1. INTRODUCTION
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1.1 Cadmium

Cadmium is a relatively rare, physiologically non-essential and toxic element. Technological use of this metal has generated much current debate as to the extent and possible consequence of its increase in the environment. Health hazards to miners and exposed industrial workers have also become a matter of increasing concern and considerable studies have therefore been undertaken and are still being pursued on the biochemical and physiological effects of cadmium (Cd).

1.1.1 Sources and distribution of cadmium in the environment

Cadmium (Cd), a crystalline, silver-white malleable metal is obtained as a byproduct in the refining of Zinc and other metals. Cadmium and its compounds have been increasingly used in industries, causing a sharp increase in environmental contamination and water pollution (1,2). Concentration of cadmium in the earth's crust is low.

The use of cadmium containing fertilizers, agricultural chemicals and pesticides might also contribute to the contamination. The human is thus exposed to toxic cadmium or its salts particularly cadmium oxide occupationally as well as non-occupationally (3).
1.1.2 Distribution of cadmium in the Tissues

Animals exposed either acutely or chronically to cadmium accumulate this metal in nearly every tissue. The tissue levels, however, are influenced by the route of administration and change as a function of time. Although Cd is easily measured in most of the major tissues such as kidney, liver, spleen, pancreas and testes shortly following exposure, it undergoes temporal redistribution such that liver and kidney become the major site of accumulation.

Hepatic cadmium

Following either an acute or chronic exposure, the liver becomes a primary site of cadmium accumulation. Cadmium administered by parenteral injection was rapidly accumulated in the liver (4,5,6,7-9). However, orally administered Cd appeared in the liver at a much slower rate (4).

Renal cadmium

Unlike liver, the kidney accumulates Cd more slowly, where it is preferentially concentrated in the cortex (10). Renal cortex continues to accumulate Cd long after other tissues reach levels of saturation and begin to slowly decline (29).

Pancreatic cadmium

A rapid accumulation of Cd occurred in the pancreas within the first 10 hours and maximum tissue levels were main-
tained for at least 25 days (9). Pancreatic Cd concentrations, measured either after single subcutaneous injection or following chronic exposure were always less than the hepatic level (9).

**Testicular cadmium**

Cadmium accumulation in testes reached the maximal levels within 10 hours after exposure, and cadmium concentrations showed a gradual decline as a function of time (9). In this organ, cadmium is primarily associated with the interstitial cells and the testicular vascular endothelium (13-16) but does not penetrate the tubules and become associated with sperm cells (17).

**Cadmium in blood**

Cadmium is rapidly accumulated and cleared from blood. Maximal concentrations were reached 5-10 minutes after either intraperitoneal or subcutaneous injection and 90% clearance occurred after several hours (11,18). Within blood, Cd is partitioned between plasma and erythrocytes (9,19).

**Cadmium in other tissues**

In addition to the major sites of Cd accumulation, the metal is found in a wide variety of other tissues like heart, spleen, lymph glands, thymus etc. though to a much lesser extent (6,8).
Cadmium protein interaction

Cadmium has been shown to interact with a variety of proteins (20), the most interesting being metallothionein, a low molecular weight metalloprotein first described by Margoshes and Vallee (12). The ratio of total metal content (Cd + Zn) to the number of titrable -SH groups suggests that three -SH groups are required for binding one metal ion (21) although Webb (22) suggests that metallothionein isolated from rat liver requires the participation of two -SH groups to bind one metal ion. The binding of Cd or Zn to metallothionein is reversible (21,23).

Data gathered from several laboratories (24-29) provide direct evidence for the inducibility of metallothionein, de novo synthesis being initiated in response to Cd$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ or Ag$^{2+}$ exposure. Cadmium bound to hepatic metallothionein was elevated 16,000 fold in rats 42 hours following a single subcutaneous dose of CdCl$_2$. The biological function of metallothionein has not been clearly identified. It has been suggested that this protein may participate in the transport and clearance of absorbed cadmium or other heavy metals. In addition, it has been inferred that metallothionein may behave as a protective agent in ameliorating acute cadmium toxicity (23,30,31).

Temporal redistribution and excretion of cadmium suggest the involvement of metallothionein as a transport protein.
Increased Cd$^{2+}$ tolerance paralleled a dose-dependent increase in the cadmium binding capacity of metallothionein as a result of either zinc or cadmium induced metallothionein synthesis (23,31).

1.1.3 Effect of cadmium on different systems

Nervous system

The neurotoxic effects associated with exposure to inorganic Cd have only recently been studied, while Cd-induced brain lesions are well documented for neonatal animals (32). Friberg et al. (33) investigated on sensory, dermal, optic and motoric chronaxie. Exposure to Cd-dust can produce complete loss of olfactory sensitivity (34). Prolonged exposure to this metal has revealed only small amounts of Cd in adult brain (35-37), indicating that the blood brain barrier effectively restricts the penetration of Cd into the central nervous system. However, studies by Lucis et al. (38) indicate that Cd penetrates the blood brain barrier with more ease in fetal rats. Moreover, Cd has also been demonstrated to accumulate in adult brain (39). Several studies have shown that cadmium produces neuropathological (40,41), neurochemical (42) and behavioral changes.

Cardiovascular system

Perhaps the most controversial issue concerning the effects of cadmium on man is the suggestion that the metal
plays a significant role in the etiology of hypertension (43–50).

Low concentrations of Cd have been shown to induce hypertension in dogs and rats, to increase retention of both sodium and potassium, and to decrease water excretion (43, 51, 52). It has been suggested that increased sodium retention may precede the onset of hypertension in Cd-treated rats (51, 52).

In rabbits and dogs, hypertension was also induced by intraperitoneal injections of Cd-acetate (46).

**Pulmonary system**

The pulmonary effects of cadmium are associated with the inhalation of airborne cadmium compounds but not with the absorption of Cd by other routes of administration.

Acute pneumonitis followed by reparative proliferation of lung tissue has been reported following exposure to Cd in animals (53, 54).

The exposure to cadmium oxide fumes cause emphysema, chronic bronchitis and bronchial carcinoma and such patients have shown higher levels of Cd in lungs, liver and kidneys (33, 55, 56).

**Renal system**

Marme' (57), was one of the first to comment on the nephrotoxic effects of Cd in animals. He reported that small doses of Cd produced a diffused inflammation of the kidneys. Several subsequent reports confirmed and elaborated on the ability of Cd to inflict morphological damage to the renal
tissue. However, it was not until mid-1900s that the effects of Cd on human renal function began to be recognized.

Reproductive system

Studies by Allsberg and Schwartz (58), as early as 1919, followed by Parizek in the 1950s (13) have demonstrated the destructive effect of Cd on testicular tissue. On parenteral administration of Cd, the testes became hemorrhagic, edematous, and in time necrotic. The sterilizing effect of Cd is very rapid and animals can become permanently sterile as early as 24 hours after injection (13,59).

Cadmium, bone and calcium metabolism

The bone abnormalities in patients with Itai-Itai disease are thought to be caused by primary kidney damage due to Cd. However it was reported that the bone lesions in Cd-treated animals occurred before kidney damage (60). Also some patients with Itai-Itai disease showed bone damage but not marked kidney damage (61). This suggests that bone damage may be due to a direct action of Cd which was further supported by the studies using embryonic chick bone in a culture system. It was revealed that Cd caused a decrease in both mineral and collagen content (62).

The amount of calcium in the feces and urine of rats exposed to continuous administration of Cd increases with duration of exposure (63). It was found (64) that Cd inhibits
the rate of Ca absorption from G.I. tract as well as its uptake into the bone. In contrast, Ca resorption from the bone was found to be greatly increased by continuous oral exposure to Cd (65). These results are indicative of a negative calcium balance that occurs due to continuous exposure of rats to Cd. A specific inhibition of Ca-transport in the intestine by Cd has also been reported (66).

**Cadmium and carcinogenesis**

A number of inorganic substances are known to be carcinogenic and impart genetic damage (67). Cadmium is carcinogenic to animals when given subcutaneously or intramuscularly as Cd-metal powder, CdSO₄, CdCl₂, CdO or CdS. Its place as a carcinogen in human is not well established. Cadmium salts given by mouth or by inhalation do not appear to give rise to malignancy. However, Takenaka et al. (68) demonstrated high incidence of lung cancer in long term experiments. Cadmium has also been shown to induce testicular tumours (14,69-71), injection-site sarcomas (14,69) and most recently prostatic tumours (70,71). Cadmium is also a suspected human carcinogen (72) and has been associated in some studies with respiratory, prostatic and renal tumours in occupationally and/or environmentally exposed individuals (73). Dietary zinc deficiency enhances carcinogenic response of the injection site of Cd, promotes the neoplastic progression of Cd induced testicular lesions, and enhances chronic progressive nephropathy (72).
1.1.4 Effect of cadmium on Hepatic Metabolism and Enzyme Systems

It is evident from the studies of various workers that liver is one of the principal target organs on which cadmium exerts its harmful effects (74-77). Klaassen et al. (78) showed that acute administration of Cd results in both time and dose dependent injury to liver. Cadmium induced hepatotoxicity occurred rapidly as evidenced by increased serum amino transferase activities. The degree of Cd-induced liver injury progresses from generalized swelling of hepatocytes to massive necrosis at later stages. Since Cd preferentially localizes in hepatocytes (79-81), the degree of liver injury is probably a cytotoxic effect of cadmium. The metal has been shown to accumulate in the nuclei of hepatocytes both in vitro and in vivo soon after exposure (82-84). Cadmium interacts with DNA in rat liver (85), and it is conceivable that such an interaction may stimulate hepatocyte cell division. The interaction of Cd with the bases in DNA may also be related to its apparent carcinogenicity (86).

The mechanism by which Cd produces liver damage is not yet well understood (87-89). A plausible mechanism for Cd-induced hepatotoxicity may involve build up of non-thionein bound Cd within hepatocytes. Cadmium, as stated earlier (81), preferentially localizes in hepatocytes after administration. Conceivably, the concentration of Cd in hepatocytes after acute exposure may exceed the capacity of intracellular constituents
(e.g. metallothionein; glutathione) to bind Cd, thus enabling the metal to interact with cellular organelles and disrupt biochemical processes. The rapid time course of liver damage observed and its relationships to dose support such a mechanism.

Few studies have focussed specifically on liver as a major organ for toxic effect of Cd after subchronic or chronic exposure. Prodan (90) observed fatty changes in liver after cats were fed with Cd in their diet or inhaled fumes containing high concentrations of cadmium oxide. Axelsson and Piscator (91) reported elevated serum aspartate transaminase (AST) activities in rabbits 17 weeks after injection with Cd (0.25 mg/kg), 5 days/week. Stowe (39) has provided morphologic evidence of Cd-induced hepatic injury after chronic poisoning. Observations, consisted of interlobular and periportal fibrosis, biliary hyperplasia, and focal infiltrates of inflammatory cells in liver of rabbits, fed 160 ppm Cd for 200 days. Interestingly, no elevation of serum enzymes indicative of hepatic damage was observed. In contrast, Faeder et. al. (92) have reported Cd-induced elevations of plasma enzymes released from liver 4-weeks after rats were injected subcutaneously (s.c.) with 0.25 mg Cd/kg, 3 days/week.

Cadmium is found in the particulate fraction of liver, which comprises mainly of mitochondria that proved to be susceptible even towards low doses of Cd (93,94). Prasad et. al. (95,96) studied the in vivo effects of Cd on liver mitochondrial enzymes of male rats after acute oral administration.
They observed that mitochondrial cytochrome c oxidase activity was significantly inhibited at all time periods after oral administration of Cd indicating that the metal interferes with the mitochondrial oxidative metabolism by inhibiting mitochondrial enzymes associated with citric acid cycle and electron transport chain.

Cadmium produces disturbances in carbohydrate metabolism as evidenced by a decrease in aldolase activity and glycogen levels, together with an increase in phosphorylase a activity of hepatic tissue (39,97). Furthermore, hyperglycaemia and glycosuria are consistently found in Cd treated animals. It is of interest that glycosuria also is a common finding in both industrially exposed workers and Itai-Itai patients (98). Chronic Cd-treatment (0.25 mg/kg and 1.0 mg/kg) for a period of 21 days and 45 days, increased the concentration of blood glucose and urea and decreased liver glycogen content. Cadmium chloride, given for 21 days at a dose of 0.25 mg/kg failed to markedly alter any of the hepatic gluconeogenic enzymes. However when the treatment was extended to 45 days, a significant rise in pyruvate carboxylase to 125%, phosphoenolpyruvate carboxy-kinase to 153% and glucose-6-phosphatase to 138% of the control values was observed (99). It may be noteworthy that the higher dose (1 mg/kg) of cadmium chloride enhanced the activities of all four gluconeogenic enzymes after 21 days. However, the degree of stimulation was generally greater in rats exposed to this dose of metal for longer period (99,100). The metabolic
alterations persisted even after discontinuation of exposure to Cd. This might be due to the fact that Cd has a long biological half-life and hence is retained by the hepatic tissue (11). Acute treatment with Cd decreased hepatic glycogen levels, and produced a significant rise in the concentration of blood glucose and serum urea levels to 415 and 232% of the control values, respectively. However, the activities of the four key gluconeogenic enzymes remained unchanged in animals exposed to Cd (99) for a short period.

Results of Merali et al. (99) showed that acute or chronic administration of Cd produces a rise in serum urea. Since urea represents the chief metabolic product of protein and amino acid catabolism, it seems to be consistent with the suggestion that Cd alters protein metabolism. Indeed, administration of Cd to rabbits has been reported to enhance the production of hepatic soluble protein, metallothionein, retard growth rate, and produce serum protein dyscrasias (39,101). The elevation of serum urea in rats exposed to Cd could also be related to enhanced glucose synthesis from proteins and amino acids.

Cadmium intoxication has also been found to induce chronic stress in animals, causing increased catecholamine production and discharge (102,103) which in turn activates phosphorylase a, promoting the conversion of glycogen stores into glucose.
It was investigated whether Cd induced changes in hepatic carbohydrate metabolism are related to the stimulation of the cAMP-adenylate cyclase system (99).

Cadmium produced a marked rise in the endogenous levels of cAMP and in the activity of basal, fluoride and epinephrine stimulated forms of adenylate cyclase (99). Since administration of cAMP increases the concentration of glucose and urea in blood, enhances synthesis of glucose from noncarbohydrate precursors and lowers the level of hepatic glycogen, it would appear that Cd-induced changes in hepatic carbohydrate metabolism might be qualitatively similar to those produced by cAMP (99). It was also revealed that changes in hepatic gluconeogenic capacity are preceded and probably triggered by alterations in the cAMP adenylate cyclase system following treatment with cadmium.

The increase in the endogenous cAMP concentration due to Cd was also reflected by a decrease in the cAMP-binding capacity of hepatic protein kinase. Although the low dose of Cd failed to produce any significant change in the activities of either cAMP dependent or independent forms of the enzyme, treatment with the higher dose markedly reduces kinase activity assayed in presence of cAMP (104).

The biological actions of metals, both essential and nonessential, are heavily conditioned by metal ion antagonism i.e., one metal may induce a biological effect by altering
the requirement for another metal (20).

The role of selenium and zinc in protecting various Cd-induced biochemical and functional alterations with respect to hepatic carbohydrate metabolism was investigated thoroughly. In one experiment, administration of Cd for 7 days elevated the activities of all the four hepatic gluconeogenic enzymes. In contrast, exposure to Se for the same length of time failed to significantly alter the four key gluconeogenic enzymes. However, when the same doses of Zn or Se were administered simultaneously with Cd (at different sites), the activities of all four gluconeogenic enzymes were significantly lower than those noted for rats given Cd alone, and were in the same range as control values (105). Similarly in the same investigation, it was shown that zinc by itself failed to alter hepatic cAMP level. However, when administered concomitantly with Cd, it completely prevented Cd induced elevation of hepatic cAMP concentration.

Both epinephrine and glucagon were able to stimulate adenylate cyclase activity in liver. However, in Cd-exposed animals, glucagon further stimulated the enzyme activity by 33% and epinephrine by 135%, indicating that the sensitivity of adenylate cyclase to these hormonal modulators is enhanced in cadmium exposed rats. It has been shown that Zn by itself does not alter the sensitivity of adenylate cyclase to epinephrine and glucagon. However, administration of Zn concurrently with Cd significantly prevented the Cd induced increase in the activity of basal as well as hormone stimulated form(s) of adenylate
cyclase (105).

Besides its effects on carbohydrate metabolism, Cd has been shown to enhance the activities of a number of hepatic enzymes. These include, δ-aminolevulinic acid dehydratase of fowl liver (106), glucose-6-phosphate dehydrogenase and malic dehydrogenase of mouse liver (107) and phosphorylase of rat liver (97).

Similarly, aldolase of rat liver (97), alcohol dehydrogenase (108), alkaline phosphatase, ALA-synthetase (109,10) and catalase of chicken liver (106) have been shown to be inhibited by cadmium.

Cadmium has been shown to alter hepatic microsomal drug metabolism (110). Hepatic mitochondrial functional changes are evident from uncoupling of oxidative phosphorylation, inhibition of electron transport and inhibition of substrate utilization (111) under in vitro conditions. Diamond and Kench (112) reported inhibition of oxygen utilization in cadmium-poisoned rat liver mitochondria.

Informations on the effect of Cd on lipid metabolism are a few. Cadmium has been reported to inhibit the activity of a number of lipid metabolizing enzymes in vitro, Viz., lecithin cholesterol acyltransferase (LCAT) (113), and acyl-Sn-glycerol-3-phosphocholine acyltransferase of rat liver microsomes (114). Cadmium has been shown both histochemically and biochemically to decrease the lipid content of liver (115,116).
It has been suggested that Cd may be an important factor in the induction and progression of atherosclerosis in regions and that it may reduce the plasma concentration of HDL and cholesterol through its effect on liver (117). Studies on phospholipid metabolism of rat liver after chronic Cd administration for 6 and 12 months have shown that inorganic $^{32}$P-incorporation into phosphatidylcholine (PC) in rat liver after ingestion of 250 ppm Cd as CdCl$_2$ in drinking water increased by 3.2 and 5.8 folds after 6 and 12 months, respectively. Incorporation of 1-{$^3$H}-glycerol into liver PC increased by 2.3 and 3.2 folds after 6 and 12 months respectively. Linoleic acid and stearic acid levels in the phospholipids were increased by Cd with a decrease in palmitic acid and decosahexamic acid [22:6] levels (118).

Evidence was obtained by Stoll et al. (85) under in vivo and in vitro conditions demonstrating both an enhancement and inhibition of RNA synthesis by Cd which was dose and time dependent and which appeared to correlate with hepatic concentrations of the metal.

A marked inhibition of DNA synthesis in regenerating liver was observed after administration of Cd (85). Moreover, Cd was also shown to differentially affect mRNA and polyuridylic acid directed L-[14C] phenylalanine incorporation into microsomal protein when the metal was administered to rats. A trend towards enhancement of mRNA activity was noted, whereas polyuridylic acid directed amino acid incorporation was uniformly
inhibited. The observed increase in mRNA activity at Cd concentrations greater than $10^{-6}$M may be explained by a possible interaction of Cd with RNA polymerase, a zinc dependent enzyme (85).

Block et al. (119) became interested in a possible role of Cd in protein kinase C, since this known carcinogen (cadmium) has been shown to exchange easily for zinc in the so-called zinc fingers of proteins (120). Cadmium interacts with signal transduction at various levels (121,122), and has recently been demonstrated to activate glucose transporter (123), which is a substrate for protein kinase C (124). Block et al. (119) have investigated on the role of Cd in activating protein kinase C at nuclear level.

Since protein kinase C has a pivotal role in the control of mitogenesis, the interaction of Cd with this enzyme seems to be an important parameter to probe into the mechanism of a possible mitogenic effect of Cd. The site of action of Cd on protein kinase C may be on the Zinc-finger domain, since it is known that Cd exchanges and substitutes for zinc in the zinc finger structure leading to an expansion of this domain (120, 125).

1.2 Riboflavin

The importance of riboflavin (vitamin B$_2$) in human nutrition has long been appreciated. It occupies a central role in
intermediary metabolism as the precursor of the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) and of flavins bound to tissue proteins, all of which catalyze a wide number of important reactions. The electron transport chain and lipid metabolism (126) require derivatives of riboflavin, as does the degradation of drugs and foreign chemicals via microsomal hydroxylation (127). Riboflavin cofactors are required for normal metabolism of folic acid (128), pyridoxine (129), and niacin (130) and are utilized by erythrocyte enzymes, glutathione reductase (131) and nicotinamide adenine dinucleotide dehydrogenase methemoglobin reductase (132), and have many other functions as well. Clinically riboflavin deficiency may be manifested by seborrheic dermatitis, cataract, glossitis, anemia and neuropathy (133). Even minor degrees of riboflavin deficiency may cause major changes of personality (134).

1.2.1 Absorption

The site of riboflavin absorption has been localized to the upper gastrointestinal tract (135,136). Studies indicate that intestinal absorptive capacity for riboflavin and its derivatives are not unlimited. When low doses of FMN are fed to normal persons, the amounts recovered in the urine are proportional to the amounts ingested. When 50 mg or more FMN is fed, however, no further increase in urinary excretion
occurs (137). Greater absorption of FMN occurs when it is given with meals than when it is given alone (138).

Phosphorylation of riboflavin to flavin mononucleotide in the intestinal mucosa was reported by Jusco et al. (138) as early as 1967. Recently, riboflavin phosphorylating enzyme flavokinase, from rat intestine was purified and its properties studied in considerable details (139). In spite of these, the physiologic importance of this reaction in the intestine for absorption of riboflavin remains unclear although this step may serve as an effective mechanism for trapping the substrate by phosphorylation.

Studies of Said and Hollander (140) have provided some insight regarding intestinal uptake of riboflavin. They have shown that the transport of the vitamin in rats occurs by an active carrier mediated process. Very recently, the uptake of luminal riboflavin into the absorptive cells of rabbit small intestine was examined by Said et al. (141) using purified brush border membrane vesicle (BBMV) preparations to eliminate the interference of intracellular metabolism that occurs to riboflavin molecule during absorption. Their results indicate that riboflavin uptake by rabbit intestinal BBMV occurs via a carrier mediated system that is Na+-independent in nature and transports the substrate by an electroneutral process. Said et al. (142) further observed that the riboflavin uptake in the intestine is regulated by the level of the vitamin in the diet.
(and/or body stores) and that the regulation is mediated via changes in the number (and/or activity) of the riboflavin uptake carriers with no change in their affinity.

1.2.2. Transport

Once absorbed from the intestinal tract a large fraction of riboflavin and FMN is bound to plasma proteins, principally albumins (143). FMN is bound more extensively than riboflavin, but its affinity is considerably less than to the various flavoprotein enzymes in tissues (143). The proportion of FMN and riboflavin bound to albumin tends to remain relatively constant throughout a wide concentration range. By contrast, in another report (144) riboflavin binding to α and β globulins was described. The factors governing the binding of riboflavin to plasma proteins and the physiological importance of "bound" vs. "free" forms of the vitamin have not been studied extensively.

1.2.3 Excretion

In man, excretion of riboflavin occurs both through urine and stool and is primarily in the form of the free vitamin rather than as the coenzyme (141,145,146). Enzymatic degradation of FMN to riboflavin occurs in the intestinal mucosa, and may account for the lack of detectable FMN in feces (147). Bacterial degradation of riboflavin in the gastrointestinal tract does not occur in physiologically appreciable amounts
The renal clearance of riboflavin exceeds the glomerular filtration rate (149), suggesting that renal tubular secretion of riboflavin probably occurs.

1.2.4 Storage and utilization of riboflavin

In most tissues, very little of the vitamin is stored as riboflavin, the majority is in the form of FMN and FAD (150) although an upper and lower limits in the tissue concentration of the flavin coenzymes appear to exist. When riboflavin is fed in large amounts to animals on a normal diet, hepatic level of FAD can be increased only to a minimal degree and not beyond a certain amount (151). Similarly, when riboflavin is eliminated form the diet, tissue concentrations of FAD do not fall below a certain point even in prolonged deficiency. The mechanism underlying these apparent limits have not been elucidated. One possibility is that excess accumulation of FAD does not occur in normal animals after riboflavin feeding, possibly due to feedback regulation of enzymes involved in FAD biosynthesis. But this possibility appears to have been ruled out by the studies of Rivlin, Gamble and Chung (152). Free FAD is more susceptible to enzymatic hydrolysis than bound FAD (153). It is likely, therefore, that when the amount of FAD synthesized exceeds the binding capacities of the flavoprotein enzymes, the unbound fraction of FAD is destroyed enzymatically rather than stored. After intraperitoneal injection of 2-14C-riboflavin, the distribution of radioactivity was found to be...
greatest in liver and kidney, with very little present in blood (146). It has been estimated that in rats under normal laboratory conditions, the tissue riboflavin has a half-life of approximately 16 days. The turnover rate is slowed in animals receiving a deficient diet, and is accelerated in rats fed a diet high in riboflavin (146). In deficient animal, the hepatic uptake of tracer riboflavin probably does not differ substantially from that in normal rats. Loss of the proteins required to bind FMN and FAD in riboflavin deficient rats may limit retention of isotopes (154).

1.2.5 Metabolic effects of riboflavin deficiency

Gross effects of riboflavin deficiency upon the livers of laboratory animals consist of increase in organ weight in relation to body weight (155) and increase in the fat and glycogen content (156,157). The latter effect has been associated with increased incorporation of alanine-1\(^{14}\)C into glycogen, and increased activity of alanine aminotransferase (158).

Loss of hepatic architecture has also been reported in mice after several weeks of deficiency. Electron microscopy of the liver from deficient animals has revealed impressive changes in the appearance of the mitochondria. They attain enormous size and exhibit increase in the number and length of cristae (159). Return of mitochondrial size and structure to normal begins within several hours of riboflavin feeding
and is completed within three days (160). Changes in the appearance of cristae without an increase in mitochondrial size were observed by other investigators (156). It is controversial whether changes in morphology of mitochondria in riboflavin deficiency are associated with diminished function. Functional abnormalities were demonstrated by one group of workers, with diminished efficiency of oxidative phosphorylation (161). Others observed, however, that oxidative phosphorylation tended to be normal in riboflavin deficiency (162,163). Because the flavin coenzymes, FMN and FAD, are involved in many facets of intermediary metabolism, the effects of riboflavin deficiency on many processes are evident. Lipid metabolism is particularly sensitive to the status of riboflavin nutrition. In moderate riboflavin deficiency, an increase in liver:body weight and in hepatic triacylglycerol content, together with changes in fatty acid profiles of hepatic phospholipids were observed (164). Mitochondria from interscapular brown adipose tissue showed a marked impairment of oxygen consumption, with palmitoyl-L-carnitine as substrate, in the riboflavin deficient pups, suggesting that the effect on oxygen consumption is more likely to be due to impaired integrity of the mitochondrial respiratory chain, than to impairment of specific capacity for uncoupling of respiration which is characteristic of brown adipose tissue mitochondria (164). Similarly, the effects of riboflavin deficiency on hepatic peroxisomal and mitochondrial
palmitoyl-CoA oxidation were examined in weanling Wistar-strain male rats where 75% depression in mitochondria at 10 weeks of deficiency was observed which is clearly indicative of the fact that hepatic mitochondria, but not peroxisomes were sensitive to riboflavin deficiency (165). These results were further supported by the studies of Brady et al. (166). They further showed that dietary fat level, however, was found to have no effect on mitochondrial substrate oxidation in riboflavin deficiency (166). However, Liao et al. (167) reported that a high fat-riboflavin deficient diet would have adverse effects on lipid metabolism.

Mitochondrial β-oxidation of fatty acids was severely depressed due to loss of activity of the three fatty acyl-CoA dehydrogenases (168). This was further confirmed by the studies of Nagao et al. (169), where immunoblot revealed severe losses of various acyl-CoA dehydrogenases and electron transfer flavoprotein in liver mitochondria of riboflavin deficient rat. FAD addition to the mitochondrial homogenates from riboflavin-deficient rat liver restored only 10-25% of the lost activity. Hence, the loss of activity was mainly due to the loss of apoproteins (170,171). If the apoenzymes were present, they should have been readily converted to the holoenzymes on FAD addition (172). These observations suggested that either in the absence of FAD ligand, the synthesis of Acyl-CoA dehydrogenases may be inhibited in riboflavin deficiency, or alterna-
tively, apoproteins are unstable in the mitochondria. Moreover, in riboflavin-deficient rats, an enhancement of hepatic peroxisomal $\beta$-oxidation was reported due to an increased activity of the FAD-dependent fatty acyl-CoA oxidase, although the activities of other peroxisomal flavoproteins, D-amino acid oxidase and glycolate oxidase, were lowered (168). In riboflavin deficiency, some loss of activity of the flavin dependent sections of the electron transport chain (complexes I and II) was also reported, but these were probably not sufficient to affect normal function in vivo (168). In riboflavin deficiency the myelin lipids, cerebrosides, and sphingomyelin, as well as phosphatidylethanolamine, a significant component of the myelin membrane, were considerably reduced in proportion. It is considered that riboflavin plays some role in the metabolism of essential fatty acids in brain lipids and the pathological effect of its deficiency is similar to that of essential fatty acid deficiency, causing fast impairment to brain development and maturation (173).

In the early stages of riboflavin deficiency, hepatic concentrations of DNA and RNA remain unchanged while in the later stages, the concentrations of both were found to be depressed (174). Concentrations of pyridine nucleotides also tend to be normal in riboflavin deficiency (161).

The effects of riboflavin deficiency upon amino acid metabolism have been studied most extensively in connection
with tryptophan. Deficient rats excrete in the urine excessive amounts of anthranilic acid, one of the tryptophan metabolites (175). There is also increased formation of xanthurenic acid from tryptophan. Certain effects of riboflavin deficiency upon tryptophan metabolism may be due to diminished availability of pyridoxal phosphate, since riboflavin is required for the conversion of pyridoxine phosphate to pyridoxal phosphate (176). Pyridoxal phosphate in turn is required as a coenzyme for several steps in tryptophan metabolism.

The pattern of hepatic flavoprotein enzymes is greatly affected by riboflavin deficiency. Activities of FMN and FAD requiring enzymes, such as xanthine oxidase, succinic dehydrogenase, triphosphopyridine [TPN]-cytochrome c reductase, glycolic acid oxidase (177,178), erythrocyte glutathione reductase and NADH-methemoglobin reductase are affected by the status of riboflavin nutrition (132). The rate at which enzyme activity is lost with the onset of riboflavin deficiency and increase in the enzyme activity after riboflavin refeeding appear to follow a characteristic pattern (155).

Riboflavin deficiency decreased glutathione reductase activity in the mitochondria to a greater extent compared to that in the cytosol. At the same time, a greater accumulation of apoenzyme in the former than that in the latter was confirmed by the amount of immunoprecipitable protein. Activation of enzyme activity by FAD in vitro as well as recovery of enzyme
activity after injection of riboflavin into riboflavin-deficient rats has also been reported (179). In contrast, addition of exogenous FAD or FMN did not restore the activity of hepatic dihydropyrimidine dehydrogenase which was found to be diminished followed by a decrease in enzyme concentration in riboflavin deficient rats (180).

Evidences for the specificity of the decreases in the activity of FMN and FAD requiring enzymes in riboflavin deficiency, both experimental and clinical is provided by the finding that many non-flavin enzymes are unchanged or actually increased in activity under similar circumstances (157,181,182).

1.2.6 Hormonal regulation of riboflavin metabolism

The effect of thyroid hormone upon riboflavin metabolism was demonstrated by Domjan and Kokai (183). They found that the hepatic level of FAD was reduced in hypothyroid rats and restored to normal by treatment with thyroxine (183). Rivlin and Langdon (184) made the same observation and suggested that the diminished level of FAD in hypothyroidism was partly due to the decreased activity of flavokinase, the enzyme responsible for the initial reaction in the two step conversion of riboflavin to FAD (185,177). Further studies demonstrated that the level of FMN and riboflavin were reduced similarly to that of FAD in hypothyroid animals and a remarkable similarity between hypothyroidism and riboflavin deficiency was noted. In hypothyroidism, hepatic flavokinase activity was diminished and concentrations of FMN and FAD were decreased.
Consequently the activities of a number of FMN and FAD requiring enzymes were decreased. In riboflavin deficiency, Nolte et al. (186) reported that serum thyroxine level was markedly decreased and basal metabolic rate was lower than that in a riboflavin control group. In the case of hyperthyroid animals Rivlin and Langdon (187) found that hepatic levels of FMN and FAD were not increased above normal despite increased activities of flavokinase and FAD synthetase responsible for their synthesis.

Studies by Lee and McCormick (188) have shown that flavokinase activity was increased in hyperthyroid animal and decreased in hypothyroid animals. Correspondence of flavokinase activity with the amount of a high affinity flavin binding protein, quantitated immunologically in hypo-, eu-, and hyper-thyroid rats, indicated that response to thyroid is caused by an increased amount of enzyme. Moreover, the concomitant decrease in a low affinity protein suggests an inactive precursor of flavokinase. FAD synthetase activity showed a similar but less pronounced trend than flavokinase. Activation of FMN-phosphatase and FAD-pyrophosphatase were not influenced by thyroid hormone. Therefore, it appears from these that the mechanism of thyroid hormone regulation of flavocoenzyme level is in the steps of biosynthesis, especially at flavokinase rather than in the degradation steps (188).

Cimino et al. (189) also studied riboflavin metabolism in the hypothyroid human adult. They found that activity of
erythrocyte glutathione reductase, an accessible FAD containing enzyme, is decreased to levels observed during riboflavin deficiency. Thyroxine therapy resulted in normal levels of this enzyme while the subjects were on a controlled dietary regimen. This demonstrates that thyroid hormone regulates the enzymatic conversion of riboflavin to its active coenzyme forms in the human adult.

Possible role of ACTH on increased conversion of \([^{14}\text{C}]\text{-riboflavin}\) to \([^{14}\text{C}]\text{-FMN}\) was investigated in kidney and liver of intact rat by Fazekas and Sandor (190). Similarly, increased formation of \([^{14}\text{C}]\text{-FMN}\) and \([^{14}\text{C}]\text{-FAD}\) from \([^{14}\text{C}]\text{ riboflavin}\) in rat kidneys following administration of aldosterone in adrenalectomised rats was also reported by Trachewsky (191). These effects may also involve induction of flavocoenzyme biosynthetic enzymes.

1.2.7 Riboflavin and cancer

Among the earliest workers to demonstrate a relationship of riboflavin to cancer were Morris and Robertson (192), who observed that in C3H mice fed a riboflavin deficient diet, the development of spontaneous mammary tumours was markedly depressed. The manipulations of dietary riboflavin content were followed by predictable changes in tumor growth rate (193). In addition, antitumor effects of riboflavin deficiency have been confirmed and extended in a number of animal species and with a variety of types of tumors (194). For example,
growth rates of lymphosarcoma in mice (195), Walker carcinoma (196) and Novikoff Hepatoma in rats (197) are all inhibited by dietary riboflavin deficiency. It is of interest that structural analogs of riboflavin that have appreciable biologic activity, such as 7-methyl-8-ethyl flavin, do not inhibit growth rate of tumors (198). These inhibitory effects have also been observed after reduced intake of zinc (199) and total calories (200), which may be mediated at least in part by alterations in immune function. In the case of riboflavin-deficiency, however, too little is known about the possible effects upon the immune system (201) to evoke this pathogenic mechanism.

Besides these, riboflavin-deficiency actually tends to enhance the carcinogenicity of certain drugs, such as the azo-dyes. These agents are degraded by the microsomal drug hydroxylation, system, which, requires flavin cofactors. Azo-dye reductase activity is markedly diminished in livers of riboflavin deficient rats (202), as are the concentrations of FAD (203), which are required for maximal enzyme activity. Under the circumstances of riboflavin deficiency, in which drug degradative capacity is diminished, the dose of carcinogen delivered to susceptible tissues is likely to be greater and the half-time of disappearance is more prolonged than in normally repleted animals. Thus, riboflavin deficiency may either decrease or increase the rate of development of tumors in experimental animals.
1.2.8 *Implications of riboflavin nutrition for the treatment of cancer*

A possible relationship of riboflavin to therapy of cancer has been explored with relation to the widely used antineoplastic agents of the anthracycline category. Drugs of this kind, of which adriamycin is the best known example, have been shown to form a complex with the riboflavin binding protein of chicken egg white (204). Treatment of rats with low doses of adriamycin inhibited the formation of FAD *in vivo* in a dose dependent manner in rat heart (205). Inhibition of biosynthesis of FAD, which is critically needed in the heart for energy metabolism and electron transport would be expected to have a profound effect upon cardiac physiology. In rats treated with adriamycin, the use of a derivative of riboflavin (Vit B₂ tetra-butyrate) reduced the lipid peroxide content of cardiac mitochondria (206).

Patients with cancer are often depressed and require antidepressant therapy. Certain drugs of this type also influence riboflavin metabolism. Chlorpromazine, a phenothiazine derivative and imipramine and amitriptyline, both of which are tricyclic antidepressants markedly interfere with the conversion of riboflavin to FMN and FAD in experimental animals (207). All of these drugs have a remarkable degree of structural resemblance to riboflavin. Chlorpromazine, in particular, depletes tissue FAD levels and promotes urinary riboflavin excretion in animals.
treated with doses of the drug comparable on a weight basis to those used clinically (208). Heart tissue appears to be particularly sensitive to the inhibition of FAD synthesis by chlorpromazine (209). Little is known of the possible role of riboflavin deficiency or excess in the cause of human cancer. On the basis of the requirement of flavin coenzymes for the microsomal drug hydroxylation system (210), deficiency of dietary riboflavin would be suspected to retard the inactivation of carcinogens, and thereby enhance their delivery to susceptible tissues. On the other hand, if the parent compound has less carcinogenic activity than its metabolites, riboflavin deficiency possibly may decrease carcinogenesis through this enzyme system.

Whether riboflavin alters the prevalence of any human malignancy is difficult to establish, particularly since deficiency of this nutrient seldom occurs.

1.3 Cadmium and vitamin metabolism

The above informations emphasize that cadmium exerts a profound effect on the structure and metabolism of different organs and liver in particular, in a large number of species. From a comprehensive search of literature, it appears that only few informations exist regarding the effects of Cd on vitamin metabolism. For example, it was reported that Cd interferes with vitamin A metabolism (211). In this study administration of Cd to female rats for 237 days significantly
reduced serum vitamin A levels. Concurrently, an increase in liver vitamin A levels was found, while absorption of vitamin A from intestine, and the release of newly absorbed vitamin A to water soluble metabolites in liver were not influenced. These findings suggest that Cd interferes with the release of the vitamin from liver into plasma. Chronic Cd exposure to rats was found to cause depletion of ascorbic acid status (212).

Stow et al. (213) have shown that concentration of Cd in liver and kidney of pyridoxine deficient rats receiving 100 mg Cd/Kg diet were significantly less than those in animals given pyridoxine supplements. However, Prasad et al. (214) have observed an increase of Cd uptake in intestine of pyridoxine and thiamine-deficient rats in vitro.

Only a few reports are available indicating that some vitamins may provide a certain degree of protection against Cd intoxication. In one such study, Tandon et al. (215) investigated the effect of vitamin B complex on Cd nephrotoxicity and hepatotoxicity in rats. The supplementation of vitamin B complex simultaneously with Cd caused less marked biological alterations. The protective effects of vitamin B-complex in Cd toxicity may be attributed to the interference by the constituents of vitamin B complex in absorption of Cd, possibly through formation of readily excretable complexes.

Recently, unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes exposed to Cd was studied
by Farris et al. (216). It was revealed that the vitamin intervenes in the critical cellular events that lead to Cd toxicity.

1.4 Aim of the Present Investigation

It appears from the available informations as depicted above that whatever studies on vitamin metabolism following exposure to heavy metals have been carried out in experimental animals were limited to either measurement of their levels in blood and tissues or their absorption in the intestine. To the best of our knowledge, no information is available on the effects of heavy metals on the metabolism of B-vitamins. Further, no attempts have been made yet to investigate the effect of Cd on riboflavin metabolism. Thus, in view of these, we became interested in studying the effects of Cd on hepatic riboflavin metabolism, using rat as an animal model.