segments in *Xenopus* tadpole requires about 8 hours (*3*). In both cases, turnover requires an extended period of darkness before light onset. During the period of darkness, *Limulus* retinular cells require an efferent input from the brain. In the absence of cyclic lighting, rod shedding in rats and *Xenopus* tadpoles (*1*, *3*) exhibits a circadian rhythm, indicating the possible existence of an extraocular control mechanism. The control of rod shedding in frog, however, lies within the eye (*6*). In sum, turnover of photoreceptive organelles in the retinas of diverse animals appears to share several common characteristics, but the control mechanisms do not.

Other invertebrate retinas exhibit evidence of turnover of photoreceptor membrane, but the detailed mechanisms may differ from those we report for *Limulus*. For example, in mosquito larvae, membrane turnover is a continuous process that depends on the state of adaptation of the retina (*14*). In shrimp and crayfish (*15*), lamellar bodies and MVB appear in the retinular cells after prolonged exposure to light, but the temporal sequence of turnover is not clear. The retina of the spider *Dinopis* may be a special case in that the first light of dawn triggers almost a complete destruction of the rhabdom, but membrane synthesis occurs in a rapid burst many hours later at nightfall (*16*).

Why do photoreceptors periodically break down and renew their photosensitive membranes? Perhaps some aspect of visual transduction is irreversible, or the membrane that supports transduction lacks long-term stability. A better understanding of the turnover process should elucidate photoreceptor function. Studies of the *Limulus* lateral eye may prove useful because rhabdom turnover is controlled in part by efferent activity transmitted from the brain.

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8. The anatomy of the *Limulus* lateral eye has been reviewed by W. H. Fahrenbach [*Int. Rev. Cytol.* **41**, 285 (1975)].
9. The degree to which the rhabdom is broken down after 15 minutes of light exposure appears to depend on how long the animal has been stored in the laboratory and on the animal's temperature during exposure. After 15 minutes of light, lamellar bodies are less numerous in animals stored for several months or exposed to light at colder temperatures. Although we generally use natural sunlight, illumination from fluorescent or incandescent lamps is also effective in triggering turnover.
10. We have inferred the temporal sequence of structures formed after the breakdown of the rhabdom from the temporal and spatial distribution of these structures and various lengths of light exposure. We have not yet carried out incorporation studies to show progressive labeling of breakdown structures.
11. The time of occurrence of the first light onset does not significantly affect rhabdom turnover. Animals first exposed to light in the early morning, midday, or late afternoon all showed similar turnover. For turnover to occur at first light onset, the preceding period of darkness must include a period of efferent activity.
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13. In these experiments, one optic nerve was cut. The animal was maintained in darkness, and the electroretinographic (ERG) responses of both eyes were monitored. In the early evening the cut nerve was shocked with a suction electrode as the sensitivity of the other eye increased due to efferent activity. Shocking was continued through the night and was terminated in the morning when the ERG response of the eye with the intact optic nerve began to drop. The animals were then exposed to light and the eyes were fixed in the normal manner (*7*).
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Erythrosin B Inhibition of Neurotransmitter Accumulation by Rat Brain Homogenate

Abstract. *A mixture of seven food dyes inhibited the accumulation of eight neurotransmitters or neurotransmitter precursors by rat brain homogenate. At a low concentration (1 microgram per milliliter), erythrosin B (FD&C red 3) was the only dye that inhibited dopamine accumulation. Erythrosin also was effective in decreasing the accumulation of all the other transmitter substances, suggesting that the inhibition is nonspecific and probably secondary to general membrane alteration.*

Food dyes are color additives utilized solely for esthetics. Except for recent concern about the carcinogenic and teratogenic potential of certain dyes, these substances have been considered to be relatively nontoxic (*1*). Questions have been raised however, about the possible behavioral effects of these and other additives in the human diet. Although not scientifically validated, there have been many claims that food dyes produce hyperactivity and other behavioral abnormalities in susceptible children (*2*). Either a pharmacologic or toxic or an immunologic or allergic mechanism could mediate such responses. Recent evidence indicates that one of these food dyes, erythrosin B (FD&C red 3), has synaptic effects at the frog neuromuscular junction (*3*). A concentration of 10 μM increased the frequency of spontaneous miniature end-plate potentials in this preparation. Our experiments were undertaken to evaluate possible chemical effects of these dyes on mammalian central nervous system (CNS) transmitters. Erythrosin B inhibited the accumulation of these substances by a CNS preparation.

The membrane transport systems for neurotransmitters or neurotransmitter (NT) precursors by CNS tissue are ac-

Table 1. Effect of dye mixture on NT accumulation.

NT	Uptake (pmole/g)*		Percent of control	P†
	Control	Dye		
Choline	79 ± 8	45 ± 10	57	<.01
Dopamine	238 ± 25	124 ± 18	52	<.001
γ-Aminobutyric acid	2072 ± 180	1146 ± 365	55	<.01
L-glutamic acid	5769 ± 205	2759 ± 471	47	<.001
Glycine	314 ± 44	115 ± 20	36	<.001
l-Norepinephrine	260 ± 28	128 ± 8	49	<.001
Serotonin	512 ± 14	359 ± 40	70	<.01
Taurine	41 ± 7	23 ± 3	56	<.02

*Uptake represents the mean ± standard deviation of quadruplicate samples in picomoles of NT accumulated per gram of tissue (wet weight) after 5 minutes of incubation at 25°C. †Significantly different from control, using the unpaired two-tailed t-test and with 6 d.f.

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Table 2. Effect of individual dyes on dopamine accumulation.

Dye*	Dopamine accumulation (% of control)
Blue 1	108.5 ± 14.0
Blue 2	104.2 ± 14.3
Red 2	98.5 ± 18.0
Red 3	56.8 ± 4.9†
Red 4	105.8 ± 12.5
Yellow 5	98.3 ± 5.8
Yellow 6	98.9 ± 16.0

*Each dye was tested in final concentration of 1 μg/ml. Results are expressed as the mean ± S.D. as percent of control dopamine accumulation. †Significantly different from control by the unpaired two-tailed t-test with d.f. = 6 at $P < .001$.

Table 3. Effect of Red 3 (final concentration, 1.0 μM) on NT accumulation. Results are expressed as means ± S.D. as percent of control accumulation.

NT	NT accumulation (% of control)
Choline	55.1 ± 8.3*
Dopamine	58.1 ± 10.9*
γ-Aminobutyric acid	52.4 ± 10.4*
L-Glutamic acid	56.7 ± 6.0*
Glycine	47.4 ± 4.7*
l-Norepinephrine	53.9 ± 15.3†
Serotonin	65.2 ± 9.2†
Taurine	52.6 ± 4.9†

*$P < .001$, two-tailed t-test with d.f. = 6. †$P < .01$, two-tailed t-test with d.f. = 6.

tive, specific processes that can be selectively inhibited by several neurally active drugs such as stimulants (for example, methylphenidate) and tricyclic antidepressants (for example, imipramine). In our study the initial NT accumulation by homogenates of adult rat brain was determined simultaneously for eight isotopically labeled putative NT's by a modification of previous techniques (*4*).

Whole brains from albino rats (200 to 300 g) were homogenized in nine volumes of 0.3M sucrose. A portion was centrifuged at 48,000g for 10 minutes, and the pellet was resuspended and diluted in a tris-buffered salt solution (pH 7.4 at 25°C) containing 1.1 mM ascorbic acid and 8.9 mM glucose. Portions of this suspension were combined with a tritium-labeled NT and either dye in buffer or buffer alone. In each 3.5-ml polypropylene tube, the final volume was 0.5 ml and contained homogenate from 1 mg of original tissue and $10^{-7}M$ exogenous NT; experimental samples contained dye (either 1 μg/ml or 1 μM). The tubes were incubated at 25°C for 5 minutes and then were rapidly cooled and centrifuged. The pellets were washed, sedimented, and subsequently assayed for accumulated radioactivity. Radioactivity accumulated in the presence of saturating amounts of unlabeled NT was subtracted to determine the net radioactivity and the number of moles of NT accumulated in the pellets.

Seven commercially utilized food dyes—namely FD&C blue Nos. 1 and 2, red Nos. 2, 3, and 4 and yellow Nos. 5 and 6 (*5*)—were used in a mixture containing a final concentration of 1 μg/ml of each. This dye mixture produced a significant but variable decrease in the accumulation of all NT to 36 to 70 percent of control values (Table 1). Although the dye solution had a blue-green color, the tissue itself was noticeably stained red. This staining did not affect the assay of radioactivity because dye or stained tissue alone produced no quenching of standard samples of tritium.

All NT's were affected by low concentrations of dye mixture. To determine which of the dyes produced this inhibition, their individual effects on single NT's were analyzed. At a final concentration of 1 μg/ml only erythrosin B (Red 3) significantly inhibited dopamine accumulation (Table 2). This was also the only dye that stained the pellets of tissue. In other experiments, the mixture from which Red 3 had been removed no longer inhibited the accumulation of dopamine.

Erythrosin B also had an effect on the other NT's. The accumulation of all NT's was significantly decreased by 1 μM (0.88 μg/ml) erythrosin B to 52 to 65 percent of control values (Table 3); under the experimental conditions used the inhibition by erythrosin therefore was not selective for a particular transmitter.

Erythrosin B and other xanthene dyes have other membrane effects (*3*, *6*). These effects have been observed at a higher concentration of erythrosin B than that effective in our studies. Some of the reported effects correlate with the lipid solubility of the different substances, and it has been suggested that they are produced by a general membrane alteration. The observed selective staining of membranes by erythrosin in our investigation demonstrates an affinity for membrane which the six other dyes do not have.

Substantial inhibition of accumulation occurs at a rather low concentration of erythrosin B, but since this is not NT specific it is difficult to predict what kind of CNS dysfunction might be produced in the intact animal. Oral administration of large quantities of erythrosin B are well tolerated by experimental animals (*1*). Much of the dye is apparently excreted unchanged in the feces, and it may be that insufficient amounts reach the central nervous system to affect NT's. However, on the basis of our observations definite neurologic effects would be predicted if, through altered absorption or other modes of administration, sufficient dye did reach the CNS. Our results do not prove that dyes can produce hyperactivity. They do suggest that any neurologic dysfunction that might be observed with these dyes could be related to the membrane effects of erythrosin B.

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