ate conditions (*16*). To test the possibility that EST 6 is involved in the production of the male lipid, extracts of males from several strains of *D. melanogaster* homozygous for the *Est* 6^S and *Est* 6^o alleles were subjected to thin-layer chromatography (*14*). The male lipid was clearly present in strains carrying both the null and active alleles, an indication that the enzyme is not involved in the production of this substance.

Gilbert *et al.* (*17*) have shown that the number of sperm stored and its utilization by females differs according to the EST 6 type of the inseminating male. Although the total number of progeny produced by females inseminated by *Est* $6^{S/S}$ or *Est* $6^{o/o}$ males is not significantly different, more sperm are stored in *Est* $6^{0/0}$ inseminated females and sperm is initially used at a greater rate by *Est* $6^{S/S}$ inseminated females. These results suggest that EST 6 may influence remating by affecting both the storage and utilization of sperm. The possibility remains that EST 6 may interact with the male lipid in the female's reproductive tract to achieve these effects.

The widespread occurrence of nonspecific esterase polymorphisms in many plant and animal populations has made them valuable tools for systematic, genetic, population genetic, and medical studies. However, an almost complete absence of information about their functions in vivo limits their utility. Our results show that *D. melanogaster* is viable without EST 6, but that the enzyme may be involved in physiological control of reproduction in *Drosophila*. Although the relation between genetic variation at the *Est 6* locus and possible differences in individual fitness are unknown (*17*), our observations provide a system in which to begin an experimental study of this relation.

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Food Dyes Impair Performance of Hyperactive Children on a Laboratory Learning Test

Abstract. *Forty children were given a diet free of artificial food dyes and other additives for 5 days. Twenty of the children had been classified as hyperactive by scores on the Conners Rating Scale and were reported to have favorable responses to stimulant medication. A diagnosis of hyperactivity had been rejected in the other 20 children. Oral challenges with large doses (100 or 150 milligrams) of a blend of FD &C approved food dyes or placebo were administered on days 4 and 5 of the experiment. The performance of the hyperactive children on paired-associate learning tests on the day they received the dye blend was impaired relative to their performance after they received the placebo, but the performance of the nonhyperactive group was not affected by the challenge with the food dye blend.*

Feingold (*1*) hypothesized that artificial food dyes are pharmacologically active substances that induce or aggravate symptoms of hyperactivity in children. Even though a controlled double-blind study of 46 hyperactive children (*2*) and a series of open trials involving 142 hyperactive children (*3*) have confirmed that about 50 percent of those tried on the Feingold diet showed a decrease in symptoms of hyperactivity, the mechanism for and the magnitude of this effect remains in question. Some studies (*4*) have failed to document that reintroduction of (or challenge with) 1 to 26 mg of artificial food dyes (*5*) elicits symptoms of hyperactivity. In other studies, the behavioral effects of the diet (*2, 6*) or the responses to challenges with food dyes (*7*) have been considerably less in magnitude than in reports based on clinical observations (*1, 8*). For these reasons, the benefits associated with the Feingold diet have been attributed to a placebo or Hawthorne effect (*9*).

The lack of response to challenges in previous studies may have been due to an insufficient dose of dye. We have used higher challenge doses—up to 150 mg, which the Food and Drug Administration (*10*) estimated to be at the 90th percentile for daily consumption of artificial food dyes by children from 5 to 12 years of age. With these larger doses we have documented a dye-induced impairment of performance on a laboratory learning test.

We tested 40 children (36 boys and 4 girls; average age, 10 years) referred to the Child Development Clinic of the Hospital for Sick Children with behavioral symptoms suggesting hyperactivity (for example, short attention span, aggressiveness, overactivity, impulsivity, distractibility). To form these groups, we selected 20 children who had shown a favorable response to stimulant medication (*11*) and 20 children in whom an adverse response had been documented. The group of favorable responders had an average score of 16.2 in the Conners Rating Scale (*12*), and the group of adverse responders had an average score of 12.3, which is under the criterion of 15 established for the diagnosis of hyperactivity. Thus, one group was composed of children on whom the diagnosis of hyperactivity had been confirmed, and the other group was composed of children for whom a diagnosis of hyperactivity had been rejected.

The children were admitted to the Clinical Investigations Unit (CIU) of the hospital in pairs matched for age and sex

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Table 1. The relative performance of the hyperactive children (N = 20) challenged at 10 a.m. with food dye blend or placebo.

Statistical tests and conditions	Time of testing			
	9:30 a.m.	10:30 a.m.	11:30 a.m.	1:30 p.m.
Dye-placebo difference* (mean + standard error)	−5.9 ± 4.18	4.2 ± 4.49	11.0 ± 3.99	9.8 ± 3.82
Value for t-test (d.f. = 19)	−1.41, $P > .10$	0.94, $P > .10$	2.75, $P < .02$	2.57, $P < .02$
Numbers of patients with				
More errors after receiving dyes	8	10	16	17
No difference	0	1	0	1
More errors after receiving placebo	12	9	4	2
Significance level†	.25	.5	.006	<.002

*For each of the six tests, the number of errors each child made after receiving the placebo was subtracted from the number of errors made after receiving the food dye blend. The mean of this difference score across patients, divided by the standard error of the mean, yielded the statistic reported in the table (Student's t-test for paired observations, two-tailed test). †Sign test.

for controlled implementation (*13*) of the Feingold diet. Any medication they had been receiving was stopped on the day before admission, and the diet was strictly maintained for 3 days. On days 4 and 5 the children received, orally at 10:00 a.m., capsules that contained either a blend of nine food dyes (*5*) or placebo (sugar), with the dye and placebo order counterbalanced across subjects. For 20 of the patients (ten in each group), the challenge dose was 100 mg of the blend; for the other 20 patients, the dose was 150 mg.

On each day of the experiment, paired-associate learning tests were administered at 9:30, 10:30, and 11:30 a.m. and at 1:30 p.m. (*14*). This test was chosen because it had been used previously (*15*) to document favorable and adverse responses to challenges with other pharmacological agents (stimulant drugs). Pictures of animals were used as stimuli, and numbers were used as the responses (*16*). The stimulus items were presented sequentially in random order, and up to 10 seconds were allowed for a response. The total number of errors made by a child before he or she attained a criterion of perfect recitation of the list was taken as the measure of performance on each of the learning tests.

A four-factor analysis of variance revealed that the challenge with the food dye blend significantly impaired performance on the learning task. The interaction of two factors—that is, challenge (dyes or placebo) and time of testing (before, 1/2 hour, 1 1/2 hours, or 3 1/2 hours after the challenge)—was significant [$F(3, 108) = 2.73, P < .05$], suggesting a rapid onset of the effect of food dyes. Neither the main effect of the dose (100 mg or 150 mg) nor any interaction with the dose factor was significant in the analysis, indicating that even the lower of the two doses was sufficiently high to elicit the response measured by the learning test.

In addition to the two-way interaction, the three-way interaction of patient type (hyperactive or nonhyperactive), challenge (dyes or placebo), and test time was significant [$F(3, 108) = 3.99, P < .01$]. As shown in Fig. 1, the food dye challenge affected children classified as hyperactive, but not the group classified as nonhyperactive. An analysis of the simple effects (that is, separate analysis of the data for the two groups) revealed a significant two-way interaction of challenge and test time in the hyperactive group [$F(3, 54) = 4.45, P < .01$] but not in the nonhyperactive group.

The data in Table 1 indicate that the effect of the high dose of food dyes took over 1/2 hour to become evident, reached its maximum by 1 1/2 hours, and lasted at least 3 1/2 hours. This pattern is consistent with the notion that the response to food dye is based on a pharmacologic or toxic mechanism, rather than an immunologic or allergic mechanism.

Our data suggest that a large dose of food dye blend decreases attention span (*17, 18*) in hyperactive children, as reflected by performance on the learning test (Table 1). The Conners Rating Scale (*12*) was also filled out twice daily, but no difference between the dye and placebo conditions was manifested on this measure of social behavior. Other investigators (*19*) have also observed that laboratory tests may be more sensitive than parent or teacher rating for documenting adverse effects of dye challenges following dietary intervention. Thd dissociation between measures of learning and social behavior has been reported previously in an evaluation of hyperactive children and their responses to stimulant drugs (*20*). However, an individualized behavioral inventory (instead of a standard rating scale) has been used in a single case design recently to document a consistent response to food dye in the natural environment of the home (*21*).

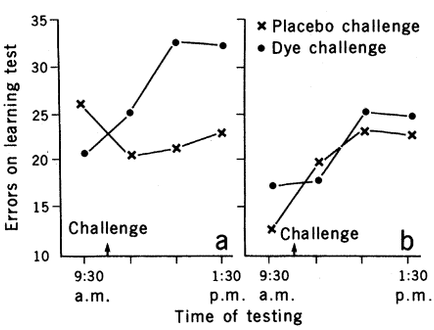

Fig. 1. Responses of (a) hyperactive children (N = 20) and (b) nonhyperactive children (N = 20) challenged with a food dye blend or with placebo.

The challenge with food dye resulted in an increase in errors on the learning test in the hyperactive children selected on the basis of severity of their symptoms and response to stimulant medication but not in the nonhyperactive children. Selection on the basis of response to stimulant medication may have established a homogeneous group of patients with a preexisting neurochemical disturbance that was further aggravated by food dye. At least one food dye (Red No. 3) affects neurotransmitter uptake in synaptosomal preparations (*22*).

The 150-mg challenge dose of food dyes is more than ten times the amount used in most previous studies (*5*). We have reported elsewhere (*23*) that no significant dye-induced impairment in performance on our laboratory learning test occurs when 26 mg of food dye blend [the Nutrition Foundation's underestimate (*5*) of daily consumption] is given in a single dose. When a blend of dyes is used, a high dose may be necessary to elicit a behavioral response in hyperactive children. This may be true for animals as well (*24*).

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14. Learning tests were also given at 2:30 p.m. and 3:30 p.m. For the children who were challenged with 100 mg of food dye blend, a second challenge was administered at 2:00 p.m. This second challenge was not administered to children receiving the 150-mg dose. The interpretation of the data from these last two tests each day is consistent with the results reported here (*23*).
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Behavioral Responses to Artificial Food Colors

Abstract. *Twenty-two young children, maintained on a diet that excluded certain foods, were challenged intermittently with a blend of seven artificial colors in a double-blind trial. Parents' observations provided the criteria of response. One child that responded mildly to the challenge and one that responded dramatically were detected. The latter, a 34-month-old female, showed a significant increase in aversive behaviors. These results further confirm previous controlled studies.*

Feingold (*1*) contends that as many as 50 percent or more of the children labeled as "hyperkinetic" or "hyperactive" can be treated successfully by eliminating from their diet synthetic colors, flavors, and certain fruits and vegetables said to contain "natural salicylates." Feingold's hypothesis emerged from clinical and parental observations, not controlled experimentation, but they were cogent enough to prompt several controlled trials.

We assessed sensitivity to artificial colors in 22 children, 15 male and 7 female, between 2.5 and 7 years old. All were enrolled in the Kaiser-Permanente Health Maintenance Organization (*2*) during the experiment. The problem behaviors of each child had been reported as improved when the child was kept on a diet that excluded artificial colors and flavors for at least the 3-month period preceding the study. None of the participants suffered from clinically significant medical or psychiatric problems; none had been diagnosed as hyperkinetic. To select an appropriate dose of colors, we obtained dietary histories on 80 children who resembled the study population. From this survey, we estimated the mean amounts of seven FD & C certified colors ingested daily, relying on published industry practices as the basis for the calculations (*3*).

The study was conducted as a double-blind trial; each child served as its own control. At a specified time on each of 77 days, the child consumed a bottle of soft drink (*4*) containing either a combination of caramel and cranberry coloring (placebo) or a freeze-dried monoblend of seven colors (Table 1) plus cranberry coloring (challenge). The two drinks were indistinguishable by sight, smell, taste, or stain color. On 8 days distributed randomly among weeks 3 through 10 of the study period, each child received the challenge drink (*5*). No parent or individual member of the study team knew whether a child was being challenged on a given day (*6*).

The children were maintained on a diet that excluded artificial colors and flavors, 14 fruits, 3 vegetables, specified spices and extracts, and the preservatives BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). Not all parents restricted the designated fruits and vegetables, claiming that their children were not sensitive to these items. We also attached identifying markers to Kaiser membership cards to alert staff to the child's special status because most pediatric drug and vitamin formulations contain artificial colors and flavors.

Parental observations provided our main data. Before a child entered the study, the parent (*7*) sorted a deck of punched cards labeled with items from several standardized behavior inventories (*8*). In successive sorts, the parent narrowed the items to seven aversive behaviors associated with infractions and three positive (typical "good") behaviors. This procedure yielded the ten target behaviors that served as response criteria for each child throughout the 11-week experimental period.

Each day of the study, the parent conducted two 15-minute observation periods, one within 3.5 hours after drink consumption and one at a later time. During these periods, the parent recorded on a form each occurrence of any of the target behaviors. Twenty-four hours after the drink was consumed the parent also recorded a global estimate, on a scale of 1 to 9, of the frequency and severity of each target behavior during that period. The parent also noted any observed or suspected dietary infractions, recorded sleep data, rated the day as a whole, completed the ten-item Conners ques-

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