Factors Influencing the Microbiological Degradation of Choline and Tryptophan in Man[1] (35722)

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In laboratory rats and in man the magnitude of trimethylamine production from choline and of indole production from tryptophan are biochemical determinants of the activity of the normal intestinal microflora (1, 2). In the laboratory rat, the total trimethylamine (TTMA) excreted in the urine after a test dose of choline is diminished by: coprophagy prevention, which limits reinoculation of the intestinal tract with bacteria; and administration of single or combined antibiotics, which suppress the normal bacterial flora of the small and large intestine (3). Similarly, indole-fed rats excrete significantly less indican in the urine when antibiotics are incorporated in their diet (4). Prolonged daily administration of choline or indole with antibacterial agents is associated in rats with a gradual rise in TTMA and indican excretion; and evidence has been obtained that this is due to escape of the choline and indole utilizing bacteria from the effects of the antibiotics (3–5).

Whether TTMA and indican excretion are useful parameters of the integrity of the intestinal microflora and its suppression by antibiotics has been investigated in normal human subjects.

TABLE I. Composition of Diets.[a]

	I	II
Calories	2782	2813
Protein (g)		
Total	99.5	102.1
Legumes	14.5	16.2
Fat (g)	109.3	111.6
CHO (g)	354.9	354.8
Choline (g)	0.12	0.12
Tryptophan (g)	1.10	1.09

[a] Basic diets I and II fed on alternate days. Calorie requirements of individual subjects met by addition of sugar.

Methodology. Diets of constant composition were fed to seven healthy male subjects, aged 22–30 years, over a period of 7 weeks. The diets were designed to contain approximately 100 mg of choline and to supply 13% of the total protein sources as vegetable protein from baked beans (Table I). The diet contained approximately 1.0 g of tryptophan. These dietary manipulations were imposed to minimize trimethylamine production from dietary choline and to promote microbial formation of indole from undigested protein reaching the large intestine.

Complete 24 hr collections of urine were analyzed for total trimethylamine (TTMA) (3), indican (6), and creatinine (7). The effect of choline loading on TTMA excretion was measured before and after penicillin G, phthalylsulfathiazole, and a combination of these drugs. The detailed plan of study, together with the drug dosage, is shown in Table II. Intestinal transit time was determined, prior to antibiotic treatment, by the use of a brilliant blue fecal dye marker (8). Records were kept of the number of bowel movements per subject per week of study and of the incidence of diarrhea during the various phases of the investigation.

Results. Intersubject variation in the response to the single or combined chemotherapeutic compounds was shown. There was a parallelism between the effect of the drugs on indican and trimethylamine excretion in three subjects (Group I) with rapid intestinal transit time. In these subjects, penicillin G alone caused a reduction in the output of TTMA and indican; and the combined drugs

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TABLE II. Time-Sequence of Investigation and Drug Dosage.[a]

Week	Days						
	1	2	3	4	5	6	7
I	0	0	0	0	0	0	0
II	0	C	C	0	0	0	0
III	P	PC	PC	0	0	0	0
IV	S	SC	SC	0	0	0	0
V	0	C	C	0	0	0	0
VI	PS	PSC	PSC	PSC	PSC	PSC	PSC
VII	PSC	PSC	PSC	PSC	C	C	C

[a] C = choline chloride, 4.0 g/24 hr (single dose fasting); P = penicillin G potassium USP (Pentid 400 tabs), 500 mg/24 hr (2 doses fasting); S = phthalylsulfathiazole (Sulfathalidine), 16.0 g/24 hr (2 doses fasting).

had a marked synergistic effect so that the output of TTMA and indican was minimized until chemotherapy was discontinued. Tenesmus and the production of frequent, bulky light-colored stools occurred in these subjects on penicillin alone and increasingly when the combined drugs were administered.

In four subjects with a more prolonged intestinal transit time (Group II), penicillin alone did not decrease indican excretion. Trimethylamine excretion was suppressed in three of the four subjects during penicillin treatment. The combined drugs caused transient reduction of TTMA excretion in all four subjects and transient decreases in indican excretion in three of the subjects. The fourth subject showed prolonged suppression of indican excretion, similar to the subjects in the first group. When transient suppression in the output of the microbial metabolites occurred, continued combined chemotherapy resulted in an output of both TTMA and indican rising to the range of pretreatment levels. The incidence of gastrointestinal symptoms was low in this group.

Intake of the sulfa drug alone had a most variable effect on indican and TTMA excretion in the subjects studied. Three subjects showed decreased output of indican and five showed decreased output of TTMA. However, the effect of this drug on the excretion of the two urinary metabolites was dissimilar in two subjects; and no relationship between intestinal transit time and the chemotherapeutic effect of the drug was determined.

Characteristic responses of subjects in Groups I and II to the different treatment modalities are shown in Figs. 1 and 2 in which the urinary output of TTMA (expressed as total trimethylamine nitrogen) and indican, during the period of study, are demonstrated. Percentage urinary losses of choline as TTMA-N and tryptophan as indican are shown in Table III. Losses of both nutrients were significantly greater in Group II than Group I on the basal diet and with penicillin G treatment; losses of choline were significantly greater in Group II when subjects received the combined antibiotics.

Discussion. It has been shown (9) that intestinal bacteria degrade choline only with the formation of trimethylamine and that no matter how low the level of urinary trimethylamine becomes, the feces do not contain significant amounts of choline in human subjects. Thus, in the present study, reduced trimethylamine excretion can be accepted as a measure of the direct or indirect antibacterial effect of the chemotherapeutic compounds administered. In man, the percentage loss of choline by microbial degradation is related inversely to the intestinal transit time. Further, the sparing effects of antibiotics, *viz.*, penicillin or penicillin plus phthalylsulfathiazole, on choline is significantly reduced in subjects exhibiting intestinal stasis (Table III). This finding may have nutritional importance in relation to dietary methionine deficiency, where adequate choline absorption is mandatory for the provision of methyl groups.

Indole, formed by the bacterial degradation of tryptophan, can be absorbed from the small as well as from the large intestine (10). After transport to the liver, via the portal circulation, it is converted to indoxyl, which is conjugated with sulfate to produce indican, the principal urinary metabolite (11). There are several lines of evidence that show that the level of urinary indican is not only a measure of the bacterial population of the small and large intestine but also reflects the mass of unabsorbed tryptophan which is available for bacterial decomposition (2, 12).

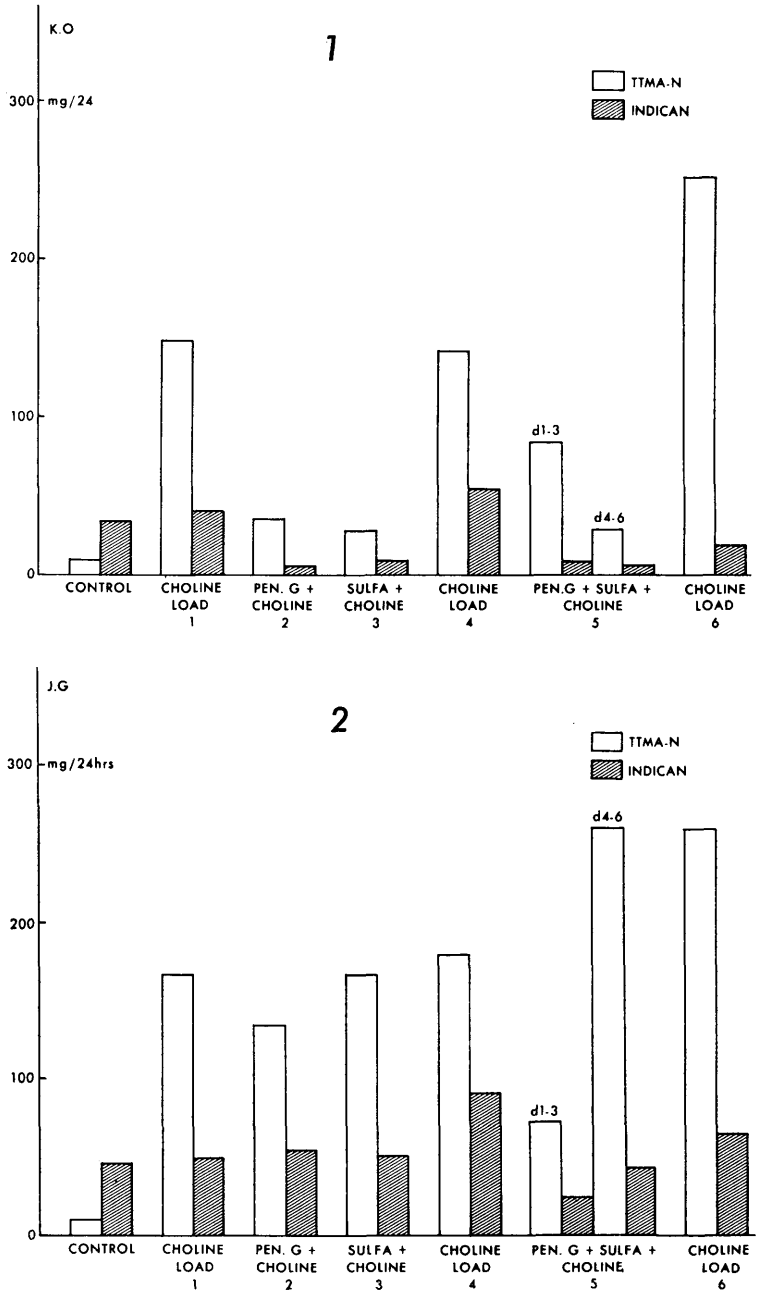

FIG. 1 and 2. Urinary excretion of TTMA-N and indican: Comparison of effects of penicillin G and phthalylsulfathiazole or a combination of these drugs on trimethylamine and indican excretion in a subject with rapid intestinal transit time (Fig. 1, group 1, K.O.) and one with slow intestinal transit time (Fig. 2, group 2, J.G.).

Under pathological or experimental conditions, which are productive of intestinal stasis, the urinary indican level is elevated (13, 14). It has further been demonstrated that protection of tryptophan from microbial degradation by use of antibacterial compounds is obviated or diminished by intestinal stasis.

TABLE III. Average Losses of Choline and Tryptophan Due to Microbiological Degradation.

Treatment	Group:	Choline lost as urinary TTMA-N (%) I[a]	Choline lost as urinary TTMA-N (%) II[b]	Tryptophan lost as urinary indican (%) I	Tryptophan lost as urinary indican (%) II
Basic diet		56.7	72.5[c]	2.4	3.3[c]
Choline loading		56.2	62.5	2.8	3.1
Pen. G + choline		25.7	45.5[c]	0.6	3.9[d]
Sulfa + choline		36.7	47.5	1.7	4.1
Pen. G + sulfa + choline	(days 1–3)	7.5	24.3	0.6	3.5
	(days 4–6)	3.8	58.3[e]	0.6	2.5

[a] Mean intestinal transit time: 24 hr (range 22–25); [b] 80 hr (range 33–152).
[c] Difference between group I and II is statistically significant: ($p < 0.05$); [d] ($p < 0.01$); [e] ($p < 0.001$).

Although the diets used in this study provide approximately 15 g of protein from poorly digestible leguminous sources, the average loss of tryptophan as urinary indican was less than 5% of the dietary tryptophan. Previous studies have established that urinary indican can be derived from dietary acetyltryptophan as well as endogenous tryptophan from bacterial and mucosal protein within the intestinal lumen (15). It appears that under the present dietary conditions, urinary indican losses are not of nutritional significance.

The subject of physiological individuality must be introduced to explain the differing effects of two chemotherapeutic agents on the intestinal microflora and thence on the excretion of the microbial metabolites, *viz.*, indican and trimethylamine. It has been shown previously that there is a marked variability in the normal intestinal population of bacteria and more particularly in the degree of colonization of the small intestine (16). Individual differences in drug as well as nutrient absorption have also been reported (17). Absorption of penicillin G after oral administration is irregular and poor, due to variable inactivation by gastric secretion as well as to the presence of intestinal penicillinase (18). In addition, rapid passage of the intestinal contents may limit penicillin G absorption so that it is present in the small and large intestine in sufficient concentration to protect both choline and tryptophan from microbial degradation. An alternative explanation of the suppressive effect of penicillin alone and penicillin plus phthalylsulfathiazole on the formation of indole and trimethylamine, in subjects with rapid intestinal transit time, is to suggest that production of diarrhea with or without malabsorption causes mechanical cleansing of the intestine with respect to its bacterial content.

The results of this investigation provide yet further support for the concept of interspecies variation or of the span from rat to man. In the rat there has been no evidence that intestinal transit time determines the percentage loss of choline as trimethylamine. Sterilization of the rat intestine cannot be produced by penicillin alone but only by the synergistic effect of the combined chemotherapeutic compounds.

Summary. Antibacterial compounds, including penicillin G and phthalylsulfathiazole or a combination of these drugs produced variable suppression of urinary trimethylamine (TTMA) and indican output in normal male subjects. Three men with rapid intestinal transit time and low initial indican values exhibited markedly reduced TTMA and indican excretion in response to either drug alone and prolonged depression with combined drug treatment; while four men with prolonged intestinal transit time and high initial indican values showed less suppression of TTMA and indican excretion with sulfa or penicillin G alone and only transient suppression of indican and TTMA with combined treatment. It has been concluded that intestinal stasis is a major determinant of the escape of intestinal microflora from the action of these drugs.

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