It is truly a great honor for me to participate in this Festschrift honouring Dr. C. Gopalan. Dr Gopalan has contributed tremendously to the field of Nutrition. His contributions as Director of National Institute of Nutrition and as Founder President of Nutrition Foundation of India are indeed phenomenal and legendary. He has trained a large number of nutrition scientists in India and has played a very significant role in the field of nutrition globally. I met Dr Gopalan for the first time in 1988 when I was invited by the Nutrition Society of India to deliver the Gopalan oration and receive the gold medal. We have remained friends for the twenty years since then, and I have truly cherished this friendship. In this contribution honouring Dr. Gopalan, I will present the historical aspects of the discovery of zinc deficiency in humans and its subsequent impact on human health.

Discovery of human zinc deficiency

Zinc deficiency in Iran

I went to Shiraz, Iran, in June 1958 after finishing my formal training in medicine at the University of Minnesota Medical School, Minneapolis, Minnesota, USA. Dr Hobart A Reimann, formerly from the University of Minnesota, Department of Medicine, was the Chief of Medicine at the Nemazee Hospital of Pahlevi University in Shiraz, Iran. He invited me to join him to set up a curriculum for teaching medicine to students and house staff. In Shiraz, I met Dr James A Halsted, who was a Fulbright Professor at Pahlevi University and was primarily involved with Saadi hospital, an equivalent to City hospital in the USA. In the fall of 1958, I was invited by Dr Halsted to discuss a patient with anaemia at the medical centre grand rounds at the Saadi Hospital. The case was presented to me by Dr M Nadimi, Chief Resident, a graduate of the Shiraz Medical School.

The patient was a 21-year-old man who looked like a 10-year-old boy. In addition to severe growth retardation and anaemia he had hypogonadism, hepatosplenomegaly, rough and dry skin, mental lethargy, and geophagia. The patient ate only bread made from unleavened wheat flour and his intake of animal protein was negligible. He consumed nearly 0.5 kg of clay daily. The habit of geophagia (clay eating) was common in the villages around Shiraz. Further studies documented iron-deficiency anaemia in the patient. There was no evidence of blood loss. Inasmuch as 10 additional similar cases were brought to the hospital for my care within a short period of time, hypopituitarism as an explanation for the observed growth retardation and hypogonadism was discarded.

The anaemia in these patients responded to oral administration of iron. The probable factors responsible for anaemia in these patients were poor availability of iron in the diet, excessive sweating (thereby causing greater iron
loss from the skin than would occur in a temperate climate) and geophagia, which further decreases iron absorption. It was difficult to explain all of the clinical features solely on the grounds of tissue iron deficiency, inasmuch as growth retardation and testicular atrophy are not seen in iron-deficient experimental animals. The possibility that zinc deficiency may have been present was considered. Zinc deficiency was known to cause growth retardation and testicular atrophy in animals. However, essentiality of zinc and its deficiency in humans was unknown. Because heavy metals may form insoluble complexes with phosphate, we speculated that some factors responsible for decreased availability of iron in these patients with geophagia may also have decreased the availability of zinc. O'Dell and Savage first observed that phytate (inositol hexaphosphate), which is present in cereal grains, markedly impaired the absorption of zinc. We published a clinical description of the Iranian cases as a syndrome in 1961 and speculated that zinc deficiency may account for growth retardation and male hypogonadism in these subjects.

**Zinc deficiency in Egypt**

I left Iran in January 1961. Subsequently I joined the department of Biochemistry and Medicine at Vanderbilt University, Nashville, Tennessee, under Dr. William J Darby. Although Dr. Darby wanted me to study porphyrin metabolism in pellagra in Egypt, I shared with him my speculation that zinc deficiency was prevalent in the Middle East and was responsible for widespread growth retardation. He was very interested in this idea. I then moved to Egypt and started my studies at the US Naval Medical Research Unit No.3 (NAMRU-3), Cairo. In Egypt, patients similar to the growth-retarded Iranian subjects were encountered. The clinical features were remarkably similar except that the Iranian patients had more pronounced hepatosplenomegaly, a history of geophagia, and no hookworm infection, whereas the Egyptian subjects had both schistosomiasis and hookworm infestations and no history of geophagia. The dietary history of the Egyptian subjects was similar to that of the Iranians. The consumption of animal protein was negligible. Their diet consisted mainly of bread and beans (Vicia fava). Based on decreased zinc concentrations in plasma, red cells, and hair, and the studies with zinc-65 that revealed, greater plasma zinc turnover, smaller 24-h exchangeable pool, and decreased excretion of zinc-65 in stool and urine in the dwarf patients as compared to the controls, we concluded that the dwarfs were zinc-deficient.

Further studies in Egypt showed that the rate of growth was greater in patients who received supplemental zinc as compared to those receiving iron instead, or those receiving only an adequate animal-protein diet. Pubic hair appeared in all the subjects within 7-12 weeks after zinc supplementation. Genitalia increased to normal size and secondary sexual characteristics developed within 12-24 weeks in all the patients receiving zinc. In contrast, no such changes were observed in the iron-supplemented group or in the group on an animal-protein diet. Thus, we related the growth retardation and gonadal hypofunction in these subjects to zinc deficiency. The anaemia responded to oral iron treatment. These studies clearly showed that severe
anaemia and iron deficiency were not causative factors for growth retardation and hypogonadism in human subjects. Thus, our studies documented for the first time that zinc is essential for humans and that zinc deficiency is prevalent in the populations of developing countries.

**Zinc deficiency in other countries**

Clinical pictures similar to those reported in zinc-deficient dwarf patients from Iran and Egypt have been observed in many other countries. Cognitive impairment has also been observed in these patients. It is believed that zinc deficiency is present in countries wherein the population consumes primarily cereal proteins. A detailed study of zinc deficiency in geophagia cases from Turkey was reported by Cavdar et al in 1983. Beneficial results of zinc supplementation on growth were observed. In 1974 a landmark decision to establish recommended dietary allowances (RDAs) for humans for zinc was made by the Food and Nutrition Board of the National Research Council of the USA National Academy of Sciences.

**Spectrum of clinical manifestations in zinc deficiency**

During the past four decades, a spectrum of clinical manifestations of deficiency of zinc in human subjects has emerged. On the one hand, the manifestations of zinc deficiency may be severe; and, at the other end of the spectrum, zinc deficiency may be mild or marginal. A severe deficiency of zinc has been reported to occur in patients with acrodermatitis enteropathica (a genetic disorder), or after total parenteral nutrition (TPN) without zinc, excessive use of alcohol, or penicillamine therapy. The manifestations of severe zinc deficiency in humans include bulbous pustular dermatitis, alopecia, diarrhoea, emotional disorders, weight loss, intercurrent infections due to cell mediated immune dysfunctions, hypogonadism in males, neuro-sensory disorders, and problems with healing of ulcers. If this condition is unrecognized and untreated, it becomes fatal. The manifestations of a moderate deficiency of zinc include growth retardation and male hypogonadism in adolescents, rough skin, poor appetite, mental lethargy, delayed wound healing, cell-mediated immune dysfunctions, and abnormal neurosensory changes.

In our studies in the experimental human male model in whom only a mild deficiency of zinc was induced by dietary means, decreased serum testosterone level, oligospermia, decreased natural killer (NK) cell activity, decreased interleukin (IL)-2 activity of T helper cells, decreased thymulin activity, hyperammonaemia, hypogeusia, decreased dark adaptation, and decreased lean body mass were observed. It is, therefore, clear that a mild deficiency of zinc in humans affects clinical, biochemical, and immunological functions adversely.

**Impact of the discovery of human zinc deficiency**

When I first started my studies in the Middle East, I knew of only three enzymes, carbonic anhydrase, alcohol dehydrogenase and carboxy peptidase,
which required zinc for their activation. Today we know of over 300 enzymes that require zinc for their activation, and over 2000 transcription factors requiring zinc for their stability have been reported thus far. Zinc is essential for cell-mediated immunity and its deficiency causes a Th1 to Th2 shift. Zinc is also an antioxidant and anti-inflammatory agent.

**Therapeutic uses of zinc**

The therapeutic uses of zinc in humans are:

- treatment of acute infantile diarrhea
- treatment of accrodermatitis enteropathica
- treatment of wilson’s disease
- treatment of sickle cell disease
- treatment of amd (age related macular degeneration)
- treatment of the common cold
- reduction in the incidence of infections in the elderly

**Acute infantile diarrhoea**

Acute infantile diarrhoea is a very serious disorder that affects millions of children in developing countries. The mortality rate associated with this condition is 60 to 80%. During the past decade, zinc in therapeutic doses has been used for treating such patients. This approach has reduced the mortality by 30 to 40%. Also, it has been observed that the incidence of pneumonia drastically decreased in patients who received zinc.

**Acrodermatitis enteropathica**

Acrodermatitis enteropathica is a relatively rare genetic disorder in which the absorption of dietary zinc is affected adversely such that the affected individuals become severely zinc deficient. If untreated, the disease becomes fatal. Mutation in the ZIP4 gene (a zinc transporter) is responsible for this disorder. Treatment with therapeutic levels of zinc is highly successful and nowadays such patients survive and lead normal lives.

**Wilson’s disease**

Wilson’s disease is a genetic disorder in which copper accumulates in liver, kidneys, intestines, brain, and other organs. In our earlier studies we observed the beneficial effect of zinc on the sickling of deoxygenated sickle cells. Later zinc administration in therapeutic doses (50 to 150 mg zinc daily given orally as acetate) was used for decreasing the pain crises in sickle cell disease patients. We observed that at this level of zinc administration, we were inducing copper deficiency in our patients. This led us to evaluate zinc as a therapeutic modality for the treatment of Wilson’s disease. Zinc acts by induction of intestinal cell metallothione in which, once induced, has a high affinity for copper and prevents the serosal transfer of copper into the blood. The intestinal cells turn over rapidly and take the complexed copper into the stool where it is excreted. Zinc blocks copper in the food and endogenously excreted copper.
through salivary, gastric and other gastrointestinal juices. As a result, zinc produces a chronic negative copper balance\textsuperscript{10}. For maintenance therapy of Wilson’s disease, zinc is the treatment of choice. Zinc has no toxic effects and it can be used for treating pre-symptomatic patients and pregnant women.

**Sickle cell disease**

Our studies in adult patients with sickle cell disease showed that nearly two-thirds of these patients were zinc deficient\textsuperscript{11}. We also related growth retardation, male hypogonadism and immune dysfunction in these patients to zinc deficiency\textsuperscript{11}. Zinc supplementation in sickle cell disorder patients in therapeutic doses has shown beneficial effects in respect of the above-mentioned clinical parameters. Chronic haemolysis in these patients causes hyperzincuria, and this leads to deficiency of zinc.

**Macular degeneration**

The age-related Eye Disease Study\textsuperscript{12} group supported by the National Eye Institute, NIH, conducted an 11-center double-masked clinical trial in patients with age-related macular degeneration (AMD). As many as 3640 participants were enrolled. Their ages ranged from 55-80 years and the average follow up period was 6.3 years. The participants were randomly assigned to receive daily oral tablets containing one of the following:

- antioxidant (vitamin C 500 mg, vitamin E 400 IU; and β carotene 15 mg);
- zinc 80 mg as zinc oxide and copper 2 mg as cupric oxide;
- antioxidants plus zinc; or placebo.

Copper was added in order to prevent copper deficiency in the zinc-supplemented group. The group taking the antioxidant-plus-zinc supplementation showed reduced risk of developing advanced AMD to the extent of ~25% and the risk of vision loss to the extent of ~19%. The group taking zinc alone showed a lower risk of developing advanced AMD to the extent of ~21% and vision loss to the extent of ~11%, whereas in the group taking the vitamins alone the risk levels for these conditions were reduced by ~17% and ~10%, respectively. No side effects were noted as a consequence of therapeutic levels of zinc supplementation. Another interesting observation was that only the zinc-supplemented group showed decreased mortality\textsuperscript{13}. The effectiveness of zinc in AMD is most likely due to its antioxidant and anti-inflammatory effects.

**Zinc in common cold**

In order to test the efficiency of zinc acetate lozenges in reducing the duration of symptoms of the common cold, we carried out a randomized, double blind, placebo-controlled trial in 50 ambulatory volunteers recruited within 24 hours of developing symptoms of the common cold\textsuperscript{14}. The participants each took one lozenge containing 12.8 mg zinc (as acetate) or placebo every 2 to 3 hours while they were awake, for as long as they had cold symptoms. Subjective symptom scores for sore throat, nasal discharge, nasal congestion, sneezing,
cough, scratchy throat, hoarseness, muscle ache, fever and headache were recorded daily for 12 days\textsuperscript{14, 15}. When compared with the placebo group, the zinc group had shorter mean overall durations of cold symptoms, cough and nasal discharge, and lower total severity scores for all symptoms\textsuperscript{14, 15}. The mechanism by which zinc may mediate the common cold is not well understood. It has been suggested that zinc may act as an antiviral agent. Another possibility is that extra-cellular zinc ions may exert their antiviral effect by stabilizing and protecting cell membranes. Zinc is known to induce production of interferon and modulate inflammatory cytokines, which in turn may result in alleviating the symptoms of the common cold.

Our recent studies in the elderly have shown that the incidence of infection, oxidative stress bio-markers and generation of inflammatory cytokines, were significantly lower in subjects who received supplements of 45 mg zinc (as gluconate) daily than in the placebo groups\textsuperscript{16}. This study demonstrates the effect of zinc, \textit{in vivo}, on immune functions, and its role as an antioxidant and anti-inflammatory agent. We have observed similar effects of zinc supplementation in patients with sickle cell disease\textsuperscript{17}.

**Conclusion**

Deficiency of zinc and its essentiality for humans was recognized nearly 45 years ago. Major manifestations of zinc deficiency include growth retardation, hypogonadism in males, immune dysfunction and cognitive impairment. Significant advances have taken place in understanding the biochemistry of zinc. Zinc is essential for the functioning of immune cells. It is estimated that nearly 2 billion subjects in the developing countries may have zinc deficiency. The therapeutic impact of zinc is also very impressive. Zinc therapy in infantile diarrhoea, Wilson’s disease, acrodermatitis enteropathica, sickle cell disease, and the common cold, and zinc supplementation for prevention of blindness in age-related macular degeneration and for the prevention of infections in elderly subjects and in patients with sickle cell disease are some of the major uses of zinc supplementation in humans.

**References**