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 strong acids and bases and with the common organic solvents.

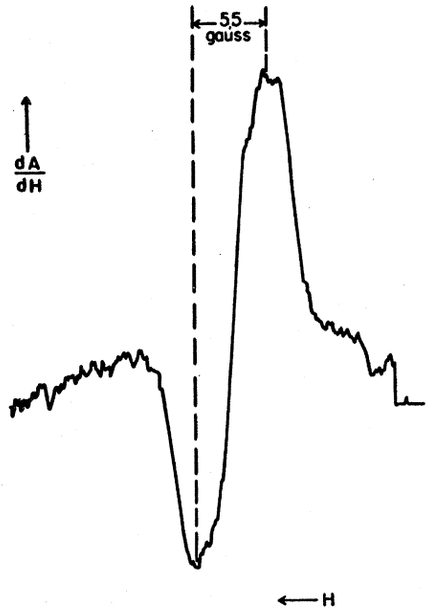

Fig. 1. ESR spectrum of the melanin particles of the eye exposed to visible light. The curve represents the derivative of microwave absorption with respect to magnetic field strength on the vertical axis plotted against the magnetic field strength on the horizontal axis. The melanin granules suspended in 0.25*M* sucrose, 0.1*M* PO_4 buffer, *p*H 6.8, were exposed to constant illumination at approximately 25°C.

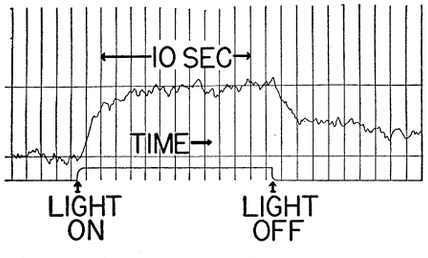

Fig. 2. Time course of ESR spectrum intensity. Magnetic field strength was adjusted to the center of the lower peak of a spectrum like that shown in Fig. 1. The upper curve shows the plot of the height of this peak as a function of time before, during and after exposure to light. Resistance-capacitance 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 H_2O at approximately 25°C were used.

Free radicals were measured by a Varian ESR spectrometer, with 9.5-kMcy/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 g and 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 g value 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 *p*H. 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

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

25 January 1962

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-

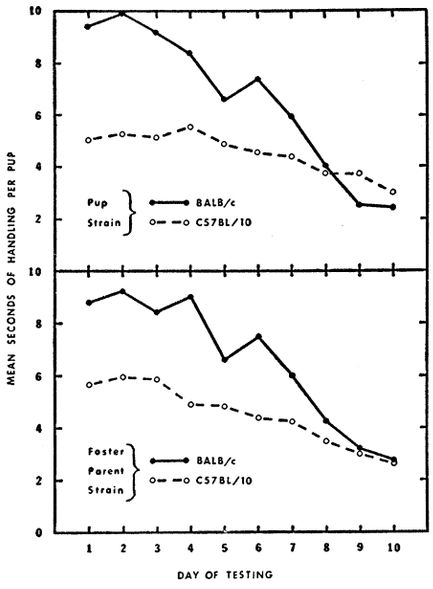

Fig. 1. Mean number of seconds of handling per pup of the two strains of pups (top) and by the two strains of parents (bottom) as functions of day of testing.

mental variable is of particular importance in an attempt to assess the purely genetic influences upon behavior.

In order to evaluate differences in handling due to the strain of the parents independently of differences in handling due to the strain of the pups, a foster rearing scheme was employed. Ten litters of the C57BL/10 strain were reared by foster parents of their own strain and ten litters were reared by foster parents of the BALB/c strain. Similarly, ten litters of the BALB/c strain were reared by foster parents of their own strain and ten litters were reared by foster parents of the C57BL/10 strain. Of the 40 litters, 33 were switched to foster parents on the day they were born and no litter was switched at more than 4 days of age. All animals were housed in aluminum pans (9⅝ by 5½ by 2¾ in.) with cedar shavings as a nesting material.

Table 1. Analysis of variance of the mean number of seconds of handling received per pup.

Source	df	MS	F
Between litters	39		
Pup strain (P)	1	421.83	9.38*
Foster parent strain (F)	1	439.72	9.78*
$P \times F$	1	10.43	.23
Error (between)	36	44.97	
Within litters	360		
Days (D)	9	128.55	14.98†
$D \times P$	9	42.39	4.94†
$D \times F$	9	19.15	2.23‡
$D \times P \times F$	9	11.56	1.35
Error (within)	324	8.58	

* $p < .005$. † $p < .001$. ‡ $p < .05$.

The measurement of parental handling began on the day after the litters were switched and continued for 10 successive days. On each test day the foster parents were removed from the nesting cage and placed in an empty cage nearby. The pups were taken from the nest and placed in the opposite end of the nesting cage. The foster parents were then returned to the nesting cage. During the succeeding 5 minutes the total number of seconds of handling of the pups by the foster parents was recorded on an electric timer which was activated by the experimenter. Handling was defined as the carrying, dragging, or oral manipulation of a pup by either of the foster parents.

The total number of seconds of handling recorded for a litter on each day was divided by the number of pups in the litter to yield a measure of the average amount of handling received per pup during each test session. The interobserver reliability of this measure for a sample of 50 sessions was found to be .98 by product-moment correlation.

Table 1 presents an analysis of variance of the average amount of handling received per pup. Significant effects were found to be due to the strain of the pups and to the strain of the foster parents. The BALB/c parents handled both strains of pups more than the C57BL/10 parents did, and the BALB/c pups received more handling from both strains of parents than the C57BL/10 pups did. In addition, the amount of handling showed a significant decline with successive days of testing (D), and the magnitude of the effects of pup strain (P) and foster-parent strain (F) decreased over the course of the 10-day period, as reflected by the significant $D \times P$ and $D \times F$ interactions. The effects of pup strain and foster-parent strain as functions of day of testing are shown in Fig. 1.

These results indicate that there is a difference in the amount of handling received by two strains of mice during infancy. The difference in parental handling due to the strain of the pups cannot be regarded as stemming from a genetic difference between the two strains of parents. Rather, the strain of the offspring must be considered an environmental variable for the parents whose behavior was being measured. In addition, the difference in the parental behavior of the two strains (irrespective of the strain of the pups) may itself be due not to a genetic difference but to a difference in the way the parents were handled as infants.

In general, behavioral differences between strains observed in adulthood should not necessarily be attributed to genetic rather than early environmental variation (*3*).

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References and Notes

1. J. L. Fuller and W. R. Thompson, *Behavior Genetics* (Wiley, New York, 1960).
2. J. A. King and B. E. Eleftheriou, *J. Comp. and Physiol. Psychol.* **52**, 82 (1959); G. J. Mogenson and D. J. Ehrlich, *Can. J. Psychol.* **12**, 165 (1958); G. W. Meier and J. L. Stuart, *Psychol. Rept.* **5**, 497 (1959); R. E. McMichael, *J. Comp. and Physiol. Psychol.* **54**, 416 (1961).
3. This research is part of a dissertation carried out under the direction of Jan H. Bruell, whose generous advice is gratefully acknowledged. It was conducted while I was a predoctoral research fellow of the U.S. Public Health Service, and it was supported in part by grant G-14410 from the National Science Foundation.

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Tracers, Transfer through Membranes, and Coefficients of Transfer

Abstract. The rate of flow of a tagged species of a material substance through a permeable membrane is proportional to the rate of flow of the substance itself when, and only when, the species mole fraction of the substance is the same on both sides of the barrier. The ratio of the osmotic transfer coefficient of a substance in a particular barrier to the exchange coefficient, determinable with a tracer, is greater than 1.

The relation between the rate of flow of a substance $\dot{n}_s$ and the rate of flow of a tagged species of the substance, $\dot{n}_{s'}$, across a plane of observation located within the barrier of the linear-transfer system phase α, membrane, phase β has been derived (*1*). A more general but less awkward derivation of the same equation is given later in this report (*2*).

The general equation for the rate of flow of s across a plane of observation may be written

$$-\dot{n}_s = \sum_k M_{sk} \frac{d\mu_k}{dx} \qquad (1)$$

Here the M_{sk}'s are the generalized admittance coefficients of a barrier and $d\mu_k/dx$ is the algebraic representation