ARTIFICIAL FOOD COLORS AND CHILDHOOD BEHAVIOR DISORDERS*

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OVER the past five years considerable public attention has been paid to the proposed hypothesis that artificial food additives (and some naturally occurring ingredients added in excess of their natural concentrations) may adversely affect learning and behavior in children. This concern has combined growing interest in so-called organic foods with continuing uncertainties over the nature of these childhood disorders, which have been varyingly labeled hyperactivity, hyperkinesis, minimal brain (or cerebral) dysfunction, or attention deficit disorder. Both the hypothesis and the syndrome, which will be called here by its more popular name, hyperactivity, have frequently been very imprecisely defined, which has undoubtedly led to difficulties in understanding and in testing any etiologic connection between them. This paper will focus rather narrowly on whether artificial food colors possess any adverse actions on biochemistry and behavior. The narrowed perspective can be justified by mutual acceptance by many participants in the scientific and medical debate that artificial food colors are testable subjects of research, that any actions of these compounds are likely to be biochemical rather than specifically immunological or allergic, and that effects on behavior may be limited to decrements in specific tasks rather than in global behavior. Feingold[1] originally proposed a less restricted hypothesis, covering many synthetic chemicals as well as the naturally occurring food constituent salicylic acid, and he has discussed possible causal links between diet and such major neuropsychiatric illnesses as depression and schizophrenia. In addition, Feingold originally suggested an analogy with allergenic mechanisms for adverse neurobehavioral reactions to artificial additives and salicylates. Although his suggestions were considered, because of the urticaria

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associated with the artificial color tartrazine (FD&C Yellow No. 5) with cross-reactivity to salicylate medication,[2,3] current investigations of the "Feingold hypothesis" have not seriously considered any notions of a "brain allergy" mechanism.

Five years after publication of the Feingold hypothesis is an appropriate time to review the present state of information. Broadly interpreted, it is in conflict. Clinical data have not demonstrated, in the words of the Nutrition Foundation National Advisory Committee, "anything like the dramatic behavior improvement [originally] described by Feingold and others."[4] In contrast, in the experimental literature, significant biochemical and neurobehavioral effects of artificial food colors are reported with increasing frequency. The major current question is, accordingly, why discrepancies exist between clinical and experimental data? What are the implications for clinical toxicology and psychiatry of the effects observed in animals?

Clinical Data

Major clinical studies, recently reviewed,[5] can be divided into two types: elimination or dietary intervention studies and challenge or pharmacological studies. In addition, there is a sizable literature of anecdotal and case study reports, many of which report significant effects of dietary intervention, usually the uncontrolled and fairly undocumented initiation by parents of the so-called Feingold diet.[1] Without considering the merits of these latter claims, it should be noted that changes in diet involve participants in many factors influencing the child's psychological situation in the family and school. Many of these changes may be beneficial, involving parents and children in positive actions toward improving their relationships, removing stimulant medication from children being inappropriately treated, focussing attention and efforts on causes less connotative of personal guilt or failure. However, none of these factors bear on the hypothesis that artificial food additives are causes of childhood hyperactivity, although they may explain the frequently reported successes of the diet and its apparently widespread endorsement by the lay public.

Dietary studies have been difficult to conduct under controlled circumstances for several reasons: first, it is very difficult to control consumption by children who move freely from home to school to a public world; second, it is hard to conduct a true double-blind experiment involving extensive changes in normal patterns of food preparation and consumption; and third, instruments to measure response, as changes in behavior, are qualitative and, in

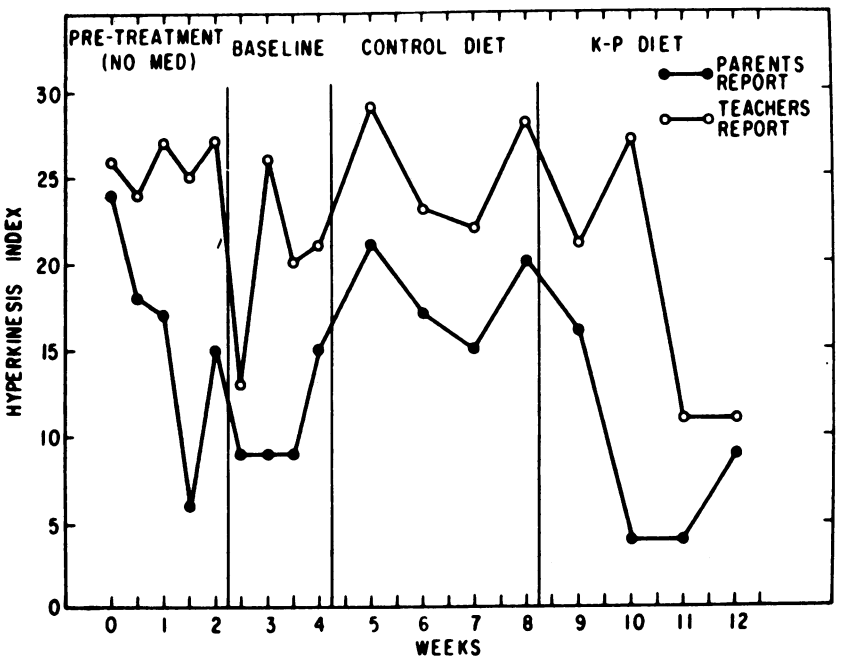


Fig. 1. Individual response to dietary intervention in a double-blind crossover design. Response was measured every week using the Conners Parent-Teacher questionnaire. Reproduced by permission from Conners, C. K., Goyette, C. H., Southwick, D. A., et al.: Food additives and hyperkinesis: a controlled double blind experiment. *Pediatrics 58*:154-66, 1976.

some cases, fairly subjective. With these considerations, dietary studies have involved approximately 100 children. Most studies have had a design, as shown in Figure 1, of a baseline period (particularly important when the child was on medication before intake into the study), followed by a double-crossover period of testing, the Feingold diet versus either an unregulated diet or, in some cases, another dietary regime in an attempt to deal with some of the nondietary factors related to restricted consumption or to preserve the ignorance of the subjects and their families. Outcome was measured primarily by use of rating scales developed for assessment by parents and by teachers. Sample questions from this measuring instrument are shown in Table I.[6] These studies, as shown for an individual example in Figure 1, have not shown dramatic changes in behavior associated with dietary intervention. The sensitivity of the Conners scales to detect relatively rapid behavioral changes may be evaluated by considering its long and successful use in

TABLE I. ABBREVIATED CONNERS TEACHER QUESTIONNAIRE AND RATING SCALE

	Degree of Activity			
Observation	*Not at all* 0	*Just a little* 1	*Pretty much* 2	*Very much* 3
1) Restless or overactive				
2) Excitable, impulsive				
3) Disturbs other children				
4) Fails to finish things he starts, short attention span				
5) Constantly fidgeting				
6) Inattentive, easily distracted				
7) Demands must be met immediately—easily frustrated				
8) Cries often and easily				
9) Mood changes quickly and drastically				
10) Temper outbursts, explosive and unpredictable behavior				

psychopharmacological studies, as shown for a double-blind crossover study of d-amphetamine (Figure 2).[7]

From the approximately 100 children so studied, 14 may be possibly identified as "responders" to the diet. Imprecision in numbers is due to possible overlap of subjects in repeated studies. However, in some studies (as shown in Figures 1 and 3), there is obvious confounding by the order of diets between control and experimental periods. In one study there was a tendency toward improvement during whichever diet was imposed first,[8] while in another apparent improvements occurred during the last testing period regardless of which diet was imposed.[9]

Failure of double-blind crossover intervention studies to support the many reports from less controlled studies may be explainable in several ways. An obvious conclusion is that the original reports erred in observation or interpretation. However, it may also be the case that the effects of dietary intervention are subtle and limited, and selection of measurement instruments, as well as methods of statistical analysis, may be important interacting variables. For example, in a study of 20 children, significant effects were observed only by mothers using the Conners rating scales; other measures (including objective performance tasks and ratings by teachers, psychiatrist and psychologist) failed to show significant changes.[10] It is also possible that

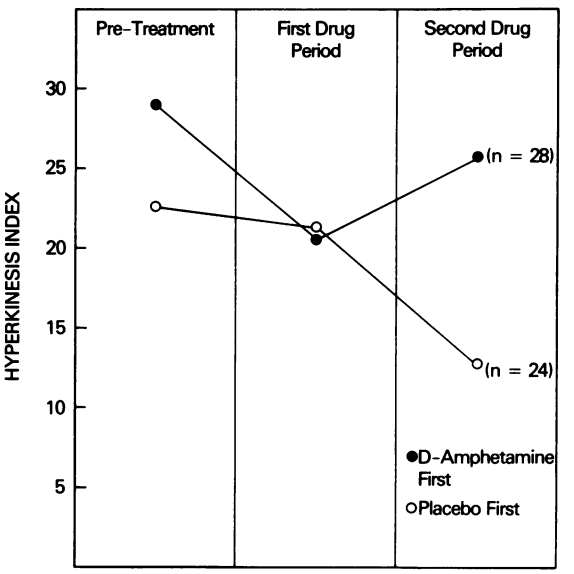


Fig. 2. Double-blind crossover design used to test effects of d-amphetamine (10 mg./day) in children diagnosed as hyperkinetic. Reproduced by permission from Conners, C. K., Eisenberg, L., and Barcai, A.: Effect of dexedrine on children. *Arch. Gen. Psychiat. 17*:478-85, 1967.

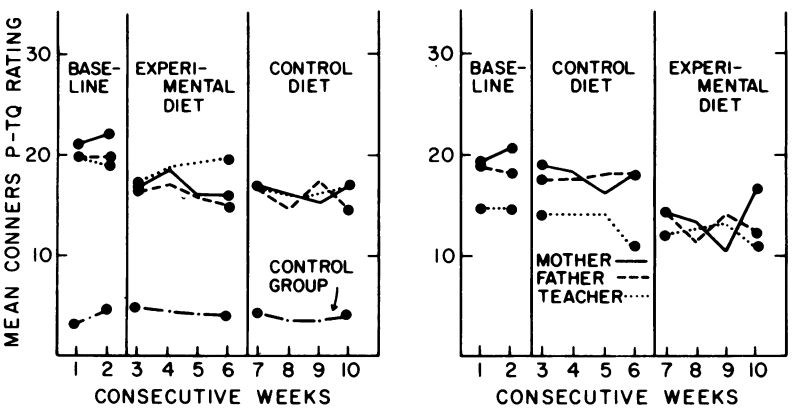


Fig. 3. Effects of diet on behavior in 10 children diagnosed as hyperactive, with diet sequence varied. Reproduced by permission from Harley, J. P., Ray, R. S., Tomasi, L., et al.: Hyperkinesis and food additives: testing the Feingold hypothesis. *Pediatrics 61*:818-28, 1978.

the apparently low success rate, on the average, may be due to undetected infractions committed during the test diet period, or to testing periods too

TABLE IIA. CONSTITUENT DYES IN THE CHALLENGE "COOKIE" DEVELOPED BY THE NUTRITION FOUNDATION

Food Color	*Percentage contained in blend*		*Amount, if 26 mg. (U.S.)*	*Amount, if 100 mg. (U.S.)*
	*U.S.**	*Canada†*		
FD & C Blue #1	3.12	3.12	0.8 mg.	3.1 mg.
FD & C Blue #2	1.70	1.70	0.4	1.7
FD & C Green #3	0.13	0.13	0.03	0.1
FD & C Red #2	—	22.27	—	—
FD & C Red #3	6.08	6.08	1.6	6.1
FD & C Red #4	0.50	0.50	0.13	0.5
FD & C Red #40	38.28	—	10.00	38.3
FD & C Yellow #5	26.91	26.91	6.99	26.9
FD & C Yellow #6	22.74	22.74	5.9	22.7
Certified Orange B	0.54	0.54	0.14	0.5

*From notarized letter to Mr. A.J. Karas, McCormick & Co., Inc. from Samuel Zuckerman, Ph.D., vice president, H. Kohnstamm & Co., Inc., dated February 20, 1976.

†From notarized letter to Mr. A.J. Karas, McCormick & Co., Inc. from Samuel Zuckerman, Ph.D., vice president. H. Kohnstamm & Co., Inc., dated February 27, 1976.

short to "wash out" body burdens of active compounds. Very little is known about the pharmacokinetics of the artificial colors, to take one class of additives, and it is not certain that 14 to 28 days is an appropriate duration for a crossover study.

Challenge studies were undertaken on the basis of assertions that discrete exposures to low levels of artificial colors could provoke rapid and overt deterioration in the behavior of children who were successful responders to dietary intervention. A cookie and a drink were formulated, by common agreement, to contain the estimated average daily intake of the 10 most commonly used artificial colors. The content was derived as shown in Tables IIA and IIB. These challenge studies utilized subjects selected by parents and physicians; no particular attempts were made to verify the child's state before dietary change, degree of compliance to the diet, or extent of improvement associated with dietary treatment. Appropriate placebos were also developed, allowing for a classic double-blind pharmacological test design.

Challenge studies, because of their acute nature, have used different outcome measures, in some cases, than intervention studies. Objective tests, such as continuous performance tasks and checklists of specific behaviors have been applied. It is not clear that these measures are relevant to the neurobehavioral dysfunctions that define hyperactivity, particularly as that syndrome is customarily assessed by parent and teacher rating scales. Challenge studies have used, aside from the Nutrition Foundation cookie or drink,

TABLE IIB. SOURCE OF DATA FOR COMPOUNDING CHALLENGE

FD & C color	*Average amount of color certified per year (lbs./year)*	*Average color intake (mg./person/day)*
Blue No. 1	143,576	0.85
Blue No. 2	78,143	0.46
Green No. 3	5,964	0.04
Red No. 2	1,025,886	6.08
Red No. 3	280,090	1.66
Red No. 4	23,206	0.14
Red No. 40	737,475	4.37
Yellow No. 5	1,239,024	7.34
Yellow No. 6	1,047,487	6.20
Orange B	24,718	0.15
		27.29 mg.

Certification data from FDA on color additives covers fiscal years 1973 and 1974 and the first six months of fiscal year 1975. The average intake values were calculated from production figures on the basis of a United States population of 210 million people.

tartrazine challenge and whole diet changes (incompletely specified and acutely applied). Of the approximately 173 children so studied, 28 have shown some adverse responses to challenge. Responses to the cookie or drink, when these occurred, were apparently rapid in onset, within one hour after ingestion and reversible within four hours, as shown in Figure 4. When these data are considered together, and statistical comparisons done on the data as a group, no significant responses to challenge are observed. However, it is arguable that this method of analysis may not be appropriate. There is some evidence for considerable heterogeneity in the population of hyperactive children, in terms of constellation of symptoms or in drug response.[11] Additionally, there may be a heritable or genetic factor,[12] possibly evidenced in the reports of increased incidence of minor physical anomalies in hyperactive boys[13] and of decreased concentrations of platelet serotonin in hyperactive children.[14] The possibility of genetic factors related to hyperactivity, specifically to responsiveness to artificial food colors, will be further discussed below.

EXPERIMENTAL STUDIES

With relative restriction of the food additive hypothesis to the artificial colors listed in Table II and rejection of the immunologic hypothesis, experimental research has recently been conducted on possible neuroactive

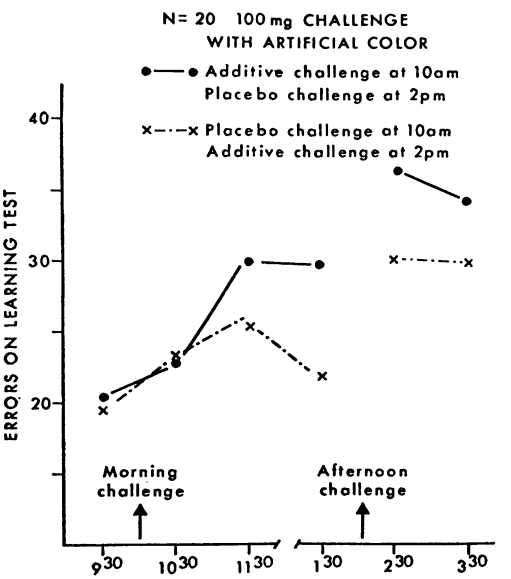
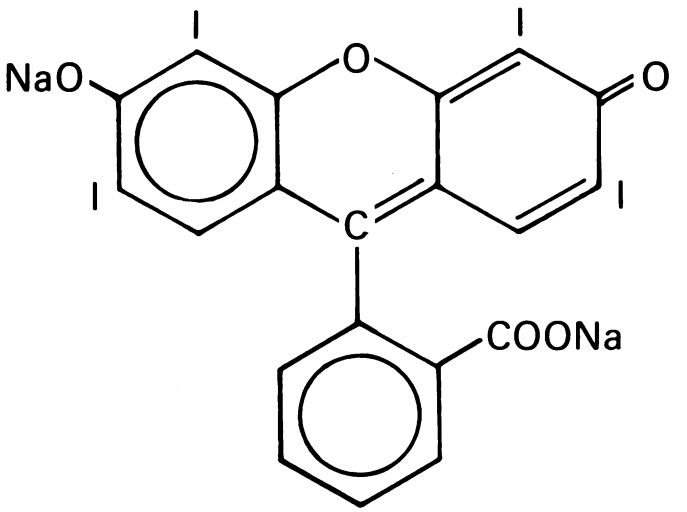


Fig. 4. Time course of responses of 20 hyperactive children administered 100 mg. artificial color (total amount containing colors in percentages as shown in Table II). Reproduced by permission from Swanson, J. M. and Kinsbourne, M. Artificial Color and Hyperactive Behavior, In: *Treatment of Hyperactivity and Learning Disorders*, Knights, R. M. and Bakker, D., editors. Chicago, University Park Press, 1979.

properties of these compounds. Of the artificial colors, erythrosin B (FD&C Red No. 3) has received most research attention.

In 1975 very little was known of the biological properties of artificial food colors. These compounds are, for the most part, on the GRAS (generally recognized as safe) list of the Food and Drug Administration, and little more than acute LD_{50} data, of unknown provenance, was known. Levitan and his coworkers had been interested in the fluorescein dyes, such as erythrosin B, eosin Y, eosin B, and rose bengal (see Figure 5) as membrane active compounds. Using various preparations of excitable tissue, including sea urchin eggs, squid giant axon, and the frog neuromuscular junction, where excitability can be measured indirectly (by fertilization of the egg) or directly (by intra- and extracellular recording techniques), these workers demonstrated that erythrosin is a fairly potent membrane active agent. The mechanism of its actions are unknown, but involve voltage-independent changes in membrane conductance, or movement, of the cations sodium and potassium.[15]

Independently, two laboratories also investigated *in vitro* neurochemical



ERYTHROSIN B

Fig. 5. Structure of erythrosin, a fluorescein dye. Erythrosin B (FD&C Red No. 3) is tetraiodofluorescein; Eosin B (D&C Red No. 21) is dibromo, dinitrofluorescein; rose bengal (Acid Red 94) is tetraiodo, tetrabromofluorescein.

effects of erythrosin B and other dyes, selecting aspects of brain chemistry thought to be relevant to hyperactivity. As shown in Figure 6, erythrosin was found to be a potent noncompetitive inhibitor of dopamine uptake by nerve endings prepared from rat brain and exposed *in vitro* to the dye for five minutes.[16] Other studies showed that this compound inhibits uptake of many other neurotransmitter and precursors as well, and that its actions could be diluted out by increasing amounts of tissue in the assay.[14,17,18] The uptake of neurotransmitters by nerve terminals is thought to be an important mechanism for removing chemicals from the synaptic cleft and thereby terminating chemically mediated neurotransmission.[19] Inhibition of uptake results in increased concentrations or prolonged presence of neurotransmitters near their receptors, and synaptic transmission is functionally augmented. Of the dyes studied, this action was limited to erythrosin B[17], and its effects were specific to that portion of uptake stimulated by sodium, as shown in Figure 6.[16] The ability of increased tissue concentrations to diminish the effects of the dye *in vitro* probably relates to its marked lipid solubility,[20] which would permit its diffusion into any proteolipid compartment. This affinity for lipid can be demon-

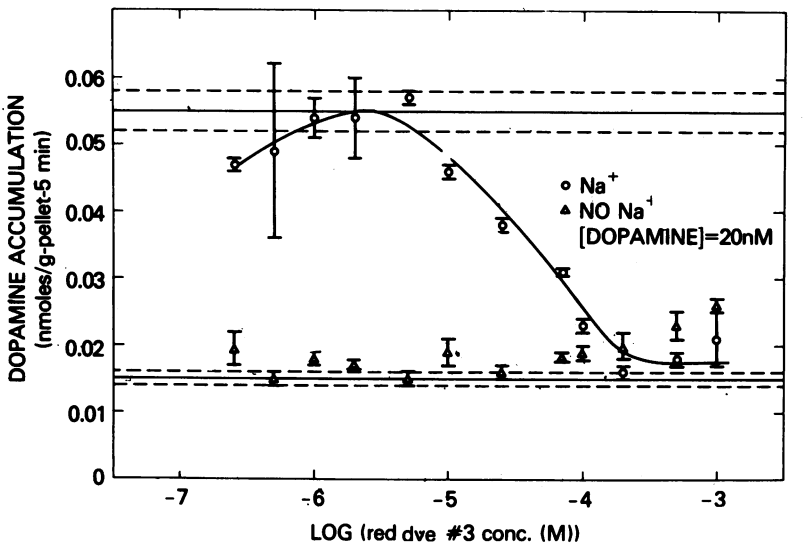


Fig. 6. Inhibition of ^{3}H-dopamine uptake by increasing concentrations of erythrosin B added *in vitro*. Reproduced by permission from Lafferman, J.A. and Silbergeld, E.K.: Erythrosin B inhibits dopamine transport in rat caudate synaptosomes. *Science 205:*410-12, 1979.

strated using artificial phosphatidylcholine vesicles (Morris and Silbergeld, unpublished data).

These studies indicated some role for an interaction between sodium and erythrosin in the neurochemical effects observed *in vitro*. A major biochemical mechanism for regulation of intracellular sodium concentration and transmembrane sodium gradients in all cells is provided by the enzyme Na,K-ATPase (sodium-potassium adenosine triphosphatase). The activity and function of this enzyme can be studied in several ways *in vitro*: by measuring the catalysis of ATP to ADP; the binding of the highly specific drugs ouabain or digitoxin to membranes, or fluxes of monovalent cations into intact cells.[21] As shown in Figure 7 and Table III, erythrosin *in vitro* is a potent inhibitor of ATP catalysis, specific ^{3}H-ouabain binding, and transport of ^{86}Rb into synaptosomes. Erythrosin is the most potent inhibitor of glycoside binding yet found outside the structural family of the digitoxin-strophanthidin molecules.

If erythrosin possesses significant digitalis-like effects, as suggested by these results, rapid and marked cardiotoxicity would be expected. Neurotoxicity of digitalis compounds has also been described,[22] but neurotoxic and cardiotoxic effects may be separable. There is evidence that while all

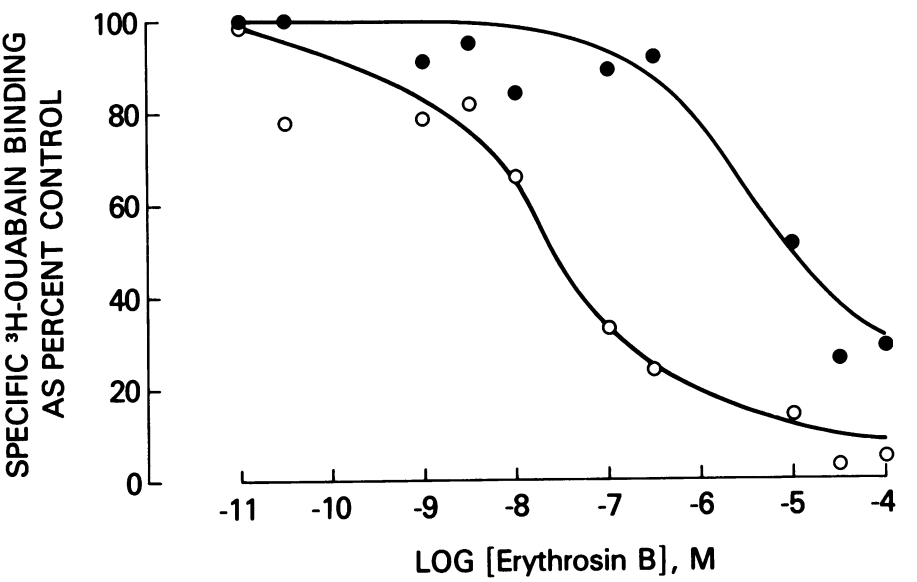


Fig. 7*a*. Effects of erythrosin B *in vitro* on specific binding of ^{3}H-ouabain to synaptic membranes prepared from rat cortex; solid circles indicate displacement of 24 nM ^{3}H-ouabain, and open circles indicate displacement of 0.13 nM ^{3}H-ouabain.

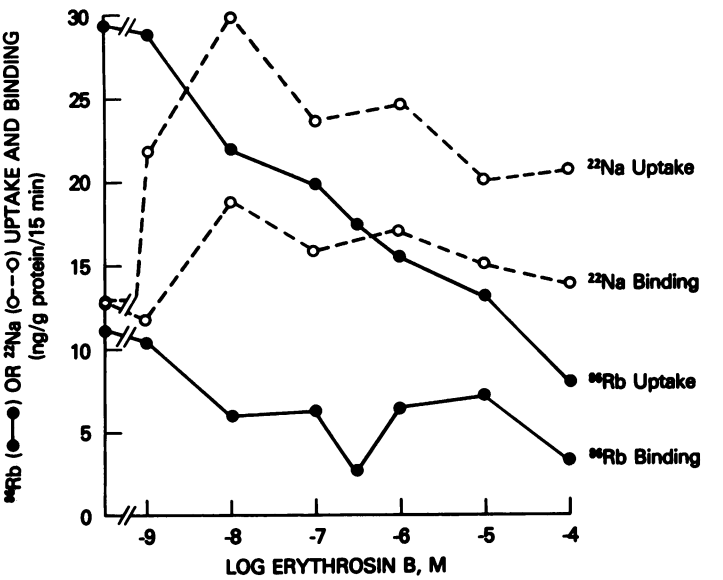


Fig. 7*b*. Effects of erythrosin B *in vitro* on transport of ^{22}Na and ^{86}Rb (a radionuclide tracer for K) by synaptosomes from rat cortex. Data are from Silbergeld, E. K.: Erythrosin B is a specific inhibitor of high affinity ^{3}H-ouabain binding and ion transport in rat brain. *Neuropharmacology 20:*87-90, 1981.

TABLE III. EFFECTS OF ERYTHROSIN, AS COMPARED TO OUABAIN, ON ATPASE CATALYSIS IN BRAIN AND RED CELL MEMBRANES

Inhibitor concentration (μM)	*Percent control activity*	
	Brain	*Red cell*
Erythrosin		
0.1	89	100
1.0	52	100
10	61	—
100	50	92
Ouabain		
1.0	76	81
10	61	52
100	31	38

cells contain a membrane-associated Na,K-ATPase, brain cells have two enzymes to regulate sodium movements. These enzymes have been distinguished by glycoside-binding studies, association-dissociation kinetics for inhibition of catalysis, and, very recently, by electrophoretic separation.[23-25] Erythrosin has the apparently unique property of acting only on one of the enzymes in brain tissue, with no effects on the ATPase presumably common to other cells, such as hepatocytes or red blood cells,[26] as shown in Table III.

Erythrosin is a photoactive agent, and, as such, some of its actions may result from such phototoxic mechanisms as the generation of highly reactive singlet oxygen molecules.[27] Its ability to block some of the effects of nerve growth factor on nerve cells in culture may be an aspect of phototoxicity (Morris and Chronwall, in preparation). However, its ouabainlike actions on brain ATPase do not depend upon photoactivation because these also occur when assays are run in the dark.

The ability of erythrosin to inhibit brain Na,K-ATPase is consistent with its inhibition of neurotransmitter uptake, described above. Ouabain interacts significantly with neurotransmitter cycling. By preventing the binding of extracellular sodium to neuronal membranes[28] or by causing release of cytosolic transmitters,[29,30] ouabain can reduce the ability of nerve terminals to take up and retain such substances as dopamine.

EXPERIMENTAL ANIMAL STUDIES

Animal studies of behavioral effects of food dyes can be divided into two classes: those in which the effects of dye ingestion are assessed by

multiple behavioral and physical measurements and studies in which effects of the dye on a specific behavior or behavioral "model" are examined. A study of the former type by Butcher and coworkers[31] measured body weight and reproductive fitness of Sprague-Dawley rat mothers given erythrosin B in their food (1, 0.5, or 0.25% of the diet) for two weeks prior to mating and during gestation and lactation. The offspring of these treated rat mothers were given eryhthrosin B in their food (1, 0.5, or 0.25% of the diet) from weaning until 90 days of age. Postnatal survival rates and body weights were recorded. During postnatal development the behavior of control and treated rat pups was measured according to a battery of tests (Table IV) developed by Butcher and coworkers[31] to provide a tool for comprehensive assessment of the psychotoxic effects of test compounds.

No obvious pattern of dose response for the effects of erythrosin B on postnatal viability or physical development were observed in this study. No behavioral differences were demonstrated between controls and rat pups presumed to have received erythrosin B during pre-and postnatal development despite evaluation of performance in numerous diverse motor and behavioral tasks. The classification of erythrosin B as a behavioral neurotoxin is not supported by these data. Several questions about this study should be addressed. First, it is not clear how much erythrosin B rat pups were exposed to during gestation and nursing; second, psychotoxic effects of erythrosin B may not be manifested in the numerous tasks assessed by these authors which are biased toward motor performance; and third, the very limited time interval during which the behavioral measurements were made may have been too restrictive to uncover transient effects or those specific to a stage of postnatal development outside of the test period.

General activity levels and T-maze and shuttle box performance were correlaries that Shaywitz et al.[32,33] used in their animal studies of hyperactivity and learning impairment. This "model" assesses changes in behavior of rats after dopamine neurons are destroyed by injections of the selective neurotoxin 6-hydroxydopamine into the cerebral ventricles at five days of age. Expected depletion of brain dopamine in 6-hydroxydopamine treated animals was confirmed by biochemical determination in rat pups killed at 30 days of age. To examine the effect of food dyes on behavior, 6-hydroxydopamine treated rats were given daily oral doses of food dyes from 5 days of age to the end of the first month of postnatal development.[33]

TABLE IV. ITEMS FOR ASSESSING DEVELOPMENTAL PSYCHOTOXICITY

Surface righting reflex	Criterion of righting: turning over from a supine to a prone position within 2 seconds
Cliff avoidance	Retraction of forepaws from over the edge of an elevated horizontal surface within 30 seconds
Pivoting locomotion	Amount of time spent pivoting during a 60-second observation period
Auditory startle	Movement of the animal elicited by an automobile horn stimulus of 0.3 seconds
Swimming development	Performance score based on direction of locomotion, body angle in the water, and action of limbs when the animal is placed in warm (27°C.) water in a rectangular swimming tank
Negative geotaxis	Time required to reorient from a head down to a head up position on a 25-degree inclined plane
Open field exploration	Latency to move, number of sections entered, and frequency of rearing during a three-minute period in a circular arena.

Reproduced by permission from Butcher, R. E., Voorhees, C. V., Wootten, V., et al.: A Survey of Early Tests for the Developmental Psychotoxicity of Food Additives and Related Compounds. In: *The Effects of Food and Drugs on the Development and Function of the Nervous System: Methods for Predicting Toxicity*, Gryder, R. M. and Frankos, V. H., editors. Rockville, Md., FDA, 1980.

The mixture of food dyes used in these studies corresponds in composition to the challenge "cookie" developed by the Nutrition Foundation (Table IIA). A food dye mixture of either 0.5, 1.0, or 2.0 mg./kg. was given orally to the rat pups from five days of age to 30 days of age, and activity levels and avoidance learning measured. Dose selection was based on an estimate of an average consumption of 1 mg./kg. per day calculated from average amounts of food coloring ingested per capita (Table IIB). Determination of activity level was made by visual observation of the amount of time an animal was active during alternating five-minute intervals scored for one hour per day at 12, 15, 19, and 26 days of age.

Escape latency was measured during 20 trials in a T-maze at 21 days of age by recording the time required for the animal to escape from a 2mA shock delivered through a floor of stainless steel rods. Shock avoidance performance (defined by moving to the safe compartment of a shuttle box) was measured at 28 days of age by recording the time required to escape a 2.5 mA shock delivered through stainless steel rods in the floor 5 seconds after a one second warning period signalled by a bell.

Rat pups treated with 6-hydroxydopamine were active during 61% of

the observation period compared to 45% for the control group. All animals were more active at the beginning of the observation period than at the end of the hour. This habituation to the testing environment was observed in all animals but was significantly less pronounced during the first 30 minutes of observation in animals receiving the highest dose of food dyes. While rat pups that were given 2 mg./kg. food coloring were 19% more active than animals given water, no clear dose response for the effects of food dyes on activity was observed.

Rat pups treated with 6-hydroxydopamine took nearly twice as much time as their vehicle injection controls to escape from the shock in the T-maze. The only effect of food colorings observed on this task was a detriment in performance in nonlesioned rat pups given the low dose of the food dye mixture. Escape latency in the shuttle box measured at 28 days of age was also much greater in the 6-hydroxydopamine treated rat pups but there was no effect of the food dyes at any of the doses studied.

Administration of food dyes produced significant increases in activity in both normal and 6-hydroxydopamine treated animals, although dose-response relations were not apparent. The most pronounced effect of food dyes on frequency of activity was manifest as a failure of animals receiving the 2 mg./kg. dose of food dyes to habituate to the test environment during the first 30 minutes of the activity observation period. Failure to habituate may indicate hyperreactivity or greater distractibility.

The effects of 6-hydroxydopamine on escape latency in both the T-maze and shuttle box were so pronounced that latency times were near the maximum time allowed in the experimental design for the completion of the task. This probably introduced a ceiling effect which prevented the measurement of an effect of food dyes on performance in these shock avoidance tests in animals pretreated with 6-hydroxydopamine. Although this ceiling effect limited the possibility of assessing a special sensitivity of 6-hydroxydopamine treated pups to food dyes, the results suggest that food dyes do adversely affect behavior in developing rat pups.

In a duplication of the previous study, both vehicle and 6-hydroxydopamine treated pups given food dyes displayed the expected increase in general activity when compared to their controls.[34] Treatment with 6-hydroxydopamine *alone* did not increase the frequency of activity during the observation period, in contrast to the earlier study.[33] As reported in the previous study, these 6-hydroxydopamine animals were slower to habituate to the testing environment.

Shuttle box performance was significantly impaired by treatment with food dyes, 6-hydroxydopamine, or both. Combination of both treatments did not result in impaired performance significantly different from that observed for either treatment alone. Any combined effect of these treatments would most likely have been obscured by a ceiling effect in the experimental design.

The behavioral effects of erythrosin B, 6-hydroxydopamine injections, or both in rats has also been measured by studying locomotion in a doughnut-shaped activity cage.[18] There was no difference between pre- and postdrug activity in young rats receiving an acute 50 mg./kg. intraperitoneal dose of erythrosin B, but adult rats given an acute intraperitoneal injection of erythrosin B were significantly more active than their saline controls. In psychopharmacology, response of rodents to such adversive stimuli as a shock is known as punished behavior and has been used to test drugs for tranquilizer-like activity. Severe suppression of behavior usually occurs when such stimuli are administered following a selected response such as bar pressing. The minor tranquilizer, chlordiazepoxide, is known to increase the rate of punished responding and thus diminish the use of punishment as a negative reinforcer. Acute intraperitoneal administration of 50, 100, or 300 mg./kg. erythrosin B to rats increased response rates and amount of punishment shocks received. This effect is comparable to the response to an 8 mg./kg. dose of chlordiazepoxide.

Lack of effect of erythrosin B on locomotion in young but not older rats reported in this study is perplexing. A developmental study of the effects of erythrosin on activity should be undertaken. Task and situation specific variation in the effects of food dyes or erythrosin B have not been investigated. The testing environment of locomotion measured in a doughnut-shaped activity cage is not the same behavioral task as open field activity. Measuring the percentage of the time the animal is active is not the same thing as counting total movements over time.

Preliminary investigations of the effects of erythrosin B on a variety of behaviors are being conducted in our laboratory. Sixty-day-old male Sprague-Dawley rats were given daily 2 mg./kg. doses of erythrosin B in their drinking water for 20 weeks. Body weight and fluid consumption for control and treated rats were equivalent. After three months of chronic exposure, no differences were observed in a 3-minute open field behavior. Nest-building behavior measured during the last four weeks of erythrosin exposure was reduced in treated animals when compared to controls

(unpublished data, Anderson and Phelan). In a pilot study of the effects of erythrosin B on open field activity, rats from several inbred strains were tested after chronic feeding of erythrosin B. These rats received 2 mg./kg. of erythrosin B in drinking water during postweaning development (25 to 60 days of age). Differences in open field behavior (three-minute test) were found only in Brown Norway rats (unpublished data, Anderson, Phelan, and Anderson). No differences in open field behavior were found, however, between pre- and postdrug treatment in 35-day-old Brown Norway rats given acute 50 mg./kg. doses of erythrosin B by gastric intubation (unpublished data, Anderson, Silbergeld, and Bradley). On the other hand, intraventricular injections of 2 nmoles of erythrosin B through an indwelling catheter result in hyperthermia and preconvulsant behavior characterized by running fits.[50]

A correspondence between the actions of cardiac glycosides and erythrosin B is suggested from their mutual inhibition of Na,K-ATPase, the inhibition of the high affinity binding of ^{3}H-ouabain to brain membranes by erythrosin B, and a similar behavioral response in rats given intraventricular injections of either erythrosin B or ouabain. We propose that the site of action of erythrosin B is Na,K-ATPase and that neurochemical variability in brain Na,K-ATPase may predispose some organisms to behavioral disorders elicited by the neurotoxic effects of erythrosin B. The enzyme Na,K-ATPase is known to be under genetic control, and a genetic variability in resistance to ouabain has been demonstrated in fibroblasts[35,36] and other non-nervous tissue cultured cell lines.[37,38] We have recently measured brain Na,K-ATPase in several inbred strains of rats and found a twofold difference in means between the highest and lowest strains (Table V). The nature of a genetic defect that may convey a predisposition to a neurotoxic response to erythrosin B is not clear, but the preceeding data suggest that such a predisposition is plausible.

There have been several investigations of a genetic etiology of hyperactivity in children. These studies have employed twin analyses,[39-41], adoption studies,[42-44] and family risk data[41,45-47] (Table VI). Despite weaknesses (small sample sizes and inconsistencies in diagnostic standards) in the research reports on a genetic etiology of the hyperactive child syndrome, consistently positive findings across studies suggest that genetic transmission is a plausible explanation for some cases of childhood hyperactivity.

Clinical studies of the Feingold hypothesis published to date have not

TABLE V. BRAIN Na,K-ATPASE ACTIVITY INBRED RAT STRAIN MEAN ± S.E.

	μ moles P_i/mg.protein/20 min.
BUF/N	2.12 ± 0.12
F344/N	2.06 ± 0.17
LOU/MnN	2.74 ± 0.12
M520/N	1.99 ± 0.23
MNR/N	3.00 ± 0.38
MR/N	2.33 ± 0.23
ODU/N	1.87 ± 0.17
RHA/N	1.95 ± 0.08
WN/N	1.48 ± 0.07
WKY/N	1.64 ± 0.06

Pilot study data (n = 4 per strain).

TABLE VI. STUDIES OF A GENETIC ETIOLOGY OF HYPERACTIVITY

Studies	*Findings*
Twin studies:	
Lopez,[39] Willerman,[40] Nichols[41]	Higher concordance rates in monozygotic than in dizygotic twins
Adoption studies:	
Safer[42]	Increased incidence of hyperactivity in full and half siblings raised apart from hyperactive probands
Morrison and Stewart,[43] Cantwell[44]	High incidence of psychopathology and previous hyperactivity in parents of hyperactive probands but no increased psychopathology or previous hyperactivity in nonbiological parents of adopted probands
Family studies:	
Morrison and Stewart,[45] Cantwell,[46] Welner et al.,[47] Nichols[41]	Increased incidence of hyperactivity in relatives of hyperactive probands

been designed to assess possible genetic variability and heterogeneity in drug response. Data analyses were not chosen to select out unusual responses. Further, the studies have neither focussed on assessing the range of responses to food dyes nor attempted to select specific groups or "types" of hyperactive children, with the exception of one study that utilized only so-called amphetamine responders.[5,48] Interestingly, this group showed significant responses to an acute dye challenge (Figure 4).

Behavioral alterations demonstrated in animal studies and in clinical trials in some children[49] provide evidence that erythrosin B is a neurotoxin. The numbers of children adversely affected by food dyes is probably only a small percentage of that first suggested by Feingold. The nature of these adverse effects may also be highly specific as suggested by the apparently specific biochemical actions of erythrosin. Publication of the Feingold hypothesis, however, did provide the impetus necessary for the first serious investigations of artificial food additives and behavior. To date, these studies have been limited to some of the FD&C artificial colors. We remain ignorant of the possible neurobehavioral effects of the potential interactions among the vast numbers of artificial colors, flavors, stabilizers, emulsifiers and other compounds added to food.

SUMMARY

Popular concern over adverse neurobehavioral effects of food additives has recently implicated artificial colors as etiologic factors in childhood hyperactivity (hyperkinesis, attention deficit disorder). However, clinical studies using total dietary elimination such as the so-called "Feingold diet" have not shown conclusive or dramatic effects when performed under controlled conditions. Challenge studies, in which identified "responders" to dietary elimination were acutely presented using a cookie or drink containing an estimated 10 common artificial colors, have suggested that some responders may exist, but that any such children are not numerous. In contrast, *in vitro* and *in vivo* exposure of rodents to erythrosin B (FD&C Red No. 3) produces significant neurochemical and behavioral effects. Experimental results identify neural Na,K-ATPase as the possible substrate of erythrosin's action. Further, these findings may suggest a genetic basis for the effects of erythrosin *in vivo*, with implications for the design of future clinical studies.

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