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Tartrazine: Solid-Phase Radioimmunoassay Studies of an Azo Dye Implicated in Allergic Reactions (Azo Dyes and Allergy) (39019)

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Tartrazine, an FD&C[1] permitted synthetic organic color additive, and aspirin have been increasingly found associated in allergic[2] reactions that range from urticaria to severe asthma (1–6). Recently, controversy has arisen over the claim that tartrazine and some other food and drug additives are responsible for some forms of hyperactivity in children (Ben F. Feingold, the Congressional Record of October 30, 1973, S. 19736–19742).

The haptenic properties of tartrazine and some structurally related food dyes have previously been examined by a quantitative hemagglutination procedure (7, 8). This report examines the haptenic properties and relationships of tartrazine, aspirin, and a pyrazolone intermediate of tartrazine using a solid-phase radioimmunoassay procedure (9). The data derived from these studies may provide information on the possible etiological basis of tartrazine and aspirin sensitivity.

Materials and methods. Haptens and reagents. Purified tartrazine (trisodium salt of 3-carboxy-5- hydroxy-1-*p*-sulfophenyl-4-*p*-sulfophenylazopyrazole), FD&C Yellow No. 5, was obtained from Allied Chemical, Morristown, New Jersey. Sulfanilic acid was obtained from Eastman Organic Chemicals, Rochester, New York. A purified preparation of sunset yellow (FD&C Yellow No. 6) was obtained from the Division of Color and Cosmetics, Bureau of Foods, Food and Drug Administration. Aspirin (acetylsalicylic acid) was obtained from Matheson, Coleman, and Bell, Norwood, Ohio.

The monosodium salt of 1-(4-sulfophenyl)-3-carboxy-5-hydroxy-pyrazole (SCHP), an intermediate in the synthesis of tartrazine, was prepared as previously described (10). The theoretical values for nitrogen and sulfur in SCHP are 9.15 and 10.47%, respectively. The synthesized material contained 9.28 and 10.29%, respectively. Further, there was a greater than 99% agreement between the calculated and actual values for titration to neutral equivalence.

Production of antisera to tartrazine. Anti-tartrazine antibodies were obtained by rabbit immunization as described previously (7, 8). The immunogen consisted of a conjugate of tartrazine and bovine serum albumin (BSA) prepared with bis-diazotized benzidine. A full description of the immunochemical specificity of the antisera has been described (8).

Preparation and iodination of tartrazine and SCHP conjugates for radioimmunoassay. Tartrazine and SCHP were coupled to rabbit serum albumin (RSA) as previously described (8). Assuming a molecular weight of 70,000 for RSA, the tartrazine conjugate contained approximately 5 *M* hapten/*M* RSA. The SCHP conjugate contained approximately 9 *M* hapten/*M* RSA. The protein moiety of the conjugates was iodinated with 4 μCi ^{125}I/μg protein by a modification of the chloramine-T method (9). This method of iodination was performed because of the technical difficulties associated with direct iodination of the haptens (unpublished data). Further, direct iodination of the haptens may have altered their reactivity with anti-tartrazine antibodies.

Solid-phase radioimmunoassay. Radioimmunoassay was carried out exactly as described (9). Various concentrations of inhibitors (in 1 ml) in phosphate-buffered saline (PBS) (0.07 *M* phosphate; 0.07 *M* NaCl),

[1] The term FD&C refers to the Food, Drug, and Cosmetic Act of 1938 as amended by the Color Additives Amendments of 1960.

[2] The term allergy as used in this paper refers to a clinical condition and not specifically to an antigen–antibody reaction.

pH 7.2, and containing 1% RSA and 0.1% sodium azide, were added to anti-tartrazine-sensitized polystyrene tubes. The tubes were incubated at 37° for 18 hr, after which 0.001 μg of ^{125}I-labeled tartrazine-RSA or ^{125}I-labeled SCHP-RSA (in 0.1 ml) was added. The tubes were shaken vigorously and incubated at 37° for 4 hr; the contents were washed once with 2 ml of PBS, and counted in a Packard Auto-Gamma Counter (Model 5320). Binding of labeled conjugates to anti-tartrazine antibodies in the absence of an inhibitor was taken as 100% binding in determining the inhibition of binding in the presence of various inhibitors. All determinations were carried out in duplicate and each sample was counted for 10 min. ^{125}I-tartrazine-RSA, in the absence of inhibitors, gave a 10-min count of 1407 while ^{125}I-SCHP-RSA gave a count of 16,350.

Results and discussion. The optimal concentration of anti-tartrazine antibody for sensitizing polystyrene tubes for radioimmunoassay was determined empirically. The salt-precipitated (9) antiserum pool used in the studies reported here was used at 1:50 dilution, representing 10 μg immunoglobulin protein/ml.

Figure 1 contains the structures of tartrazine, SCHP, sunset yellow, sulfanilate, and aspirin. Solid-phase radioimmunoassay inhibition data with these haptens are presented in Fig. 2.

Inhibition data with ^{125}I-tartrazine-RSA and anti-tartrazine are presented in Fig. 2A. The most effective inhibitor in the system was tartrazine, followed by SCHP. Sunset yellow, which has the *p*-azobenzene-sulfonate structure, was a much less effective inhibitor, and sulfanilate, lacking the diazo group, was even less effective. These results suggest that the specificity of the anti-tartrazine antibody is strongly directed toward the SCHP moiety of tartrazine. Aspirin was non-inhibitory, even at a concentration that was 29,000 times greater than that of tartrazine, which resulted in approximately 60% inhibition of ^{125}I-tartrazine-RSA binding. Bis-diazotized benzidine-coupled RSA (8), 10 μg/ml, had no inhibitory effect on the system, thus indicating that bis-diazotized benzidine specificities are not involved. Tartrazine, at the highest concentration employed in the present studies, had no inhibitory effect on an unrelated solid-phase radioimmunoassay system (9). Only 4% of the ^{125}I-tartrazine-RSA label was bound by anti-tartrazine-coated tubes in the absence of inhibitors. This result was probably due to the slow release of noncovalently bound tartrazine from the RSA. Preliminary data indicate that this strong noncovalent binding and slow release continues indefinitely, even in the presence of 1 *M* NaCl (unpublished results). This effect is probably the reason that passive hemagglutination of tartrazine-coupled sheep red cells by anti-tartrazine was not possible in previous studies (7, 8). Nonetheless, the binding was sufficient here to define the haptenic specificities.

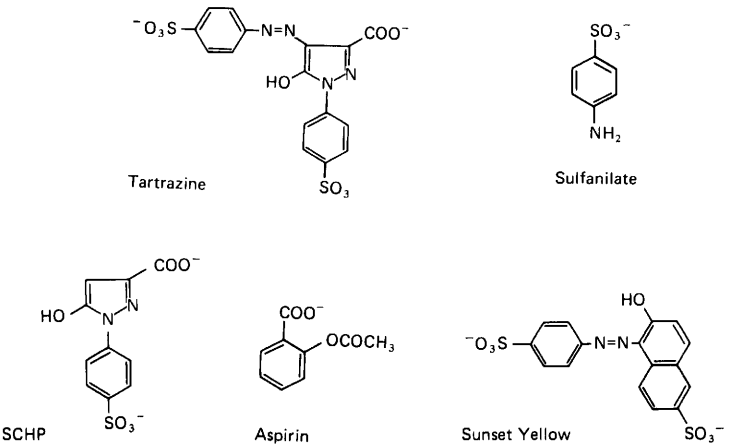

FIG. 1. Structure of haptens.

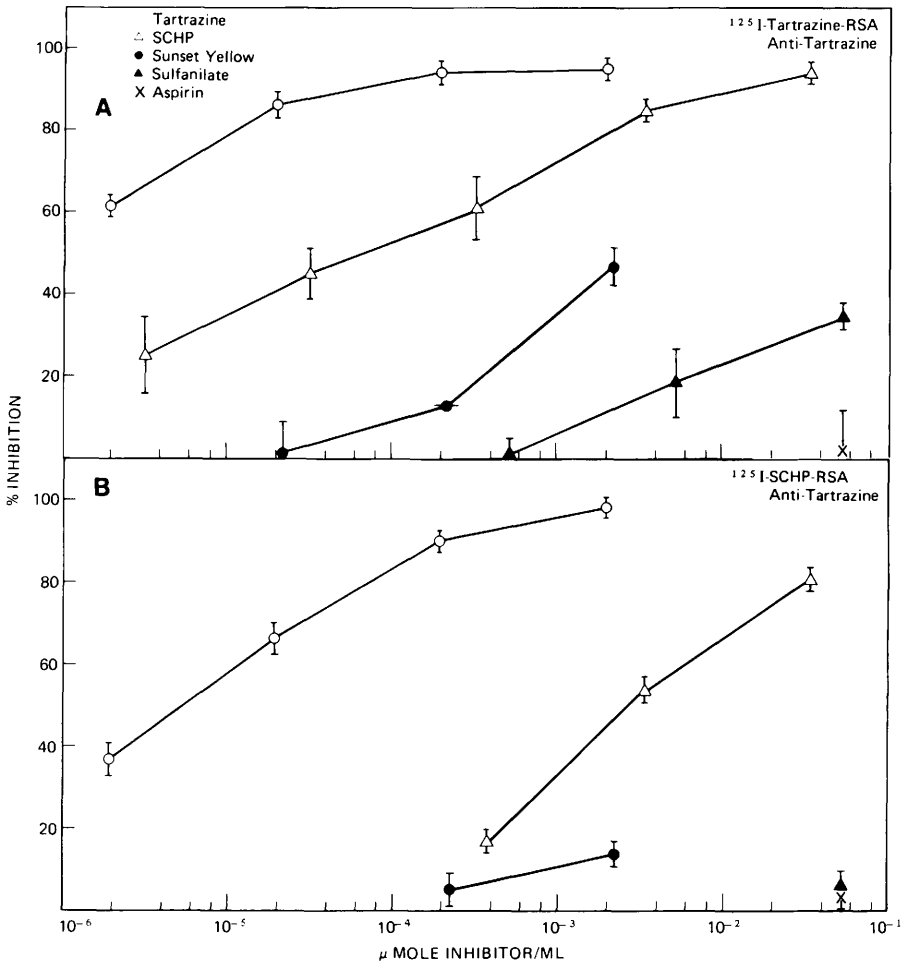

FIG. 2. Solid-phase radioimmunoassay inhibition of anti-tartrazine binding of (A) ^{125}I-tartrazine-RSA and (B) ^{125}I-SCHP-RSA. Data plotted as percentage of inhibition ±SE.

A similar pattern of inhibition (Fig. 2B) was also obtained with ^{125}I-SCHP-RSA as the radiolabel against anti-tartrazine. Tartrazine was again the most effective inhibitor, followed by SCHP. These results suggest that even though the SCHP moiety is probably dominant in the haptenic specificity of tartrazine, other functional groups probably also contribute to the specificity. Again, aspirin was non-inhibitory. Twenty-eight percent of the ^{125}I-SCHP-RSA label was bound by anti-tartrazine-coated tubes in the absence of inhibitors. The greater binding of ^{125}I-SCHP-RSA as opposed to ^{125}I-tartrazine-RSA was probably due to the lack of a strong noncovalent association of SCHP with RSA (unpublished data). Thus, a built-in inhibitor, slowly released from the conjugate, was not present in the conjugate to diminish the binding to antibody-coated tubes.

Numerous attempts to produce antibodies specific for aspirin (11) were unsuccessful, so this aspect of the haptenic relationship of tartrazine and aspirin was not examined.

Clinically, tartrazine and aspirin sensitivities are associated (1–6). The lack of a haptenic relationship between the two substances, as studied here, suggests that mechanisms other than antigen–antibody reactions could be responsible for aspirin and tartrazine sensitivity. This nonantigen–antibody aspect of aspirin sensitivity has been pre-

viously suggested (3). The data presented here provide immunochemical support for such a thesis. Studies are in progress to ascertain whether humans with tartrazine sensitivity possess anti-tartrazine antibodies. These studies would more precisely define the nature of the adverse reaction of some people to tartrazine.

Finally, tartrazine has been shown to undergo reductive scission in the gut after oral administration (12). An important product of this scission is 1-(4-sulfophenyl)-3-carboxy-4-amino-5-hydroxy-pyrazole, which differs from 1-(4-sulfophenyl)-3-carboxy-5-hydroxy-pyrazole (SCHP) only in that it contains an amino group. Further studies therefore seem warranted on the biological and haptenic properties of SCHP.

Summary. A solid-phase radioimmunoassay procedure was adapted for the haptenic study of tartrazine, an azo dye implicated in various forms of allergy. Further, the haptenic relationship of tartrazine and aspirin was investigated, since sensitivity of individuals to the two substances is often clinically associated. The specificity of antibody to tartrazine was directed strongly toward a pyrazolone intermediate of the molecule, 1-(4-sulfophenyl)-3-carboxy-5-hydroxy-pyrazole. Aspirin did not cross-react with anti-tartrazine, suggesting that the clinical association of aspirin and tartrazine sensitivity in patients is a nonimmunological phenomenon.

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