INDICAN AS A "DIAZOTIZABLE AMINE" IN HUMAN URINE

BY

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Introduction

The metabolism of tryptophan, an essential amino acid, and its relationship to bladder cancer and other pathological states in humans has been the object of intensive study in the Cancer Research Hospital Laboratories. Quanitative methods have been developed (1) for the measurement in urine of several of these metabolites. In one of these methods, acidified samples of urine were placed on a column of Dowex 50 cation exchange resin, and the bound constituents were eluted with increasing concentrations of hydrochloric acid in five Fractions, A through E. Aliquots of these Fractions were then diazotized and coupled, according to the procedure of Bratton and Marshall (2) for primary aromatic amines, and measured spectrophotometrically. It was found (1) that Fraction B contained the glucuronide of anthranilic acid; Fraction C, o-aminohippuric acid; Fraction D, anthranilic acid and acetylkynurenine; and Fraction E, kynurenine, all of which are metabolites of tryptophan (Figure 1). The diazotizable component of Fraction A was unknown at the start of this study (1,3,4). This project was undertaken to identify the diazotizable component of Fraction A, and to study its possible relationship to the metabolism of tryptophan and related compounds.

Recently it was shown that administration of acetyl-L-tryptophan to human subjects resulted in a considerable increase in the excretion of the diazotizable material of Fraction A (3), but this observation could not be explained. Subsequently, in preliminary studies, it was found that administration of indole to rats resulted in a sizable increase in the urinary diazotizable amine of Fraction A. In examining the literature concerning the known metabolic pathways of indole in mammals, it was found that indole has been shown to yield indoxyl, which, in turn, was conjugated with sulfate or glucuronic acid to produce, respectively, indican (indoxyl potassium sulfate) or indoxyl glucuronide (Figure 2). These metabolites were then excreted in the urine (5). Since indican had been identified as a major metabolite of indole in mammals (6), it appeared possible that this compound might be the unknown "diazotizable amine" of Fraction A.

Indole and tryptophan are closely related. Structurally, they are both composed of a benzopyrrole nucleus, with tryptophan, in addition, containing an alanine side chain. Metabolically (Figure 3), it has been known for a long time that Escherichia coli, a normal inhabitant of the human intestinal tract, can convert tryptophan into indole (7). In contrast, Neurospora, a fungus, can transform indole, in the presence of serine, into tryptophan (8). Indole can also be metabolized through several intermediate steps into anthranilic acid by certain bacteria (9), and conversely Neurospora (8) and certain tryptophan auxotrophic strains of Escherichia coli (10) are able to utilize anthranilic acid to synthesize indole. In mammals, tryptophan, through the intermediate kynurenine, is the chief natural source of anthranilic acid.

In rats fed a diet containing tryptophan and 2-acetylaminofluorene a high incidence of bladder tumors was produced, while ingestion of these compounds separately failed to have this effect (11). Very recently, it was reported that indole, added to the diet in place of tryptophan and fed with 2-acetylaminofluorene, would also result in production of an 83 per cent incidence of bladder tumors (12).

Although some controversy has arisen in recent years concerning the production of urinary indican in mammals and man, the traditional doctrine that urinary indoles are derived from intestinal putrefaction, or more specifically from tryptophan degradation continues to prevail (5). The chief basis for this assumption seems to be the work of Ellinger and Gentzen (13) and Asayama (14), who demonstrated that direct injection of tryptophan into the caecum of rabbits was followed by increased urinary excretion of indican, while subcutaneous injection or oral administration of tryptophan was not followed by increased indican excretion. Underhill (15) reported that indican excretion was increased in dogs fed a lean meat diet, and that only a trace of indican was excreted following a gelatin diet which was low in tryptophan content. On the other hand, Sherwin and Hawk (16) reported that indican excretion continued in a dog which was fasted for 117 days.

The *in vivo* production of indican by means other than putrefaction and from compounds other than indole or its immediate derivatives has been reported by Houssay et. al. (17), Bohm and

Gruner (18) and Stoppani (19). The compounds studied were chiefly derivatives of the o-nitro and o-aminobenzyl group, and resulted in increased urinary indican excretion in dogs and other animals.

Conflicting reports have been published concerning the effect of antibiotic intestinal sterilization on urinary indican excretion. Muller (20) found that indican excretion disappeared after oral administration of large doses of streptomycin to children. Makino and Umezu (21) discovered that oral administration of 0.05 per cent terramycin to rats resulted in a low indican excretion, even after the intestinal bacterial count had risen. Brummer and Kasanen (22) showed that ingestion of neomycin and bacitracin, oxytetracycline, and chloramphenicol by human subjects resulted in diminished urinary indican excretion. On the other hand, Conochie (23) reported that urinary excretion of indican was unaffected in cows fed streptomycin and sulfathiazole although Escherichia coli could no longer be cultured from the feces. Finally, Wooldridge et. al. (24) demonstrated that sterilization of the intestine of human subjects with streptomycin and glucuronolactone was followed by no decrease in indican excretion, although both the aerobic and anaerobic cultures from the feces showed no growth of organisms.

This report will present evidence indicating that the major diazotizable component of Fraction A in man is indican; that oral administration of indole or acetyl-L-tryptophan is followed by an increased excretion of this metabolite; and finally, that L-tryptophan administration through an intestinal tube placed in the middle or lower ileum will result in increased excretion of urinary indican by man.

Materials and Methods

The indole was obtained from commercial sources. The acetyl-L-tryptophan was prepared from L-tryptophan by the method of du Vigneaud and Sealock (25). The indican was isolated from the urine of indole-fed rats by the method of Hoppe-Seyler (26), and it was purified according to the method of Ellinger (27) with white glistening crystals corresponding to those described being obtained. Elemental analysis[1] of this isolated substance revealed:

$C_8H_6NO_4SK$. Calculated. N 5.60, S 12.80
Found[1]. N 5.42, S 12.79

In preliminary studies, 0.855 millimoles of indole was administered orally to two male rats following the collection of two 24 hour basal urine samples, and three 24 hour specimens collected following indole supplementation.

For the indole and acetyl-L-tryptophan studies, four normal subjects, male and female laboratory personnel, were used. The indole was weighed into gelatine capsules and administered as a single oral dose. The acetyl-L-tryptophan was administered as a single oral dose suspended in water. After collecting one 24 hour

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1. Analyses by Clark Microanalytical Laboratory, Urbana, Illinois.

urine sample, the supplement was administered. Two more 24 hour urines were collected, and aliquots of all samples were stored at 0° C. until analyzed, usually within 2 to 5 days.

The intestinal intubation studies were carried out as follows. The tube was constructed out of 10 feet of Intramedic Polyethylene tubing, PE 240 (I.D. 0.066" x O.D. 0.095"). On one end two rubber finger cots were superimposed, with 4 ml. of mercury placed inside to serve as a weight. A knot was tied in the distal end of the tubing, and the finger cots tied to the end of the tube with surgical silk. The knots were then covered with rubberized liquid cement. Several small holes were cut into the distal end of the tube over a distance of 6 inches to provide an outlet. Three normal male subjects were used. The tube was inserted <u>per os</u>, and permitted to pass through the gastro-intestinal tract until the proximal end was at the mouth. Fluoroscopy was used to demonstrate the passage of the mercury bag through the pylorus of the stomach, and in all cases, at the termination of intubation, demonstrated the presence of the mercury bag near the sacrum. It could be shown that the distal end of the tube was lying within the ileum, but more accurate localization was not possible. After collecting two 24 hour urine samples, the supplement of 1.47 millimoles of L-tryptophan, dissolved in distilled water, was injected into the proximal end of the tube, the tube washed with an excess of water, and then removed slowly over a period of two hours from the mouth. Five more 24 hour urine samples were collected, and aliquots of all samples were stored at 0° C. until analyzed.

Analytical Methods

Fraction A was prepared as described by Brown and Price (1). Briefly, 1 per cent of the 24 hour urine sample was acidified to 0.1 N HCl, diluted to 40 ml. with water, placed on a column of Dowex 50 cation exchange resin, and washed with two 20 ml. aliquots of 0.1 N HCl, making a total volume of 80 ml. Duplicate 3 ml. aliquots were diazotized and coupled as has been previously described (1).

The method of Rose and Exton (28) using 5 per cent hexylresorcinol in absolute ethyl alcohol and 0.5 per cent cupric bromide in concentrated HCl was utilized for the determination of indican. To duplicate samples containing 0.02 per cent aliquots of the 24 hour whole urine, or 1.5 ml. of Fraction A, 0.05 ml. of the hexylresorcinol reagent was added, followed by 2 ml. of the cupric bromide reagent. After allowing 3 minutes for the reaction to take place, the samples were extracted with 3 ml. of amyl acetate, transferred to matched colorimeter tubes, and read immediately at 555 millimicrons in the Beckman DU spectrophotometer adapted for the use of these tubes.

The method described by Sharlit (29) for the quantitative determination of indican in urine was also utilized as modified by Meiklejohn and Cohen (30). Duplicate samples of whole urine and Fraction A, prepared as described above for the method of Rose and Exton, were diluted to 5 ml. with water in 15 ml. capacity graduated centrifuge tubes. 0.5 ml. of a 1 per cent $K_2S_2O_8$ solution followed by 0.5 ml. of a 1 per cent thymol solution in 95 per cent ethyl alcohol were added, and the tubes well mixed. 5 ml. of 25 per cent trichloroacetic acid and concentrated HCl was added, and the tubes placed in a hot water bath for 45 minutes. Following cooling at room temperature, the tubes were centrifuged, and the supernatant liquid removed and discarded. The colored complex remaining was diluted to 3 ml. with glacial acetic acid, transferred into matched 13 x 100 mm. colorimeter tubes, and read immediately at 540 millimicrons in the Beckman DU spectrophotometer. A blank was prepared in the above manner for each sample analyzed, except that 0.5 ml. of 95 per cent ethyl alcohol was substituted in place of the thymol-alcohol reagent. A comparison of the spectral curve of the purified indican, following the thymol reaction, with that reported by Townsend (31) showed a good correlation, with both peaks of maximal light transmittancy being at 540 millimicrons (Figure 4).

Standard curves of purified indican, prepared for the three colorimetric tests described above, obeyed Beer's law. The useful range for the thymol and hexylresorcinol tests was found to be 0 to 30 micrograms per tube, and for the diazotization and coupling 0 to 75 micrograms per tube. Since aromatic amine Fraction A was obtained from the Dowex 50 columns in dilute acid, and acid hydrolysis of Fraction A destroyed the indican, all analyses for indican reported here were completed within 1 to 4 hours following the preparation of Fraction A.

Previous attempts at paper chromatography of Fraction A were reported to be unsuccessful (1,3). However, satisfactory chromatograms were obtained by neutralization of aliquots (50 ml.) of Fraction A to pH 7 with solid potassium bicarbonate before the samples were concentrated to dryness in a vacuum desiccator over $CaCl_2$ and NaOH. The residue was then extracted with a few drops of hot 95 per cent ethyl alcohol and spotted on Whatman No. 1 filter paper. After exposure to ammonia, the chromatograms were developed as described by Price and Brown (3), examined with ultraviolet light, and then sprayed with Ekman's reagent for diazotizable aromatic amines and with diazotized sulfanilic acid by the procedures described by Dalgliesh (32). Paper chromatograms were also run on whole urine.

Results

The administration, in preliminary studies, of 0.855 millimoles of indole orally to two male rats resulted in a huge increase in the diazotizable component of Fraction A, from about 20 micromoles per day in the basal day to 534 micromoles of indican in the post-indole day, an increase of nearly 26 times over the basal day value (Table I). Fractions B through E also increased somewhat in diazotizable component over their basal day values, but the change was not as striking quantitatively.

A comparison of the basal with the post-indole whole urine, utilizing the technique of paper chromatography, revealed that in the basal day there was only one spot which diazotized and coupled, while in the day following indole administration, four new reactive spots appeared, and the spot noted in the basal day increased in size and intensity of reaction (Figure 5). None of the reactive spots found in the whole urine followed any of the chromatographic criteria given by anthranilic acid, acetylkynurenine, o-aminohippuric acid, kynurenine, or indole. No satisfactory method existed at this time for the paper chromatography of Fraction A, but Fractions B through E were desiccated in vacuo and quantities of these applied to paper. After chromatography, the materials in these fractions corresponded roughly in R_F to each other and to one of the spots in the whole urine, and diazotized and coupled; but bore no relation to any of the known diazotizable amines contained in these fractions, or to indole. It therefore seemed reasonable to believe that these new spots represented unidentified diazotizable metabolites of indole.

The administration of various doses of indole orally to human subjects resulted in a large increase of indican in whole urine and Fraction A, as measured by the thymol and hexylresorcinol tests (Table II). The diazotizable amine component of Fraction A also increased, but to a greater degree than that expected from the results of the thymol and hexylresorcinol tests. When indican was added to water or urine it was quantitatively recovered in Fraction A by all three colorimetric procedures. No increase in either diazotizable substance or thymol and hexylresorcinol reactive substance was found in any of the other four fractions from the Dowex 50 columns so these data were not included in Table II.

The thymol and hexylresorcinol tests on the whole urine and

Fraction A were usually in agreement with each other, but the values obtained from Fraction A were generally somewhat lower than the corresponding values for the whole urine. The diazotizable amine values of Fraction A were quantitatively greater than the values obtained by the thymol and hexylresorcinol tests on whole urine. However, the thymol test for indican accounted for between 43 and 113 per cent (average 71 per cent) of the diazotizable component. Thus indican appears to be the major "diazotizable" component of Fraction A.

Paper chromatograms of the whole urine and of Fraction A showed only one diazotizable spot in the basal and supplemented urines from these normal subjects. This spot increased in intensity and size following administration of indole, and corresponded in R_F value, fluorescence, and Ekman reaction to purified indican.

Acid hydrolysis of purified indican, whole urine, and Fraction A resulted in complete disappearance of thymol and hexylresorcinol reactive substance. The diazotizable component of Fraction A was reduced to a negligable amount following hydrolysis and rechromatography on Dowex 50.

The ingestion of acetyl-L-tryptophan resulted in a marked increase in indican in Fraction A (Table III). The amount of indican found by the thymol method accounted for 64 per cent of the increase in diazotizable amine. Paper chromatography of Fraction A and whole urine indicated a considerable increase in the intensity of fluorescence of the spot corresponding to indican, and a spot apparently identical with acetyl-L-tryptophan in R_F value and fluorescence was evident. The latter spot reacted slowly and faintly with Ekman's reagent. Acetyl-L-tryptophan did not interfere with the thymol or hexylresorcinol tests for indican. Large amounts (5 mg.) of acetyl-L-tryptophan did diazotize and couple slowly, but the color produced was slight. Thus the increased diazotizable color reaction appearing in Fraction A following administration of acetyl-L-tryptophan orally to human subjects appears to be caused by increased excretion of indican.

The administration of L-tryptophan via intestinal intubation resulted in an increase in indican in Fraction A (Table IV). The peak of indican excretion was usually observed in the second day following injection of the tryptophan into the tube, and the amount of indican increase accounted for 13 to 19 per cent (average 17 per cent) of the tryptophan dose. Paper chromatography of Fraction A and whole urine indicated a moderate increase in the intensity of the spot corresponding to indican, and no evidence of unmetabolized urinary tryptophan could be demonstrated by this method. No increase in diazotizable substance was found in any of the other four fractions from the Dowex 50 columns so these data were not included in Table IV. Since previous studies (1,3,4) have shown that larger doses of tryptophan administered orally to humans causes no increase of diazotizable component in Fraction A, it was concluded that if tryptophan is placed in the lower intestinal tract, it can serve as a precusor of urinary indican.

Discussion

The diazotization and coupling of indican under the conditions described apparently has not been observed previously. However, a reaction of this type was employed by Eckert (33) in the quantitative determination of tryptophan. Even with large amounts of indican there was no evidence for the formation of indigo red or indigo blue in the presence of the nitrous acid. When the coupling agent was added, the rate of color development with indican was similar to that of Fraction A or aromatic amines. On paper chromatograms containing intensely fluorescent spots of indican, spraying with Ekman's reagent resulted in two distinct zones of color, a central grey-blue zone (indigo blue) (34) surrounded by a red color typical of diazotizable aromatic amines. With smaller amounts of indican only the red color was observed.

An increase in the diazotizable component of Fraction A was always accompanied by an increase in the content of indican as measured by the thymol and hexylresorcinol tests. Any treatment of Fraction A which would destroy indican (e.g. acid hydrolysis) resulted in loss of the diazotizable component. Since added indican appeared quantitatively in Fraction A and paper chromatography provided qualitative and semi-quantitative confirmation of all the colorimetric analyses, it appears likely that the major diazotizable substance in Fraction A is indican. The detection of indican in normal human urine confirms the recent observations of Dalgliesh (34) who detected indican in concentrates of normal human urine by paper chromatography.

It was difficult to decide which of the three colorimetric procedures provide the most accurate measure of the amount of indican in urine. The determination based on diazotization and coupling usually gave higher values than those obtained by the thymol or hexylresorcinol methods. It is probable that small amounts of other diazotizable substances occur in Fraction A.

In previous studies Fraction A has been calculated as anthranilic acid (1,3,4). A comparison of the standard curves for anthranilic acid and indican revealed that data calculated as micromoles of anthranilic acid per day may be converted to micromoles of indican by multiplication by a factor of 7.6. Previous data (1,3,4) for indican in Fraction A were in agreement with the range of values found in the present study after application of this factor.

Sakamoto, Uchida, and Ichihara (9) reported that certain strains of bacteria converted indole to anthranilic acid. After ingestion of indole by man or the rat, there was no increase in the diazotizable amines in the fractions known (1) to contain anthranilic acid, o-aminohippuric acid, or anthranilic acid glucuronide. Thus it would appear that little, if any, anthranilic acid was formed from indole by man or the rat, since this amine was excreted in the urine almost quantitatively by these species when it was administered in relatively small doses (35).

The increase in the urinary excretion of the "aromatic amine" found in Fraction A following ingestion of acetyl-L-tryptophan by human subjects (3) appears to be the result of an increase in indican excretion. No increase in the diazotizable amine in this fraction was observed after ingestion of L-tryptophan, D-tryptophan, acetyl-D-tryptophan, or D,L-tryptophan by man (1,3,4). The metabolism of acetyl-L-tryptophan differs in other respects from the metabolism of L-tryptophan (1,3,36). However, the fact that 5 to 10 per cent of an oral dose of acetyl-L-tryptophan was found unchanged in the stool (36) may be of particular significance. Part of the acetyl-L-tryptophan may be converted to indole by bacteria in the lower gastrointestinal tract, followed by absorption of the indole and excretion in the urine as indican. Acetyl-D-tryptophan, which appears to be metabolically inert in man (3,36) might not be expected to enter into this reaction, even though very large amounts of an oral dose were eliminated in the stool (36).

An increase in urinary indican excretion appears to result from administration of L-tryptophan into the middle or lower ileum of human subjects via intestinal intubation. The fact that no increase in the diazotizable amine of Fraction A was observed after oral ingestion of L-tryptophan in doses 8 to 40 times greater (1) than those given via intubation, suggests that this amino acid is absorbed in the duodenum, jejunum, and upper ileum before reaching an area in the lower ileum or caecum where high concentrations of intestinal bacteria, capable of metabolizing L-tryptophan to indole (7), are present.

The results obtained following administration of L-tryptophan via intestinal intubation, while highly suggestive, are not conclusive in demonstration a uniform increase in the urinary excretion of indican. In two of the three subjects used, maximal indican excretion was observed on the second day following L-tryptophan administration, while the third subject demonstrated maximal indican excretion on the fourth day following intubation. However, the recovery of 13 to 19 per cent (average 17 per cent) of administered L-tryptophan as urinary indican by this method agrees well with the increased indican excretion observed by Ellinger and Gentzen (13) (34 and 18 per cent) and Asayama (14) (11 per cent) following direct injection of tryptophan into the caecum of rabbits.

The dose of L-tryptophan administered (1470 micromoles) was chosen on the basis that 5 to 10 per cent of an oral dose of acetyl-L-tryptophan was found unchanged in the stool (36). This dose was also equivalent to that injected into the caecum of rabbits (13,14). It is probable that somewhat greater doses of L-tryptophan would have resulted in a more significant elevation of indican excretion.

Two significant factors were observed which may have played an important part in this type of experiment. First, there was much difficulty associated with the determination of the location of the distal end of the intestinal tube. While the mercury bag could be seen in all cases near the sacrum (on fluoroscopic examination) prior to administration of L-tryptophan, the exact location within the ileum of the distal end of the tube could not be ascertained. The lack of uniformity of results with different subjects suggests that the area in the ileum in which the L-tryptophan is placed may be very critical, and that administration of this substance either proximal or distal to this area will result in inconclusive alterations of urinary indican excretion. Perhaps in future studies of this type, this factor can be circumvented by attempting to place the distal end of the tube accurately into the caecum, either _per os_ or _per rectum_, then letting the tube pass through the anus instead of withdrawing it _per os_.

The second factor of consequence was the appearance of symptoms of diarrhea 8 to 48 hours after administration of the tryptophan. From one to three watery stools were noted, but no other gastrointestinal or systemic symptoms were noted. It is possible that the increased intestinal motility may have prevented the maximal conversion of L-tryptophan to indole by the intestinal bacterial flora, thus altering the level of urinary indican excretion.

While highly suggestive, these experiments are not conclusive alone in demonstrating a uniform increase in urinary indican excretion following administration of L-tryptophan via intestinal intubation. However, when viewed with the results obtained from injection of tryptophan into the caecum of rabbits (13, 14), it appears that in man, under the proper conditions, tryptophan is capable of serving as a precursor of urinary indican.

Summary

Oral administration of indole to two male rats resulted in a marked elevation of the diazotizable component of a previously unidentified urinary Fraction A. Aliquots of 24 hour human urine collections for one day before and two days after the administration of single oral supplements of indole or acetyl-L-tryptophan were analyzed for indican by the thymol and hexylresorcinol methods. The ingestion of either supplement was invariably followed by an increase in the excretion of indican (indoxyl potassium sulfate). Increases in excretion of indican were always accompanied by an increase in the previously unidentified "diazotizable aromatic amine" in Fraction A. Evidence was presented that the chief diazotizable component of Fraction A was indican. In human subjects indican appeared to be a metabolite of acetyl-L-tryptophan or indole but not of acetyl-D-, D-, DL-, or L-tryptophan ingested orally.

Suggestive evidence was presented indicating that in human subjects an increase in urinary indican excretion will occur following administration of L-tryptophan via gastrointestinal intubation into the middle or distal ileum.

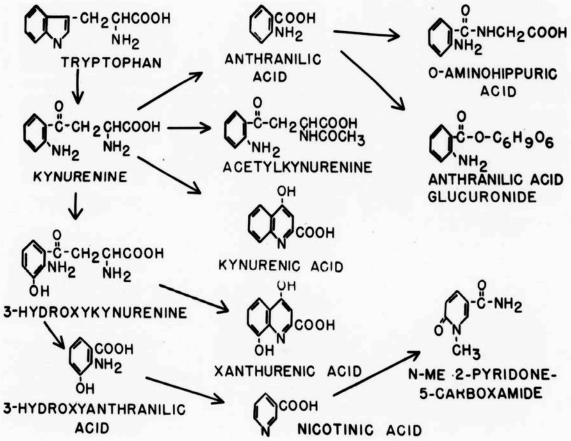
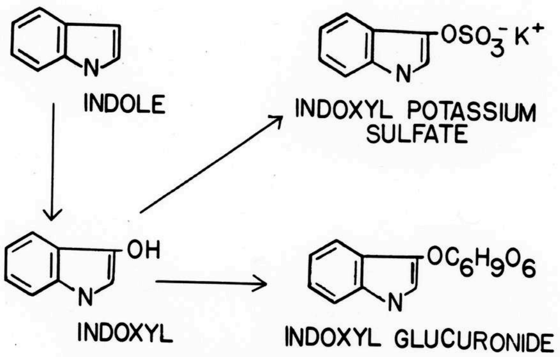


Figure 1

Metabolic Pathway of Tryptophan



KNOWN METABOLIC PATHWAYS OF INDOLE IN MAMMALS

Figure 2

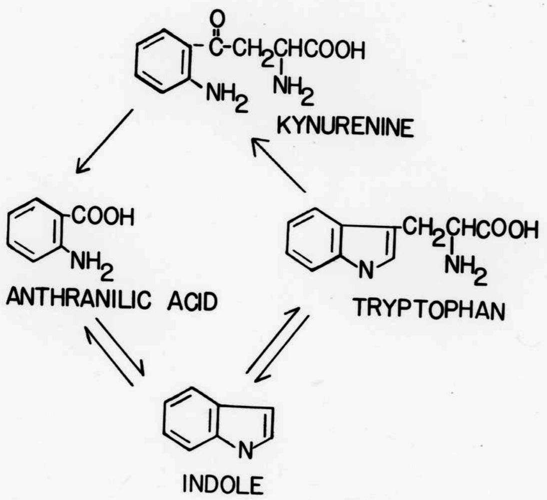


Figure 3

Metabolic Relationship of Indole and Tryptophan

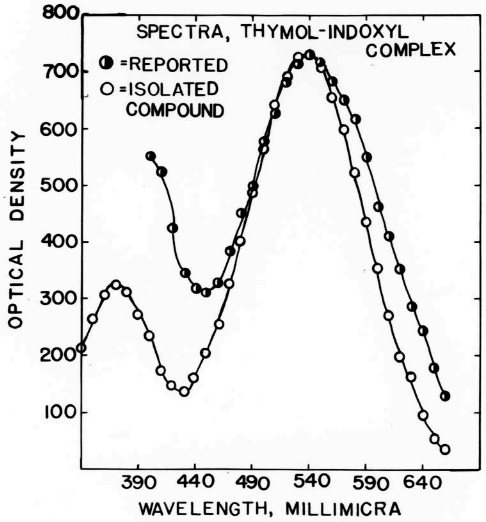


Figure 4

A comparison of spectral curve of thymol-indoxyl complex given by isolated indican with that reported by Townsend (31)

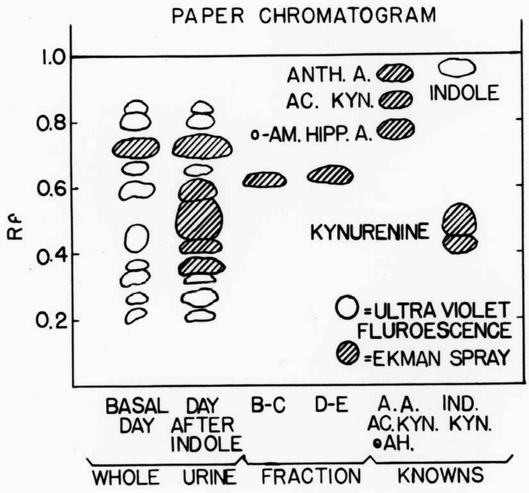


Figure 5

Comparison of basal and post-indole rat urines with respect to diazotizable materials present (Ekman spray)

Table I

Excretion of diazotizable amines in urine of rats following indole administration

All of the data have been expressed as micromoles excreted per 24 hours. The indole was disolved in 2.5 ml. of corn oil and administered by stomach tube to two male rats at the start of the third 24 hour period of urine collection.

Day	Dose indole, mmoles	Micromoles excreted in urine per day				
		A^{x}	B^{o}	C*	D^{a}	E^{y}
1		20.7	0.48	0.96	1.16	2.33
2		19.8	0.38	0.96	0.67	1.55
3	0.855	534.0	10.2	10.2	11.6	11.6
4		22.2	0.56	1.02	1.34	1.75
5		15.3	0.33	0.90	0.61	1.26

The following abbreviations are used: A - aromatic amine Fraction A; B - aromatic amine Fraction B; C - aromatic amine Fraction C; D - aromatic amine Fraction D; E - aromatic amine Fraction E.

x Calculated as indican
o Calculated as anthranilic acid
* Calculated as o-aminohippuric acid
a Calculated as acetylkynurenine
y Calculated as kynurenine

Table II

Urinary excretion of indican before and after administration of indole to human subjects

The data have been expressed as micromoles of the potassium salt excreted per 24 hours. Three subjects were used, and the supplement was administered upon completion of the first day of urine collection.

Dose, mmoles	Day	Micromoles indican excreted per day				
		In whole urine		Found in Fraction A		
		T.M.	H.M.	T.M.	H.M.	D.A.M.
0.171	1	152	102	121	—	195
	2	182	141	158	--	260
0.430	1	301	288	205	—	482
	2	437	484	408	—	783
0.426	1	262	186	248	173	406
	2	436	460	426	392	595
	3	329	411	250	206	347
0.663	1	236	127	248	132	219
	2	481	454	464	458	625
	3	308	334	322	249	340

The following abbreviations are used: T.M. - Thymol method; H.M. - Hexylresorcinol method; D.A.M. - Diazotizable amine method.

Table III

Indican excretion as measured in Fraction A before and after administration of acetyl-L-tryptophan to human subjects

The data have been expressed as micromoles excreted in urine per 24 hours. Four subjects were given this supplement and the average results have been presented. The supplement was administered upon completion of the first day of urine collection.

Dose, mmoles	Day	Micromoles of indican excreted in urine per day		
		Thymol Method	Hexylresorcinol Method	Diazotizable amine Method
	1	305	206	334
9.8	2	555	418	728
	3	496	316	575

Table IV

Indican excretion as measured in Fraction A before and after administration of L-tryptophan via intestinal intubation to human subjects

The data have been expressed as micromoles excreted in urine per 24 hours. Three subjects were given this supplement and the average results have been presented. The supplement was administered upon completion of the second day of urine collection.

Dose, mmoles	Day	Micromoles of indican excreted in urine per day		
		Thymol Method	Hexylresorcinol Method	Diazotizable amine Method
	1	258	241	300
	2	232	232	278
1.47	3	254	244	293
	4	384	345	437
	5	283	268	335
	6	320	287	373
	7	240	223	294

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