

KRAMADHATI VENKATA GIRI

(1907-1958)

Elected F.N.I. 1956

BIRTH, PARENTAGE AND CHILDHOOD

KRAMADHATI VENKATA GIRI was born in the year 1907 in a remote village near Madanapalle in Andhra Pradesh where his father, Shri K. Surappa lived. Giri at a very young age ventured out of his village to Madanapalle for his early education.

FORMATIVE INFLUENCE ON THE YOUNG SCIENTIST

As a young student in the Madras University, Giri set for himself high standards of morality and dedication in achieving his goals, preparing himself to obtain the best possible education to enable him finally to become one of the leading biochemists of his time.

SCHOOL AND UNIVERSITY EDUCATION AND RESEARCH

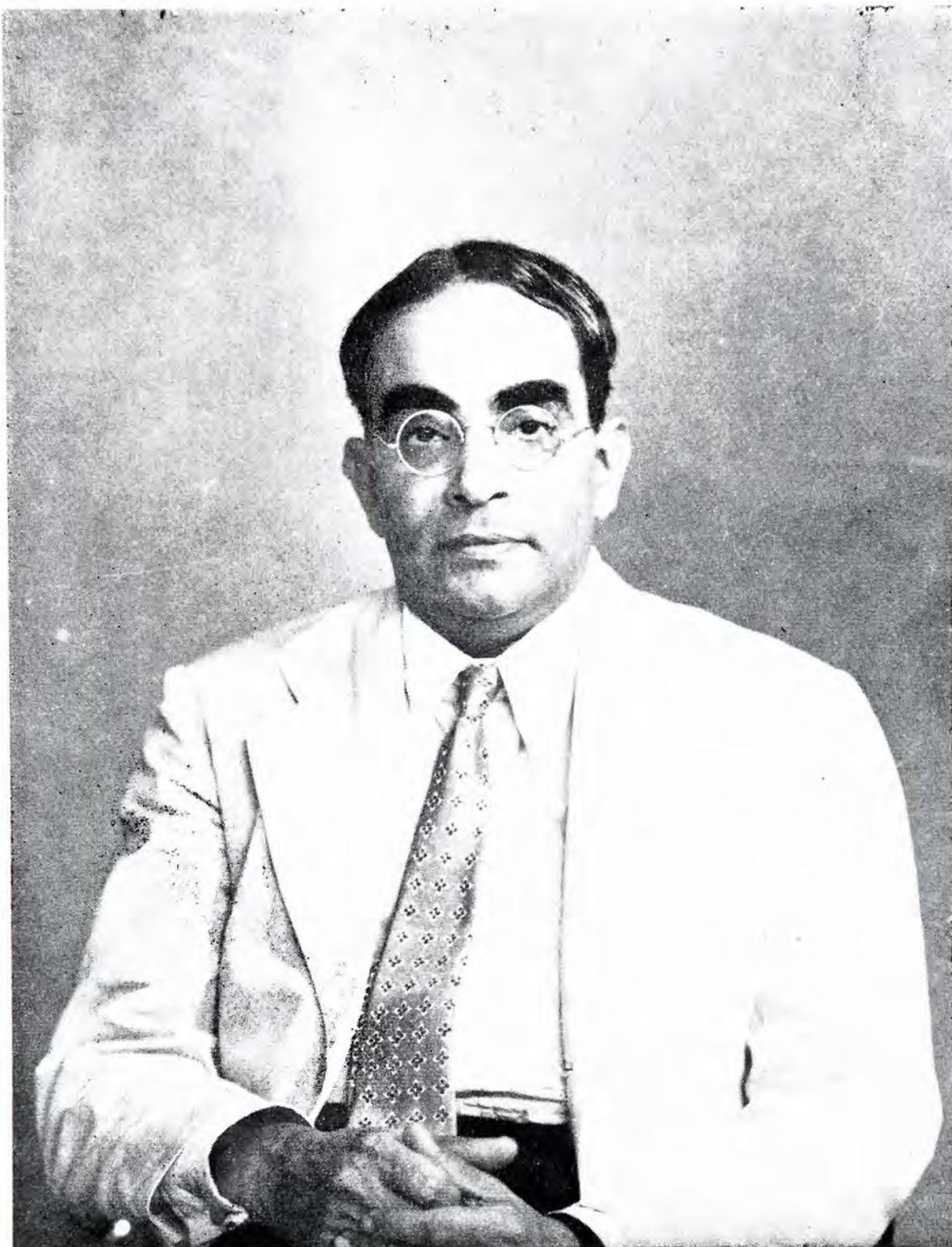
Giri obtained the B.A. degree of the Madras University from the St. Joseph's College, Tiruchirappally. He took the M.Sc. degree of the University of Calcutta and then joined the Department of Biochemistry, Indian Institute of Science, Bangalore in 1929. His early work on 'Investigations on Enzymes' gained for him the degree of Doctor of Science of the Calcutta University in 1938.

PROFESSIONAL

After two years of service as Enzyme Chemist at the Nutritional Research Laboratories, Coonoor (1939-40) and three years as Gowthami lecturer and later Reader in Biochemistry at the Andhra University, Dr. K. V. Giri returned to the Institute at Bangalore to become a lecturer in Biochemistry in 1943. He was elevated in 1950 to the Professorial Chair in Biochemistry, a post he held till the end. During 1949-50, Prof. Giri was associated with Prof. H. von Euler at the University of Stockholm, Sweden and visited Norway, Denmark and England. He presided over a session of the International Symposium on 'Enzyme Chemistry' held in Tokyo in the autumn of 1957. Prof. Giri was actively associated with executive and advisory committees of several universities, research institutions and technical bodies, both in India and abroad.

Till the very end, Prof. Giri was greatly devoted to science and in the spread of biochemical knowledge for exploitation of abundant vegetable and animal





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resources. He held the firm conviction that to achieve this end, economical and highly simplified biochemical techniques are necessary pre-requisites. He spared no pains to popularise these techniques through his writings, lectures, radio-talks visual demonstrations by charts and cine films. He took great delight in assiduously collecting photographs and life sketches of famous scientists, particularly, Nobel Laureates. His chief pleasure was in the laboratory where he preferred to work himself rather than to entrust to others. Until his passing away, he had great admiration for his colleagues and students all of whom he treated with extreme affection in the laboratory and his home

The sudden demise of Prof. Giri on 17 July 1958 in Madras took away from our midst a well-loved personality and a distinguished biochemist of our country.

CONTRIBUTIONS TO NEW KNOWLEDGE

Water-soluble vitamins

Thiamine (Vitamin B₁): A simple and rapid method for the estimation of vitamin B₁ in foodstuffs was developed and the amounts of this vitamin in ground-nut, during germination and under different conditions of manuring, were estimated. Investigations were also carried out on its biosynthesis in molds and by the intestinal flora of rats under different dietary supplements like milk, curds, fats or on the administration of sulphaguanidine, *p*-aminobenzoate and penicillin.

Biotin: It was shown that administration of excess amounts of biotin to rats leads to an increased synthesis and accumulation of cholesterol in tissues and that these effects could be reversed by giving inositol.

Ascorbic Acid: Giri's studies on the analytical chemistry of ascorbic acid and on its stability under varying environmental conditions, are also worthy of mention. The mechanism of the inhibitory action of ascorbic acid on important enzymes like amylases and phosphatases was also investigated.

Riboflavin (Vitamin B₂): Of considerable interest is his observation that a mutant of yeast, *Saccharomyces cerevisiae*, excreted considerable amounts of this vitamin into the medium. This work on riboflavin eventually led to extensive investigations on the flavin coenzymes.

Enzymes

Professor Giri was a pioneer in enzyme research and his earliest studies were concerned with purification and documentation of the properties of some key enzymes in intermediary metabolism, viz., phosphatases, amylases, lipases and proteases. Much of this work was published in *Zeitschrift fur Physiologische Chemie*, *Biochemische Zeitschrift*, *Biochemical Journal*, *Nature* and *Enzymologia*.

Carbohydrate Metabolism

A systematic search for phosphorylase in Indian pulses showed that green gram was a good source of the enzyme. The enzyme was purified and its nature and behaviour towards various substances of biological importance were studied. A method was developed for the preparation of glucose-1-phosphate, using green



gram extract as a source of phosphorylase. In addition to phosphorylase, another enzyme with the branching factor or Q enzyme which takes part in the synthesis of starch was isolated from green gram and its characteristics were studied.

Evidence in support of the existence of Embden Meyerhof glycolytic pathway in green gram was advanced through identification and isolation of all the enzyme systems involved. Phosphorylated glyceric acids were shown to be very effective precursors for sucrose synthesis in green gram.

An enzyme capable of building up glucofructosans from sucrose was detected in water extracts of the tubers of *Crinum longifolium* and *Crinum latifolium*. It was concentrated and purified from associated carbohydrates and its characteristics were determined. Normally, sucrose acts as the acceptor as well as the donor of fructosyl residues. Raffinose also was found to act as a substrate; it builds up two oligosaccharides when acted upon by this enzyme. It was shown that neither glucose nor fructose acts as an inhibitor.

The penicillin producing strain *P-chrysogenum* Q 176 was capable of synthesizing various types of oligosaccharides when grown in a medium containing maltose and sucrose. Enzymes capable of synthesizing these oligosaccharides were prepared in pure form by passing through starch column and by subsequent alcohol precipitation. All the oligosaccharides synthesized from maltose were identified and characterized by means of periodate oxidation, physical constants, mobility on paper chromatograms and by comparison with acid hydrolysates of known polysaccharides.

Nitrogen Metabolism

A systematic survey of the free amino acid content of several edible leaves and leaves of the citrus family was made. Citrus leaves contain a good percentage of proline. A high concentration of asparagine was observed in betel leaves. Hydroxyproline was found to occur in the leaves of sandal in high concentrations; the flowers and pericarp contained the highest percentage. This amino acid was isolated by a combination of ion-exchange and circular paper chromatography and was characterized by physical and chemical methods as allo-hydroxy-L-proline. This is the first report of free hydroxyproline in the plant kingdom.

Cell-free extracts of green gram were found to synthesize glycine from a variety of amino acids by transamination with glyoxylic acid.

Partial purification of the enzyme, arginase, from field beans (*Dolichos lablab*) was effected and the kinetics of metal ion activation was studied. The enzyme activity was unaffected by copper and cadmium, but was inhibited by borate, citrate, cystine, ornithine and lysine. The presence of transaminases, viz., glutamic acid-alanine and glutamic acid-aspartic acid, in some of the Indian pulses was detected by paper chromatography. The glutamic-aspartic transaminase was inhibited by pterygospermin, an antibiotic isolated from the roots of the drumstick tree (*Moringa pterygosperma*).

An enzyme which converts riboflavin to flavin mononucleotide (FMN) was purified from green gram extracts, with a recovery of about 40%. The conversion of riboflavin to FMN was nearly 60%.



An enzyme which hydrolyses flavin adenine dinucleotide (FAD) to FMN and adenosine-5'-phosphate (AMP), was obtained in crystalline state from green gram seedlings. Its optical pH was 9.0–9.4. At the ammonium sulphate stage of purification and on treatment with glutathione (GSH), this enzyme gave riboflavin, pyrophosphate and adenosine. This alteration of the site of attack is inhibited by phosphate ions. Further, the inhibition of the original activity (i.e., FAD to FMN and AMP) together with a change in the spectrum of the enzyme, on the addition of phosphate indicates the key role played by phosphate ions in this activity.

The FAD hydrolase was purified 300-fold from germinating seedlings of green gram. It was inhibited by EDTA and the inhibition could be reversed only by Zn^{2+} and Co^{2+} ions. Zinc ions at concentrations above 1×10^{-4} M inhibited the EDTA-treated as well as the untreated enzymes. This effect of Zn^{2+} ions is similar to that observed in the case of well-known regulatory enzymes which are inhibited either by excess substrate or co-factor. Similarly, this enzyme was inhibited by pyrophosphate, ATP and AMP, EDTA and activation of the EDTA-inhibited enzyme by Zn^{2+} followed a typical allosteric pattern.

AMP is an allosteric inhibitor for the crystalline FAD-hydrolase from mung bean (*Phaseolus radiatus*). One of the sites of AMP inhibition could be desensitized by P-chloromercuri-benzoate (PCMB) or N-ethyl maleimide or by ageing. A partially inactivated enzyme could be reactivated by low concentrations of adenosine diphosphate (ADP).

Riboflavin Metabolism in Plants

It is well known that the B-vitamins occur abundantly in higher plants. With the demonstration that some of the B-vitamins like nicotinic acid and riboflavin function as components of enzymes, increasing attention was devoted to the study of the role of these vitamins in plants. Investigations were carried out on the forms in which riboflavin occurs in plants and on the enzymatic synthesis of the flavin coenzyme nucleotides.

Starting with riboflavin, FMN is synthesized by the enzyme flavokinase by an irreversible reaction with ATP. The partially purified (75-fold) enzyme functions optimally at a temperature of $55^\circ C$ and pH 8.6. The high temperature optimum, although very unusual for enzymes in general, is not uncommon in plants. The substrates (riboflavin and ATP) exert a protective action against heat denaturation and this would, to a certain extent, explain the activity at high temperature. It is highly specific for the flavin, but not so specific for the phosphate donor.

FAD is synthesized from FMN and ATP and this reaction is catalysed by the enzyme, FAD synthetase, which functions optimally at a temperature of $37^\circ C$ and pH 7.5. The 83-fold purified enzyme is specific for its substrates FMN and ATP, and it showed some hydrolytic activity which was very much less than that of the crude extract; this suggested the possibility that in addition to the hydrolysis by the reversal of FAD-synthetase, FAD may also be hydrolysed by a specific enzyme.



Studies on Blood Coagulation

Prof. Giri was one of the leading research workers of his time who initiated a systematic study of the mechanism of blood coagulation. His painstaking work on the separation of various protein factors involved in blood coagulation, the study of their interactions and the isolation of anti-coagulant factors from indigenous plant sources, represented his major work in this area.

Paper Chromatography

Prof. Giri and his associates made important contributions to the development of paper chromatography as a powerful tool in biochemical research. Particular mention may be made of the development of a paper chromatographic technique for the separation and identification of enzymes, for the qualitative and quantitative analysis of a variety of biochemically important compounds like amino acids, sugars, organic acids, etc.

It was shown that enzymes could be made to move on paper employing aqueous acetone or alcohol or salt solutions as the developing solvents. The position of the enzymes on the developed chromatograms was located by the agar plate method.

The circular paper chromatographic technique developed by him has proved to be a simple, elegant and rapid method for the separation and identification of many biological metabolites. The wide application of this method in diverse fields is indicative of its advantages, in certain respects, over the conventional methods.

Agar Electrophoresis

Prof. Giri and his colleagues also developed a simple technique of agar electrophoresis for the separation of serum proteins. In this modification of the well-known paper electrophoresis method, the filter paper is replaced by agar gel layered between glass plates, as the medium. The method is simple, quick and efficient in the resolution of protein components without adsorption. Further, this technique affords the separation in the serum of a larger number of components than those obtained by paper electrophoresis.

A simple method of two-dimensional agar electrophoresis was developed by which mucoproteins could be separated into well-defined zones. The mucoprotein spots could be identified by fuchsin sulphate spray after periodate oxidation. This technique is useful for the examination of the changes in mucoproteins in pathological samples of blood and urine.

Prof. Giri felt the need to spread biochemical knowledge widely and made a hobby of devising scientific films. He wanted every medical practitioner in India to have access to clinical biochemical services and this objective was stressed through his films, writings, radio talks and lectures.

MARRIAGE, WIFE AND PRIVATE LIFE

Prof. Giri married the daughter of Shri H. Krishna Sastri, the first Indian Chief Epigraphist to the Government of India. His wife, Smt. Janakamma took a deep



interest in his work and helped to translate many of his publications into Telugu and Kannada.

Prof. Giri has four children, two sons and two daughters. His eldest son, Dr. K. V. Ravi, has specialised in material science and is now working as the Technical Director of MOBILE-TYCO laboratories in U.S.A. and is directing research programmes in the area of solar energy. His second son Shri K. V. Chandrasekhar, is Managing Director of M/S. Sekhar Electronic Industries Pvt. Ltd., a company engaged in the manufacture of electronic components in Bangalore.

His first daughter, Smt. Usha is a qualified Chartered Accountant and his second daughter, K. V. Amba is a Computer Scientist.

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