hemoglobin type, ionic composition, glucose metabolism and enzyme activity (Blunt, 1975).

Plasma glucose may not then be appropriate for assessing energy status in the young lamb. The response of plasma FFA but not blood glucose to ovine growth hormone supports this viewpoint (Lindsay and Leat, 1975). Rowlands (1980) concluded that in sheep, unlike in cattle, "FFA concentrations correlate better with energy intake than do glucose concentrations."

As expected, plasma glucose was not affected ($P>0.05$) by iron treatment (95.3±3.0 mg/dℓ vs. 94.9±4.7 mg/dℓ in control and iron-treated groups).

Total Protein, Albumin and BUN

Plasma total protein, albumin, and BUN (blood urea nitrogen) are typically included as indices of protein metabolism in the metabolic profile. Results are frequently ambiguous as these parameters are not sensitive to small quantitative or qualitative dietary changes. For example, decreased serum protein turnover has been observed to maintain serum levels for animals on a low or zero-protein diet. Serum albumin, total protein, Hb and PCV are found to variably and slowly respond to protein deficiencies. Protein intake seems to affect serum albumin but not globulin in sheep (Rowlands, 1980).

In this study, total protein, albumin and BUN were normal. There was no reason to anticipate significant treatment responses.

Total protein values averaged 5.98±0.09 g/dℓ, with no significant treatment effect at $P>0.05$. Typical mean values in the literature average 5.81±0.54 g/dℓ (Kuttler and Marble, 1960) and 5.46 g/dℓ (Irfan, 1967). Total protein increases with age, mainly because of increasing gamma globulins, whereas albumin decreases proportionately less (Dimopoullos, 1970). Consequently, serum protein ranges from 5.70-9.10 g/dℓ in sheep (Mitruka and Rawnsley, 1977), depending on the age of the animal.

Mean plasma albumin was 3.24±0.06 g/dℓ. Literature values of albumin vary from 2.70-4.55 g/dℓ (Mitruka and Rawnsley, 1977).

The mean BUN was 16.0±0.8 mg/dℓ. Controls and iron-treated lambs did not differ significantly ($P>0.05$), with means of 16.4±1.2 vs. 17.4±1.1 mg/dℓ. BUN has been reported to range from 15.0 to 36.0 mg/dℓ in normal sheep (Mitruka and Rawnsley, 1977).

Cholesterol

Sheep have a low plasma lipid concentration compared to other ruminants, usually less than 200 μg/dℓ. Cholesterol esters are a major component, and with a similar amount of phospholipid, total from 70-80% of the plasma lipid (Nelson, 1969; Lindsay and Leat, 1975). Workers in Germany have measured serum cholesterol to predict several metabolic diseases in early lactation possibly associated with liver malfunction (Manston and Allen, 1981), however its use in ovine nutrition studies remains to be clarified.

Mean plasma cholesterol was 137±7 mg/dℓ, which is higher than the mean of 57.8±8 mg/dℓ reported by Smith et al. (1978) and 64.6±3.3 reported by Hackett et al. (1957), but within the range of 50.0-140 (Mitruka and Rawnsley, 1977). The high level of cholesterol is probably an age effect, as plasma cholesterol decreases when adult rumen function develops.

Alkaline Phosphatase

Iron treatment significantly ($P<0.05$) affected plasma alkaline phosphatase. Plasma AP activity was much greater in the control lambs, with a mean of 755±40 IU/ℓ compared to 627±40 IU/ℓ in the iron-treated lambs. Healy (1975c) mesured mean AP activity of 741±66 IU/ℓ in 28 lambs at 4 weeks of age.

Age, isoenzyme source, blood group, nutrition, feed intake, and rate of growth are among the major sources of variation known to affect alkaline phosphatase activity. In the current study, breed, sex, and rearing were not significant, while blood group was not isolated as a source of variation.

The range in AP activity may be maximum at birth. Serum AP activity ranged from 220-8500 IU/ℓ in newborn lambs, at which time virtually all activity was of skeletal origin (Healy, 1975b). AP activity peaks at 24 hours as intestinal AP enters the circulation. Serum alkaline phosphatase activity tends to decrease with increasing age in sheep. The isoenzymes also vary with age. At 2 weeks of age, isoenzyme analyses showed that 79% of serum AP activity was skeletal type, the remainder intestinal. The high AP activity in lambs and the gradual fall with age are considered to reflect the changing rate of osteoblastic activity associated with skeletal development. By maturity, liver and/or intestinal isoenzymes predominate (Healy, 1975a).

Many nutritional factors affect plasma alkaline phosphatase. When diets varying in the ratio of wheat to alfalfa were fed at maintenance levels, both total AP activity and the proportion of serum heat resistant AP (intestinal isoenzyme) were affected by diet and blood group (Healy and Davis, 1975). Healy and McInnes (1975) also observed that dietary intakes influenced serum AP activity in lambs fed to gain at different rates on the same diet, and in spite of the absence of isoenzyme studies, concluded that the AP response reflected the influence of the dietary intake on skeletal development. Serum AP is reduced in zinc deficiency in pigs (Furugouri, 1972) and calves (Miller et al., 1965) and could be useful in the diagnosis of zinc deficiency in ruminants (Blackmon et al., 1967). Alkaline phosphatase also responds quickly to changes in dietary phosphorus in calves, with activity varying inversely to serum P_i (Wise et al., 1958). The relationship between iron deficiency and plasma AP has not been investigated.

Lactate Dehydrogenase and Aspartate Transaminase

Plasma lactate dehydrogenase (LDH) and aspartate transaminase (AT or GOT) are used to diagnose selenium and vitamin E deficiency diseases.

Mean plasma levels of lactate dehydrogenase (LDH) and aspartate transaminase (AT or SGOT) were 578±31 IU/ℓ and 108.9±2.8 IU/ℓ, respectively. Smith et al. (1978) reported a mean of 311±55 IU/ℓ of LDH in adult ewes, and 71±26 IU/ℓ of AT. Data from Horton et al. (1978) were similar, with lamb serum containing higher amounts of LDH and AT. Mean LDH and AT values were 643 IU/ℓ and 35 IU/ℓ in the lambs injected with vitamin E and selenium, and 869 IU/ℓ LDH and 176 IU/ℓ AT in the control lambs (Horton et al., 1978). The lambs in the current study did not have AT and LDH levels indicative of selenium and/or vitamin E deficiency. Iron treatment did not alter activity of either enzyme.

WEIGHT

Weight data are given in Appendix 3. At the time of initial treatment, iron-treated lambs averaged 4.2±0.3 kg compared to 3.9±0.2 kg in the control group, but the difference was not significant. Between 2 days and 1 week of age the iron-treated group gained almost twice as fast as the control group (1.1 kg vs. 0.6 kg). The weight difference continued to increase between the iron-treated and control groups until 7 weeks, resulting in a significant ($P>0.05$) iron treatment effect from 6 weeks to 9 weeks of age.

Rearing significantly ($P<0.01$) affected lamb weight throughout the study. At 2 days, single lambs weighed 4.5 kg and twins, 3.6 kg. At 11 weeks, singles weighed 56.5 kg and twins, 47.3 kg. Dorsets and Finns

weighed 4.0 kg and 4.1 kg at birth, and 23.6 and 23.4 kg at 11 weeks. A significant sex X rearing interaction was observed from 2 days to 11 weeks ($P<0.05$). Male singles were on average heavier than female singles, but male twins were lighter than female twins.

TRIAL 2 LEVEL OF IRON SUPPLEMENTATION

Effect of Iron Level on Hemoglobin, PCV, and Plasma Iron

Mean Hb and PCV values did not differ in the three groups of lambs at 2 days of age, just prior to treatment. At 16, 30 and 44 days of age differences between the control and iron-treated groups were significant ($P<0.001$) for both Hb and PCV. Hb and PCV were higher ($P<0.05$) for those lambs which received 500 mg of iron compared to those which received 250 mg, at 30 and 44 days of age.

In the control group, Hb fell from 13.6 g/dℓ at 2 days to a low of 10.3 g/dℓ at 30 days, and then increased to 11.8 g/dℓ at 44 days (Figure 3). Similarly, PCV decreased from 38.0% at 2 days to 29.2% at 30 days, and reached 34.0% at 44 days (Figure 4). In contrast, mean Hb and PCV values for both iron-injected groups increased from 2 days to 30 days, then decreased slightly. (Data are given in Appendix 4.)

The maximum differences between the control and high iron (500 mg) groups occurred at 30 days in this study. At this time, mean Hb and PCV values were 40.7% and 40.1% higher respectively in the high iron group. Results in Trial 1 and those reported in the literature indicate that minimum Hb and PCV values are reached at 3 weeks in suckling lambs. PCV and Hb probably continued to decrease to 3 weeks, and then increased, instead of plateauing from 16 to 30 days of age as shown in Figures 3 and

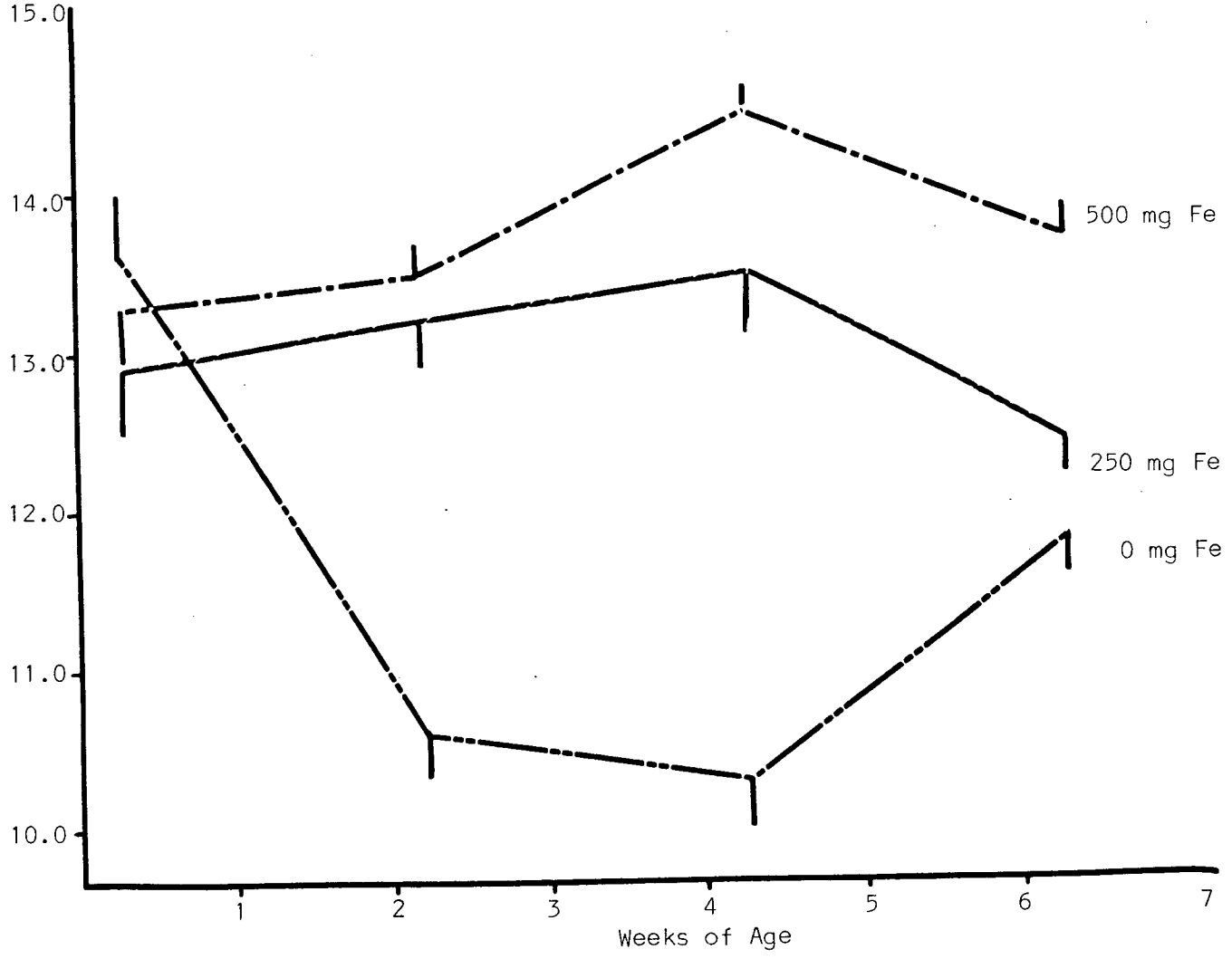

Figure 3. Effect of iron level on hemoglobin (Trial 2).

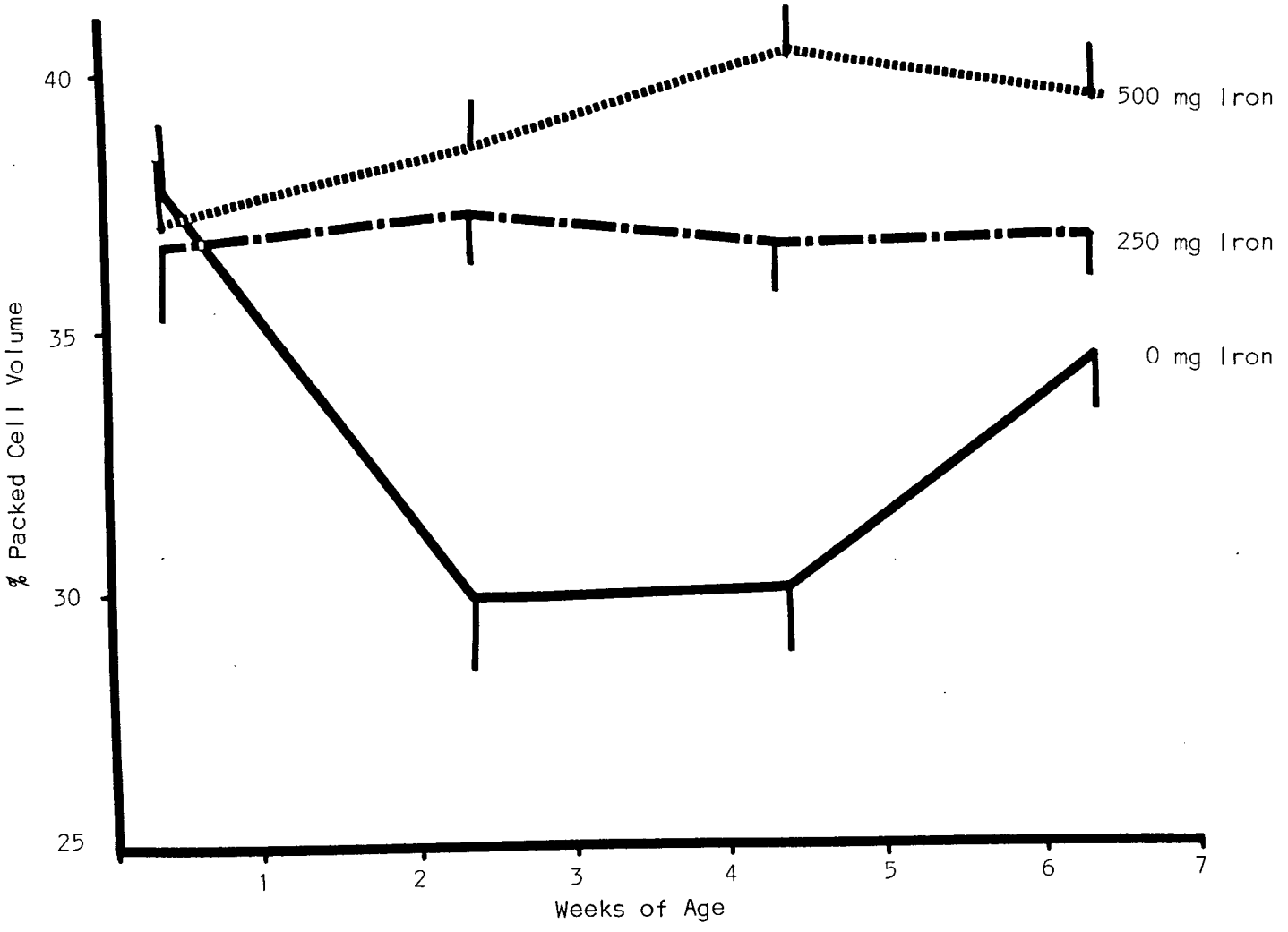

Figure 4. Effect of iron level on packed cell volume (Trial 3).

4. Minimum levels of Hb and PCV could well have been even lower at 3 weeks than observed at the 2 and 4 week sampling periods.

Birth weight was used as a covariable in the statistical analysis. The significant effect of birth weight on Hb at 16 and 44 days, and on PCV at 16 and 30 days, indicates that birth weight is related to iron stores. This appears to be confirmed by the relationship between birth weight and plasma iron.

Plasma iron increased with iron dosage. Treatment means at 30 days of age were 132±22, 204±18, and 230±14 μg/dℓ for the control, low and high iron groups. Orthogonal contrasts comparing the control vs. iron groups, and the low vs. high iron levels, were both significant at $P<0.05$.

Dorsets had significantly lower plasma iron levels than Finn cross lambs ($P<0.05$, 171±12 vs. 224±18 μg/dℓ), however more of the Finn cross lambs were either twins or triplets and thus their slower growth rate could have contributed to this effect.

The significance of birth weight as a covariable ($P<0.05$) suggests that lamb birth weight affects iron stores, as occurs in humans (Betke, 1970). Even though plasma iron was measured at 30-31 days of age, birth weight still had a major influence on plasma iron, according to the regression analysis. Significant partial correlation coefficients with plasma iron ($P<0.05$) were 0.3289 for birth weight, 0.4839 for hemoglobin, and 0.4772 for PCV (Appendix 5).

Thirteen out of 65 lambs had plasma iron levels lower than 100 μg/dℓ at 30-31 days of age, in spite of the fact that iron status is expected to improve from 21 days as lambs start to eat creep feed. The 13 lambs included 10 out of 20 control lambs, and 3 out of 23 lambs injected with

250 mg of iron at birth. Thus if the purpose of iron injection is to maintain plasma iron levels above 100 μg/dℓ from birth to 4 weeks, a dosage of between 250 and 500 mg iron is warranted. It was assumed that 100 μg/dℓ plasma iron is at or above the threshold for unrestricted erythropoiesis in lambs, as in humans. However, the optimum plasma iron level in sheep is unknown. As previously mentioned, normal levels of 8 week old lambs are higher than 200 μg/dℓ (Hidiroglou and Jenkins, 1971) and that level may be optimum.

Hemoglobin and PCV are more commonly used to assess anemia in farm animals than is plasma iron. Schalm et al. (1975) have defined anemia as being characterized by a 20% reduction in either PCV or hemoglobin. The data from this study were assessed on this basis assuming normal values of 35.0% for PCV and 11.5 g/dℓ for hemoglobin (Schalm et al., 1975).

At 4 weeks of age 42% of the control lambs could be considered anemic with PCV values below 28%. On the basis of hemoglobin, 21% of the control lambs could be considered anemic with levels below 9.2 g/dℓ. The difference between Hb and PCV for assessing anemia was caused by the arbitrary levels set for "normal" Hb and PCV values, and not by the actual techniques. Apparently, the normal level of 11.5 g/dℓ Hb was too low for the UBC flock, based on average values for 8 week old lambs (12.4 g/dℓ Hb and 36.4% PCV).

Plasma Profile

Plasma profile data were analyzed by regression as well as covariance techniques. The effects of weight gain and birthweight on the metabolites were of major interest, but both variables could not be included in the covariance model, due to confounding. Consequently, regression analysis was used separately to investigate correlations between metabolites and

birth weight, plasma iron, hemoglobin, PCV and most importantly, average daily gain to 30 days.

Many plasma constituents may be affected by growth rate. Glucose, BUN, P_i, globulin, albumin and serum iron have been correlated with plane of nutrition and growth rate in cattle (Kitchenham et al., 1975; Little et al., 1977; Kitchenham et al., 1977). Also, plasma AP is positively correlated with growth rate in lambs (Healy and McInnes, 1975). In the current study, glucose was positively correlated with weight gain, whereas cholesterol and AT were negatively correlated with weight gain ($P<0.05$). Results of regression analysis are given in Appendix 5 and 6.

Covariance analysis of the plasma profile data indicated that iron treatment had a significant effect ($P<0.001$) on ten out of eleven plasma constituents measured (Table II). The significance of Trial 2 compared to Trial 1 results may be related to the higher overall growth rate, especially considering the higher incidence of twins; sampling at 30 instead of 24 days of age; much larger number of experimental units per treatment; use of orthogonal contrasts for means separation; and virtual absence of hemolysis in blood samples.

Calcium

Plasma calcium data were not available. The vacutainer tubes were apparently contaminated with disodium EDTA, or mislabelled as containing sodium heparin as anticoagulant.

Phosphorus

The response of plasma P_i to iron injection was small but significant ($P<0.001$). Means were 8.9±0.2, 8.7±0.3, and 8.4±0.1 mg/dℓ for the

TABLE II.

Effect of 3 levels of iron treatment on plasma profile[1] at 30-31 days of age (Trial 2)

	TREATMENTS			
	0 mg Fe	250 mg Fe	500 mg Fe	Significance of Contrasts[2]
	$\bar{X}$ ± SE			
n	20	22	24	
Inorganic Phosphate (mg/dℓ)	8.9 ± 0.2	8.7 ± 0.3	8.4 ± 0.1	C1***, C_2*
Glucose (mg/dℓ)	104.9 ± 2.5	101.3 ± 2.6	92.9 ± 2.0	C_1***, C_2***
BUN (mg/dℓ)	17.0 ± 0.8	15.6 ± 0.8	18.4 ± 0.7	C_2***
Cholesterol (mg/dℓ)	96.1 ± 4.5	108.5 ± 3.1	123.5 ± 5.8	C_1***, C_2*
Total Protein (g/dℓ)	5.57 ± 0.06	5.49 ± 0.15	5.20 ± 0.06	C_1***,C_2***
Albumin (g/dℓ)	2.91 ± 0.06	2.86 ± 0.08	2.90 ± 0.06	C_1***, C_2*
Alkaline Phosphatase (IU/ℓ)	1098 ± 116	881 ± 84	725 ± 43	C_1***, C_2*
Lactate Dehydrogenase (IU/ℓ)	570 ± 34	608 ± 39	563 ± 30	C_1***
Aspartate Transaminase (IU/ℓ)	146 ± 38	116 ± 6	111 ± 6	C_1*, C_2*
Plasma Fe (μg/dℓ)	132 ± 22	204 ± 18	230 ± 14	C_1***, C_2*

[1] Calcium data not available due to manufacturer's contamination of vacutainer tubes with disodium EDTA.

[2] Predetermined orthogonal contrasts were as follows:
C1 = Control vs. both levels of iron treatment;
C2 = Low (250 mg Fe) vs. High (500 mg Fe) levels;
*P<0.05; **P<0.01; ***P<0.001

control, low, and high iron groups. P_i tends to decrease during increased carbohydrate utilizaton (Simesen, 1970, p. 319). This would indicate more efficient carbohydrate utilization in the iron-teated groups, probably through increased synthesis of iron-dependent enzymes. While plasma inorganic phosphate levels are "intimately related to the intermediary metabolism of glucose", these changes are said to occur independently of blood glucose (Latner, 1975, p. 46). Regression analysis confirmed that plasma P_i was not significantly correlated with blood glucose, nor with weight gain, Hb, PCV or plasma iron, in spite of the significant response to iron treatment.

Glucose

Plasma glucose means were 104.9±2.5, 101.3±2.6, and 92.9±2.0 mg/dℓ for the control, low and high iron treatments. The difference between the control and both iron treatments, and the low and high levels of iron treatment, were both significant ($P<0.001$).

There may be two aspects to the relationship between iron and glucose. As previously discussed in Trial 1, plasma glucose changes appear to be related to the shift in erythrocyte metabolism in young lambs. Iron supplementation would enhance the shift by accelerating the production of adult types of red cells. This hypothesis is supported by the significant correlation ($P<0.05$) of Hb, PCV, and plasma iron with plasma glucose at 4 weeks of age, with partial correlation coefficients of -0.3055, -0.2533, and -0.3981 (Appendix 5). Alternatively, iron supplementation may prevent a slight elevation of blood glucose associated with anemia. In anemia, the

transfer of glucose from blood to tissues is retarded. However, no disturbance of tissue glucose utilization is involved (Latner, 1975, p. 65). Knight et al. (1983) observed a significant depression in fasting serum glucose for iron-injected piglets compared to controls.

Breed and rearing were also significant sources of variation ($P<0.05$) for plasma glucose. The positive relationship between intake and growth rate with plasma glucose explains the higher glucose levels in single compared to twin lambs (104.7 vs. 98.1 mg/dℓ). Means were 103.2 mg/dℓ for the Dorsets and 91.6 mg/dℓ for the Finn cross lambs.

Total Protein

Total plasma protein was significantly depressed with increasing iron dosage. Mean total protein values were 5.57±0.06, 5.49±0.15, and 5.20±0.06 g/dℓ for dosages of 0, 250 and 500 mg or iron. The difference between control and iron-treated groups, and between the low and high iron groups, were both significant ($P<0.001$). Regression analysis confirmed a strong negative correlation of TP with plasma iron, and less so with hemoglobin. Partial correlation coefficients were -0.4243 and -0.3276 ($P<0.001$ and $P<0.01$). Iron may indirectly affect plasma total proteins by enhancing the synthesis of hemoglobin.

Albumin

Albumin was significantly depressed ($P<0.001$) by iron treatment. Mean values were 2.91±0.06, 2.86±0.08, and 2.90±0.06 g/dℓ for the dosage levels of 0, 250 and 500 mg iron. As there was no linear trend with treatment, these results may not be meaningful, although one would expect a

depression in albumin similar to that in total protein. However, albumin was not significantly correlated with plasma iron, nor with total protein.

BUN

BUN was significantly affected by iron treatment ($P<0.001$), but because of the major influence of birth weight, the trend was not linear. Means were 17.0±0.08, 15.6±0.8, and 18.4±0.7 mg/dℓ. BUN was highly correlated with birth weight, with a partial correlation coefficient of 0.4595 ($P<0.001$); BUN was also positively correlated with plasma iron, with a partial correlaton coefficient of 0.2651 ($P<0.05$).

Cholesterol

Cholesterol was significantly increased by iron treatment ($P<0.001$). Means were 96.1±4.5, 108.5±3.1, and 123.5±5.8 mg/dℓ. The response to iron may be explained by the positive correlation of plasma cholesterol with packed cell volume. Practically all human patients with severe hypochromic anemia demonstrate hypocholesterolemia. The rise in plasma cholesterol upon iron treatment is proportionate to the rise in reticulocyte concentration. The basis of the response is not clear. The reduced cholesterol in anemia is associated with other changes in plasma lipids, including reduced phospholipids (Latner, 1975, p. 142).

Cholesterol was lower in single than twin lambs ($P<0.01$; 99.0 vs. 113.4 mg/dℓ). Regression analysis confirmed the negative correlation of cholesterol with weight gain (Appendix 5). Cholesterol was also lower in Dorset lambs than in Finn cross lambs ($P<0.05$, 105.9 vs. 119.5 mg/dℓ). The breed difference may be genetic, reflecting the differences in lipid metabolism between the Finn breed and the various British breeds (Boylan *et al.*, 1976).

Alkaline Phosphatase

Alkaline phosphatase was sensitive to several of the factors tested. Breed affected AP ($P<0.05$). The 45 Dorset lambs had a mean plasma AP activity of 987 IU/ℓ, compared to a mean of 680 IU/ℓ in the 20 Finn cross lambs. Iron treatment and birth weight were also major sources of variation. Regression analysis demonstrated a significant negative correlation ($P<0.0005$) of AP with birth weight, plasma iron, Hb, and PCV. Birth weight and hemoglobin were the most important regression coefficients (Appendix 6).

The response of plasma AP to iron supplementation cannot be readily explained. Means were 1098, 881, and 725 IU/ℓ for control, low and high iron lambs. Clinically, an increase in plasma AP reflects osteoblastic proliferation or increased activity, usually implicating inadequate mineralization. This occurs in rickets and osteomalacia, for example (Latner, 1975, p. 562). Possibly iron deficiency also affects plasma AP through a disturbance in bone metabolism. Iron deficiency is known to cause skeletal abnormalities in both man and experimental animals, proportionate to the severity of the deficiency (Beutler and Fairbanks, 1980). More commonly, iron deficiency in children is frequently associated with temporary episodes of high blood alkaline phosphatase, presumably caused by a temporary disturbance of bone metabolism.

Isoenzyme analyses of plasma AP are necessary to determine the source of the increased AP activity of iron deficiency - whether hepatic, skeletal, or intestinal. If skeletal, it is important to distinguish between osteoblast and marrow sources. AP is known to be primarily localized in the osteoblast, particularly in growng bone and cartilage (McComb et al.,

1979, p. 579), but vascular and reticuloendothelial sources, including marrow, are thought to make a substantial contribution to plasma AP levels (Wolfe, 1970). One can speculate that increased AP activity in marrow may accompany the erythroid hyperplasia of anemia.

Lactate Dehydrogenase and Aspartate Transaminase

Lactate dehydrogenase and aspartate transminase values were within the normal range as in Trial 1. Grand means were 580±2.1 IU/ℓ for LDH, and 123±12 IU/ℓ for AT. For both enzymes, the orthogonal contrast comparing the control treatment with the combined iron-injected groups, was significant ($P<0.00001$), yet there was no difference between low and high iron treatments ($P>0.05$). LDH means by treatment were 570±34, 608±39, and 563±30 IU/ℓ for the control, 250 mg, and 500 mg iron groups. The data were extremely variable, ranging from a low of 339 IU/ℓ in a control lamb with 48 μg/dℓ plasma iron, to highs of 1110 and 1020 IU/ℓ in two lambs from the low iron group, which had plasma iron levels of 196 and 221 μg/dℓ. LDH was not significantly correlated with weight gain, Hb or plasma iron using linear regression analysis. All in all, a true response of LDH to iron treatment was not evident.

Aspartate transaminase activities varied markedly within treatments, apparently because of a significant correlation with weight gain. Means for the control, low and high iron treatment groups were 146±38, 116±6, and 111±6 IU/ℓ AT. In spite of the apparent trend, it could be erroneous to conclude that iron reduced plasma AT. For instance, the maximum AT activity was 226 IU/ℓ, in a high iron lamb with 245 μg/dℓ plasma iron. The minimum activity was 78 IU/ℓ, in a control lamb with 31 μg/dℓ plasma iron.

Multiple linear regression identified weight gain and plasma iron as the major regression coefficients. Plasma iron was positively related to AT, whereas weight gain was negatively related to AT activity (Appendices 5 and 6). These results demonstrate the importance of accounting for unfixed factors such as individual growth rate which affect plasma parameters.

WEIGHT

Age in days, and actual birth weight, were covariables used in analyzing weekly weight data from 2 days to 51 days. Exact age and birth weight were significant covariables ($P<0.0001$) at time of treatment between 0-3 days of age. Birth weight still had a significant effect ($P<0.001$) at the final sampling at 7 weeks of age.

Initial weight and growth rate to 4 weeks were both higher in Trial 2, than in Trial 1, in spite of the higher percentage of twin lambs (79% and 54% twins, respectively).

Rearing was the only significant main effect. Single lambs were heavier ($P<0.05$) than twin lambs at 1 week, (8.0 kg compared to 6.1 kg). The difference was significant ($P<0.0005$) at 30 days, at which time singles weighed 15.3 kg compared to 11.2 kg for twins. The increased milk intake of single lambs during the first month of age accounts for this effect; after 30 days, solid food consumption increases markedly and twin weight gain begins to catch up.

Iron treatment had no effect on lamb weight (Appendix 7). Average weights for the control and high iron groups of lambs were identical from initial to final samplings. The low-iron group was only 1 kg heavier than the control group at 51 days. At no time were differences significant.

A weight response to iron supplementation was observed in Trial 1 and, as will be shown later, also in Trial 3. The reason for the lack of response in growth rate in this trial cannot be readily explained.

TRIAL 3 IRON AND/OR SELENIUM SUPPLEMENTATION

Effect of Treatment on Hemoglobin and PCV

Combined data from the three replicated experiments in Trial 3 were analyzed by analysis of variance (Appendices 8 and 9). The four treatments were control, selenium, iron and iron plus selenium. Multiple range tests and orthogonal contrasts were used for means separation. The contrasts tested were iron effect, selenium effect, and interaction of iron and selenium. They were necessary as iron and selenium were not considered as separate factors in the design, but were combined together under treatment.

Figures 5 and 6 illustrate the effects of treatments on Hb and PCV for lambs from 2 days to 8 weeks of age. Hemoglobin and PCV decreased from 2 days to 3 weeks of age in control and selenium treated lambs. The values then increased slowly from 3 to 8 weeks of age. In lambs treated with iron or iron plus selenium, the Hb and PCV values declined from 2 days to 1 week, then increased reaching a plateau at 3 weeks of age.

As in the previous trials, the maximum difference between the iron treated lambs and those not treated with iron occurred at 3 weeks of age. Hb was 11.3% higher at 1 week, 24.5% higher at 2 weeks, 25.6% higher at 3 weeks, and 20.5% higher at 9 weeks in iron-treated lambs. Mean Hb levels at 3 weeks were 10.9±0.2, 10.6±0.3, 13.6±0.2 and 13.4±0.2 g/dℓ for control, selenium, iron and iron plus selenium treated lambs respectively. Corresponding PCV values were 31.2±0.4, 31.9±0.4, 41.4±0.3, and 39.2±0.4%.

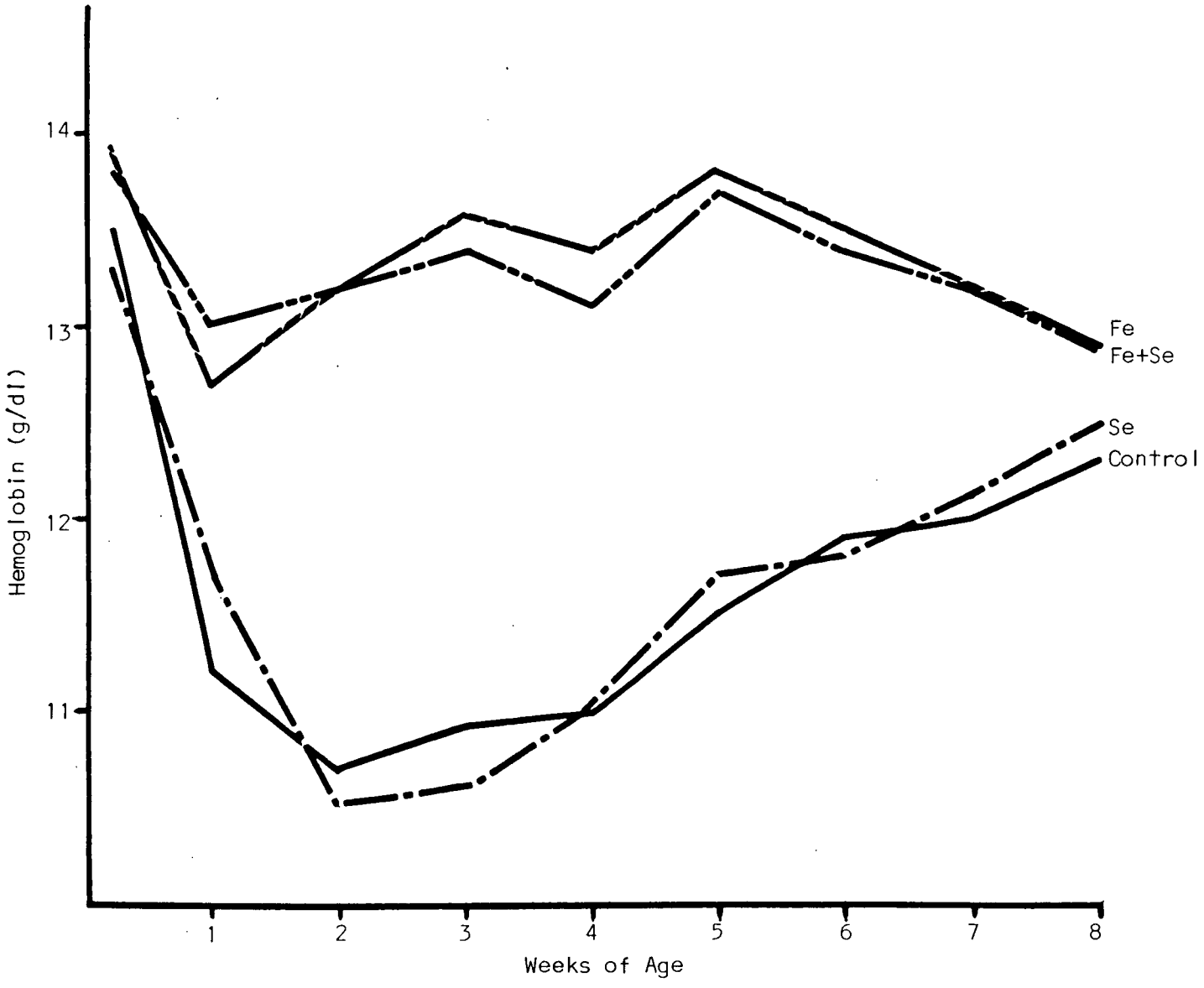

Figure 5. Effect of iron and selenium supplementation on hemoglobin (Trial 3).

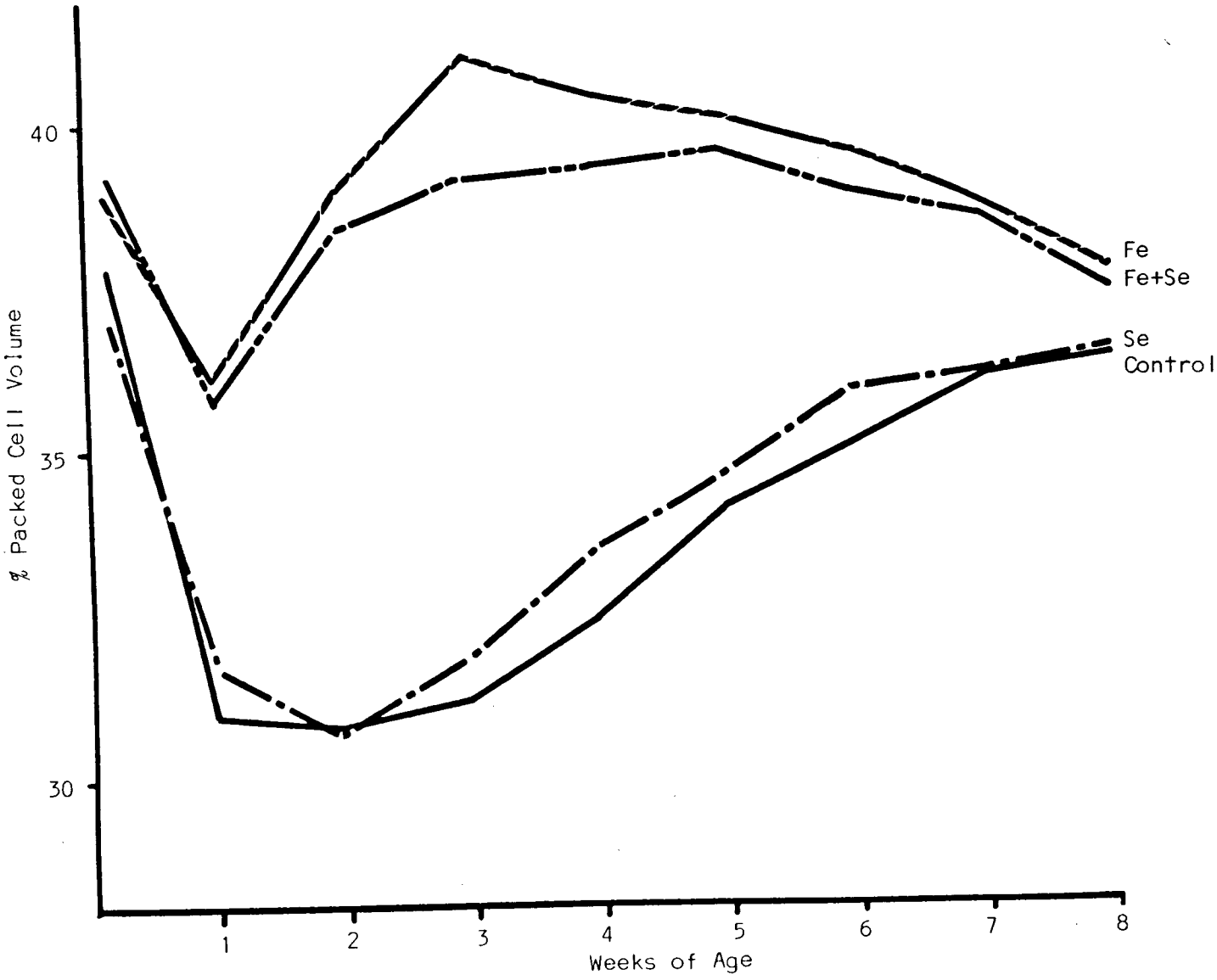

Figure 6. Effect of iron and selenium supplementation on blood packed cell volume (Trial 3).

On the basis of Hb and PCV response to iron treatment, virtually all control and selenium lambs were deficient in iron, and a significant number could be classed as anemic. As previously, the criteria of <9.2 g/dℓ Hb or <28% PCV were used to assess the proportion of control lambs anemic at any time. Using this arbitrary Hb level, 22% of controls were anemic at 3 weeks, and 11% at 4 weeks. Similarly, using the level of <28% PCV as the definition of anemia, 25% of control lambs were anemic at 2 weeks, 23% at 3 weeks, and 17% at 4 weeks. Iron-injected lambs were never anemic.

Selenium did not affect Hb or PCV at any time between 2 days and 8 weeks, nor was an interaction with iron evident ($P<0.05$). The data therefore did not support the results of Horton et al. (1978) and Buchanan-Smith et al. (1969), who found selenium supplementation depressed Hb levels. The use of regression analysis of their data would have eliminated a growth response to Se as the reason for the Hb depression. The current study also failed to confirm the erythropoietic response to selenium observed by Niyo et al. (1980) and by Fontaine et al. (1977a, 1977b).

The absence of a hematological response to selenium is not conclusive. It only suggests that the selenium status of the control lambs in Trial 3 was adequate for hemoglobin production and/or red cell maintenance, possibly because vitamin E levels were good. On the other hand, blood hemoglobin was probably a crude if not inappropriate technique to assess the importance of Se in heme metabolism. The effect of selenium status on heme synthesis and catabolism in bone marrow has not yet been thoroughly investigated, although limited information is available for liver, spleen and muscle (Burk et al., 1974; Burk and Correia, 1978; Whanger et al., 1977).

Hemoglobin versus Hematocrit

Much discussion in the literature on iron deficiency concerns the comparative efficiency of hemoglobin and hematocrit for detection of anemia. Different results are frequently obtained, depending on which parameter is used.

The hematocrit test is cheaper and easier than the hemoglobin test, but can be less effective than hemoglobin in identifying anemia. For example, the hematocrit fails to detect 20-50% of children who would be considered anemic based on hemoglobin levels (Graitcer et al., 1981).

The large amount of data collected on lamb hematology in this trial provided an opportunity to compare the Hb and PCV tests. Hemoglobin was plotted against PCV, and regression equations were developed with and without outlier rejection. All equations given were derived using 5% outlier rejection.

The correlation between hemoglobin and hematocrit was very high from birth to 6 weeks of age. The coefficient of linear correlation was 0.93 at 2 days of age, 0.92 at 1 week, 0.96 at 2 weeks, 0.94 at 3 weeks, 0.89 at 4 weeks, 0.93 at 5 weeks, 0.86 at 6 weeks, 0.75 at 7 weeks, and 0.74 at 8 weeks. The regression equations for prediction of Hb from PCV according to these age groups are given in Appendix 10.

The regression analyses showed that the relationship between Hb and PCV was close at birth (Figure 7) and even closer at 4 weeks (Figure 8). As Figure 9 illustrates, the relationship between PCV and Hb was not as tight by 8 weeks.

When data from all sampling periods of Trial 3 were analyzed together, the coefficient of linear correlation was 0.9084. Excluding the

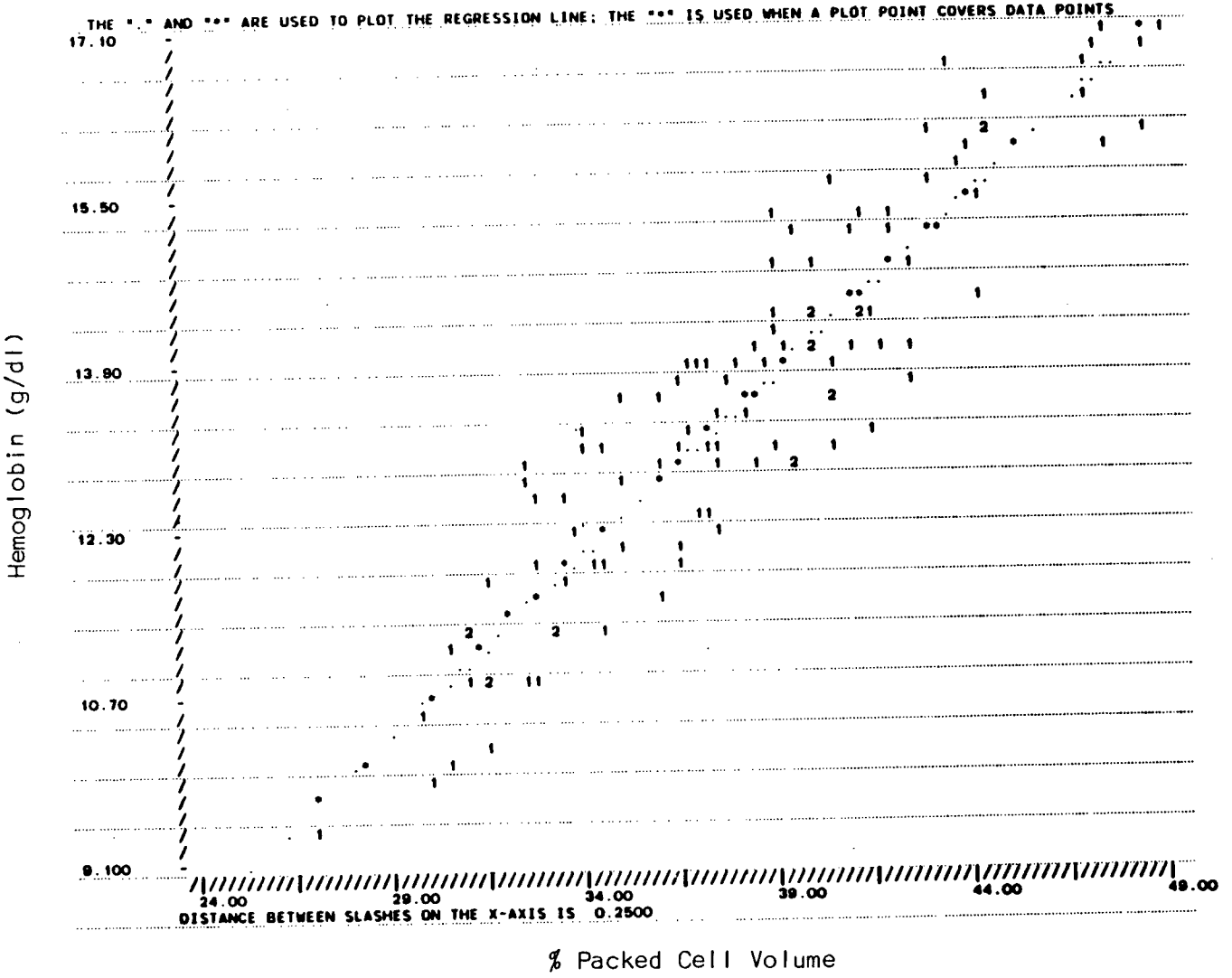

Figure 7. Hemoglobin versus PCV at 2 days (Trial 3).
$\hat{Y} = 0.4851 + 0.3419X \qquad R^2 = 0.8624$

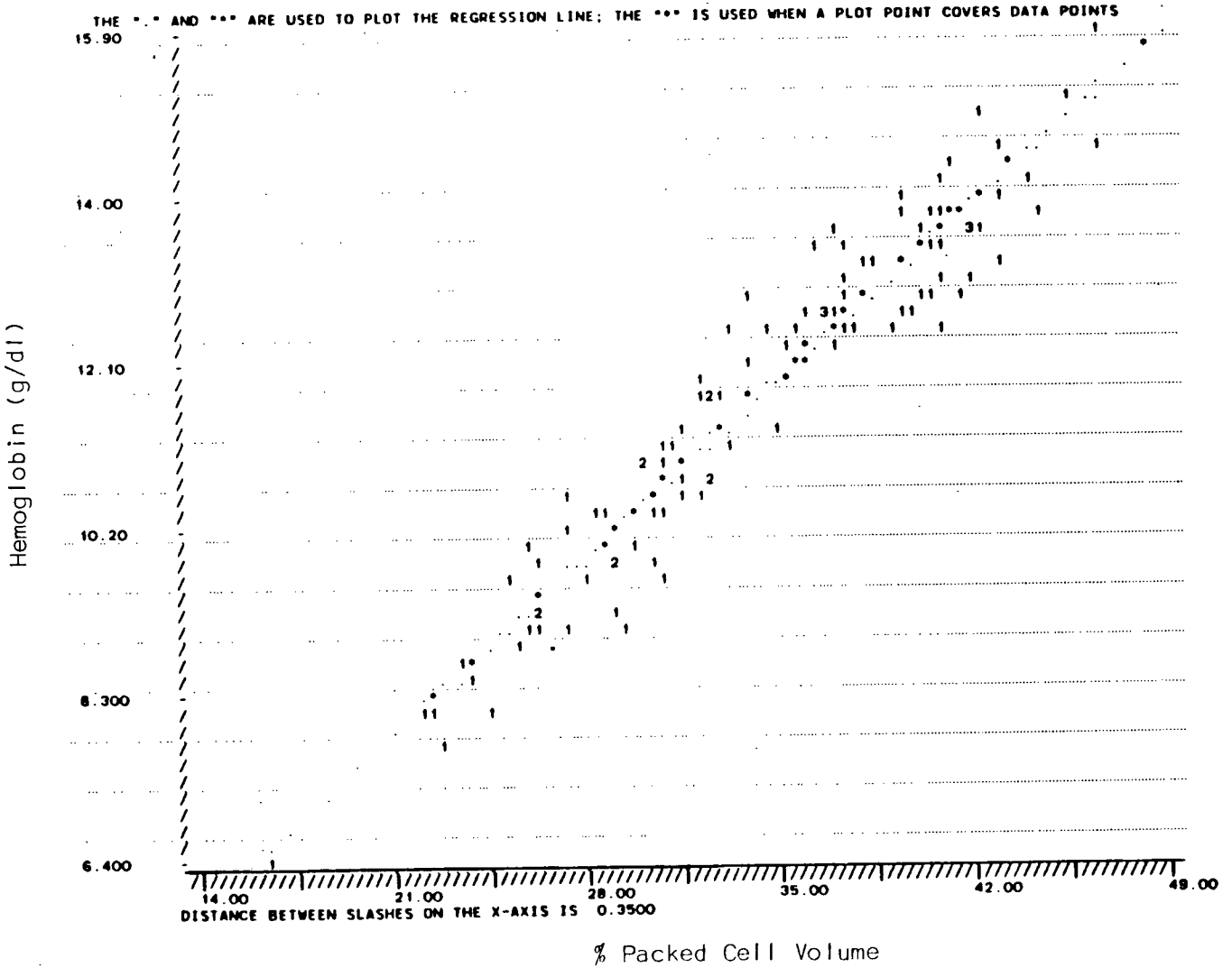

Figure 8. Hemoglobin versus PCV at 4 weeks (Trial 3).
$\hat{Y} = 0.2120 + 0.3346X \qquad R^2 = 0.9228$

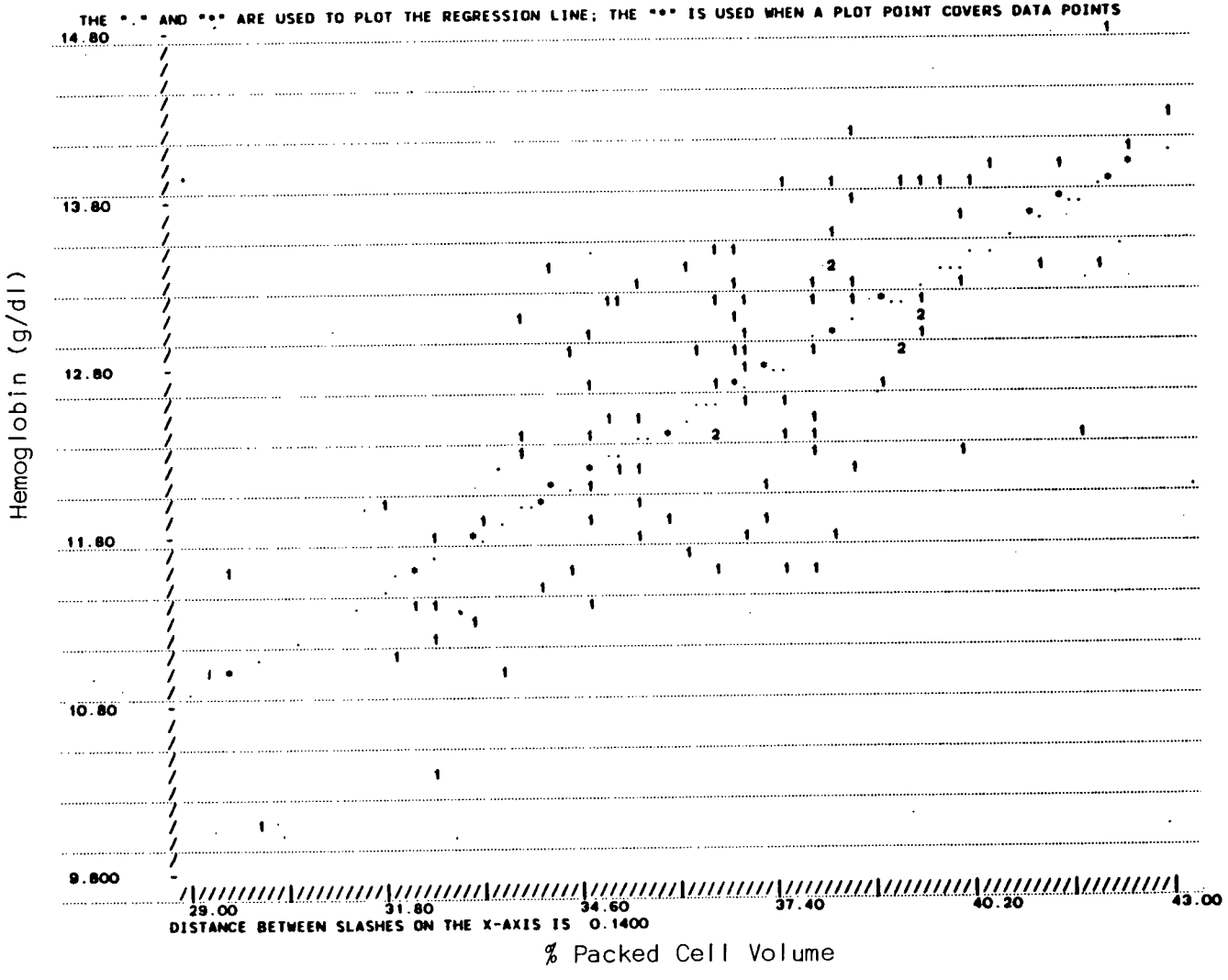

Figure 9. Hemoglobin versus PCV at 8 weeks (Trial 3).
$\hat{Y} = 4.084 + 0.2334X \qquad R^2 = 0.5409$

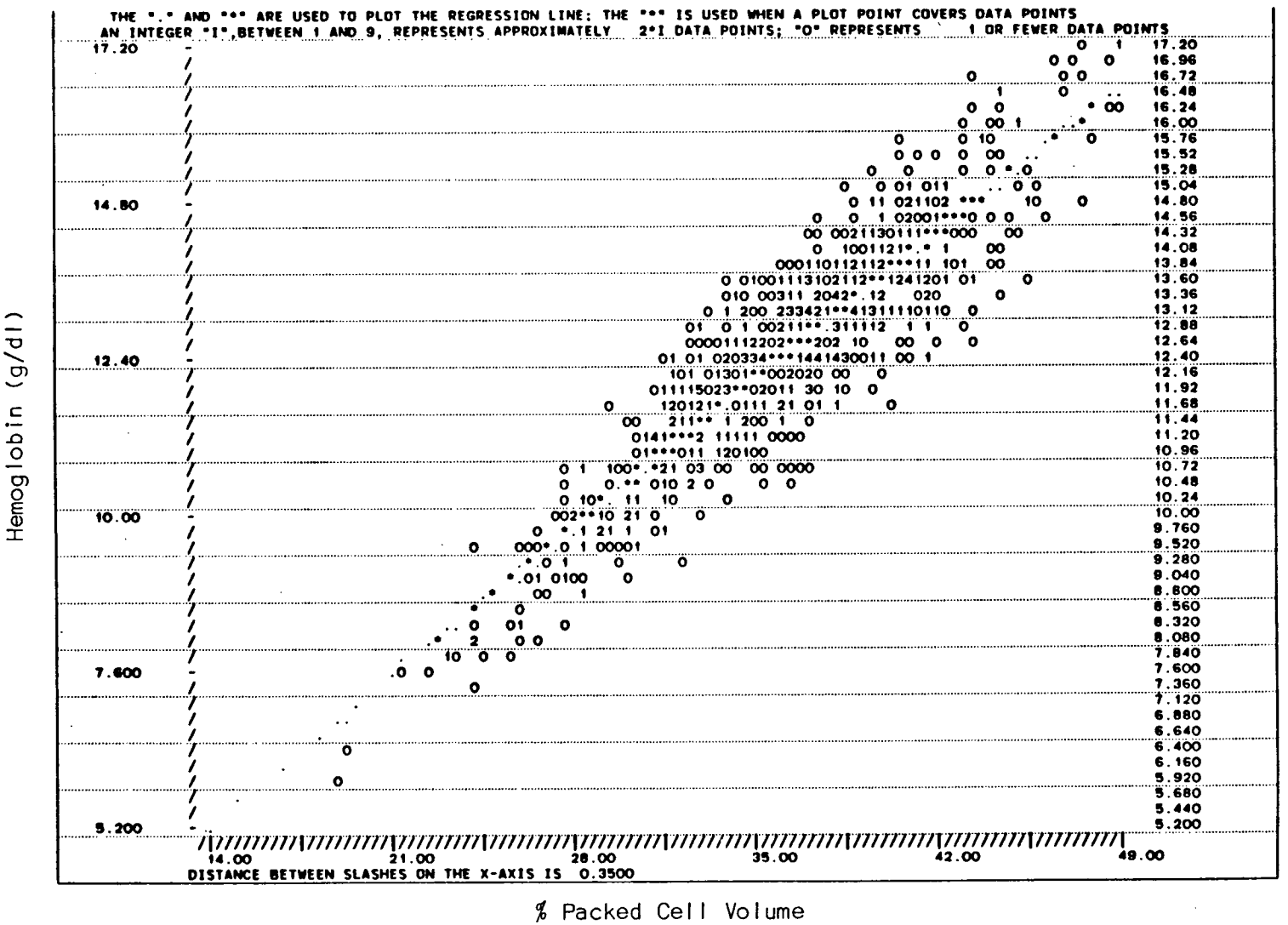

Figure 10. Hemoglobin versus PCV, all Trial 3 data (5% outlier exclusion level)
$\hat{Y} = 0.8015 + 0.3221X \qquad R^2 = 0.8251$

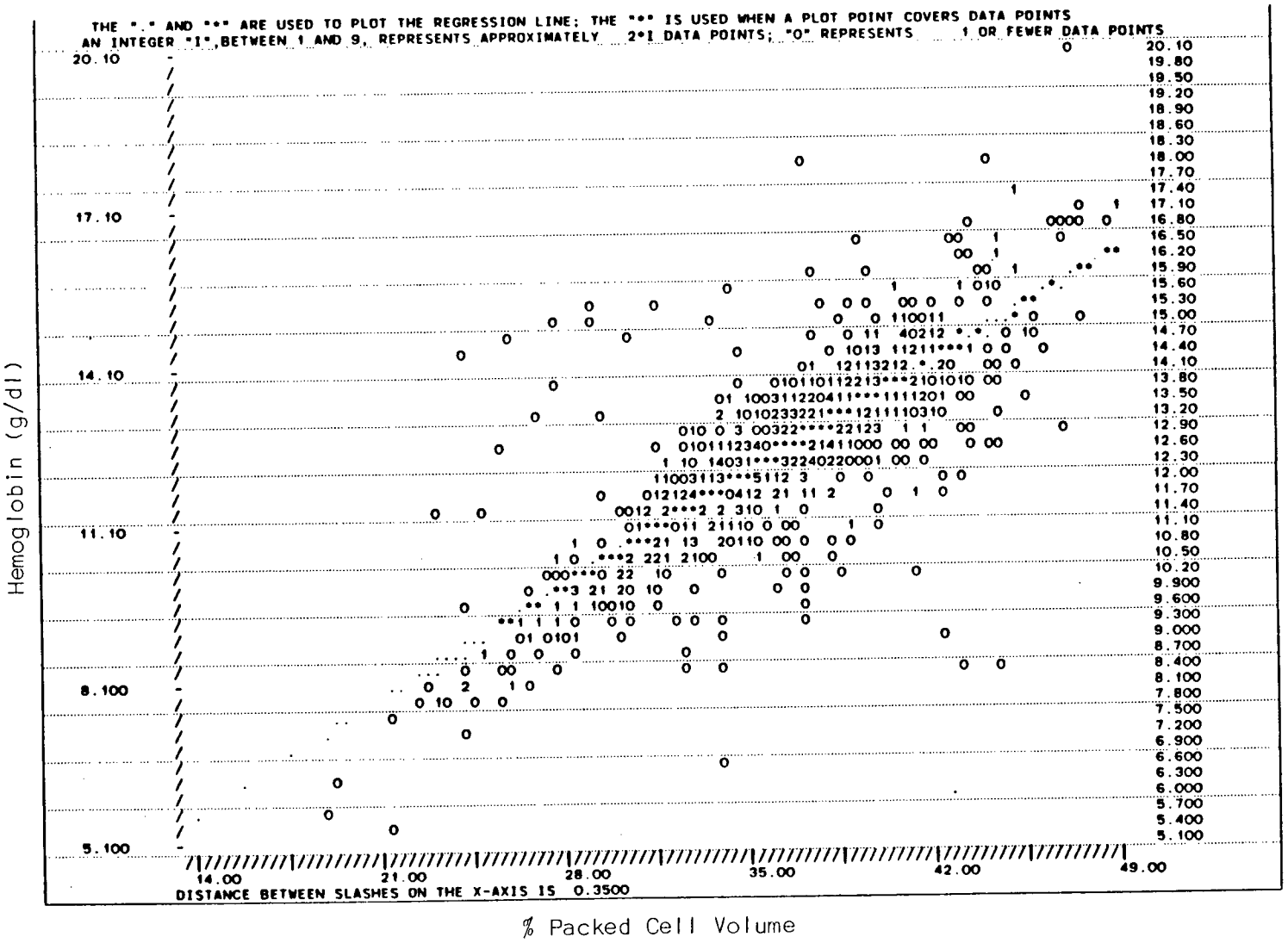

Figure 11. Hemoglobin versus PCV for all Trial 3 data.
$\hat{Y} = 1.613 + 0.2996X \qquad R^2 = 0.6571$

outliers, 1081 complete pairs of observations were used in the analysis. The simple linear regression equation derived from this data was

$$Y = 0.8015 + 0.3221X.$$

where Y = the dependent variable hemoglobin

X = the independent variable PCV.

The plot of Y vs. X and of the regression line is shown in Figure 10. Figure 11 shows the same data plotted without exclusion of the outliers.

The hematocrit test appeared to be a good estimator of lamb iron status between birth and 8 weeks. However, the hemoglobin technique was preferable for lambs over 6 weeks of age, depending on the accuracy desired. After 6 weeks of age, the relationship between the two variables deteriorated, and it was not known if the correlation would become even lower after 8 weeks. Since the concentration of Hb in the erythrocyte is relatively constant except during anemia, this may not occur.

PLASMA SELENIUM

Plasma selenium data were obtained for 39 lambs in Replicate 2, and 36 lambs in Replicate 3 of Trial 3. Mean selenium values were 0.092±0.004 ppm in Replicate 2, and 0.091±0.004 ppm in Replicate 3. As replicates did not affect plasma selenium ($P<0.05$), data from the replicates was combined.

Selenium treatment significantly increased plasma Se levels at 4 weeks ($P<0.05$). Means were 0.086 ppm Se for the 38 control lambs, and 0.098 ppm Se for the 37 selenium-injected lambs.

The range in plasma selenium values was large, extending from deficient to normal levels within both treatments (Figure 12). The assumption that the flock had a marginal selenium status was justified by plasma selenium values in the control lambs. Control lambs had levels ranging from 0.016-0.118 ppm Se. Between 0.080 and 0.500 ppm Se is considered adequate for serum selenium levels in sheep in B.C. (Puls, 1981). Based on this criterion, 25 out of 38 controls, or 66%, had adequate plasma selenium levels at 4 weeks. The level of .08 ppm is much higher than that considered normal in some other studies (ie. 0.02 ppm Se may be adequate), but was chosen as levels of $\leq$ 0.07 ppm Se were currently associated with Se-responsive WMD in the UBC flock. However, no WMD occurred in any lamb used in Trial 3.

Corresponding ranges for selenium-injected lambs were 0.029-0.170 ppm Se. A larger percentage of selenium-injected lambs had plasma levels within the nromal range. Out of 37 injected lambs, 30 animals or 81% could be considered to have adequate plasma selenium values. Due to the very low selenium levels in some individuals, the recommended Se injection dosage of 1.5 mg selenium as sodium selenite may not ensure adequate selenium levels in all treated lambs.

According to Thompson *et al.* (1976), sheep appear to form two distinct groups, one having high blood selenium levels ranging from 133-249 ng/mℓ, and the other having low levels ranging from 21-67 ng/mℓ. Blood selenium data from the present study appeared to have a normal distribution (Figure 12).

Breed, sex and rearing had no effect on plasma selenium levels ($P>0.05$).

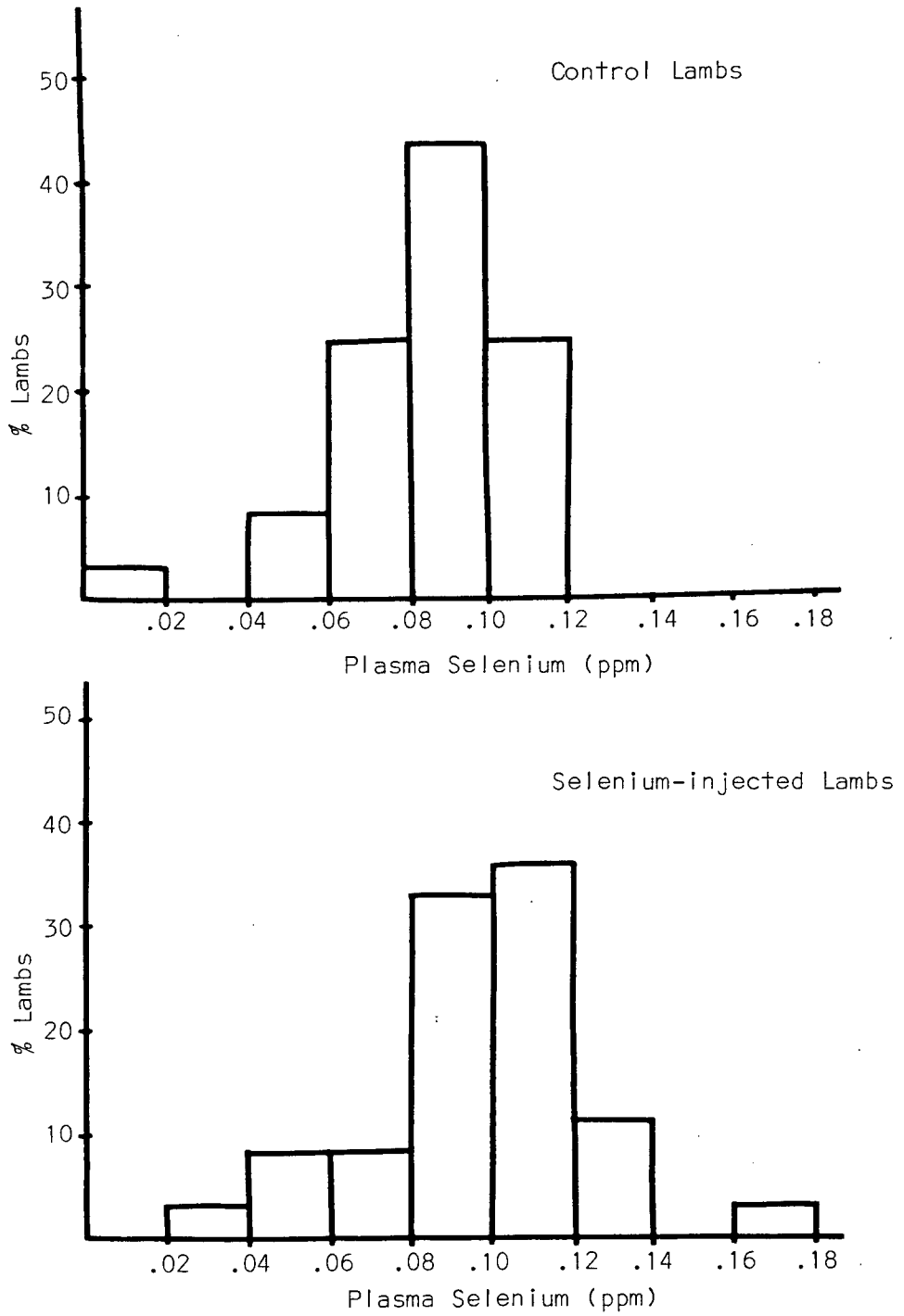

Figure 12. Effect of selenium treatment at birth on plasma selenium at 4 weeks.

The interaction of iron with selenium was significant ($P<0.05$). Selenium levels were significantly higher in those lambs injected with selenium and not injected with iron, than in the other three treatment combinations ($P<0.05$, Newman-Keuls multiple range test). Means were 0.085 ppm (-Fe-Se); 0.086 ppm (+Fe-Se); 0.107 ppm (-Fe+Se); and 0.088 ppm Se (+Fe+Se), with 18 to 20 lambs per treatment combination.

These differences may reflect a difference in the partitioning of blood selenium but not necessarily in total selenium levels. Selenium is incorporated into the erythrocyte only during its formation, mainly as GSH-Px (Ganther et al., 1976). As selenium was injected simultaneously with iron, which greatly stimulated erythropoiesis, possibly more selenium was incorporated into the erythrocyte fraction of +Se+Fe lambs than in +Se-Fe lambs. Analyses of Se and GSH-Px in both plasma and red cells would provide valuable information.

Iron deficiency, but not anemia per se, may restrict the synthesis of GSH-Px (Rodvien et al., 1974). Iron deficiency anemia is associated with decreased erythrocyte GSH-PX activity, and iron supplementation induces a rapid increase in GSH-Px. A study with humans indicated that the decrease in GSH-Px was proportional to the decrease in Hb (Macdougall, 1972). However, a study with rabbits showed that erythrocyte GSH-Px activity was markedly depressed by iron deficiency, even when expressed per unit of hemoglobin (Rodvien et al., 1974).

The significant interaction in the current study between iron and selenium treatments on plasma selenium levels suggests a role for iron in selenium metabolism. As GSH-Px does not contain iron (Ganther et al., 1976), it is likely that iron-containing enzymes may be involved in the

synthesis or regulation of GSH-Px. Alternately, plasma selenium could be decreased in +Se+Fe lambs merely because of the increased red cell mass containing GSH-Px.

Plasma Profile

The plasma profile included the same parameters as in the previous two trials. The statistical analysis tested replicate, treatment, breed, sex, rearing and interactions, using orthogonal contrasts to isolate the effects of iron and selenium.

Replicate significantly affected every parameter, as can be seen in Table III. However, mean values of each parameter were within normal ranges. Replicate means were highest for P_i, BUN, LDH and AT in Replicate 2, and lowest in Replicate 1. Means were highest for calcium, glucose, cholesterol and AP in Replicate 1, and lowest in Replicate 2. All Replicate 3 means were intermediate.

The data indicated that Replicate 2 samples suffered some deterioration in storage, and Replicate 3 samples a lesser amount of deterioration. Plasma samples were stored in a freezer at -10°C, but due to temperature fluctuations the samples were often found to be in a semi-frozen state upon removal for analysis. Trial 2 samples were stored for the longest time and therefore were the most affected.

While twenty of the most commonly measured plasma constituents, including glucose, are stable when frozen for three or more years, repeated freezing and thawng must be avoided (Caraway, 1962).

Alkaline phosphatase was the only parameter significantly affected by iron in all replicates of Trial 3 (Table IV). As in Trials 1 and 2, the activity of plasma alkaline phosphatase was consistently lower in plasma of

TABLE III.

Effect of replicate on plasma profile at 4 weeks (Trial 3)

METABOLITE	$\bar{X}$ ± SEM	R1	R2	R3	Significant Main Effect[1],[2]
Calcium (mg/dℓ)	10.82 ± .12	11.56	10.41	10.60	Rep**, Rear*
P_i (mg/dℓ)	11.27 ± .15	10.14	12.10	11.44	Rep**
Glucose (mg/dℓ)	49.8 ± 3.7	94.6	18.1	42.4	Rep***
BUN (mg/dℓ)	19.3 ± 0.5	16.9	22.6	18.1	Rep***
Cholesterol (mg/dℓ)	123.1 ± 3.6	132.6	106.5	126.8	Rep**
TP (g/dℓ)	6.20 ± 0.05	6.98	6.45	6.17	Rep*
Albumin (g/dℓ)	3.72 ± 0.04	3.24	4.00	3.87	Rep***
AP (IU/ℓ)	605 ± 18	691	527	604	Rep*, Fe*
LDH (IU/ℓ)	696 ± 21	578	756	775	Rep***, Sex*
AT (IU/ℓ)	161 ± 10	109	188	180	Rep**
n	(111)	(33)	(37)	(41)	

[1]Main effects tested were replicate, treatment, breed, sex and rearing.

[2]Predetermined orthogonal contrasts were as follows:
FE. Control + Se treatments vs. Fe + Fe/Se treatments
SE. Control + Fe treatments vs. Se + Fe/Se treatments
FEXSE Control + Fe/Se treatments vs. Se + Fe treatments

[3] *P<0.05; **P<0.01; ***P<0.001

TABLE IV.

Effect of iron and selenium supplementation on plasma profile (Trial 3)

PLASMA METABOLITE	REPLICATE 1				REPLICATE 2				REPLICATE 3			
	Control	Se	Fe	Se/Fe	Control	Se	Fe	Se/Fe	Control	Se	Fe	Se/Fe
Calcium (mg/dℓ)	11.76	11.70	11.71	11.06	10.82	10.50	10.13	10.13	10.06	11.02	10.85	10.40
P_i (mg/dℓ)	10.44	10.22	9.97	9.96	11.94	12.57	11.67	12.23	11.85	11.26	11.28	11.39
Glucose (mg/dℓ)	97.0	93.5	94.8	93.0	19.5	12.0	25.8	15.1	40.2	43.4	41.5	45.0
BUN (mg/dℓ)	17.2	15.6	18.4	16.2	22.2	21.2	23.6	23.7	18.3	17.0	20.1	16.6
Cholesterol (mg/dℓ)	148.0	135.7	129.4	134.5	111.3	94.0	114.2	105.9	120.8	123.8	133.0	128.4
TP (g/dℓ)	6.12	5.97	6.02	5.80	6.42	6.41	6.39	6.58	6.01	6.5	6.13	6.02
Albumin (g/dℓ)	3.26	3.29	3.23	3.18	3.95	3.97	4.08	4.01	3.84	3.94	3.78	3.94
AP (IU/ℓ)	799	717	612	643	553	538	507	508	645	646	570	556
LDH (IU/ℓ)	552	585	582	593	794	730	733	762	775	735	726	725
AT (IU/ℓ)	110	100	112	115	262	150	172	159	176	172	204	160
n	8	8	9	8	10	9	9	9	10	10	12	9

iron-treated lambs. Mean AP in 56 control lambs was 645±28 compared to 565±28 IU/1 in 56 iron-treated lambs.

Selenium treatments were not associated with any changes in plasma parameters indicative of white muscle disease. The deterioration of muscle tissue that occurs in WMD induces a host of biochemical changes in plasma as well as muscle tissue. For example, inorganic phosphate and alkaline phosphatase are increased (Koval'skii and Ermakov, 1970), while serum calcium and magnesium are unaffected (Godwin et al., 1974). Plasma levels of tissue enzymes are sensitive to muscle damage, increasing even during subclinical WMD. Consequently, plasma malate dehydrogenase, alanine aminotransferase, lactate dehydrogenase, and aspartate aminotransferase are commonly used to diagnose WMD in ruminants (Whanger et al., 1969b; Boyd, 1973).

Except for plasma selenium and GSH-Px, few plasma parameters respond to variations in selenium status in the absence of WMD. Glucose and fatty acid metabolism may be affected at various levels of selenium availability. Selenium status influenced rate of glucose metabolism, rate of fatty acid metabolism, and tissue fatty acid content in one study (Fischer and Whanger, 1977). Supplementing dietary Se to an adequate Se diet decreased blood sugar, pyruvic acid, and P_i, and increased tissue glycogen and muscle ATP (Koval'skii and Ermakov, 1970, p. 68). On the other hand, Whanger et al. (1969b) were unable to measure a response in blood glucose, lactate or cholesterol to WMD, although tissue cholesterol is presumed to increase.

The high variability contributed by replicate and other sources of variation may have obscured iron and selenium treatment effects. Contrary

to expectation, Se did not significantly ($P>0.05$) affect LDH and AT levels. The difference in selenium status at 4 weeks between the control and selenium treatments may have been too narrow to be reflected in plasma metabolites. The scanty literature on the subject indicates that a sub-clinical Se deficiency may be expected to have only a subtle effect on plasma constituents. Hence plasma profile analysis, except for certain muscle enzymes, may be of doubtful value for investigating selenium even under carefully controlled experimental conditions.

Weight

The weight data in Trial 3 was taken from a total of 121 lambs. There were 35, 43, and 43 lambs respectively in replicates 1, 2 and 3.

Replicate was a significant main effect from 1 week to 8 weeks of age. However, differences between replicates were slight. Initial weights were 4.05±0.99, 3.84±0.88, and 3.90±0.98 kg in Replicates 1, 2 and 3. Final weights at 8 weeks were 18.6±4.4, 19.0±4.0, and 19.4±3.9 kg.

Rearing significantly affected weight throughout the trial ($P<0.001$). The 46 single lambs were heavier than the 75 twin lambs from birth (4.47±0.97 vs. 3.59±0.77 kg) to 8 weeks (21.8±3.9 vs. 17.3±3.1 kg).

Initial weights were 3.79±0.75 kg for 63 females, compared to 4.06±1.10 kg for 58 males. Final weights at 8 weeks averaged 17.9±3.2 kg for females, and 20.2±4.5 kg for males, however the difference was not significant ($P>0.05$).

Iron treatment significantly affected lamb weight gain from 2 weeks to the end of the trial (Appendix 11). As shown in Figure 13, the difference between iron-treated lambs and other categories increased slowly throughout the trial.

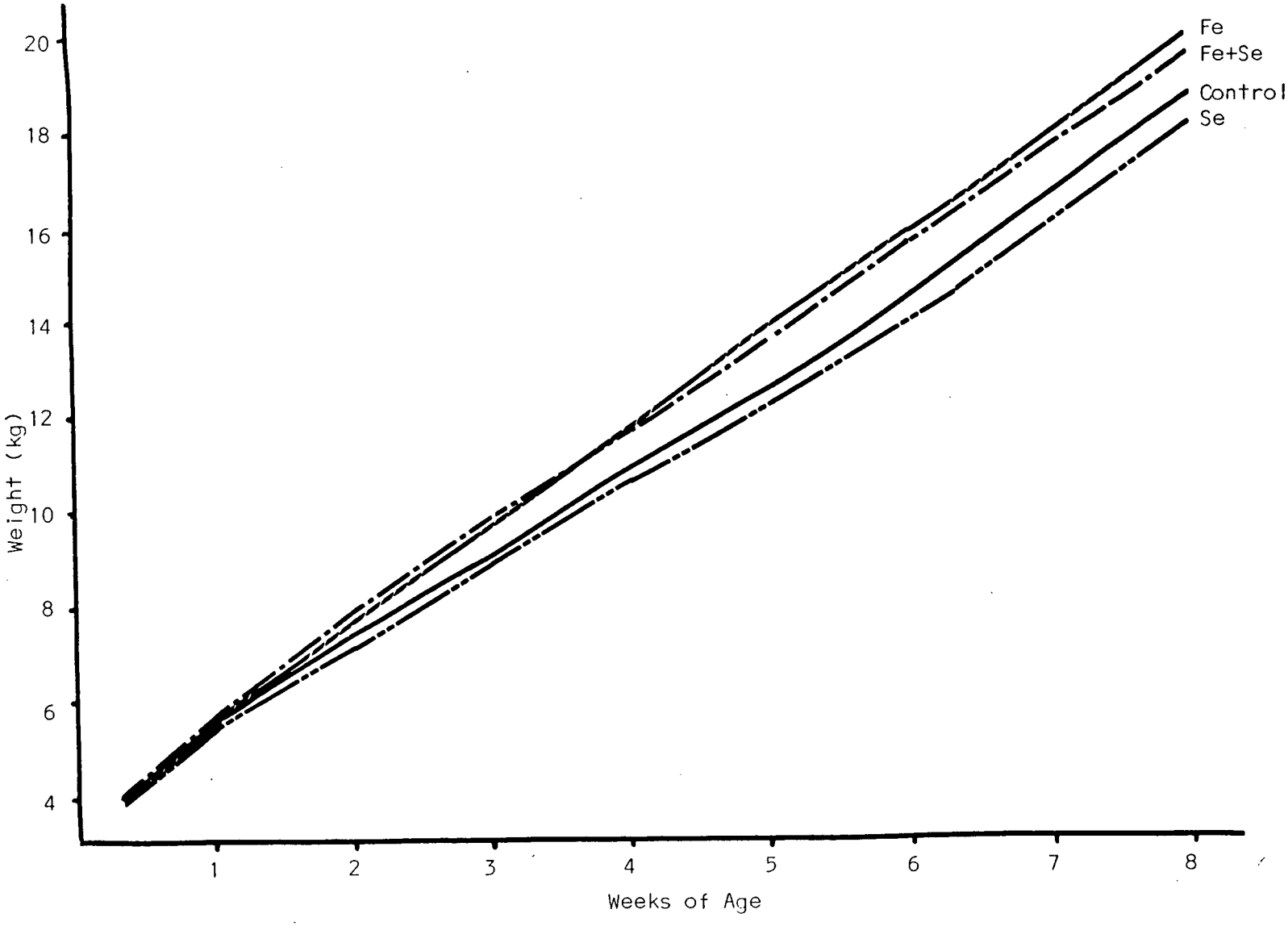

Figure 13. Effect of iron and selenium supplementation on lamb weight.

The treatment interactions with sex and with rearing were not significant ($P>0.05$), undoubtedly because iron and selenium combinations were considered together as treatments. However, if the effects of selenium are ignored (selenium having no apparent influence on weight gain at $P>0.05$), some very interesting results emerge. The fastest gaining categories of lambs - single lambs rather than twins, and males rather than females - appear to respond most favourably to iron treatment.

The 29 iron injected male lambs gained 17.2 kg., while the 29 male lambs not injected with iron gained 15.1 kg. This was a difference of 2.1 kg due to iron treatment of male lambs. In comparison, 31 female iron-treated lambs gained 14.3 kg compared to 13.9 kg for 32 female controls, a difference of only 0.4 kg.

Similarly, weight gain was improved by iron more markedly in single than in twin lambs. The 25 iron-injected single lambs gained 18.2 kg, while the 21 control singles gained 16.2 kg, a difference of 2 kg. The 35 iron-injected twins gained 13.9 kg, which was only 0.3 kg more than the 40 control twins gained.

These results indicate that fast-growing lambs may benefit most by iron treatment, particularly single and/or male lambs. Under conditions where intake is restricted, iron may not become limiting for growth. The lambs in this study were Dorset and Dorset/Finn crosses. Suffolk or Hampshire lambs might demonstrate a greater response to iron due to their high growth rate.

Iron supplementation may enhance growth rate in several ways. Logically, the availability of iron for hemoglobin, myoglobin and enzyme synthesis prevents a restriction in growth. Additionally, several deleterious side-effects of iron deficiency are avoided.

The gastrointestinal mucosa is especially susceptible to iron deficiency in young animals of most species so far studied, including human, dog, and pig. Atrophy of the gastrointestinal mucosa and deficiencies in tissue enzymes are common in iron deficient animals and may impair absorption of iron, vitamin A, and other nutrients (Beutler and Fairbanks, 1980; Guha et al., 1968). This problem has not been studied in the lamb. Another side-effect of iron deficiency is increased susceptibility to scours and enteritis, which could well contribute to growth depresson (Larkin and Hanran, 1983).

Plasma Protein Electrophoresis

The electrophoresis of plasma proteins yielded some interesting results, although the technique is not commonly used in nutrition studies. While serum proteins are sensitive to nutritional influences, in most cases the changes are subtle and difficult to detect and interpret (Kaneko, 1975). Over one hundred plasma proteins have been described. However, only five plasma protein bands are obtained from most species by agarose gel electrophoresis. Thus a change in a specific protein is rarely of sufficient magnitude to produce a clinical change in the associated protein band (Latner, 1975, p. 200).

As in the plasma profile samples, some storage deterioration was evident, which may have caused some protein denaturation. The total protein values were higher than those obtained in the plasma profile.

Difficulty in distinguishing peaks may have contributed to the experimental error. Fibrinogen trails the beta-globulin fraction (Kaneko,

1975), which tends to obscure the boundary between the beta- and gamma-globulin bands when plasma is used instead of serum. In a useful discussion of agarose gel electrophoresis, Johansson (1972) suggested adding heparin to the plasma sample to improve separation of beta lipoproteins. Otherwise, use of serum instead of plasma might have improved the resolution of the beta and gamma bands.

The densitometric tracings were very similar to those obtained by Keay and Doxey (1982). The alpha-1 and alpha-2 globulin zones could be easily subdivided, and the alpha-2 globulin zone was much greater quantitatively (Figure 14). In the present study, the alpha-1 zone was even smaller, and in some cases non-existent. The major difference was a noticeably smaller gamma globulin peak in the current study. As Keay and Doxey (1982) did not quantitate their results, the data couldn't be compared. Comparison of results with other literature values are summarized in Table V.

Many of the protein fractions were highly correlated with the total protein covariable (Table VI). Total protein was consistently related to albumin ($P<0.001$) and gamma globulin ($P<0.05$) levels.

Data from Replicate 2 were available only from 4 week old lambs. Resolution of bands was rather poor, compared to replicate 3, possibly because of longer time in storage. As a result, treatment effects were obscured. Data from Replicate 2 is shown in Table VII, but the discussion will center around the results from lambs at 2, 4 and 6 weeks of age in Replicate 3.

Gamma globulin ranged from 0.10-1.21 g/dℓ at 2 weeks, and 0.10-0.62 g/dℓ at both 4 and 6 weeks of age. Overall gamma globulin levels declined from 0.69 g/dℓ at 2 weeks to 0.37 g/dℓ at 6 weeks, reflecting the

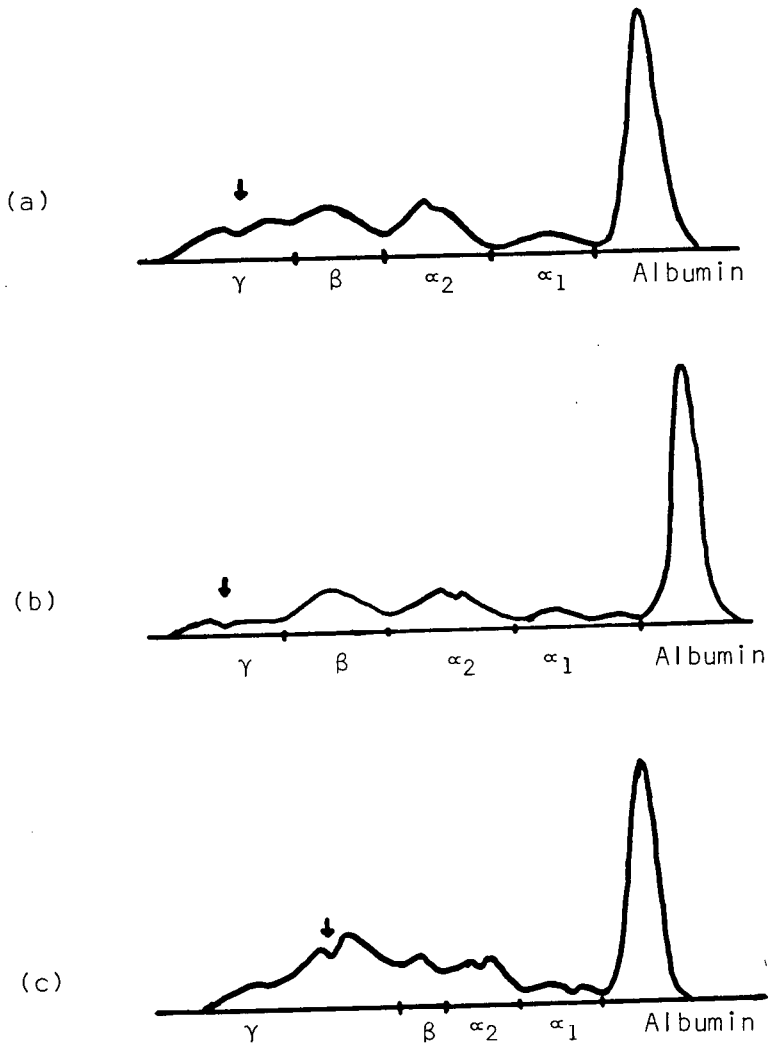

Figure 14. Densitometric traces of plasma proteins. Arrow indicates sample application slit.
a. sample from 2-week old lamb
b. sample from 4-week old lamb showing low γ-globulin content
c. comparative trace from normal sheep, Keay and Doxey (1982)

TABLE V.

Plasma protein electrophoresis results compared to literature values

PROTEIN (g/dℓ)	REPLICATE 3 RESULTS[a]			REPLICATE 2[a]	MITRUKA & RAWNSLEY[b]			IRFAN[c]
	2 weeks (40)[d]	4 weeks (42)	6 weeks (39)	4 weeks (39)	Normal male sheep	Normal female sheep	Normal range	3 months (10)
TOTAL	7.83 ± 0.16	7.70 ± 0.16	7.28 ± 0.15	6.41 ± 0.15	6.80 ± 0.30	7.20 ± 0.31	5.70 - 9.10	5.46
ALBUMIN	4.37 ± 0.12	4.52 ± 0.13	4.52 ± 0.10	3.66 ± 0.13	3.70 ± 0.35	3.81 ± 0.33	2.70 - 4.55	3.10
α-1 globulin	0.41 ± 0.03	0.38 ± 0.02	0.38 ± 0.02	0.43 ± 0.02	0.33 ± 0.08	0.38 ± 0.06	0.15 - 0.50	0.35
α-2 globulin	1.40 ± 0.08	1.39 ± 0.07	1.26 ± 0.05	0.99 ± 0.06	0.96 ± 0.13	0.73 ± 0.12	0.45 - 0.12	0.48
β-globulin	1.02 ± 0.06	0.93 ± 0.06	0.76 ± 0.07	0.77 ± 0.04	0.52 ± 0.10	0.91 ± 0.13	0.25 - 1.20	0.50
γ-globulin	0.69 ± 0.04	0.43 ± 0.02	0.37 ± 0.02	0.54 ± 0.03	1.33 ± 0.20	1.37 ± 0.25	0.82 - 1.90	1.03
ALBUMIN/ GLOBULIN	1.30 ± 0.05	1.50 ± 0.06	1.73 ± 0.07	1.36 ± 0.05	1.19 ± 0.20	1.12 ± 0.21	0.70 - 1.60	1.314

[a]Variation expressed as S.E.

[b]Mitruka, B.M. and Rawnsley, H.M. 1977. Data summarized from the literature; variation expressed as S.D.

[c]Irfan, M. 1967.

[d]Number of sheep samples

TABLE VI.

Effect of age and treatment on plasma proteins (Trial 3, Replicate 3)

Protein Fraction (g/dℓ)	Control	Se	Fe	Se/Fe	S.E.M.	Significance of Contrasts[1,2]	Significance of Main Effects[1,3]
2 weeks							
Gamma globulin	0.73	0.77	0.64	0.57	NS	NS	TP*
Beta Globulin	1.25	1.03	1.00	0.76	0.06	Se*, Fe**	TP*
Alpha-2 globulin	1.32	1.38	1.45	1.46	0.08	NS	TP*
Alpha-1 globulin	0.40	0.31	0.48	0.45	0.03	NS	NS
Albumin	4.42	4.29	4.44	4.29	0.12	NS	TP***
Albumin/Globulin	1.52	1.48	1.74	1.73	1.07	NS	Sex*
Covar. (TP)	7.83	7.80	8.00	7.61	0.16		
4 weeks							
Gamma globulin	0.40	0.50	0.43	0.41	0.02	NS	TP*
Beta globulin	1.17	1.02	0.86	0.57	0.06	Fe***	Sex*
Alpha-2 globulin	1.29	1.31	1.52	1.45	0.07	NS	TP***, Sex*
Alpha-1 globulin	0.37	0.46	0.34	0.34	0.02	NS	NS
Albumin	3.84	4.50	4.93	4.89	0.13	Fe***	TP***
Albumin/Globulin	1.48	1.94	1.94	2.20	0.09	Fe*	NS
Covar. (TP)	7.12	7.76	8.09	7.82	0.16		
6 weeks							
Gamma globulin	0.32	0.31	0.41	0.43	0.02	Fe*	TP**
Beta globulin	0.78	0.71	0.80	0.71	NS	NS	
Alpha-2 globulin	1.16	1.34	1.10	1.53	0.05	NS	Rear*
Alpha-1 globulin	0.41	0.35	0.34	0.43	0.02	NS	Rear*
Albumin	4.43	4.56	4.47	4.67	0.10	NS	TP***
Albumin/Globulin	2.22	2.11	2.23	1.98	0.08	NS	NS
Covar. (TP)	7.04	7.29	7.15	7.72	0.15		
n	11	11	12	8			

[1]Covariable = Total Protein; Main Effects tested were treatment, breed, sex, rearing.

[2]FE = Fe + Se/Fe vs. Control + Se treatments.
SE = Se + Se/Fe vs. Control + Fe treatments.
SE X FE = Control + SE/Fe vs. Fe + Se treatments.

[3]NS P>0.05, *P<0.05; **P<0.01; ***P<0.001.

TABLE VII.

Effect of treatment on plasma proteins at 4 weeks Trial 3 (Trial 3, Replicate 2).

Protein (g/dℓ)	Control	Se	Fe	Se/Fe	S.E.M.	Significant Effects[a,b]
Gamma globulin	0.51	0.62	0.50	0.52	0.03	TP**
Beta globulin	0.73	0.88	0.68	0.78	0.04	TP***
Alpha-2 globulin	1.01	1.02	1.08	0.88	0.66	TP***
Alpha-1 globulin	0.40	0.49	0.39	0.42	0.02	TP*
Albumin	3.51	3.57	3.96	3.60	0.13	TP***
Albumin/Globulin	1.75	1.62	1.95	1.85	0.16	NS
T.P. (Covariable)	6.14	6.59	6.69	6.23	0.15	
n	9	9	10	11		

[a]Iron, selenium, breed, sex, and rearing were non-significant sources of variation; TP = total protein (covariable).

[b]*P<0.05; **P<0.01; ***P<0.001.

catabolism of maternal antibodies and the apparent immaturity of the lambs' lymphoid system.

Sheep serum contains 3 major immunoglobulins: IgA, IgG, and IgM. IgG is quantitatively the most important, accounting for 89% of total serum immunoglobulin, compared to 1.5% and 9.5% for IgA and IgM respectively (Smith et al., 1975). The so-called gamma-globulin band in electrophoresis may contain only IgG, as IgA and IgM may extend into the beta-globulin band (Laurell, 1972).

The plasma gamma-globulin concentration in the young lamb may not be related to neonatal nutrition, except that the consumption of colostrum determines initial levels. The young lamb is born with negligible levels of serum immunoglobulins, so the ingestion of colostrum provides immunoglobulins, mainly IgG, which are important for the first weeks of life (Curtain, 1975). These maternal antibodies probably depress the endogenous antibody production, as occurs in calves (Husband and Lascelles, 1975).

Selenium deficiency is known to depress gamma-globulin levels in sheep (Keeler and Young, 1961). Given the major contribution of maternal gamma-globulin to lamb plasma levels, a response to selenium supplementation was not expected. Also, the dosage of selenium used was not sufficient to ensure optimum selenium levels in all treated lambs. Selenium did not significantly affect gamma globulins at 2, 4 or 6 weeks, although very low gamma globulin levels were found only in non-selenium supplemented lambs.

Surprisingly, iron significantly increased globulin levels at 6 weeks ($P<0.05$). Means were 0.43 ± 0.2 g/dℓ for the nineteen iron-treated lambs, and 0.32 ± 0.02 g/dℓ for twenty lambs not injected with iron.

As expected, the beta-globulin fraction was significantly lower in iron-treated lambs at 2 weeks ($P<0.01$) and 4 weeks ($P<0.001$). Transferrin is a major component of the beta-globulin band, and increases markedly during iron deficiency (Fielding, 1980). Transferrin was not affected at 6 weeks because of the recovery of plasma iron levels in control lambs by this time. With the exception of transferrin in iron deficiency, increases in beta-globulins are extremely rare (Kaneko, 1975).

The effect of iron status on plasma proteins with the exception of beta-globulin is obscure. In this study, iron very significantly ($P<0.001$) increased plasma albumin levels at 4 weeks, but not at 2 or 6 weeks. Hypoalbuminemia is common in iron deficient infants, and may be caused by a malabsorption syndrome (Naiman et al., 1964).

Very little information is available on the effects of selenium on plasma proteins, other than the dramatic increase in certain plasma enzymes during white muscle disease. Keeler and Young (1961) found a marked increase in alpha-globulin and a decrease in beta-globulin as well as gamma-globulins in selenium-deficient sheep. Mice deficient in selenium, vitamin E and cystine had decreased fibrinogen and total plasma protein levels (Ganther et al., 1976).

The significant depression ($P<0.05$) of the beta-globulin fraction at 2 weeks by Se treatment was virtually of the same magnitude as the effect of iron on transferrin. Means were 1.25 g/dℓ for control lambs, 1.03 g/dℓ for +Se lambs, 1.00 g/dℓ for +Fe lambs, and 0.76 g/dℓ for +Se+Fe lambs. Selenium treatment also tended to reduce beta-globulin levels at 4 weeks, but due to the high variability this effect was not significant ($P<0.05$). It is possible that a larger dosage of selenium might give more conclusive

results. If selenium treatment does depress beta-globulin levels, this would conflict with the results of Keeler and Young (1961).

The influence of selenium cannot be explained at this point. A measureable effect of selenium on the beta-globulin band would most likely involve fibrinogen, transferrin, hemopexin, or complement fractions (C_3, C_4, and others), as these are the major proteins in this band. Possibly a major increase in plasma enzymes might increase the beta fraction in selenium deficient lambs, but there was no evidence of clinical WMD, nor were AT and LDH significantly increased in the plasma profile at 4 weeks.

Selenium also appeared to affect many of the other protein fractions, but results were never significant. For example, alpha-2 globulin means at 6 weeks were 1.13±0.05 g/dℓ for non-Se lambs, and 1.43±0.05 g/dℓ for +Se lambs, with $P<0.06$.

The limitations of the electrophoresis technique, condition of the samples, number of lambs compared to treatments, and the low selenium dosage were some of the problems faced. The absence of significant Se treatment effects for most plasma proteins was therefore not conclusive. More information is needed on this subject, using a larger number of lambs to compensate for the high variability in the data, and using clearly differentiated levels of selenium. The effects of iron on gamma globulin and albumin, and of selenium on beta globulin, were unexpected and would bear further investigation.

Effect of Selenium on Epidemiology of Soremouth

Throughout Replicate 3 of Trial 3, lambs were observed weekly during sampling for the presence of soremouth lesions. Severity was evaluated

subjectively on the scale of 0-5. Because of the subjective nature of these observations a statistical analysis was not carried out. However, some interesting trends were evident, and further investigation with a greater number of lambs is warranted.

Soremouth is an infectious poxvirus disease of sheep and goats, in which pustular lesions develop on the lips, oral mucous membranes, and udder. Synonyms include contagious pustular dermatitis and orf. Transmission occurs through minor abrasions or trauma. Pustules develop within 4 days of infection, scabs build up, and healing takes about 3 weeks. Soremouth may be debilitating in the case of severe secondary bacterial infections, or through interference with eating and drinking (Mohanty and Dutta, 1981).

Iron did not appear to have any effect on soremouth. The same number of lambs treated with iron were infected as those not treated. This was contrary to expectation as mouth lesions are commonly observed in iron deficient people (Fletcher et al., 1975) and also in iron deficient calves (Blaxter et al., 1957).

Selenium seemed to influence the incidence, duration and severity of the disease (Table VIII). Selenium-injected lambs were less likely to develop soremouth, and appeared to contract the disease at a slightly older age, and recover faster.

The mean plasma selenium level was greatest in lambs which did not develop soremouth, in both the +Se and -Se groups. The mean Se level was 0.112 ppm in healthy +Se lambs, compared to .087 ppm in soremouth-infected +Se lambs. Additionally, in +Se lambs with soremouth lesions, selenium level appeared to be negatively correlated with the severity of the disease.

TABLE VIII.

Effects of selenium treatment on epidemiology of soremouth infection

Treatment	+ Selenium	Control
Number of lambs	20	23
Incidence		
-2 weeks of age	10.0%	17.4%
-3 weeks	15.0%	34.8%
-4 weeks	20.0%	47.8%
-5 weeks	30.0%	56.5%
-6 weeks	15.0%	21.7%
-7 weeks	15.0%	17.4%
-8 weeks	5.0%	0.0%
Severity of disease in infected lambs[a]		
-2 weeks	2.00	2.25
-3 weeks	2.66	2.75
-4 weeks	2.00	2.45
-5 weeks	1.50	2.30
-6 weeks	1.30	1.60
-7 weeks	4.30[b]	1.25
-8 weeks	1.00	-
Avg. duration of disease in infected lambs	2.3 weeks	2.9 weeks
Avg. age at infection	4.4 weeks	3.6 weeks
% lambs infected	50% (10/20)	70% (16/23)
Avg. 4 week plasma Se in soremouth lambs	0.087 ppm	0.076 ppm
Avg. 4 week plasma Se in healthy lambs	0.112	0.093

[a]Severity ranked on sale of 0 (no soremouth lesions) to 5 (severe lesions).

[b]High score due to 2 lambs with +5 scores due to late development of soremouth.

While these results are far from conclusive, they do suggest that selenium has an impact on the epidemiology of soremouth. Possibly a higher dosage of selenium resulting in optimum plasma selenium levels, could significantly affect the resistance of lambs to certain diseases. The influence of selenium on diseases of high morbidity and low mortality such as scours or soremouth could explain the benefit of selenium treatment to unthrifty flocks and/or growth rate when obvious deficiency does not occur.

Hemagglutinaton Results

The hemagglutination titer of lambs injected with chicken red blood cells (CRBC) from four to eight weeks of age was influenced by sex, duration of stimulus, interactions of selenium with sex and possibly iron. Data are given in Appendix 12 and Appendix 13.

The sheep responded immunologically to repeated antigenic stimulus from CRBC with increasing hemagglutination titers. At the time of the initial injection at 4 weeks of age, the titer was 0. With progressive injections, the mean hemagglutination titer increased to 4.8±0.4, 15.9±0.4, 32.7±0.3, and 42.1±0.3 at 5, 6, 7, and 8 weeks of age. Because of the very slow initial increase in hemagglutination titer, it may be necessary to continue the experiment for a longer period of time in order to accurately assess treatment effects.

Iron and selenium did not significantly ($P>0.5$) affect titer at any time, as indicated by figures 15 and 16. However, selenium-treated lambs had higher mean titers throughout the trial. Means for controls compared to +Se lambs were 4.1±0.5 vs. 5.6±0.6 at 45 weeks; 15.6±0.4 vs. 16.2±0.7 at 6 weeks; 28.5±0.4 vs. 38.2±0.4 at 7 weeks; and 36.6±0.4 vs. 48.6±0.4 at 8

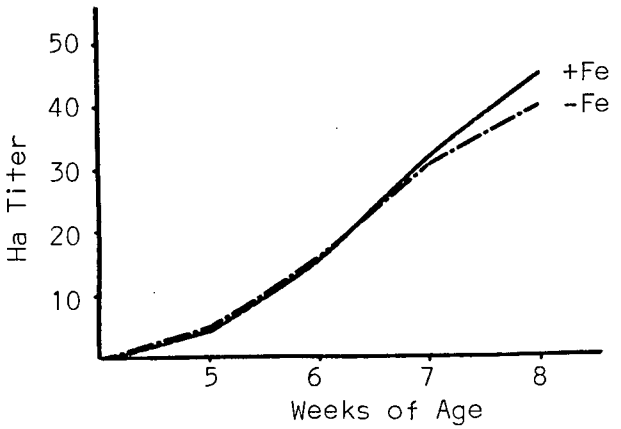

Figure 15. Effect of iron on HA titer.

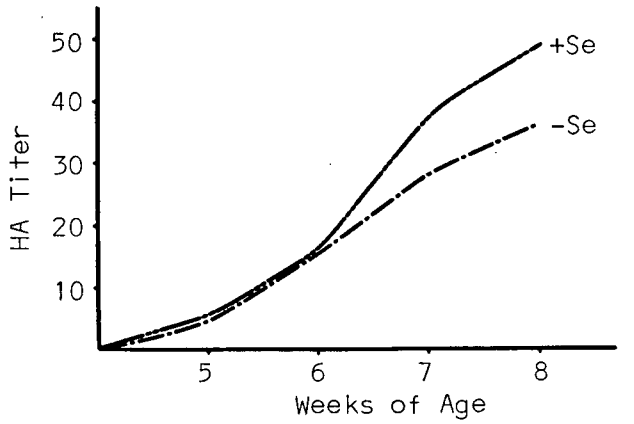

Figure 16. Effect of Se on HA titer.

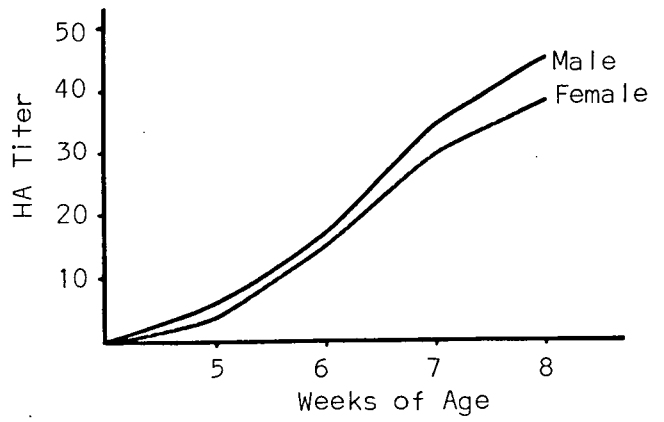

Figure 17. Effect of sex on HA titer.

weeks. Not surprisingly, in view of the large standard error and also the lack of effect of selenium on female lambs, the effect of selenium failed to be significant ($P>0.05$).

Breed and rearing did not influence titers, but sex resulted in significant effects ($P<0.05$) at 5 and 7 weeks of age (Figure 17). Male lambs had higher hemagglutination titers at all times. Means were 3.4 ± 0.5, and 5.9 ± 0.5, for females and males at 5 weeks. By 8 weeks, mean titer in females was 38.6 ± 0.4, compared to 45.4 ± 0.4 in males. An interaction between selenium and sex may account for this effect. Selenium seemed to have little if any effect on titer in females, but markedly increased titer in males (Figure 18). Males not treated with Se had titers similar to those of females. Thus the mean titer was highest for selenium-treated males throughout the trial.

While iron did not affect titer, the selenium X iron interaction may have become significant if the experiment had been continued. Iron and selenium are both involved in the immune response, and may possibly interact additively or synergistically (Figure 19).

Selenium and/or vitamin E significantly raised the hemagglutination titer to sheep red blood cells in a study with weanling swine (Peplowski *et al.*, 1980). Peplowski *et al.* (1980) found that an immediate source of these nutrients, such as injections or a high dietary concentration provided on a short-term basis, enhanced the immune response in young pigs marginally deficient in Se and/or vitamin E. A higher titer was obtained with high dietary Se than with the high Se dosage injected.

The difference in response to selenium between our results and those of Peplowski *et al.* (1980) can be explained by several factors, mainly the

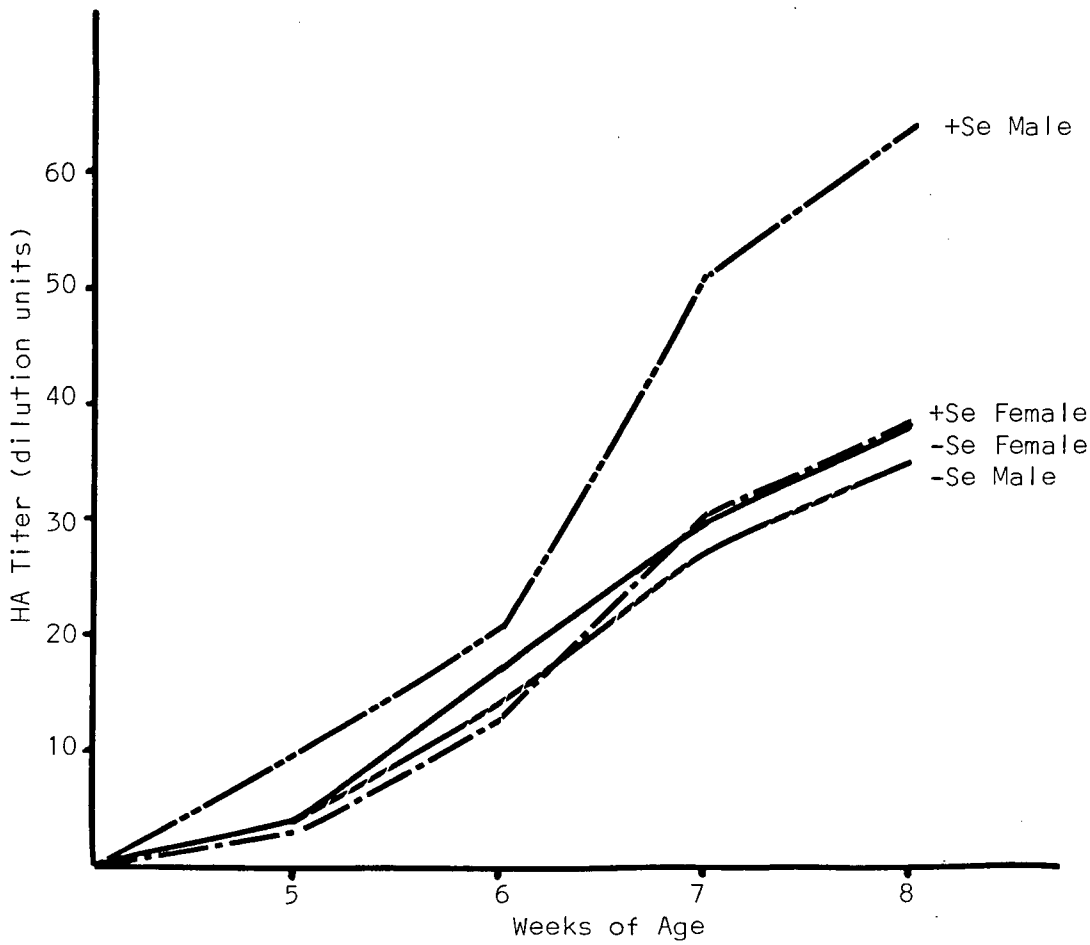

Figure 18. Effect of selenium x sex interaction on hemagglution titer.

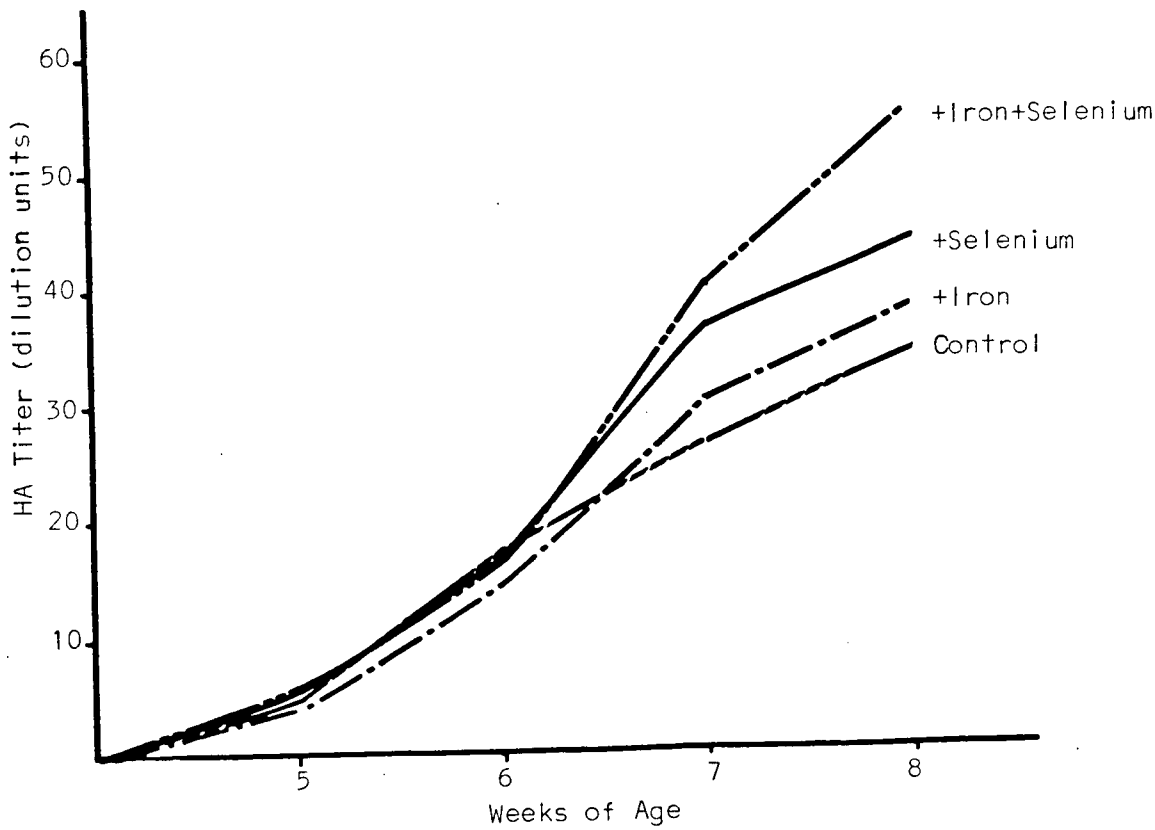

Figure 19. Effect of iron x selenium interaction on HA titer.

dosage and timing of selenium use, in addition to the presence of Se interactions. In the present study 1.5 mg Se was injected at birth, and CRBC antigen was injected at 4-4.5 weeks of age and weekly thereafter; by this time, while plasma selenium was still significantly higher in the +Se group, many lambs in both groups were marginally deficient in Se status. In contrast, Peplowski et al. (1980) used weaned swine of the same age, the same schedule of antigen injections using a similar antigen, but the Se treatment simultaneously provided 0.50 ppm Se, which is five times the dietary requirement. Similarly, Se dosage for injected weanling pigs was 6.0 mg, which was four times the level we used for lambs of similar size. Work with mice which demonstrated an immunologic response to selenium, also involved high levels of dietary selenium (Spallholz et al., 1973; Spallholz et al., 1974; Spallholz et al., 1975).

The hemagglutination technique is very sensitive but important limitations include the "occasional lack of reproducibility, qualitative rather than quantitative nature, and occasional non-specificity" (Stavitsky, 1954). According to Stavitsky (1954), its sensitivity can be a liability, in that small amounts of heterologous antibody or antigen in antisera or test antigen may confuse the estimation of the major antibodies.

The adaptation of the hemagglutination test for this experiment, specifically the use of CRBC antigen, probably induced production of heterologous antibody. Because the maximal size of a specific antigenic determinant is equivalent to four to six amino acids or simple sugars, the potential number of different combining sites on the red cell membrane is extremely large (Garvey et al., 1977, p. 133). In effect, then, a

multitude of potential antigens, each at extremely low and variable concentrations, were then injected in each weekly dose of 1 ml cell suspension.

The presence of heterologous antibodies frustrated the interpretation of the serial dilution results. Instead of a relatively clear cut distinction between positive and negative readings, frequently as many as 4 or 6 dilutions in sequence would appear intermediate between positive and negative. This contributed to poor reproducibility of the technique.

CONCLUSIONS

This study has investigated several aspects of iron and selenium supplementation of newborn lambs. Iron supplementation had a profound influence on lamb metabolism, apparently affecting most blood metabolites measured, as well as beta-globulin (transferrin), plasma iron, Hb, and PCV. Iron supplementation unexpectedly depressed plasma selenium levels, and also enhanced gamma globulin production at 6 weeks of age, indicating that lymphoid tissues may be particularly vulnerable to preweaning iron deficiency, as in rats (Baggs and Miller, 1973). However, iron had little effect on hemagglutination titer except in selenium-treated lambs.

Injection of 500 mg Fe significantly ($P<0.05$) increased hemoglobin from 2 to 11 weeks of age in Trial 1, and from 1 to 8 weeks in Trial 3. While iron dosages of either 250 or 500 mg prevented the depression of Hb and PCV from birth to 30 days, plasma iron was significantly higher ($P<0.05$) at 4 weeks in lambs receiving 500 mg Fe.

A significant proportion of control lambs were anemic at 3-4 weeks of age in all studies. The number of lambs defined as anemic varied according to the trial, and the parameter used. For example, 42%, 21% and 50% of the control lambs were anemic in Trial 2 based on the criteria of <28% PCV, <9.2 g/dℓ Hb, and <100 µg/dℓ plasma iron. The assessment of anemia should therefore be based on normal values for the flock rather than literature values. PCV is highly correlated with Hb in lambs 0-6 weeks of age; consequently, PCV can be measured instead of Hb to assess iron status.

The two selenium treatments did not adequately contrast levels of selenium status. As plasma selenium levels in both the selenium-injected

lambs and control lambs varied from marginal to adequate levels, the effects of selenium treatment could not be considered conclusive. Probably some selenium treatment effects were obscured.

Selenium treatment did not affect Hb and PCV values significantly ($P>0.05$). While selenium may have a role in heme metabolism in the bone marrow, the circulating hemoglobin concentration may not be affected except at markedly deficient levels of Se.

A weight response to iron treatment, but not selenium treatment, was observed in Trials 1 and 3. In Trial 3, iron-treated lambs were slightly heavier than controls from 2 weeks ($P<0.05$) to 8 weeks ($P<0.01$) of age. Trial 1 was continued to 11 weeks, and indicated that control lambs began to catch up to iron-treated lambs after 8 weeks of age. A weight response to iron treatment was consistently observed in the faster growing lambs, ie. male and/or single lambs, and not in twin lambs. Iron treatment did not influence average weight gain in Trial 2, possibly because of the large numbers of twin lambs. The very low incidence of disease during that particular trial may also have been a factor.

Preliminary information was provided on the effect of iron deficiency on the lamb plasma profile. Surprisingly, no comprehensive study of the plasma profile in iron deficiency has apparently been published for any species, including man. However, comparison of the data with the literature on human medicine indicates that iron deficiency affects plasma metabolites similarly in lambs and humans. In both species, iron deficiency increases plasma glucose and alkaline phosphatase, while decreasing plasma iron and cholesterol. Other comparative data was lacking for humans.

Iron had a major impact on the plasma profile in Trial 2, and P_i, glucose, cholesterol, total protein, AP, AT, and plasma iron responded linearly to iron dosage. Many parameters (glucose, BUN, cholesterol, TP, AP, and AT) were also significantly correlated with plasma iron. These results suggest that iron status may broadly influence metabolism through multiple roles in tissue enzyme systems. Many iron enzymes are reduced at an early stage of iron depletion, and therefore would be affected by even a mild degree of deficiency. Levels of iron sufficient for Hb maintenance may not provide sufficent iron for other functions, as Hb may have priority for iron. A weight response to iron supplementation may depend on the provision of adequate iron for tissue enzymes, which may explain conflicting results of iron on weight at dose levels of 150 and 300 mg Fe used in earlier studies.

Iron and selenium supplementation affected plasma protein fractions. As expected, iron deficiency resulted in significantly higher ($P<0.01$) β-globulin levels at 2 and 4 weeks of age, due to increased transferrin productin. Iron injection increased ($P<0.001$) albumin at 4 weeks, and also increased γ-globulin levels at 6 weeks of age. An earlier effect of iron may not be observed as γ-globulin production is depressed in the first weeks of life; therefore it would be interesting to follow γ-globulin levels after 6 weeks. Selenium treatment significantly ($P<0.05$) depressed β-globulin levels at 2 weeks, and the reason for this was unknown.

Effects of iron and selenium on disease resistence were measured by susceptibility to soremouth, and anti-CRBC hemagglutination titer. Selenium treatment appeared to influence susceptibility of lambs to soremouth infection. The response of lambs to antigenic challenge from chicken RBCs

was apparently influenced by selenium as well, even though Se status of the Se-injected lamb was suboptimal at time of challenge. Iron treatment had a lesser influence, possibly because lambs were already eating creep-feed containing adequate dietary iron during this part of the trial. Iron may be relatively more important in resistance to such diseases as E. coli scours, in which phagocytosis is more crucial than humoral immunity. Scours occurred in most lamb crops, but the relationship of scours to iron treatment, and its effect on growth, could not be measured.

The data presentd in this study suggest that iron deficiency in suckling lambs affects the overall metabolism, growth rate, and health of the lambs. Anemia per se does not appear to be the major concern in iron deficiency. Other effects of iron deficiency include suboptimal functioning of many iron enzyme systems, impairment of various immune systems, and possibly malabsorption syndromes and/or gastrointestinal lesions. Quantitatively, these conditions may be more important and more persistent than the anemia. The demonstration of anemia in suckling lambs is an indication that less visible, concurrent iron deficiency syndromes may reduce productivity and compromise health.

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APPENDIX

APPENDIX 1.

Composition of creep-feed

A. Ingredient Composition

Barley	80.0%
Soybean Meal	10.0%
Butterfields 32% Cattle Supplement, Reg. No. 5273	10.0%

B. Nutrient Composition, As-fed Basis (calculated)

Protein[1]	17.0%
Fiber	5.5%
Calcium	0.33%
Phosphorus	0.20%
Iron	95 ppm

[1]not more than 2% equivalent protein from urea.

APPENDIX 2.

Procedures used in analysis of plasma constituents

METABOLITE	REFERENCE
Calcium	Kessler, G. and Wolfman, M. 1964. Clin. Chem. 10:686
Inorganic Phosphate	Hurst, R.O. 1964. Can. J. Biochem. 42:287
Glucose	Bondar, R.J.L. and Mead, D.C. 1974. Clin. Chem. 20:586.
BUN	Marsh, W.H., Fingerhut, B. and Miller, H. 1965. Clin. Chem. 11:624.
Cholesterol	Levine, J., Morganstern, S. and Vlastelica, D. 1967. Automation Anal. Chem., Technicon Symp. 1967.
Total Protein	Skeggs, L.T. and Hochstrasser, H. 1964. Clin. Chem. 10:918.
Albumin	Doumas, B.T., Watson, W.A. and Biggs, H.G. 1971. Clin. Chem. Acta 31:87.
Alkaline Phosphatase	Morganstern, S., Kessler, G., Averbach, J. Flor, R.V., and Klein, B. 1965. Clin. Chem. 11:876.
Lactate Dehydrogenase	Hochella, N.J. and Weinhouse, S. 1965. Analytical Biochem 13:322.
Aspartate Transaminase	Morganstern, S., Oklander, M., Averbach, J., Kaufman, J. and Klein, B. 1966. Clin. Chem. 12:95.
Plasma Iron	Carter, P. 1971. Analytical Biochem. 40:450.

APPENDIX 3.

Effect of iron dextran injection on lamb weight, hemoglobin and packed cell volume from birth to 11 weeks (Trial 1).

	WEIGHT (kg ± SE)			HEMOGLOBIN (g/dℓ ± SE)			P.C.V. (% ± SE)		
Age	Control	Iron		Control	Iron		Control	Iron	
	n=17	n=18		n=17	n=18		n=17	n=18	
Birth	3.9 ± 0.2	4.2 ± 0.3	NS†	13.7 ± 0.6	14.5 ± 0.4	NS	37.8 ± 1.5	40.2 ± 1.2	NS
1 week	4.5 ± 0.3	5.3 ± 0.3	NS	12.3 ± 0.5	12.9 ± 0.2	NS	33.5 ± 1.1	35.8 ± 0.8	NS
2 weeks	6.2 ± 0.4	7.4 ± 0.5	NS	10.9 ± 0.4	13.3 ± 0.2	***	31.2 ± 1.1	38.8 ± 0.8	***
3 weeks	8.1 ± 0.4	9.5 ± 0.6	NS	10.7 ± 0.4	13.5 ± 0.2	***	30.9 ± 1.3	39.4 ± 0.8	***
4 weeks	9.9 ± 0.5	11.4 ± 0.7	NS	11.1 ± 0.3	13.7 ± 0.3	***	32.2 ± 0.9	39.5 ± 0.7	***
5 weeks	11.5 ± 0.6	13.5 ± 0.8	NS	11.4 ± 0.3	13.7 ± 0.2	***	32.8 ± 0.9	39.0 ± 0.7	***
6 weeks	13.4 ± 0.6	15.6 ± 1.0	*	11.9 ± 0.3	13.7 ± 0.3	***	34.4 ± 0.8	39.3 ± 0.7	***
7 weeks	15.2 ± 0.7	17.9 ± 1.1	*	12.0 ± 0.2	13.2 ± 0.2	***	34.9 ± 0.6	38.3 ± 0.5	***
8 weeks	17.2 ± 0.8	19.8 ± 1.2	*	12.0 ± 0.2	12.8 ± 0.2	**	35.4 ± 0.7	37.3 ± 0.6	*
9 weeks	18.8 ± 0.8	21.4 ± 1.2	*	12.2 ± 0.1	12.6 ± 0.2	*	35.5 ± 0.4	36.9 ± 0.7	*
10 weeks	20.5 ± 0.8	23.3 ± 1.3	NS	12.3 ± 0.2	12.8 ± 0.2	*	36.0 ± 0.7	36.8 ± 0.6	NS
11 weeks	22.3 ± 0.9	24.6 ± 1.3	NS	12.0 ± 0.3	12.6 ± 0.2	*	35.2 ± 0.7	36.9 ± 0.6	*

†NS $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$

APPENDIX 4.

Effect of three levels of iron treatment on hemoglobin and packed cell volume between birth and weaning (Trial 2)

	TREATMENTS			Significance of contrasts[1]
	Control	250 mg Fe	500 mg Fe	
	20	24	24	
Hemoglobin (g/dℓ ± SE)				
2 days	13.6 ± 0.4	12.9 ± 0.4	13.3 ± 0.4	NS
16 days	10.6 ± 0.3	13.2 ± 0.3	13.5 ± 0.2	C_1***
30 days	10.3 ± 0.3	13.5 ± 0.4	14.5 ± 0.2	C_1***,C_2*
44 days	11.8 ± 0.2	12.4 ± 0.2	13.7 ± 0.2	C_1***,C_2***
P.C.V. (% ± SE)				
2 days	38.0 ± 1.0	36.6 ± 1.0	37.4 ± 1.1	NS
16 days	29.3 ± 1.0	37.2 ± 0.7	38.6 ± 0.6	C_1***
30 days	29.2 ± 0.9	37.2 ± 0.7	41.0 ± 0.7	C_1***,C_2*
44 days	34.0 ± 0.7	36.7 ± 0.5	40.0 ± 0.7	C_1***,C_2**

[1]Predetermined orthogonal contrasts were as follows:
C_1 = Control vs. both Fe treatments
C_2 = 250 mg Fe vs. 500 mg Fe
NS = not significant, $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

APPENDIX 5.

Regression analysis of Trial 2 Plasma Profile Data: Partial Correlation Coefficients

Metabolite n=65	POTENTIAL INDEPENDENT VARIABLES						
	Birth Weight	Weight Gain	Hb	PCV	Iron	Glucose	T.P.
P_i	NS	NS	NS	NS	NS	NS	-
Glucose	NS	NS	-0.306*	-0.253*	-0.398***	-	-
B.U.N.	0.460***	NS	NS	NS	0.265*	-	-
Cholesterol	NS	NS	0.359**	0.432***	0.336**	-0.336**	-
Total Protein	NS	NS	-0.328**	-0.292*	-0.424***	-	-
Albumin	NS	NS	NS	NS	NS	-	NS
A.P.	-0.476	NS	-0.427***	-0.417***	-0.468***	-	-
L.D.H.	NS	NS	NS	NS	NS	-	-
A.T.	NS	-0.298*	NS	NS	0.288*	-	-
Iron	0.329**	NS	0.484***	0.477***	-	-	-

*P<0.05
**P<0.01
***P<0.001

APPENDIX 6.

Regression Analysis of Trial 2 Plasma Profile Data: Regression Equations

METABOLITE[1]	REGRESSION EQUATION	R-SQUARED	F-PROBABILITY
Glucose	$\hat{Y} = 98.1274 + 0.6981W - 0.0611\ I$	0.231	0.000291
Cholesterol	$\hat{Y} = 60.8949 - 1.5701W + 2.1871P$	0.258	0.000094
Total Protein	$\hat{Y} = 5.8606 - 0.0024I$	0.180	0.000427
Alkaline Phosphatase	$\hat{Y} = 2503.7841 - 85.6757B - 67.5236H$	0.349	0.000002
Aspartate Transaminase	$\hat{Y} = 130.1291 - 2.2732W + 0.1211I$	0.200	0.000993
Iron	$\hat{Y} = -126.3880 + 10.7648B + 17.1745H$	0.297	0.000018

where $\hat{Y}$ = dependent variable (glucose, cholesterol, etc.)
W = independent variable weight gain (weight at sampling - birth weight)
I = independent variable plasma iron
P = independent variable packed cell volume
B = independent variable birth weight
H = independent variable hemoglobin

[1] P_i, Albumin and LDH not included due to lack of significant correlations.

APPENDIX 7.

Effect of iron level on lamb weight (Trial 2).

Age	Mean	TREATMENTS			Significance of Contrasts and Main Effects[1,2]
		Control	250 mg Fe	500 mg Fe	
	$\bar{X}$ ± S.E. (kg)				
2 days	4.42 ± 0.13	4.4 ± 0.3	4.4 ± 0.2	4.4 ± 0.2	Age***, B.Wt.***
9 days	6.5 ± 0.2	6.4 ± 0.3	6.6 ± 0.3	6.4 ± 0.4	Rear*, Age**, B.Wt.***
16 days	8.5 ± 0.3	8.3 ± 0.3	8.8 ± 0.3	8.3 ± 0.5	Rear**, Age*, B.Wt.***
3 days	10.5 ± 0.3	10.2 ± 0.5	10.9 ± 0.5	10.2 ± 0.5	Rear**, B.Wt.***
0 days	12.0 ± 0.3	11.8 ± 0.5	12.6 ± 0.6	11.8 ± 0.6	Rear***, B.Wt.***
8 days	14.0 ± 0.4	13.7 ± 0.6	14.6 ± 0.7	13.8 ± 0.7	Rear**, TrxSex*, Age**, B.Wt.***
44 days	16.0 ± 0.4	15.6 ± 0.6	16.5 ± 0.7	15.8 ± 0.9	Rear**, B.Wt.***
1 days	18.0 ± 0.5	17.6 ± 0.6	18.6 ± 0.8	17.7 ± 0.9	Rear**, B.Wt.***
n	68	20	24	24	

[1]Covariables were age and birthweight; main effects were treatment, breed, sex, rearing.

[2]*P<0.05; **P<0.01; ***P<0.001.

APPENDIX 8.

Effect of iron and selenium on hemoglobin (Trial 3).

Age	TREATMENTS				Significance of Contrasts[1]	Significant Main Effects[2]
	Control	SE	FE	SE + FE		
	$\bar{X}$ ± S.E. (g/dℓ)					
n	31	30	31	29		
2 days	13.5 ± 0.4	13.3 ± 0.4	13.8 ± 0.3	14.0 ± 0.3	NS	NS
1 week	11.4 ± 0.4	11.7 ± 0.3	13.0 ± 0.3	12.7 ± 0.3	FE***	NS
2 weeks	10.7 ± 0.3	10.5 ± 0.3	13.2 ± 0.2	13.2 ± 0.2	FE***	NS
3 weeks	10.9 ± 0.3	10.6 ± 0.5	13.6 ± 0.3	13.4 ± 0.2	FE***	NS
4 weeks	11.0 ± 0.3	11.0 ± 0.3	13.4 ± 0.3	13.1 ± 0.3	FE***	NS
5 weeks	11.4 ± 0.2	11.7 ± 0.3	13.8 ± 0.2	13.7 ± 0.2	FE***	NS
6 weeks	11.9 ± 0.2	11.8 ± 0.2	13.5 ± 0.2	13.4 ± 0.2	FE***	NS
7 weeks	12.0 ± 0.2	12.0 ± 0.2	13.2 ± 0.2	13.2 ± 0.2	FE***	NS
8 weeks	12.3 ± 0.2	12.4 ± 0.2	12.9 ± 0.2	12.9 ± 0.2	FE**	NS

[1]Predetermined orthogonal contrasts were as follows: FE = Control + Se vs. Fe + (Se + Fe); SE = Se + (Se + Fe) vs. Control + Fe; SE X FE = Control + (Se + Fe) vs. Fe + Se; NS>0.05; *P<0.05; **P<0.01; ***P<0.001.

[2]Main effects tested were replicate, treatment, breed, sex, and rearing.

APPENDIX 9.

Effect of iron and selenium on packed cell volume (Trial 3).

Age	TREATMENTS				Significance of Contrasts[1]	Significant Main Effects[2]
	Control	SE	FE	SE + FE		
	$\bar{X}$ ± S.E. (% PCV)					
n	31	30	31	29		
2 days	37.9 ± 0.9	37.0 ± 1.0	39.0 ± 0.8	39.3 ± 0.9	NS	NS
1 week	31.0 ± 0.7	31.7 ± 0.8	36.2 ± 0.7	35.8 ± 0.6	FE***	Rear***
2 weeks	30.8 ± 0.6	30.6 ± 0.8	39.1 ± 0.6	38.5 ± 0.7	FE***	Rear*
3 weeks	31.2 ± 0.7	31.9 ± 1.0	41.1 ± 0.6	39.2 ± 0.7	FE***	NS
4 weeks	32.3 ± 0.7	32.5 ± 0.8	40.5 ± 0.6	39.4 ± 0.6	FE***	NS
5 weeks	34.1 ± 0.7	34.7 ± 0.7	40.2 ± 0.5	39.6 ± 0.6	FE***	Rep*
6 weeks	35.1 ± 0.6	35.9 ± 0.6	39.6 ± 0.5	39.0 ± 0.6	FE***	Sex*
7 weeks	36.1 ± 0.6	36.1 ± 0.5	38.8 ± 0.4	38.6 ± 0.6	FE***	Sex*
8 weeks	36.3 ± 0.5	36.5 ± 0.5	37.8 ± 0.5	37.4 ± 0.5	FE*	Sex*

[1]Predetermined orthogonals contrasts were as follows: FE = Control + Se vs. Fe + (Se + Fe); SE = Se + (Se + Fe) vs. Control + Fe; SExFE = Control + (Se + Fe) vs. Fe + Se; NS>0.05; *P<0.05; **P<0.01; ***P<0.001.

[2]Main effects tested were replicate, treatment, breed, sex, and rearing.

APPENDIX 10.

Regression equations for hemoglobin versus packed cell volume (Trial 3).

†AGE	REGRESSION EQUATION	*R^2
2 days	$\hat{Y} = 0.4851 + 0.3419X$	0.8624
4 days	$\hat{Y} = 0.4429 + 0.3738X$	0.8829
6 days	$\hat{Y} = -2.000 + 0.4128X$	0.9136
8 days	$\hat{Y} = -0.0625 + 0.3609X$	0.8436
2 weeks	$\hat{Y} = 0.2120 + 0.3346X$	0.9228
3 weeks	$\hat{Y} = -0.2123 + 0.3397X$	0.8840
4 weeks	$\hat{Y} = 0.6801 + 0.3187X$	0.7882
5 weeks	$\hat{Y} = 0.5235 + 0.3292X$	0.8568
6 weeks	$\hat{Y} = 1.1992 + 0.2861X$	0.7342
7 weeks	$\hat{Y} = 3.417 + 0.2467X$	0.5614
8 weeks	$\hat{Y} = 4.084 + 0.2334X$	0.5409

where $\hat{Y} = a + bX$

and $\hat{Y}$ = dependent variable (Hb)
a = constant, or intercept
b = regression coefficient
X = independent variable (PCV)

*R^2, the coefficient of determination, represents the proportion of the total treatment sum of squares accounted for by regression.

†Number of observations each at 4 days and 6 days = 43 lambs. All other periods include data from all 121 lambs in Trial 3.

APPENDIX 11

Effect of iron and selenium on lamb weight (Trial 3).

Age	TREATMENTS				Significance of Contrasts[1]	Significant Main Effects[2]
	Control	SE	FE	SE + FE		
	$\bar{X}$ ± S.E. (kg)					
n	31	30	31	29		
2 days	3.9 ± 0.2	3.8 ± 0.1	4.1 ± 0.2	3.9 ± 0.2	NS	Rear***
1 week	5.5 ± 0.2	5.4 ± 0.2	5.8 ± 0.3	5.7 ± 0.3	NS	Rep***, Rear***
2 weeks	7.4 ± 0.3	7.2 ± 0.3	8.0 ± 0.3	7.8 ± 0.4	FE*	Rep***, Rear***
3 weeks	9.1 ± 0.3	8.9 ± 0.3	9.9 ± 0.5	9.7 ± 0.5	FE*	Rep**
4 weeks	10.9 ± 0.4	10.6 ± 0.4	11.7 ± 0.5	11.8 ± 0.6	FE*	Rep*, Rear***
5 weeks	12.6 ± 0.4	12.2 ± 0.4	14.0 ± 0.7	13.6 ± 0.6	FE*	Rep*, Rear***
6 weeks	14.6 ± 0.5	14.0 ± 0.5	15.7 ± 0.7	15.6 ± 0.7	FE**	Rep*, Rear***
7 weeks	16.7 ± 0.6	16.0 ± 0.5	17.9 ± 0.8	17.5 ± 0.7	FE**	Rep*, Rear***
8 weeks	18.5 ± 0.6	18.1± 0.5	19.9 ± 0.9	19.5 ± 0.9	FE**	Rep*, Rear***

[1] Predetermined orthogonal contrasts were as follows: FE = Control + Se vs. Fe + (Se + Fe); SE = Se + (Se + Fe) vs. Control + Fe; FExSE = Control + (Se + Fe) vs. Fe + Se
*P<0.05; **P<0.01; ***P<0.001.

[2] Main effects tested by Analysis of Variance were replicate, treatment, breed, sex and rearing.

APPENDIX 12.

Trial 3. Effect of iron, selenium and sex on hemagglutination titer

	HA TITER ± S.E. (log_2 units)		HA TITER ± S.E. (dilution units)[1]	
IRON TREATMENT	-FE	+FE	-FE	+FE
AGE - 5 weeks	1.62 ± 0.18	1.50 ± 0.22	5.1 ± 0.5	4.5 ± 0.6
6 weeks	2.82 ± 0.20	2.71 ± 0.21	16.7 ± 0.5	15.0 ± 0.6
7 weeks	3.44 ± 0.15	3.54 ± 0.11	31.2 ± 0.4	34.3 ± 0.4
8 weeks	3.68 ± 0.11	3.80 ± 0.16	39.5 ± 0.4	44.7 ± 0.5
Number of lambs	22	21	22	21
SELENIUM TREATMENT	-SE	+SE	-SE	+SE
AGE - 5 weeks	1.42 ± 0.19	1.72 ± 0.20	4.1 ± 0.5	5.6 ± 0.6
6 weeks	2.75 ± 0.14	2.78 ± 0.25	15.6 ± 0.4	16.2 ± 0.7
7 weeks	3.35 ± 0.13	3.64 ± 0.13	28.5 ± 0.4	38.2 ± 0.4
8 weeks	3.60 ± 0.13	3.88 ± 0.14	36.6 ± 0.4	48.6 ± 0.4
Number of lambs	23	20	23	20
SEX	FEMALE	MALE	FEMALE	MALE
AGE - 5 weeks	1.24 ± 0.19	1.77 ± 0.18	3.4 ± 0.5	5.9 ± 0.5
6 weeks	2.70 ± 0.20	2.81 ± 0.20	14.9 ± 0.6	16.7 ± 0.5
7 weeks	3.40 ± 0.12	3.56 ± 0.14	30.0 ± 0.4	35.2 ± 0.4
8 weeks	3.65 ± 0.11	3.82 ± 0.16	38.6 ± 0.4	45.4 ± 0.4
Number of lambs	20	23	20	23

[1] Converted from log_2 (natural log) of dilution units.

APPENDIX 13.

Trial 3. Effect of selenium interactions with iron and sex on hemagglutination titer.

	HA TITER ± S.E. (LOG_2 UNITS)				HA TITER ± S.E. (DILUTION UNITS)[1]			
TREATMENT	CONTROL	+SE	+FE	+SE+FE	CONTROL	+SE	+FE	+SE+FE
AGE - 5 weeks	1.49 ± 0.27	1.75 ± 0.27	1.35 ± 0.27	1.68 ± 0.38	4.4 ± 0.8	5.8 ± 0.7	3.9 ± 0.8	5.4 ± 1.0
6 weeks	2.85 ± 0.22	2.78 ± 0.35	2.64 ± 0.20	2.78 ± 0.41	17.3 ± 0.6	16.2 ± 0.9	14.1 ± 0.6	16.2 ± 1.1
7 weeks	3.28 ± 0.21	3.60 ± 0.20	3.41 ± 0.16	3.70 ± 0.16	26.6 ± 0.6	36.4 ± 0.6	30.3 ± 0.5	40.4 ± 0.5
8 weeks	3.55 ± 0.14	3.78 ± 0.17	3.64 ± 0.21	4.01 ± 0.25	34.7 ± 0.5	44.0 ± 0.5	38.2 ± 0.6	55.0 ± 0.7
number of lambs	11	12	11	9	11	12	11	9
TREATMENT	-SE FEMALE	-SE MALE	+SE FEMALE	+SE MALE	-SE FEMALE	-SE MALE	+SE FEMALE	+SE MALE
AGE - 5 weeks	1.42 ± 0.30	1.42 ± 0.24	1.12 ± 0.25	2.32 ± 0.16	4.1 ± 0.9	4.1 ± 0.7	3.1 ± 0.7	10.2 ± 0.5
6 weeks	2.88 ± 0.23	2.68 ± 0.19	2.58 ± 0.31	3.01 ± 0.41	17.8 ± 0.7	14.5 ± 0.5	13.1 ± 0.8	20.4 ± 1.1
7 weeks	3.39 ± 0.14	3.32 ± 0.20	3.41 ± 0.20	3.93 ± 0.12	29.8 ± 0.5	27.7 ± 0.6	30.2 ± 0.6	50.9 ± 0.5
8 weeks	3.64 ± 0.25	3.58 ± 0.15	3.66 ± 0.10	4.16 ± 0.28	38.2 ± 0.7	35.7 ± 0.5	38.8 ± 0.4	64.1 ± 0.8
number of lambs	9	11	14	9	9	11	14	9

[1] Converted from log_2 (natural log) titers.