central-peripheral pairs of areas so tested.

In another experiment, in a single receptive field the effect of illumination at the center was compared with illumination of three areas peripheral by various distances. The threshold rises evoked centrally by peripheral stimulation, and vice versa, did not decrease rapidly with increased separation (as would be expected if caused by scattered light), but remained roughly the same over the whole range of separations (300–810 μ).

For a fixed duration of light adaptation, it was found that $I_{2t}/I_{1t} \approx kI_a/I_{1i}$; where I_{2t} is the post-adaptation threshold (measured after 1 minute in the dark) and I_{1t} is the pre-adaptation threshold, both for the tested area; k is a constant; I_a is the adapting intensity; and I_{1i} is the pre-adaptation threshold of the illuminated area. If illumination and testing are done on the same area, I_{1t} and I_{1i} become the same. This relation held no matter which was the illuminated and which the tested area in the given receptive field. It held, and with the same constant of proportionality, for four receptive fields, all "on-off" type, each in a different retina, in which a total of 31 adaptations were measured.

These findings indicate that the fraction of excitation reaching the ganglion cell from illuminated receptors anywhere in the receptive field is reduced in proportion to the amount of activity just previously sent toward the ganglion cell from illuminated receptors in the same or a different region of the receptive field. The resultant threshold rise found on testing any group of receptors is not dependent on their previous exposure to light (and any consequent changes in their visual pigments), but apparently is a change in the efficiency of excitation transmission along the neural pathways to the ganglion cell (*7*).

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Plaque Reduction, a Sensitive Test for Eastern Encephalitis Antibody

Abstract. Serologic surveys of vertebrates to determine rates of eastern encephalitis infection were made to discover the most likely disseminators of virus in nature. Of the techniques available, neutralization is the most specific, and antibody is known to persist for many years. This communication reports a fivefold increase in sensitivity of neutralizing-antibody detection by the application of a plaque-reducing technique.

Itoh and Melnick (*1*) were able to detect antibody to ECHO type 4 virus by plaque reduction when standard tube techniques failed to reveal it. They defined the titer of a serum as the highest dilution which reduced the number of plaque-forming units by 80 percent. The use of this method for detecting eastern encephalitis antibody was suggested by Henderson and Taylor (*2*).

Adequate numbers of sera, in adequate amounts, were not available for testing from natural infections; only 11 human sera, collected 1½ to 22 years after infection with the virus, were available. To induce infection, five wild rabbits, five crows, two blue jays, four pigeons, five chickens, five frogs, and four squirrels were inoculated subcutaneously with eastern encephalitis virus. All animals which became viremic produced plaque-reducing neutralizing antibody and maintained it throughout the 9 months of serial bleedings (group 1). The animals in which viremia was not demonstrated failed, with one exception, to produce antibody, and blood samples from these animals, together with samples from pre-inoculation bleedings, comprised the controls (group 2). Blood samples from these various sources plus 91 samples from field specimens of birds and mammals (group 3) made a total of 229 sera examined in parallel by two methods.

Chick embryo tissue cultures were prepared, as described previously (*3*). The planting medium, Hanks-Eagle-pyruvate 1-percent fresh egg albumin (HEPA), was supplemented with 1-percent horse serum to obtain a continuous sheet of cells in 60-mm plastic petri dishes. After 48 hours of incubation the medium in tube cultures was replaced with "change medium" HEPA, buffered with tris hydroxymethyl amino methane (*p*H 7.8) (no bicarbonate and no serum). The planting medium was removed from the plates just before inoculation.

Sera were diluted 1:5, inactivated at 56°C for 30 minutes, and incubated for 1 hour at room temperature with an equal volume of eastern encephalitis virus at a dilution calculated to contain 200 $TCID_{50}$ per 0.1 ml. Two plates and two tube cultures were inoculated with 0.1 ml of each serum-virus mixture. After 1 hour of adsorption at 36°C, the plates were overlaid, without washing, with 4 to 5 ml of "change medium" containing 1 to 1.5 percent of agar. After 48 to 72 hours' incubation at 36°C, the plates were stained with neutral red (1:10,000) in saline.

The plaque-forming units of several control plates were averaged in each of nine experiments; the average number of units ranged from 25 to 80. In tube cultures, hemagglutination of goose red blood cells by supernatant fluids (*4*) served to differentiate specific viral cytopathogenic effects from other tissue-destroying factors, such as toxic sera.

Table 1. Detection of eastern encephalitis neutralizing antibody: results of tests by two methods, in parallel, of serum-virus mixtures. Group 1, sera (N = 11) from human beings who had recovered from eastern encephalitis, and sera (N = 97) from inoculated animals after viremia had been demonstrated. Group 2, sera from animals before inoculation, and after inoculation when no viremia had been demonstrated. Group 3, sera from field specimens (birds and mammals).

Plaque-reduction neutralization*			Tube-culture neutralization†			
Positive	Equivocal	Negative	Positive	Equivocal	Negative	Total
			Group 1, infected			
82			18	11	53	82
	16		0	1	15	16
		10	0	0	10	10
			Group 2, controls			
3‡			0	0	3	3
	0		0	0	0	0
		27	0	0	27	27
			Group 3, unknowns			
7			0	2	5	7
	2		0	0	2	2
		82	0	0	82	82
			Totals			
92	18	119	18	14	197	229

* Categories for reduction of plaque count of the serum-virus mixtures are as follows: (positive) 0 to 20 percent of the average count of plaque-forming units of virus controls; (equivocal) 21 to 30 percent; (negative) 31 percent or more. † (Positive) 2:2 cultures, no virus growth; (equivocal) 1:2 cultures, no virus growth; (negative) 0:2 cultures, no virus growth. ‡ Three blood samples from a single inoculated wild rabbit; presumably viremia was missed.

The following technical points contributed to success in the tests. (i) Replacement of tris buffer in the overlay with sodium bicarbonate (0.35 g/lit.) and incubation in 5-percent carbon-dioxide increased the contrast of staining and the size of the plaques. (ii) The volume of inoculum for test and control plates was always kept the same, since in pilot experiments multiples in excess of five of the standard inoculum (0.1 ml) gave a lower count of plaque-forming units than was expected; this was ascribed to the reduction in number of cell hits by virus particles with increasing depth of fluid. With the small volume of inoculum, a humid atmosphere during adsorption was essential to prevent destruction of the cell sheet by drying.

Of 92 sera found positive by the plaque-reduction technique, only 18 (20 percent) were found positive by the tube assay of the same serum-virus mixtures (Table 1). Sixteen of these positive tube tests were on sera which reduced the plaque-forming units to the lowest level—that is, strongly positive sera. The 11 sera collected from seven human beings who had recovered from eastern encephalitis were all strongly positive according to the plaque-reducing test, but only five were positive according to the tube test. The inefficiency of tube cultures in antibody detection is believed to be due to the high sensitivity of such cultures to a few unneutralized particles of infectious virus. Dissociation of virus from antibody in the fluid medium may be a factor.

Correlation of results of the plaque-reducing test with the history of the serum donor was good. There were no false positive results in these tests in the control group. Only four sera could be considered "false-negatives"—that is, sera in which the plaque-reducing test failed to reveal antibody when the animal had previously been shown to be producing antibody.

We hope in the future to get more accurate information, through the plaque-reducing method, on immunity to eastern encephalitis in human beings in endemic areas and in animals collected in the field (*6*).

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Nondiscriminated Avoidance Behavior in Human Subjects

Abstract. College students were required to learn a plunger-pulling response to postpone the occurrence of a shock or to avoid the loss of a monetary reward. Marked individual differences in the response patterns appeared in the first hour and persisted through 20 hours of testing. These differences overshadowed those produced by moderate alterations in the schedule or value of the aversive event.

Since Sidman (*1*) described the experimental schedule in which each response postpones the occurrence of an aversive event, it has been used in numerous studies of rats, cats, monkeys, and pigeons (*2*).

Recently, Hefferline *et al.* (*3*) reported conditioning human adults to make a tiny, involuntary twitch of the thumb in order to turn off or postpone an aversive noise. Baer has used the same avoidance-escape schedule with preschool children (*4*) who worked to escape interruptions in the presentation of cartoons.

The subjects in this experiment were 33 paid volunteer college students. Two kinds of motivating conditions were used: under the shock-avoidance condition, the subject had disk electrodes strapped to the front and back of his forearm on the nonpreferred side. The aversive stimuli were alternating-current pulses of 30-msec duration which were individually adjusted to the highest level judged endurable (which ranged from 0.3 to 1.0 ma). Subjects at both ends of the shock current continuum described the shock as "a sharp pinprick." Under the coin-loss condition, the subjects were shown 100 pennies in a display magazine and told that all pennies remaining at the end of the session would be added to their base pay of $1.50 per hour. The aversive stimulus under this condition was the disappearance of a penny from the magazine, with the accompaniment of a loud clang. Under both conditions the aversive events occurred at 20-second intervals unless the appropriate response was made.

Two Lindsley manipulanda (*5*) protruded from the front of the fully enclosed relay rack that also served to support the penny magazine. Pulling and releasing the left-hand plunger was the correct response, while manipulating the right-hand lever was entirely irrelevant.

Six subjects were run for ½ hour under the shock-avoidance condition, followed by ½ hour under the coin-loss one, and five were run in the reverse order, coin-loss followed by shock-avoidance. The remaining 22 subjects were tested under the coin-loss condition only. The first 23 subjects were told only that something they could do in the experimental room would influence how many aversive stimuli were presented. The last 10 were told that the plungers on the front of the apparatus would control the occurrence of the aversive stimuli, but were not told how to use them.

Of the 33 subjects, 23 developed a stable avoidance response within the first hour. However, only nine achieved the most efficient pattern of responding in that (i) their rate of responding on the correct plunger approached three responses per minute, and (ii) the rate on the irrelevant plunger was zero. Of 11 subjects exposed to both loss of coin and shock as aversive stimuli, 82 percent developed an avoidance response; of those exposed to loss of coins only, 64 percent did so. The difference, not statistically significant, appeared to be due to a resurgence in exploratory behavior after the experimenter entered

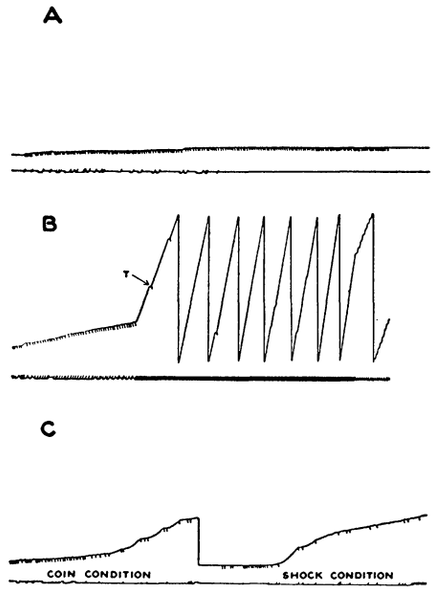

Fig. 1. Representative records from first hour of testing. Each response by subject moves pen vertically. Full excursion is 500 responses. Small pips on record indicate occurrence of aversive event—loss of coin in all cases but *C*. The horizontal line indicates responses on the irrelevant lever.