Tartrazine induced histamine release *in vivo* in normal subjects

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Although the use of tartrazine (E102) as a colouring agent in food has been thought to provoke both urticaria and asthma [1], there is little evidence that it does so by an immunological mechanism.

Evidence obtained from animal studies [2], isolated tissues [3] and isolated cell populations suggests that a pharmacological action is more likely. We have therefore studied the overall effect of this compound on histamine release in healthy subjects, as judged by histamine levels in plasma and urine.

The maximum daily intake of tartrazine probably does not exceed 100 mg [4], with a considerably lower intake for other azo colours. Some investigators have, however, used cumulative doses in excess of 150 mg as a diagnostic tool in an attempt to reproduce clinical symptoms in subjects purporting to be sensitive to these colours. The aims of this study were therefore to examine the clinical and biochemical effects of the ingestion of tartrazine in normal healthy adults, both at doses within the range of common experience and at 'supramaximal' doses.

Materials and methods

Patient selection

Ten healthy adults were selected on the basis of a negative history of adverse reactions to foods and food additives. Atopic status was determined by a positive skin prick test to one or more common inhalant allergens. The group composition with regard to age, sex, weight is shown in Table 1.

Table 1. Composition of study group.

Sex	5 male, 5 female
Age mean	31.1 yrs (22–45 yrs)
Weight mean	59.6 kg (51–70 kg)
Atopic status	5 atopic and 5 non-atopic

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Dietary restrictions

For 72 hours prior to and throughout the study period, all subjects were asked to exclude foods known to be high in histamine, ie alcoholic and fermented products, cheese, yoghurt, aubergines, spinach and smoked or tinned fish and meat. They were also asked to avoid foods containing azo dyes and other colouring agents.

Capsule challenges

Without being told the capsule content, subjects were given tartrazine capsules of 5 mg at 10.30 am followed at two-hourly intervals by 50 mg and 150 mg. On subsequent days either tartrazine at 50 mg or carmoisine at 200 mg was given as a single capsule at 10.30 am. On control days a placebo (150 mg lactose) was given three times, according to the protocol in Fig. 1.

Blood sampling

A Venflon cannula was inserted into a peripheral vein at the beginning of the day for blood sampling at half-hourly intervals from 9.00 am to 6.00 pm. The cannula was kept patent with heparin and saline. A 2 ml blood sample was drawn to waste and a further 2 ml blood sample placed in an EDTA blood collection bottle, mixed, and spun within 5 min of sampling. The top 50 per cent of the plasma was removed and stored at $-20°C$ until the time of assay.

Urine collection

Urine samples were collected into urine collection pots containing 1 ml of chlorhexidine gluconate 20% w/v on days -2, -1 and throughout the test day. An enforced liquid diuresis of at least 3 litres was maintained, allowing urine samples to be collected on an hourly or a more frequent basis.

Histamine and creatinine measurements

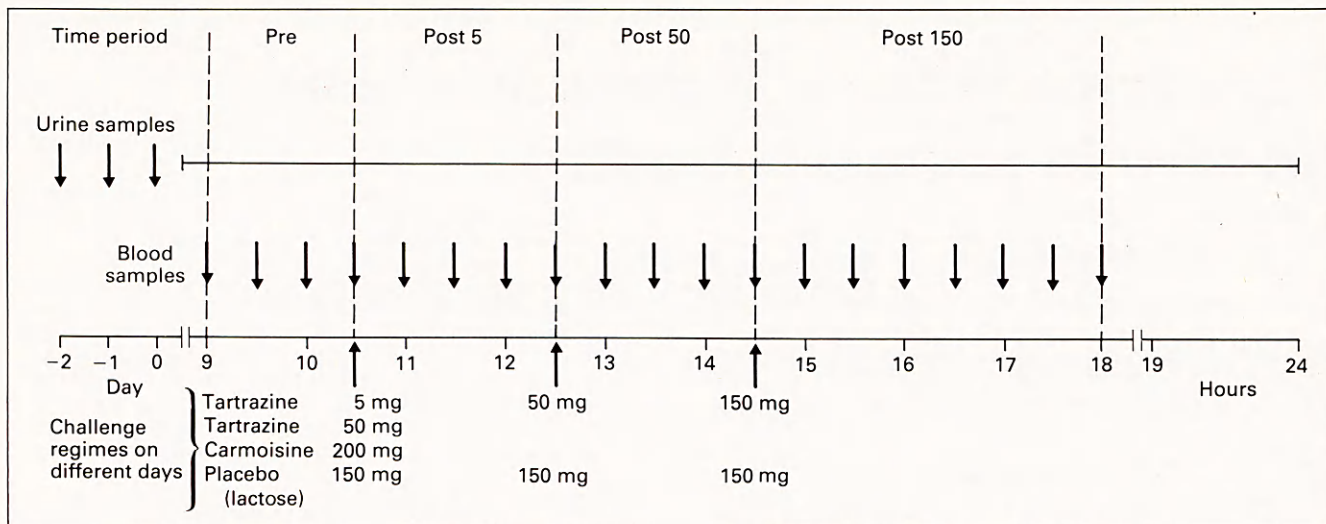Plasma samples were assayed blind for histamine according to the method of Guilloux *et al.* [5] and Church *et al.*

Fig. 1. *Protocol for the 4 days of the study. Graphical representation refers to time blocks as stated.*

[6], with modifications according to Belcher *et al.* [7]. Aliquots of urine were assayed for their histamine content employing the unmodified method of Myers *et al.* [8]. 1-Methyl histamine, the primary metabolite of histamine, was assayed using the unmodified methods of Granerus and Wass [9]. Creatinine was measured spectrophotometrically by the Jaffé reaction [10].

Washed leukocyte challenges

Washed leukocyte preparations were obtained from eight of the subjects after completion of the study. The leukocytes were challenged with tartrazine, carmoisine, sulphanilic acid and anti-human IgE at doses of 1ng–10μg, as used previously [7].

Results

Plasma histamine results

Figure 2 shows the effect of three doses of tartrazine (5, 50 and 150 mg) on plasma and urine histamine levels in one subject on both challenge and placebo days. This subject remained symptomless, but 60 minutes after the ingestion of 150 mg tartrazine the plasma histamine level rose from a baseline of less than 0.2 ng/ml and reached 1.2 ng/ml within a 90-min period. This rise was not seen on the control day. These findings were paralleled in the urine studies, which showed baseline histamine levels of 10 ng/mg creatinine, rising to 50 ng/mg creatinine 150 min after challenge. As with the plasma, urine histamine levels did not rise on the placebo days.

Figure 3 shows plasma histamine data for the 10 subjects on active and control days. Baseline histamine levels for the pre-ingestion samples were 0.27 ng/ml (± 0.04 ng/ml SEM), rising significantly to 0.92 ng/ml (± 0.16 ng/ml SEM) after the ingestion of 150 mg tartrazine ($p < 0.005$). Similar rises were not seen after the 5 or 50 mg doses. Levels on the placebo day indicated no signifi-

cant diurnal or other fluctuations in the baseline. In the nine volunteers who reacted, the mean interval from ingestion to maximum plasma level was 100 min, with a range of 30–200 min.

Urinary levels of histamine and metabolites

Figure 4 shows urinary histamine levels expressed as ng histamine/mg of creatinine to correct for the enforced diuresis and urine volume fluctuations. Results show a significant rise in urinary output of histamine following the ingestion of 150 mg of tartrazine (but not after 5 mg or 50 mg) from a resting level of 16 ng/mg ± 4 ng/mg to 36 ng/mg ± 7.2 ng/mg. These results were statistically significant at the $p < 0.05$ level. On the control day no significant differences were found between urinary histamine levels in the pre-challenge period (9.8 ± 3.4 ng/mg creatinine) and at the end of the day (14 ± 11 ng/mg creatinine).

Measurements of 1-methyl histamine were used in order to provide confirmation of the endogenous production of histamine, since 1-methyl histamine is not subject to the artefacts that may be introduced by bacterial production of histamine in the urogenital tract. These measurements corroborated the urinary histamine trends for both the tartrazine and carmoisine study days (data not shown).

Further tartrazine and carmoisine challenges

Since there was considerable variation in the time interval between ingestion and maximum response, it seemed possible that a large challenge dose after an interval of 120 min might in some cases have obscured the response to the earlier capsule. We therefore carried out a further study in which 50 mg doses of tartrazine were given at the beginning of the day and plasma and urine followed throughout. There were no significant changes in plasma histamine levels between the morning (0.29 ng/
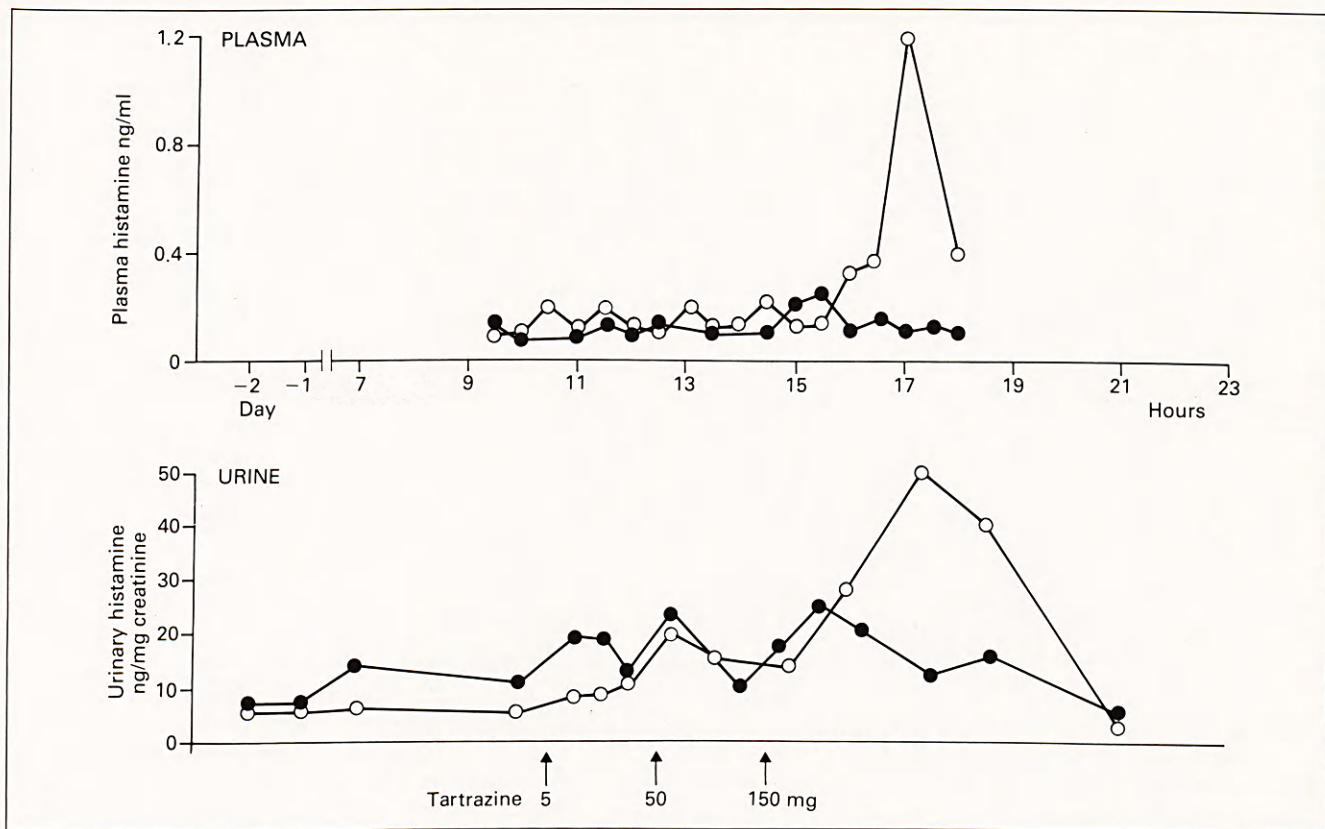
Fig. 2. *Response in a healthy volunteer to increasing doses of tartrazine (O) or placebo (●).*

ml ± 0.048 SEM) and late afternoon (0.32 ng/ml ± 0.045). Urinary histamine was unaffected (data not shown).

The azo colour carmoisine is chemically related to tartrazine. Subjects were given a single bolus of 200 mg and followed throughout the day. There was no significant change in histamine levels, in the plasma (0.155 ng/ml ± 0.045 SEM as compared with 0.3 ng/ml ± 0.058) or urine (9.6 ± 4.2 ng/mg as compared with 13.5 ± 5.6 ng/mg).

Washed leukocyte studies

Washed leukocytes from eight subjects were challenged with tartrazine, carmoisine and sulphanilic acid at doses 1, 10 and 100 ng, 1 and 10 μg/ml. Only one subject reacted to tartrazine and sulphanilic acid with significant histamine release. Significant release was determined as the mean + 2 SD of spontaneous release in 30 control subjects. All eight subjects released histamine in a dose-dependent manner upon challenge with anti-human IgE (Miles-Yeda) at 1:100 and 1:500.

Discussion

Very little work has been done on the pharmacological effects of azo dyes in man, despite the evidence of such effects in animals and *in vitro* systems [2,3]. Our study showed that, in nine of the 10 subjects studied, the ingestion of a cumulative dose of 200 mg tartrazine resulted in a significant rise in plasma and urinary histamine levels 30 min to three hours after ingestion, with a mean time of 100 min. While the plasma rises were substantial—from mean levels of 0.27 ng/ml to 0.92 ng/ml, they did not result in systemic symptoms attributable to histamine. Our evidence thus establishes that large doses of tartrazine can induce histamine release *in vivo*, but it remains to be established whether it is tartrazine or a metabolite that is responsible. Tartrazine is a hydrogen-bonded keto hydrazone which undergoes reductive scission by intestinal flora in the large intestine. Indeed, in the rat, sulphanilic acid conjugates in the faeces may account for 50 to 55 per cent of azo dyes given orally, whereas tartrazine administered intraperitoneally is excreted unchanged in the urine [11]. The time course for the histamine release which we observed seemed too early and possibly too transient to be initiated by metabolites of azo dyes arising as a result of bacterial action in the colon. Furthermore, although it is known that other azo dyes are metabolised in similar fashion [12], carmoisine, a synthetic azo colour, did not provoke histamine release in our subjects.

Little is known about the absorption characteristics of the azo compounds [12], but it has been suggested that less than two per cent of an oral dose is likely to enter the systemic circulation unchanged. This assumes that ab-
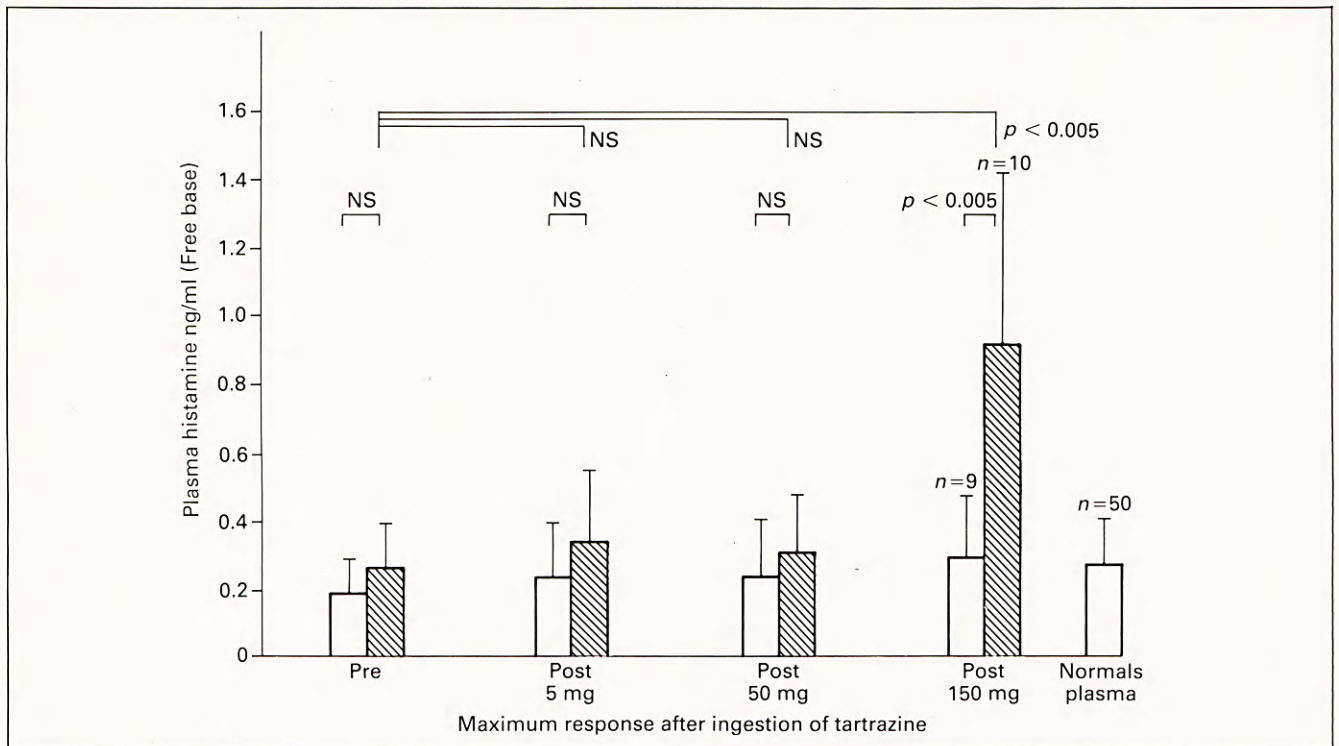
Fig. 3. *Maximum plasma histamine levels (mean ± SEM) in 10 symptomless volunteers following ingestion of tartrazine (shaded) or placebo (unshaded).*

sorption does not vary. It is, however, known that intestinal permeability to some small molecules, as measured by selective sugar absorption [13], increases dramatically as a result of parasitic infection [14] or viral gastroenteritis [15]. The threshold doses at which pharmacological effects are obtained may therefore need further evaluation in a variety of conditions, including those which affect intestinal permeability or changes in the histamine content of the diet.

The histamine release which we have demonstrated in normal subjects, with 200 mg of tartrazine, would appear to occur through a pharmacological and not an immunological mechanism. In seeking to explain the transience of many adverse clinical reactions to tartrazine [1], it is necessary to define the circumstances in which non-immunological reactions involving histamine release can either be potentiated or suppressed and also the circumstances in which they are accompanied by clinical symptoms.

The rise in plasma histamine seen after challenge with 200 mg of tartrazine raises questions concerning both the validity of diagnostic challenge tests with large doses of additives and the adequacy of the safety factors that determine the levels permitted in food. It remains to be established whether the pharmacological effects of large doses can provide a valid indication of the likely clinical effects of normally ingested quantities. As yet, this evidence is lacking.

References
1. Juhlin, L., Michaelsson, G. and Zetterstrom, O. (1972). *Journal of Clinical Allergy and Immunology,* **50,** 92.
2. Safford, R. J. and Goodwin, B. F. J. (1984). *International Journal of Immunopharmacology,* **6,** 233.
3. Hedman, S. E. and Anderson, R. G. G. (1984). *Acta Pharmacologica et Toxicologica.* **52,** 153.
4. Fisher, C. (1986). Personal communication.
5. Guilloux, L., Hartmann, D. and Ville, G. (1981). *Clinica Chimica Acta,* **116,** 269.
6. Church, M. K., Pao, GJ-K. and Holgate, S. T. (1982). *Journal of Immunology,* **129,** 2116.
7. Belcher, N. G., Murdoch, R. D., Dalton, N. *et al.* (1987). *American Review of Respiratory Disease* (In Press).
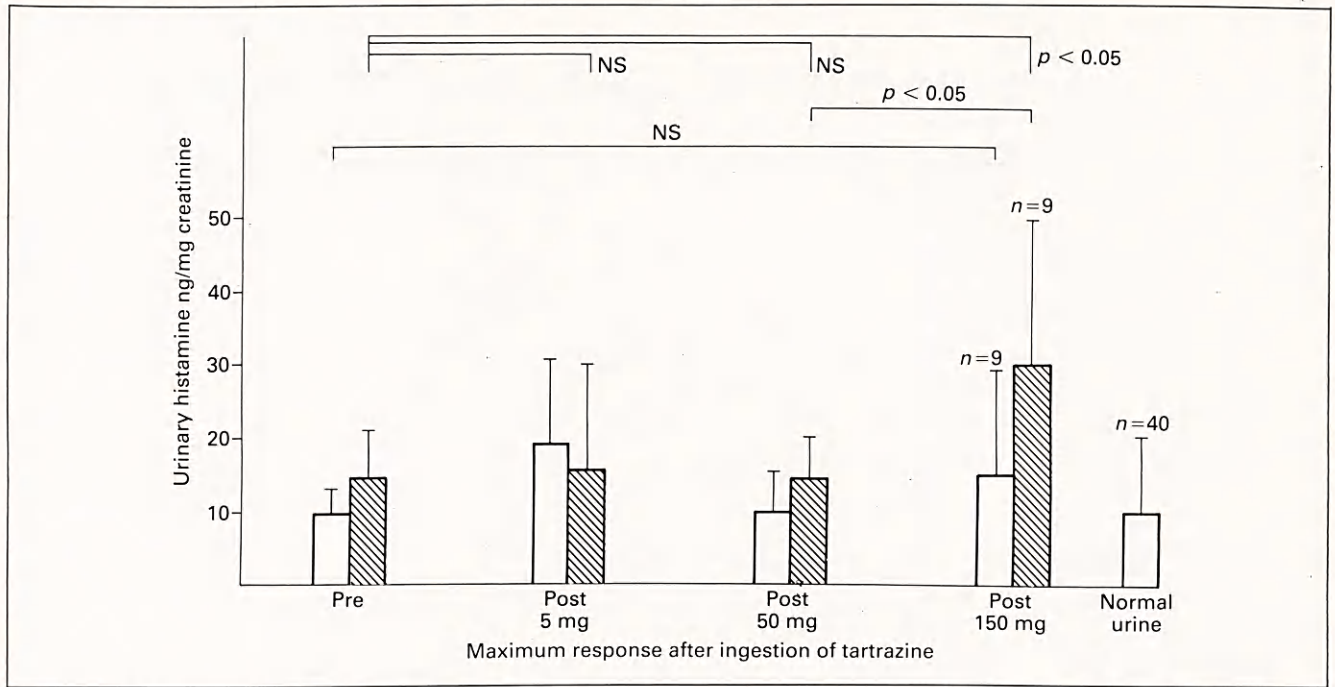
Fig. 4. *Maximum urinary histamine levels (mean ± SEM) in 10 symptomless volunteers expressed following ingestion of tartrazine (shaded) or placebo (unshaded).*

8. Myers, G., Donlon, M. and Kaliner, M. (1981). *Journal of Allergy and Clinical Immunology.* **67**, 305.

9. Graneus, G. and Wass, U. (1984). *Agent and Actions,* **14**, 341.

10. Wooton, I. D. P. (1986). In *Microanalysis in medical biochemistry* (ed E. D. Wooton) p 67. Edinburgh: Churchill Livingstone.

11. Roxon, J. J., Ryan, A. J., Welling, P. G. and Wright, S. E. (1967). *Food and Cosmetic Toxicology,* **5**, 447.

12. Walker, R. (1970) *Food and Cosmetic Toxicology,* **8**, 659.

13. Maxton, D. G., Bjannason, I., Reynolds, A. P. *et al.* (1986). *Clinical Science,* **71**, 71.

14. Cobden, I., Rothwell, J. and Axon, A. T. R. (1979). *Gut,* **20**, 716.

15. Noone, C., Menzies, I. S., Banatvala, J. and Scopes, J. W. (1986). *European Journal of Clinical Investigation,* **16**, 217.