A STUDY OF TRYPTOPHAN METABOLISM IN MAN

by

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Thesis

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INTRODUCTION

Tryptophan is one of the essential amino acids required by the mammalian body for protein anabolism. In recent years increasing interest has arisen in its intermediary metabolism. This is evidenced by the mass of literature which has appeared describing various aspects of its degradation. A number of pathways of tryptophan breakdown have been described and partially clarified, but only a portion of the total tryptophan intake by the mammalian body can be accounted for by the excretion of its known metabolites.

Numerous studies have been made on variations in the urinary excretion of individual metabolites or on the appearance in the urine of metabolites detected in the urine of normal subjects. The abnormality appears to be related to certain disease conditions. Inborn errors in tryptophan metabolism have also been discovered as a result of excretion of individual metabolites in excess of normal.

None of these studies have been concerned with any one pathway of tryptophan degradation by including determinations of a number of intermediary metabolites. The work reported herein is primarily concerned with a study of the normal blood and urine concentrations of tryptophan and several of its metabolites and the effect of oral administration of this amino acid on their concentrations.

REVIEW OF THE LITERATURE

Three pathways of tryptophan metabolism have been described. In essence they involve: (a) the modification of the sidechain of tryptophan resulting in the production of auxin and related substances; (b) the hydroxylation of the indole nucleus and modification of the sidechain giving rise to serotonin and related substances; (c) the oxidative cleavage of the indole ring and recycling to form either quinolines or pyridines.

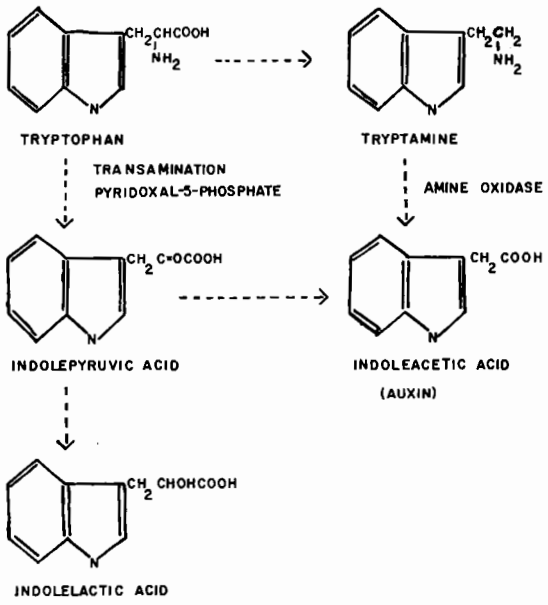
A. Tryptophan Metabolism Via the Auxin Pathway.

As illustrated in Figure 1, conversion of tryptophan to auxin (indoleacetic acid) could take place either by decarboxylation to tryptamine followed by oxidation to an aldehyde or by transamination to indolepyruvic acid with subsequent decarboxylation to an aldehyde. Oxidation of the aldehyde would then yield indoleacetic acid. Further degradation of indolepyruvic acid can give rise to indolelactic acid.

1. Tryptophan Degradation to Indoleacetic Acid.

Mehler (1) considers the pathway involving indolepyruvic acid the more probable one since decarboxylation of tryptophan in mammals has not been demonstrated. Dalgliesh (2) suggests 'that much of the indoleacetic acid (in human urine) is derived from bacterial degradation of tryptophan in the gut, and is then absorbed into the bloodstream and to a large extent conjugated with glycine in the liver'. Indoleaceturic acid is found only in freshly voided urine specimens. On standing, hydrolysis appears to take place and only free indoleacetic acid is found (3).

Most of the knowledge of tryptophan degradation to indoleacetic acid has been gained from data accumulated during the investigation of this pathway of metabolism in plants and moulds. Thimann (4) found that



TRYPTOPHAN METABOLISM - PATHWAY TO AUXIN

Figure 1.

the auxin formed by Rhizopus was indoleacetic acid and that the production of this plant hormone was dependent on the presence of tryptophan in the medium.

2. Oxidative Deamination of Tryptamine.

When tryptamine was administered to dogs there was a marked increase in indoleacetic acid excretion (5). As much as 84.6% of tryptamine administered to rats has been recovered as total (free and conjugated) indoleacetic acid in urine. Normally rat blood does not contain detectable amounts of auxin but after subcutaneous or intramuscular injection of tryptamine, indoleacetic acid can be demonstrated (6). In the mouse C^{14}-tryptamine studies revealed that the major degradation is catalyzed by monoamine oxidase resulting in the urinary excretion of labelled indoleacetic acid, chiefly as its glycine conjugate (7). Tryptamine is so suitable a substrate for monoamine oxidase that Sourkes et al (8) have made use of the changes in its absorption spectrum due to oxidation, as a basis for estimation of monoamine oxidase activity.

3. Excretion of Indoleacetic Acid and Other Indole Derivatives.

It has long been known that indoleacetic acid is excreted in the urine of man (9) (10). The excretion is increased after muscular exercise (11). Abnormal amounts of indoleacetic acid occur in urine of patients with phenylketonuria (12). However, the indole derivative present in the greatest concentration is indolelactic acid, as much as 20-150 mg./g. urinary creatinine being excreted. Normally indolelactic acid (13) or indolepyruvic acid (14) appear in urine only after administration of dl-tryptophan. Indolelactic acid is presumably formed, by reduction, from indolepyruvic acid (2).

Tryptophan Metabolism Via the Serotonin Pathway.

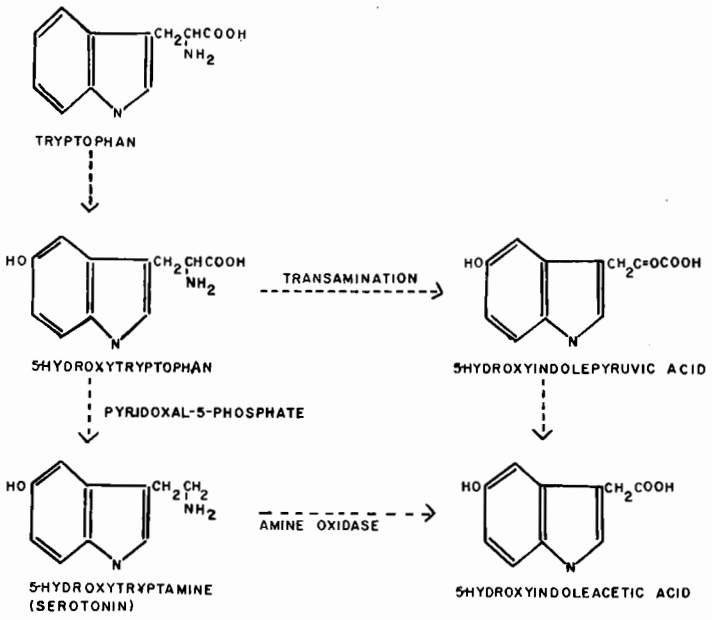
As illustrated in Figure 2 the first step in the degradation of tryptophan along this pathway is the hydroxylation of the indole nucleus. The 5-hydroxytryptophan thus formed may be decarboxylated to 5-hydroxytryptamine which is deaminated to 5-hydroxyindoleacetic acid. 5-hydroxytryptophan may also be degraded by transamination to 5-hydroxyindolepyruvic acid which is then decarboxylated to 5-hydroxyindoleacetic acid.

1. 5-hydroxytryptophan Formation.

No details are as yet available for the mechanism of the conversion of tryptophan to 5-hydroxytryptophan in mammals. Udenfriend and his co-workers (15) have demonstrated the conversion of tryptophan to 5-hydroxy-l-tryptophan by Chrombacterium violaceum. When they administered 2-C^{14}-dl-tryptophan orally to toads, they were able to extract from the venom glands labelled 5-hydroxytryptophan (16)

2. 5-hydroxytryptamine (Serotonin) Formation.

When C^{14}-5-hydroxytryptophan was administered to rabbits, C^{14}-serotonin was found in the body depots (17). Administration of 5-hydroxytryptophan resulted in the appearance in the brain of 5-hydroxytryptamine (18). Assuming that only the l-form of 5-hydroxytryptophan is metabolized, 20% of a load of this substance administered to dogs was recovered as 5-hydroxytryptamine in the urine (16). The enzyme catalyzing the decarboxylation to 5-hydroxytryptamine is highly specific for the l-form of 5-hydroxytryptophan and requires pyridoxine as a coenzyme (19). It occurs exclusively in the non-particulate fraction of cells (20). It is present in high concentration in kidney, liver and gastro-intestinal tissues and in low concentration in spleen, platelets and bone marrow.



TRYPTOPHAN METABOLISM - PATHWAY TO 5-HYDROXYINDOLES

Figure 2.

Since 5-hydroxytryptamine is a very potent vasoconstrictor, a great deal of attention has been directed to the investigation of its biosynthesis and distribution in the mammalian body. Rapport et al (21-26) have succeeded in isolating and identifying the vasoconstrictor principle as a creatinine-sulfate complex of 5-hydroxytryptamine. Enteramine (27)(28) and thrombocytin (29) have also been identified as 5-hydroxytryptamine. Enteramine is believed to be a true hormone regulating the flow of blood and lymph through the kidney by action on the afferent vascular bed of the glomerulus, causing spasm (27).

Several theories for the site of 5-hydroxytryptamine formation have been postulated. Erspamer's assumption that enteramine is produced by the enterochromaffin cell system is corroborated by the increased 5-hydroxytryptamine content in carcinoid tumors (30)(31) and in the blood of patients with malignant carcinoid (32). The ability of blood platelets, which are the source of serum serotonin (33)(34), to absorb 5-hydroxytryptamine from the surrounding medium (35) suggests that the 5-hydroxytryptamine found in them is not formed in situ but rather is accumulated by the platelets as they circulate through the tissues. Another hypothesis for the source of serotonin is that 5-hydroxytryptamine is synthesized and incorporated at the site of platelet formation (36) but this is not in accord with the low 5-hydroxytryptophan decarboxylase activity of bone marrow (20).

3. 5-hydroxytryptamine Degradation.

Catalysis by monoamine oxidase is considered the major pathway of degradation of 5-hydroxytryptamine in animals (39). When 5-hydroxytryptamine is incubated with kidney or liver homogenates, it is rapidly metabolized and approximately 30% can be accounted for as 5-hydroxyindoleacetic acid. After administration to dogs, 40% has been recovered in the urine as 5-hydroxyindoleacetic acid (37). Monoamine oxidase activity has been found to

vary in degree in different tissues (38). Semicarbazide will block the formation of 5-hydroxyindoleacetic acid suggesting that 5-hydroxyindole-acetaldehyde is an intermediary metabolite (37)(16). It has been suggested that the aldehyde, a highly reactive compound, may undergo other reactions in addition to oxidation to 5-hydroxyindoleacetic acid; for example it may well undergo oxidation and polymerization to form some of the melanin pigments (16).

4. 5-hydroxyindoleacetic Acid Formation.

5-hydroxyindoleacetic acid is a normal constituent of human urine, 2-8 mg. being excreted daily (40)(41). Patients with malignant carcinoid (argentaffinoma) excrete vastly increased amounts, 200-600 mg./24 hrs. (16) (31). Normally only about 1% of the dietary intake of tryptophan is accounted for by urinary 5-hydroxyindoles. In patients with malignant carcinoid tumors as much as 60% of the daily tryptophan intake is diverted to this pathway. There is a conversion from what is normally 'a minor pathway of tryptophan metabolism into a predominant route of metabolism' (32).

When 2-C^{14}-dl-tryptophan was administered orally to a patient with malignant carcinoid the excreted 5-hydroxyindoleacetic acid was found to be radioactive (31). Feeding large amounts of unlabelled amino acid to normal dogs produced a variable but slight increase in the excretion of 5-hydroxy-indoleacetic acid (16). Other indoles were administered to these animals and the excretion of 5-hydroxyindoles was studied. Tryptamine did not influence this excretion, evidence that it is not an intermediate in 5-hydroxyindole formation. Presuming that only the l-forms are metabolized, 30% of an administered dose of 5-hydroxytryptophan and 25-30% of an administered dose of 5-hydroxytryptamine were recovered as 5-hydroxyindoleacetic acid in urine (16). Intravenous administration of 5-hydroxyindoleacetic acid resulted in 50-75% being excreted in 1 hour; 5-hydroxytryptamine must be

rapidly metabolized since little if any appeared in the urine; 5-hydroxytryptophan resulted in a large proportion being slowly excreted in the urine as 5-hydroxyindoleacetic acid as well as 5-hydroxytryptamine. It has been suggested (16) that 5-hydroxytryptophan is taken up by the tissues and slowly released as 5-hydroxytryptamine which in turn gives rise to 5-hydroxyindoleacetic acid. The inability to demonstrate the presence of 5-hydroxytryptophan in mammalian tissue is possibly due to the presence of large amounts of decarboxylase. Apparently the rate limiting step in the conversion of tryptophan along this pathway is one of hydroxylation. If this step normally operates at a maximum, it would explain why an increased tryptophan intake shows little effect on 5-hydroxyindoleacetic acid excretion.

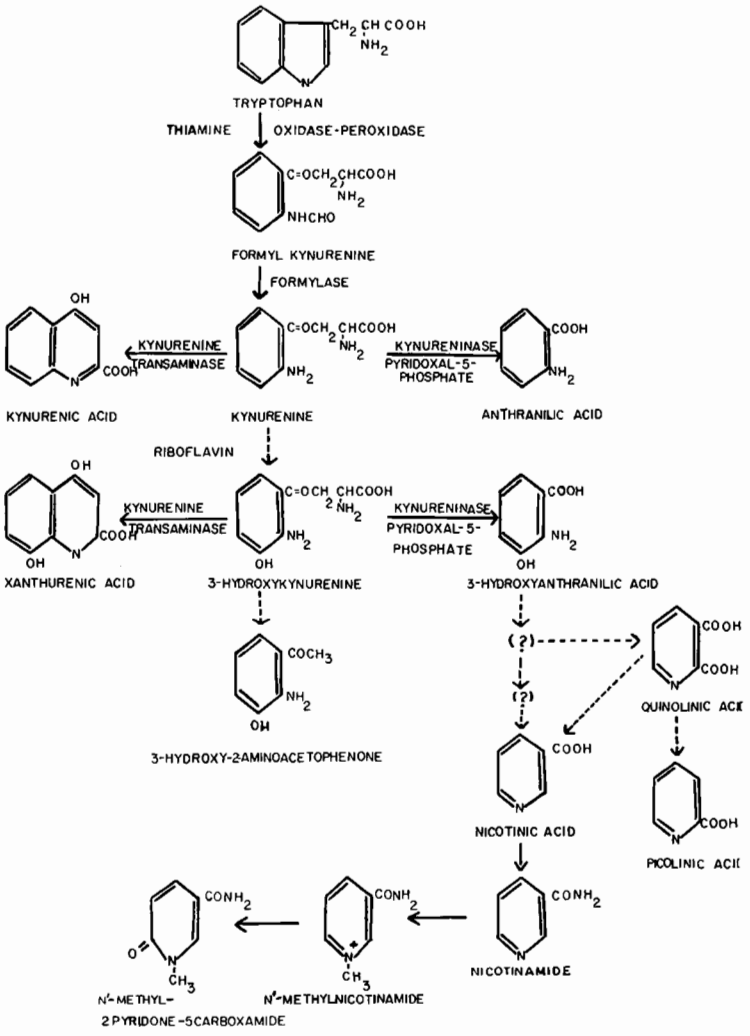
It has also been suggested that some 5-hydroxyindoleacetic acid may be derived by decarboxylation of 5-hydroxyindolepyruvic acid which may arise from 5-hydroxytryptophan by transamination (40).

5. Effect of Hormones.

The influence of the endocrines on this pathway of tryptophan metabolism has as yet not been extensively investigated. One report (42) has appeared on the effect of insulin and adrenaline on 5-hydroxytryptamine in the mammalian brain. When either hormone was injected subcutaneously into puppies of the same litter, no effect was seen on the 5-hydroxytryptamine content in the brain.

C. Tryptophan Metabolism Via the Nicotinic Acid Pathway.

As illustrated in Fig. 3 tryptophan metabolism along this pathway proceeds by the cleavage of the indole ring giving rise to formylkynurenine. Hydrolysis of the formyl group yields kynurenine. Kynurenine may then be degraded by transamination and recycling to kynurenic acid, by splitting of the sidechain to yield anthranilic acid, or by hydroxylation to 3-hydroxykynurenine. Breakdown of 3-hydroxykynurenine may proceed by transamination



TRYPTOPHAN METABOLISM – PATHWAY TO NICOTINIC ACID

Figure 3.

and recycling to xanthurenic acid, by hydrolysis of the sidechain to 3-hydroxyanthranilic acid, or by hydrolysis of the sidechain to 3-hydroxy-aminoacetophenone. It is postulated that 3-hydroxyanthranilic acid may give rise to an intermediate which may cycle to form quinolinic acid or which may be decarboxylated to a second intermediate which cycles to form nicotinic acid. Quinolinic acid may be decarboxylated to nicotinic acid or picolinic acid. Nicotinic acid is amidated to nicotinamide which is then methylated to form N'-methylnicotinamide. This methyl derivative is further oxidized to form N'-methyl-2pyridone-5 carboxamide.

1.Kynurenine Formation.

The first step in the metabolism of tryptophan via the oxidative cleavage of the indole ring is the formation of formylkynurenine. Knox, Mehler et al (43) have established that the liver contains a coupled peroxidase-oxidase enzyme system which is specific for l-tryptophan. If formylase is removed from the liver system oxidizing tryptophan, formyl-kynurenine accumulates. The formylase which catalyzes the second step along this pathway has also been isolated and partially purified (43). This formylase activates the hydrolysis of formylkynurenine giving rise to kynurenine. Heidelberger et al (44), using labelled tryptophan, established that, in the mammalian body, the β-C of tryptophan gives rise to the β-C of kynurenine. The tryptophan peroxidase-oxidase system is an adaptive one increasing after administration of tryptophan (45). Formylase is present in liver in considerable excess relative to tryptophan peroxidase-oxidase and therefore formylkynurenine is not normally found in tissues or excreted in urine.

2. Kynurenine Degradation.

There are three pathways for kynurenine degradation. Kynurenine is converted to kynurenic and anthranilic acids, both of which are end products

in mammalian metabolism. These are excreted in the free form or as conjugates of glycine, glucuronide or sulfate. The third pathway is the oxidation of kynurenine to 3-hydroxykynurenine.

(a) The β-C of tryptophan has been shown to give rise to the 3-C of kynurenic acid (44). Kynurenic acid is formed by the action of kynurenine transaminase (46) on kynurenine in the presence of ∝ keto-glutarate or pyruvate (47). Under optimal conditions for kynurenine transaminase activity, liver homogenates show only about 1/2 the activity of kidney preparations (48). Pyridoxal-5-phosphate is required for optimal activity. Experimental conditions which are optimal for ∝ ketoglutarate-kynurenine transamination are not optimal for pyruvate-kynurenine transamination (49).

(b) Kynurenine may be hydrolyzed by kynureninase to anthranilic acid and alanine (50). Kynureninase purified from Neurospora acts on l-kynurenine, 3-hydroxy-l-kynurenine and N-formyl-l-kynurenine. Magnesium ions increase the rate of the reaction (51). Liver is the primary site of kynureninase activity (52), which is not increased adaptively by administration of tryptophan or kynurenine. Kynureninase is a pyridoxal dependent enzyme (53). In pyridoxal deficiency the potential kynureninase activity is reduced by half (52). As a possible explanation of this phenomenon it has been suggested that a Schiff base is formed between pyridoxal phosphate and kynurenine (54); when there is a pyridoxine deficiency this reaction is limited.

(c) No enzyme system has yet been isolated which will oxidize kynurenine to 3-hydroxykynurenine. Dalgliesh (2) suggests that the hydroxylation stage of kynurenine metabolism is one of oxidative phosphorylation rather than simple oxidation.

3. Hydroxykynurenine Degradation.

Musajo et al (55) administered dl-kynurenine to rats and recovered 9 - 11% as xanthurenic acid in the urine. When equimolar amounts of 3-hydroxykynurenine were administered, 20 - 28% was accounted for as xanthurenic acid. 3-hydroxykynurenine forms xanthurenic acid by action of kynurenine transaminase. Although in vitro kynurenine transaminase requires pyridoxal phosphate, in vivo studies after administration of tryptophan first revealed xanthurenic acid excretion in the presence of pyridoxine deficiency (56).

Xanthurenic acid is considered an abnormal excretion metabolite of tryptophan although Kotake et al have reported finding minute amounts of conjugated xanthurenic acid in normal human urine (57). Xanthurenic acid is excreted after administration of a dose of tryptophan (56) or in pyridoxine deficiency with a tryptophan-rich diet. Xanthurenic acid may be excreted in the free form or conjugated, probably as its glucuronide or o-sulfate (58). It may also be further degraded by xanthurenicase in the liver, possibly giving rise to a phenolic derivative by scission of the pyridine ring (59).

3-hydroxykynurenine is hydrolized to 3-hydroxyanthranilic acid by the action of kynureninase (51) which also catalyzes the scission of the sidechain in the formation of anthranilic acid from kynurenine. The 3-C of tryptophan gives rise to the carboxyl C of 3-hydroxyanthranilic acid (44). Pyridoxal phosphate is required as a coenzyme (60).

At this stage a small amount of 3-hydroxy-2 aminoacetophenone is believed to be formed by the hydrolytic scission of the sidechain of 3-hydroxykynurenine. This product is excreted as a phenolic sulfate (60).

4. Hydroxyanthranilic Acid Degradation.

The mechanism of conversion of 3-hydroxyanthranilic acid to nicotinic acid has not been completely clarified. Henderson et al (61), on feeding tryptophan to rats, found an increased excretion of quinolinic acid and concluded that it was formed from tryptophan. Similarly, administration of tryptophan to man (62) resulted in increased excretion of nicotinic acid, methylnicotinamide and quinolinic acid. The last was present in the greatest amount. 3-hydroxyanthranilic acid was established as an intermediate before quinolate formation by this group as well as by Schweigert et al (63). The hypothesis set forth was that the benzene ring is split by enzymatic oxidation and spontaneous cycling gives rise to quinolate, while another, less active enzyme converts this to picolinic acid (64). Dalgliesh (2) proposes that 3-hydroxyanthranilic acid is catalyzed by "enzyme A" to form "intermediate A" which, on the one hand, spontaneously cycles giving rise to quinolinic acid, and on the other hand, catalyzed by "enzyme B", is decarboxylated giving rise to "intermediate B", which spontaneously cycles to form nicotinic acid. Quinolinic acid may also give rise to nicotinic or picolinic acid by decarboxylation.

5. Nicotinic Acid Degradation.

Metabolism of nicotinic acid proceeds via N'-methylation in carnivorous and omnivorous species (65). Heidelberger et al (44) established that, in the rat, tryptophan is converted to nicotinic acid. The 3-C of tryptophan becomes the C of the carboxyl group. The nicotinic acid is excreted chiefly as its N'-methyl derivative. At first trigonelline (N'-methylnicotinic acid) was believed to be formed by man as a derivative of nicotinic acid (66). Later it was shown that trigonelline is not an

excretory metabolite of nicotinic acid (67). For a time N'-methyl-nicotinamide was the only metabolite estimated as an end product of nicotinic acid (68) (69). However excretion of nicotinic acid and N'-methylnicotinamide accounted for only 30-35% of administered nicotinic acid. Knox and Grossman (70) found a new metabolite of nicotinamide, the 6-pyridone or N'-methyl-2 pyridone-5 carboxamide. The ratio of excretion of N'-methylnicotinamide to pyridone was found to be 1:1.37 (65). Only recently has a comparatively simple and specific method been developed for determination of this metabolite (71) and Price et al (72) have confirmed that N'-methyl-2 pyridone- 5 carboxamide is indeed an important excretion product of tryptophan metabolism.

Judging by excretion of nicotinic acid and its derivatives, Goldsmith et al (73) found that 55 mg. of tryptophan will replace 1 mg. of nicotinic acid in the dietary requirements of man. Snyderman et al (74) investigated the site of tryptophan conversion to nicotinic acid in man to answer the question whether or not intestinal flora were responsible for nicotinic acid biosynthesis. They found the rate of excretion of nicotinic acid derivatives by infants given 1 g. tryptophan to be the same whether the administration was by the oral route or by slow intravenous drip. Studies, with the aid of isotopes, of the conversion of nicotinic acid to N'-methylnicotinamide in the rat, showed that little N'-methylnicotinamide was derived from other sources. Under the conditions of the experiment, 95.7% of the radioactive C was recovered (75). Earlier experiments by Huff and Perlzwieg (76) revealed that N'-methylnicotinamide was the end product of metabolism of nicotinic acid or its amide rather than trigonelline (N-methylnicotinic acid) (66).

Huff and Perlzwieg found that the liver was the site of methylation in the rat (77). Coulson et al (78) investigated factors affecting methylation of nicotinic acid in newborn babies and found that though N'-methylnicotinamide excretion is increased tenfold after administration of nicotinamide, the added administration of methionine was not followed by any alteration in the excretion of the methylated product. They also found that excretion of N'-methylnicotinamide, after ingestion of nicotinamide by the newborn, is equivalent to that of adults given the same doses. They therefore concluded that the absolute weight of the liver is not a factor in methylation.

Price et al (72) investigated the conversion of potential precursors to N'-methyl-2 pyridone-5 carboxamide and found that 80% of the physiological doses of nicotinic acid or its amide, administered to man, could be accounted for by the excreted pyridone. Judging by the percentage of pyridone excreted after administration of the potential precursors, it would appear that conversion of nicotinic acid takes place first by amidation, second by methylation, and third by oxidation.

6. Metabolism of d-forms.

Most enzyme studies have been carried out with the l-forms of tryptophan and its metabolites. The d-forms are metabolized to a much lesser extent. Kidney slices will degrade d-tryptophan giving rise to indolepyruvic acid (53). d-tryptophan administered to rats is recovered as unmetabolized tryptophan and indolepyruvic acid. Small amounts of kynurenic acid are formed from d-kynurenine by kidney slices (48), but administered d-kynurenine is apparently not metabolized in vivo (53). Anthranilic acid is produced from l-tryptophan and l-kynurenine only. Kynureninase is highly specific for the l-form of kynurenine (51).

Langer and Volkmann (79) studied the fate of ingested acetyl-l and -d-tryptophan in human subjects. They found that approximately 75% of the administered acetyl-d-tryptophan was excreted in the feces while only 5 - 10% of the administered acetyl-l-tryptophan was thus recovered. Purified acylase preparations from liver and kidney of various animals was found to be specific for the l-form. Kallio and Berg (80) administered d and l-tryptophan and d and l-acetyltryptophan to rats and measured 24 hour urinary N'-methylnicotinamide excretions. They found that acetylation retarded niacin formation from the l-tryptophan and completely blocked it from the d-form. The increment in the N'-methylnicotinamide excretion following d-tryptophan was two thirds that following l-tryptophan. However in man, Sarett and Goldsmith (81) found that only the l-lsomer of tryptophan was effective as a nicotinic acid precursor.

7. Role of B Vitamins.

(a) Pyridoxine.

The pathway of tryptophan metabolism resulting in the formation of nicotinic acid, a member of the complex of B vitamins, is greatly influenced by other vitamins of this complex. For instance, patients receiving daily injections of desoxypyridoxine showed a lower excretion of N'-methylnicotinamide. After a tryptophan load test, they excreted increased amounts of xanthurenic acid. This response was depressed by administration of pyridoxine (82). Increased xanthurenic acid excretion after tryptophan will appear long before clinical symptoms of pyridoxine deficiency are manifested (83). Snyderman et al (84) increased urinary excretion of N'-methylnicotinamide two to three times in infants by intravenous administration of 1 g. l-tryptophan. When pyridoxine deficiency was induced there was no conversion. The ability to convert tryptophan to

N'-methylnicotinamide disappears long before clinical symptoms of B_6 deficiency appear and lags behind institution of therapy and clinical remission (84).

One of the first observations of abnormality in tryptophan metabolism was made when xanthurenic acid was found to be excreted by pyridoxine deficient rats fed tryptophan (56). No xanthurenic acid was found in normal human urine until tryptophan was administered. With pyridoxine deficiency this excretion was markedly increased. Treatment with pyridoxine resulted in a decided decrease of xanthurenic acid excretion after tryptophan (85)(86). Wachstein and Guaditis (87) found large amounts of xanthurenic acid to be excreted by pregnant women after a test dose of tryptophan. Here again administration of a large dose of pyridoxine completely suppressed the abnormal xanthurenic acid excretion. Similar observations were made by Zartman et al (88) who used xanthurenic acid excretions as an indirect measure of pyridoxine deficiency. Further studies on man (89) showed that the amount of xanthurenic acid excreted by diabetic subjects was significantly greater than that excreted by normal subjects. When pyridoxine was administered to the diabetics the xanthurenic acid excretion was reduced.

Henderson et al (90) found pyridoxine deficiency to result in a marked reduction in the conversion of 3-hydroxykynurenine to quinolate. Experiments with rats led Dalgliesh to state that pyridoxine is concerned at the kynurenine to anthranilic or 3-hydroxykynurenine to 3-hydroxyanthranilic conversion site. Henderson et al (91) concluded that 'lack of pyridoxine impairs cleavage of the side chain'.

(b) Riboflavin

In an early experiment to determine whether rats, deficient in vitamins other than pyridoxine, were able to convert tryptophan to nicotinic acid, Junquierra and Schweigert (92) found that there was a reduction in excretion of nicotinic acid and N'-methylnicotinamide when caloric intake was restricted, as well as in pantothenic acid, thiamine and riboflavin deficiencies. The authors suggested that the restriction of caloric intake resulted in reduction of effective enzyme concentrations in the tissues which in turn was reflected in the decreased excretion of metabolites. Since these experiments the importance of the B-complex vitamins in tryptophan metabolism has been more definitely established.

Porter et al (93) found that when tryptophan was administered to normal rats, approximately 17% appeared in the urine as kynurenine, kynurenic acid, and xanthurenic acid. In riboflavin deficiency the proportion of the dose appearing as these metabolites increased. The increase was present chiefly as kynurenic acid, which overbalanced a decrease in xanthurenic acid excretion. Henderson et al (94) found a marked reduction in quinolinic acid excretion by riboflavin deficient rats when fed tryptophan and only a moderate reduction in quinolate excretion by these rats when fed kynurenine, and no reduction after administration of 3-hydroxyanthranilic acid. The reductions were more striking in pyridoxine deficient rats fed tryptophan or kynurenine, but the excretion was not influenced after a dose of 3-hydroxyanthranilic acid. An increased excretion of anthranilic acid was found to occur in the urine of a child with congenital hypoplastic anemia (95). Administration of riboflavin decreased anthranilic acid excretion in this and similar cases.

Administration of tryptophan to riboflavin deficient rats resulted in considerable excretion of anthranilic acid and its conjugates (96). Since riboflavin deficiency results in increased excretion of anthranilic and kynurenic acids and decreased excretion of quinolinic and nicotinic acids it would appear that the site of action of riboflavin is at the hydroxylation of kynurenine. In support of this theory, Henderson et al (90)(91) found that riboflavin deficiency reduced quinolinic acid conversion from tryptophan to one tenth and had no effect on quinolate excretion after 3-hydroxykynurenine. Charconnet-Harding et al (97) suggests that the normal oxidation of kynurenine is one of oxidative phosphorylation and that riboflavin is concerned with phosphorylation rather than the oxidation.

(c) Biotin.

As yet biotin has not been directly linked with tryptophan metabolism. Dalgliesh fed rats tryptophan and found no difference in the pattern of excreted metabolites between control and biotin deficient animals (98)(99).

(d) Thiamine.

From his experiments, Henderson (94) concluded that the reduction of the conversion of tryptophan to quinolinic acid during thiamine deficiency appeared to be largely due to inanition. Dalgliesh (99) found that as thiamine deficiency became established, the excretion of tryptophan metabolites by tryptophan-fed rats ceased. When thiamine deficiency was imposed on pyridoxine deficiency, the metabolite excretory pattern typical of B_6 deficiency was almost abolished. The author concluded that the formation of substrate for kynureninase reaction was inhibited. When kynurenine was fed at this point, there was a marked

excretion of metabolites. No formylkynurenine was excreted when tryptophan was administered to rats with simple thiamine deficiency. The author therefore proposed that thiamine is concerend in the conversion of tryptophan to formylkynurenine.

8. Carbohydrates.

Albanese et al (100) found that the administration of glucose resulted in a transient fall in the level of total urinary tryptophan as well as aminonitrogen and total nitrogen in adults and infants. Munro et al (101) however, found that although glucose and fat lowered the plasma levels of aminoacid nitrogen, they did not decrease urinary excretion of tryptophan.

When adrenalectomized rats were fed tryptophan or anthranilic acid, their lifespan was prolonged from 4 - 5 days to 20 - 27 days. Oral administration of nicotinic acid did not prolong the survival time of these animals. Tryptophan and anthranilic acid were found to assist in maintaining the liver glycogen content at near normal levels (102). Xanthurenic acid has also been shown to have a pronounced effect on glucose metabolism (103). Administration of xanthurenic acid first results in a period of hyperglycemia with an accompanying decrease in liver and skeletal muscle glycogen. This is followed by a hypoglycemia period with somewhat increased liver and heart glycogen but continuing decrease in skeletal muscle. Then a second period of hyperglycemia follows with a decreased liver and skeletal muscle glycogen and increased heart glycogen. Mirsky et al (104) reported a hypoglycemic response resulted after oral administration of l-tryptophan to rats. One hour after indoleacetic acid was administered there was a significant hypoglycemia produced. A similar response occurred two hours after administration of nicotinic acid and a lesser response two hours after anthranilic acid. Studies made with I^{131}-insulin on mice and rat liver

extracts have led the authors to suggest that the hypoglycemic action of nicotinic acid is related to decreased activity of insulinase while that of auxin is related to the release of insulin from the pancreas.

9. Endocrine - Tryptophan Relationships.

Little work has been done on the possible action of hormones on tryptophan metabolism. Ichiharo et al (105) studied liver homogenates from thyroidectomized and sham-operated rabbits. After thyroidectomy there was a decrease in kynurenine transaminase activity as well as a decrease in kynurenine formation from tryptophan and in anthranilic acid formation from kynurenine. Addition of ATP, α ketoglutarate and pyridoxal phosphate, singly or combined, resulted in increased activity of the kynurenine transaminase but did not elevate the decreased kynurenine and anthranilic acid formation.

Wachstein and co-workers (106) have demonstrated that 3-hydroxykynurenine, acetyl-3-hydroxykynurenine and xanthurenic acid appear in urine of patients with hyperthyroidism after administration of 10 g. dl-tryptophan. Oral administration of pyridoxine hydrochloride before the test dose of tryptophan suppressed the excretion of abnormal metabolites. The authors suggest that tryptophan metabolism is disturbed by a B_6 deficiency due to the disease.

Rosen et al (89) found that the amount of xanthurenic acid excreted by diabetic subjects after a load of tryptophan was significantly greater than that excreted by normal subjects. Administration of pyridoxine reduced the xanthurenic acid excretion in the diabetic patients. Kotake et al (57) also found increased excretion of xanthurenic acid in rats by feeding sodium butyrate and tryptophan (107). The excretion of xanthurenic acid was decreased by insulin injection (108), which also resulted in an increased excretion of anthranilic acid and kynurenic acid.

The authors concluded that insulin has the ability to regulate tryptophan metabolism.

It has been shown that potential kynureninase activity is reduced by half in pyridoxine deficiency (52). It is, therefore, conceivable that the disturbance in tryptophan metabolism due to altered hormonal states, can be overcome to some extent by flooding the system with pyridoxine. On the other hand, it may be that utilization of available pyridoxine is impaired by disorders of endocrine function and therefore massive doses of pyridoxine or administration of the necessary hormone allows for the normalization of tryptophan metabolism. A great deal of work will have to be done before this problem is unravelled.

Another site at which insulin may have an effect is at the conversion to nicotinic acid. The normal increase in N'-methylnicotinamide excretion after a tryptophan load is reduced in alloxan diabetic rats (109). Administration of insulin returned the N'-methylnicotinamide excretion to normal levels. After feeding 3-hydroxyanthranilic acid this excretion was also decreased in diabetic animals. The livers of these rats have been shown to have an increased concentration of the enzyme which converts the oxidation product of 3-hydroxyanthranilic acid to picolinic acid, while the tryptophan and 3-hydroxyanthranilic acid oxidizing systems remain unchanged (110). Treatment with insulin decreased the level of the enzyme catalyzing picolinic acid formation. It is, therefore, suggested that the decreased nicotinic acid production is due to an increase of 3-hydroxyanthranilic acid breakdown through the picolinic acid pathway.

EXPERIMENTAL WORK

A. Introduction.

Insulin plays an important role in carbohydrate metabolism. The effect of insulin on protein metabolism is not as clear cut. In the absence of insulin, the upset in protein metabolism may be secondary to the disturbance in carbohydrate metabolism. Such a hypothesis, however, does not account for all the observed changes in protein metabolism after insulin administration.

Insulin is known to lower blood plasma αamino nitrogen levels, to decrease urinary amino acid excretion and to increase the conversion of amino acids into tissue proteins. More specifically, insulin has been involved in the metabolism of tryptophan (See pages 22 & 23).

The ultimate objective of this investigation is a study of tryptophan metabolism in diabetes. However it was necessary first to establish the pattern of tryptophan metabolism in normal man, on which to base such a study. Accordingly, experiments were set up to follow the degradation of tryptophan along what appeared to be the major pathway of metabolism, namely that of niacin formation. The choice of this pathway for investigation was also influenced by the fact that insulin had already been implicated in its regulation.

B. Subjects.

The subjects used for these experiments were young men and women of the laboratory personnel who were in good health. Seven-day dietary histories were collected on all subjects and analyzed (153)(154)*(155). Only those subjects, whose food intake of the B vitamins met the requirements of the Canadian Recommended Standards, were included in this study. Experiments on the female subjects were arranged to take place approximately two weeks after onset of their last menses. This was done in an attempt to

* Diet analyses made by Miss J. Billette.

avoid possible variations due to metabolic changes during different periods of the menstrual cycle.

C. Analytical Methods.

1. Handling of Specimens.

The volume of all urine specimens was measured. Rinsings of the specimen collection bottles were used to dilute the urine volume to a rounded figure. The urines were filtered and aliquots taken for estimation of tryptophan, kynurenine, and anthranilic acid. The urines were then acidified and aliquots taken for α amino nitrogen estimations. Large aliquots were stored in the deep freeze at -7°C for further investigation at a later date.

The blood specimens were allowed to clot at room temperature and the serum removed after centrifuging at 3000 rpm. for ten minutes. Aliquots of serum were then removed for tryptophan, kynurenine, anthranilic acid and α amino nitrogen estimations. The remainder of the serum was also stored in the deep freeze for further investigation.

The aliquots of serum and urine were immediately treated with trichloracetic acid or sodium tungstate and sulfuric acid and the resulting protein-free filtrates stored in the refrigerator overnight. All α amino nitrogen, tryptophan, kynurenine and anthranilic acid estimations were made the following day.

With the thought in mind that diabetics, who would eventually be the subjects investigated, might have proteinuria, all methods to be used had to be applicable to protein-free filtrates. The following methods were investigated and set up for routine use in these experiments.

2. ∝Amino Nitrogen Estimations in Serum.

Folin (111) developed a method for the estimation of ∝ amino nitrogen using β-naphthoquinone-sulfonic acid. A red color is produced with amino acid nitrogen and ammonia due to the o-quinone group. This procedure was later modified by Frame et al (112) and Russell (113).

The last authors found no interference produced by amide-N, peptide-N, tertiary amines, unsaturated pyrimidine, pyridine, imidazole, thiazole, guanidino group, urea, creatinine, and amino-purines. Primary aromatic amines yield as much color as amino acids. Uric acid and allantoin yield a red color in acid solution. Tungstate acid filtrates were found to give the best results.

To test this method, it was applied to serum and the color produced was found to obey Beer's Law in the range of 8 - 30 µg. of ∝amino nitrogen. Recoveries of ∝amino nitrogen from serum ranged from 98 - 106%. (Table 1).

TABLE 1

Recoveries of ∝Amino Nitrogen Added to Serum.

Original ∝ AN (mg. %)	Added ∝ AN (mg. %)	Recovered ∝AN (mg. %)	% Recovery
4.0 (serum 1)	9.3	13.3	100
	5.6	9.7	101
	4.7	9.2	106
	1.9	6.2	105
5.6 (serum 2)	9.3	15.0	100.6
	5.6	11.0	98
	4.7	10.1	98
	1.9	7.6	98.7

3. α Amino Nitrogen Estimation in Urine.

Cagan et al (114) adapted the Frame-Russell method for the determination of urinary α amino nitrogen. Attempts to apply this procedure to protein-free filtrates resulted in clouding. This was probably due to precipitation of sulfur from the thiosulfate bleaching agent used to eliminate excess quinone reagent. Addition of duponal, gum acacia, cetyl alcohol or gum ghatti did not eliminate the turbidity. To eliminate the possibility that the clouding was due to impurities in the quinone reagent, the β naphthoquinone-sulfonic acid was repurified according to Folin (115). However, clouding persisted and another method for estimating urinary amino nitrogen was adopted.

The purple color produced when α amino groups react with ninhydrin to form diketohydrindylidene-dikethydrindamine (DYDA) can be used as a quantitative measure for amino acids. Moore and Stein (116) first applied this principle to estimate amino acids, separated by column chromatography from protein hydrolysates. The Troll and Cannan modification (117) avoids the use of stannous chloride as a reducing agent and eliminates the necessity of storing ninhydrin reagent under nitrogen. Yemm and Cocking (118)(119) further simplified the procedure by the use of an organic solvent to accelerate color development. The authors found that most amino acids produce colors equivalent to 97 - 102% of pure DYDA on an equimolar basis. The exceptions were tryptophan (83%), lysine (108%), tyrosine and phenylalanine (89%).

When this method was tested, standard curves obtained with glutamic acid and glycine were found to be almost identical and to obey Beer's Law in the range of 1 - 5 μg. α amino nitrogen/ml. aliquot. This method was applied to tungstate protein-free filtrates of diluted urine (1:10) and recoveries of glutamic acid ranged from 96 - 101% (Table 2).

TABLE 2

Recoveries of Glutamic Acid ∝Amino Nitrogen Added to Urine

Original ∝AN (µg.)	Added ∝AN (µg.)	Recovered ∝ AN (µg.)	% Recovery
5.41 (urine 1)	1.0	6.31	98.4
	5.0	10.4	100.
	10.0	14.9	96.9
3.45 (urine 2)	1.0	4.43	99.5
	2.5	5.87	98.7
	4.0	7.52	100.7
	5.0	8.47	100.2

Recoveries of Tryptophan ∝ Amino Nitrogen Added to Urine.

Original ∝AN (µg.)	Added ∝AN (µg.)	Recovered ∝ AN (µg.)	% Recovery
1.68 (urine 3)	1.82	2.81	80.3
	3.65	4.15	77.9

∝amino nitrogen values obtained by this method from normal sera were consistently lower than the normal range estimated by other methods quoted in the literature. Therefore, this method was not used for the serum ∝amino nitrogen determinations.

4. Estimation of Tryptophan, Kynurenine and Anthranilic Acid.

Eckert (120) developed a colorimetric method for estimating tryptophan without the necessity of previously removing other amino acids. Tryptophan undergoes diazotization with sodium nitrite and coupling with N-(1-naphthyl) ethylenediamine. Spies (121) found no interference with diazotization by such non-related substances as glucose and fructose. Mason and Berg (53) applied this principle to the determination of kynurenine and anthranilic acid which also form diazonium salts. They found no interference from other tryptophan metabolites such as kynurenic acid, xanthurenic acid or indolepyruvic acid. Ichihara et al (105) further modified this method, introducing temperature control, in applying it to liver enzyme extracts. 3-hydroxy-kynurenine and 3-hydroxyanthranilic acid

were found not to interfere in these estimations.

With a slight modification this method was applied to trichloracetic acid protein-free supernatants of urine and serum. Since the concentration of material to be measured in serum is low, 2 ml. of supernatant were used instead of 1 ml. as in urine. The total volume was kept constant by reducing the amount of ammonium sulfate,used to eliminate excess oxidizing reagent, from 4 ml. to 3 ml. in tryptophan estimations or by reducing the volume of water used for dilution, from5ml. to 4 ml. in kynurenine and anthranilic acid estimations.

For each metabolite standard solutions and reagent blanks were treated as serum or urine. Standard curves were found to obey Beer's Law in the range of 10 - 250 µg. for tryptophan, 50 - 105 µg. for kynurenine and 10 - 50 µg. for anthranilic acid. Recoveries of tryptophan from urine and serum ranged from 93 - 104%; of anthranilic acid from urine 93 - 99%; and of kynurenine from urine 92 - 99% as shown in Table 3.

Attempts were made to store specimens in the deep freeze. Recoveries of tryptophan, kynurenine and anthranilic acid from urines stored from one to four months, ranged from 74 - 98% for tryptophan, 84 - 136% for kynurenine and 91 - 166% for anthranilic acid. Such a scatter made it impossible to store specimens. All estimations were made on fresh specimens.

Details of method:

(1) 2 ml. urine, serum, standard solution or water (for blank). Add 8 ml. 10% trichloracetic acid and mix vigorously. Centrifuge and decant clear supernatant.

(2) Reagents are added according to the following schedule;

	Anthranilic Acid		Kynurenine (plus Anthranilic Acid		Tryptophan (plus Anthranilic Acid plus Kynurenine)	
	Urine	Serum	Urine	Serum	Urine	Serum
Test solution	1 ml.	2 ml.	1 ml.	2 ml.	1 ml.	2 ml.
HCL	1 ml. (0.2 N.)	1 ml.	1 ml. (0.2 N.)	1 ml.	1 ml. (2 N.)	1 ml.
$NaNO_2$	1 ml. (0.1%)	1 ml.	1 ml. (0.1%)	1 ml.	1 ml. (1%)	1 ml.
Time	3 minutes at room temperature				30 min. at 30°C.	
$NH_4SO_3NH_2$ (4%)	1 ml.	1 ml.	1 ml.	1 ml.	4 ml.	3 ml.
Time	1 hr. @ 37°C.		3 min. @ room temperature		10 min. at 30°C.	
H_2O	5 ml.	4 ml.	5 ml.	4 ml.	---	----
0.1% N-(1-naphthyl)-diethylene dihydrochloride						
	1 ml.	1 ml.	1 ml.	1 ml.	3 ml.	3 ml.
Time	1 hr. @ 30°C.		1 hr. @ 30°C.		30 min. @ 30°C.	

(3) Read in the Beckman DU spectrophotometer at 560 mμ against a reagent blank.

The optical density (OD) of tryptophan is obtained by subtracting from the OD of the three substances that of kynurenine plus anthranilic acid. Similarly, the OD of anthranilic acid is subtracted from the kynurenine - anthranilic OD to give the OD of kynurenine. The recoveries of these substances are shown in Table 3.

TABLE 3

Original Content (µg.)	Added Standard (µg.)	Total Expected (µg.)	Total Recovered (µg.)	% Recovery
Recoveries of Tryptophan Added to Urine				
55.5 (urine 4)	10.9	66.4	62.2	93.8
	17.5	73.0	73.2	100.2
	21.9	77.4	77.7	100.3
	40.0	95.5	95.5	100.0
	43.7	99.2	95.5	96.4
23.1 (urine 5)	13.95	37.05	37.2	100.3
	27.90	51.00	50.9	99.8
	41.86	64.96	65.2	101.0
	55.81	78.91	81.8	104.0
	83.72	106.82	104.5	97.8
Recoveries of Tryptophan Added to Serum				
21.85 (serum 3)	139.5	161.4	163.5	101.3
	279.4	301.3	303.0	100.8
	418.6	440.4	427.5	97.0
Recoveries of Anthranilic Acid Added to Urine				
7.16 (urine 6)	11.28	18.44	18.15	98.4
	22.56	29.72	28.58	95.4
	45.12	52.28	48.7	93.3
4.67 (urine 7)	11.28	15.95	15.7	98.4
			15.2	95.4
			15.8	99.0
4.07 (urine 8)	11.28	15.35	14.35	93.8
Recoveries of Kynurenine added to Urine				
7.83 (urine 9)	44.8	52.63	51.8	98.6
	89.6	97.43	91.8	94.2
16.6 (urine 10)	19.9	36.5	33.4	91.6
14.3 (urine 11)	16.8	31.1	28.7	92.3
	33.6	47.9	45.2	94.4
	50.4	64.7	59.5	92.0

5. Estimation of Xanthurenic Acid

The formation of a green color-complex between xanthurenic acid and ferric salts was the basis of a method of xanthurenic acid measurement described by Rosen et al (122). This was modified by Glazer et al (123) who added ferric alum in alkaline medium to urine to form the green color specific for xanthurenic acid.

The amount of this acid normally excreted in urine is low, except after administration of tryptophan. However, the authors found that unidentified chromogenic metabolites in urine produce a brown color which gave false optical density (OD) readings.

The method of Glazer was adapted for use with trichloracetic acid protein-free urine filtrates. The interfering chromogens were diluted but not eliminated. Comparison of the color complex produced by chromogens in two hour urine specimens on one day in any individual, and in two hour specimens on different days, showed no statistically significant difference ($p > 0.1$). The specimens obtained on a normal day were used as urine blanks. Their OD readings were subtracted from the OD readings obtained with specimens of comparable periods from the same individuals after administration of tryptophan.

The proportion of carbonate mixture used was increased to neutralize the acid protein-free filtrate. Ferric hydroxide which formed during the procedure, resulting in turbidity, was eliminated by centrifugation.

The standard curve obtained with this modified method does not obey Beer's Law. However recoveries of xanthurenic acid from urine, calculated from the plotted curve were in the range of 95 - 108%, for concentrations of 17 - 308 µg. (Table 4).

Xanthurenic acid was added to urine specimens and these were stored for one month in the deep freeze. Recoveries were increased 3 - 4%. All xanthurenic acid determinations were carried out on specimens stored for less than one month.

Details of Method:

(1) 5 ml. trichloracetic acid protein-free filtrate (prepared as for the tryptophan estimations).

(2) Add 3 ml. carbonate mixture (saturated $NaHCO_3$: saturated Na_2CO_3 - 7:3) Mix well.

(3) Add 0.1 ml. 1.7% $FeNH_4(SO_4)_2.12H_2O$ and mix.

(4) Add 1 drop phenolphthalien. If pink color appears, add N.HCl drop by drop, shaking vigorously after each addition till pink just disappears.

(5) Dilute to 10 ml. with water.

(6) Centrifuge at 3500 - 4000 rpm. for ten minutes.

(7) Read supernatant immediately (color begins to fade after fifteen minutes) in the Beckman DU spectrophotometer at 620 mμ against a reagent blank.

TABLE 4

Recoveries of Xanthurenic Acid Added to Urine

Added Xanthurenic Acid (μg.)	Recovered Xanthurenic Acid (from standard graph) (μg.)	% Recoveries
17 (urine 12)	17	100
31	31	100
51	50	98
85	83	97.6
102	97	95
127	137	107.9
308	307	99.7

6. Estimation of N'-methylnicotinamide.

N'-methylnicotinamide condenses with acetone in alkaline medium producing a highly fluorescent derivative (124). Huff and Perlzweig (125) developed a method for estimating N'-methylnicotinamide in urine based on this principle. The authors found that in pure solution the fluorescence of the acetone compound is a linear function of N'-methylnicotinamide for amounts from 0.2 to 2.5 µg. Similar results were obtained from urine except that absolute galvonometer readings varied from specimen to specimen, making the use of an internal standard for each specimen a necessity.

The authors found that no fluorescing derivatives were formed with acetone by urea, creatine, caffeine, uric acid, aspirin, salicylic acid, ammonia, nicotine, thiamine, trigonelline, nicotinic acid, nicotinamide, nicotinuric acid or coramine. Riboflavin results in a fluorescence which is chiefly removed by the charcoal and corrected for by the urine blank. The oxidative metabolite of pyridoxine, 4 pyridoxic acid, which is normally present in small amounts gives rise to a fluorescing lactone on heating with acid and is corrected by the blank.

When this method was tested,both pure solutions of N'-methylnicotinamide and urine containing added N'-methylnicotinamide were found to yield fluorescence which obeyed Beer's Law. However, the fluorescence was not found to be stable. The most reproducible results were obtained if readings were made on the Coleman Electronic fluorometer within one to one and a half hours. It was also found that urine filtrates, obtained after treatment with charcoal to remove fluorescing constituents of urine, could not be stored in the refrigerator overnight. The results obtained with these filtrates were lower than with freshly prepared ones.

Recoveries of N'-methylnicotinamide added to urine ranged from 94 - 104%, for concentrations of 4 to 40 µg/ml. as seen in Table 5.

TABLE 5

Recoveries of N'-methylnicotinamide Added to Urine

Original Content (µg)	Added N'-methyl-nicotinamide (µg)	Recovered N'-methylnicotin-amide (µg)	% Recovery
4 (urine 13)	4	8.25	103.1
	12	16.25	101.6
	20	23.5	97.9
	32	34	94.4
	40	44.5	101.1
5 (urine 14)	20	25	100
	32	35.75	96.6
0.24 (urine 15)	4	4.16	98.1
	20	20.24	100
	32	32.24	100
	40	41.6	103.4

N'-methylnicotinamide was added to urine specimens and these were stored in the deep freeze for one month. Recoveries ranged from 83 - 91%. With such a great loss due to storage, estimations should be made on fresh specimens. The determinations of N'-methylnicotinamide were made on specimens which had been stored one to four months and therefore were not a true picture of excretion of this metabolite in the following experiments. The results obtained were at least 10 - 20% lower than the actual values.

D. EXPERIMENT 1

1. Purpose.

A study of tryptophan metabolism in normal man was undertaken to establish blood concentration and urinary excretion patterns for tryptophan and a number of its metabolites under normal conditions and after oral administration of tryptophan.

2. Protocol.

The procedure followed was the same for each subject. The details of the schedule follow.

The subjected fasted overnight. The first morning urine was discarded. Two hours later blood and urine (basal) specimens were collected. On the control day, the subject then drank 300 ml. fruit juice while, on the tryptophan load day, 4 g. (19.6 mM) of l-tryptophan were administered with the fruit juice. For the following six hours, at two hour intervals, blood and urine specimens were collected. Three hours after taking the fruit juice, the subject was served a specially prepared lunch. The caloric, protein, carbohydrate, fat and tryptophan contents were kept approximately constant for all experiments. For the remaining eighteen hours of each day, the subject kept a record of all food taken during this interval and attempted to duplicte the diet of the control day on the tryptophan load day. At the end of the twenty-four hour period, a final post-absorptive blood specimen was taken.

The standard lunch consisted of the following:

Tomato juice or clear broth	4 oz.
Chicken or steak	3 oz. for female subjects. 6 oz. for male subjects.
* Vegetable B (peas, carrots, etc.)	1/2 cup.
Vegetable A (salad)	1/2 cup.
Fat	1 tbsp.
Potato	1.
Bread (or 4 arrowroot biscuits)	1 slice.
Fruit (canned ∠ 2 tbsp. syrup)	* 1 exchange.
Tea, lemon, black coffee	1 cup.
Sugar	1 tsp. (if not used, replace with one soda cracker).

For male subjects, the lunch contained 850 Calories and 600 mg. tryptophan. For female subjects, the lunch contained 625 Calories and 400 mg.tryptophan.

* The terms, vegetable A, vegetable B and exchange are used in the same way as in the "Meal Planning and Exchange List" (1949) of the Joint Committee of the American Diabetic Association, The American Dietetics Association and the Diabetic Section of the U. S. Public Health Service.

3. Results.

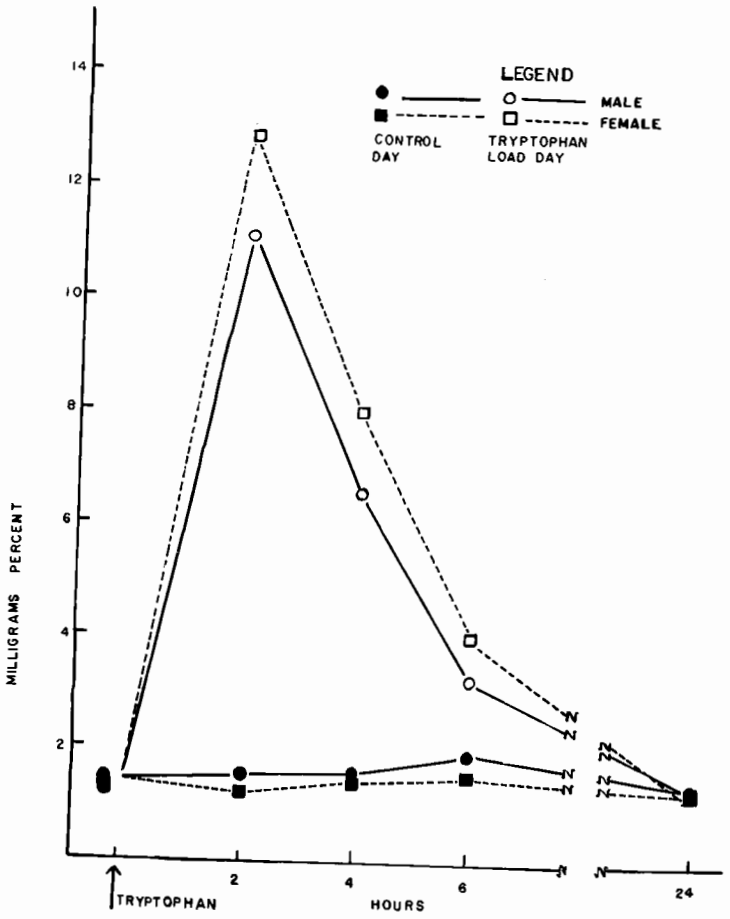
The results of the serum analyses for each of the subjects are shown in Tables 6 to 13. All concentrations are expressed as mg.%, the term commonly used to mean mg. per 100 ml. of serum. The composite Tables, 14 and 15, show the mean concentrations, and the Standard Errors of the means, of the serum ∝amino nitrogen and tryptophan levels. Figure 4 is a graphic illustration of the same data.

(a) Control day.

(1) Serum levels in male subjects.

The ∝amino nitrogen@ concentrations in the serum specimens varied slightly after food intake. As seen in Table 14, the fasting level was 4.72 ∠ 0.13 mg.%. Two hours after the fruit juice, there was a slight increase in the concentration. One hour after lunch, the serum

@The determinations of ∝amino nitrogen, xanthurenic acid and N'-methylnicotinamide were carried out with the assistance of Mrs. M. Aikman.



BLOOD TRYPTOPHAN LEVELS

Figure 4.

level had decreased below the fasting level. However, three hours after lunch a peak level of 6.00 $\pm$ 0.15 mg. % was obtained. Twenty-four hours after the onset of the experiment the α amino nitrogen concentration in the serum was 4.64 $\pm$ 0.41 mg. %.

The serum tryptophan levels remained fairly constant, with an elevation seen only in the six hour specimen. As shown in Table 15, the fasting concentration was 1.40 $\pm$ 0.13 mg. % and the peak concentration, three hours after lunch, was 1.91 $\pm$ 0.23 mg. %, a statistically significant increase. ($p < 0.01$)(155). The twenty-four hour serum level was again 1.40 $\pm$ 0.20 mg. %.

Neither kynurenine nor anthranilic acid were present in measurable concentrations in the serum.

(2) Serum levels in female subjects.

The fasting serum level of α amino nitrogen in the female subjects was 4.89 $\pm$ 0.27 mg. %. The peak level reached three hours after lunch was 5.50 $\pm$ 0.34 mg. %. At twenty-four hours, the level had returned to 4.53 $\pm$ 0.59 mg. %.

The tryptophan level in the female subjects differed slightly from that of the male subjects. From the fasting concentration of 1.41 $\pm$ 0.14 mg. %, there was a drop to a level of 1.22 $\pm$ 0.11 mg. % two hours after the fruit juice. The peak, seen three hours after lunch, was 1.53 $\pm$ 0.19 mg. %. This was not as high as the one obtained in the male subjects. None of these variations with time were statistically significant ($p > 0.1$).

As found with the male subjects, kynurenine and anthranilic acid were not present in the serum in measurable concentrations.

(3) Urinary excretions.

Tables 16 to 23 show in detail the amounts of the metabolites excreted by individual subjects. The mean values of these excretions and their Standard Errors are compiled in Tables 24 to 26.

The urinary excretion of α amino nitrogen varied greatly from one two hour period to the next as seen in Table 24. There was a similar twenty-four hour excretion by male and female subjects, 311.10 $\pm$ 33.01 mg. and 317.57 $\pm$ 60.00 mg.

The data collected, for male and female subjects, on the excretion of tryptophan and its metabolites was subjected to an analysis of variance (Table 27) (156)(157)(158). This revealed that the excretion of tryptophan varied among individuals. The differences were significant at the 1% level. There were also significant differences ($p < .05$) in the excretions among intervals on the same day. Examination of the raw data indicated that the significance was probably due to the variation in the female subjects.

For kynurenine, anthranilic acid and xanthurenic acid (chromogens) excretions, there were differences among individuals significant at the 1% level. The excretion differences among two hour intervals were not significant at the 5% level, for kynurenine and anthranilic acid and just significant at the 5% level for xanthurenic acid.

Basal urine specimens were collected from each subject on each of four separate days. These were examined for day to day variations in excretion of tryptophan and its metabolites. Analysis of variance on the data obtained (Table 28) revealed that there were no differences significant at the 5% level among the amounts of tryptophan excreted on different days. Similarly, there were no differences significant at the 5% level for theexcretions of kynurenine, anthranilic acid and xanthurenic acid on different days. There were differences significant at the 5% level among individuals, among the

amounts of tryptophan excreted, but not among those of kynurenine and anthranilic acid. The differences among individuals were significant for xanthurenic acid at the 1% level.

The N'-methylnicotinamide results were not included in the analysis because the specimens had been stored for one to four months before examinations were carried out and the values obtained were at least 10 to 20% lower than the actual concentrations at the time of collection.

(b) Tryptophan load test day.

(1) Serum levels in male subjects.

After administration of l-tryptophan, the amino nitrogen levels in the sera of the male subjects showed a slight decrease. When the theoretical α amino nitrogen concentration contributed by the tryptophan measured was subtracted from the total α amino nitrogen content of the serum, the decrease was accentuated, as seen in Table 14. The only decrease which was statistically significant ($p < 0.01$) occurred six hours after ingestion of tryptophan.

The peak tryptophan concentration measured in the serum occurred in the specimens collected two hours after intake of the tryptophan (Table 15) (Figure 4). This level, 11.10 ± 1.34 mg. % was almost seven times as high as the fasting level. There was a gradual decrease in the serum tryptophan concentration and, at six hours, the serum level of 3.27 ± 0.69 mg. % was little more than twice as high as the level measured at twenty-four hours, 1.47 ± 0.12 mg. %.

Only one male subject showed a measurable amount of kynurenine in his serum after ingestion of tryptophan. The peak concentration of 0.78 mg.% (Table 6) occurred six hours after ingestion of tryptophan and coincided with the period of greatest kynurenine excretion.

The same male subject was also the only one in whose serum anthranilic acid appeared in measurable concentrations after tryptophan administration. Here the peak level, 1.97 mg. %, coincided in time with the peak tryptophan level two hours after ingestion of the amino acid but did not coincide with the period of increased excretion of anthranilic acid in the urine.

(2) Serum levels in female subjects.

The concentrations of α amino nitrogen in the serum of the female subjects, after the ingestion of tryptophan, showed very little change from those observed during the control day (Table 14). When the theoretical α amino nitrogen, contributed by the tryptophan measured in the serum, was subtracted from the total α amino nitrogen measured, there was also no statistically significant difference seen ($p > 0.1$).

After administration of tryptophan, the serum tryptophan levels reached a peak of 12.86 ± 1.38 mg. %, as measured in the specimens collected two hours after ingestion of the dose (Table 15). The decrease of serum tryptophan concentration proceeded at a slightly slower rate than that observed in the male subjects. By six hours after tryptophan the concentrations were down to 4.00 ± 0.51 mg. %, i. e. three times the level of the twenty-four hour specimens, 1.33 ± 0.09 mg. %.

Only one female subject (Table 10) showed a measurable amount of kynurenine in the serum after tryptophan. The peak concentration, 1.08 mg. %, occurred at four hours after ingestion of the dose. This coincided in time with the period of greatest excretion of kynurenine.

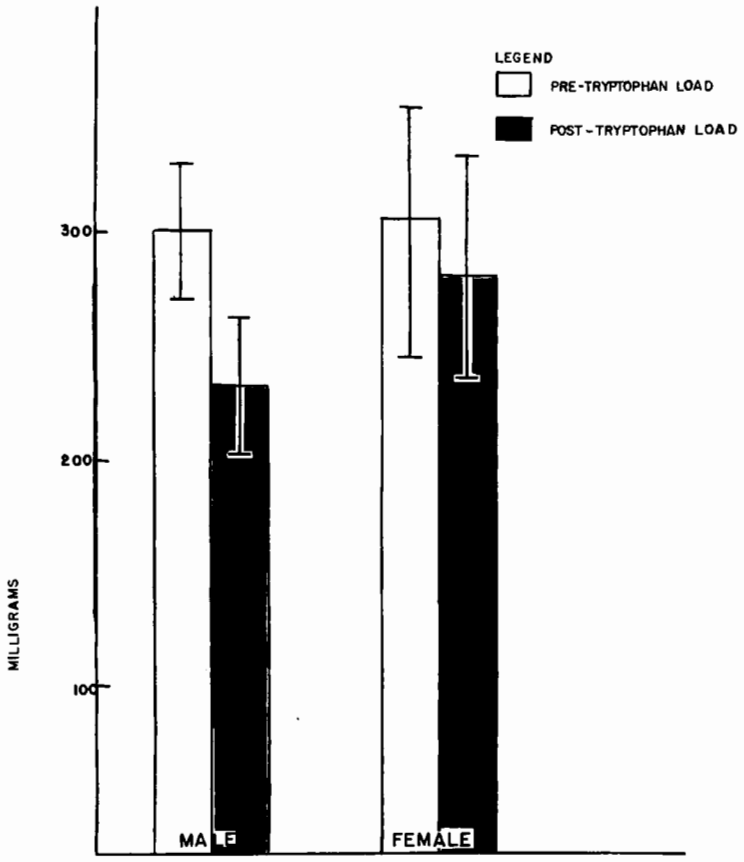
The same female subject showed a barely measurable concentration of anthranilic acid in the blood specimen collected two hours after tryptophan. As with the male subject, the peak concentration coincided with the peak tryptophan level, but not with the period of maximum anthranilic acid excretion.

(3) Urinary excretions of male subjects.

Very little difference was seen in the amounts of α amino nitrogen excreted by male subjects when the three 2 hour intervals of the control day were compared with their equivalent periods of the test day (Table 24). However, the amount excreted during the 6 - 24 hour interval decreased from 251.26 $\pm$ 31.90 mg. to 189.59 $\pm$ 27.58 mg. When the theoretical α amino nitrogen contributed by the excreted tryptophan and kynurenine was subtracted from the total α amino nitrogen measured, the excretion of "non-tryptophan" amino nitrogen during twenty-four hours fell from 302.11 $\pm$ 31.71 mg. on the control day to 241.00 $\pm$ 29.46 mg. after administration of tryptophan (Figure 5).

There was no delay in the increase of urinary tryptophan excretion (Table 25), the peak increment occurring in the specimens voided two hours after administration of the amino acid (Table 25) (Figure 6). The increase at this time amounted to 31.73 $\pm$ 8.86 mg. The total twenty-four hour increase in tryptophan excretion, 96.29 $\pm$ 18.72 mg., accounted for 2.40 $\pm$ 0.47% of the administered dose.

Kynurenine excretion was also increased in the first two hour urines after administration of tryptophan (Table 25). However, the greatest increment in excretion, 13.84 $\pm$ 4.76 mg., occurred in the urines collected two to four hours after tryptophan (Table 26) (Figure 7). The total twenty-four hour increase in kynurenine excretion was 38.69 $\pm$ 17.67 mg., accounting for 0.99 $\pm$ 0.39% of the ingested tryptophan.



24 HR. URINE ∝AMINONITROGEN (MINUS CONTRIBUTION BY TRYPTOPHAN & KYNURENINE)

Figure 5.

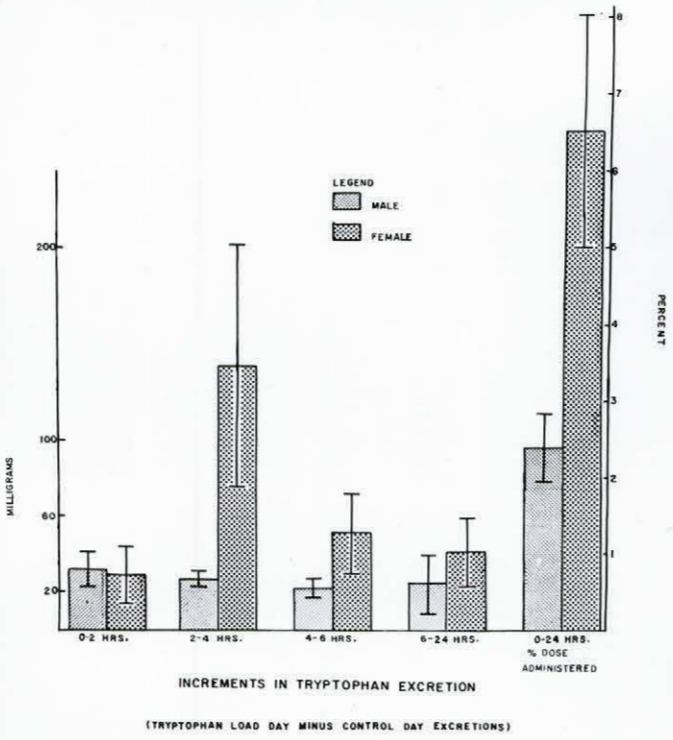

LEGEND
MALE
FEMALE
MILLIGRAMS
200
100
60
20
PERCENT
8
7
6
5
4
3
2
1
0-2 HRS.
2-4 HRS.
4-6 HRS.
6-24 HRS.
0-24 HRS.
% DOSE
ADMINISTERED
INCREMENTS IN TRYPTOPHAN EXCRETION
(TRYPTOPHAN LOAD DAY MINUS CONTROL DAY EXCRETIONS)

Figure 6.

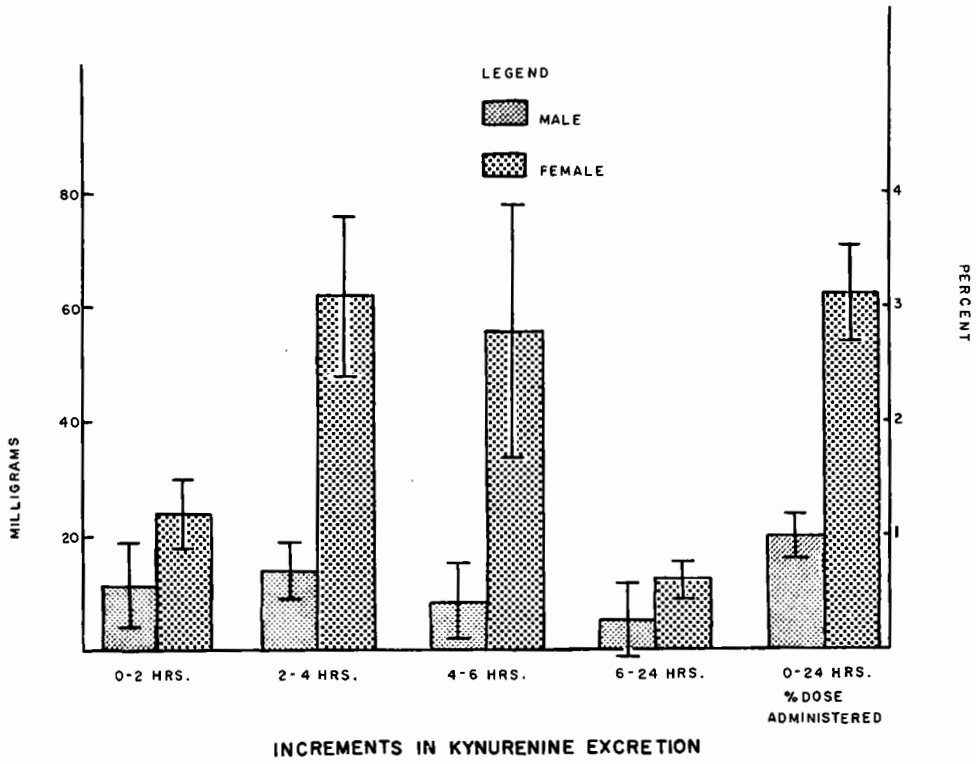
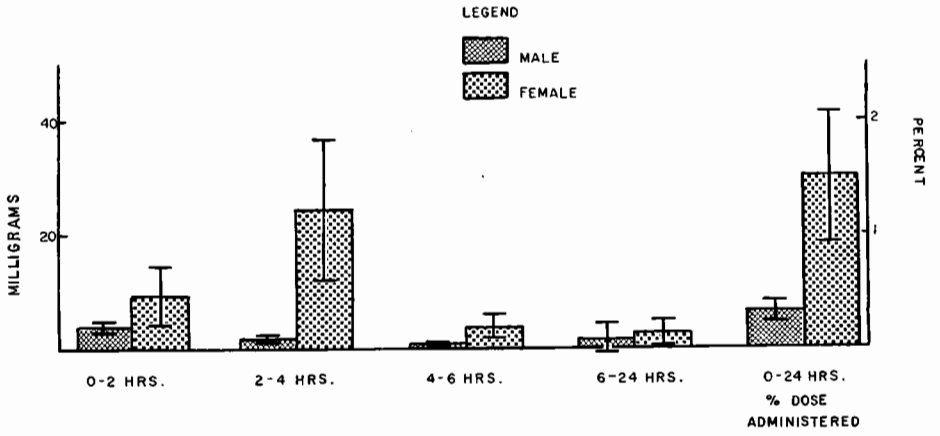

LEGEND
MALE
FEMALE
80
60
40
20
MILLIGRAMS
4
3
2
1
PERCENT
0-2 HRS.
2-4 HRS.
4-6 HRS.
6-24 HRS.
0-24 HRS.
% DOSE
ADMINISTERED
INCREMENTS IN KYNURENINE EXCRETION
(TRYPTOPHAN LOAD DAY MINUS CONTROL DAY EXCRETIONS)

Figure 7.



INCREMENTS IN ANTHRANILIC ACID EXCRETION

(TRYPTOPHAN LOAD DAY MINUS CONTOL DAY EXCRETIONS)

Figure 8.

The major increase in anthranilic acid excretion, 2.98 ± 1.08 mg., occurred in the urines voided two hours after administration of tryptophan (Table 26)(Figure 8). The total twenty-four hour urinary increase of 6.98 ± 3.33 mg. accounted for 0.28 ± 0.10% of the ingested amino acid.

Although xanthurenic acid appeared in the first urine specimens after tryptophan administration, the peak excretion, 8.88 ± 1.42 mg., occurred in the 4 - 6 hour urines (Table 26) (Figure 9). The total xanthurenic acid excreted in twenty-four hours was 24.73 ± 5.66 mg. and accounted for 0.59 ± 0.15% of the oral dose of tryptophan.

(4) Urinary excretions of female subjects.

There was no difference between the total urinary excretion of α amino nitrogen on the control day, 317.57 ± 60.0 mg., and that on the tryptophan load day, 321.70 ± 52.59 mg. (Table 24). When the theoretical α amino nitrogen, contributed by the excreted tryptophan and kynurenine, was subtracted from the total α amino nitrogen measured, a small decrease in the "non-tryptophan" nitrogen from 306.83 ± 65.96 mg. on the control day to 282.82 ± 54.66 mg. on the test day was seen (Figure 5). This was not significant at the 5% level.

When the twenty-four hour excretions of tryptophan in the two days were compared, an almost threefold increase in excretion was found, i. e. from 162.29 ± 28.07 mg. on the control day to 423.15 ± 167.97 mg. on the test day (Table 25)(Figure 6). This increase accounted for 6.52 ± 1.50% of the dose administered. (Table 26).

Similarly, comparison of urinary kynurenine on the two days revealed a thirteen-fold increase, from 11.23 ± 3.03 mg. excreted on the control day to 144.18 ± 34.13 mg. after tryptophan (Table 25) (Figure 7). This increase accounted for another 3.26 ± 0.85% of the administered amino acid (Table 26).

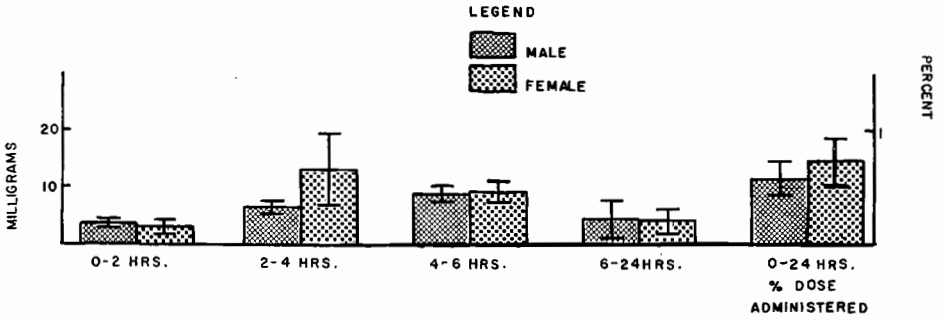
There was almost a fourfold increase in anthranilic acid excretion, from 14.23 ± 4.17 mg. during twenty-four hours on the control day, to 54.92 ± 17.71 mg. on the test day (Table 25)(Figure 8). Here the increase accounted for 1.51 ± 0.58% of the administered tryptophan (Table 26).

Administration of tryptophan to the female subjects resulted in an excretion of 29.69 ± 8.45 mg. xanthurenic acid in twenty-four hours (Table 26)(Figure 9). Excretion of this metabolite was in the same range as that of the male subjects studied and accounted for 0.74 ± 0.20% of the administered tryptophan.

(5) Differences in urinary excretions.

Table 26 and Figures 6-9 illustrate the increments in the excretion of tryptophan and its metabolites due to the test dose of tryptophan. In the male subjects the peak increment in tryptophan excretion, 31.73 ± 8.86 mg. occurred in the 0 - 2 hour period while in the female subjects the peak, 139.16 ± 62.74 mg. occurred in the 2 - 4 hour period.

In both male and female subjects the peak increment in kynurenine excretion occurred in the 2 - 4 hour period. However, while the maximum increment for the male subjects was 13.84 ± 4.76 mg., that of the female subjects was 62.46 ± 14.27 mg.



INCREMENTS IN XANTHURENIC ACID EXCRETION

(TRYPTOPHAN LOAD DAY MINUS CONTROL DAY)

Figure 9.

In the male subjects, the increment in anthranilic acid excretion, as in tryptophan excretion, showed a peak, 2.98 $\pm$ 1.08 mg., in the 0 - 2 hour interval. The maximum increment in anthranilic acid excreted by the female subjects, 24.69 $\pm$ 12.54 mg. occurred during the same interval as the peak excretion for tryptophan and kynurenine.

The peak increments of xanthurenic excretion also differed in time between male and female subjects. For the males, the level of increased excretion was 8.88 $\pm$ 0.42 mg., and occurred in the 4 - 6 hour interval, while for the females, the time of the greatest increase in excretion, 12.98 $\pm$ 6.26 mg., was, as with all the other metabolites measured, in the 2 - 4 hour interval.

Although the N'-methylnicotinamide excretions are tabled, the reliability of these results is invalidated by the long storage of the urine prior to the measurements as noted on page 35.

(6) Analysis of increments in excretion of tryptophan and its metabolites.

From Table 26 and Figure 10, it can be seen that the major portion of the twenty-four hour increments in urinary excretion of metabolites occurred during the first six hours after the ingestion of tryptophan. An analysis of variance (Table 29) was made on the six hour urinary excretion increments of tryptophan, kynurenine, and anthranilic acid between male and female subjects. The analysis revealed that at the 5% level there was a significant difference in the excretions of tryptophan, kynurenine and anthranilic acid between male and female subjects. For xanthurenic acid there was no significant sex difference in the amounts excreted.

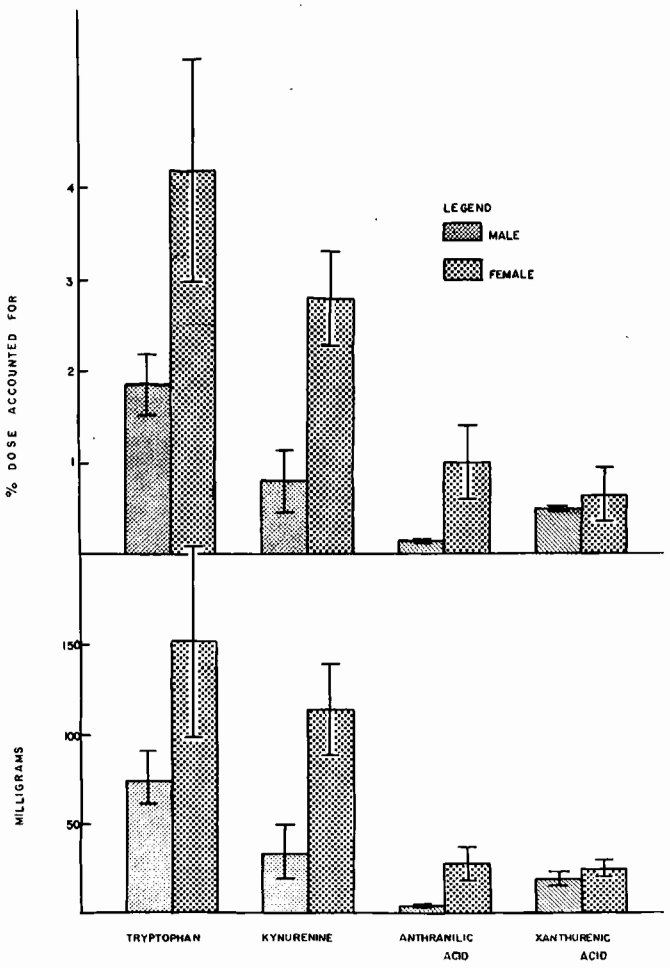

LEGEND
MALE
FEMALE
% DOSE ACCOUNTED FOR
4
3
2
1
MILLIGRAMS
150
100
50
TRYPTOPHAN
KYNURENINE
ANTHRANILIC ACID
XANTHURENIC ACID
6HR. INCREMENTS IN URINE EXCRETION

Figure 10.

Increments in urinary excretion of tryptophan and the metabolites measured, together accounted for 4% of the administered tryptophan in the male subjects and for 12% in the female subjects. In order to compare the patterns of excretion between male and female subjects an analysis of variance was made (Table 30). The total percentage of the dose accounted for by the twenty-four hour excretions of tryptophan and metabolites was considered as 100%. Excretion of each metabolite was calculated as a percentage of the total. As seen in Table 30, tryptophan excretion amounted to 57.73 $\pm$ 10.46% of the total increment in male subjects and 55.21 $\pm$ 3.01% in female subjects. Kynurenine excretion in male subjects accounted for 22.26 $\pm$ 7.74% and in female subjects for 27.22 $\pm$ 3.99%. Anthranilic acid constituted 6.71 $\pm$ 2.41% of the total dose accounted for by the male subjects and 11.18 $\pm$ 2.11% by the female subjects while xanthurenic acid amounted to 13.49 $\pm$ 2.08% for male subjects and 6.40 $\pm$ 0.96% for female subjects. The analysis of variance revealed that although the actual amounts of excreted metabolites differed, their proportion, in relation to the total dose accounted for, formed a pattern which was the same for both sexes.

4. Discussion.

(a) Comparison of results with data in the literature.

Normal twenty-four hour tryptophan excretions, as measured by the chemical method employed in these experiments, were 120.76 $\pm$ 23.41 mg. for the male subjects and 162.29 $\pm$ 28.07 mg. for the female subjects. These measurements are greater than those obtained by microbiological assay as seen in Table 31. However, the values obtained

by this chemical method are not as high as those, 281 ± 30 mg., obtained by the chemical method used by Albanese and his co-workers (130). In the present work, the excretions measured represent approximately 10% of the dietary intake of tryptophan in the male subjects and 20% in the female. Denko and Grundy (135) found that their measurements of tryptophan excretion by microbiological assay represented 1 - 3% of dietary tryptophan in normal male subjects.

The postabsorptive serum tryptophan values obtained here compare well with the results obtained by various groups using microbiological assays. This can be seen in examing these levels listed in Table 32.

Kynurenine excretions, as seen in Table 33, have not been investigated by many workers. Brown and Price (140) measured tryptophan metabolites excreted in urine of man. When a dose of 19.6 mM of l-tryptophan was administered to normal male subjects, excretions of these metabolites increased as seen in Table 34.

Recovery of the administered dose of tryptophan as kynurenine and its conjugate amounted to 0.79% while in the present experiments, in male subjects, it was 0.99% of the administered dose.

The only data available on anthranilic acid excretion were included in this work by Brown and Price (140) in which the twenty-four hour excretion of anthranilic acid conjugates accounted for 0.33% of the administered dose of l-tryptophan. In the present experiment the excreted anthranilic acid accounted for 0.28% of a tryptophan load of the same magnitude.

TABLE 34

	Normal 24 hour excretion (μM)	24 hour excretion after dose (μM)	Increment (μM)	% of dose administered
anthranilic acid-glucuronide	6	14	8	
o-aminohippuric acid	29	86	57	
			65	0.33
acetyl-kynurenine	8	56	48	
kynurenine	15	121	106	
			154	0.79
xanthurenic acid	70	103	33	0.17

Xanthurenic acid excretion in the experiments of Price and Brown accounted for 0.17% of the administered tryptophan while the excretions of normal male subjects in the present experiment accounted for 0.49%. The other xanthurenic acid excretion measurements listed in Table 33 are not comparable since the amount and form of the administered tryptophan differs. However, the amounts of xanthurenic acid excreted after tryptophan in these experiments, 24.73 $\pm$ 5.66 mg. for the male subjects and 29.69 $\pm$ 8.45 mg. for the female subjects would fall within the wide range listed as normal by various research groups (88) (140).

Although the N'-methylnicotinamide results obtained in these experiments can not be included with any accuracy in the discussion of tryptophan metabolite excretions, it is interesting to note that the increment as measured accounts for 0.10% of the administered dose for male subjects and 0.17% for female subjects. These values are at least

20% lower than the actual values because of the difficulties in nicotinamide measurement previously discussed. Perlzweig et al (69) measured twenty-four hour N'-methylnicotinamide excretion in male and female subjects before and after the administration of 5 g. dl.-tryptophan. Male subjects showed an increase in N'-methylnicotinamide excretion from 5.9 to 12.9 mg., while the excretion of females increased from 7.2 mg. to 13.9 mg. This metabolite accounted for 0.41% of the administered load in male subjects and 0.39% in the female subjects. It is assumed that only l-tryptophan was metabolized to nicotinic acid and was, therefore, the source of the increased excretion. If, however, as Kallio and Berg (80) have shown, administration of d-tryptophan does result in an increase in N'-methylnicotinamide excretion equivalent to 67% of the increment induced by the l-form, then these increments would account for 0.24% of the administered dose in the male subjects and 0.23% in the female subjects.

(b) Experimental observations.

As previously shown in the results of this experiment for each metabolite examined (Tables 27 and 28), there was a constancy in the basal excretion of tryptophan and its metabolites from day to day as well as in excretions of these substances during intervals on the same day. Therefore, it was valid to compare excretions on the control and tryptophan load days and attribute the differences in the excretions as increments due to ingested tryptophan.

In order to see if the administered tryptophan was being handled as dietary tryptophan would be, the proportions of tryptophan and its metabolites excreted on the control day were compared with those on the load day. Xanthurenic acid excretion accounted for 7.39 $\pm$ 2.11%

of the tryptophan and metabolites excreted by the male subjects and 4.28 ± 0.54% excreted by the female subjects (Table 35).

TABLE 35

Metabolites Excreted in 24 Hours as Percentage of Total 24 Hour Excretions Measured.

	Male Subjects		Female Subjects	
	Control Day	Tryptophan Load Day	Control Day	Tryptophan Load Day
Tryptophan	77.41 ± 4.73	67.95 ± 6.56	83.26 ± 3.72	63.71 ± 2.70
Kynurenine	8.81 ± 3.94	14.81 ± 4.93	5.44 ± 0.90	20.53 ± 2.91
Anthranilic Acid	13.78 ± 1.80	9.85 ± 1.57	11.30 ± 3.05	11.48 ± 2.11
Xanthurenic Acid	0.	7.39 ± 2.11	0.	4.28 ± 0.54
Total	100	100	100	100

In the male subjects, the differences in the proportions of the metabolites excreted between the two days were not statistically significant ($p > 0.1$). There was a significant change in the proportions of metabolites excreted by the female subjects. Although the variation in the proportion of anthranilic acid was not significant ($p > 0.1$), there was a significant decrease in the proportion of tryptophan excreted and an increase in the proportion of kynurenine excreted ($p < 0.01$).

The order of formation of metabolites along the pathway of tryptophan degradation studied (Figure 3) would suggest a sequence of increased excretion, after a load of tryptophan, in the following order:- 1. tryptophan, 2. kynurenine, 3. anthranilic acid and 4. xanthurenic acid. Where measurable amounts of anthranilic acid appeared in the blood the peak levels were observed in the two hour specimens. In the male subjects the peak excretion increments for tryptophan and anthranilic acid occur first, then kynurenine and finally xanthurenic acid. Except for the early

appearance of anthranilic acid, the increments in excretion in the male subjects would be compatible with the metabolic scheme for tryptophan. It is possible that, by the time the peak serum kynurenine level was reached, the kynureninase system converting kynurenine to anthranilic acid, which is not an adaptive one (52), had reached its maximum rate of activity and therefore there was no further increase in serum anthranilic acid concentrations or urine excretion levels. Since the same enzyme also converts 3-hydroxykynurenine to 3-hydroxyanthranilic acid, there may also have been competition for the available enzyme.

The male subjects appeared to metabolize the dose of tryptophan in anormal manner, since the proportions of metabolites excreted on the control and load days did not vary significantly. The degradation of the tryptophan load by the female subjects was markedly different from the normal breakdown of dietary tryptophan. The time of peak excretion of tryptophan and metabolites after administration of the amino acid was not compatable with the accepted metabolic scheme. The peak excretion increments for all the measured metabolites occurred simultaneously. It would almost appear as if all the enzyme systems involved reached a high rate of activity within two hours in order to eliminate the large quantity of tryptophan absorbed into the bloodstream from the intestinal tract.

E. Experiment 2.

1. Purpose -

This experiment was carried out to study the effect of inadequate riboflavin intake on tryptophan degradation in human subjects.

2. Introduction.

As a result of the diet history analysis made on each subject (Table 36), the opportunity arose to study tryptophan metabolism in two subjects whose diets met the minimal requirements of the Canadian

Standards (155) except for the content of riboflavin. One subject was further studied for changes in the pattern of tryptophan metabolism after a month of multiple vitamin therapy. (The daily vitamin intake is listed in Table 10).

3. Protocol.

The procedure for this experiment was the same as for Experiment 1. Vitamin therapy was discontinued on the day prior to the test.

4. Results.

(a) Serum determinations.

The individual serum measurements are listed in Tables 37 and 38. The results of the test on Subject 5 after vitamin therapy, as shown in Tables 10 and 20, were included with Experiment 1 since all the criteria for a normal subject were then met.

The most striking difference seen when the results of the serum determinations of these two subjects were compared with those of the normal females in Experiment 1, was the high concentration of kynurenine found after administration of tryptophan. In Subject 5a a peak of 2.10 mg. % was reached and in Subject 6, 3.33 mg. %.

(b) Urinary excretion determinations.

The urinary excretion results for these subjects are listed in Tables 20, 39 and 40. Both subjects excreted more kynurenine after tryptophan than did the normal females. The twenty-four hour increments, 351.49 mg. and 341.96 mg., lay 6 Standard Deviations from the mean of 132.95 $\pm$ 35.01 mg. (S.E.) for normal female subjects.

Subject 5a also excreted greatly increased twenty-four hour increments of tryptophan, 836.66 mg., and of anthranilic acid,

11.59 mg. After tryptophan, the twenty-four hour increase in urinary excretion by normal females was 260.86 ± 62.17 mg. for tryptophan and 40.69 ± 15.73 mg. for anthranilic acid. These increments lay outside 8 S.D. and 4 S.D. of the respective means.

(c) Effect of vitamin therapy.

Comparison of control day results before and after vitamin therapy in Subject 5 revealed no change in tryptophan excretion, a 50% decrease in kynurenine excretion and a 13% decrease in anthranilic acid excretion. On the tryptophan load day, the tryptophan excretion was decreased by 53% after vitamin therapy, kynurenine by 49% and anthranilic acid by 45%.

After vitamin therapy and the administration of tryptophan, the peak serum tryptophan level was 7.51 mg.% compared with 11.13 mg.% prior to therapy. The serum kynurenine levels were also decreased after therapy. The peak measurement decreased from 2.10 mg.% to 1.08 mg.%, a fall of 51%.

Excretion increments of tryptophan, kynurenine and anthranilic acid were also decreased. The twenty-four hour increments were 338.20 mg., 217.99 mg. and 58.29 mg. respectively. These results all fell within 2 S.D. of the mean for normal females.

5. Discussion.

The dietary survey (Table 36)(153)(154)(155) showed that these two subjects did not meet the dietary requirements, in their intake of riboflavin, recommended by Canadian Standards. As mentioned in the reviw of the literature, Dalgliesh and his co-workers (2)(97) have placed the site of riboflavin action at the point of hydroxylation

of kynurenine. The increased excretion of kynurenine seen in these subjects is compatible with the experimental evidence for the site of riboflavin action and would suggest a partial block at this point of metabolism due to riboflavin deficiency. The increase of 111.7% in xanthurenic acid excretion after therapy would also be in accord with an increased production of 3-hydroxykynurenine and would be compatible with the role assigned to riboflavin at this step in metabolism. There is the possibility that riboflavin insufficiency may have resulted in a decrease of tryptophan degradation by other pathways thereby resulting in increased kynurenine production. However,there was also an appreciable increase in N'-methylnicotinamide excretion after vitamin therapy, adding evidence in favor of the proposed site of riboflavin action.

The increased excretion of tryptophan and anthranilic acid as well as kynurenine in Subject 5a could be indicative of the severity of the riboflavin deficiency. The experiment after vitamin therapy would have furnished more direct evidence of riboflavin action if the therapy had not included other vitamins of the B complex.

F. Experiment 3.

1. Purpose.

The purpose of this experiment was to study a short term tryptophan load test which would be a more practical method for the investigation of tryptophan metabolism than that used in Experiment 1.

2. Introduction.

A modification of the test protocol of Experiment 1 was necessary for the study of tryptophan metabolism in man under disease conditions. A procedure which involved forty-eight hours and the willing cooperation of laboratory personnel might not be manageable with bed-

ridden and uncooperative patients. A major difficulty often encountered in clinical investigation is the proper collection of twenty-four hour urine specimens. Another factor was the need for a test which would not require the hospitalization of ambulatory patients.

Examination of the data collected in Experiment 1 revealed that 70 - 90% of the excretion increments occurred in the first six hours after administration of tryptophan (Table 41). The analysis of variance (Table 27) of the two hour excretions on the control day in Experiment 1 suggested that the basal excretion multiplied by 3 could be used to predict the excretion for the following six hours. It was decided to limit the test to a basal two hour period plus a period of six hours following administration of tryptophan.

There appeared to be a wide scatter in the excretion results obtained from the four female subjects studied in Experiment 1. Three more subjects were studied by the abbreviated procedure in order to obtain data on a larger group of female subjects.

3. Protocol.

(a) Recalculation of data from Experiment 1.

The data of Experiment 1 was recalculated on the following basis: The basal excretion values multiplied by 3 were subtracted from the excretion values of the six hour interval after tryptophan ingestion. The differences were considered as the excretion increments induced by the administered amino acid.

(b) Additional female subjects.

The subjects were instructed not to partake of any food after supper on the evening prior to the test. The first morning

TABLE 42

SUBJECT # 10

Sex - female Age - 24 years Height 5' 4" Weight - 130 lbs. (5th day of menstrual cycle)

Food Intake- 1465 Calories; 76 g. protein; 144 g. carbohydrate; 65 g. fat; 700 mg. tryptophan.

BLOOD SERUM LEVELS

Time	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
fasting	1.18	--	--
2 hours	12.07	0.42	--
4 hours	8.09	0.66	--
6 hours	3.67	0.28	--

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
Basal 2 hours	44	100	15.28	0.94	1.13	2.60	0.61
0 - 2 hours	75	150	24.21	43.95	1.78	8.48	0.51
2 - 4 hours	65	110	71.25	59.08	15.04	18.70	0.41
4 - 6 hours	72	160	36.52	17.34	4.50	11.20	0.57
6 - 24 hours	305	500	26.18	3.77	3.04	7.50	2.74
Total			158.16	124.14	24.36	45.88	4.23
0 - 6 hours (/ tryptophan)			131.98	120.37	21.32	38.38	
Theoretical-0 - 6 hours (3 x basal)			45.84	2.82	3.39	7.80	
Increase over theoretical			86.14	117.55	17.93	30.58	
Increase in mM			0.4218	0.5646	0.1308	0.1490	
% dose accounted for			2.15	2.88	0.67	0.75	

TABLE 43

SUBJECT # 11

Sex - female Age - 26 years Height 5' 2" Weight - 108 lbs. (5th day of menstrual cycle)

Food Intake- 1800 Calories; 58 g. protein; 148 g. carbohydrate; 75 g. fat; 580 mg. tryptophan.

BLOOD SERUM LEVELS

Time	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
Fasting	1.99	--	--
2 hours	16.23	0.28	--
4 hours	14.19	0.38	--
6 hours	7.82	0.14	--

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
basal 2 hours	31	60	7.15	0.73	0.71	1.95	0.57
0 - 2 hours	245	260	31.88	4.18	1.99	6.76	0.57
2 - 4 hours	150	300	61.65	10.49	2.30	9.75	1.14
4 - 6 hours	155	300	21.02	24.48	3.24	9.00	1.05
6 - 24 hours	740	1000	75.92	14.40	0.49	26.00	9.43
Total			190.47	53.55	8.02	51.51	12.19
0 - 6 hours (- tryptophan)			114.55	39.15	7.53	25.51	
Theoretical 0 - 6 hours (3 x basal)			21.45	2.19	2.13	5.85	
Increase over theoretical			93.10	36.96	5.40	19.66	
Increase in mM			0.4559	0.1775	0.0394	0.0958	
% dose accounted for			2.33	0.91	0.20	0.49	

TABLE 44

SUBJECT # 12

Sex - female Age-24 years Height - 5' 3 1/2" Weight - 128 lbs. (25th day of menstrual cycle)

Food Intake - 1644 Calories; 58 g. protein; 218 g. carbohydrate; 60 g. fat; 765 mg. tryptophan.

BLOOD SERUM LEVELS

Time	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
Fasting	1.45	--	--
2 hours	14.22	0.82	--
4 hours	8.38	1.63	--
6 hours	4.28	0.38	--

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
BASAL 2 hours	73	150		7.95	1.30	0.55	3.00	0.23
0 - 2 hours	130	200		26.82	11.91	3.76	6.50	0.45
2 - 4 hours	110	250		60.26	137.04	18.03	14.13	0.65
4 - 6 hours	110	200		11.81	4.41	0.64	5.20	0.19
6 - 24 hours	1500	2000		144.62	11.52	11.10	52.00	4.46
Total				243.51	164.88	33.53	77.83	5.75
0 - 6 hours (- tryptophan)				98.89	153.36	22.43	25.83	
Theoretical 0 - 6 hours (3 x basal)				23.85	3.90	1.65	9.00	
Increase over theoretical				75.04	149.46	20.78	16.83	
Increase in mM				0.3674	0.7178	0.1515	0.0820	
% dose accounted for				1.87	3.66	0.77	0.42	

urine was discarded and two hours later basal urine and blood specimens were collected. The subjects were then given 4 g. l-tryptophan as in Experiment 1. Blood and urine specimens were collected in two hour intervals. Three hours after ingestion of tryptophan the subjects were served the standard lunch. This time the tests were not arranged to occur at any specific time in the menstrual cycle.

4. Results.

(a) Comparison of actual and calculated six hour excretion increments.

The six hour excretion increments calculated by the short procedure (i.e. excretion after tryptophan minus 3 times basal) were compared with the actual six hour increments obtained by the two day procedure of Experiment 1 for male and female subjects separately. There were no significant differences among the results obtained.

(b) New subjects.

The results of the tests on the three new subjects are in Tables 42 - 44. The data obtained from the tests on the additional female subjects were pooled with the data of the female subjects previously studied after recalculation as explained above. Comparison of Table 45 with Table 15 shows that the values of the serum tryptophan concentrations previously obtained were confirmed by the larger group. Five of the eight female subjects studied showed measurable amounts of kynurenine in their blood after tryptophan. The mean values and their Standard Errors are shown in Table 45.

(c) Comparison of urinary excretions of males and females.

Table 46 shows urinary excretions of all subjects in Experiments 1 and 2 recalculated according to the method described. Table 47 shows the urine excretion results, as recalculated, of subjects studied in Experiment 1, together with the results obtained in the present experiment. The six hour increments in tryptophan

excretion by male subjects was 74.24 ± 14.00 mg. representing 1.86 ± 0.34% of the administered tryptophan (Table 47). In the female subjects the tryptophan excretion increment amounted to 154.20 ± 57.63 mg. or 4.21 ± 1.26% of the administered dose.

The six hour excretion increment of kynurenine for the male subjects was 34.70 ± 14.59 mg. which accounts for 0.81 ± 0.34% of the ingested tryptophan. Kynurenine excretion in the female subjects was much greater. The increment amounted to 114.50 ± 22.84 mg. and represented a recovery of 2.81 ± 0.52% of the administered tryptophan.

The increment in anthranilic acid excretion by the male subjects accounted for 0.14 ± 0.05% of the test dose. The increment itself amounted to 3.85 ± 1.48 mg. Excretion of anthranilic acid in the female subjects was also much greater. The increment of 28.20 ± 11.06 mg. represented 1.05 ± 0.40% of the ingested tryptophan.

The range of xanthurenic acid excretion did not differ appreciably between male and female subjects. The excretion increment for the males was 19.17 ± 4.11 mg. while that of the females was 25.49 ± 5.17 mg. The dose accounted for was 0.48 ± 0.09 and 0.63 ± 0.32% respectively.

5. Discussion.

Since there was no significant difference among the results obtained by the two day procedure and the abbreviated eight hour one, this short term test met the requirements desired for the study of tryptophan metabolism.

The value of this shortened procedure for the evaluation of tryptophan metabolism lies in the savings it involves. Firstly the duration of the test is cut from 48 hours to 8 hours. Secondly only four specimen collection periods are involved as compared

with ten periods in the two day test. This "short-cut" can be used without an appreciable loss of information. As seen in Table 41, most of the information gathered from the two day test can be garnered in the six hour period after tryptophan. The tryptophan load test, in this abbreviated from,can be a practical tool for routineclinical investigation.

GENERAL DISCUSSION

A. Normal Metabolism of the Tryptophan Load.

These experiments were undertaken in order to establish serum concentration and urinary excretion patterns for tryptophan and a number of its metabolites, under normal conditions and after oral administration of tryptophan. The results obtained have already been briefly discussed (Pages 53-58, 60-61, 64-65).

There was a marked difference between male and female subjects in the amounts of tryptophan and its metabolites excreted in the urine after ingestion of this amino acid. There are several possible explanations for this difference between the sexes: (1) metabolism may have proceeded at a higher rate in the male subjects so that the metabolites excreted represented only a fraction of the dose which was degraded along the pathway to nicotinic acid derivatives; (2) in the male subjects a greater portion of the tryptophan may have been diverted along another pathway of degradation; (3) in the male subjects a greater portion of tryptophan may have entered the body amino acid pool and tissues and was not available for degradation. If protein anabolism was increased it would explain the observation that there was a greater decrease in the excretion of amino acids other than tryptophan by male subjects than by female.

This decrease in α amino nitrogen excretion encountered after administration of tryptophan introduces a point of interest. Since total nitrogen excretion determinations were not made, this decrease can not be taken as a definite indication of nitrogen sparing due to the administered amino acid. It may well be that the decrease in α amino nitrogen excretion was due to increased amino acid catabolism. On the other hand, indoleacetic acid has been shown to stimulate amino acid transfer into cells (152). The observed decrease in α amino nitrogen excretion may have been an indirect result of such action.

The male subjects appeared to metabolize the administered tryptophan in the same manner that they did dietary tryptophan since the excretion patterns were the same on the control and test days. In the female subjects the pattern of excretion of tryptophan metabolites after a dose of this amino acid was different from that observed on the control day when the only source of tryptophan was the diet. It would be of importance to determine if this apparent difference was a consequence of the large amount of tryptophan administered. It is proposed to clarify this point by administering isotopically labelled tryptophan in an amount which does not exceed normal dietary intake of tryptophan.

B. Variations in the Metabolism of the Tryptophan Load.

Since the B vitamins play a vital role in the metabolism of tryptophan it is not surprising to find that an inadequate vitamin intake was associated with disturbances in the metabolism of an administered load of tryptophan. In Experiment 2 the subjects studied did not have an adequate dietary intake of riboflavin. The

increment in urinary kynurenine excretion after tryptophan was more than twice that found in normal females. This is compatible with the concept of a decrease in the hydroxylation of kynurenine as a result of a decreased amount of riboflavin. Similarly, other investigators have found that after administration of tryptophan, the excretion of xanthurenic acid is greater in patients with pyridoxine deficiency than in normal subjects (Table 32). Vitamin deficiencies due to malnutrition or inability to utilize adequate vitamin intake may cause other disturbances in tryptophan metabolism. Before disturbances in tryptophan metabolism can be attributed to any other cause, the possibility of vitamin deficiency must be considered. A tryptophan load test could be used to demonstrate an inadequate vitamin intake which may not be great enough to give rise to clinical symptoms.

Disorders in tryptophan metabolism have been encountered in hypo- and hyperthyroidism, as well as in diabetes (105) (106)(89)(57)(108). Administration of pyridoxine partially corrected the disturbances. The former method of treatment may have corrected a dietary deficiency which was associated with the disease state. On the other hand, the correction may have been due to a mass action of the vitamin which overcame a relative pyridoxine deficiency secondary to the hormonal disturbance. The action of insulin would imply that the disturbance in tryptophan metabolism was not due to a simple pyridoxine deficiency.

The role of insulin in tryptophan metabolism could be investigated by a series of experiments: firstly in the uncontrolled diabetic state before any therapy has been instituted; secondly after the diabetes has been brought under control with insulin and a proper dietary regimen instituted; and finally after discontinuing insulin for

a period long enough to produce insulin insufficiency while continuing the proper dietary regimen.

This report does not include the results of chromatographic studies which are at present under investigation. Many metabolites of tryptophan are present in urine in such small amounts that they can be detected only by chromatography; others appear in minute concentrations only in the disease state (Table 48). This method of investigation may provide further differences in tryptophan metabolism between normal and diabetic subjects.

SUMMARY

1. An investigation of tryptophan metabolism in normal male and female subjects has been carried out in the form of a two day test, preliminary to a proposed study of tryptophan metabolism in diabetes. Blood serum concentrations and urinary excretions of tryptophan and several of its metabolites were compared, at regular intervals, on a control day with those on a tryptophan load test day. A distinct pattern of urinary excretion increments were established. The patterns differed between male and female subjects in actual amount of individual metabolites excreted. The increments in urinary excretion of tryptophan and its metabolites measured accounted for 4% of the administered tryptophan in male subjects and 12% in female subjects. However, when the total dose accounted for was considered as 100%, there was no difference between male and female subjects in the proportion contributed by each metabolite.

2. Two subjects whose dietary intake of riboflavin did not meet the minimal requirements set by the Canadian Standards were investigated by the two day tryptophan load test. Their urinary excretion increments of kynurenine, after ingestion of tryptophan, were greater than those obtained in normal female subjects. In one of these subjects, the amounts of urinary metabolites excreted were decreased to the normal range after vitamin therapy.

3. It was found that basal two hour urinary excretions did not vary from day to day and that a two hour basal period could be used to predict the urinary excretion for the following six hours. After administration of tryptophan 70 - 90% of the twenty-four hour increment in excreted metabolites occurred within the first six hours. The combination of these observtions led to the modification of the two day tryptophan load test to an eight hour test. Recoveries of the administered amino acid as urinary metabolites amounted to approximately 3% in the male and 9% in the female subjects. The ratios of the recoveries were the same in the two day and eight hour tests. Thus, a practical method for the short term study of tryptophan metabolism has been developed.

4. The results obtained in these experiments were compared with results obtained by other workers as reported in the literature.

5. The possible causes for variations in the metabolism of a test dose of tryptophan were discussed.

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TABLE 6

SUBJECT # 1

Sex - male Age - 30 years Height 5' 8" Weight - 135 lbs.

Food Intake -

Control day : 1620 Calories; 69 g. protein; 212 g. carbohydrate; 55 g. fat; 675 mg. tryptophan.

Tryptophan load day: 1620 " ; 69 g. " ; 212 g. " ; 55 g. " ; 675 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting		1.44	--	--
2 hours		1.44	---	--
4 hours		1.30	--	--
6 hours				
24 hours		1.49	--	--
TRYPTOPHAN LOAD DAY				
Fasting		1.79	--	--
2 hours		14.64	--	1.97
4 hours		10.00	0.54	1.55
6 hours		5.10	0.78	1.03
24 hours		1.79	--	--

TABLE 7

SUBJECT # 2

Sex - male Age - 33 1/2 years Height 6' 1" Weight - 185 lbs.

Food Intake -

Control day: 2500 Calories; 110 g. protein; 268 g. carbohydrate; 110 g. fat; 1145 mg. tryptophan.
Tryptophan load day: 2400 " ; 113 g. " ; 253 g. " ; 105 g. " ; 1230 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	4.57	1.02	--	--
2 hours	6.08	1.44	--	--
4 hours	4.16	1.50	--	--
6 hours	6.01	1.50	--	--
24 hours	3.81	0.90	--	--
TRYPTOPHAN LOAD DAY				
Fasting	3.81	0.90	--	--
2 hours	5.19	8.29	--	--
4 hours	3.89	4.54	--	--
6 hours	4.52	2.32	--	--
24 hours	4.21	1.21	--	--

TABLE 8

SUBJECT # 3

Sex - male Age 33 1/2 years Height - 6' 1" Weight - 180 lbs.

Food Intake -

Control day : 2375 Calories; 128 g. protein; 184 g. carbohydrate; 125 g. fat; 1640 mg. tryptophan.
Tryptophan load day: 1945 " ; 115 g. " ; 102 g. " ; 115 g. " ; 1585 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	4.50	1.46	--	--
2 hours	4.27	1.46	--	--
4 hours	4.48	1.64	--	--
6 hours	5.89	1.93	--	--
24 hours	5.10	1.31	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.85	2.42	--	--
2 hours	4.35	11.31	--	--
4 hours	4.97	6.35	--	--
6 hours	4.73	3.58	--	--
24 hours	4.76	1.50	--	--

TABLE 9

SUBJECT # 4

Sex - male Age - 29 years Height - 5' 11" Weight - 174 lbs.

Food Intake -

Control day : 2844 Calories; 145 g. protein; 253 g. carbohydrate; 140 g. fat; 1185 mg. tryptophan.
Tryptophan load day: 2460 " ; 133 g. " ; 182 g. " ; 133 g. " ; 1490 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	5.08	1.69	--	--
2 hours	4.71	1.69	--	--
4 hours	4.71	1.85	--	--
6 hours	6.11	2.30	--	--
24 hours	5.01	1.91	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.89	1.24	--	--
2 hours	5.26	10.10	--	--
4 hours	4.53	5.41	--	--
6 hours	5.30	2.08	--	--
24 hours	4.84	1.38	--	--

TABLE 10

SUBJECT # 5b

Sex - female Age - 30 years Height - 5' 6 1/2" Weight - 124 lbs. (20th day of menstrual cycle)

Food Intake:-

Control day : 2102 Calories; 76 g. protein; 210 g. carbohydrate; 110 g. fat; 705 mg. tryptophan.
Tryptophan load day: 2107 " ; 74 g. " ; 194 g. " ; 115 g. fat; 755 mg. " .

After 1 month vitamin therapy of daily doses of: 15,000 IU. Vit.A; 2400 IU. Vit.D; 112.5 mg. Vit.C; 205 mg. liver yeast; 9 mg. B_1; 6 mg. B_2; 105 mg. B_6; 3µ B_{12}; 6 mg. pantothenol; 75 mg. nicotinamide. Vitamin therapy was discontinued one day prior to experiment.

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	4.70	1.13	--	--
2 hours	4.49	0.92	--	--
4 hours	4.20	1.29	--	--
6 hours	4.87	1.46	--	--
24 hours	4.28	1.21	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.28	1.21	--	--
2 hours	4.63	7.51	0.50	0.20
4 hours	4.11	5.38	1.08	--
6 hours	4.51	2.14	0.18	--
24 hours	3.62	1.21	--	--

TABLE 11

SUBJECT # 7

Sex - female Age - 25 years Height - 5' 3 1/2" Weight - 120 lbs. (17th day of menstrual cycle)

Food Intake -

Control day : 2068 Calories; 101 g. protein; 191 g. carbohydrate; 100 g. fat; 1095 mg. tryptophan.
Tryptophan load day: 2106 " ; 89 g. " ; 216 g. " ; 94 g. " ; 1115 mg. " .

BLOOD SERUM LEVELS

Time	α-Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	4.26	1.32	--	--
2 hours	4.75	1.26	--	--
4 hours	5.37	1.78	--	--
6 hours	5.39	1.78	--	--
24 hours	3.19	1.72	--	--
TRYPTOPHAN LOAD DAY				
Fasting	3.19	1.72	--	--
2 hours	3.34	13.11	--	--
4 hours	3.37	7.46	--	--
6 hours	6.06	3.90	--	--
24 hours	4.87	1.16	--	--

TABLE 12

SUBJECT # 8

Sex - female Age - 26 years Height 5' 5" Weight - 122 lbs. (15th day of menstrual cycle)

Food Intake -

Control day : 2178 Calories; 91 g. protein; 213 g. carbohydrate; 106 g. fat; 800 mg. tryptophan.
Tryptophan load day: 1856 " ; 67 g. " ; 226 g. " ; 76 g. " ; 755 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	5.25	1.72	--	--
2 hours	4.32	1.15	--	--
4 hours	5.34	1.38	--	--
6 hours	6.65	2.07	--	--
24 hours	6.72	1.49	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.92	1.26	--	--
2 hours	5.68	14.86	--	--
4 hours	4.82	8.63	--	--
6 hours	6.14	4.46	--	--
24 hours	4.89	1.67	--	--

TABLE 13

SUBJECT # 9

Sex - female Age - 22 Height - 5' 2" Weight - 130 lbs. (13th day of menstrual cycle)

Food Intake -

Control day : 1682 Calories; 73 g. protein; 181 g. carbohydrate; 74 g. fat; 820 mg. tryptophan.
Tryptophan load day: 1690 " ; 80 g. " ; 189 g. " ; 69 g. " ; 805 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	4.53	1.80	--	--
2 hours	4.23	1.62	--	--
4 hours	4.37	1.38	--	--
6 hours	4.80	0.90	--	--
24 hours	4.48	0.84	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.23	1.81	--	--
2 hours	4.88	15.03	--	--
4 hours	4.83	10.70	--	--
6 hours	5.04	5.21	--	--
24 hours	5.41	1.26	--	--

TABLE 14

BLOOD ∝ AMINO NITROGEN LEVELS (mg. %)

				Minus Theoretical Tryptophan ∝AN	
		Control Day	Tryptophan Load Day	Control Day	Tryptophan Load Day
fasting	♂	4.72 ± 0.13	4.52 ± 0.33	4.62 ± 0.17	4.41 ± 0.35
	♀	4.89 ± 0.27	4.20 ± 0.26	4.79 ± 0.28	4.10 ± 0.27
2 hours	♂	5.02 ± 0.54	4.94 ± 0.31	4.91 ± 0.56	4.27 ± 0.30
	♀	4.44 ± 0.11	4.86 ± 0.44	4.36 ± 0.02	3.98 ± 0.42
4 hours	♂	4.45 ± 0.16	4.46 ± 0.33	4.34 ± 0.09	4.09 ± 0.30
	♀	4.81 ± 0.25	4.44 ± 0.29	4.72 ± 0.22	3.89 ± 0.27
6 hours	♂	6.00 ± 0.15	4.85 ± 0.23	5.87 ± 0.15	4.67 ± 0.21
	♀	5.50 ± 0.34	5.57 ± 0.33	5.40 ± 0.32	5.31 ± 0.33
24 hours	♂	4.64 ± 0.41	4.60 ± 0.21	4.55 ± 0.38	4.50 ± 0.20
	♀	4.53 ± 0.59	4.66 ± 0.31	4.44 ± 0.59	4.57 ± 0.30

TABLE 15

BLOOD TRYPTOPHAN LEVELS (mg. %)

		Control Day	Tryptophan Load Day
fasting	♂	1.40 ± 0.13	1.59 ± 0.36
	♀	1.41 ± 0.14	1.47 ± 0.12
2 hours	♂	1.51 ± 0.06	11.10 ± 1.34
	♀	1.22 ± 0.11	12.86 ± 1.38
4 hours	♂	1.57 ± 0.11	6.58 ± 1.19
	♀	1.40 ± 0.11	8.05 ± 0.86
6 hours	♂	1.91 ± 0.23	3.27 ± 0.69
	♀	1.53 ± 0.19	4.00 ± 0.51
24 hours	♂	1.40 ± 0.20	1.47 ± 0.12
	♀	1.31 ± 0.14	1.33 ± 0.09

TABLE 16

SUBJECT # 1

URINE EXCRETIONS

Time	Urine (ml.)	Volume diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
0 - 2 hours		100	18.4	11.8	3.54	2.09	1.80	0.52
2 - 4 hours		100	11.1	10.2	2.74	1.45	1.25	0.36
4 - 6 hours		100	27.7	10.2	3.01	1.23	1.50	0.36
6 - 24 hours		400	156.8	28.4	9.35	4.33	12.00	2.87
Total			214.0	60.6	18.64	9.10	16.55	4.11
TRYPTOPHAN LOAD DAY								
0 - 2 hours		100	17.6	23.4	7.20	2.86	3.25	0.34
2 - 4 hours		100	6.4	26.0	24.80	3.52	4.85	0.17
4 - 6 hours		200	62.4	21.3	31.20	2.38	27.60	1.29
6 - 24 hours		800	148.0	92.0	9.80	9.10	20.80	6.11
Total			234.4	162.7	73.00	17.86	56.50	7.91
Difference			∤ 20.4	∤ 102.10	∤ 54.36	∤ 8.76	∤ 39.95	∤ 3.80
Basal 2 hours	78	100		14.03	2.01	1.05	2.75	1.08
" 2 "	64	100		9.98	0.91	1.24	2.75	0.80
" 2 "	80	110	28.92	9.17	0.13	1.39	1.80	0.43
" 2 "	70	100	19.83	9.71	0.09	1.22	1.50	0.54

TABLE 17

SUBJECT # 2

URINE EXCRETIONS

Time	Urine (ml.)	Volume diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Aeid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
basal 2 hours		100	31.30	7.48	0.55	0.61	2.60	1.04
0 - 2 hours		150	34.20	9.45	0	0.61	3.90	1.19
2 - 4 hours		100	10.00	6.30	1.00	0.21	1.80	0.41
4 - 6 hours		200	27.61	13.46	0	0.70	3.60	0.87
6 - 22 hours		1200	225.98	98.84	4.96	6.25	24.00	3.34
22 - 24 hours		150	32.75	13.94	0.21	1.05	2.70	1.18
Total			330.54	137.99	6.17	8.87	36.00	6.99
TRYPTOPHAN LOAD DAY								
basal 2 hours		150	32.75	13.94	0.21	1.05	2.70	1.18
0 - 2 hours		200	45.34	48.60	34.40	6.55	9.60	1.22
2 - 4 hours		200	23.03	40.80	18.80	2.22	10.20	0.74
4 - 6 hours		200	23.75	17.30	4.20	1.37	6.00	1.52
6 - 24 hours		1600	191.92	125.00	24.70	12.78	36.80	8.00
Total			284.04	231.70	82.10	22.92	62.60	11.48
Difference			-46.50	/ 93.71	/75.93	/14.05	/26.60	/4.49
Basal 2 hours	128	180	49.92	10.47	1.06	1.16	4.68	0.93
" 2 "	200	230		11.99	0.61	1.70	4.60	0.83

TABLE 18

SUBJECT # 3

URINE EXCRETIONS

Time	Urine (ml.)	Volume diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
basal 2 hours	27	50	8.20	3.39	0.64	0.46	0.75	0.51
0 - 2 hours	70	100	19.30	8.18	1.06	1.06	2.00	0.91
2 - 4 hours	81	100	24.60	8.65	1.06	1.22	2.00	1.11
4 - 6 hours	129	150	26.00	13.15	0.80	1.55	2.25	1.01
6 - 24 hours	1090	1200	268.86	82.73	10.91	12.40	18.00	6.17
Total			338.80	112.71	13.83	16.23	24.25	9.20
TRYPTOPHAN LOAD DAY								
basal 2 hours	90-	150	38.26	9.22	2.47	2.98	2.98	0.56
0 - 2 hours	86	110	11.63	60.42	3.20	3.29	5.50	0.65
2 - 4 hours	52	100	9.67	37.51	2.91	1.80	7.40	0.46
4 - 6 hours	96	130	17.94	38.70	1.03	1.76	7.35	1.13
6 - 24 hours	1020	1200	151.63	116.31	0.	7.31	21.60	7.39
Total			190.87	252.94	7.14	14.16	41.85	9.63
Difference			-147.93	∤140.23	-6.69	-2.07	∤17.60	∤0.43
Basal 2 hours	60	100		8.76	1.23	0.91	3.25	0.65
" 2 "	71	140		7.76	1.34	0.43	3.64	1.01

TABLE 19

SUBJECT # 4

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	α Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide
CONTROL DAY								
basal 2 hours		150	30.13	19.58	1.27	2.21	2.70	1.52
0 - 2 hours		150	21.88	22.11	1.73	2.58	3.00	1.06
2 - 4 hours		100	14.46	14.29	1.08	1.77	2.30	0.76
4 - 6 hours		100	31.09	18.90	2.70	2.33	5.10	0.78
6 - 24 hours	1420	1500	293.64	116.44	0.	16.10	34.50	7.00
Total			361.07	171.74	5.51	22.78	44.90	9.60
TRYPTOPHAN LOAD DAY								
basal 2 hours	75	100	28.93	11.69	0	2.98	2.75	1.35
0 - 2 hours	99	150	23.44	40.05	8.77	5.48	7.28	0.95
2 - 4 hours	74	150	20.17	44.75	11.94	4.06	10.88	0.90
4 - 6 hours	69	130	17.55	24.88	4.33	2.75	7.02	0.77
6 - 24 hours	1180	1300	266.79	105.07	11.61	17.79	29.90	8.61
Total			327.95	220.75	36.65	30.08	55.08	11.23
Difference			-33.12	↑49.01	↑31.14	↑7.30	↑10.18	↑1.63
Basal 2 hours		110	57.59	10.02	3.11	1.95	4.40	0.91
" 2 "		120	52.44	8.05	0.74	1.94	3.60	1.04

TABLE 20

SUBJECT # 5b

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝ Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
0 - 2 hours	152	190	27.75	15.77	0.17	1.14	5.70	2.54
2 - 4 hours	110	150	14.72	11.64	0	0.77	3.90	1.43
4 - 6 hours	56	100	31.24	13.90	1.26	1.17	3.25	1.00
6 - 22 hours	605	720	101.55	52.01	2.60	4.50	23.40	3.45
22 - 24 hours	43	80	21.62	14.50	0.58	1.26	4.12	0.91
			196.88	107.82	4.61	8.84	40.37	9.33
TRYPTOPHAN LOAD DAY								
basal 2 hours	43	80	21.62	14.50	0.58	1.26	4.12	0.91
0 - 2 hours	74	200	19.67	20.12	35.92	11.41	8.00	1.26
2 - 4 hours	57	200	24.18	297.23	73.23	34.44	13.90	1.56
4 - 6 hours	168	400	58.79	65.20	102.89	11.74	17.20	2.75
6 - 24 hours	670	900	215.48	63.47	10.56	9.54	24.75	9.84
Total			318.12	446.02	222.60	67.13	63.85	15.41
Difference			∤121.24	∤338.20	∤217.99	∤58.29	∤23.48	∤6.08
basal 2 hours	64	100		8.02	0.69	0.92	6.10	0.82
" 2 "	84	100		10.18	0.69	2.08	4.25	0.73

TABLE 21

SUBJECT # 7

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
basal 2 hours	71	100	22.70	19.31	2.12	1.90	3.85	0.56
0 - 2 hours	204	250	20.16	23.56	1.53	2.64	6.50	0.43
2 -4 hours	68	100	17.50	21.61	1.89	2.37	3.50	0.33
4 - 6 hours	114	150	26.05	23.62	2.91	2.90	6.38	0.33
6 - 22 hours	990	1100	255.90	126.54	11.65	13.32	38.50	1.10
22 - 24 hours	74	100	18.93	15.16	1.30	1.68	3.50	0.22
Total			338.54	210.49	19.28	22.91	58.38	2.41
TRYPTOPHAN LOAD DAY								
basal 2 hours	74	100	18.93	15.16	1.30	1.68	3.50	0.22
0 - 2 hours	74	100	23.26	95.87	34.38	26.58	13.25	0.32
2 - 4 hours	75	130	35.23	226.41	98.52	58.47	36.40	0.31
4 - 6 hours	75	150	28.02	105.52	10.73	2.65	13.88	0.34
6 - 24 hours	660	800	274.52	174.56	22.26	11.32	49.72	2.74
Total			361.03	602.36	165.89	99.02	113.25	3.71
Difference			⁄22.49	⁄391.87	⁄146.81	⁄76.11	⁄54.87	⁄1.30
Basal 2 hours	210	230		17.31	3.48	3.53	5.29	0.53
" 2 "	40	80		15.51	1.66	1.46	4.52	0.88
" 2 "	71	140		21.09	3.17	1.36	4.55	0.61

TABLE 22

SUBJECT # 8

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
basal 2 hours	73	100	35.85	21.49	5.30	0.89	3.50	0.91
0 - 2 hours	330	350	39.95	25.04	4.95	0.19	2.63	0.47
2 - 4 hours	71	90	34.50	14.07	1.91	0.66	1.80	0.65
4 - 6 hours	54	75	41.69	13.38	1.91	0.54	2.25	0.64
6 - 24 hours	578	650	324.18	158.49	1.20	4.12	21.13	4.43
Total			439.32	210.98	9.97	5.51	27.81	6.19
TRYPTOPHAN LOAD DAY								
basal 2 hours	27	50	20.99	9.94	1.26	0.77	1.50	0.70
0 - 2 hours	63	100	26.14	53.37	24.64	2.29	3.25	0.52
2 - 4 hours	56	100	20.11	49.26	50.65	4.08	9.60	0.81
4 - 6 hours	300	400	38.21	64.00	29.88	3.37	12.00	0.63
6 - 24 hours	890	1000	245.11	208.10	23.18	10.32	26.00	7.24
Total			329.57	374.73	128.35	20.06	50.85	9.20
Difference			-109.75	∤163.75	∤118.38	∤14.55	∤23.04	∤3.01
basal 2 hours	33	60		12.22	1.29	1.61	2.67	0.34
" 2 "	36	50		9.04	1.03	2.29	2.83	0.99

TABLE 23

SUBJECT # 9

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide
CONTROL DAY								
0 - 2 hours	142	190	30.47	19.35	0.17	2.46	3.80	1.29
2 - 4 hours	43	70	17.31	6.71	1.11	1.60	2.70	0.76
4 - 6 hours	48	80	21.47	14.38	1.48	1.49	3.10	0.88
6 - 22 hours	785	900	386.94	72.25	7.95	13.22	29.30	5.80
22 - 24 hours	36	50	23.50	7.19	0.35	0.87	2.55	0.72
Total			479.69	119.88	11.06	19.64	41.45	9.45
TRYPTOPHAN LOAD DAY								
basal 2 hours	87	110	26.22	12.67	1.29	1.69	2.86	0.66
0 - 2 hours	134	170	25.78	30.79	8.20	4.10	6.80	0.90
2 -4 hours	64	120	27.40	37.78	32.33	7.15	3.90	1.15
4 - 6 hours	70	130	30.84	36.37	9.48	3.21	9.10	1.50
6 - 24 hours	1020	1200	378.26	164.56	9.88	18.99	39.00	12.94
Total			462.28	269.50	59.89	33.45	58.80	16.59
Difference			-17.41	⁄149.62	⁄48.83	⁄13.81	⁄17.35	⁄7.14
basal 2 hours	70	100		6.35	0.77	1.55	4.80	0.79
" 2 "	76	100		12.95	1.29	2.50	4.00	0.91

TABLE 24

URINE ∝AMINO NITROGEN EXCRETION (mg.)

		Control Day	Tryptophan Load Day
Basal 2 hours	♂	31.48 ± 5.11	
	♀	30.08 ± 3.98	
0 - 2 hours	♂	23.45 ± 3.65	24.50 ± 7.36
	♀	28.23 ± 3.43	25.89 ± 2.46
2 - 4 hours	♂	15.04 ± 3.32	14.82 ± 4.01
	♀	18.65 ± 4.23	27.00 ± 2.50
4 - 6 hours	♂	28.10 ± 1.07	30.41 ± 11.13
	♀	21.77 ± 6.01	33.49 ± 7.67
6 - 24 hours	♂	251.26 ± 31.90	189.59 ± 27.58
	♀	245.52 ± 59.95	235.31 ± 51.03
0 - 24 hours	♂	311.10 ± 33.01	259.31 ± 29.77
	♀	317.57 ± 60.00	321.70 ± 52.59
Theoretical ∝AN due to:			
Tryptophan	♂	8.25 ± 1.59	14.58 ± 1.30
	♀	9.98 ± 1.59	26.39 ± 4.53
Kynurenine	♂	0.74 ± 0.21	3.35 ± 1.15
	♀	00.72 ± 0.15	12.27 ± 3.31
Measured 24 hour ∝AN - theoretical			
	♂	302.11 ± 31.71	241.00 ± 29.46
	♀	306.83 ± 65.96	282.82 ± 54.66

TABLE 25

24 HOUR TRYPTOPHAN LOAD TEST

Urine Excretion of Tryptophan and Its Metabolites.
(4 male subjects and 4 female subjects)

Control Day.

		Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.) / Chromogens	N'-methyl-nicotinamide (mg.)
Mean basal	♂	10.33 ± 1.06	1.03 ± 0.18	1.46 ± 0.26	2.97 ± 0.32	0.90 ± 0.12
	♀	12.57 ± 1.89	1.53 ± 0.43	1.49 ± 0.20	3.84 ± 0.46	0.72 ± 0.04
0 - 2 hours	♂	12.89 ± 3.16	1.58 ± 0.74	1.59 ± 0.45	2.68 ± 0.47	0.92 ± 0.14
	♀	20.94 ± 2.06	1.71 ± 1.12	1.61 ± 0.57	4.66 ± 0.87	1.18 ± 0.49
2 - 4 hours	♂	9.86 ± 1.68	1.47 ± 0.42	1.18 ± 0.32	1.84 ± 0.21	0.66 ± 0.17
	♀	13.51 ± 3.10	1.23 ± 0.44	1.35 ± 0.39	2.98 ± 0.45	0.79 ± 0.23
4 - 6 hours	♂	13.93 ± 1.81	1.63 ± 0.73	1.45 ± 0.33	3.11 ± 0.79	0.76 ± 0.13
	♀	16.32 ± 2.44	1.89 ± 0.36	1.53 ± 0.49	3.75 ± 0.87	0.71 ± 0.15
6 - 24 hours	♂	85.09 ± 20.39	6.36 ± 2.69	10.03 ± 2.54	22.80 ± 4.93	5.14 ± 0.91
	♀	111.54 ± 22.67	6.41 ± 2.64	9.74 ± 2.80	30.63 ± 4.36	4.16 ± 1.06
0 - 24 hours	♂	120.76 ± 23.41	11.04 ± 3.19	14.25 ± 3.31	30.43 ± 6.25	7.48 ± 1.25
	♀	162.29 ± 28.07	11.23 ± 3.03	14.23 ± 4.17	42.00 ± 6.28	6.85 ± 1.65

TABLE 25 (cont.)

After 4 g. l-tryptophan

		Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.) ± Chromogens	N'-methyl-nicotinamide (mg.)
0 - 2 hours	♂	44.62 ± 7.73	13.39 ± 7.09	4.55 ± 0.88	6.41 ± 1.34	0.79 ± 0.19
	♀	50.04 ± 16.77	25.79 ± 6.36	11.10 ± 5.52	5.33 ± 3.90	0.75 ± 0.20
2 - 4 hours	♂	37.27 ± 4.03	14.61 ± 4.36	2.90 ± 0.53	8.33 ± 1.38	0.57 ± 0.15
	♀	152.67 ± 64.69	63.68 ± 14.31	26.04 ± 12.85	15.95 ± 7.11	0.96 ± 0.26
4 - 6 hours	♂	24.05 ± 4.95	10.19 ± 7.04	2.07 ± 0.30	11.99 ± 5.21	1.18 ± 0.15
	♀	67.77 ± 14.23	38.25 ± 22.23	5.24 ± 2.17	13.05 ± 1.68	1.31 ± 0.53
6 - 24 hours	♂	109.59 ± 7.14	11.53 ± 4.88	11.75 ± 2.31	27.32 ± 3.67	7.53 ± 0.52
	♀	152.67 ± 31.16	16.47 ± 3.61	12.54 ± 2.18	34.87 ± 5.90	8.19 ± 2.15
0 - 24 hours	♂	217.02 ± 19.30	49.72 ± 17.26	21.26 ± 3.43	54.01 ± 4.35	10.06 ± 0.83
	♀	423.15 ± 167.97	144.18 ± 34.13	54.92 ± 17.71	71.69 ± 14.10	11.23 ± 2.98

TABLE 26

DIFFERENCES IN EXCRETIONS (TRYPTOPHAN LOAD DAY minus CONTROL DAY)

(4 male subjects and 4 female subjects)

		Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N^1-methyl-nicotinamide (mg.)
0 - 2 hours	♂	31.73 ± 8.86	11.31 ± 7.68	2.98 ± 1.08	3.68 ± 0.88	-0.13 ± 0.06
	♀	28.61 ± 14.78	24.08 ± 6.38	9.44 ± 5.23	3.17 ± 1.29	-0.43 ± 0.29
2 - 4 hours	♂	27.41 ± 4.03	13.84 ± 4.76	1.75 ± 0.38	6.49 ± 1.28	-0.09 ± 0.21
	♀	139.16 ± 62.74	62.46 ± 14.27	24.69 ± 12.54	12.98 ± 6.26	0.17 ± 0.08
4 - 6 hours	♂	11.62 ± 4.88	8.56 ± 6.59	0.61 ± 0.20	8.88 ± 1.42	0.45 ± 0.23
	♀	51.45 ± 24.47	55.78 ± 22.48	3.72 ± 2.37	9.30 ± 1.73	0.79 ± 0.42
6 - 24 hours	♂	24.51 ± 15.90	5.17 ± 6.63	1.70 ± 2.59	4.48 ± 3.34	2.36 ± 0.59
	♀	41.14 ± 18.31	12.10 ± 3.32	2.80 ± 2.21	4.24 ± 2.41	4.03 ± 1.16
0 - 24 hours	♂	96.29 ± 18.72	38.69 ± 17.67	6.98 ± 3.33	24.73 ± 5.66	2.57 ± 0.95
	♀	260.86 ± 62.17	132.95 ± 35.01	40.69 ± 15.73	29.69 ± 8.45	4.38 ± 1.35
mM (24 hours)	♂	0.4714 ± 0.0916	0.1939 ± 0.0784	0.0549 ± 0.0211	0.1150 ± 0.0311	0.0189 ± 0.0068
	♀	1.2773 ± 0.2994	0.6386 ± 0.1680	0.2968 ± 0.1182	0.1447 ± 0.0414	0.0320 ± 0.0097
% Recovery of tryptophan	♂	2.40 ± 0.47	0.99 ± 0.39	0.28 ± 0.10	0.59 ± 0.15	0.10 ± 0.03
	♀	6.52 ± 1.52	3.26 ± 0.85	1.51 ± 0.58	0.74 ± 0.20	0.17 ± 0.04

TABLE 27

ANALYSIS OF VARIANCE

1. Tryptophan Excretion during 2 hour intervals on the Control Day.

Variation	SS	DF	MS
Between individuals	669.7822	7	95.6832
Between time intervals	130.9560	3	43.6520
Residual	229.1021	21	10.9096
Total	1029.8403	31	----

Variance Ratios	
Between individuals/Residual	8.7706
Between intervals/Residual	4.0012

2. Kynurenine Excretion during 2 hour intervals on the Control Day.

Variation	SS	DF	MS
Between individuals	29.1529	7	4.1647
Between intervals	3.9833	3	1.3278
Residual	12.6604	21	0.6029
Total	45.7966	31	----

Variance Ratios	
Between individuals/Residual	6.9078
Between intervals/Residual	2.2023

3. Anthranilic Acid Excretion during 2 hour intervals on the Control Day.

Variation	SS	DF	MS
Between individuals	14.3115	7	2.0445
Between intervals	0.6197	3	0.2066
Residual	2.6030	21	0.1240
Total	17.5342	31	----

Variance Ratios	
Between individuals/Residual	16.4879
Between intervals/Residual	1.6661

TABLE 27

ANALYSIS OF VARIANCE (cont.)

4. Xanthurenic Acid (Chromogens) Excretion during 2 hour intervals on the Control Day.

Variation	SS	DF	MS
Between individuals	38.0797	7	5.4400
Between intervals	7.3471	3	2.4490
Residual	16.4094	21	0.7814
Total	61.8362	31	----

Variance Ratios	
Between individuals/Residual	6.9619
Between intervals/Residual	3.1341

TABLE 28

ANALYSIS OF VARIANCE

1. Basal Excretion of Tryptophan on Different Days.

Variations	SS	DF	MS
Between individuals	220.3879	7	31.4840
Between days	75.0214	3	25.0071
Residual	233.5331	21	11.1206
Total	528.9424	31	

Variance Ratios	
Between individuals/Residual	2.8311
Between days/Residual	2.2487

2. Basal Excretion of Kynurenine on Different Days.

Variations	SS	DF	MS
Between individuals	11.8877	7	1.5554
Between days	4.2555	3	1.4188
Residual	21.7048	21	1.0336
Total	37.8480	31	-----

Variance Ratios	
Between individuals/Residual	1.5048
Between days/Residual	1.3727

3. Basal Excretion of Anthranilic Acid on Different Days.

Variations	SS	DF	MS
Between individuals	5.4437	7	0.7780
Between days	1.0842	3	0.3614
Residual	10.9951	21	0.5236
Total	17.5230	31	----

Variance Ratios	
Between individuals/Residual	1.4859
Between days/Residual	0.6902

TABLE 28

ANALYSIS OF VARIANCE (cont.)

4. Basal Excretion of Xanthurenic Acid (Chromogens) of Different Days.

Variations	SS	DF	MS
Between individuals	20.7534	7	2.9648
Between days	5.1324	3	1.7108
Residual	16.8910	21	0.8043
Total	42.7768	31	-----

Variance Ratios	
Between individuals/Residual	3.6862
Between days/Residual	2.1271

TABLE 29

TOTAL 0 - 6 HOUR EXCRETION INCREMENTS OF TRYPTOPHAN AND ITS METABOLITES IN 4 NORMAL MALE 4 NORMAL FEMALE SUBJECTS

		Control Day	Tryptophan Load Day	Increment
Tryptophan (mg.)	♂	36.67 ± 6.24	110.18 ± 38.19	73.51 ± 13.54
	♀	50.73 ± 6.62	270.48 ± 73.87	219.75 ± 76.06
Kynurenine (mg.)	♂	4.68 ± 1.76	38.19 ± 13.32	33.52 ± 16.09
	♀	4.82 ± 1.67	130.46 ± 35.94	125.64 ± 36.47
Anthranilic Acid (mg.)	♂	4.21 ± 1.06	9.51 ± 0.76	5.30 ± 1.21
	♀	4.48 ± 1.42	42.36 ± 18.53	37.88 ± 17.67
Xanthurenic Acid (mg.)	♂	7.63 ± 1.34	26.73 ± 3.24	19.13 ± 4.07
	♀	11.38 ± 2.08	36.82 ± 9.79	25.44 ± 7.94

Analysis of Variance (Increments)

Variation	SS	DF	MS
Between excretions	84312.6083	3	28104.2028
Between sexes	38505.9001	1	38505.9001
Interaction	23591.1070	3	7863.7023
Within classes (error)	94496.2456	24	3937.3436
Total	240905.8610	31	-------

Variance ratios

excretions/error	7.1379
sexes/error	9.7797
interaction error	1.9972

TABLE 30

DISTRIBUTION PATTERN OF EXCRETED TRYPTOPHAN AND METABOLITES EXPRESSED AS % OF TOTAL DOSE ACCOUNTED FOR IN 24 HOURS

(4 normal males and 4 normal females)

	Male	Female
Tryptophan	57.73 ± 10.46	55.21 ± 3.01
Kynurenine	22.26 ± 7.74	27.22 ± 3.99
Anthranilic Acid	6.71 ± 2.41	11.18 ± 2.11
Xanthurenic Acid	13.29 ± 2.08	6.40 ± 0.96

Analysis of Variance

Variation	SS	DF	MS
Between excretions	11824.4916	3	5761.1480
Between sexes	0	1	0
Interaction	196.7786	3	65.5929
Within classes (error)	2519.7774	24	104.9907
Total	14541.0476	31	-------

Variance ratios

excretions/error	54.8729
sexes/error	0
interaction/error	0.6247

TABLE 31

URINARY TRYPTOPHAN EXCRETION IN MAN

Reference number	Conditions of Experiment	Total tryptophan (mg./24 hours) Males	Total tryptophan (mg./24 hours) Females	Free tryptophan Males	Free tryptophan Females	Method of Estimation
126	normal diet			18	14.1	microbiological assay
127	normal diet	41.4 ± 17.5		24.6 ± 11.3		microbiological assay
128	25 g. protein (0.31 g.tryptophan)	31(22-46)				microbiological assay
	100 g. protein (1.16 g.tryptophan)	41(29-70)				
	200 g. protein (2.37 g.tryptophan)	33(22-42)				
129	normal diet	22.9(17-32)	16.6(12-26)			microbiological assay
130	normal diet	281 ± 30				chemical
131	AM fasting			1.0-3.4/2 hrs.		microbiological assay
132	N(g.) tryptophan(mg.)					microbiological assay
	9.8 885		30.6(22.7-53.6)		10.8(6.8-17.6)	
	7.22 530		25.9(22.7-32.5)		10.3(7.1-12.9)	
	8.42 616		26.6(23.1-32.0)		11.3(9.4-13.5)	
	9.51 595		36.1(34.6-37.6)		14.4(13.6-15.2)	
	8.85 552		27.7(24.4-31.1)		8.5(7.3-9.7)	
133	normal diet				12.6-30.5	microbiological assay
134	30 g. protein				6.9(5.5-9.8)	microbiological assay
135	70 g. protein (813 mg. tryptophan)			8-22		microbiological assay

TABLE 32

TRYPTOPHAN LEVELS IN HUMAN BLOOD (mg. %)

Reference number	Conditions of Experiment (Postabsorptive)	Serum Males	Plasma Males	Plasma Females	Method of Estimation
136		1.21(0.95-1.40)	1.14(0.85-1.30)		Microbiological assay
137			1.11 ± 0.23	0.98 ± 0.11	Microbiological assay
138			1.27 ± 0.24		Microbiological assay
128	25 g. protein (0.31 g. tryptophan)		1.5(1.0-1.8)		Microbiological assay
	100 g. protein (1.16 g. tryptophan)		1.7(1.6-1.8)		Microbiological assay
	200 g. protein (2.37 g. tryptophan)		2.0(1.6-2.3)		Microbiological assay
131			1.78		Microbiological assay
139	Aged adult		1.03 ± 0.20	0.91 ± 0.23	Microbiological assay
	Young adult		1.17 ± 0.18	1.18 ± 0.22	Microbiological assay

TABLE 33

METABOLITES OF TRYPTOPHAN EXCRETED IN HUMAN URINE

Reference number	Metabolite	Condition of Experiment	Condition of Subject	Values (mg./24 hours)
140	Acetylkynurenine	normal diet	normal	8 μM.
		19.6 mM. l-tryptophan	"	56 μM.
	Kynurenine	normal diet	"	15 μM.
		19.6 mM. l-tryptophan	"	121 μM.
141	Kynurenine	normal diet	"	0.2/100 ml.
142	Kynurenine	1.5 g. l-tryptophan	normal	0.38/100 ml./12 hrs.
			malignancy	0.93/100 ml./12 hrs.
14	Kynurenine	2 g. d-tryptophan	normal	80-200 (d-form)
143	Xanthurenic Acid	100 mg. l-tryptophan	normal infant	0.58
			normal baby	7.64
			normal female	94.12
88	Xanthurenic Acid	10 g. dl.-tryptophan	normal male & female	17(4-41)/10 hrs.
			normal pregnancy	251(116-741)/10 hrs.
		10 g. dl.-tryptophan + 50 mg. B_6	normal pregnancy	13/10 hrs.
		10 g. dl.-tryptophan + 200 mg. niacin	normal pregnancy	221(78.3-452.3)/10 hrs.
		1 g. dl.-tryptophan	newborn	0-97
87	Xanthurenic Acid	10 g. dl.-tryptophan	normal	14 ± 2.3
			pregnancy	195 ± 14
			toxic pregnancy	285 ± 37
89	Xanthurenic acid	10 g. dl.-tryptophan	normal	9.76(0-20.35)
			diabetic	25.02(6.06-54.23)
			diabetic + retinopathy	38.22(15.31-84.26)

TABLE 33 (cont.)

METABOLITES OF TRYPTOPHAN EXCRETED IN HUMAN URINE

Reference number	Metabolite	Condition of Experiment	Condition of Subject	Values (mg./24 hours.)
140	Xanthurenic Acid	normal diet	normal	70 µM.
		19.6 mM. l-tryptophan	"	103 µM.
140	Anthranilic Acid-glucuronide	Normal diet	normal	6 µM.
		19.6 mM. l-tryptophan	normal	14 µM.
140	o-aminohippuric Acid	normal diet	normal	29 µM.
		19.6 mM. l-tryptophan	"	86 µM.
62	Quinolinic Acid	normal diet	normal	3.1-5.5
		* 5 x 1 g. dl.-tryptophan	"	10.6-11.9
69	N'-methylnicotinamide	normal diet	normal female	7.2
		5 g. dl.-tryptophan	normal female	13.9
		normal diet	normal male	5.9
		5 g. dl.-tryptophan	" "	12.9
144	N'-methylnicotinamide	normal diet - 2 hours after breakfast	normal	5.75-10.6 µg./ml.
145	N'-methylnicotinamide	normal diet	normal	5.5(3.1-8.0)
		50 mg. nicotinamide	"	13.8(8.7-22.7)
65	N'-methylnicotinamide	normal diet	normal	7(4-12)
74	N'-methylnicotinamide	1 g. l-tryptophan (per os or IV)	2.24 month infants	1.7

* Administration of tryptophan took place in five separate doses at four hour intervals.

TABLE 33 (cont.)

METABOLITES OF TRYPTOPHAN EXCRETED IN HUMAN URINE

Reference number	Metabolite	Condition of Experiment	Condition of Subject	Values (mg./24 hours)
78	N'-methylnicotinamide	normal diet	1 day old	3.2
		100 mg. nicotinamide	baby	36.7(11.5-95)
		100 mg. nicotinamide + 0.3 g. methionine	baby	32.4
68	N'-methylnicotinic Acid	normal diet	corn diet	19.6 - 20-6
		5 g. dl.-tryptophan	" "	27.6-30
		normal diet	wheat diet	2.5-16.0
		5 g. dl.-tryptophan	" "	13.8-25.1
65	N'-methyl-2 pyridone-5 carboxamide	normal diet	normal	8.6(3.6-12)
71	N'-methyl-2 pyridone-5 carboxamide	normal diet	"	6.7-21
			diabetic	3.3-20.4
			epilepsy	19.5
			Tuberculosis	9.9
			cancer	3.3
72	N'-methyl-2 pyridone-5 carboxamide	normal diet	normal	18-20
146	N'-methyl-2 pyridone-5 carboxylic Acid	normal diet	normal	3-6
40	5-hydroxyindoleacetic Acid	normal diet	normal	7(2-8)
			argentaffinoma	< 350
31		normal diet	normal	2-9
			carcinoid	76-580

TABLE 33 (cont.)

METABOLITES OF TRYPTOPHAN EXCRETED IN HUMAN URINE

Reference number	Metabolite	Condition of Experiment	Condition of Subject	Values (mg./24 Hours)
41	5-hydroxyindoleacetic Acid	normal diet	normal male	2.00 $\pm$ 0.10 µg./mg. creatinine
			normal female	3.15 $\pm$ 0.36 µg./mg. creatinine
			psychiatric ill male	2.67 $\pm$ 0.26 µg./mg. creatinine
			psychiatric ill female	3.42 $\pm$ 0.37 µg./mg. creatinine
			schizophrenic male	2.16 $\pm$ 0.16 µg./mg. creatinine
			schizophrenic female	3.21 $\pm$ 0.30 µg./mg. creatinine
13	indolelactic acid	normal diet	phenyl ketonuria	20-150 mg./g. creatinine.

TABLE 36

RESULTS OF DIET HISTORY SURVEY ON SUBJECTS

Subject	Food Intake							Recommended Riboflavin Intake Based on Caloric Intake - Can. Standards.
	CHO	Protein	Fat	Calories	Thiamine	Riboflavin	Niacin	
#1 ♂	299	109	101	2538	1.11	2.82	16.21	1.27
2 ♂	332	151	156	3330	1.43	2.78	22.3	1.66
3 ♂	125	70	87	1494	0.59	1.55	13.4	0.17
4 ♂	215	87	100	2108	0.93	1.52	15.0	1.05
5 ♀	153	69	71	1527	0.66	0.54	13.5	0.76
6 ♀	234	78	82	1982	0.88	0.96	16.4	0.99
7 ♀	146	67	71	1505	0.60	1.25	13.9	0.75
8 ♀	195	76	73	1736	0.79	1.18	12.7	0.87
9 ♀	162	73	60	1475	0.71	1.58	9.9	0.84
10 ♀	164	75	89	1752	0.98	1.48	10.1	0.88
11 ♀	212	63	60	1643	0.94	1.28	10.8	0.82
12 ♀	242	77	76	1963	0.95	1.34	12.1	0.98

TABLE 37

SUBJECT # 5a

Sex - female Age - 30 years Height - 5' 6 1/2" Weight - 124 lbs. (12th day of menstrual cycle)

Food Intake -

Control day : 1930 Calories; 44 g. protein; 202 g. carbohydrate; 100 g. fat; 400 mg. tryptophan.
Tryptophan load day: 1600 " ; 39 g. " ; 155 g. " ; 80 g. " ; 400 mg. " .

BLOOD SERUM LEVELS

time	αAmino N (mg. %)	Tryptophan (mg. %)	Kynuranine (mg. %)	Anthranilic Acid (mg. %)
CONTROL DAY				
fasting		1.81	--	--
2 hours		1.36	--	--
4 hours		1.58	--	--
6 hours		1.20	--	--
24 hours		1.16	--	--
TRYPTOPHAN LOAD DAY				
fasting		1.16	--	--
2 hours		11.13	1.17	--
4 hours		5.90	2.10	--
6 hours		2.15	0.85	--
24 hours		1.05	--	--

TABLE 38

SUBJECT # 6

Sex - female Age - 24 years Height - 5' 6" Weight - 106 lbs. (15th day of menstrual cycle)

Food Intake -

Control day : 1270 Calories; 43 g. protein; 151 g. carbohydrate; 55 g. fat; 440 mg. tryptophan.
Tryptophan load day: 1388 " ; 55 g. " ; 119 g. " ; 78 g. " ; 670 mg. " .

BLOOD SERUM LEVELS

Time	Amino N (mg. %)	Tryptophan (mg. %)	Kynurenine (mg.%	Anthranilic Acid (mg. %)
CONTROL DAY				
Fasting	5.72	1.10	--	--
2 hours	4.42	1.15	--	--
4 hours	4.78	1.15	--	--
6 hours	5.80	1.46	--	--
24 hours	3.99	1.31	--	--
TRYPTOPHAN LOAD DAY				
Fasting	4.36	1.36	--	--
2 hours	5.77	13.80	1.16	--
4 hours	5.05	8.06	3.33	--
6 hours	6.10	4.28	3.18	--
24 hours	4.53	1.36	--	--

TABLE 39

SUBJECT # 5a

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
basal 2 hours		50	27.05	9.31	0.89	1.26	4.38	0.64
0 - 2 hours		100	7.62	8.92	1.33	1.06	3.50	0.59
2 - 4 hours		150	7.62	13.21	0	1.62	4.13	0.99
4 - 6 hours		150	4.19	10.12	0	1.24	3.90	0.51
6 - 22 hours		500	199.50	71.50	7.45	5.64	32.00	4.31
22 - 24 hours		50	22.55	7.81	0.43	0.56	2.70	0.58
Total			241.88	111.64	9.21	10.12	46.23	6.98
TRYPTOPHAN LOAD DAY								
basal 2 hours		50	22.55	7.81	0.43	0.56	2.70	0.58
0 - 2 hours		50	26.50	191.80	42.60	18.57	5.10	0.41
2 -4 hours		100	32.50	582.50	212.00	83.50	16.25	0.53
4 - 6 hours		100	40.20	87.40	99.00	14.57	10.40	1.12
6 - 24 hours		400	178.00	88.60	7.10	5.07	25.60	8.00
Total			277.20	950.30	360.70	121.71	57.35	10.06
Difference			⁄85.32	⁄838.66	⁄351.49	⁄111.59	⁄11.12	⁄3.08
basal 2 hours		55	30.08	8.02	0.32	1.06		

TABLE 40

SUBJECT # 6

URINE EXCRETIONS

Time	Urine (ml.)	Volume Diluted	∝Amino N (mg.)	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
CONTROL DAY								
0 - 2 hours		500	22.8	11.0	0.61	1.15	5.00	0.43
2 - 4 3/4 hours		300	9.2	11.0	0.73	0.99	3.00	0.72
4 3/4 hours - 6 hours		250	6.4	7.3	0.61	0.64	2.50	0.26
6 - 24 hours		850	95.0	49.8	6.19	4.32	19.55	1.63
Total			133.4	79.1	8.14	7.10	30.05	3.04
TRYPTOPHAN LOAD DAY								
0 - 2 hours		215	34.6	42.3	39.40	5.33	6.45	0.34
2 - 4 3/4 hours		200	28.1	53.8	208.50	16.14	14.40	0.81
4 3/4 - 6 hours		400	11.6	41.7	86.30	4.50	14.00	0.22
6 - 24 hours		1000	63.2	94.5	15.90	6.74	27.50	6.43
Total			137.5	232.3	350.10	32.71	62.35	7.80
Difference			∤ 4.1	∤153.2	∤341.96	∤25.61	∤32.30	∤4.76

TABLE 41

URINE EXCRETION OF TRYPTOPHAN AND METABOLITES

(Tryptophan load day - control day)

Expressed as % of total 24 hour difference

		0 - 2 hours	0 - 4 hours	0 - 6 hours
Tryptophan	♂	32.49 ± 7.10	63.32 ± 13.87	74.09 ± 13.11
	♀	13.02 ± 3.67	54.25 ± 10.38	75.05 ± 9.99
Kynurenine	♂	26.71 ± 8.67	65.85 ± 10.06	83.24 ± 9.90
	♀	16.64 ± 1.75	69.72 ± 7.05	93.15 ± 2.99
Anthranilic Acid	♂	41.27 ± 13.29	63.34 ± 12.72	71.00 ± 11.58
	♀	18.03 ± 3.12	68.17 ± 10.70	82.20 ± 8.80
Xanthurenic Acid	♂	18.19 ± 5.39	50.52 ± 15.20	79.60 ± 7.78
	♀	9.11 ± 3.05	43.93 ± 9.20	79.79 ± 7.78
N'-Methyl-nicotinamide	♂			21.83 ± 10.02
	♀			6.30 ± 2.20

TABLE 45

SHORT TERM TEST

8 Female Subjects.

BLOOD TRYPTOPHAN LEVELS (mg. %)

fasting	1.50 ± 0.10
2 hours	13.35 ± 0.95
4 hours	8.86 ± 0.92
6 hours	4.47 ± 0.57

BLOOD KYNURENINE LEVELS (mg. %)

fasting	0
2 hours	0.40 ± 0.14
4 hours	0.86 ± 0.40
6 hours	0.52 ± 0.38

TABLE 46

SHORT TERM TEST

URINE EXCRETIONS

Time	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)
SUBJECT # 1				
0 - 6 hours (/ tryptophan)	70.70	63.20	8.76	35.70
Theoretical 0 - 6 hours (3 x basal)	28.32	2.37	3.69	4.95
Increase over theoretical	42.38	60.83	5.07	30.75
Increase in mM	0.2075	0.2922	0.0370	0.1499
% dose accounted for	1.06	1.49	0.19	0.76
SUBJECT # 2				
0 - 6 hours (/ tryptophan)	106.70	57.40	10.14	25.80
Theoretical 0 - 6 hours (3 x basal)	41.82	0.63	3.15	8.10
Increase over theoretical	64.88	56.77	6.99	17.70
Increase in mM	0.3177	0.2438	0.0510	0.0863
% dose accounted for	1.62	1.24	0.26	0.44
SUBJECT # 3				
0 - 6 hours (/ tryptophan)	136.73	7.14	6.85	20.25
Theoretical 0 - 6 hours (3 x basal)	27.66	7.41	8.94	8.97
Increase over theoretical	109.07	0	0	11.28
Increase in mM	0.5341	0.00	0.00	0.550
% dose accounted for	2.73	0	0	0.28
SUBJECT # 4				
0 - 6 hours (/ tryptophan)	115.68	25.04	12.29	25.18
Theoretical 0 - 6 hours (3 x basal)	35.07	3.84	8.94	8.25
Increase over theoretical	80.61	21.20	3.35	16.93
Increase in mM	0.3947	0.1018	0.0244	0.0825
% dose accounted for	2.01	0.52	0.12	0.42
SUBJECT # 5a				
0 - 6 hours (/ tryptophan)	832.57	320.60	111.79	28.29
Theoretical 0 - 6 hours (3 x basal)	23.43	1.29	1.68	8.10
Increase over theoretical	809.14	319.31	110.11	20.19
Increase inmM	3.9621	1.5336	0.8030	0.0984
% dose accounted for	20.21	7.82	4.10	0.50

TABLE 46

SHORT TERM TEST (cont.)

URINE EXRETIONS

Time	Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)
SUBJECT # 5b				
0 - 6 hours (/ tryptophan)	382.55	212.04	57.59	39.10
Theoretical 0 - 6 hours (3 x basal)	43.50	1.74	3.78	12.36
Increase over theoretical	339.05	210.30	53.81	26.74
Increase in mM	1.6602	1.010	0.3924	0.1303
% dose accounted for	8.47	5.15	2.00	0.66
SUBJECT # 6				
0 - 6 hours (/ tryptophan)	137.80	334.20	25.97	34.85
0 - 6 hours (control)	29.30	1.95	2.78	10.50
Increase	108.50	332.25	23.19	24.35
Increase in mM	0.5313	1.5957	0.1691	0.1187
% dose accounted for	2.71	8.10	0.86	0.61
SUBJECT # 7				
0 - 6 hours (/ tryptophan)	427.80	143.63	87.70	63.53
Theoretical 0 - 6 hours (3 x basal)	45.48	3.90	5.04	10.50
Increase over theoretical	382.32	139.73	82.66	53.03
Increase in mM	1.8721	0.6771	0.6028	0.2585
% dose accounted for	9.55	3.45	3.08	1.32
SUBJECT # 8				
0 - 6 hours (/ tryptophan)	166.63	105.17	9.74	24.85
Theoretical 0 - 6 hours (3 x basal)	29.82	3.78	2.31	4.50
Increase over theoretical	136.81	101.39	7.43	20.35
Increase in mM	0.6699	0.4870	0.0542	0.0992
% dose accounted for	3.42	2.48	0.28	0.51
SUBJECT # 9				
0 - 6 hours (/ tryptophan)	104.94	50.01	14.46	19.80
Theoretical 0 - 6 hours (3 x basal)	38.01	3.87	5.04	8.58
Increase over theoretical	66.93	46.14	9.42	11.22
Increase in mM	0.3277	0.2216	0.0687	0.0547
% dose accounted for	1.67	1.13	0.35	0.28

TABLE 47

SHORT TERM TRYPTOPHAN LOAD TEST

Urine Excretion of Tryptophan and Its Metabolites
(4 male subjects and 7 female subjects)

		Tryptophan (mg.)	Kynurenine (mg.)	Anthranilic Acid (mg.)	Xanthurenic Acid (mg.)	N'-methyl-nicotinamide (mg.)
basal 2 hours	♂	11.07 ± 1.11	1.19 ± 0.95	2.06 ± 0.53	2.52 ± 0.30	0.95 ± 0.18
	♀	11.86 ± 1.27	1.06 ± 0.11	1.11 ± 0.14	2.79 ± 0.33	0.56 ± 0.13
0 - 2 hours	♂	44.62 ± 7.73	13.39 ± 7.09	4.55 ± 0.88	6.41 ± 1.34	0.79 ± 0.19
	♀	40.44 ± 10.07	23.31 ± 5.80	7.42 ± 3.43	7.58 ± 1.13	0.65 ± 0.12
2 - 4 hours	♂	37.27 ± 4.03	14.61 ± 4.36	2.90 ± 0.53	8.33 ± 1.38	0.57 ± 0.15
	♀	114.66 ± 38.97	65.91 ± 15.92	19.93 ± 7.65	15.22 ± 3.94	0.86 ± 0.17
4 - 6 hours	♂	24.05 ± 4.95	10.19 ± 7.04	2.07 ± 0.30	11.99 ± 5.21	1.18 ± 0.15
	♀	48.63 ± 12.11	27.03 ± 16.28	4.19 ± 1.32	11.08 ± 1.45	1.00 ± 0.33
0 - 6 hours	♂	107.45 ± 13.77	38.19 ± 13.32	9.51 ± 1.14	26.73 ± 3.24	2.54 ± 0.34
	♀	203.91 ± 50.56	117.68 ± 22.75	31.54 ± 11.25	33.86 ± 5.64	2.51 ± 0.61
3 x basal	♂	33.22 ± 3.31	3.56 ± 1.24	6.18 ± 1.59	7.57 ± 0.88	2.85 ± 0.56
	♀	35.42 ± 3.91	3.17 ± 0.34	3.34 ± 0.51	8.37 ± 1.00	1.67 ± 0.28
Increment	♂	74.24 ± 14.00	34.70 ± 14.59	3.85 ± 1.48	19.17 ± 4.11	-0.32 ± 0.41
	♀	154.20 ± 57.63	114.50 ± 22.84	28.20 ± 11.06	25.49 ± 5.17	0.84 ± 0.41
Increment (mM)	♂	0.3633 ± 0.0688	0.1595 ± 0.0577	0.0281 ± 0.0108	0.0934 ± 0.0200	
	♀	0.8250 ± 0.2475	0.5509 ± 0.1097	0.2057 ± 0.0802	0.1242 ± 0.0252	
% Recovery of tryptophan	♂	1.86 ± 0.34	0.81 ± 0.34	0.14 ± 0.05	0.48 ± 0.09	
	♀	4.21 ± 1.26	2.81 ± 0.52	1.05 ± 0.40	0.63 ± 0.32	

TABLE 48

TRYPTOPHAN METABOLITES IN HUMAN URINE DETECTED CHROMATOGRAPHICALLY

Reference number	Metabolite	Condition of Subject	Condition of Experiment	Results
95	anthranilic acid	congenital hypoplastic anemia		increased
			/ 1.6 g.l-tryptophan	"
			/ riboflavin	decreased
147	3-hydroxyanthranilic acid	carcinoid		increased
147	3-hydroxyanthranilic-sulfate	carcinoid		decreased
148,149	3-hydroxyanthranilic acid	pulmonary tuberculosis	/ 5 g.l-tryptophan	increased
60	2-amino-3-hydroxyacetophenone (and -sulfate)	leukemia, anemia		increased
150	indoleacetic acid	normal		present
11	" "	normal	after exercise	increased
150	" "	phenylketonuria		increased
13	" "	phenylketonuria		"
150	5-hydroxyindoleacetic acid	normal		present
150	" "	carcinoid		increased
13	" "	phenylketonuria		"
150	5-hydroxyincoleacetic-sulfate	carcinoid		"
13	indolelactic acid	phenylketonuria		present
150	indoxyl sulfate	normal		present

TABLE 48

TRYPTOPHAN METABOLITES IN HUMAN URINE DETECTED CHROMATOGRAPHICALLY (cont.)

Reference number	Metabolite	Condition of Subject	Condition of Experiment	Results
147	3-hydroxykynurenine	carcinoid		increased
149,151	"	fever		present
106	"	(hyperthyroidism (fever (lupus erythematosus	/ 10 g. dl-tryptophan	present
106	acetyl-3-hydroxykynurenine	(hyperthyroidism (fever (lupus erythematosus	/ 10 g. dl-tryptophan	present
147	3-hydroxykynurenine-sulfate	carcinoid		decreased
150	N'-methyltryptamine	normal		present
150	5-hydroxytryptamine	carcinoid		variable