CHAPTER II

REVIEW OF LITERATURE ON STUDIES WITH COPPER FOR RUMINANTS
Copper is probably the first metal to be extensively used by man. Articles of copper were made as early as 6,000 years ago. Copper was obtained by Romans and Greeks from the Island of Cyprus. The Latin name 'Acs cyprium' or 'Cyprian copper', afterwards became 'Cuprium' and finally "Cuprum". The alchemists associated the metal with planet Venus, and designated it by the symbol 9. Copper as a metal has been used since very early times, the oldest specimens of cast copper from Egypt and Babylonia dates back to 4,000 B.C. As a remedy for various ailments in man, it has been used since the time of Egyptians 1,500 B.C. The Greeks in the time of Hippocrates prescribed copper compounds in pulmonary affections.

In India, this metal is known since "vedic" times. It is considered to be sacred metal as its use is mentioned as a container for water storage in religious functions as well as for human consumption. Copper was used in Ayurvedic medicine, since the time of Charaka and Sushruta, 1,800 to 1,400 B.C. The preparations and uses of copper in the form of 'Tamrabh-asma' is mentioned by these authors. Ayurvedic preparations containing copper compounds are prescribed in liver and spleen enlargements, inflammation of external secretary glands and in ascitis. It was also used in anaemia diarrhoea and dysentery.
Copper forms also an ingredient of other preparations of
aurvedic medicines. Copper as copper sulphate appears to have
been introduced by Wagbhat in Aurvedic medicine since 1,300 A.D.
It was indicated for use in dressing wounds and in skin diseases,
especially in syphilis in combination with other drugs.

In the western world, Paracelsus recommended copper for the
treatment of mental diseases and lung affections and by 18th
century, copper compounds were in wide clinical use. In veteri-
nary practice, copper was used extensively in anti-helminthic
preparations.

During the later part of 19th century, copper was recognised
as a normal constituent of blood. Its presence was established
much earlier in plant life. The real importance of this element
in the nutrition of mammals was not appreciated, however, until
1920's, when Hart and co-workers demonstrated that the sucklings
suffering from anaemia responded to copper supplements and that
it was an essential element for the well being. As a result of
this pioneer work, the copper deficiency in animals was studied
in many countries including India, in 1930 onwards. Considerable
amount of literature are available on this subject in the last 40
years. A review of the same follows in these pages.

2. Biological significance of copper:

Copper as a metal has been known for centuries and though
occurring in soil, the presence of this metal in biological
material and realisation of its importance in biological acti-
vities, however, remained undetected scientifically, until the
beginning of the nineteenth century. Bucholz (1816) and Meissner (1817) were probably the first to establish definitely the presence of copper in plant life. Later, Sarzeau (1830) detected the presence of copper in animals and determined the amount quantitatively. He found ox blood to contain 700 μg./l. While studying the copper content of vegetables, Deschamp (1848) observed a relationship between the copper in plants and that in the soil on which they were grown. The presence of copper in animal tissue was for the first time detected by Sarzeau (1830) and was recognized well over a one and quarter century ago by Boutigny (1833). Chevruel (1868), in a review on the copper content of biological materials, suggested that the metal was quite widely distributed in organic matter, but offered no explanation for its presence. Its occurrence was assumed to be accidental. Lehman (1395) summarized the then known facts regarding the distribution of copper and added many results from his own investigations.

Bertrand (1920), Guerithault (1920), Fleurent and Levi (1920) established the universal distribution of copper in plant life and suggested that copper should be placed in the group of catalytic elements found in the plants. The results obtained by Fleurent and Levi showed that leaves contain the maximum amount, whereas Guerithault found seeds to be the richest source of copper. Maquenne and Demoussy (1920) detected the presence of copper in all the samples of plant they analysed, the proportion being greatest in young shrubs and leaves. They concluded that copper in some way or the other aids in the vital functions
and was an essential element in plant metabolism. They observed that treatment of soil with copper sulphate improved the yield and quality of the plants grown on it. The essentiality of copper as plant nutrient was established in 1931 by Sommer (1931) and Lippman and Mackinney (1931).

In animal kingdom, the concentration of this metal was considered to be higher in lower invertebrates than in plants and higher vertebrates. Harless (1847) detected the presence of copper in Helix Pomatia (Snails) molluscan blood and showed that it existed in combination with blood protein. Fredericq (1873) established the fact that the copper containing pigment (haemocyanin) of the blood of octopus, behaved as a respiratory pigment. Lehman (1895) showed the presence of copper in whole blood and in serum. Church (1889, 1892) reported the discovery of pigment turacin in the feathers of the South African turaco birds. It was shown that turacin belongs to the group of porphyrin derivatives, normally found in plants and animals. This is the only species in which copper occurs combined with porphyrin. Dhere (1915) has reviewed in detail the literature on the copper contents of marine animals. Rose and Bodansky (1920) suggested that possibly copper is an essential constituent of marine fish tissue.

Even though the presence of copper in animal tissues was first demonstrated by Sarzeau (1830) and Boutigny (1833) and reviewed by Chevrue (1868), its occurrence was assumed to be accidental. But it was not until the investigations of Bodansky (1921) and McHargue (1925, 1926, 1927), however, that copper be-
came recognised as other than an accidental constituent and gave an indication that it might have an essential function in nutrition and physiological processes. McHargue published a series of papers which have demonstrated a role for copper in animal life. In 1925, he showed that copper is a constituent of blood of all animals and in 1928 he reported that copper is required for the formation of haemoglobin. The first conclusive evidence that copper is an essential dietary component emerged from studies initiated at Wisconsin in 1924 on haemoglobin regeneration in rats suffering from milk anaemia by Hart and co-workers (1928). They announced that copper, in addition to iron, was necessary for haemoglobin formation in rats. Data of copper in the whole body as well as in the separate organs of rats fed varying amounts of copper was published by Lindow et al. (1929). Specificity of copper as a supplementation to iron in regeneration of haemoglobin has been demonstrated by Elvehjem and Hart (1929). Copper deficiency brings about bone changes and makes it brittle and fragile in animals (Bennetts, 1932). Subsequent investigations at Wisconsin and elsewhere confirmed this new and exciting discovery and established the fact that copper is essential for haematopoiesis in a range of animal species (Elvehjem, 1935).

This classic work of Hart and associates initiated the trace elements era in the modern science. Following the stimulus obtained by this work on the essential role of copper in haematopoiesis, attention was then turned to biological functions and mode of action of copper at the cellular level, in the tissues of the animal body. A number of copper protein compounds from
both plants and animals were isolated. Many of them were shown to be enzymes with oxidative functions, such as tyrosinase, uricase, ascorbic acid oxidase, laccase and cytochrome oxidase, though the mechanism was not known (Keilin and Mann, 1938; Krebs, 1938; and Kubowitz, 1937). The specificity of copper as a supplement to iron in the regeneration of haemoglobin has since been demonstrated by Keil and Wilson (1931), Hamilton et al. (1933), Meyers et al. (1934), Potter et al. (1938) and Frost et al. (1940). A specific effect on red blood cell production has been attributed to copper by Oda (1932), Schultz (1932) Stein and Lewis (1933), Binnet and Strumza (1934), Somogyi (1935) and others.

The livestock malady which has been found to be associated with the copper deficiency has been reported from widespread regions of the world in early 1930's. Investigations on naturally occurring diseases of grazing sheep and cattle showed that affected cattle responded to copper or copper and cobalt therapy. Neal and associates (1931) after their studies on "salt sick" cattle in Florida were the first to suggest that copper deficiency occurs naturally in livestock. The work was subsequently confirmed by Becker et al. (1931). Two years later Sjollema (1933) from Holland showed that copper deficiency is a casual factor of a wasting disease of sheep and cattle, called 'Lechtucht', characterised by anorexia, emaciation, anaemia and diarrhoea. In 1938, he attributed the salt sick condition in cattle to the poor quality of hay and widespread disease of grain crops and observed that this condition was
checked by the copper sulphate fertilizer. Fens (1941) reported in Holland, a similar condition which too was remedied by copper administration.

Bennetts and Chapman (1937) found that a usually fatal condition of ataxia in new born lambs, enzootic in certain parts of Western Australia was associated with a deficiency of copper in the grazing mother and could be avoided by treating the ewes with the oral copper during the period of gestation. Confirmation of their discovery was reported from South Australia by Bull et al (1938) and from England by Dunlop and Wells (1938), wherein a similar malady known as 'sway back' occurred in certain restricted areas. Marston et al (1938) observed that 'ataxia' in lambs occurred on calcarious soil and he confirmed Sjollema's findings. A report of de-pigmentation, a new dietary deficiency disease, cured by copper supplementation has been made by Gorter (1935) and McCollum et al (1939).

Black et al (1937) reported that adequate amount of copper in diet showed a more efficient utilization of food, greater weight gain, greater energy storage and greater accumulation of fat and protein. Bennetts and Beck (1942) in their bulletin, reported low copper content of pastures in Australia and parts of New Zealand. Bennetts and Hall (1939) and also Bennetts et al (1942-48) described in the same area a disease of dairy cattle 'falling disease', which is characterized by loss of condition, fibrosis of myocardium and a seasonal incidence of sudden death. Innes and Sheaver (1940) showed that the disease 'sway back' in lambs, wide spread in Great Britain was pathologically same as that of 'enzootic ataxia noticed in Australia,
except for the fact that the copper contents of the grasses ranged from 7 to 33 ppm. Diarrhoea in cattle, varying in severity from a mild and transient condition to an acute, persistent and debilitating scouring occurred under conditions of copper deficiency. In Holland it was called 'scouring disease' (Brouwer et al. 1938). In New Zealand, acute scouring condition was popularly known as 'peat scour' (Chunningham, 1946).

Davis et al. (1946) stated that in Florida, cattle developed deficiency within 60 to 90 days and on copper supplementation, scour was arrested in 24 to 48 hours. Withdrawal of copper resulted in the reappearance of scour, within 2 to 4 weeks. Supplementation of ration with 3 gm of copper sulphate once in a fortnight or 0.5 to 2.0 gm of copper sulphate given daily maintained the animals in good condition.

Washburn (1946) in Colorado observed that symptoms of lameness, crooked feet and legs fragile bones, soft and eroded bone-articulative surfaces and occasional sterility showed by the animals could be prevented or cured by supplementation of copper at the rate of 15 ppm of the feed. The animals fed copper were more alert and active and presented a more desirable show and appearance. Smith and Ellis (1947) reported that copper deficiency in rabbit resulted in opacity, dermatitis, achirometric trichia, which could be cured by copper supplements.

These pioneer observations of Hart and associates, Neal and co-workers, Sjollema, Bennetts and Chapman etc., were
followed by the demonstration of copper deficiency of varying extent in many parts of the world, affecting both crop and livestock, and the laboratory studies with the different animal species revealed that copper participates in a remarkable varieties of functions in the living system. Simultaneously, the copper was shown to be vitally concerned in the processes of pigmentation, keratinization of wool, bone formation, myelination of spinal cord, reproduction and enzyme functions, in addition to that of hematopoiesis. It was possible to carry out these investigations, only because of the concurrent development of reliable analytical methods of sufficient sensitivity and precision to estimate the minute quantities of trace elements and their distribution in plant and animal tissues and their metabolic movements to be followed. All these gave a fillip to the overall knowledge of the problem and further development.

Indian workers were fascinated by these research findings abroad, initiated work in the country. The available references have been reviewed.

Capt. MacGuck (1931) commented on practical feeding tables for dairy cattle in India and suggested that the danger of mineral deficiency is ever present in the country. He suggested mineral mixture containing ground limestone, steamed bone meal and common salt besides a provision of Rock-salt by the side of the animal to overcome the mineral deficiencies.

Chaudhary (1931) mentioned the practice of feeding to cattle a very acid curd (dahi), in which the copper coims are
put, which results in the colour of the same to turn to blue. Krishnan and Iyer (1934) stated that copper induces better assimilation of lime. Krishnappa (1936) envisaged the possibility of the occurrence of 'ataxia' or 'sway back' in lambs in two villages. The affected 47 lambs showed shivering, convulsion and died in two days. Haemorrhages in brain and degeneration of spinal cord was also observed.

A systematic investigation on copper in relation to cattle nutrition was undertaken at Indian Veterinary Research Institute, Izatnagar in 1944. Results given in Annual Reports of 1946-47, 1952-53 and 1953-54 indicated that the

1. Normal copper requirement of cattle was estimated to be 45 to 77 mg per day. It will vary according to region (Sahai and Kehr, 1946-47),

2. Average copper content of cow and buffalo blood per 100 ml was found to be 137 ± 8.6 μg and 144.5 ± 7.7 μg, and that of sheep blood was 86.4 ± 10.9 μg,

and (3) Haemoglobin values were discovered to be low on certain farms, and it was suggested that copper deficiency may be one of the causes for this. Effect of blood forming elements iron, copper and cobalt on fluorine toxicity was studied. Trace elements feeding to the animals, which had developed fluorine toxicity resulted in beneficial effect. The animals improved in their condition and amelioration of toxic symptoms was brought about.

Lal (1947) has mentioned common deficiency diseases of animals and suggested the likelihood of prevalence of mineral deficiencies in India. He suggested remedies such as the inclusion of copper and cobalt in mineral mixture. Sahai and Kehr (1951) determined the copper content of 72 common feeds and feeding stuffs as well as blood and tissues of six normal
Mahadevan (1959) in the final report of the scheme 'Studies on the influence of trace elements on the health and productive capacity of livestock in India' recorded that it is reasonable to assume that sub-acute copper deficiency is widespread throughout the length and breadth of the country. From the study of copper intake by cattle in 50 farms and the analysis of the copper content of 437 samples of feeds and fodders consisting of some 90 different items fed in these farms, these observations were made.

Deshpande (1959) reported the incidence of copper deficiency of a far widespread nature in the former Bombay state. Existence of sub-clinical deficiency manifesting in stunted growth, low fertility and late maturity was reported.

Gopalkrishna and Mahadevan (1959) concluded from the digestibility studies on pigs that the digestibility of crude fibre improved significantly by the supplementation of the ration with copper. They observed linear relationship between intake and retention of copper.

Pachalag et al (1972) reported that the supplementation of copper and cobalt together in the ration had no effect on the packed cell volume, red blood cell count and haemoglobin in weaner lambs. A higher white blood cell count was recorded in the animals given the supplements.

3. Metabolism of copper

The importance of copper in the nutrition of farm animals and its role in the prevention of certain nutritional diseases
is considerable. However, very little is known of the mechanism of the absorption, distribution, retention, functions and excretion of copper in the body as compared to that of iron. It appears to be absorbed at least by two processes, one being a first order reaction and the other through an enzyme mechanism, (Giflin et al, 1960). The absorption takes place markedly in pronounced acid reaction part of the gut. This was evident by the marked depression in the absorption of this element in sheep by high dietary intake of calcium carbonate, which rendered the reaction to alkaline side, as shown by Dick (1956). This indicates that absorption takes place from the upper part of the small intestine. Lassiter and Bell (1960) showed that the availability of copper to sheep is influenced by the chemical and physical form in which it is administered. Under the conditions of these experiments, the copper in the elemental and oxide form was found to be less available than in the sulphate, chloride, nitrate or carbonate forms. More soluble compounds are very well absorbed. As shown by Mills (1954, 1955, 1966) copper in the herbage and pastures exists to a major extent in the bound form of water-soluble organic complexes, and small quantity in the free ionic form, or positively charged complexes. He suggested that copper may be transported through the intestinal mucosa in both the forms mentioned above. The nutritional importance of it cannot yet be evaluated, but he suggested that the form in which copper occurred in feed and fodder is important and it needs more study, especially the aspect of the net available copper from the feed and fodder to the animal although the total copper content of these are sufficiently high.
There are many unrecognised dietary factors which can exert some influence on the absorption and utilization of copper. It has been shown by Lewis (1960) that the deficiency of copper in cattle and sheep occurred due to primarily the wide variations in the reaction of individual animals and secondarily the molybdenum and inorganic sulphate and copper contents of feeds and fodders. In fact he observed excessive copper storage in housed sheep on diets containing the concentrations of these three constituents similar to those of herbage on which neonatal ataxia can occur in lambs.

The absorbed copper is transported by the blood plasma in a loosely bound form with plasma protein to the orange and tissues of storage, utilization and excretion. The copper is either stored in these sites as liver, spleen, kidney or released for the incorporation of this into the various copper containing enzymes of the cells and such other compounds as hemocuprein and caeruloplasmin or it is secreted into the bile and excreted via this route to the intestinal contents. It may be excreted out or reabsorbed from the intestine. Little quantity may also pass directly back from the plasma through the intestinal wall in the intestine and little into the urine.

Comar (1950) found only 6% of a single dose of interavenous injected copper to be excreted by cattle in the first 5 days, as compared to 78% of a similar oral dose, which indicated that injected copper is better retained by the body than orally administered one. This finding was later confirmed by Lassiter and Bell (1960) in sheep and Bowland et al (1961)
in pigs. Their findings also indirectly confirmed that copper once absorbed, is excreted slowly. The main channel of excretion is intestinal tract via bile. Normally under routine dietary conditions, 90% or more of ingested copper appears in the faeces, which is unabsorbed. Less than one percent of copper is excreted through urine, and negligible amount is lost in sweat (Mitchell and Hamilton, 1949).

Gubler et al (1962-63) found that copper from gastrointestinal tract reaches the liver through 'caeruloplasmin', which is a blood enzyme. Since it acts on paraphenylenediamine and p-phenols but not on mono-phenols and mono-amines, it has been classified as laccase. Most, if not all, of the copper in normal human or animal serum is stated to be present in the form of this blue protein. There is a rapid shift of copper from the plasma into the cells, but there is no vice-versa shift. In the blood, the ratio of plasma copper to cell copper remains constant at about 6:1, even when copper is injected.

Joshi (1984) conducted metabolism trials under different dietary regimes to see the absorption, excretion and utilization of copper under acid and alkali producing feeds. Feeding of paddy straw, concentrate mixture and calcium carbonate gave a positive copper balance of 4.92 mg/day, when the total intake was 16.11 mg per day. The main channel of outgo was found to be faeces. About 70% passed through faeces, 30% was absorbed and 1.6% was excreted through urine. Of the absorbed copper, 98% was retained in the body. Based on these, he concluded that the feed of calves need to be supplemented with 40 mg of copper
per head per day. Mahadevan and Zubairy (1969) conducted balance trial on Harlana cows and found that retention of copper was 144 mg per day in animals given copper supplements as against 14 mg per day in animals receiving no supplement. Gopalakrishna and Mahadevan (1969) observed a significant linear relationship between copper intake and retention in the body of pigs.

Reddy (1966) studied the effect of copper supplementation on digestibilities of nutrients in lactating cows. In this study, the animals of control group received 6.99 - 8.23 ppm of copper in their feed, whereas the animals of experimental group received 21.96 - 32.49 ppm of copper in their feed. It was observed that the ingestion of dry matter, total digestible nutrients and water consumption by the animals were less in the experimental group compared with those of control group. The high level of copper feeding was observed to reduce significantly the digestibilities of crude protein and ether extractives. The digestibilities of dry matter and nitrogen-free extract were also found to be depressed slightly by the higher level of copper intake. However, digestibility of crude fibre was not affected. Copper supplementation was observed to favour significant retention of copper, calcium and nitrogen. The phosphorus seemed to be unaffected, though the average retention by the animals in the supplemented groups was little less than those in the control group.

While studying the growth rate of cow and buffalo calves on pastures, Gupta (1963) supplemented the feed of the calves with copper, cobalt and iodine at the rates of 1.5, 0.05 and 0.1 g,
respectively per week per head. It was observed that the introduction of trace elements did not bring about any change in the digestibility co-efficients of the nutrients either in the cow or buffalo calves.

Kapoor and Mahadevan (1970b) studied the effect of supplementation of the feed with copper alone and copper plus cobalt on the digestibilities of the nutrients and their retention in Hariana calves. The calves of two experimental groups were given the supplements at the rate of 12.64 mg copper and 12.64 mg copper plus 1.32 mg cobalt per 100 kg body weight. The supplementation of either copper alone or copper plus cobalt had no significant effect on either the balances of nitrogen, calcium, phosphorus, copper and cobalt or the intake of dry-matter, D.C.P. and T.D.N.

4. Copper in animal tissues and organs:

Rusoff (1941) noticed a marked differences in the distribution of copper in two new born calves. However, despite their great variability, the findings emphasized that large amounts of copper can exist in the bones, muscles, skin and liver of animals. Heart, brain and kidney were the other important organs of copper storage. Dick (1954) found the distribution to be 72-79% in the liver, 8-12% in the muscles, 9% in the skin and hairs and about 2% in the skeleton of adult sheep. The susceptibility of the individual tissues to variations in dietary copper intake also varies considerably. The endocrine glands, the muscles, the heart, the brain and the skin are not susceptible to constant...
change, whereas the copper concentrations in the liver, kidney, spleen, and lungs can be greatly increased by high copper intakes and those of liver, kidney, spleen and blood readily reduced under conditions of dietary deficiency (Underwood, 1963). High concentration of copper occurs in the eye tissue, especially the pigment portion of many species. It is associated particularly with the pigments (melanin) in the eye and its role over there is not yet understood, (Bowness et al 1952). Copper content of hair (wool) has got special importance and is reviewed elsewhere.

Since the liver is the main storage organ of the body for copper, and the levels in the liver provides an useful index of the copper status of the animal, liver copper concentrations have been extensively studied from all the angles in a wide range of animal species. The liver copper levels are related to four main factors, namely, the species, the age of the animal, the nature of its diet, and the occurrence of certain disease conditions. Individual variations in liver copper concentration is high in all species. The review of this subject is beyond the scope of these studies. However, it is worth mentioning that normal liver copper content of adult cattle is 100 to 300 ppm on dry matter basis (Cunningham, 1946).

5. Copper in blood

Mann and Keilin (1933) isolated a copper-protein from the red blood cells of the cattle. They called this compound as 'haemocuprein' and showed that it contained 0.34% copper in the cupric form. It has a molecular weight of 35,000 and 2 atoms of
copper per molecule. It is a bluish pigment. Later, Tompsett (1940) suggested that copper in the red blood cells may occur in the cuprous form, combined with glutathione. Red cell copper can be increased to very high levels following the administration of massive doses of copper to animals (Cartwright, 1950). The function of haemocuprein is not yet fully understood.

Copper in the blood plasma exists in the form of a blue copper-protein compound known as 'caeruloplasmin' as reported by Cartwright (1930) and Wintrobe (1953). This compound is an $\alpha_2$-globulin with a molecular weight of 151,000 and it contains 8 atoms of copper per molecule. It is a blue protein having true oxidase activity. Similar to haemocuprein, the important physiological function of caeruloplasmin is not known. The remaining copper in blood plasma reacts directly with dithizone, is nondialyzable and is loosely bound to protein, probably serum albumin. However, on addition of acid, it becomes dialyzable and can be completely extracted with tri-chloro acetic acid. (Bowlan et al, 1961; Wintrobe et al, 1953).

In cattle, Eden and Green (1939) showed that copper in blood is normally divided equally between cells and plasma. Plasma copper is more labile than cellular copper and is more reliable indicator of changes in the copper status of an animal than whole blood copper content. Concentration of copper in the whole blood of healthy animal ranges widely. Beck (1956) reported for a wide range of species of animals, the copper content of whole blood to be 0.5 to 1.5/mg per litre. Physical exertion increases the whole blood copper level, while sex or
the pregnancy does not show any effect on whole blood or plasma copper contents.

Copper concentration of whole blood as well as that of plasma are influenced by the level of dietary copper as well as the ratio of this copper to molybdenum and inorganic sulphate intakes. Hypocupremia was reported in sheep and cattle grazing on copper-deficient pastures by Eden (1940) and Bennett and Beck (1942). Values as low as 10 μg per 100 ml of whole blood have been reported in sheep and cattle. Hypocupremia can also result from heavy infestation of intestinal parasites due to both blood loss and interference with copper absorption (Bremner, 1959).

Hypercupremia has been reported to be caused either by high dietary copper intakes or transiently by single high dose of injection of copper (Boyden et al, 1938; Cunningham, 1946; and Eden, 1939-40). It can also be produced by very high intakes of molybdenum and inorganic sulphate (Dick, 1952, 1953, 1954).

The report of Indian Veterinary Research Institute (Anon 1946-47) indicated that the copper content of blood of Kumboni bullocks was within the range recorded for cattle. Supplementation of the feed with copper sulphate increased their blood copper levels.

Dunkley et al (1963) studied the effect of injecting the cows with copper glycinate on blood copper levels and found a prolonged elevation of blood copper level i.e., up to 175 μg per 100 ml. Later, Dunkley et al (1968) further investigated the
influence of dietary copper and ethylene-diamine-tetra-acetate (EDTA) on copper concentration in blood and concluded that treatments did not influence the concentration of copper in the blood of the cows. Feeding of the supplements other than copper such as molybdenum or sulphate was found to be accompanied by changes in the concentration of copper in blood and also milk (Riest et al, 1967).

The normal copper content of blood of Kankrej and Gir breed of cattle was reported to be 170 μg and 131 μg per 100 ml respectively, by the Western Regional Animal Nutrition Station (1961-62, 1962-63). Deshpande (1959) reported the blood copper values for normal healthy field animals to be 90 to 130 μg per 100 ml and that of sub-clinical deficient animals to be 60 to 90 μg per 100 ml. He observed an increase in blood copper level from 50 μg to 120 μg per 100 ml within a month by supplementation of the feed of six heifers with 5 g copper sulphate per day.

The annual report of Indian Veterinary Research Institute (Anon., 1953-54) indicated the average copper content of blood of 15 cow calves and 15 buffalo calves to be 137 ± 8.6 and 144.5 ± 7.7 μg per 100 ml respectively. The later report of the institute (Anon., 1954-55) gave the values to be 18.4 to 61.8 μg copper per 100 ml of blood of calves. The effect of trace element mixture supplementation to calves on the blood copper level was studied in the same institute in the year 1955-56. It was seen that the blood copper level increased due to the supplementation.
The blood samples collected from 19 sterile cows in Bombay state were found to contain 71.56 to 85.26 μg of copper per 100 ml. Out of these 4 samples were having the blood copper below normal values. Similarly 60% of the 80 blood samples collected from animals in Assam had low copper contents, indicating sub-acute type of deficiency. About 50% calves on a farm were found to have sub-acute copper deficiency status (Anon, 1958-59).

Kumar (1960) analysed the blood of 27 male and 27 female calves and 26 cows for their copper content. He reported the range of blood copper contents to be 87 to 113 and 91 to 107 μg per 100 ml in the blood of male and female calves respectively. The blood copper level ranged in lactating and pregnant cows from 77 to 116 and 93 to 107 μg per 100 ml respectively. He concluded that copper content of the blood of calves of various age groups did not reveal any significant difference nor did they differ according to sex-wise. Dwarkanath et al (1962) showed the increase in blood copper levels of hypocuprimic cows by supplementation with dietary copper. This observation was later confirmed by Agarwal and Majumdar (1963).

Reddy (1966) reported that the copper supplementation increased significantly within a month the whole blood copper content, which showed a decline later on. The blood copper concentration of the animals after 1, 2 and 3.5 months after copper supplementation for the supplemental group were 90.1 ± 1.7, 104.5 ± 4.0, 99.9 ± 4.4 whereas that of control group
were $62.0 \pm 5.0$, $91.4 \pm 4.2$ and $86.3 \pm 4.9 \mu g$ per $100$ ml, respectively. Copper supplementation resulted in an increase of red blood cell count, haemoglobin and white blood cell counts significantly. However, Gupta (1938) observed no significant difference in haemoglobin, R.B.C., and W.B.C. counts in the blood of either cows calves or buffalo calves by supplementing their feed with trace mineral mixture containing copper, cobalt and iodine.

Kapoor and Mahadevan (1970a) observed a highly significant co-efficient of correlation between total copper in plasma and caeruloplasmin copper in the bovine blood.

6. Copper and milk:

The copper content of milk, as it is secreted by the animal is influenced by species, stage of lactation and the level and form of copper intake by the animal. Individual variations within the species exists for the reason unknown, but much of the individual variations can be attributed to faulty analytical methods and substantial contamination of the metal during milking sampling, handling and processing (Davis, 1936 and Mulder et al 1964).

Presence of traces of metal compounds in milk is of fair importance from several points of view. Besides their physiological importance in nutrition, they are important for the activity and growth of micro-organisms in curd and cheese. They are also important in chemical processes as catalyst during processing, ripening and storage of dairy products.
The milk of rats appears to be unique in that it has the highest copper content compared to the milk of other mammals examined so far. Cox and Muller (1937) reported the copper content of milk of rat to be 7 mg per litre of pooled samples taken from rats at four different stages of lactation, whereas cow's milk contained only 0.6 mg copper per litre of milk similarly obtained and analysed. The quoted figures of human milk is 0.5 mg copper per litre.

Even though there is no variation during one milking or between morning and evening milking, definite variation does exist during lactation. In all species colostrum is significantly richer in copper and in most cases there is a progressive fall throughout the lactation. According to Mulder et al (1964) the published figures from 1920 onwards are very highly variable and most unreliable due to high copper contamination. Hence, it is difficult to compare the results. However Beck (1941) found the copper content of the milk of normal ewes to vary from 0.20 to 0.64 mg per litre in early lactation to 0.04 to 0.16 mg per litre several months later. Similar fall of copper content was observed in the milk of cow during lactation by Mulder and Koppejan (1953). They also observed a tendency of the copper content of milk to increase at first for a period of 7 days of lactation and then to decrease in a later stage upto 6 weeks. The general trend observed was that colostrum contained about 100 \( \mu \)g of copper per litre, which upto 7 days then increased to 200 \( \mu \)g of copper per litre of milk and again decreased as the lactation advanced. After one month the copper
content is mostly between 40 to 80 μg per litre, which decreases
to a level of 20 to 40 μg copper per litre and then onwards
remains steady. Broek and Wolff (1935) were the first to give
reliable figures for the copper content of cow's milk at three
different stages of lactation. Recently King and Dunkley (1959)
and King and Williams (1963) confirmed the earlier findings.

Broek and Wolff (1935) measured the copper content of cow's
milk along with other trace elements. They found that neither
feed nor season influenced the copper content of milk, which
varied from 15 to 51 μg of copper per litre of milk. Similar
findings were made by Kohler et al (1935). Krauss and Wash-
burn (1936) studied over a period of one year the copper and
iron contents and the anaemia producing properties of mixed
Jersey and Shorthorn milk and confirmed that it neither varied
appreciably with the season nor with feed, even though the
copper and iron intake of cows during stall feeding was about
three times that when they were on pasture. The copper content
of milk was shown to be 140 to 190 μg per litre.

Elvehjem at al (1929) found the copper content of cow's
milk to be about 100 μg per litre under normal feeding. After
the copper content of the feed of cows was increased by 5 folds,
no increase in the copper content of milk was found. Broke
(1935) reported a feeding experiment in which cows received 3.0 /
gm of copper sulphate per day per head. The natural copper
content of the milk before the supplementation was 32 μg per
litre and was about 35 μg per litre after 7 weeks. The copper
content of the milk had not changed. He also reported that
there was no change in the copper content of milk when the cows were turned to pasture from stall feeding and vice-versa.

Kiermeier and Steger (1962) found a statistically significant dependence of the copper content of milk on the acidity of the soil on which the milk was produced. The copper content of the milk from an acid soil was about 47% lower than that of the milk from neutral or alkaline soil.

King and Dunkley (1969) observed no difference between pasture and dry feeding in the natural copper content in the resulting milk. They reported natural copper content of cow's colostrum to be 200 μg per litre and that for milk to be 20 to 40 μg per litre.

Mulder et al. (1964) observed no effect of the feed on the copper content of milk. One of the authors added such quantities of copper sulphate to the feed of the cows that they showed unmistakable symptoms of copper poisoning. Even then the copper content of milk, however, remained unchanged. And also according to previous findings of Mulder and Koppejan (1963) cows when changed from pasture to stall feeding or vice-versa, the copper content of milk was not affected. By feeding copper, the deficiency symptoms disappeared in the cows when they were fed 2 gm copper sulphate per head per day. But the copper content of milk did not increase. On the other hand, deficient intake of copper were reflected in sub-normal levels in the milk of cows and ewes as shown by Beck (1941). He found the copper content of milk of cows and ewes grazing on copper deficient pasture containing 1 to 3 ppm copper on dry basis, fell to as low level as 10 to 20 μg per litre.
Contrary to the above findings, King and Dunkley (1959) observed that when the cows were drenched with large amounts of copper sulphate (10 gm) there was an increase of variable extent in the copper content of the milk, which ranged between 10 - 15 to 70 µg per litre of milk.

Dunkley et al (1964) reported that estrus cycle of cows had no effect on the natural copper content of milk.

Copper content of ewes' milk as reported by Thomas (1937); Bennetts and Chapman (1937); and Crimmer (1948) ranged from 40 to 64 µg per litre and that of goats' milk ranged from 150 to 900 µg per litre of milk, as reported by Grendel (1930), Penny (1932); and Maeno and Ardate (1951), which is very high as compared to that of cow or ewe's milk.

Significant differences were observed by Dunkley et al (1960) in the copper contents of milk produced by the cows fed rations containing alfalfa hay and oats hay, as the roughages. This was due to higher dietary copper provided by the alfalfa hay ration, which was 2.5 times that of hay ration. Dunkley et al (1963) reported that the natural copper content of milk increased for at least 4 weeks as a result of administering 300 mg of copper as copper glycinate sub-cutaneously. Significant increase in the copper contents of milk was observed by feeding supplements of copper such as copper sulphate (P < 0.05) and copper EDTA (P < 0.01). Copper content of milk ranged from 40 to 60 µg per litre.

Melnikova and Ilyukhina (1969) showed that there was little increase in mean daily milk yield but not the fat percentage by
The copper supplementation was found to increase the butter fat percentage within 3 days and the increase being significant \((P < 0.05)\) after 18 days of supplementation. The increase in butter fat percentage was temporary and the same was not significant after 1 month of supplementation. However, the total milk yield of the animals were unaffected by copper supplementation (Reddy and Mahadevan, 1966).

Mirolyubov (1973) studied the mean daily milk yield, fat and protein contents of milk, before and after supplementation of the ration of Russian Black Pied cows with copper sulphate along with an enzyme preparation "Glyukavamorin P 10 Kh" during the 5th and 6th months of lactation and observed that the supplementation helped in the maintenance of milk yield as well as fat and protein percentages.

7. **Symptoms of copper deficiency**:

Copper deficiency symptoms as described by various workers are mainly of two types, namely, severe or acute and mild or sub-acute in nature. In general, ruminants are reported to develop copper deficiency much more readily than horses and swine. The copper deficiency diseases in animals are classified as follows:

1. **Absolute copper deficiency in feed or uncomplicated copper deficiency** (Underwood, 1962)

2. **Conditioned or physiological copper deficiency**, not due to lack of copper in feed, but due to inefficient utilization of copper by other interfering dietary factors having associative effects on or complicated copper deficiency (Abdellaifi, 1986)
Copper deficiency is a regional disease and symptoms shown by the affected animals, as well as the name of the disease varies from region to region and species to species of animal. The terminology of the disease either due to copper deficiency or molybdenum and sulphate interference varied in different places. For instance in Australia it is known as

Ataxia, Neonatal ataxia and Enzootic ataxia in lambs (Bennetts & Chapman, 1937)

Falling disease in cattle (Bennetts, 1939) and

Coast disease in sheep of south Australia

In New Zealand it is called as Peat scours in cattle (Cunningham, 1944) and in Holland, Scouring disease in cattle and Lechsuchf in sheep and cattle (Sjollema, 1933)

In England it is known as Sway-back or swing back in calves (Inns & Shearer, 1940), Teart, Wart in sheep and cattle (Ferguson, et al, 1938).

It is termed as Salt sick in cattle (Neal et al, 1931) in Florida (USA)

A wide varieties of symptoms have been associated with dietary deficiency of copper or the disease which responds to the copper therapy (Davis, 1951). They include anemia, depressed or stunted growth, loss of condition, deprived appetite, a characteristic pained and tucked up appearance and lethargic when driven (Bennetts et al, 1941). Rough and faded hair coat, depigmentation of hair or wool, slow keratinization and abnormal
wool growth are also seen. Bone disorders with split or broken and fragile bones, particularly pelvic and rib bones are the common sites. Fibrosis of myocardium and heart failure are also seen. Gastro-intestinal disturbances and diarrhoea may vary from intermittent to severe are noticed (Beecher et al 1963). Impaired reproductive performance, due to suppressed oestrus, sub-normal blood and liver copper levels and copper-containing enzymes, low haemoglobin levels, microcytic hypochromic anaemia, have been observed.

In young animals, beading of ribs and enlargement of the ends (epiphysis) of the lung bones occurs. Young calves may have straight pasterns and stand forward on their toes. Stunted growth and 'Goggled eye' are symptoms of copper deficiency in growing calves. General debility leading to sudden fall and death after little or no struggle, has been reported. The deficiency symptoms are marked after the weaning stage. Haemoglobin levels fall to 6 gm or below per 100 millilitres.

In sub-acute deficiency the animals remain in debilitated condition, appetite is fair and slight fading of body coat takes place. In females the oestrus cycle may be irregular and conception rate may be low. In males there is a destruction of germinal tissue of the testicles, which may be irreparable. Although only minute traces of copper are required, a deficiency of copper in feed and fodder and factor interfering in its assimilation will produce clinical symptoms of dietary deficiency, as mentioned by Allman and Hamilton (1968). Apart from the above mentioned symptoms, there may be the muscular
Incoordination, pica, dead or weak offspring, decreased or cessation of growth. The extent to which one or more these dysfunctions are revealed depends upon the species of animal and its age, and also upon the environmental factors and the severity and duration of the copper deficiency, whether it is uncomplicated or conditioned by other dietary factors (Underwood, 1962).

Dunlop and Wells (1938) investigated the disease known as 'away back' or 'swing back', 'warf' and 'Gingin rickets', which are widely distributed throughout Great Britain. They described the disease in North Derbyshire as being confined almost exclusively to lambs during the first month of life. Most were affected at birth and were unable to raise themselves to obtain milk from their dams. Others sway about in a peculiar manner and collapse or turn somersault, if they attempt to raise or walk. This was shown to be due to low copper level in the animals' body caused by interfering factors such as high amounts of lead, iron, calcium and zinc, but not due to the deficiency of dietary copper in pastures, which contained 12 to 27 ppm of copper.

The scouring disease on 'taart pastures' in cattle and sheep in Britain was found to be due to high molybdenum content, which ranged from 120 to 130 ppm while copper content of these pastures were 11 to 18 ppm. The scouring disease in cattle was also due to calcium deficiency. Pasture was not deficient in copper and also the molybdenum content was not abnormal, but it was found to be low in magnesium content.
However, the copper supplementation of the diet in all the above conditions mitigated the copper deficiency condition resulted either from low dietary intake or induced by interfering factors such as molybdenum, sulphate, heavy metals or even very high calcium and phosphorus ratio.

Subnormal levels of copper both in blood and liver and also occurrence of a whole range of copper deficiency symptoms were reported by Beck (1941) in both sheep and cattle grazing on pastures containing 3 to 4 ppm or less of copper and less than 1.5 ppm of molybdenum.

Despite the wide-spread use of copper licks, sway-back was found to prevail in North Derbyshire. Hunder, Eden and Green (1945) demonstrated the importance of ingestion by the ewes of the required amount of copper and indicated that some factors in the natural herbage other than the quantity of copper is responsible for the low copper status of grazing ewes.

Russel and Duncan (1956) observed a disease known as 'Enzootic ataxia' associated with anaemia and also stringiness of wool and attributed these to uncomplicated copper deficiency status in ewes and lambs, grazing on pasture containing less than 5 ppm copper. Sudden death and falling disease in Australia were seen in animals grazing on pastures containing less than 2 ppm copper. Similarly, in Europe licking disease in cattle, sheep and goat was found to be due deficiency of copper in pastures containing less than 5 ppm of copper.
Allcroft and Parker (1949) described hypocupraemia as a condition characterised by diarrhoea, low fertility, poor milk production and stunted growth. Cunningham (1950) reported the change of coat colour of red animals to be bleached to yellowish-red. The hair coat in adult cattle was found to be rough and staining and the colour of black animals to become tinged with dull red or grey colour and specially ground around the eyes. Scouring was particularly bad in animals fed on the bush pasture of spring and to a lesser state when the rains come in autumn. Marston (1953) listed specific coat changes in which the hair lost its capacity to produce pigment and became harsh and dishevelled. In mature cattle, heart failure may result due to atrophic lesions and fibrosis of myocardium.

Lately, a genetic influence on copper metabolism in sheep has been shown by Wiener et al (1968, 1969). In a flock, the level of copper in blood and liver as well as the susceptibility of new born lambs to sway back were related significantly to breed. Anke (1967) proposed a similar method of detecting copper deficiency in dairy cows. He proposed that 7 ppm copper in black hair is a border-line level for copper intake index. But Chauvaux et al (1965) concluded, after examining 536 hair samples from 31 farms, that copper levels in the hair were not effective for the diagnostic purposes. Seekles and Claessens (1967) indicated that copper content of the hair may be influenced by dietary copper intake. They also reported that positive correlation existed between the levels of blood and hair copper levels. The copper content
of the hair was found to be influenced by pregnancy (Anke, 1965) and hair colour (O'Mary, 1969). Binot et al (1970) found that the copper contents of hair and blood in cattle varied directly with the copper content of forage and inversely with the amount of iron and sulphate content of forage. Anke (loc cit) obtained a positive correlation between the molybdenum content of the pasture and the black hair of the cattle and reported that symptoms of molybdenosis resulted when molybdenum content of hair exceed 1 ppm.

8. Copper, blood formation and anaemia

The well established need of copper in the diets of farm animals and its value in the prevention of certain nutritional diseases and the historical findings of Hart et al (1928) have stimulated the interest in the subject. Hart and co-workers established that copper had a definite function in the utilization of iron for the formation of haemoglobin. McHargue in the same year supported Hart's findings. In the absence of copper, dietary iron is absorbed and deposited in the liver or elsewhere but normal regeneration of blood does not occur, resulting in anaemia, which is to some extent is a frequent manifestation of copper deficiency in all species. When copper is added to the diet, haemoglobin synthesis is resumed.

The exact manner in which the copper plays its role and its absence affects haemoglobin formation is not yet completely understood. Present evidences suggest that a certain minimum supply of copper to the bone marrow is necessary for
the production and maturation of red cells, that have a normal survival time in the circulation and also for the synthesis of the haem-moiety of the haemoglobin.

The classical work of Hart et al. (1929) at Wisconsin showed that nutritional anaemia could be produced by the exclusively feeding of milk, established beyond doubt that copper in addition to iron is essential for blood formation, i.e., haemoglobin and red blood cells. The development of the work on milk anaemia and the validity of these claims have been fully discussed by Elvehjem and Hart (1929). They have found out the relation of iron and copper to haemoglobin synthesis in the chick. It is probable that the role of copper in the formation of blood is secondary to its effect on cells.

Schultze and his associates (1936) suggested the catalytic action of copper in haematopoiesis following their work on young swine. They suggested that rapid and continuous haematopoiesis cannot take place unless the copper content of the blood is maintained above a certain minimum level. Later this level has been suggested to be about 20 ìg per 100 ml of blood in pigs (Lahey et al., 1952). In sheep a level of 10 to 12 ìg copper per 100 ml of blood has been found to limit blood formation (Beck, 1941; and Marston et al., 1948). If such low level is maintained for long time, anaemia occurs and death follows. Rats, pigs and dogs placed on a milk plus iron diet, quickly become anaemic due to the rapid fall in blood copper below the limiting levels.
The nature of anaemia caused by copper deficiency varies with the species. In cattle and ewes, it was found to result in hypochromic and macrocytic types of anaemia (Bennetts & Beck, 1942; Cunningham, 1946; and McDonald, 1947). Bennetts and Beck (1942) also showed that in lambs it is hypochromic and microcytic anaemia. Barer and Fowler (1937) found that some reticulocyte response occurred in milk anaemia on feeding copper alone (but not iron alone), which indicated that copper acts as catalyst both for the incorporation of iron into haemoglobin and for erythrogenesis and is not restricted especially to either function alone. Further evidence that copper is concerned in the erythrocyte maturation process comes from the observation that copper anaemic pigs and dogs contained a lower percentage of reticulocytes in their blood than those with iron (Lahey et al., 1952). As well, Smith and Medlicott (1944) showed that addition of copper to the diet of rats, rabbits and pigs suffering from anaemia due to combined iron and copper deficiencies showed marked and persistent reticulocyte response, whereas iron has little effect on reticulocyte. Kumaoni bullocks fed on copper deficient diet had low erythrocyte count and showed a tendency towards development of what may be classified as macrocytic and hyperchromic type of anaemia (Anonymous, 1946-47).

Schultz (1939, 1941) showed for the first time that there was a marked loss of cytochrome oxidase in the tissue (bone and liver) of the copper deficient rat, pig and chick. His findings later were supported by Gubler et al. (1957),
who indicated that copper was especially concerned in the synthesis of the prosthetic group of cytochrome oxidase and that this must be regarded as one of the basic function of copper, unrelated to iron metabolism. Recently, Mills and Dalgarno (1970) and Poole (1970) also showed that the liver cytochrome oxidase activity decreased during copper deficiency and suggested that this could serve as an copper status of the animal.

Bus and associates (1966) observed that the survival time of the erythrocytes is shorter in copper deficient animals than normal ones. They suggested that copper was an essential component of adult red blood cell and that certain minimum of copper must be available both for their production and maintenance of their integrity in the circulation. Decrease in the absorption of iron from gastrointestinal tract was also claimed by them under copper deficiency.

9. Influence of copper on iron metabolism:

Even though the amount of iron in the body is very small, its functions are very vital. The chromatic substance of the nucleus of every cell is an iron protein compound. The haemoglobin constitutes the outstanding material of the red blood cells and has the most important function of transport of oxygen to the nook and corner of the body and assisting in the maintenance of neutrality. Iron constitutes nearly one third of the haemoglobin. Hogen and Nierman (1927) showed that about 0.004 percent, by weight of animal body consists of this element.
It is also an indispensable constituent of enzymatic oxidation system (Linton, 1950). Iron alone or in combination with other trace elements such as copper, cobalt, etc., prevented the occurrence of anaemia in growing animals (Krauss, 1929; Supplee et al, 1930; Knoop et al, 1935 and Thomas and Okamoto, 1953).

Cunningham (1931) and Joseph (1932) did not observe any significant difference between the total body iron content of rats fed on milk diet plus iron and that of those on milk diet plus iron and copper. Later in 1935, Elvehjem reviewed the literature with regards to functions of copper in the absorption, utilization of iron and concluded that it is not concerned with the assimilation of iron except for the transformation of the ingested iron into haemoglobin in the animals. Five years later, Schultze (1940) concluded that copper is not necessary for the absorption and storage of iron in tissues, but it facilitates or is necessary for the utilization of iron by the blood forming organs and for the mobilisation of iron from the tissues. Marston and Allen (1967) observed that the amount of radioactive iron (Fe$^{59}$) absorbed was favoured by amounts of dietary copper upto levels of 0.25 to 0.50 μg per rat per day. Above this amount, there was no greater iron absorption and on the other hand a some what reduction in the absorption of iron was observed. The influence of copper on the absorption of iron is not due to the simultaneous administration of copper with iron but appears to be correlated with the level of copper in the tissues. One of the specific effects of copper on iron metabolism appears to rest in the synthesis of the 'heme'
(iron-porphyrin), which is the prosthetic group of haemoglobin, cytochromes and other important biochemical compounds containing iron. They postulated that copper was required for the release of iron from its storage sites in the liver.

On the other hand, Houk et al. (1946) found that addition of copper to copper deficient diets increased the dietary iron utilisation from about 30 to 70% as measured by the total body iron content of rats. Later, Chase and associates (1953) employing radio-iron observed that copper supplementation increased the iron absorption in rats and also the absorption of iron from gastro-intestinal tract was markedly reduced in copper deficient pigs. It was shown by Gubler et al. (1952) that the deficiency of copper impaired the ability to mobilize iron from the tissues and to utilize iron in haemoglobin synthesis. Further more, from the subsequent studies of Bush et al. (1956) and Jensen et al. (1956) it became apparent that the transport and mobilization of iron was not impaired in copper deficient pigs as thought by the earlier workers. However, they still maintained that copper deficient pigs had a reduced ability to absorb iron from the intestinal tract.

Studies on the parallel lines as in rats and pigs have not been conducted with ruminant and hence little is known of copper - iron inter-relation in these species. On the other hand, the heavy deposits of iron that occur in the liver and other tissues of cattle and sheep confined to the pastures or areas which are deficient in copper suggested that copper does not markedly influence the absorption of iron in ruminants (Marston et al., 1948). Recently, as reported by Underwood
(1968) copper is apparently not directly involved in iron absorption, mobilization and transport to the bone marrow, as claimed by earlier workers (Matrone, 1960; Underwood, 1962). He further claimed that haemoglobin synthesis was blocked in copper deficient animals, so that absorbed iron could only enter the tissue iron reservoirs which in time causes a 'mucosal block', thereby decreasing iron absorption. Earlier, he felt that copper was apparently not necessary for proto-porphyrin synthesis, since normal or higher than normal amounts of free proto-porphyrin was found in the mature erythrocytes of copper deficient animals. Also there was no clear evidence that copper was concerned in the incorporation of iron into proto-porphyrin to form heme. The evidence so far available are therefore, not adequate to implicate copper unquestionably with any stage in the biosynthesis of haemoglobin. Therefore, the anaemia of copper deficiency could be explained for the time being, on the basis of impaired erythrocyte maturation and a reduced survival time of the mature erythrocytes produced. It should be emphasised nevertheless, that any factor such as copper that limits erythropoiesis also limits haematopoiesis, because haemoglobin is confined to the red blood cells and the haemoglobin content of the single cell cannot be increased beyond certain normal limit.

The studies of Standish et al (1969) demonstrated that excessive levels of dietary iron depressed liver copper in cattle, whereas Chapman and Kidder (1964) indicated that iron accumulated in the liver when copper was deficient. Rats fed
on copper deficient diet accumulated excessive liver iron, while those fed on iron deficient diet stored high levels of copper in their livers (Sourkes et al, 1963).

Pal et al (1970) supplemented the ration with copper of growing hariana calves and did not observe any appreciable difference in the total red blood cell count, haemoglobin, packed cell volume and serum calcium and phosphorus. However, copper and cobalt mixed supplementation increased the white blood cell count. The increased white blood cell count was supposed to have resulted from the increased ceruloplasmin copper and low free reacting copper.

10. Copper, Enzymes and Vitamins:

The only common characteristic which all the traces elements have is their capacity to function in very minute quantities in the body. It is in this capacity that they must act as catalysts involved in enzyme, vitamin, hormone systems, either as constituent parts of the molecules of these or as enzyme activators. In fact, as Green (1941) suggested 30 years ago that 'enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects'. Many examples of trace elements functioning in these ways are now known. Copper and iron are known to be irreplaceable compounds of the molecule of several oxidative enzymes. Cobalt has also been shown to be an integral part of vitamin B12. These findings have little doubt that these trace elements function as activators or as catalysts in the
living cell, and that they lie at the root of living process. The metal ions, which include many of the trace elements, appear to act in two different ways in enzyme systems. They may be an indispensable part of the protein from which they can only be dissociated with difficulty, viz., iron complex of the 'heme-protein' and the cobaltic complex of vitamin B₁₂. In these cases, it would be better, as Williams (1963) has suggested, to describe the function of metal ions, which are highly specific as one of activators rather than that of catalyst of an enzymatic reaction. In these cases, ions are readily dializable from the enzyme, after which they become less active. The activating metal is loosely bound and can get readily lost during processing.

As described by Bowen (1966) the enzymes containing copper come under metallo-enzymes and due to their better defined chemical nature, they have been more intensively studied than metal activated enzymes. They are numerous in number and many more probably await discovery. Metallo-enzymes are divided into metallo-protein, metallo-prophyrin and metallo-flavin enzymes. Metallo-protein enzymes, which contain copper, are phenol oxidase, tyrosinase, urichase, laccase, mono-amine oxidase, ascorbic acid oxidase, ceruloplasmin, galactose oxidase, β-γ-ε-sulphurase and benzylamine oxidase. The metal ions are often the catalysts for the reactions they take part in. For example, cupric ions catalyse the oxidation of phenols and ascorbic acid. Metallo-porphyrin enzyme containing copper is cytochrome oxidase having one atom each of copper and iron.
with one protohaem as porphyrin, which is most important in the haemoglobin formation (Mahler, 1961). Cytochrome oxidase is reported to be reduced in the brain tissues of lambs with 'ataxia' due to 'conditioned' copper deficiency (Mills and Quarterman, 1963). Metallo-flavin enzymes containing copper atom are hyponitrite reductase, nitrous oxide reductase, and nitrite reductase type II (Mahler, 1961).

Furthermore, Bowen (1966) listed the metallo-proteins, other than enzymes. The copper containing metallo-proteins are haemocyanin, cytocyanin, erythrocuprin, hepatocuprin, cerebrocuprin, milk copper-protein etc. Cytocyanin and haemocyanin are known as respiratory pigments. Therefore, the importance of copper in enzyme system is well understood from the above information. Hence its deficiency is bound to affect the normal physiological body processes.

Second only to the haemoglobin formation, is the role of copper in the activation of oxidation-reduction enzyme. Schultz (1939, 1941) from a series of experiments demonstrated that a high cytochrome activity of bone marrow was intimately associated with haematopoiesis. In copper deficiency, cytochrome oxidase activity of bone marrow was lowered. Upon starting the copper therapy, there was a rapid response, which reached a maximum in about 24 hours. A similar observation was made on the cytochrome oxidase activity of the liver and heart. Further, studies of Schultz and Juiteen (1941) suggested that the catalase activity of liver, kidney and blood was greatly influenced by copper and was found to be
decreased with copper deficiency and rapidly increased with copper therapy. Tyrosinase, associated with pigment formation is an enzyme activated by copper and deficiency of copper was shown to be exhibited through this by depigmentation of hair and wool.

Dawson (1950) reported that copper was concentrated within a limited area of its site of action in the body and was firmly bound to a specific or a type of protein of the enzyme. Lerner (1950) showed that certain compounds which combine with copper inhibit tyrosinase activity. The failure of pigmentation in copper deficiency resulted from a breakdown in the conversion of tyrosine to melanin, since that conversion is catalysed by copper-containing poly-phenyl oxidases (Raper, 1928). Mahler (1963) identified butyl CoA dehydrogenase as a cupro-flavo-protein in which copper occurs as a part of prosthetic group. The activities of this enzyme was found to be dependent on the copper they contain. The mode of its binding with the protein, the way in which it functions and also takes part in the oxidative process in the intact living cell are not yet fully understood.

Relation of copper to enzyme activity is complicated by other deficiencies. In a comprehensive study of enzyme activities in copper deficient rats, Gallagher (1957) found that anaemia developed after ten weeks of starting the experiment and death occurred after fifteen weeks. Fourteen different enzymes were assayed, of which only cytochrome oxidase, the terminal enzyme of biological oxidation, showed a marked
decline as a result of copper deficiency. It was of interest that addition of copper salts in vitro did not restore the cytochrome oxidase activity, while normal liver cytochrome activity was resumed by feeding copper for ten days.

Copper had a role in the synthesis of iron-containing heme-prosthetic group of cytochrome oxidase and D.P.H. cytochrome-c-reductase, a flavoprotein which contains iron, but not in heme form. The activities of these enzymes were not affected due to copper deficiency. In case of copper deficiency, it appeared that synthesis of the heme-prosthetic group of cytochrome oxidase was affected earlier, while that of haemoglobin somewhat later, and the catalase was found to be least sensitive. The exact mechanism of the action of copper in the synthesis of the iron porphyrin is not known (Mahler, 1958).

An indication that the copper is tied up with a vitamin such as pantothenic acid and ascorbic acid was observed by Singer (1949) and Singer and Davis (1950). This concept was somewhat expanded by the work of Hundley and Ing (1950), who suggested that pantothenic acid deficiency actually produced grey hair by blocking utilisation of copper in hair growth and melanin formation.

11. Copper and bone formation

Changes in bones and deformities of bone have been reported to be very common in pigs, dogs, foals and chicks (Lahey et al, 1952; Teague and Carpenter, 1951; Bennetts,
1932; and Gallagher, 1957), but not in laboratory animals. A less incidence of spontaneous bone fracture was observed in cattle and sheep grazing on copper-deficient pastures in some areas (Bennetts and Beck, 1942; Cunningham, 1950; and Davis, 1960). The factured bones are almost normal in appearance except for the osteoporosis and brittleness. Detailed study of the bones of the copper deficient dogs and pigs revealed a marked failure of deposition of bone in the cartilage matrix, accompanied by normal growth of cartilage. The bones were characterised by abnormally thin cortices, deficient trabeculae and wide epiphyses together with normal ash, calcium, phosphorus and CO₂ contents. These changes were distinct from those seen in rickets. It was suggested that studies of the levels of cytochrome oxidase or other copper enzymes in the ossification process may reveal the role of copper in bone formation (Underwood, 1962).

Copper is apparently necessary for the normal development of bone (Davis, 1950, 1951). Marston (1952), reported that in copper deficient areas, normal bone metabolism was inhibited and the cattle were unable to form bones properly without copper supplementation, with the result, that a condition 'very similar to ricket' was seen in young calves, whereas osteoporosis and rarification of bones resulted in older animals. In Australia and New Zealand, the bones of calves and lambs were found to have a tendency to fracture easily due to copper deficiency (Bennetts, 1932; Bennetts et al., 1942; and Cunningham, 1950).
One of the most striking effects of the deficiency of copper is the loss of pigmentation in the hair coat. The achromotrichia of the hair is the earliest and most sensitive index of copper deficiency in all the animal species, except the pig. A related effect, apparently, is the interference with normal wool formation in sheep with loss of crimp, defective keratinization, lack of pigment production in black-wooled sheep and production of so-called 'steely or stringy wool' (Lee, 1951). This depigmentation during deficiency and pigmentation on an adequate copper level responds so quickly that, pigmented and depigmented bands in same fleeces are seen (Garter, 1935; and McCollum, 1939). Even on fairly high copper intakes the functioning of copper in pigmentation processes can be blocked within two days by raising the molybdenum and inorganic sulphate intakes sufficiently (Dick, 1952, 1953 and 1954). The action of copper in the skin has been repeatedly emphasised and apparently this is one of the main sites of copper action. (Ferguson, 1943; Davis et al., 1946; Hundley, 1950; Commar et al., 1948, 1949; Singer, 1949; Marston, 1950, 1952; and Davis, 1951).

According to Smith and Ellis (1947), the achromotrichia, alopecia and dermatosis syndrome is a more sensitive index of copper deficiency in rabbits as well as sheep, rather than the anaemia. The biochemical mechanism involving copper in the pigmentation process has not been defined. Raper (1928) suggested that breakdown in the conversion of tyrosin to
melanin may occur, since this is known to be catalyzed by copper containing poly-phenol oxidases. Bennetts (1932) and Marston et al (1938) observed decline in quality and quantity of wool in copper-deficient sheep. An increase in quantity and improvement in quality was noticed by Marston and Lee (1948). This was attributed to the improved keratinization after copper supplementation. The abnormalities described were more obvious in the wool of breeds that are heavily crimped such as Merinos of Australia. However, they were demonstrated experimentally in Great Britain breeds of sheep (Lee, 1956). This type of lesions were not seen on copper-deficient pasture, other than those in Australia. These observations indicated that some other dietary factors in addition to copper may be involved and responsible for such abnormalities in that particular environment, where the lesions occur. Utilization of copper in hair growth and melanin formation was suggested to be blocked due to the deficiency of pantothenic acid (Hundley and Ing, 1950).

The copper content of hair and wool was determined by Kikkawa et al (1955), who found that it was significantly higher in those of pigmented (black) hair than those of white hair. However, Goss and Green (1965) were unable to confirm this finding. They examined black and white hairs of healthy animals of ten different species and reported 10 to 47 ppm of copper in the washed dry hairs. No relationship between pigmentation and copper concentration was observed. However a decrease in copper content was correlated with an increase
in sulfhydryl groups and a decrease in disulphide linkages of the wool (Marston, 1952 and Burley, 1954).

Reddy and Mahadevan (1966) examined twenty-four poll hair samples and blood from twelve cows, before and after copper supplementation of the feed and concluded that poll hairs can be used for assessing the copper status of the animals.

13. Copper and 'scouring' of cattle:

Severe diarrhoea is a predominant symptom of enzootic disease, prevailing in certain parts of Holland and is known as 'scouring disease'. This was shown to occur in copper deficient areas and not in other parts (Sjollema, 1933). In New Zealand, similar diarrhoea symptoms were observed in the animals grazing on pastures on reclaimed peat land. This disease was known as 'peat scours'. In 'teart' disease, Allcroft and Parker (1949) in England observed similar severe and chronic diarrhoea in cattle grazing on pastures. Intermittent diarrhoea in cattle was reported by Bennett and Hall (1939) in the falling disease areas of Western Australia. In all these cases, the scouring was observed in the animals of low copper status of blood and tissues. This was found to be cured or prevented by the administration of copper supplement orally or by injection, as well as treatment of the pastures with copper-containing fertilizers.

On the other hand, no such symptoms were reported in animals grazing on copper-deficient pastures in Florida,
even though the other typical deficiency symptoms were noticed (Davis, 1950). Moreover, sheep and horses grazing on similar pastures did not scour. Laboratory animals, dogs and pigs did not develop diarrhoea even under severe copper deficiency status. However, scouring was reported in goats maintained on Dutch pastures that induced scouring in cattle (Underwood, 1966).

Molybdenum level in pastures was suggested to be the factor concerned in 'peat scours' (Cunningham, 1960). However, other dietary factors may also be involved, apart from deficiency of copper and higher levels of molybdenum in the pastures.

14. Copper, Molybdenum and Sulphate Inter-Relationship:

Interest in the metabolic significance of molybdenum arose first from the investigations on the severe scouring disease of cattle, known as 'tart' in England, caused by excessive intake of molybdenum from the herbage by the cattle. This could be prevented or controlled by the treatment of animals with massive doses of copper supplements (Ferguson et al., 1938, 1943). Dick and Bull (1945) showed that copper assimilation and retention in sheep and cattle could be limited by molybdenum. The interaction between these two elements and the inorganic sulphate is of great complexity, in which other elements and nutrients are reported to be involved. The quantitative relationships involved amongst these three constituents are yet to be fully understood. The inter-relationship between copper and molybdenum i.e., the toxicity due to a combination of excessive levels of molybdenum
and low levels of copper in forage was reported in Florida and California (Britton and Goss, 1946; and Davis, 1950).

Subsequently, peat scours of cattle in New Zealand was established as a molybdenosis, conditioned by a relative deficiency of copper (Cunningham, 1944). Simultaneous investigations on another problem of chronic copper poisoning of sheep in eastern Australia revealed further metabolic interrelations between molybdenum, copper and inorganic sulphates. In Great Britain, Jamieson and Russel (1946), Allcroft (1946, 1949) and Blakemore and Vearn (1950) reported deficiency of copper in animals maintained on field, which were found to contain sufficient levels of copper. This was attributed to high level of molybdenum in the herbage. Green (1951) did not observe any relationship between the levels of copper and molybdenum and hypocupremia. However, Dick (1952, 1953, 1954) showed that molybdenum exerted its limiting effect on copper retention in sheep only in the presence of inorganic sulphate and also neither molybdenum nor sulphate alone did interfere with copper retention. The effectiveness of either was found to be increased to a maximum as the intake of the other was increased. He further observed the molybdenum excretion in sheep to be profoundly affected by the dietary inorganic sulphate level. This finding was subsequently confirmed by Spais (1959).

Chronic copper poisoning associated with extremely high liver copper levels in sheep was observed under conditions of moderate copper intake and very low dietary levels of
molybdenum and sulphate. Conversely, depletion of animals' copper reserves, even to the extent of clinical copper deficiency was observed to take place after several months on normal copper and high molybdenum and sulphate intakes (Wynne, 1956). The administration of high doses of molybdenum (50 and 100 mg molybdenum per day as molybdate) to sheep, grazing on pasture with normal copper content, was found to decrease the copper concentration of the liver. However, a significant decrease in rate of depletion of liver copper, maintenance of significantly higher concentrations of copper in blood and precipitation of signs of severe copper deficiency were noticed in sheep grazing on copper-deficient pastures and receiving molybdenum supplementation. Dick (1952, 1954) demonstrated the characteristic lesions of copper deficiency in wool of sheep immediately after administering very high doses of both molybdenum and sulphate, despite the fact that liver copper was not depleted and blood copper levels were elevated above normal. These diverse and apparently conflicting observations were explained by Dick (1956) on the hypothesis that a membrane whose permeability to molybdenum is impeded by sulphate blocks copper transport.

Mills (1961) administered Cu\textsuperscript{54} intravenously to sheep and demonstrated that high intakes of molybdate and sulphate did not increase the rate of copper excretion. He suggested that these anions restrict copper utilization in sheep by depressing copper solubility within the digestive tract through precipitation as insoluble cupric sulphide. However, subsequently many drawbacks were noticed in this concept.
The other possibilities put-forth to explain the molybdenum induced metabolic disturbances involving copper were either inhibition of the activity of copper containing enzymes or the fixing of copper in an unavailable form in the tissues by the molybdeno-sulphur complexes (Underwood, 1962). There are indications that other dietary factors can exert a modifying influence on the inter-relationship between molybdenum and copper. Dick (1956) observed that high manganese intakes can block the limiting effect of molybdenum on retention of copper in sheep. He further showed that high protein diet with manganese and molybdenum together exert a severely limiting effect on copper retention.

The anti-molybdenum toxicity effect of copper has been repeatedly demonstrated in United States of America (Bennetts and Hall, 1939) and other parts of the world and it was found to be the only satisfactory method of overcoming the effect of high molybdenum intakes (Allcroft and Parker, 1949). It has been well demonstrated that higher intake of copper was necessary to prevent the toxic effect due to higher intake of molybdenum. With adequate copper and minimum molybdenum intake, the copper storage was found to be accentuated although the copper was not physiologically available to the animal.

Later studies were conducted by Vanderveen and Keener (1964) to evaluate the inter-relationship between these three constituents. It was observed that the heifers receiving diets low in copper and containing as much as 50 ppm of molybdenum and without added sulphate did not develop any
symptoms of molybdenum toxicity. Similar results were obtained with the diets containing 5 to 20 ppm molybdenum and 0.3% added sulphate sulphur. But the heifers receiving the diets containing 50 ppm molybdenum and 0.3% added sulphate sulphur developed severe symptoms of molybdenosis, which were alleviated by the supplementation of the diets with copper. Goodrich and Tillman (1966) obtained a lower body weight gains by increasing the sulphate sulphur level from 0.1 to 0.4% of the diets, which contained 2 ppm molybdenum and 10 ppm copper. This did not occur, however, when the diets contained 8 ppm molybdenum. Liver copper was lowest when 0.4% sulphate sulphur was fed in combination with 8 ppm molybdenum. Working with sheep, Marci-lese et al. (1969) reported a response with 0.4% dietary sulphate as similar to that with the control diet as measured by liver copper accumulation, plasma clearance and urinary excretion of intravenously injected radio-active copper. The sulphate plus 50 ppm molybdenum, however, resulted in reduced liver copper accumulation, reduced ceruloplasmin formation, a delayed plasma clearance of the injected copper and increased urinary excretion of both stable and radio-active copper. Similar results were obtained with sulphate plus molybdenum (Suttle and Field, 1968; and Smith et al., 1969).

Hogan et al. (1969) from a study with sheep concluded that under conditions which facilitate excessive storage of liver copper, molybdenum plus sulphate depressed the storage of the same. Dowdy and Matrone (1968 and & b) suggested the presence of copper-molybdenum complex, which renders copper
unavailable to the animal for metabolism. Hartmans and Van Der Grift (1964) observed the liver copper content of yearling cattle to be similar when they were fed either calcium or sodium sulphate and the liver copper levels were significantly lower than those of control group receiving no sulphate supplements.

Lambs born to ewes grazing on copper deficient pastures often developed enzootic ataxia or sway-back. Mills and Fell (1960) reported this condition when ewes were maintained on diets high in sulphate and molybdenum. Suttle and Field (1968) recorded liver and brain copper concentration in the lambs similar to those of ataxia by feeding either a low copper diet or a molybdenum plus sulphate supplement to the pregnant ewes. Effects were more severe when the two treatments were combined.

Butler and Barlow (1963) and Hogan et al (1966) failed to produce toxicity symptoms in sheep by feeding high dietary sulphate and molybdenum. Differences in the basal diet and breed of sheep were suggested as the probable causes for the dissimilar results, apart from some unknown dietary factor. However, some stiffness and incoordination of hind limbs as well as non-specific lesions of the central nervous system similar to those of ataxia were seen in young lambs with initial low copper status when they were administered orally large amounts of molybdate and sulphate by Butler et al (1964).

15. Copper and other dietary factors:

The simple or uncomplicated deficiencies or excess of trace elements rarely occur in animals under natural
conditions. They are frequently ameliorated or accentuated i.e., 'conditioned' by other dietary factors peculiar to the conditions and environment where the disease occurs. Some of them have already been reviewed. Nevertheless it is apparent that dietary balance is no less important with regard to the trace elements than it is with other nutrients and the metabolic studies with these elements can be frustrating and misleading unless the nature of the whole diet is taken into account.

The possible relationship of copper to calcium and phosphorus metabolism was suggested due to the occurrence of fragile bones in copper deficient animals. This led to the investigations on this aspect of copper metabolism. Shirley et al (1964) demonstrated that high levels of molybdenum and low copper intakes by the cattle resulted in an interference with phosphorus metabolism, with an excessive excretion of the same. Davis et al (1947) in a study of alkaline blood phosphatase discovered that when the liver copper was depleted, there was a sudden rise in inorganic phosphorus and phosphatase activity of the blood. This was often rapidly followed by fractures of bones and even death of the animal, if copper therapy was not instituted promptly.

High intakes of zinc were reported to induce copper deficiency in rat, probably by reducing its absorption (Smith and Larson, 1946). Dynna and Harve (1961) observed a complex zinc-copper deficiency in both grazing and stall-fed cattle. The symptoms were typical of those expected for deficiencies
of both zinc and copper, and the condition was aggravated by the administration of zinc alone. A positive response was observed in cattle when supplemental zinc and copper were provided.

The interference of cobalt in copper absorption was observed by Marston et al. (1948). The Merino sheep grazing on the highly calcareous soils of South Australia, where herbage contained about 3 ppm copper, rapidly developed signs of copper deficiency when supplied with additional cobalt.

Bosman (1966) observed that intake of high digestible crude protein combined with a low starch equivalent increased the sulphide content of rumen liquor of grazing cows. This enhanced the copper sulphide formation resulting in poor absorption of copper. He suggested that high blood urea level associated with a surplus of organic sulphur in the rumen indicated the presence of high concentrations of sulphide in the rumen and serve as evidence of a copper deficiency in the animal. Increase of dietary protein resulted in reduced levels of liver copper in sheep, presumably by decreasing copper absorption. Soybean meal had a greater depressing effect on liver copper than did casein (Ammerman et al., 1963; McPherson and Hemingway, 1965). Similar results were reported with swine by Combs et al. (1966). Goodrich and Tillman (1966) obtained higher liver copper in sheep when urea served as the nitrogen supplement than with the purified soybean protein. High dietary protein intakes ameliorated toxic effects of
copper in rats and pigs (Wallace et al, 1960). Methionine also was found to be effective in this way.

Research of Hartmans and Bosman (1970) in Netherland, indicated that the availability of copper to the animal was more in the dry forages than in the fresh ones. Despite having a lower copper content, mature grass was more effective in maintaining liver copper level in cattle than young grass.

The continued ingestion of fluorine at the level of 3 mg per kg live weight per day interfered with copper metabolism by increasing the minimum copper requirement to 133 percent (Anonymous, 1946-47). Kaufman et al (1951) studied the relationship of copper to choline and found that choline either increased absorption and deposition of copper or else inhibited its excretion. Indications were there to show that carbohydrate metabolism may also be associated with copper absorption. The increased metabolic rate during pregnancy resulted in an increase of copper storage in both the dam and the foetus (Kozelka and Penderco, 1952).

16. Copper in relation to Reproduction

The suppression of oestrus with low fertility and temporary sterility in cattle was associated with copper deficiency in several areas as reported by Bennetts and Hall (1939), Bennetts et al (1942, 1948), Russell (1944), Allcroft and Parker (1949) and Bennetts (1959).

A high incidence of infertility was reported by Bennetts et al (1948) to be associated with 'falling disease' in areas
where copper levels of the pastures were less than 3 ppm, which could be overcome by giving dietary copper supplements. Allcroft and Parker (1949) noticed low fertility in copper deficient cows and raised their blood copper levels by feeding copper sulphate supplement. Cunningham (1950) also considered that the reproductive capacity of cattle was reduced during copper deficiency condition. Hignett (1959) stated that hypocuprosis was sometimes, but not always, associated with reproductive disorders in female cattle. Then fertility was affected, he found that administration of copper either orally or parenterally, usually resulted in a rapid and dramatic improvement in breeding performance. This was in agreement with the report of Van Hensburg (1961), but is not supported by the observations of Russell (1944), Cunningham (1950) and Bennettts (1959). Dutt and Mills (1960) in a preliminary study with rats fed on low copper diet, found that only one out of eighteen females produced a live litter and this rat immediately started to eat her young ones. Resorption of embryo was also observed. Eight rats receiving added copper at the rate of 2 ppm produced normal litters of 3 to 7 pups. However, no abnormality in the oestrus cycle of affected rats was noted. In South Africa, Van Hensburg (1961) recorded increased fertility in cows by the administration of copper.

Lower liver and blood copper levels were noticed in the dairy cattle given no supplements than compared with those given supplements of either copper alone or trace elements plus copper. However, differences were not observed in the growth, production
and reproductive performances of the animals of control or supplemented groups (Engel et al, 1964).

Donaldson et al (1964) studied the effect of copper and cobalt supplementation of the ration of Shorthorn heifers on their growth rate and fertility. A condition of ill-thriftiness was noticed in weaner cattle. A response was obtained by copper but not cobalt therapy. There was a significantly greater number of pregnancies in copper supplemented groups. Also there was no evidence of delayed onset of oestrus in non-pregnant heifers. The circumstances under which a supplement of copper improved the reproductive performance were reported. But the problem was found to be complex one according to Mills (1967), who pointed out that situations were known in which copper deficiency in young stock was severe and yet no adverse effects on breeding performance was apparent. Tossell Robin (1967) reviewed the subject on the effects of the minerals on the reproductive performances of sheep and cattle.

Sane et al (1958) described an interesting out-break of herd infertility in India, apparently due to deficiency with the typical symptoms of hypo-cupraemia in Gir cattle. There was a good response to the feeding of one gramme of copper sulphate daily per head. Kehar et al (1964) found that feeding mineral supplements including copper resulted in reduced inter-calving period. Further investigations at Indian Veterinary Research Institute, Izatnagar and National Dairy Research Institute, Bangalore, indicated that extra copper supplements may help in inducing early oestrus in heifers and dry cows (Ray, 1967).
Sampath et al (1969) observed a retention of 25% of the supplemented copper, fed to heifers. Consequently, the blood copper levels were also found to be increased. The animals receiving the copper supplement came into oestrus and conceived earlier than those receiving no supplement. Amrit Kumar et al (1973) studied the effect of feeding copper and cobalt supplements to cross-bred cattle at Madhavaram Milk Colony, Madras where low fertility was reported. They reported that feeding supplement of copper and cobalt resulted in 72% of heifers becoming pregnant and 75% animals settled within 1 to 6 months.

A batch of six heifers which had not conceived in spite of having regular oestrus cycle, were given daily 5 gm copper sulphate along with concentrate mixture. All of them were inseminated after 2 to 8 weeks of starting the supplementation. More than one insemination was not required except in case of one cow, which was served twice. All were found carrying when examined after 3 months (Deshpande, 1959).

Kehar et al (1959) observed the lower calving intervals by supplementing the ration with copper of cows, which were thought to be deficient in copper.

Mahadevan et al (1961) and Mahadevan (1963) found that the cows came into heat within 23 to 90 days of giving copper supplement. Mathur (1960-61) reported that Sindhi heifers aged about three years came into heat within 46 to 98 days of copper supplementation of their ration. Mathur (1960-61) and Mahadevan (1958, 1959) observed lower copper levels of 56
to 84 and 93/μg per 100 ml respectively, in the blood of Sindhi and Mariana heifers, ranging in age from 30 to 48 months. The low blood copper levels were associated with anoestrus. They stressed that the adequate level of copper was required for normal reproduction.

The continuous feeding of 0.5 gm copper sulphate per day per head for over six years to Mariana cows had no toxic or adverse effects. But it was found to be very beneficial in inducing oestrus and reducing the inver-calving period. The blood copper level was also found to be significantly higher than those receiving no supplement (Mahadevan and Zubery, 1969).

Reddy (1969) concluded from his studies that supplementation of copper at the rate of 0.5 gm copper sulphate per day per animal for a period of 2 to 3 months after parturition to be beneficial for increasing the productive efficiency of the cattle in copper deficient or marginally deficient areas. Maciver and Foot (1972) observed higher (P < 0.005) percent of the embryo from the copper sulphate and steroid treated groups developed in vitro into blastocysts than those from L.H. treated group (92.0% vs 76.6%).

17. Copper toxicity:

The copper toxicity in ruminants is an important nutritional problem. It can be divided primarily into two types, namely (1) Natural chronic copper poisoning which is generally seen in sheep (Bull, 1949) due to (a) high copper content of soils and pastures, (b) normal copper content of soils and
pastures, but low level of molybdenum (Dick, 1952, 1953) and (c) in association with liver damage due to poisonous plants disturbing normal physiological function of liver and thereby copper storage and poisoning subsequently (Trautner and Mu- 
field, 1949). (2) Accidental copper poisoning as a result of ingestion of excessive amounts of copper due to increased use of copper compounds in agriculture and veterinary practices (Boughton and Hardy, 1934) and indiscriminate use of copper in the treatment of the various copper-deficiency diseases of the stock that occur in some areas (Underwood, 1962).

In all animals, continued ingestion of copper in excess of the requirement leads to some accumulation with the tissues, especially the liver. In cattle and sheep, liver has a re-
markable ability to take up and retain very large amounts of ingested copper up to certain levels, which varies greatly among individuals and species, without any physiological harm to the animal. Above these levels, a catastrophic liberation of a high proportion of the liver copper into the blood stream occurs, with resultant extensive haemolysis and jaundice followed by death. Ammerman (1970) in his recent review has divided the chronic copper poisoning into two distinct phases, namely, (a) a period of passive accumulation of copper in the tissue (especially liver) during which no symptoms of toxicity are exhibited and (b) the toxic phase, which is an acute ill-
ness, frequently referred to as the 'copper crisis syndrome' or 'haemolytic crisis', which can cause death within few hours to 2 - 3 days (Guilbide, 1963). It is suggested that the
high liver copper concentration result in erythrocyte destruction in that organ (Goldberg et al., 1956). The common characteristic features are sudden hemoglobinemia and hemoglobinuria associated with marked acute hemolytic icterus, widespread necrosis of liver, high levels of copper in liver and blood in fatal cases.

Sheep are more susceptible than cattle to copper poisoning. Cattle tolerate high dietary intake of copper than other species that have been studied. Very few references on chronic copper poisoning in the bovines are found. Adult cows were fed 1.2 to 2.0 gm copper sulphate daily for 5 to 10 weeks without harm (Ferguson, 1943). Cunningham (1946) fed 0.8 to 5.0 gm copper sulphate per day per head for 9 months to calves and adult cows without any ill effects. On the other hand, Kidder (1949) induced generalised haemolysis, icterus, haemoglobinuria and death in 500 lb steer by feeding 5.0 gm hydrated copper sulphate for 122 days. Shand and Lewis (1957) noticed typical signs of chronic copper poisoning in calves fed on milk substitute powder containing 115 ppm copper.

In large doses copper is extremely toxic and heavy losses due to copper poisoning were reported by Boughten and Hardy (1934) and Eden (1940). The toxic level, however, was reported by Eden (1940) and Pullar (1940) to vary with the type of diet as also with the species. Cunningham (1946) gave copper sulphate upto 80 gm in a single dose to yearling heifers, which did not develop toxic symptoms. A single dose of 400 gm of copper sulphate was lethal to all cattle, whereas the average lethal
dose (single) ranged between 200 to 400 gm. Border line toxic level for cattle was found to be approximately 1 gm of copper sulphate per 100 lb body weight (Boughten and Hardy, 1934).

Natural chronic copper poisoning can be alleviated by molybdate containing 'licks', which reduced the liver copper levels (Dick and Bull, 1954; Pierson and Aanes, 1968). The value of dosing with ammonium molybdate and sodium sulphate in preventing chronic copper poisoning in sheep was demonstrated by Ross (1964).

McCloskey (1968) made a detailed study on blood copper during chronic copper poisoning in sheep. During the first stage, there was a transient elevation in red blood cell copper and in the second stage the level of whole blood copper essentially doubled from increases in red blood cell copper and also direct reacting copper. Plasma colour changed by the presence of bilirubin and there was a reduction in haematocrit value. A high level of 190 µg copper per 100 ml blood appeared during the 'hemolytic crisis' first in plasma as direct reacting copper and later in the red blood cells. Similar observations with calves subjected to chronic copper poisoning were reported byTodd and Thompson (1965) and Weiss and Baur (1968). The later authors reported dystrophy and cirrhosis of the liver and levels of copper in the liver at the time of death to vary between 898 to 2,091 ppm on a dry tissue basis.

The determination of serum glutamic oxaloacetic transaminase (SGOT) activity was suggested to be an useful index in
diagnosing and evaluating the treatment of copper poisoning (Ross, 1966; and Todd and Thompson, 1963). Concentrations of SGOT increased rapidly during approximately 4 weeks before death and decreased in relation to the effectiveness of the copper toxicity treatment. The author emphasized the value of plasma arginase as an aid in differential diagnosis, since this enzyme increased in liver damage while SGOT concentrations increased with either liver or other tissue damage. Todd and Thompson (1964) demonstrated that a single dose of intravenously injected copper (80 to 160 mg per head) caused death in sheep within a few hours to several days after injection and resulted in symptoms similar to gastro-enteritis rather than chronic copper poisoning. Repeated injections of smaller amounts of copper resulted in characteristic symptoms of copper toxicity.

The copper toxicity of sheep under natural grazing conditions in Western Australia was suggested by Beck and Bennetts (1963) to result from factors including native plants containing high levels of copper and hepatotoxic principles of fungal origin. The other toxic principles causing liver degeneration and thus, pre-disposing the animal to copper toxicity were recently discussed by Clemetson (1966) and Proyor (1966).

The degree of toxicity was found to be influenced by the form of copper administered. About 12 gm copper sulphate per head daily when administered as a drench to steers caused death in approximately 60 days, whereas same amount of copper sulphate administered in dry form in a gelatin capsule even for a period
of over a year did not indicate any toxic symptoms (Champman et al, 1962).

18. Copper Requirements and Supplementation:

Due to the presence of many dietary factors, which greatly influence the assimilation and utilization of copper in the body, the assessment of copper requirements based solely on copper intakes are of limited values. The copper requirements vary according to the presence or absence of these factors, and to the extent they are present in the normal diet of animals. Therefore, it is difficult to prepare a master table of the requirements of such a inter linked essential element. Secondly, the contamination as well as intrinsic and complex physiology of the element concerned pose problems in the assessment of its actual requirements. Apart from this, the feeding of purified diet to large animals to overcome these drawbacks, is not practicable and also this does not give the exact picture when the feeding is done under natural conditions.

The studies similar to the one undertaken will be more of practical value in overcoming the previously mentioned problems in the assessment of the requirement of the trace elements.

A basic minimum requirement can be assessed when all the other dietary conditions influencing copper absorption assimilation are at the optimum and balanced. Such an exact requirement has not yet been given. However, attempts have been made to get as closer as possible to the requirement.
The minimum copper requirement of cattle and sheep were assessed to be 4 ppm of the diet (on dry matter basis) on pastures with less than 1 ppm of molybdenum and not containing calcium carbonate. Marino sheep, however, required nearly 6 ppm of copper on such pastures (Beck, 1951). Dick (1964) demonstrated that an intake of as low as 0.5 mg of molybdenum per day adversely affected the copper retention in sheep, provided the sulphate intake was high. This study indicated that if other interfering factors were present in the diet, they may adversely affect the absorption and retention or increase the excretion of copper. Such dietary factors were observed to be operative in many environments. (Marston and Lee, 1948a,b; Marston et al, 1948; Anonym, 1942-46, 1946-47, 1948, 1952, 1953). Under such particular conditions, where there was high consumption of calcium carbonate and moderate intakes of molybdenum as well as sulphate from the herbage, it appeared that the minimum copper requirements of wool sheep were close to 10 mg per day (10 ppm of the diet).

A sub-normal status of copper in cattle (Allcroft and Parker, 1949; Lewis and Allcroft, 1960) and in sheep together with 'sway-back' in lambs (Allcroft, 1952; Allcroft and Lewis, 1957; Hunter et al, 1945; Innes and Sheaver, 1940; Sheaver et al, 1940; Sheaver and McDougall, 1944) were shown to arise on pastures containing 8 to 14 ppm copper or even more and normal levels of molybdenum and sulphate. It is evident that the assessment of the minimum copper requirement of sheep and cattle is one of great complexity.
Bennetts et al. (1941) and Maynard (1951) reported that the minimum requirement of copper for cattle and sheep to be 50 - 60 mg and 5 mg respectively per day. The animals were observed to be healthy on forages containing 5 - 8 ppm copper when the interference from molybdenum and sulphate were minimum.

The copper requirement of the cattle was not known as reported by the National Research Council Committee on Animal Nutrition, U.S.A. (1950). The addition of one percent of copper sulphate in salt, in copper deficient areas was recommended by the committee. However, on residual muck and high molybdenum mineral soils, higher levels of supplementation of copper were recommended.

Investigations carried out at the Indian Veterinary Research Institute, Izatnagar, (Annon, 1946-47) on trace elements with Kumaoni bullocks revealed that the minimum requirement of copper for these bullocks ranged from 6.56 to 6.87 with an average of 6.69 mg of copper per 100 lb live weight per day. Further, it was noticed that optimum results were obtained by supplementation of the feed consisting of wheat straw and rape cake with copper at the rate of 7.38 mg of copper per day per 100 lb live weight. It was almost 10 percent higher than the minimum copper requirement recommended. It was further recommended that the minimum level of copper required by the cattle to be 7.3 to 8.0 ppm of the ration and the levels less than this was likely to result in the manifestation of the symptoms of copper deficiency. Based on the metabolism trials on cattle with different levels of copper intakes, Sahai (1947) suggested the requirement of this mineral to be 6.5 mg per 50 kg body weight.
The requirements of these species for copper in deficient areas can be met by any one of the three suggested methods: (i) by the application of copper containing fertilizers to the soil, which raises the copper content of the herbages to the adequate levels, (ii) by providing copper containing salt licks, drenching the animals at suitable intervals with soluble copper salts, or addition of 0.5 to 2% of copper sulphate to salt/mineral mixture and (iii) by injecting various organic complexes of copper intramuscularly or sub-cutaneously at about 3 months intervals in doses of 120 to 240 mg for cattle (Allcroft and Uvarov, 1959) and 30 to 40 mg for sheep (Cunningham, 1969; Moule et al, 1969) and Sutherland et al, 1966). For this purpose, copper glycinate was found to be safe and effective. Though this method of copper supplementation is not widely used, except in Great Britain, the workers in Australia also indicated its effectiveness (Alexander et al, 1967, Cook et al, 1966; Donaldson, 1964; and Dunkley et al, 1963). Injectable copper glycinate, copper methionate and also Cu-EDTA, were effective in maintaining normal blood copper levels in pregnant ewes (Hemingway et al, 1970). The injectable Cu-EDTA complexes currently available generally result in less localized reaction than that occur with copper glycinate. But they may have a greater toxicity potential than with glycinate (Ishmael et al, 1970).

Copper deficiency or molybdenum toxicity was successfully treated by the use of dietary supplements of copper (Dye and O'Harra, 1959; Eide, 1962; Underwood, 1966). Top dressing of
copper deficient forages with copper salts was of limited success in various geographic locations (Allcroft and Uvarov, 1959; Underwood, 1960; and Spencers et al, 1958). The incorporation of copper salts in water or ration was often practiced in Western countries. However, the administration of dietary supplements of copper was found to be impracticable in the case of animals under pasture and range conditions. Hence, with the advent of injectible organic compounds such as copper glycinate suspensions, many ranchers and stock owners switched over to this preventive method of overcoming the deficiency of copper in their stock (Harvey and Sutherland, 1963; Cunningham, 1957; and Dye and O’Harra, 1959).

Many copper compounds were used as dietary supplements. The uptake of Cu$^{64}$ by the blood of sheep was found to be greater in the case of labelled cupric chloride than either cupric nitrate or sulphate, administered orally (Lassiter, and Bell, 1960). Similarly, cupric carbonate was better utilized than cupric or cuprous oxide. Chaapan and Bell (1963) while working on Hereford steers with radioactive copper compounds, noted that copper from cupric nitrate, cupric sulphate, cupric chloride and cupric carbonate were absorbed to a similar extent. More absorption into blood stream was found from cupric oxide in the powder form than from the needle form. The authors concluded that cupric sulphate was the most suitable source, out of those tested for use as a dietary copper supplements, from both physical and biological points of view. Buescher et al (1961) and Bunch et al (1961, 1965) also obtained similar results with the pigs.
19. Copper in Animal Feed and Fodder:

The copper content of animal feeds such as straws, green fodders and concentrates, which constitute the principle basal diet for the livestock in various parts of India, was estimated by Sahai and Kehar (1951). Seventy-two common feeding stuffs were analysed for their copper contents, of which some are listed in Table A (Appendix). The copper content of feeds ranged from 3.6 mg in oat straw to 17.8 mg per kg in Stereocarpus spurius (Podal) leaves. A critical examination of the results suggest that a tentative grouping of the feeds based on their copper contents is possible. When arranged in an ascending order of copper contents, cereal straws with 3.6 to 4.7 mg per kg constitute the first group. Green feeds containing 5.9 to 11.9 mg of copper per kg from the second group. The third group includes oil cakes, grains, seeds and their by-products, having a copper content of 9.2 to 17.2 mg per kg.

Similarly the data of the copper contents of pertinent animal feeds, published by Kanwar and Randhawa (1967) and also Elvehjem and Hart (1927) are also included in the Table A (Appendix).

The copper contents of crops could be increased within certain limits by manuring with a copper salt (Elvehjem and Hart, 1927; Wark, 1954; Joshi and Joshi, 1957 and Bhumbla et al, 1963-65). The copper content in the grasses was reported to be increased as a result of heavily fertilizing the
soils with the nitrogen or nitrogen and potash (Midd, 1970). And this level of copper in grasses was in accordance with the Australian standards for the requirements of dairy cows. But only in few cases, it exceeded the levels prescribed.

Levels of copper that gave satisfactory results in farm animals (Blaxter, 1952) are given in the following table.

<table>
<thead>
<tr>
<th>Copper content of pastures in relation to requirements of sheep and cattle</th>
<th>ppm of copper in</th>
<th>ppm of copper in</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Estimated mineral requirement</td>
<td>5</td>
<td>HERBAGE</td>
</tr>
<tr>
<td>2. Normal grazing, Great Britain</td>
<td>7 to 14</td>
<td></td>
</tr>
<tr>
<td>3. Normal grazing, Australia</td>
<td>7 to 12</td>
<td></td>
</tr>
</tbody>
</table>

For the areas where the diseases are cured by the administration of copper:

1. Sway-back area, Australia                                              | 1 to 14          |
2. " " Derbyshire                                                         | 12 to 27         |
3. " " Scotland                                                          | 4.4 to 9.0       |
4. Peat-scour area, New Zealand                                           | 3.6              |
5. " " England                                                           | 8 to 26          |
6. Copper-pine areas, Caithness                                           | 6 to 21          |
7. Falling disease areas, Australia                                       | less than 2      |
8. Polder disease areas, Holland                                          | 5 to 11          |

Deshpande (1959) analysed the samples of straws, greens and hay for their copper contents and reported the values to be 3.0 to 6.85 ppm for grasses and 3.39 to 5.23 ppm for cereal straws. Green jowar and maize contained 8.30 and 9.20 ppm copper, respectively.
Patel et al (1968) analysed about thirty samples of feeds and fodders collected from the different sources in Gujarat state, for their trace mineral contents. Their findings are also incorporated in Table A (appendix). Sawhney and Bedi (1972) and Sawhney et al (1973) analysed about hundred samples of feeds, fodders, tree leaves of hill area for their trace mineral contents. They reported that straws contained 5.8 to 15.8; grasses 5.8 to 19.8; green fodders 10.2 to 21.0; and tree leaves 7.8 to 17.0 ppm of copper on dry matter basis. Pertinent findings of theirs are also listed in Table A (appendix). Their study indicated that the distribution of copper varied widely in feeds and fodders grown in different regions. The concentration of copper in majority of fodders from hilly regions was found to be comparatively lower than those from the plains.