

Fig. 1. Distress vocalizations to shock while subject was held and while it was not held and resting on a bare surface.

and then ten shocks in the other condition. This procedure was repeated at progressively higher levels of shock, with intensity increasing in 0.50-ma steps to 5.50 ma. A comparable descending series of shocks terminated the session.

Figure 1 shows the percentage of total shocks that elicited vocalization as a function of intensity and holding conditions. The curves show that vocalization was uniformly low at all shock levels when the subject was held, whereas, when the subject was not held, vocal responses increased with increasing shock. The total percentage of vocalization was 8.1 and 46.0 for the held and not-held conditions, respectively, and for each subject the difference between conditions was significant (p < .01) as determined by Wilcoxon tests comparing total vocalizations at each shock level.

The second experiment began when the subjects were between 110 and 123 days of age; it sought to determine the effects of holding on the threshold for initial vocalization to shock. A further purpose was to investigate the relationship of hunger to vocal responsiveness and to holding. Thresholds were established by the method of limits. In each test session the shock was set initially at a level which did not elicit vocalization and increased in 0.50-ma steps until vocalization occurred on two successive presentations. A descending series was then performed which terminated when vocalization failed to occur on two successive presentations. Shock duration was 2 seconds unless vocalization occurred. Four threshold determinations, each including an ascending and a descending series, were made in each session, two while the subject was held, and two while it was on a bare surface. There was a total of eight testing sessions; half of these occurred before the morning feeding when the subjects had been deprived of formula for approximately 10 hours, and half occurred within 1 hour after the regular morning feeding. The order of the holding and the food deprivation conditions was balanced over sessions.

All animals exhibited higher thresholds when held; on 62.5 percent of the determinations, the threshold was arbitrarily recorded at 6.50 ma, the upper limit of shock, because this level was reached without eliciting vocalization. Vocalization failed to occur on only five determinations under the not-held condition, and this failure occurred entirely with one subject. Mean thresholds for the held and not-held conditions (combined over deprivation conditions) were 6.03 ma and 3.51 ma, respectively. The difference between conditions was statistically significant (p =.01, Wilcoxon tests) for two infants, and results for the third animal were consistent with this outcome, but could not be established statistically. Hunger had no apparent effect on the threshold for vocalization when the infants were held. For the not-held condition, however, thresholds were lower for all subjects when they were hungry than when they were fed (mean thresholds: hungry, 2.88 ma; fed, 4.13 ma), although the overall effect of deprivation conditions was not statistically significant.

These results indicate that the stimulus conditions provided by holding inhibit vocal responses to aversive stimuli. Although our findings do not rule out the possibility that this effect is specific to vocal activity, we believe it more likely that there is a general decrease in arousal associated with holding which will be reflected in other response measures, for example, muscle tension. According to this interpretation, the arousal-reducing characteristics of physical contact that are present in the neonate should constitute an important factor in the tendency of older infants to seek the mother or an appropriate substitute (3) in moments of distress (4).

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References and Notes

- 1. R. M. Yerkes and M. I. Tomilin, J. Comp. Psychol. 20, 321 (1935).
- Psychol. 20, 321 (1935).
 It should be noted that conditions involving terry cloth permitted grasping.
 T. L. McCulloch, J. Psychol. 7, 305 (1939).
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Generation by Visible Light of Labile Free Radicals in the Melanin Granules of the Eve

Abstract. The melanin granules of the eye generate a free radical when irradiwith visible light under approxiated conditions. mately physiological The rapid generation in light and decay in dark of the free radical as indicated by electron spin resonance studies, coupled with the anatomical proximity of the melanin granules to the rods and cones of the eye, suggest that these granules may play an important role in the visual The investigation of this free process. radical offers a possible approach to connect the photochemical events in the eye with the subsequent electrophysiological events in the nerve.

Electron spin resonance (ESR) offers a powerful approach to the study of molecules in short-lived excited states (1). In both the free radical and triplet states, molecules have an extra degree of reactivity which allows functions forbidden to molecules in the ground state. Conceivably the photochemical events in the eye could be translated into a nerve signal via the pathway of photoactive molecules converted to the free radical state. With this in mind, we inaugurated ESR investigations of the visual process. An ESR signal was found to be generated by visible light in the melanin granules of the eye under approximately physiological conditions. Some aspects of this reaction are detailed below.

Beef eyes were obtained immediately after slaughter and transported to the laboratory in a light-tight, iced container. The eyes were opened under dim red light and the retinas were excised. The choroid layer, Bruch's membrane, and the pigment epithelial layer of the retina were removed intact, and were shaken vigorously with successive washes of isotonic sucrose to bring the melanin granules into suspension. After the fibrous material was removed by filtration through fine silk screening, the filtrate containing the melanin was homogenized in isotonic sucrose with a Potter homogenizer and centrifuged at 1000g for 10 minutes. The melanin granules that precipitated at this speed were washed by repeating the suspension and centrifugation procedures. The material then was suspended in isotonic sucrose, layered over 2.0M sucrose, and centrifuged at 25,000g for a period of 30 minutes.

The precipitate from this treatment, when resuspended in various aqueous solutions, was used as source material

for the free radical studies. These suspensions appeared black and showed an essentially flat absorption spectrum throughout the visible range. Under the microscope, these preparations appeared as homogeneous suspensions of black particles. The particles remained insoluble even after treatment with



Fig. 1. ESR spectrum of the melanin particles of the eye exposed to visible light. The curve represents the derivative of microwave absorption with respect to magnetic field strength on the vertical axis plotted against the magnetic field strength on the horizontal axis. The melanin granules suspended in 0.25Msucrose, 0.1M PO₄ buffer, pH 6.8, were exposed to constant illumination at approximately 25°C.



Fig. 2. Time course of ESR spectrum intensity. Magnetic field strength was adjusted to the center of the lower peak of a spectrum like that shown in Fig. 1. The upper curve shows the plot of the height of this peak as a function of time before, during and after exposure to light. Resistance-capacitance filtering with 0.3-sec. time constant was used on the ESR signal. The lower curve represents the onset and termination of the illumination measured by a photoresistor with response time of 2 msec or better. Eye melanin granules suspended in H₂O at approximately 25°C were used.

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strong acids and bases and with the common organic solvents.

Free radicals were measured by a Varian ESR spectrometer, with 9.5kMcv/sec microwaves with a magnetic field strength in the region of 3300 gauss and with 100 kcy/sec modulation of the magnetic field. The granules in aqueous suspension were put in a flat quartz flow cell which was oriented in the microwave cavity to have its flat face parallel to a series of slots cut in one side of the cavity. Light from a 750-watt, tungsten-filament projection lamp was focused on the sample through the slots after passing through glass lenses which removed ultraviolet light and through water which removed infrared radiation. We plotted ESR spectra as the derivative of microwave absorption with respect to magnetic field strength (dA/dH) versus magnetic field strength (H). The time course of radical generation and decay was followed by adjusting magnetic field strength to correspond to the lower peak of the ESR spectrum, and then peak height was recorded before, during, and after the application of light with a 40-msec-response time recorder. Resistance-capacitance filtering with time constant 0.3 to 1.0 sec had to be applied to the ESR signal to improve the signal-to-noise ratio. Values for gand line widths of the ESR spectra were measured by standard methods with a proton magnetic resonance gaussmeter and a resonant cavity type microwave wavemeter.

The melanin granules isolated as described showed a relatively small ESR spectrum in the dark which was greatly intensified in the light. A typical ESR spectrum of melanin granules in the light (Fig. 1) shows a single absorption peak of line width 2.75 gauss (peak to middle of derivative curve) and a gvalue of 2.004. The time course of the height of the lower peak (Fig. 2) shows a rapid generation of free radical after the onset of light to a stable equilibrium plateau and a decay of this free radical over a period of seconds after the light was turned off.

The radical generated by light was stabilized at alkaline pH. This fact, and the polyquinone nature of other melanins, suggests that the light-induced free radical in eye melanin is a semiquinone. Melanins from other sources have previously been found to generate free radicals when exposed to ultraviolet light, but the phenomenon of reversibility was not described (2). The presence of rapid and reversible photoactive free radical-generating melanin granules in the eye in close proximity to the rods and cones suggests that they may play a more important role in the visual process than merely to absorb stray light. Extensive studies along these lines are in progress.

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References

D. J. E. Ingram, Free Radicals as Studied by Electron Spin Resonance (Butterworth, London, 1958).
 H. S. Mason, D. J. Ingram, B. Allen, Arch. Biochem. Biophys. 86, 225 (1960).

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Parental Handling in Two Strains of Mice Reared by Foster Parents

Abstract. The amount of handling received in ten daily tests by infant mice reared by foster parents was significantly affected by the strain of pups and by the strain of foster parents. This finding suggests that at least some behavioral differences between highly inbred strains may be due to early environmental rather than genetic variation.

Recent experiments (1) have shown that differences exist in the behavioral characteristics of highly inbred strains of animals. Under the assumption that environmental conditions have been held relatively constant or have varied in only a random fashion, the results of these studies have been viewed as demonstrations of a direct genetic influence upon behavior. However, among experimental animals normally reared with their own parents, both the prenatal and the postnatal environment during rearing are confounded with genotype. Consequently, variations in parental environment which are correlated with genotype must be identified if genetic effects are to be properly evaluated.

The experiment reported here was designed to gain information about possible differences between two inbred strains of mice in their handling of offspring. Since variations in handling received by animals during infancy have been shown to influence a number of behavioral characteristics in adulthood (2), consideration of this environ-