tral America. During the first four months of the year, another current begins at Panama, where the Equatorial Counter Current and the California Current meet, and flows south toward the Ecuadorian coast, where it merges with the westward-flowing Humboldt Current. If these currents have not changed their paths markedly in the past 5000 years, they would have brought a drifting vessel almost inevitably to the part of the Ecuadorian coast where the Valdivia culture appears.

Whether the influence originated in Japan or on the Asiatic mainland is still questionable. While the resemblances to Middle Jomon are numerous, many Valdivia features are duplicated in pottery of continental eastern Asia, by which Jomon was also influenced. Further field work in Ecuador and more extensive consultation with experts on Asiatic archeology are being undertaken in an effort to delimit the place of origin more specifically than can now be done.

EMILIO ESTRADA
Museo Víctor Emilio Estrada, Guayaquil, Ecuador

BETTY J. MEGGERS
CLIFFORD EVANS
Smithsonian Institution, Washington, D.C.

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2 June 1961

Retinal Cholinesterase and Glycolysis in Rats Raised in Darkness

Abstract. Rats that are raised in the dark from birth to 17 weeks of age have significantly lower acetylcholinesterase activities in the retina than control rats raised under standard conditions. Pseudocholinesterase and the glycolytic enzymes are not affected.

Raising animals in the dark provides an experimental situation for an investigation of cytological and biochemical correlates of visual deprivation. Riesen (*1*) has reported loss of retinal ganglion cells in chimpanzees raised in darkness for over 1 year. Cats show no cell losses but do have narrowed inner plexiform layers after they are deprived of light for 3 months to 3 years. No cytological changes have been described for rabbits reared to maturity in the dark (*2*). The decreasing cytological sensitivity of an animal to visual deprivation as the phylogenetic scale is descended requires the use of sensitive biological measures to detect the effects of raising lower mammals in the dark.

This is a report on the effect of long-term light deprivation on the cholinesterase and glycolytic activities of rat retina. These biochemical parameters were chosen because of their important roles in retinal function.

Transmission of nerve impulses in the retina may involve the acetylcholine-cholinesterase system (*3*). The retina contains both acetylcholine and its synthesizing enzyme, cholineacetylase. A high concentration of cholinesterase occurs in the vertebrate retina where it is located primarily in the inner plexiform layer among the synapses connecting the bipolar and ganglion cells.

Glycolysis is required for normal visual function. Injection of iodoacetate, a potent inhibitor of glycolysis, into a rabbit, produces a complete loss of the electroretinogram and selective destruction of rod and cone cells (*4*). Neonatal rats and rabbits show a sudden increase in glycolytic activity as their eyes open and the retina begins to function (*5*).

Twenty age-matched rats of the S-3 Tryon strain were used in the experiment. Ten of these rats were raised in the normal light-and-dark conditions of the animal room while the other ten were put into the dark with their mothers at age 3 days (before their eyes opened). At 17 weeks of age, the rats were killed and their retinas were quickly removed. For the cholinesterase experiments, 28 retinas (from seven dark-raised and seven normally raised rats) were weighed and individually homogenized in 0.1*M* phosphate buffer (*p*H 8.0); 1 ml of buffer was used for each 8 mg of tissue. Cholinesterase assays were performed by the method of Ellman *et al.* (*6*) with acetylthiocholine and butyrylthiocholine used to determine total cholinesterase (that is, acetylcholinesterase plus pseudocholinesterase) activity and pseudocholinesterase activity, respectively. Glycolytic experiments were performed with six retinas each from the dark-raised and normally raised groups. Single, whole retinas were incubated in 2 ml of Krebs-Ringer phosphate buffer with glucose (20 mmole) for 1 hour at 37°C. Lactic-acid production was determined by the Barker and Summerson technique (*7*) and is expressed on a dry-weight basis.

Table 1. Total cholinesterase, pseudocholinesterase and glycolytic activities in retinas from dark-raised and normally raised rats (mean plus or minus standard error of the mean).

Total cholinesterase activity*	Pseudocholinesterase activity†	Glycolytic activity‡
	Dark-raised rats	
88.8 ± 2.1	3.6 ± 0.7	1.25 ± 0.12
	Normally raised rats	
108.7 ± 5.0§	4.5 ± 0.6	1.29 ± 0.07

*Moles of acetylthiocholine hydrolyzed per minute per milligram (wet weight) × 10^{10}. †Moles of butyrylthiocholine hydrolyzed per minute per milligram (wet weight) × 10^{10}. ‡Micromoles of lactic acid formed per hour per milligram (dry weight). §$p < .01$.

The retinal cholinesterase and glycolytic activities of dark-raised and normally raised rats are shown in Table 1. No difference in glycolysis between the two groups was found. This indicates that the glycolytic process, necessary for normal visual function, is not influenced by lowered retinal excitation. Total cholinesterase activity in retina of dark-raised rats is 19 percent lower than that in retina of normally raised rats ($p < .01$). The activity measured represents both acetylcholinesterase and pseudocholinesterase, but in the retina more than 95 percent of the total activity is made up by acetylcholinesterase (see Table 1). Since pseudocholinesterase activity does not differ between the two groups, the lower total activity in the retina of dark-raised rats must be the result of an effect on acetylcholinesterase, the enzyme intimately involved in nerve transmission.

Animals raised in the dark have less visual stimulation than their normally raised counterparts. Presumably this lower level of stimulation results in less acetylcholine being released in the retina. Chang *et al.* have reported lower levels of acetylcholine in the retina of a dog who had one eye blindfolded for 30 weeks (*8*). If the synthesis and maintenance of acetylcholinesterase are dependent upon the level of acetylcholine, then the lower acetylcholinesterase activity found in the retina of dark-raised rats would be explained. There is evidence that supports the hypothesis that acetylcholinesterase activity is directly related to the amount of acetylcholine present. For example, Burkhalter *et al.* (*9*) have shown that

acetylcholinesterase in cultivated chick embryonic lung can be increased 2 to 6 times by adding 0.02*M* acetylcholine to the culture medium. Preliminary results from this laboratory indicate that mice treated with anticholinesterase drugs (to increase the level of acetylcholine in tissues) show increased cholinesterase activities in the liver, lung, skeletal muscle, and blood. Indirect evidence that cholinesterase synthesis may be altered in the retina of dark-raised rats is found in reports that ribonucleic acid, required for enzyme synthesis, is decreased in the retinas of animals deprived of light for varying periods (*1, 10, 11*).

ROBERT LIBERMAN
Department of Pharmacology, University of California School of Medicine, San Francisco

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Simazine: Degradation by Corn Seedlings

Abstract. The herbicide 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) is converted to 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) in vivo by corn seedlings and in vitro by corn extracts. Hydroxysimazine is considered to be a detoxified form of the herbicide. In vitro conversion was produced by reacting simazine with either a cyclic hydroxamate (2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine) or its glucoside. The latter compounds may mediate at least some of the in vivo conversion in corn.

The herbicide 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) is used as a selective pre-emergence spray to control annual weeds in corn fields. The tolerance of corn to simazine has been associated with an unidentified nonenzymatic substituent (*1, 2*) which converts simazine to 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) which is nonphytotoxic. While the formation of hydroxysimazine in vivo has been postulated (*1*), its isolation from treated plants has not been previously reported. The objectives of our investigation were to study the initial steps in the degradation of the simazine molecule both in vivo and in vitro.

Hydroxysimazine is the major degradation product in short-term experiments with intact corn seedlings (*Zea mays* var. Dixie 82). Seedlings were grown in quartz sand (in a greenhouse in 1-quart containers). When they were 6 days old, 400 μg of ring-labeled C^{14}-simazine (specific activity 0.83 mc/mmole) was applied to the surface of the sand. Plants were harvested 10 days after treatment. Degradation products were isolated separately from roots and shoots by solvent fractionation. The distribution of radioactivity in the various fractions is presented in Table 1.

The chloroform-soluble fractions were chromatographed on Whatman 3 MM paper (ascending) in either *i*-amyl alcohol saturated with 3*N* hydrochloric acid or 65-percent 2,6-lutidine; C^{14}-simazine and C^{14}-hydroxysimazine were used as standards. All of the radioactivity in the chloroform-soluble basic fraction chromatographed as hydroxysimazine. The chloroform-soluble nonbasic labeled material was not resolved satisfactorily from the chlorophyll and carotenoid pigments.

The aqueous fractions from the roots and shoots (Table 1) were pooled and passed through an Amberlite IR-120 (H^+) column. The basic components were eluted with 2*N* ammonium hydroxide (72,246 counts per minute). Samples of the eluent were cochromatographed in five solvent systems [65-percent lutidine; *i*-amyl alcohol saturated with 3*N* hydrochloric acid; *n*-butanol, ammonium hydroxide, and water (8:1:1); *n*-butanol, acetic acid, and water (4:1:5); and 30-percent acetic acid on kerosene-soaked paper] with C^{14}-simazine and C^{14}-hydroxysimazine. The major C^{14}-labeled degradation product appeared to be hydroxysimazine.

Roth [as cited by Gysin and Knüsli (*1*)] found a "phenol-like" substance in corn plants which could degrade simazine in vitro. This substance has an R_F of 0.78 in *n*-butanol, acetic acid, and water (4:1:5). A cyclic hydroxamate and its 2-glucoside were recently isolated from corn seedlings.

Table 1. Fractionation of the radioactivity accumulated by corn seedlings treated with C^{14}-simazine. Liquid samples were applied to 1-inch diameter glass sand-blasted planchets for radioactivity determinations. The density of the dried ethanol-insoluble residues was less than 1 mg/cm². Thin layers of the ground residue (30 mg) were counted in 1-inch diameter aluminum planchets, and the counts were corrected for infinite thinness. Results are presented as total counts per minute for ten seedlings. Fresh weight of ten roots was 11.2 g, and fresh weight of ten shoots was 7.6 g.

Fraction	Radioactivity (count/min)	
	Roots	Shoots
Insoluble in 80% ethanol	8,349	2,867
Soluble in 80% ethanol	75,654	52,901
Chloroform-soluble (total)	17,260	12,640
Nonbasic	553	420
Basic	16,707	12,220
Aqueous fraction	46,860	43,120

The hydroxamate's structure has been established as 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (*3, 4*). The aglucone has an R_F similar to Roth's simazine-degrading substance (*3, 4*). Both a crystalline sample of this cyclic hydroxamate (*3*) and the 2-glucoside dechlorinate simazine in vitro. The glucoside was isolated from cold acetone-*n*-butanol extracts of 5-day-old corn roots (*Zea mays* var. Dixie 82).

About 2 μmole of the aglucone and 2 μmole of the glucoside were incubated separately with 0.02 μmole of C^{14}-simazine at 37°C in a total volume of 1 ml. After 4 hours the incubation

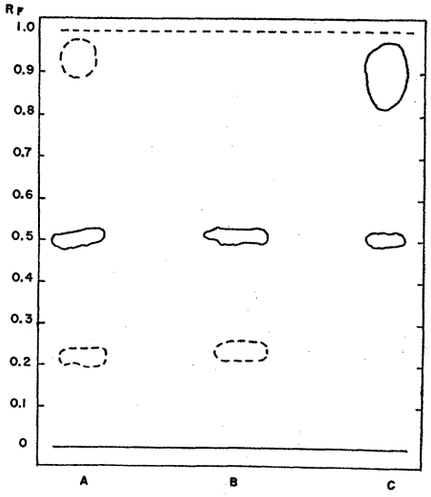

Fig. 1. An autoradiogram made after chromatography of incubation mixtures of (*A*) C^{14}-simazine plus 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine; (*B*) C^{14}-simazine plus 2-glucosyl-4-hydroxy-3-keto-7-methoxy-1,4-benzoxazine; and (*C*) C^{14}-simazine (upper spot) and C^{14}-hydroxysimazine (lower spot).