URINARY INDICAN IN HEALTHY INDIAN SUBJECTS*

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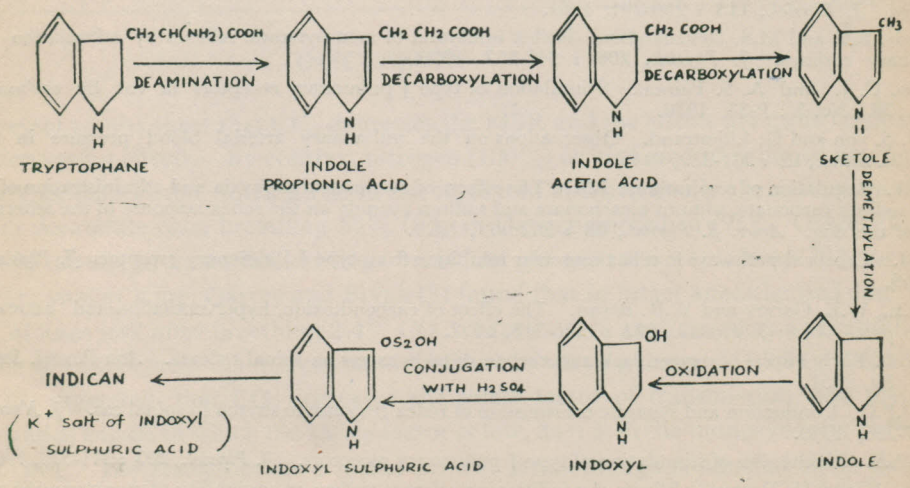
Summary: Forty normal subjects have been taken for the present study. The mean Indican excretion was 40.45 *mg*/24 hrs. The mean jejunal count was $1.96 \times 10^3 \pm 5.39 \times 10^3$ organisms/*ml* and 40% of the jejunal aspirates were sterile. Wide range of bacteria were cultured but the coliform organisms were obtained in only 16.6%. There was a significant correlation between Indican excretion and total bacterial count ($P<.01$).

Key words: Indicanurea intestinal bacterial flora

INTRODUCTION

Bacterial decomposition in the intestine is characterised by their action upon the aminoacids which have been liberated from the proteins (11). Their action upon tryptophane produces large number of substances which are excreted as such or in detoxified form in urine or faeces (11).

Enzyme tryptophanase catalyses the transformation of tryptophane into Indole probably through intermediate products by series of chemical reactions as depicted in Fg. 1. After being



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absorbed from midgut Indole is oxidised into Indoxyl in the liver and is then conjugated with sulphuric acid to form Indoxyl sulphuric acid. Indican is the potassium salt of this metabolite and is primarily excreted in the urine.

The idea of using Indican excretion as measurement of bacterial overgrowth in small bowel comes from the observation of Jeffe (9) who found that obstruction of small bowel produces greater Indican urea than large bowel obstruction.

Diagnostic intestinal intubation for assessing intestinal bacterial overgrowth is rather inconvenient and not all patients with malabsorption suffer from bacterial overgrowth. Therefore, a simple screening test like estimation of urinary indican for high count of faecal organism in upper gut has been devised.

MATERIALS AND METHODS

Forty healthy subjects were selected on the basis of criteria of Cohen and Shock (2). Routine investigations like blood for TLC, DLC, Hb% and ESR; urine for albumin, sugar and microscopic examination, stool for ova, cyst and occult blood and liver function tests were done. Jejunal bacterial studies were performed by collecting jejunal fluid by specially designed polyvenyl pyrillidone tube under strict aseptic conditions. First sample equivalent to the capacity of the tube was rejected. The second sample was collected. The third sample obtained after injecting 10 *ml* of air was also discarded and the fourth sample was collected. Jejunal fluid was cultured aerobically on nutrient agar, milk agar, Mchonkey's media, blood sugar, Saboraud's media and anaerobically on sodium thioglycolate, nutrient agar and milk agar.

Organisms were indentified by their morphology, character of the colonies and on the basis of fermentation reaction. Bacterial count was performed by serial dilution technique using colony counter and represented as total number of colony forming units/*ml* of jejunal fluid.

Urinary indican excretion was measured while patients were taking standard ward diet contaning 60—100 *gms* of proteins 464 *gms* carbohydrate; and 76 *gms* fat a day. The urine was collected in Winchester bottles for 24 hrs containing 10 *ml* of chloroform as preservative. Indican was estimated by the Fenton and Martin method (5).

RESULTS

The forty healthy subjects in the series consisted of 31 males and 9 females of 15 to 61 years (Mean 33.9 years). The results of haematological, urine, stool, and liver function tests were within normal limits (Table I).

Jejunal fluid was greenish yellow in colour. The fluid was sterile after 48 hours of incubation in 16 cases (40%). Mean bacterial count was $1.96 \times 10^3 \pm 5.39 \times 10^3$ organism/*ml* of jejunal fluid. In two cases the count was 10^4/*ml*, in fourteen 10^3/*ml*, in four 10^2/*ml* and

in two $10^1/ml$ of jejunal fluid. Table II shows the percentage of the various organisms and the wide range of bacteria present in jejunal fluid.

Indican excretion ranged from 26 to 56 *mg*/24 hrs with a mean of 40.45 ± 8.16 (Table III). Only four subjects showed growth of coliform organisms and their Indican excretion ranged from 49 to 54 *mg*%. There were others with similar Indican levels but without these organisms. A statistical comparison of urinary Indican and jejunal bacterial flora shows a significant correlation ($t=2.12$, $p<0.01$) (Table IV).

TABLE I: Normal findings in healthy subjects.

Hb %	12.5 ± 1.7 *gm*%
Serum Bilirubin	0.635 ± 0.12 *mg*%
Alkaline phosphatase	11.1 ± 1.96
Normal faecal fat	< 5 *gm*

TABLE II: Jejunal bacterial flora

Total no. of cases	*Total no. of sterile cases*	*Range of organ/ml. of Jej. fl.*	*Organism*	*No. of cases*	%
40	16	10^1-$10^4/ml$	Strep. Vir.	24	100.0
			Staph. Aib.	24	100.0
			Micrococci	24	100.0
			Aerobic Lac. B.	22	91.3
			Strep. Haem.	8	33.3
			Staph. Citr.	4	16.6
			E. Coli.	4	16.6
			Staph. Aur.	4	16.6
			Anaer. Lac. Bac.	2	8.33
			Kleb.	2	8.33
			Strep. Feac.	2	8.33
			Bacteroides	2	8.33

DISCUSSION

The measurement of urinary Indican as an indirect assessment of the bacteria of intestinal tract has been studied by many workers (3, 11, 7, 8). Not only there is a disagreement regarding the diagnostic value of urinary indican but even the normal levels are variably

reported. Hamilton *et al.* (8) reported its levels as 140, 199 and 223 *mg*/24 hrs in the 3 subjects with no evidence of bowel disease; in 12 other normal subjects studied by them Indican level varied from 10-160 *mg* and yet other two had indican excretion of 46 *mg*/24 hrs in the same series. These authors could not find a clearcut upper limit of Indican excretion in normal subjects as their results showed a skew distribution. Curzon and Wales (3) have reported in 28 ambulatory patients values of Indicanurea as 48 *mg*/24 hrs$\pm$19 *mg*/24 hrs.

TABLE III: Urinary Indican levels (*mg*/24 *hrs*).

No. of cases	*Percentage*	*Indican levels*	*Mean* $\pm$*S.D.*	*Range*
6	15	50—56	40.45$\pm$8.16	26—56
16	40	40—50		
16	40	30—40		
2	5	<30		

TABLE IV: Showing coefficient correlation and statistical significance between faecal fat, urinary indican and jejunal bacterial flora.

	Faecal fat Urinary Indican	*Faecal fat VS bacterial flora*	*Bacterial Flora VS Urinary Indican*
n	18	18	18
r	—0.13	0.000616	0.453
t	0.68	0.0025	2.12
p	0.05	0.05	<0.01
Significance &	Insinificant	Insignificant	Significant

Hamilton *et al.* (8) showed a mean value of 38 *mg*/24 hrs in normal subjects, although there was considerable variation. Our results are more in keeping with those of Greenberg *et al.* (7) and Tabagchali *et al.* (12) who reported mean values of 38$\pm$5 *mg*/24 hrs and 48 *mg* $\pm$20 *mg*/24 hrs respectively. The variation in the Indican values in our study is much less as compared to other reports (8).

Neale and Tabagchali (11) found a good correlation of Indican excretion with malabsorption, specially of those complicated by bacterial proliferation in the small bowel. The correlation between Indicanurea and jejunal bacterial population in normal cases has not been reported. We have found that Indican excretion was comparatively high in normal subjects

harbouring cliform organism, and there was a significant correlation between Indicanurea and jejunal bacterial count.

Indicanurea increases after infusion of casein into the colon (6) and in Hartnup disease (1 and 10) and in other malabsorption states. Dixon (4) followed the fate of bacteria inoculated in exteriorised loops of intestine in rats by including an unabsorbed Cr^{51} marker in the inoculum. The results of the inoculum indicated that bacteria moved rapidly onwards and were soon removed from the small intestine by peristaltic action but there was no indication of bacteriocidal action. He concluded that mechanical removal by peristaltic action aided by secretion of mucous is probably the main way, by which the organisms entering the small intestine were dealt with.

The bacterial population in the small intestine would thus reflect the population in the large bowel where the main organisms found are coliforms. A higher excretion of Indican in such cases would therefore reflect high population not only in small bowel but also in the colon due to diminished peristalsis. On the one hand certain studies (7,8) have failed to correlate the levels of indicanurea with findings of jejunal aspirate but on the other hand Tabagchali and Booth (12) have demonstrated significant correlation between these two.

It therefore appears that levels of Indicanurea not only depends upon the upper bowel bacterial overgrowth but on certain other factors also including the availability of trytophan and site of cleavage by Indole-forming organsims.

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