BIOSYNTHESIS AND METABOLISM OF TRYPTOPHAN IN THE RUMINANT AND IN REED CANARY GRASS

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Paper 2 Tryptophan absorption and metabolism in sheep. 129
(To be published in Can. J. Animal Sc. in August of 1970)

ABSTRACT

Candlish, Violet Elizabeth, PhD., The University of Manitoba, October 1970. Biosynthesis and metabolism of tryptophan in the ruminant and in reed canary grass. Major Professors: Lucien J. LaCroix, Department of Plant Science; Thomas J. Devlin, Department of Animal Science.

A continuous flow artificial rumen apparatus was constructed to investigate the utilization of DL-tryptophan-^{14}C (U) -benzene ring labelled material by the rumen microorganisms. Half of the labelled material left the rumen sac in the first six hours. After a 24 hour period, less than 4 % of the label was associated with either protozoal or bacterial fraction of the rumen fluid.

Biosynthesis of tryptophan by rumen microorganisms was investigated using the in vitro apparatus. L-serine-^{14}C (U) was incubated with rumen microorganisms. Half of the labelled material diffused into the saline before 4.5 hours had elapsed. The maximum amount of label associated with protozoa was 2.1 %; the similar value for bacteria was 1.5 %. These values were obtained only after the 42 hours of incubation. Serine was a much better substrate than tryptophan for volatile fatty acid production by the microorganisms. The synthesis of tryptophan was indicated by appearance of label in the tryptophan area of the thin layer chromatograms. In all but one sample, the percent of label appearing in the tryptophan was less than one.

Labelled compounds appeared in the urine within 10 minutes after injecting DL-tryptophan -^{14}C (U) -benzene ring labelled into the rumen of 2 sheep. Label appeared in portal blood prior to its appearance in jugular blood. Most of the label in the blood samples occurred in tryptophan. Urine samples were examined for label appearing in tryptamine, indoleacetic acid and tryptophan. Less than 20 % of the label in the urine was in the analysed compounds with tryptamine showing negligible activity.

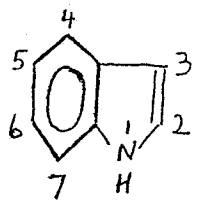
Rapid absorption of tryptophan from the rumen and reticulum of two other sheep was shown. After injection of labelled tryptophan into the rumen, 26.7 % was absorbed using the washed rumen technique, and 68.8 % was absorbed when the whole rumen technique was employed. When labelled sodium acetate was injected into the rumen and the washed rumen technique used, 49.0 % was absorbed.

Reed canary grass was injected with L-serine -^{14}C (U) and DL-tryptophan -^{14}C (U) -benzene ring labelled, prior to flowering. After seed ripening (33 days postinjection) plants were harvested and analysed. An average of 2.17 % of injected radioactivity from serine treated plants, and 13.67 % of injected radioactivity from tryptophan treated plants, appeared in the ethanol extract. The average percent of injected label which was recovered in tryptophan was 0.119 % of the labelled serine and 0.412 % of the labelled tryptophan. The alkaloid fraction of high alkaloid level plants contained 0.7 % of injected tryptophan label and 0.1 % of injected serine label.

INTRODUCTION

The importance of tryptophan to living cells was realized when proteins were shown to contain this amino acid, as a primary structural unit. The nutritional significance of tryptophan was evident when it was characterized as an essential amino acid for monogastric animals, including man.

Interest in tryptophan increased when it was established that the chemical structure of this amino acid was different and more complex than any of the other twenty amino acid building blocks involved in protein synthesis. Although phenylalanine and tyrosine are also aromatic amino acids, tryptophan reacts distinctly as a result of the indole ring.



The 2 and 3 positions of the indole ring have reactive characteristics which permit condensation with other compounds to yield intensely coloured complexes. This reactivity has been used in colour tests for identifying proteins and to study enzyme action.

Research involving amino acids has in many instances neglected the study of tryptophan due to the necessity of separate analysis as tryptophan is destroyed during the normal acid hydrolysis procedure. Nevertheless researchers working

in the area of tryptophan biosynthesis and metabolism have been rewarded for their efforts by the association of tryptophan and tryptophan metabolites with an increasing number of living processes.

Prior to the 1950's, ruminant nutritionists had accepted the view that microorganisms in the rumen provided all the amino acids necessary for the ruminant animal and microorganisms could synthesize amino acids from a simple carbon source and non-protein nitrogen. Subsequently several research reports indicated that the "quality" of dietary nitrogen had an effect on animal performance. The rates at which various proteins and amino acids were attacked by rumen microorganisms differed considerably. The assumption that amino acids were not absorbed through the rumen wall but passed to the abomasum before moving into the body blood stream was questioned.

The sparse literature available concerning tryptophan metabolism in ruminants indicated that tryptophan might be attacked slowly by rumen microorganisms. The fact that this amino acid was essential to most nonruminant mammals led to the question of whether or not tryptophan was essential to ruminants.

In addition to nutritional studies with tryptophan in animals, chemical studies of tryptophan and related indole compounds were carried out with plants. Indole alkaloids present in Phalaris tuberosa L. were thought to

be the toxic agent involved in Phalaris staggers, a disease first described in sheep in Australia. Reed canary grass (Phalaris arundinacea L.) is not palatable to ruminants but grows extensively in pasture areas. If the reason of the unpalatiblity and toxic nature of this plant could be located, the use of reed canary grass for ruminant feed might be possible. Tryptophan is also considered a precursor of the plant hormone, indoleacetic acid. Knowledge of the interrelationships of tryptophan and these important plant metabolites may solve the problems associated with the use of these plant materials as animal feeds.

The research reported in this thesis was concerned with examination of the biosynthesis and metabolism of tryptophan by rumen microorganisms using a continuous flow in vitro apparatus. The results obtained were compared with results of in vivo studies using labelled tryptophan injected into the rumen of sheep. Biosynthesis and metabolism of tryptophan were also studied in maturing reed canary grass. The possibility of incubating labelled reed canary grass with rumen microorganisms in the continuous flow in vitro apparatus was also considered.

TRYPTOPHAN BIOSYNTHESIS AND METABOLISM IN RUMINANTS.

LITERATURE REVIEW

Importance of tryptophan to animals

Tryptophan has been classified as an essential amino acid for the monogastric animal. Recent research has suggested a role for tryptophan in the protein synthesizing machinery of the body in addition to its function as a protein constituent. Munro (1968) reported studies with rats and mice fed amino acid mixtures deficient in one essential amino acid. Only the amino acid mixture lacking tryptophan gave rise to a reduction in liver polysome aggregates and accumulation of oligosomes. Sidransky et al (1968) tube-fed fasted mice a complete amino acid mixture and a mixture devoid of tryptophan They found that only the mice fed the amino acid mixture lacking tryptophan behaved like the fasted controls in causing polysome disaggregation. Tryptophan supplementation produced a response similar to that induced by the complete amino acid mixture. Methionine, threonine and isoleucine were also tested in a similar manner and did not shift the hepatic polysomes from lighter to heavier units. Freedman et al (1968) incubated rabbit reticulocytes under conditions of tryptophan deficiency and obtained results confirming Sidransky's findings. They speculated that polysome disaggregation was due to the location of

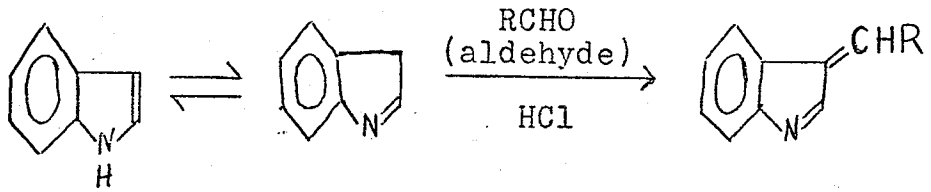
tryptophan near the amino terminal ends of rabbit hemoglobin. Since growth of the peptide chain normally proceeds from the amino terminal end, during tryptophan deficiency, the rate of translation of the messenger RNA was retarded near the proximal end, at the sites of the tryptophan codons. Beyond the tryptophan codon sites, the rate of translation was normal. The failure of the system to maintain a steady state number of ribosomes on messenger RNA resulted in polyribosome disaggregation.

According to Munro (1968), most proteins contain less tryptophan than other amino acids and therefore would contain considerable stretches of peptide chain not including tryptophan. Rate of translation would not be inhibited in non-tryptophan containing areas. Tryptophan could then readily become the limiting factor for protein synthesis. Munro also suggested that a low level of tryptophanyl-sRNA was in the cell due to tryptophan being the least abundant amino acid. Protein synthesis would be limited because insertion of tryptophan at the correct site in the peptide chain would involve the greatest delay in translation of the message.

Supporting evidence for the importance of tryptophan in protein synthesis was published by Yamashita et al (1969). Supplementation of a 20% casein diet with tryptophan, increased rat liver threonine dehydratase activity. Puromycin, an inhibitor of protein synthesis, inhibited tryptophan

stimulated threonine dehydratase activity, whereas actinomycin, an inhibitor of RNA synthesis, had no inhibitory effect. These authors concluded that the enzyme induction by tryptophan was based on de novo synthesis of protein at the translation step. Other amino acids did not increase the enzyme activity.

Chan and Schellenberg (1968) concluded from observations made on pig heart mitochondrial malate dehydrogenase that tryptophan was present at the active site of the enzyme. The tryptophanyl residues of some dehydrogenases were thought to participate in the hydrogen transfer through an intermediary indolenine structure.



The synthesis and metabolism of tryptophan has been found to be under a close metabolic control which differs from one species to another. Leklem et al (1969) found induction of tryptophan oxygenase in rats with less response in cats. Several urinary metabolites of tryptophan were known to be bladder carcinogens in mice. The urinary excretion of tryptophan metabolites was greater in rats than in cats and less spontaneous incidence of bladder cancer was observed in cats.

The above findings illustrate the diverse nature of tryptophan research and diseases which interfere with tryptophan metabolism further indicate the importance of

this compound to the body. Disturbance of tryptophan metabolism appears as a secondary effect in hyperhistinemia, phenylketonuria and maple syrup urine disease. Few direct tryptophan deficiency diseases are known in man, probably because a deficiency leads to general protein deficiency. Hartnup disease is a hereditary pellagra believed to be caused by defective absorption of tryptophan from the upper gut. In this disease there is increased excretion of indole derivatives and decreased excretion of metabolites of the nicotinic acid pathway.

An oral dose of tryptophan was found effective in producing interstitial pulmonary emphysema in experiments reported by Carlson et al (1968). The concentration of tryptophan in the plasma was not directly related to the development of the syndrome in cattle. Evidence indicated that some product of ruminal metabolism of tryptophan might be the causative factor. As sheep were not susceptible to tryptophan-induced interstitial pulmonary emphysema and the naturally occurring acute bovine pulmonary emphysema had not been reported in sheep, the authors concluded that the tryptophan-induced syndrome was species specific and that a difference in tryptophan metabolism might exist between cattle and sheep.

Impaired metabolism of tryptophan was shown in bovine tympanites or bloat (Johnson and Dyer 1968). The tympanitic bovine tended to excrete greater quantities of most of the tryptophan metabolites measured. The monoamines, 5-hydroxy-tryptamine (serotonin) and tryptamine, were thought to be

the most likely metabolites involved in the tympanitic syndrome. Previously Dyer et al (1964) had shown a difference in the metabolism of tryptophan and tyrosine by the normal and bloater bovine. More tryptophan was excreted in the urine by the bloater and it was excreted more rapidly than by the normal bovine.

The different metabolism of tryptophan in bloat is believed to have its effect through the tryptophan metabolite serotonin. Serotonin is a hormone involved in smooth muscle contraction, antidiuresis, depression of central ganglionic transmission and vasoconstriction. Barnes and Lowman (1968) showed that serotonin also protected mice against x-ray lethality.

Kuhn et al (1968) showed that sleep deprivation in humans led to a disturbance of tryptophan metabolism. An increased 5-hydroxy indoleacetic acid excretion in urine was explained by serotonin being bound in blood cells by ATP. Sleep deprivation led to a drop in level of ATP and subsequent release of serotonin from blood cells in the brain. On release, the serotonin was deaminated and then excreted as 5-hydroxy indoleacetic acid, considered to be a stress manifestation of sleep deprivation.

Two other tryptophan metabolites, 3-hydroxykynurenine and kynurenine, were found to be precursors of pigment in insect eyes (Fruton and Simmonds 1958). Mutagenic effects were produced by 3-hydroxykynurenine, along with 3-hydroxy anthranilic acid, which induced chromosomal rearrangements

in human primary embryonic tissue (Kuznetsova 1969).

The most thoroughly investigated metabolite of tryptophan in the animal body is nicotinamide. This B vitamin is an essential part of the enzyme system concerned with hydrogen transport in the living cell. Nicotinamide is the functional group of two coenzymes which act with flavoprotein enzymes in the electron transport chain.

General amino acid studies of ruminants

General reviews on the subject of nitrogen metabolism in the ruminant have been published by Moir (1957); Weller et al (1962) and Smith (1967). Less information is available concerning amino acid nutrition of the ruminant than the accumulated knowledge concerning other animals.

In the past, ruminants were thought to be independent of dietary sources of "essential" amino acids through the symbiotic relationship between the ruminant and rumen microorganisms. The assumption was made that much of the food nitrogen, whether of protein or non-protein nature, was synthesized by the bacteria into their own cellular protein; the protozoa utilized bacterial protein for growth; the ruminant then digested the protozoal and remaining bacterial protein. Black et al (1952 and 1955) provided evidence that the tissues of the cow synthesized only non essential (to the rat) amino acids. Therefore, the balance of amino acids absorbed from the gastrointestinal