largely into planar forms. Such "flattened" membranes would clearly tend to scatter light much less readily than those with curved surfaces which predominate in the control material. Thus there is a correspondence between the measurements of light scattering and the structural change visualized in electron micrographs.

Preliminary studies reveal a correlation between the structural change and a cyanide-insensitive oxidation of ascorbic acid. When the ascorbate in a reaction cuvette is exhausted, the change in light scattering ceases. Similarly, if at any point the ascorbate is rapidly degraded by the addition of an equivalent of an oxidant which by itself has no effect on the system, for example, ferricyanide or cytochrome *c*, there is an immediate cessation of the change. Moreover, under anaerobic conditions there is complete inhibition of the ascorbate effect, suggesting that the change is dependent on a continuous transport of electrons, rather than on a static reduction, with oxygen as the final acceptor. If anaerobiosis is introduced after a decrease in light scattering has occurred there is considerable reversal of the change. As yet, no other conditions for reversal have been found. The possible "coupling" of ascorbate oxidation to the structural transformation appears to be very complex since chlorpromazine (50 μM) will completely inhibit the change in light scattering but allow the oxidation of ascorbate to continue unhampered.

Although changes in cellular conformation occur in tissue culture it remains a question whether the structural changes in isolated microsomal membranes have a counterpart in intact cells or in vivo. Several substances thought to affect brain function—namely serotonin, norepinephrine, phenothiazines, and thyroxine—are potent inhibitors of the ascorbate-induced changes in brain microsome structure (*7; 8*).

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Eyeball Retraction: Classical Conditioning and Extinction in the Albino Rabbit

Abstract. *A comparison of the percentage-performance curve of a classical conditioning group with those of three control groups provided unequivocal evidence of successful conditioning of the retractor bulbi response to an auditory conditioned stimulus.*

Stimulation of the cornea of the rabbit's eye with a puff of air is accompanied by a closure of the outer eyelids, retraction of the eyeball into its orbit, and a sweeping of the nictitating membrane across the surface of the cornea. Although the mechanism of movement and the innervation of the nictitating membrane remain subject to controversy, eyeball retraction is accomplished by the retractor bulbi or choanoid muscle, activated by the sixth cranial nerve (*1*). With regard to the rabbit's nictitating membrane, Last (*2*) contends it has no connection with any muscle and simply moves passively across the cornea when the eyeball retracts. On the other hand, Lierse (*3*) reports the presence of a striated muscle connected to the upper temporal portion of the membrane which acts to pull the membrane forward.

Although we have conditioned the eyelid (*4*) and nictitating membrane response (*5*) in the albino rabbit, the conditioning of eyeball retraction may possibly offer certain advantages. If, in fact, the membrane moves across the eye only as a concomitant of the retractor bulbi response, it follows that the latter is the more direct and perhaps more stable of the two response systems. Furthermore, it is improbable that attenuation of the air puff (that is, the unconditioned stimulus) could occur from anticipatory retractions of the eyeball (that is, conditioned responses), thus aborting an argument which is sometimes used against eyelid conditioning.

The conditioning apparatus and the manner in which the rabbit was restrained have been described (*4–6*). To record eyeball retractions, a transducer was constructed of a balanced lever, on one end of which was mounted a 9-mm diameter loop made of PE 60 polyethylene tubing. A nylon string fastened to the other end of the balanced lever was connected to the shaft of a gravity-return rotary potentiometer. To permit unimpeded recording of eyeball retractions the upper and lower eyelids of the rabbit's right eye were taped open and the nictitating membrane held back by a metal hook fastened to a loop of nylon that had been sutured in the membrane. The polyethylene loop was then placed on the cornea of the prepared eye, and retractions of the eyeball caused the shaft of the potentiometer to rotate. The signals from the potentiometer were then amplified and graphically recorded by an ink-writing penmotor.

The conditioned stimulus (CS) was a 600-msec 3500-cy/sec tone at a sound pressure level of 72 db (relative to 2×10^{-4} dyne/cm^2) and the unconditioned stimulus (UCS) was a puff of compressed nitrogen of 100-msec duration with an intensity of 80 mm-Hg at its point of delivery to the right eye. The orifice of the air jet was adjusted to deliver the air puff through the center of the polyethylene loop at a distance of about 5 mm from the cornea.

Twenty-four albino rabbits, 80 to 100 days of age, were assigned to one of four groups for 2 days of adaptation, 8 days of acquisition training, and 3 days of extinction. On each of the 2 days of adaptation neither conditioned nor unconditioned stimuli were presented and during these sessions a measure of spontaneous eyeball movement was obtained for all groups by recording the frequency of responses in time intervals corresponding to the 70 CS-UCS acquisition trials presented to the classical conditioning group. In acquisition, one control group received the CS alone (group CS-Alone), another received the UCS alone (group UCS-Alone), and a third group received random presentations of the CS alone and the UCS alone (group CS-UCS Mixed).

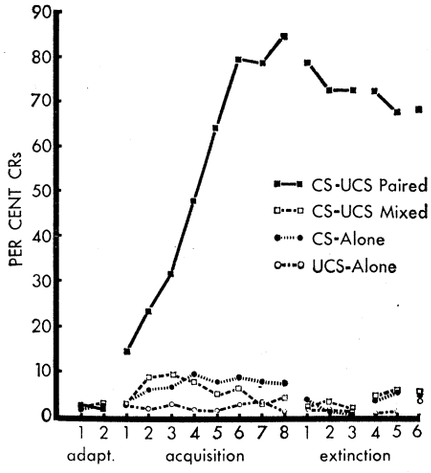

Fig. 1. Mean percentage of responses plotted in 70 trial blocks for adaptation, acquisition, extinction, and spontaneous recovery. CRs = conditioned responses. Blocks of trials are indicated along the abscissa.

The fourth group (group CS-UCS Paired) was the classical conditioning group and it received paired presentations of the CS and UCS at a CS-UCS interval of 500 msec. Twelve rabbits were assigned to the classical conditioning group and four to each of the control groups. Groups CS-UCS Paired, CS-Alone, and UCS-Alone received 70 acquisition trials per day at randomized intertrial intervals of 20, 30, and 40 seconds (mean of 30 seconds); whereas group CS-UCS Mixed received 70 CS alone and 70 UCS alone trials, randomized with the restriction that no more than two CS alone or two UCS alone trials occurred consecutively; these trials were presented at randomized intertrial intervals of 10, 15, and 20 seconds (mean of 15 seconds). During extinction, all groups received 210 presentations of the CS alone on the first day, 140 on the second, and 70 on the third.

In adaptation and acquisition each eyeball retraction was recorded, provided it was at least 1 mm in amplitude and occurred no later than 525 msec after "CS onset"; and in extinction the recording interval was extended to 600 msec. During acquisition the responses of rabbits in group CS-UCS Mixed were recorded only on trials in which the CS was presented, and for group UCS-Alone the responses were recorded in an interval from 500 msec before to 25 msec after onset of the UCS. Figure 1 shows the results of plotting the percentage of eyeball retractions for all groups in the latency range of 55 to 525 msec (defined as the latency range of conditioned responses) for blocks of 70 trials. Examination of the figure reveals that for adaptation the percentage of responses was about 2 percent in all groups. In acquisition, none of the control groups demonstrated a level of responding which exceeded 9 percent on any day, whereas group CS-UCS Paired showed a steady increase in the percentage of conditioned responses and attained a level of 85 percent on day 8. The extinction curves show that the control groups did not demonstrate more than a 5-percent level of responding. On the other hand, group CS-UCS Paired showed considerable resistance to extinction by responding at a level of 79 percent on trial-block 1 and showing only a small decline to 68 percent on trial-block 6.

The performance curves obtained in the investigation clearly indicate that the course of acquisition and extinction of the retractor bulbi response in the classical conditioning group cannot be attributed to changes in the spontaneous frequency of eyeball retractions resulting from UCS presentations (group UCS-Alone); pseudoconditioning or sensitization (group CS-UCS Mixed); nor can they reflect the occurrence of alpha (reflex) responses to the CS (group CS-Alone), particularly since examination of the latency distribution of responses revealed no evidence of alpha responses (that is, the few responses observed were unsystematic in their distribution). Although the rate of conditioning of eyeball retractions does not appear to be appreciably different from that observed for the nictitating membrane (*5*), the response does demonstrate substantially greater resistance to extinction. Whether this increased resistance to extinction is attributable to the retractor bulbi response system or to the differences in methods of detecting the responses has not yet been ascertained (*6*).

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Mosaic Histocompatibility of Skin Grafts from Female Mice

Abstract. *The histoincompatibility determined by one or more genes on the X chromosome of the mouse effects a complete rejection of skin of the (C57BL/6 ♀ × BALB/c ♂) F_1 hybrid male grafted onto the reciprocal type F_1 hybrid male, but only an incomplete rejection of either reciprocal type F_1 hybrid female skin, grafted onto the same type of male host. The resulting mosaic survival pattern of the female graft is interpreted as support for the Lyon hypothesis of X-chromosome inactivation.*

Lyon (*1*) has postulated that either the maternally derived or the paternally derived X chromosome of each somatic cell in the normal female mouse becomes genetically inactivated at the time in embryogeny when it also becomes heteropyknotic, a cytological phenomenon described by Ohno and Hauschka (*2*). Furthermore, the particular X chromosome that is inactivated is initially determined at random but remains inactivated in subsequent cell generations so that cells of the two X inactivation types will be distributed with equal frequency and as clones in adult tissues. Inactivation of X, she hypothesized, is a normal method of gene-dosage compensation, permitting the genes of only one X chromosome to be effective in each cell of the female, as in the male.

The bulk of evidence supporting the hypothesis comes from sex-linked genes (or normally autosomal genes translocated onto the X chromosome) which through local gene action affect either the structure or color of the coat. The observed effect for such genes in the heterozygous female is a curious mosaic pattern of gene expression. Sex-linked genes with nonlocal action, on the other hand, show variable gene expression in the heterozygous female. Such observations on both local and diffuse acting genes to date have been consistent with the hypothesis. Lyon has recently reviewed the supporting evidence (see *3*) and has extended the hypothesis to cover mammals in general.

A further test of the hypothesis was made possible by a recently reported discovery of one or more histocompatibility genes located on the X chromosome of the mouse (*4*). The existence of these genes was demonstrated by the rejection of grafts exchanged