MODIFICATION OF CADMIUM TOXICITY IN BIOLOGICAL SYSTEMS BY OTHER METALS

ARCHANA SHARMA, ANITA MUKHERJEE and GEETA TALUKDER
Centre for Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35 Ballygunj Circular Road, Calcutta 700019, India.

ABSTRACT

The information available of the interaction of cadmium with other metals, leading to modifications of the action of cadmium on living systems indicates that the ultimate expression of the interaction may be antagonistic, synergistic or simply additive. It depends on a number of external factors, amongst which the nature of the organism, the concentrations applied and the mode of administration are the more important ones. This interaction has considerable importance in counteracting the biotoxic activity of cadmium and its compounds.

INTRODUCTION

Toxic effects of cadmium on biological systems have invited progressive interest following the increase in global use of this element, particularly in industries and its association with certain human disabilities, addictions and dietary deficiencies. Considerable information is available on these aspects of the problem[1-6].

In this review, the available information on the interaction of cadmium with other metals, leading to modifications of the toxic action of cadmium on living systems, has been summarised.

The action of cadmium salts on the living systems has been studied both as a single toxic entity and in combination with other chemicals, including other trace elements. The latter have been shown to interact with a number of toxic heavy metals, reducing their activity[7]. The importance of metal–metal interactions in both enhancement and inhibition of metal carcinogenesis has also been emphasized[7,8].

Toxicity may be altered by antagonistic interactions between the toxic and essential cations[9-13]. Thus, pretreatment with or simultaneous administration of, excess of essential ions also protects against many of the acute effects of Cd[14-16]. The antagonism between orally ingested Cd and essential cations leads to certain effects which depend upon the relative contents of the latter in the diet. The expression of such antagonism may not be necessarily simple but may be confounded by interactions between the essential ions themselves. Such interactions, for example, between Zn and Cu, Cu and Fe, Fe and Zn are well known in animal nutrition[17-19]. It follows, therefore, that the administration of a specific metal to counteract the toxicity of Cd may interfere with the metabolism of others.

In plants, Cd has been shown to affect other soil constituents or introduced pollutants. For example, Cd depresses Mn and P uptake by plants[20,21]. The uptake of Cd could be reduced by a high level of Se[22] or an excess of Al, K, Ca or P[20]. Tolerance induced, following pretreatment with various metals, to the harmful action of Cd has been extensively studied[20,22-24]. Earlier work had suggested that such tolerance is highly metal specific[25], and that tolerance to more than one elements was not always specific[24,26]. Possibility of co-tolerance between a naturally occurring divalent cation and a metal pollutant forms the basis of the phenomenon of such antagonism.

FACTORS AFFECTING INTERACTION

Numerous factors affect the interactions between metals[27,28]. Species, strain and individual variations may enable an organism to metabolise compounds rapidly and thus prevent the

accumulation of a metal to an effective level in blood and tissue. Metabolism of the same compound in another organism may be so slow that the cumulative level of the metal in the tissue is toxic[29]. Sex, age and weight are other factors influencing the degree of toxicity of the metals[30]. Weaning rats excrete more Cd in the faeces during the first 24 hours than the normal young adult animals[27]. Both environmental and nutritional states play vital roles in detoxification. The mode of administration has a decisive effect on the degree of interaction. Parenteral administration leads to more drastic effect than oral feeding. Among the different routes of parenteral administrations, subcutaneous (sc) injection is the most effective, closely followed by intraperitoneal (ip) or intra-venous (iv) modes[28]. The few studies of interaction of metals on plants show that the edaphic factors and the physiological conditions of the plant are largely responsible for Cd uptake.

INTERACTIONS WITH OTHER METALS

Cadmium and Zinc

Zinc attenuates many toxic effects of Cd including testicular damage[31-35], inhibition of hepatic microsomal drug metabolism[36], carcinogenesis[37], cytotoxicity[38], inhibition of oxidative phosphorylation[39] and lethality[40-42].

In *Escherichia coli*, the addition of Zn shortened the lag phase in the growth of the bacteria when treated with Cd, pretreatment with Zn being even more successful[43]. The inhibitory effect of Cd on the growth of yeast has been counteracted by Zn as well[44]. Antagonism between Cd and Zn was reported in *Euglena gracilis* with respect to the morphology and cell division[45]. In hydroponic experiments with corn, toxic effects of Cd were ameliorated by the addition of Zn to the nutrient growth solution[46]. On the other hand, during *Allium* test, Zn and Cd acted independently in their effects on divisional frequency and chromosome aberrations[47].

Dosage mortality curves indicated that equal concentrations of Cd and Zn in mixture, applied to *Daphnia magna*, were less toxic than the individual metals[48].

Combined toxicity of the two metals in mixture was additive in *Salmo gairdneri* – a rainbow trout exposed artificially to a mixture of the metals[49].

In embryonic chick bone tissue culture, Zn prevented a decrease in *hyaline* phosphate (Hy-p) content caused by Cd. Mixture of the two metals, applied to intravitelline membrane of chicken embryo for three days, reduced the frequency of malformations induced by Cd when given alone[51]. In marine birds, toxic effects of Cd were reduced by Zn and there was a positive correlation between the amounts of Zn and Cd present in the kidney[52].

At clastogenic and mutagenic levels, human lymphocyte culture revealed a higher incidence of chromatid type of aberrations and gaps in cultures exclusively treated with Cd, which was decreased appreciably in combination treatments with acetates of Zn and Cd[53]. The uptake of Zn by *HeLa* cells *in vitro*[54] is inhibited by Cd, while the toxicity of the latter cation in mouse cells is reduced proportionately with the enhancement of the Zn:Cd ratio in culture medium[55].

Morphological and biochemical evidences demonstrate that pretreatment with Zn markedly decreases the hepatotoxic activity of Cd[56]. The results are confirmed by studies on hepatic microsomal drug metabolism[36]. Cell swelling and cytoplasmic eosinophilia were predominant in rats treated first with Zn and then with Cd[56]. Cell swelling is generally regarded as reversible[57]. Zn affords protection against Cd-induced inhibition of pancreatic function and stimulation of hepatic gluconeogenic enzymes[58]. Cd-induced anemia in rats, fed with a diet containing mixtures of Cd and Zn, was prevented by Zn[59].

Pretreatment with Zn protects the mammalian testis against Cd salts[32,60], as also the ovary. $ZnCl_2$ (200 times the equimolecular dose of $CdCl_2$) prevented the gonadal changes caused by $CdCl_2$. Administration of the two salts in equimolar dose (0.04 m mol/kg) was ineffective[61]. Zinc acetate, when given prior to $CdCl_2$ treatment, checked histological damage and reduced polyamine content in rat testis[62].

Decreased spermine and spermidine levels were altered. Antagonistic interaction between Cd and Zn was observed following intravenous injection of the acetate salts in rabbits, after six weeks[63]. Pretreatment of mice, with repeated small doses of Cd, protected against the acute testicular necrosis caused by a single large dose of Cd[64].

The *teratogenic action* of Cd salts has also been shown to be appreciably altered by Zn salts[16]. Foetal death or resorption induced by Cd was reversed by a combination of zinc acetate and $CdCl_2$[65,66]. A combination treatment with Zn and Cd of pregnant rats on gestation days nine[67] and 12–15 prevented the fetotoxicity caused by Cd alone[68]. Excess dietary Zn protected against fetal growth retardation induced by Cd in rats[69].

At *carcinogenic* level the reports are conflicting. Concurrent injection of zinc acetate protects the testicular vessels from acute damage and inhibits the ultimate development of testicular tumour produced by subcutaneous (sc) injection of Cd[40]. On the other hand Zn dust failed to check the tumour produced by Cd dust, when both the compounds were injected intramuscularly[70].

Cadmium and Selenium

Se has been shown to protect against damage induced by Cd including lethality in rats[71] and in mice[40], testicular necrosis[40,71], pancreatic changes and alterations in gluconeogenic enzymes[58] and reductions in plasma and testicular glutathione peroxidase activity in rat[72,73].

Sensitivity of the fresh water mollusks *Lymnea stagnalis* to Cd was nearly halved in presence of the sub-lethal amounts of Se, while sub-lethal amounts of Cd, gave additional protection against the toxic effects of Se[74]. The level of antagonism depends, among other factors, on the organism concerned since it could not be observed in estuarine forms. For example, concurrent exposure to equal amounts (400 ppb) of Se and Cd for 72 hr did not significantly alter the overall accumulation of Cd in whole oysters (*Crassotrea virginica*), though the gills contained lower levels of Cd than in animals exposed to Cd alone[75].

Amongst plants, *Allium sativum* when treated with Cd and Se salts, in different combinations, showed additive effects, as measured by cytotoxic and clastogenic activities[76].

In broiler chicken, sodium selenite (10. or 20 ppm) in feed decreased the negative action of cadmium acetate (30 ppm). Total tissue content of Cd was lowered by Se and *vice versa*[77].

Hepatotoxicity, in rats induced by Cd, shown by decreased activity of the microsomal monooxygenase enzyme system could be prevented by Se *in vivo* and *not in vitro*[73,78]. Alterations in glucose tolerance and insulin release responses to a glucose load were reversed by Se[58].

The inhibition of the toxic action of Cd by Se is correlated with tissue sulphydryl content and glutathione reductase activity. Se pretreatment (13 μ mol/kg) prevented the lowering effect of sulphydryl content by Cd significantly in liver and testis[79].

Equimolecular weight of sodium selenite (0.04 m mol/kg) was ineffective to reverse the Cd-induced characteristic degenerative changes in testis, while ovarian changes caused by $CdCl_2$ could be prevented by a dose of Se higher than $CdCl_2$[61]. Increased vascular permeability leading to haemorrhagic necrosis in the testis, in the ovaries of female rats in persistent oestrus and damage to the lactating mammary gland could be reversed by Se[80,81].

In vitro experiments on human erythrocytes showed that Se at $\leqslant$ 0.1 m M concentration inhibited the Cd uptake while the latter was enhanced at concentrations greater than 0.3 m M Se[82].

Cadmium and Lead

The types of interaction of Cd and Pb vary according to the nature of organism and dosage applied. The primary production of phytoplankton inhibited by Cd was increased, indicating antagonism, when concentration of Pb exceeded that of Cd[83]. Synergism was observed in solutions where the concentration of Cd was greater

than that of Pb. Low concentrations (0.1 to 1.0 mg l) of Pb increased the toxicity of Cd (0.1 mg l). In experiments with individual heavy metals, phytoplankton was not adversely affected, but a combination of the same concentrations of the metals showed a synergistic adverse effects on the plants[84].

Wheat and corn seedlings grown in the presence of Pb and Cd resulted in a decreased accumulation of the elements, with a consequent reduced effect on the plants[85]. In *Lolium hybridum*, the presence of Pb in soil enhanced the concentration of Cd. On the other hand, increase in Cd concentrations in soil decreased concentration of Pb in tissues of both *Lolium hybridum* and *Avena sativa* straw[86]. The toxic action of Cd was enhanced in presence of Pb in the blue green algae *Anacystis nidulans*[87]. A combination of cadmium chloride and lead acetate caused senescence in the three submerged aquatics *Potamogeton pectinatus*, *Vallisneria spiralis* and *Hydrilla verticillata*, by decreasing chlorophyll, DNA, RNA, protein and dry weight and increasing free amino acid, tissue permeability and activities of protease and RNAase and the ratio of acid to alkaline pyrophosphatase activity[88]. In *Lepidium sativum*, no synergism with concomittant application of Cd and Pb could be observed[89]. Chlorides of Pb and Cd in combination reduced growth in *Saccharomyces cerevisiae* inhibiting the enzymes (malic, glutamic and glyceraldehyde 3-phosphate dehydrogenase). No interaction was, however, recorded with regard to the rates of fermentation and respiration[90]. In the liver of Langhorn chicken, embryo investigations were carried out 15 days after a single injection of Cd and/or Pb (0.25 μg/g) as acetate into the egg or their combination into egg yolk[91]. The administration of Cd significantly increased the hepatic uptake of Pb and Cu. Significantly enhanced Cd, Pb and Zn levels were detected in the embryonic liver from the eggs injected simultaneously with Cd and Pb, showing their synergistic activity[91–94].

The toxic effects of Pb (500 ppm) and Cd (100 ppm) fed simultaneously in the ratio 5 : 1 were additive on the growth of rats[95]. Cd inhibited the retention of Pb (or stimulated its excretion) whereas Pb did not affect accumulation of Cd. Rats treated intraperitoneally with Cd and Pb daily for 10 to 60 days showed more severe damage to kidney and liver on day 30 as compared to day 60 following injection of Cd alone[96]. Chronic exposure to Pb and Cd decreased the incidence of virus-induced mortality and other effects produced by exposure to each metal alone[97]. Cell injury due to Cd was also reduced by Pb[98]. The combined action of Pb and Cd was synergistic on testicular damage and prostatic cytology[99]. Although no tumours were formed, the replacement of columnar epithelium by squamous cells suggested progressive, precancerous changes.

Increased number of red blood cells was observed in male rats exposed to dietary Pb and Cd alone or in combination, for ten weeks, the enhancement being greater in the combined group[100]. A reduction in blood and tissue levels of Pb with severe anemia occurred in animals simultaneously exposed to the two metals. Similar synergism was observed in rats treated intragastrically with Pb and Cd, leading to loss in body weight and increase in relative liver, brain and adrenal weights[101]. In rabbits, during and after six weeks intravenous administration of lead acetate and cadmium acetate in various combinations, a synergistic effect was observed when these were given together[63].

A shorter interval between the onset of clinical changes and death occurred in ponies fed hay contaminated by the two metals[102].

In some cases, the effects were additive[103,104]. Cardiac phosphocreatine and hepatic sugar phosphate concentrations were depressed following long term exposure to low levels of Cd and Pb through drinking water[103]. Risk of mortality related to cardiac failure in humans was seen in 80 percent of cases due to combined effects of Pb and Cd[104]. The relationship with age was significantly high.

Antagonistic interaction between the two metals was manifested by the reversal of *in vitro* inhibitory effects of Pb on human erythrocytic 8-aminolevulinic acid dehydratase (δ-ALAD) activity through low concentrations of Cd[105]. Rats fed on Pb (0.5 %) showed a marked reduction of

reticulocytosis induced when toxic Cd diets were administered[106].

No interaction between the two have been reported[107] on the renal function of a group of workers exposed simultaneously to Pb and Cd. Simultaneous administration of Pb and Cd did not induce any additive toxic effects on carbohydrate metabolism[108]. Pb also did not protect against the decrease in collagen content caused by Cd in embryonic chick bone culture[50].

Clastogenic and mutagenic information on the interaction of Pb and Cd is extremely sparse, and results obtained are mainly speculative. Analysis of peripheral lymphocytes of workers occupationally exposed simultaneously to Cd, Pb and Zn gave an increase in chromosomal aberrations as compared with the effects of only Pb[109] or Cd[110]. Higher incidences of chromatid type were recorded in unstimulated human lymphocytes from cultures exclusively treated with Cd, than in those treated with Cd and Pb[111].

Cadmium and Mercury interactions are variable. In lower organisms, effects on growth showed mixed synergism and antagonism depending upon the actual concentrations used. Irrespective of the sequence of administration, Cd decreased Hg toxicity in mussels—*Mytilus edulis*. Association of the two metals had no significant effect on the accumulation of Hg, but decreased Cd in the body[112]. Hg and Cd in combination on *Anabaena inequalis* showed synergistic action towards photosynthesis and nitrogenase activity[113].

Pretreatment of female rats with $CdCl_2$ (2, 2.4 mg/kg) protected against the nephrotoxic effect of Hg (0.5, 1.0 and 1.5 mg/kg) given six days after the second dose of Cd. male rats were more sensitive in the case of $HgCl_2$ and could be protected only upto against 0.5 mg/kg. Protection by Cd was not associated with decreased accumulation of Hg in the kidneys[114]. Prior injection with $HgCl_2$ was more efficient as an antagonist than post-treatment[115]. Toxicity was decreased in the acute intoxication. Injection of $CdCl_2$ in low doses before treatment caused an initial reduction and then an enhancement of toxicity induced by $HgCl_2$. Retention of Hg was raised in rat liver and kidney; effect on organ retention of Cd was noted[116]. Due to the upper threshold of the ability of the kidney to bind Hg, with an increasing dose of Hg, the relative amount deposited in the kidneys decreased. The urinary excretion of Hg rose at the same time[117–119].

Cadmium and copper mainly give additive or synergistic effects on lower organisms. In *Tisbe holothuriae*, a marine copepod, the mortality rate was found to be higher than expected on a purely additive basis when the two metals were administered together[120]. *Salmo gairdneri*, a rainbow trout, exposed artificially to a mixture of Cu and Cd showed that the combined toxicity to be additive[49]. Cd contents of herring eggs were shown to be reduced by Cu[121].

Cd, added in the M-sucrose medium, suppressed frond growth in *Lemna paucicostata* 6746, while Cu suppressed both multiplication and frond growth. In a medium containing both salts, inhibition of multiplication depended on the concentrations of both metals adsorbed. Frond growth however was affected only by the concentration of Cu[122].

Various combinations of Cd and Cu in drinking water administered to mice did not alter tissue Zn, Fe and Cu levels[123]. Subcutaneous injection of 1 mg $CdCl_2$/kg to rats caused anemia associated with reduction of levels of Cu and Fe in the blood, erythrocytes, leucocyte and thrombocyte counts, and haemoglobin values. These effects were neutralized by concomitant oral administration of 0.5 mg $CuSO_4$/day[124]. The interaction of Cu and Cd thus depends on a specific rates of metal.

Cadmium and Iron

Reversal of Cd toxicity by Fe in batch cultures of diatoms *Thalassiosira weissflogii* indicates antagonistic interaction between the two[125]. Iron transport in duodenal loops is reduced significantly in chicks maintained on a diet supplemented with 75 μg Cd[126]. In Japanese quail, haemoglobin levels are reduced in blood and iron concentrations in liver after 4 weeks on a purified soybean protein diet, supplemented with Cd[127].

Excessive exposure to Cd of growing laboratory animals leads to a hypochromic microcytic anemia and growth retardation associated with increased plasma transferrin levels and decreased body iron[127-130]. These effects[131] can be prevented by either parenteral or increased dietary administration of iron[10,128-130,132-134]. However, at higher levels of dietary Cd (> 50 ppm $CdCl_2$) Fe could not completely prevent the increase of liver and kidney Cd levels[134]. Chronic Cd exposure in diet causes almost linear time-dependent depletion of Fe from the livers of rats[135].

Cadmium and Calcium

Calcium, introduced either as sulphate or carbonate, protected brook trout against Cd toxicity[136]. In Japanese quail, continual dietary exposure to Cd decreases the retention of Ca, resulting in poor bone mineralisation[137] either through Fe deficiency, or reduced food intake rather than to a direct effect of Cd on Ca utilization[138]. In cultures of chick embryo tibiae addition of Cd decreases the uptake of Ca[139].

In the whole animal Cd inhibits Ca transport through the intestinal mucosa and in pregnant rats causes a dose-dependent delay in foetal skeletal calcification[140]. Tissue uptake of Cd is enhanced in animals on Ca-deficient diets[141], through higher synthesis of intestinal Ca binding protein[142]. This protein in the rat duodenal mucosa is inactivated by oral administration of Cd[143]. Accumulation of Cd in the kidneys of rats was lowered and mortality was decreased by enriching soft tap water (hardness 2.8°) with $CaCl_2$ to a hardness of 25° [144].

Experimental animals and industrial workers exposed to Cd, as well as patients with *Itai Itai* disease[145], who may accumulate appreciable amounts of Cd in their bones[146], develop osteoporosis and osteomalacia[147-149]. These effects are secondary ones due to renal tubular dysfunction and the failure to reabsorb Ca.

Dietary deficiency of protein, coupled with Ca and vitamin D, may be responsible for the dysfunction in *Itai-Itai* patients. Such deficiencies also aggravate the skeletal changes due to chronic Cd poisoning in experimental animals[150]. Earlier observations on well nourished industrial workers with Cd-induced renal tubular damage but with high intakes of Ca, did not show bone abnormalities[151].

MODE OF INTERACTION

The antagonism exhibited by other metals to the activities of Cd may be through three separate processes:

(i) Competition for membrane sites.
(ii) Diversion of Cd from low molecular weight protein to high molecular ones.
(iii) Alterations in organ distribution.

The membrane phospholipids are the essential active sites for reversing the binding of the two metals[60,152] *in vivo*. Evidences indicate a *competitive inhibition* between the metals, particularly from the same group of periodic table[153]. Membrane phospholipids form essential components of membrane binding sites and therefore cell membrane permeability would be altered by lipid peroxidation resulting in specific lesions[154-157]. This view point is supported by the fact that *in vivo* effects of Cd were prevented by injection of sodium selenite before administration of Cd[72].

In the marine organisms, competition between chemically similar ions for binding sites can significantly affect bioaccumulation of Cd, since the latter depends upon the existence of metal binding ligands in the proteins, capable of forming highly stable complexes with Cd. If the Cd can move across the permeable membrane and bind to the intercellular ligands, the metal will continue to accumulate until all the binding sites are occupied. Intracellular mobilization can occur simultaneously. The uptake process was linearly related to the time of exposure and concentration of Cd, indicating that the process of interaction is also diffusion controlled and obviously the divalent cations would compete in the process[153].

Metallothionein, an inducible protein in several organs[158], including plant[159], can sequester large amounts of Cd following exposure[4]. Zn

administration can induce the synthesis of MT[160]. Following Cd-induced synthesis of hepatic MT, Zn and Cd are bound to the protein[161]. Se[162] and Hg[163] can induce *de novo* synthesis of MT, which presumably due to its high sulphydryl contents then sequesters the metal[164].

Such a mechanism does not apply to rat testis—a target for Cd carcinogenesis. The protein previously thought to be testicular MT[165] has been shown to be a different one[166] and not inducible following Cd exposure[158,167–169].

Another possible explanation for the protective effect involves the ability of the protecting metal to alter tissue distribution of Cd. The cation may be diverted from the target to less sensitive binding sites through non-specific incorporation into various proteins[168]. The presence of specific proteins of unknown function (molecular weight 30,000 daltons) in the testis was reported with a high affinity for Cd[169]. During the protection afforded by selenite against Cd, the first step in the metabolic conversion of selenite occurs in the red blood cells and leads to an intermediate which interacts with plasma proteins (s) and with Cd in 1 : 1 atomic rates[170]. Endogenous Se is not available for this interaction, which diverts Cd from its normal carrier protein fraction (molecular wt. 77,000) in the plasma to proteins of higher molecular weight. Se was observed to be significantly increased in the blood and testis but reduced in the liver and kidney[171]. Thus the Cd in soluble fraction of testis of rats was diverted by Se from low molecular weight proteins to larger ones during the protection of testes[78]. Increased Cd levels in testicular cytosolic fraction have been redistributed from low (15,000–34,000) to high molecular weight (110,000–115,000)[73,169], as well as in the hepatocytes[170]. Similar subcellular distribution and chemical forms of Cd are changed by Zn[50].

Alternatively, the preventive effects of physiological essential metals against metal-induced carcinogenesis may result from reduced binding at target sites[171] or may be mediated by other biochemical mechanisms[3]. Cd in many respects acts as an antimetabolite to Zn and thus can influence the Zn metabolism. It may act as one promotor among others in the complex development of prostrate cancer[3].

The formation of chemical complex of any two elements like Cd and Se has been suggested as a mechanism of detoxification. In this case, the inhibitory effect of one element should be decreased in the presence of the other[78]. Such effect could not be observed *in vitro*[172].

In the interaction between cadmium and other metals one or more of these mechanisms may be effective. The ultimate expression—antagonistic, synergistic or simply additive—depends, as mentioned earlier, on a number of extraneous factors, amongst which the nature of the organism, the concentration applied and the mode of administration are more important.

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ANNOUNCEMENTS

BIOTECHNOLOGY IN FOOD PROCESSING

The Department of Food Science and Nutrition at the University of Minnesota will sponsor an International Symposium on Biotechnology in Food Processing, October 7–9, 1985, at the Radisson University Hotel, Minneapolis, Minnesota. This is the first major biotechnology symposium to focus specifically on food processing applications. A brochure describing the program in detail is enclosed.

The purpose of this symposium is to provide an opportunity for participants from academia, government and industry to explore the present and future applications of biotechnology in the food processing industry.

For additional information, contact: Lynette Marten, Office of Special Programs, 405 Coffey Hall, 1420 Eckles Avenue, University of Minnesota, St. Paul, Minnesota 55108 USA.

NAGAMMA DATTATREYA RAO DESAI AWARD

Dr Srikant Kulkarni has received the Nagamma Dattatreya Rao Desai award in recognition of his outstanding research contributions on Rice and Wheat Pathology as also on Ragi which have helped to get high yields in these respective crops in Karnataka. The University of Agricultural Sciences, Bangalore has awarded this for the year 1984. The Prize carries cash Rs. 2,500/- and a citation.