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BIO-METABOLISM OF GREEN S AND INDIGO CARMINE THROUGH CAECAL MICROFLORA OF RATS

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Bio-metabolism of two permitted food colours viz., Green S and Indigo Carmine, through caecal microflora of rats was investigated. Incubation of Green S with caecal flora resulted in one coloured and two fluorescent metabolites with the corresponding Rf values of 0.83, 0.33, 0.22, respectively. Indigo carmine was metabolized by caecal microflora to flour fluorescent metabolites with Rf values of 0.09, 0.27, 0.5 and 0.72. The DI 50 value of Green S was considerably higher (456 min) as compared to that of Indigo carmine (54 min). These results suggest that Green S and Indigo carmine are biotransformed to a variety of metabolites by rat caecal microflora.

Green S and Indigo carmine belonging to triaryl methane and indigoid class, respectively, are permitted food colour in India (1) and many other parts of the world. Both the dyes have not been shown to produce abnormalities in a short term and teratological study in experimental animals (2-5). Oral administration of these dyes to rats and mice for more than 80 weeks produce no signs of carcinogenic response (6-9). Green S and Indigo carmine showed no signs of mutagenicity in many of the assay system while with metabolic activation system Green S showed signs of a week mutagen which is attributed due to the presence of impurities

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(10). Recent studies suggest that Indigo carmine enhances the mutagenicity of Try-P-1 in the Salmonella-microsome assay (11).

Absorption, distribution and excretion studies suggest that Green S is not absorbed or metabolzied, as most of the dye was excreted unchanged in faeces within 72 hrs (12). Similarly, Indigo carmine administered orally is also excreted in faeces (60-80%) indicating poor absorption (13).

Gut microflora plays an important role in the bio-disposition of various azo dyes (14). However, their role in the metabolic disposition of triaryl methane, indigoid and xanthene class of dyes is not clearly understood. In this communication we report the bio-metabolism of Green S and Indigo carmine through caecal microflora of rat which may help in delineating the mechanism of safety of these permitted food colours.

MATERIALS AND METHODS

Chemicals: Green S was a gift from Williams Ltd., Honslow, U.K. and Indigo carmine was procured from Vesco Product Company, Calcutta, India. Peptone and yeast extract were obtained from DIFCO Laboratories, USA.

Preparation of rat caecal extract: Adult male wistar albino rats (160± 20 gm) obtained from Industrial Toxicology Research Centre, Lucknow animal breeding colony, were kept under standard laboratory conditions and fed pellet diet (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. The animals were fasted overnight and sacrificed by cervical dislocation.

The content of caecum was suspended in a sterilized nutrient broth containing 0.5% each of glucose, peptone and yeast extract in 0.1 M phosphate buffer pH 7.0 to give 10% v/v suspension. This mixture was kept for 10 minutes to settle the food particles and other course sediments and then decanted off to give a uniform suspension (15).

Incubation system for caecal extract: The incubation system containing 1 mg of Green S or Indigo carmine was mxied with rat caecal extract (0.2 ml) in a total of 2.3 ml of nutrient broth. The reaction was stopped at different time interval by 0.2 ml of methanol. The comparative degradation pattern of the dyes was estimated at different time interval and expressed as degradation index 50 (DI 50) value i.e. the time period required to degrade 50% of the original dye concentration present in the incubation system.

Resolution of metabolites: Paper chromatographic resolution of Green S, Indigo carmine and their microfloral metabolites was

performed on Whatman No. 1 paper sheets, in descending manner, using Isopropanol: Ammonia (25%): Water (7:2:1, v/v) as the developing solvent system. The separated fluorescent spots were visualized under ultraviolet lamp and relative movements of individual spot (Rf values) of Green S, Indigo carmine and their metabolites were recorded.

RESULTS AND DISCUSSION

Figure 1 shows the paper chromatography tracing of the biotransformation of Green S and Indigo carmine by rat caecal microflora. Lane 1 shows the spot of Green S incubated in the absence of caecal flora with a Rf value of 0.77. It is interesting to note that Green S in the presence of caecal microflora showed as many as 4 spots (Lane 2). One colorued spot having an Rf vlaue of 0.77 matched with Green S while one coloured metabolite spot was detected at Rf 0.85 (Lane 2). The developed paper chromatogram seen under UV light detected two

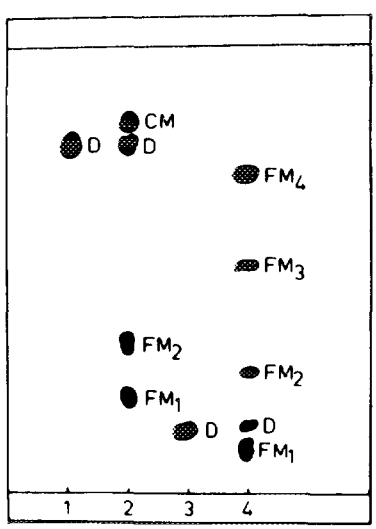

Figure 1. Tracing of paper chromatographic resolution of Green S, Indigo carmine and their metabolites.
Lane 1: Green S
Lane 2: Green S metabolites
Lane 3: Indigo carmine
Lane 4: Indigo carmine metabolites
CM = Coloured Metabolite
D = Original Dye
FM = Fluorescent Metabolite

fluorescent metabolite spots having Rf vlaue of 0.22 and 0.33 (Lane 2 and Table 1). Lane 3 depicts a single coloured spot of Indigo carmine in the absence of caecal microflora and showed the Rf value of 0.14. However, Indigo carmine in the presence of caecal microflora showed 5 spots as indicated in lane 4. Of these only one coloured spot with an Rf of 0.14 matched with the parent dye, Indigo carmine, while 4 fluorescent metabolite spots with Rf vlaues of 0.09, 0.27, 0.51 and 0.72 were detected (lane 4 and Table 1). The degradation of Green S and Indigo carmine by rat caecal microflora is shown in Figure 2. The maximum degradation of Green S (64.7%) and Indigo carmine (72.6%) occurred after 18 and 6 hrs, respectively. Indigo carmine showed faster degradation having DI50 value of 54 min (Fig. 2b), while the metabolism of

Table 1

Rf Values of the Green S and Indigo Carmine dye and their metabolites

Dye/Metabolites	Rf values[a]
Green S	0.77
Green S Fluor Meta I	0.22
Green S Fluor Meta II	0.33
Green S Fluor Meta III	0.83
Indigo Carmine	0.14
Indigo Carmine Fluor Meta I	0.09
Indigo Carmine Fluor Meta II	0.27
Indigo Carmine Fluor Meta III	0.51
Indigo Carmine Fluor Meta IV	0.72

Data from a typical experiment repeated five times with similar results.

[a] The paper was developed using a solvent system Isopropanol : Ammonia : Water (7 : 2 : 1) and Rf values calculated.

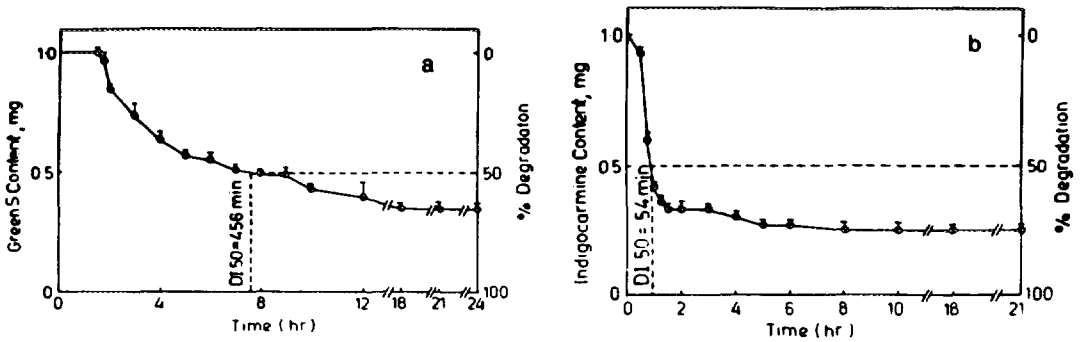

Figure 2. Time dependent degradation of (a) Green S and (b) Indigo carmine by rat caecal microflora.

Green S was relatively very slow with a DI50 value of 456 min (Fig. 2a).

Earlier studies of Daniel (16) reported that approximately 30% of Green S was recovered in the faeces and 0.34% in the urine after oral administration of the dye at a dose level between 250 and 400 mg/kg to rats. Another study showed that all the administered Green S (25 g/litre) was recovered in the faeces of pigs and calves within 5 days and 1-2 weeks, respectively (17). However, our present data suggest that Green S is biotransformed to 3 metabolite through caecal microflora, one of which is a green coloured metabolite and 2 are fluorescent. However, earlier studies of Phillips et al. (12) showed that Green S was not absorbed or metabolized in the gastrointestinal tract of rats, mice and guinea pigs as less than 0.15% of administered dose of Green S (100 mg/kg) was excreted in the rat bile in over 5 hrs.

Our study also indicates that Indigo carmine is found to be biotransformed to 4 fluorescent metabolites. Earlier, Lethco and Webb (15) and Gaunt et al. (4) reported poor absorption of orally administered Indigo carmine in gastrointestinal tract and much of the orally administered dose was excreted unchanged. Furthermore, Lethco and Webb (15) suggested that breakdown of Indigo carmine

in vivo. However, they were unable to account for the number of metabolite(s) in urine and their source (15).

In conclusion, our study indicates that Green S and Indigo carmine are biotransformed by rat caecal microflora to 3 and 4 metabolites, respectively.

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