Food Dyes Produce Minimal Effects on Locomotor Activity and Vitamin B-6 Levels in Postweanling Rats[1]

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ABSTRACT We investigated the effects of food dye consumption on locomotor activity, brain neurotransmitters, tissue vitamin B-6 levels, and hepatic cytochrome P-450 concentrations in postweanling rats. Animals were individually housed in stabilimeter-type activity cages for 4½ weeks, and fed ad libitum a semipurified basal diet containing graded levels (4, 2, 1, 0.5 or 0%) of a blend of all seven Food, Drug and Cosmetic (FD & C) food dyes. Rats in the 4% dye group were significantly ($P < 0.001$) less active during the first 3 weeks of dietary treatment, but no significant differences existed among groups during the final 10 days. Similarly, although dye ingestion depressed food intake ($P < 0.0025$) and body weight ($P < 0.05$) when averaged for all animals, the differences among groups disappeared by the last week of the experiment. Postmortem tissue analyses revealed no significant effect of dyes on brain tissue levels of serotonin, dopamine, norepinephrine, 5-hydroxyindoleacetic acid or homovanillic acid. Moreover, no significant differences were detected in either plasma and brain tissue levels of pyridoxal phosphate or in hepatic cytochrome P-450 concentrations. These results demonstrate that animals may adapt to the chronic consumption of food dyes and do so with minimal evidence of toxicity. Our data also suggest that previously reported behavioral abnormalities attributed to food dyes are probably unrelated to altered vitamin B-6 metabolism. J. Nutr. *114*: 1402–1412, 1984.

INDEXING KEY WORDS food dyes • locomotor activity • vitamin B-6 • neurotransmitters

The possibility that common food dyes may alter central nervous system metabolism and modify behavior remains a controversial and unresolved issue. Results of several clinical trials have indicated that the ingestion of food dyes impaired learning and aggravated symptoms in hyperkinetic children (1, 2) but other studies showed only minimal effects when susceptible children consumed dye-containing cookies (3, 4). Variability in dye dosages and subject selection criteria possibly contributed to the conflicting results. Among the few reported animal studies, the administration of food dyes to developing rat pups appeared to increase spontaneous locomotor activity and to affect cognitive function (5, 6). Although the mechanisms underlying the reported behavioral responses to food dyes were not clear, several researchers have suggested that

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effects on brain neurotransmitters are operative. Logan and Swanson (7) demonstrated in vitro that food dyes exert a neurotoxic effect in rat brain membrane preparations that prevent the uptake of dopamine and other neurotransmitters, and Lafferman and Silbergeld (8) reported that erythrosine B inhibited dopamine uptake in rat caudate synaptosomes.

We have conducted experiments to determine whether food dye consumption influences behavior and brain chemistry. We were particularly interested in the possibility that dyes could affect tissue levels of pyridoxal 5'-phosphate (PLP), the major coenzyme form of vitamin B-6, which is prominent in central nervous system metabolism. PLP is a cofactor for the aromatic-l-amino acid decarboxylase enzymes in serotonin and catecholamine biosynthesis. A number of drugs as well as naturally occurring substances are antagonistic to PLP (9, 10). We therefore postulated that food dyes might also be chemically reactive with PLP and that behavioral abnormalities reportedly due to food dyes might be secondary to disturbed PLP metabolism. In the present study we fed postweanling rats various levels of a food dye blend and monitored their daily spontaneous locomotor activity for 4½ weeks. At the end of the study period neurotransmitters were assayed in brains and PLP concentrations were determined in plasma, brain and liver. We also measured hepatic cytochrome P-450 to determine whether food dyes produced a "drug-like" response characteristic of a mixed function oxidase inducer.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 45–50 g were obtained at 21 days of age. During a 3-day adaptation period they were provided a semipurified basal diet (Teklad Test Diets, Madison, WI) (table 1) and distilled water ad libitum. When the animals were 24 days old, they were randomly assigned to individual stabilimeter-type activity cages (11) where they remained for the duration of the experiment.[5] The animals were maintained on the basal diet for nine more days while individual activity counts were recorded. Beginning at 33 days of age, the animals were divided into five dietary groups, eight rats per group, evenly distributed among the horizontal and vertical rows of the activity cages. Each group was administered a different level (4.0, 2.0, 1.0, 0.5 or 0%, wt/wt) of a blend of all seven Food, Drug and Cosmetic (FD & C) approved food dyes incorporated into the basal diet (table 2). The individual dyes in the blend were proportional to their estimated occurrence in American foods. The animals were maintained on the dye-containing diets for 4½ weeks. A diurnal fluorescent lighting cycle of 12 hours (lights on at 0800) was maintained in an animal room with an ambient temperature of 23°C.

Experimental procedures. Throughout the experimental period food intake and

TABLE 1

Composition of diet

Ingredient	Amount
	g/kg
Casein, vitamin free test	250.0
Cornstarch	318.98
Sucrose	318.98
Cottonseed oil	60.0
Nonnutritive fiber (cellulose)	10.0
Sodium selenite	0.0001
Mineral mix, Bernhart-Tomarelli[1]	40.0
Vitamin mix[2]	2.0398

[1]Supplied in grams/kilogram of mineral mix: calcium carbonate, 21.0; calcium phosphate dibasic, 735.0; magnesium oxide, 25.0; potassium phosphate dibasic, 81.0; potassium sulfate, 68.0; sodium chloride, 30.6; sodium phosphate dibasic, 21.4; cupric citrate, 0.46; ferric citrate, 5.58; manganese citrate, 8.35; potassium iodide, 0.0072; zinc citrate, 1.33; citric acid, 2.2728.
[2]Supplied in grams: *p*-aminobenzoic acid, 0.01; biotin, 0.0003; vitamin B-12 (0.1% trituration in mannitol), 0.2; calcium pantothenate, 0.02; choline chloride, 1.5; folic acid, 0.002; inositol, 0.1; niacin, 0.04; riboflavin, 0.01; thiamin · HCl, 0.005; pyridoxine · HCl, 0.006; dry vitamin A palmitate (500,000 U/g), 0.04; ergocalciferol in corn oil (400,000 U/g), 0.0075; DL-α-tocopherol (1100 U/g), 0.1; menadione, 0.005.

[5]Conventional cages were modified to tilt slightly in response to animal locomotor activity. Cage movements were detected by a piezoelectric crystal transducer, and the amplified signals were fed into electronic digital counters.

TABLE 2

Composition of food dye blend

FD & C no.	Dye class	Common name	% of blend
Blue #1	Triphenyl-methane	Brilliant Blue FCF	3.12
Blue #2	Indigoid	Indigotine	1.70
Green #3	Triphenyl-methane	Fast Green FCF	0.13
Red #3	Xanthene	Erythrosine	6.08
Red #40	Azo	Allura Red AC	38.96
Yellow #5	Azo	Tartrazine	27.09
Yellow #6	Azo	Sunset Yellow FCF	22.92
Total			100.00

body weight were recorded every other day and activity counts, during a period of 22 hours, were recorded daily. At 65 days of age, animals were killed by decapitation, and blood was drained into heparinized (0.1 ml 1% aqueous heparin, 1000 USP/ml) 15-ml screw-cap culture tubes wrapped with aluminum foil to minimize light exposure. Plasma was separated from cells by centrifuging at 1500 × g for 20 minutes and stored at −20°C. Brains were quickly removed, cut along the midsagittal plane into halves, frozen in liquid nitrogen in less than 1 minute and stored at −80°C. Neurotransmitters in individual brain halves were assayed within 3 weeks, alternating the left and right halves of successive animals within each dietary group. The remaining eight half brains in each group were used for the determination of PLP. Livers were excised, weighed and minced. After rinsing with an excess of isotonic KCl to facilitate removal of blood, the minced liver was frozen in liquid nitrogen and stored at −20°C. A microsomal suspension was prepared from about half of each liver within 2 days of death and assayed for cytochrome P-450 and protein. The remainder of the liver and the plasma were analyzed for PLP.

Analytical techniques. Neurotransmitters were assayed by fluorometric procedures (12–15). The assay permitted the simultaneous extraction and quantitation of three neurotransmitters (serotonin, norepinephrine and dopamine) and two metabolites (5-hydroxyindoleacetic acid and homovanillic acid) from the same brain sample. PLP was assayed by a radioisotopic enzymatic method based on the decarboxylation of L-[1-^{14}C]tyrosine by the PLP-dependent enzyme tyrosine apodecarboxylase (16). PLP extracts were prepared from deproteinated plasma (17) and brain (18). Liver samples were deproteinated following homogenization with cold isotonic saline (4×, wt/vol) in a Potter-Elvehjem tissue grinder. A 0.1-ml aliquot of the liver homogenate was transferred to 15-ml screw-cap culture tubes, and 9.9 ml of 10% (wt/vol) trichloroacetic acid was added. Tubes were applied to a vortex mixer for 10 seconds and incubated at 32°C for 30 minutes. Following the incubation, the tubes were applied to the vortex mixer for 5 seconds and centrifuged for 30 minutes at 1500 × g. Aliquots of 1.0 ml were removed and extracted by the same procedure as was used for plasma (17). All operations during the PLP assay were performed under subdued yellow light. Liver microsomal suspensions were prepared as described previously (19) and assayed for cytochrome P-450 (19) and protein (20). The microsomal suspension was diluted 1:50 with 0.1 M potassium phosphate buffer (pH 7.0) prior to the protein assay, and bovine serum albumin (fraction V) was used for the standards.

Statistical analysis of the data. All data were analyzed by using the S.A.S. statistical software package with an IBM 370 computer (IBM Corp., White Plains, NY). Food intake, body weight and locomotor activity were expressed relative to a baseline. For each of these parameters, we used the General Linear Models (GLM) procedure to develop statistical models representing data averaged over all experimental groups throughout the observation period. The best-fit model for the "overall data" was tested by evaluating the sample variance and residual plot, which indicated whether a transformation of the data was appropriate. The GLM Type I sum of squares was used to test significance of main effects and interactions. Because of the daily fluctuations in locomotor activity, we plotted the computer-predicted means, rather than the actual means at each time point. The predicted means were the points falling on the regression line derived from the actual

means. We also utilized an ANOVA to detect daily significant differences in food intake, body weight and locomotor activity. Post hoc analysis of the biochemical parameters was performed using the Tukey Honestly Significant Difference test (21).

RESULTS

The dye-containing diets were tolerated by the animals despite the immediate onset and persistance of diarrhea in the 1, 2 and 4% groups. Food intake, body weight and locomotor activity generally were inversely proportional to the dye level consumed. These parameters were all expressed relative to a baseline to facilitate comparisons of groups before and after dye consumption. Because a steady state did not exist during the period before dye adaptation for food intake and body weight, which continued to increase since the animals were growing, the day prior to dye supplementation (age 32 days) was chosen as the baseline. A more representative baseline for locomotor activity, due to the variability of these data, was the mean activity counts for the preceding 2 days (ages 31 and 32 days). Since the magnitude of absolute changes may have depended on the initial levels, the data were all expressed as a ratio to baseline. This relative change compensated for innate differences among the animals and for slight variations in cage sensitivity. We developed statistical models using the GLM procedure to describe the effects of dye consumption on food intake, body weight and locomotor activity. The best-fit models for body weight and locomotor activity were achieved with logarithmic transformations of the data. The sample variance and residual plot generated by the GLM procedure confirmed that the models and baseline values selected provided the best fit for the data.

The animals in the 2 and 4% groups initially rejected their diets (fig. 1*A*), but 5 days after the addition of dyes (age 38 days) the ANOVA showed no significant differences in food consumption among the groups. Throughout most of the experiment, however, the ANOVA indicated that the daily differences among groups were significant ($P < 0.05$), with the 1, 2 and 4% groups eating consistently less. The daily food intake of the 4% group fluctuated the most. There were significant main effects of dye ($P < 0.0025$) and age ($P < 0.0001$) on food intake (table 3*A*) as well as a significant dye × age interaction ($P < 0.0001$).

Growth curves of the 0, 0.5, 1 and 2% dietary groups were all similar (fig. 1*B*). Except for a few isolated days, when there were no significant differences between groups, the body weight of the 4% dye group was significantly lower ($P < 0.05$) than the other dietary groups between ages 34 and 54 days. The GLM analysis (table 3*B*) showed a significant effect ($P < 0.05$) of dyes on body weight that was age-dependent ($P < 0.001$). A separate GLM analysis (not shown) with Type IV sum of squares suggested that the lower body weight of the 4% group was related to decreased food consumption ($P < 0.001$) and not to an intrinsic effect of the dyes ($P > 0.482$). However, it is also possible that the diarrhea or a threshold toxic effect of the dyes not detected by the statistical analysis was responsible for the lower body weight of this group.

Locomotor activity was more variable than either food intake or body weight. Because of the daily fluctuations in activity counts, we utilized a regression model and a log transformation to generate the predicted means (fig. 1*C*). The ANOVA showed that the 4% dye group was significantly ($P < 0.001$) less active than the other groups only between ages 35 and 53 days. The corresponding GLM analysis (table 3*C*) indicated two distinct geometric patterns: the 0, 0.5, 1.0 and 2.0% dye groups all had a quadratic shape over time (age × age term significant, $P < 0.0001$) and the 4% dye group had a cubic shape (age × age × age term significant, $P < 0.001$). This analysis also demonstrated that the significant ($P < 0.0025$) effect of dyes on locomotor activity was derived entirely from the 4% group. If this group were omitted from the analysis, the dye effect was not significant ($P > 0.250$).

The animals eventually seemed to adapt to the dye-containing diets. On the final two measurement days (ages 62 and 64 days) there were no significant differences in food intake among groups. Similarly, body weight and locomotor activity did not differ significantly among groups after 54 days of

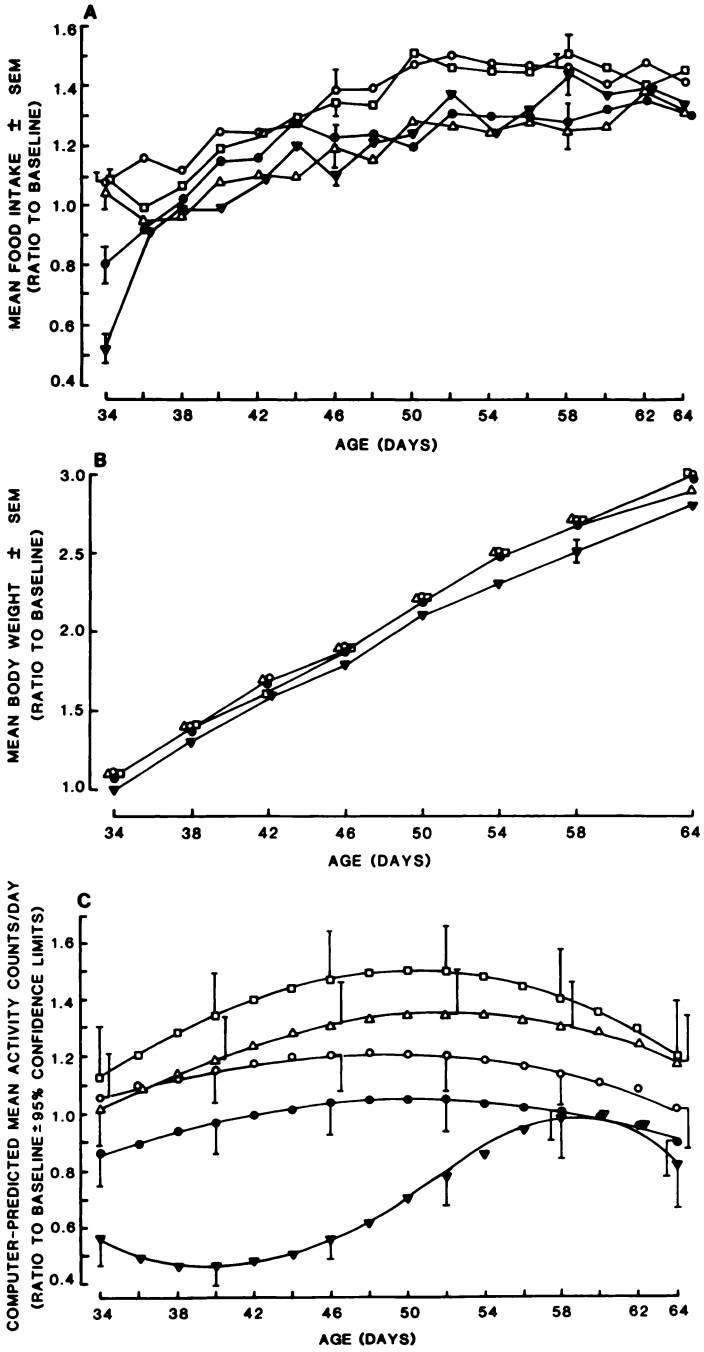

Fig. 1 Effect of various dietary levels of a food dye blend on food intake (1A), body weight (1B), and locomotor activity (1C) in post-weanling rats. Percent dye blend (wt/wt) contained in basal diet: (○) 0%, (□) 0.5%, (△) 1.0%, (●) 2.0%, (▼) 4.0%.

TABLE 3

GLM analysis of food intake (A), body weight (B) and locomotor activity (C)[1]

Variable	df	F	P > F
A. *Food intake*			
Dye	4	5.63	0.0025
Animal (dye group)	35	13.38	0.0001
Age	1	950.22	0.0001
Age × age	1	159.09	0.0001
Dye × age	4	11.20	0.0001
Dye × age × age	4	5.52	0.0002
		R^2 = 0.767	
B. *Body wt*[2]			
Dye	4	2.83	0.0500
Animal (dye group)	35	57.39	0.0001
Age	1	99999.99	0.0001
Age × age	1	3025.42	0.0001
Dye × age	4	5.91	0.0001
Dye × age × age	4	0.59	0.6733
		R^2 = 0.995	

	All dye groups			Omits 4% dye group		
	df	F	P > F	df	F	P > F
C. *Locomotor activity*[2]						
Dye	4	5.49	0.0025	3	1.45	0.2500
Animal (dye group)	35	35.52	0.0001	28	37.77	0.0001
Age	1	51.95	0.0001	1	2.21	0.1378
Age × age	1	31.19	0.0001	1	43.59	0.0001
Age × age × age	1	10.28	0.0014	1	0.40	0.5282
Dye × age	4	32.51	0.0001	3	1.36	0.2527
Dye × age × age	4	2.53	0.0389	3	0.50	0.6844
Dye × age × age × age	4	7.08	0.0001	3	0.96	0.4116
		R^2 = 0.652			R^2 = 0.566	

[1]GLM, General Linear Models. [2]Model based on logarithmic transformation of the data.

age. The food dyes also had minimal effects on biochemical parameters. There were no significant differences among dietary groups in concentrations of brain neurotransmitters, neurotransmitter metabolites, plasma PLP, brain PLP, microsomal protein or cytochrome P-450 (tables 4, 5, 6), but an unexpected decrease in liver PLP was observed in the 1% group (table 5). The final liver weight and liver weight:body weight ratios decreased with increasing levels of food dyes (table 7), but the liver weight:body weight ratios of the 2 and 4% groups were not significantly different from the 0% group.

Postmortem inspection of internal tissues revealed interesting information: two rats from the 4% dye group and one each from the 2, 1 and 0.5% groups had dark livers that were discolored (greenish gray) and foul smelling ("fishy" odor). This indication of metabolic abnormality was suggestive of possible hepatic accumulation of dye or dye metabolites. However, the animals in this subgroup did not deviate significantly from their respective group means in any of the parameters measured. The abnormal livers appeared to be an isolated effect of chronically ingesting large dye doses, and was not

TABLE 4

Effect of various dietary levels of a food dye blend on brain neurotransmitters and metabolites[1,2]

% dye blend	Serotonin	Norepinephrine	Dopamine	5-Hydroxyinole-acetic acid	Homovanillic acid
			μg/g brain		
0	0.196 ± 0.029 (7)	0.164 ± 0.017 (7)	0.471 ± 0.087 (7)	0.456 ± 0.040 (7)	0.038 ± 0.013 (7)
0.5	0.211 ± 0.022 (8)	0.185 ± 0.018 (8)	0.443 ± 0.063 (8)	0.412 ± 0.023 (8)	0.037 ± 0.014 (8)
1.0	0.179 ± 0.028 (7)	0.170 ± 0.020 (7)	0.505 ± 0.077 (7)	0.423 ± 0.035 (7)	0.032 ± 0.011 (7)
2.0	0.229 ± 0.027 (8)	0.195 ± 0.023 (8)	0.725 ± 0.071 (8)	0.408 ± 0.021 (8)	0.030 ± 0.012 (8)
4.0	0.229 ± 0.037 (8)	0.219 ± 0.026 (8)	0.641 ± 0.101 (8)	0.395 ± 0.013 (8)	0.042 ± 0.014 (8)

[1]Data are means ± SEM, *n* in parentheses [2]No significant differences.

characteristic of the groups as a whole. No overt abnormalities were noted in any other internal organ.

DISCUSSION

The data presented here indicate a remarkable lack of biological and behavioral toxicity in rats consuming large daily doses of food dyes, even at dietary levels as high as 4%. The principal results of dye consumption were the early onset of diarrhea in the 1, 2 and 4% groups which persisted throughout the experiment, and an initial reduction in locomotor activity and food intake in the 4% group. At the end of the 32-day period of dye consumption, however, there were no significant differences among groups in food intake, body weight or locomotor activity, indicating that an adaptation had occurred. Furthermore, the ingestion of food dyes had no significant effect on levels of brain neurotransmitters, tissue PLP, hepatic microsomal protein or hepatic cytochrome P-450. Possible metabolic aberrations resulting from dye consumption were indicated by discolored livers from several rats, by the lowered liver weight:body weight ratios of the 2 and 4% groups and by elevated activity levels in the 0.5 and 1.0% groups, but these differences failed to reach statistical significance.

We were not able to support our hypothesis that behavioral affects attributed to food dyes were linked to altered pyridoxine or PLP metabolism. Growth curves of all dietary groups were similar, and the ability of PLP to serve as a cofactor in plasma, liver and brain was apparently not altered by the dyes. We speculated that dye compounds might behave similarly to drug antagonists, interfering with the body's ability to utilize the vitamin (22). The azo dyes would have most likely exerted this effect since their azo-reductive cleavage products are readily absorbed, and some are structurally similar

TABLE 5

Effect of various dietary levels of a food dye blend on tissue pyridoxal phosphate (PLP)[1,2]

% dye blend	Plasma PLP	Liver PLP	Brain PLP
	ng/ml	*μg/g liver*	*μg/g brain*
0	99.271 ± 16.977 (5)	5.774 ± 0.220[b] (8)	0.927 ± 0.021 (8)
0.5	154.271 ± 30.662 (6)	5.257 ± 0.334[ab] (8)	0.975 ± 0.043 (8)
1.0	144.352 ± 18.629 (8)	4.473 ± 0.298[a] (8)	1.008 ± 0.034 (8)
2.0	137.496 ± 24.558 (8)	5.231 ± 0.162[ab] (8)	0.981 ± 0.029 (8)
4.0	108.994 ± 16.076 (8)	5.233 ± 0.222[ab] (8)	0.959 ± 0.013 (8)

[1]Data are means ± SEM, *n* in parentheses. [2]Means not sharing a common superscript are significantly different by Tukey's multiple comparison test at $P < 0.05$.

TABLE 6

Effect of various dietary levels of a food dye blend on hepatic microsomal protein and cytochrome P-450[1,2]

% dye blend	Microsomal protein	Cytochrome P-450	
	mg/g liver	*nmol/g liver*	*nmol/mg protein*
0	31.007 ± 1.702 (8)	22.694 ± 1.448 (7)	0.712 ± 0.032 (7)
0.5	32.883 ± 2.183 (8)	22.088 ± 1.654 (8)	0.672 ± 0.023 (8)
1.0	32.455 ± 1.645 (8)	22.198 ± 1.577 (8)	0.684 ± 0.033 (8)
2.0	33.676 ± 2.391 (8)	21.220 ± 1.521 (8)	0.634 ± 0.029 (8)
4.0	29.402 ± 2.674 (8)	19.374 ± 1.684 (8)	0.669 ± 0.031 (8)

[1]Data are means ± SEM, *n* in parentheses. [2]No significant differences.

to known antagonists (10, 23). We also found no evidence that dyes could accelerate the photodecomposition of pyridoxine, as had been previously suggested (24).

Locomotor activity. The patterns of locomotor activity we observed may be stress-related responses of the central nervous system (fig. 1C). In the days immediately after supplementation of dyes to the basal diet, the activity level of the 4% dye group decreased sharply and remained significantly ($P < 0.001$) below the other groups until 53 days of age. Beyond this time, differences among groups were no longer significant. The sigmoidal or cubic shape of this curve conforms to paradigms describing an adaptive ability of organisms chronically exposed to toxicants (25, 26). An underlying principle is that the central nervous system exhibits plasticity, which allows a behavioral effect to become diminished or eliminated with time unless extensive damage to brain tissue or neurochemical abnormalities have occurred. There was no evidence of neurological damage insofar as we found no significant differences in brain levels of neurotransmitters or PLP among the dietary groups, and brain weight was also not affected. We speculated that if the dyes had extensively inhibited PLP cofactor activity or diminished permeability of the blood-brain barrier for PLP, a functional pyridoxine deficiency might have occurred in the brain, possibly resulting in a reduction in neurotransmitters.

One of the inherent problems in testing a behavioral response to food dyes is that the central nervous system is more sensitive to many toxicants in developing rats than in mature ones (27). Hence, Shaywitz et al. (5) fed 5-day-old developing rat pups a mixture of dyes similar to our blend, and found that the dyes caused an increase in activity. These authors administered the dye solutions by intubation as a single bolus each day. To overcome this potentially confounding procedure Goldenring et al. (6) performed a similar experiment in which artificially reared pups were fed a continuous gastric infusion of a liquid diet with or without a food dye blend. These investigators also found increased activity in pups receiving dyes. The use of mature postweanling rats in our experimental design avoided the problems of artificial rearing and intubation. Animals were able to maintain their normal ad libitum dietary patterns, and we were able to monitor the daily locomotor activity of individual animals

TABLE 7

Effect of various dietary levels of a food dye blend on liver weight and liver weight:body weight ratio[1,2]

% dye blend	Liver wt	Liver wt:body wt
	g	
0	14.59 ± 0.68[ab]	0.0406 ± 0.0015
0.5	15.80 ± 0.43[b]	0.0435 ± 0.0012
1.0	14.43 ± 0.62[ab]	0.0419 ± 0.0011
2.0	13.56 ± 0.80[ab]	0.0383 ± 0.0018
4.0	12.61 ± 0.53[a]	0.0384 ± 0.0010

[1]Data are means ± SEM; $n = 8$. [2]Means not sharing a common superscript are significantly different by Tukey's multiple comparison test at $P < 0.05$.

over a 32-day period. However, the absence of significant chronic effects from the food dyes in this study might be attributable to the age of the animals or to differences in central nervous system properties.

Food dye blend. By testing a blend of dyes, we were able to screen all seven FD & C food dyes at once to determine whether any produced a positive response. A formulation similar to ours was employed in a number of clinical studies designed to test behavioral responses to food dyes (1–4, 28). Although utilizing a mixture precludes identification of individual dyes that might be responsible for an observed effect, it is a realistic representation of how dyes exist in the food supply. The standard dose-response levels we used were not relevant to human intake, but were chosen to maximize the amount of dyes absorbed, thus increasing the likelihood of observing an effect. No dye-related dose-response effects were observed.

We did not investigate the extent to which the dyes were absorbed nor attempt to identify possible target organs of the absorbed dyes. Other researchers, however, have found that orally administered food dyes are absorbed together with their metabolites in varying amounts (29–32). Based on these reports, and considering the large dietary levels we administered, we assume that some absorption of dyes or metabolites did occur.

The capacity of a dye group to be absorbed, and therefore its potential for exhibiting biological activity is directly proportional to its prevalence in the food supply. The azo dyes account for about 90% of the dyes consumed in the U.S. Tartrazine, a widely used azo dye, has long been implicated in producing allergic-type reactions such as asthma and urticaria (33). Although the azo dyes are poorly absorbed intact from the gastrointestinal tract, they are readily cleaved at the unstable azo linkage by intestinal bacteria, and their metabolites, primarily sulfanilic acid, are extensively absorbed (34, 35). The next most prevalent dye, erythrosine, is slightly absorbed and has also been shown to affect biological systems (8, 36–38). The poorly absorbed indigoid and triphenylmethane dyes are present in exceedingly small amounts in the common diet and have not been linked to any metabolic perturbation.

There is little information concerning the metabolic fate of food dyes or their metabolites following absorption. Of particular importance regarding behavioral studies is the ability of dye compounds to enter the central nervous system or cross the blood-brain barrier. It was recently reported that ^{35}S-labeled sulfanilic acid could enter the brains of neonatal rats by diffusion (34), but results of in vitro studies suggestive of a neurotoxic effect of dyes may not apply in vivo (39).

Our data indicate that chronic food dye consumption causes minimal physiological and biochemical effects in postweanling rats. The principal dye effect we observed was on locomotor activity. Although animals in the 4% group showed a significant acute reduction in locomotor activity, there were no significant differences among groups by week 4. We urge caution in generalizing these data among species, however, since differences in absorption, metabolism and central nervous system properties undoubtedly exist (40).

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