1. **INTRODUCTION**

1.1 The history of tryptophan may be traced to the observation of Tiedman and Gmelin (1831) that pancreatic juices of a dog gave a violet colour with chlorine water. The substance responsible for this reaction was regarded as the "protein chromogen". The name 'Tryptophane' was suggested by Neumeister (1890) for the colour forming fraction obtained from proteins by putrefaction with pancreas. A colour reaction for proteins was described by Adamkiewicz (1874) which Hopkins and Cole (1901) showed to be a reaction of the "protein chromogen" with glyoxylic acid. Hopkins and Cole found that the chromogenic substance of proteins survived hydrolysis and that it was, relatively simple substance. They discovered that mercuric sulphate precipitated it from a strongly acidified protein hydrolysate solution. By the use of this reagent they isolated tryptophan from a pancreatic digest of casein.

The structural relationship of tryptophan to indole was established by Nencki (1895), who showed that the substance formed with chlorine water gave indole and skatole on fusion with alkali. The substance isolated by Hopkins and Cole also gave the pyrrole pine splinter test and liberated indole and skatole on heating. On oxidation with ferric chloride it gave $\beta$-indole aldehyde. Ellinger and Flammand (1907) synthesised it from $\beta$-indole aldehyde by the hippuric acid condensation and gave it its present constitution.
1.2 OCCURANCE AND DISTRIBUTION IN PROTEINS.

The isolation of tryptophan stimulated the early investigations on the role of amino acids in nutrition. Wilcock and Hopkins (1906) made the important discovery that tryptophan was an essential amino acid for nutrition. Although the amino acid is present in proteins only in small amounts, it is a constituent of most of them. Fibrin is a rich source of tryptophan, containing 3.5 per cent. Casein is the protein generally used for its isolation. It is present only in traces in zein and gelatin. For a general appreciation of the tryptophan content of various proteins a reference may be made to the monograph of Block and Bolling (1951) on this subject and Abderhalden's Biochemisches Handlexikon (1911).

1.3 ESTIMATION OF TRYPTOPHAN.

The early methods developed for the determination of tryptophan were chiefly colorimetric. Spectrophotometric and microbiological methods have come into use recently and these have helped very much in obtaining accurate data. The glyoxylic acid method was used by Hopkins (1901) and similar colorimetric methods using formaldehyde (Rosenheim 1906) and p-dimethylaminobenzaldehyde (Voisenet 1905, Rhode 1905) were used by other investigators. Folin and Looney (1922) used the phenol reagent (phosphomolybdc, phosphotungstic acid) while Lugg (1937) employed the Millon's reagent for the colorimetric estimation. The method of Eckert (1943) based on the determination of colour formed by diazotised tryptophan with N(1-naphthyl)ethy-
lene diamine HCl and the method of Gordon and Mitchell (1949) which measures the green fluorescence produced by the reaction of tryptophan with perchloric acid were introduced later. The spectrophotometric method employed by Holiday (1936) and by Goodwin and Morton (1946) based on the specific absorption of ultraviolet light at 280 nm were valuable additions to the earlier methods. More recently microbiological methods based on the nutritional requirements of tryptophan by lactic acid bacteria have been developed for the estimation of tryptophan in proteins by many investigators. (Greene & Black 1944, Wooley & Sebrell 1945, Kuiken, Lyman and Hale 1947).

1.4 THE LIBERATION OF TRYPTOPHAN FROM PROTEINS.

The estimations of tryptophan by the aldehyde and spectrophotometric methods are generally carried out directly on the protein but for the other methods it is necessary to liberate the amino acid from the protein by hydrolysis. Hydrolysis of proteins with strong mineral acids results in almost complete destruction of tryptophan, while hydrolysis with alkali destroys it partially and brings about racemisation. Homer (1915) carried out hydrolysis of casein with various concentrations of barium hydroxide while Lugg (1937) carried out the hydrolysis with sodium hydroxide in the presence of stannous chloride. The liberation of tryptophan from proteins by pancreatic enzymes, a fact known since the discovery of this amino acid, was studied in detail by Hunter (1925) and later by Ragins (1928) who showed that trypsin was the most active enzyme for the libera-
tion of tryptophan while pepsin and erepsin were inactive. The complete liberation of tryptophan from proteins by enzymic hydrolysis still requires experimental proof which can be shown only by the isolation of the quantity known to be present by estimation.

1.5 THE ISOLATION OF TRYPTOPHAN.

Hopkins and Cole (1901-1902) isolated tryptophan from pancreatic digests of casein and obtained a yield of 1.5 per cent based on the weight of casein. Dakin (1918) introduced butanol extraction as an additional step to the procedure of Hopkins and Cole and obtained a yield of 1.7 per cent from casein. Onslow (1921) similarly obtained yields of 1.7 per cent. Abderhalden and Kempe (1907) however, recovered only 0.53 per cent and Cox and King, 0.7 to 0.8 per cent. According to Plimmer (1917) a yield of 1.0 per cent from casein is generally obtained. Tryptophan was isolated from fibrin by Neuberg and Popowsky (1907) and by Bergmann and Niemann (1937). It has been isolated from several other proteins as well.

1.6 ISOLATION OF PEPTIDES FROM PARTIAL HYDROLYSATES OF PROTEINS.

Hofmeister (1902) suggested that the amino acids may be linked in the protein molecule with the bond R-Co-NH-R, which was later termed by Fischer as the peptide linkage. The existence of this configuration in the protein molecule was established by Fischer and Abderhalden and associates who isolated a number of peptides and peptide anhydrides from partially hydrolysed proteins (Synge, 1943).
Glycine L-alanine anhydride, glycine L-tyrosine, L-alanyl glycine, and L-alanylglycyl L-tyrosine were isolated from partial hydrolysates of silk fibroin; glycine L-leudine anhydride, glycine L-valine anhydride, L-alanyl-L-leucine and L-leucyl-L-alanine were isolated from elastin, and found to be identical with the compounds prepared by synthesis. Abderhalden (1908) reported the isolation from cotton seed globulin, of a dipeptide of tryptophan and glutamic acid and a tripeptide of tryptophan, glutamic acid and leucine. The study of the phosphorus containing group of casein (Damodaran and Ramachandran 1941) resulted in the isolation of a phosphopeptone containing phosphoserine, glutamic acid and isoleucine.

The methods employed in the past for the separation of the products of partial hydrolysis of proteins were inadequate and therefore no appreciable progress was made in this field. The recent introduction of methods based on adsorption, liquid liquid extraction, chromatography and electro-phoresis, and the methods of end group analysis (The Proteins, Neurath & Bailey, 1953) have widened the field of investigation in the chemistry of protein structure. Therefore, it has now become possible to reinvestigate some of the old problems, which have not yielded consistent results.

1.7. THE PRESENT INVESTIGATION.

The object of the present investigation was to study the methods of estimation and isolation of tryptophan with the improved techniques now available for the study of
products of protein hydrolysis. A careful reinvesti-
gation has been made of the usual method for the isolation
of tryptophan with a view to make a systematic study of
the substances precipitated by mercuric sulphate from a
pancreatic digest of casein. A fractionation of these
substances has been carried out to isolate simple peptides
of tryptophan and of other amino acids. Tryptophan
peptides were synthesised in order to study the analytical
behaviour of tryptophan in different peptide combinations
in the estimation of tryptophan by the colorimetric
glyoxylic acid method and the microbiological methods of
assay. The work is described in two parts, part I
dealing with the estimation of tryptophan and part II,
the isolation of tryptophan and its peptides from tryptic
digest of casein.