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 carbondioxide 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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6. *Note added in proof.* The following recent papers on the use of plaque reduction for antibody measurement have come to our attention: E. Cutchins, J. Warren, W. P. Jones, *J. Immunol.* **85**, 275 (1960); J. S. Porterfield, *Bull. World Health Organization* **22**, 373 (1960).

17 October 1960

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

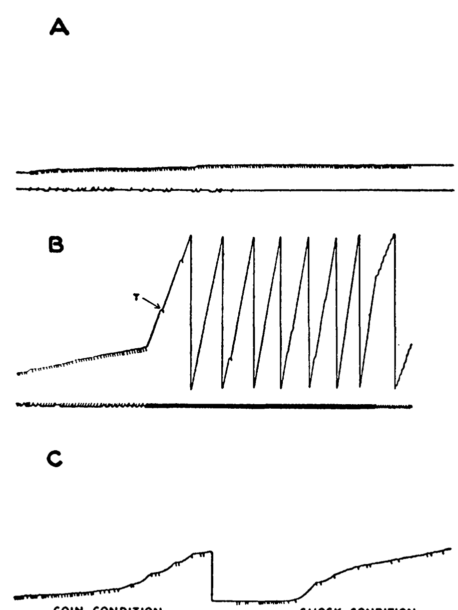

Fig. 1. Representative records from first hour of testing. Each response by subject moves pen vertically. Full excursion is 500 responses. Small pips on record indicate occurrence of aversive event—loss of coin in all cases but *C*. The horizontal line indicates responses on the irrelevant lever.

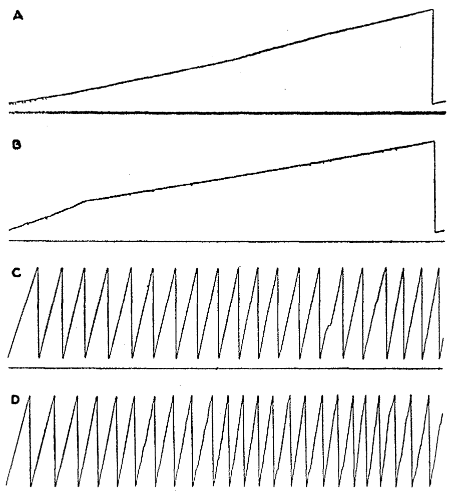

Fig. 2. Records of selected 2-hour test sessions from two subjects. *A*, 3rd session; *B*, 9th session of subject with lowest mean rate; *C*, 3rd session; *D*, 9th session of subject with highest mean response rate. The session previous to that shown in *B* required 12 responses per minute.

the room halfway through the period to give new instructions. On inquiry, 7 of the 11 subjects who experienced both conditions stated that losing money was the more distasteful, only three said the shock was more distasteful, and one was indifferent. Avoidance responses were developed equally often by those who were instructed to use the plunger and by those who were not. Those who were not so instructed sometimes displayed such bizarre behavior as standing in the corner or standing on the heads. The proportions of males and females who learned the avoidance response did not differ.

Three representative records from the first hour of testing are shown in Fig. 1. The record of an individual who did not develop an avoidance response is shown in Fig. 1*A*. Characteristically, some responses were made on each plunger during the first half-hour of the session, but all efforts were abandoned after the pennies were exhausted about midway in the session. Figure 1*B* shows a common pattern of abrupt change in response rate. Also common was the pause such as occurred at the point marked *T* in the record. Note that the irrelevant right-hand plunger was pulled throughout the session, and also that satiation phenomena appeared near the end of the hour. The individual of Fig. 1*C* showed initially overcomplex behavior that was later eliminated, but he continued to manipulate the irrelevant plunger throughout. Also of interest is his lack of generalization of the response between the two conditions; such a failure was the rule, occurring in five of six opportunities. Some individuals eliminated responses on the irrelevant plunger, but maintained an inappropriate rate, while others achieved an appropriate rate but failed to eliminate the irrelevant responses. No subject developed the most economical response pattern within the first hour's training.

After the first experimental session, 10 subjects were selected at random to participate in 20 additional hours of testing, and seven of them completed the entire series of tests. The loss of coins was used throughout this series as the aversive event, to investigate the effects of variation in the length of test session, in the interval between loss of coins, and in the magnitude of each loss.

All these subjects acquired the avoidance response by the end of the second hour, although some persisted with inappropriate rates and responses on the irrelevant lever throughout the entire 20 hours of testing. Typically from the third session on, each subject entered the experimental room and commenced immediately to pull the plunger at his characteristic rate. Only one subject showed the so-called warm up phenomenon that is often encountered in rats working under this schedule. Records of the third and ninth testing sessions (during which the response-loss interval was 20 seconds, and each aversive event cost 2 cents) for the subjects with highest and lowest response rates are shown in Fig. 2. Differences in response rates between individuals far exceeded those produced by alterations in the experimental conditions. Mean response rates for subjects under all conditions ranged from 6.21 to 119.8 per minute. In contrast, the median responses per minute for the three conditions of coin value were: 2 cents, 7.91; 10 cents, 8.22; and 50 cents, 8.88. The interval between a response and the loss of the next coin had a somewhat greater influence: at a 5-second interval the median rate was 22.84 responses per minute (12 was the minimum rate to protect all coins). Rates for the 20- and 80-second intervals were 4.46 and 3.06 respectively.

The basis for the large individual differences observed in the performance of nondiscriminated avoidance behavior by human subjects is now being investigated in this laboratory (*6*).

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Electroretinogram in Response to X-ray Stimulation

Abstract. The retina of the grass frog, *Rana pipiens,* responds to flashes of high-intensity x-rays and produces an electroretinogram indistinguishable in form from the electroretinogram produced in response to light stimulation at low and intermediate intensities. At higher intensities the form changes and, for maximal responses, the electroretinogram in response to x-rays shows a lower amplitude and a longer latent period than that in response to light. The prolonged latent period indicates additional intermediate reactions for the x-ray response.

Invisibility is generally emphasized as one of the properties of x-radiation (*1*). The ability of x-rays to evoke some sort of retinal response, however, has been known from the fact that, in early years, men looked into x-ray beams and reported various visual sensations. Once the harmful nature of x-rays was recognized, this activity was suddenly curtailed.

Two successful attempts to produce electroretinograms by x-rays have been reported in the literature. Himstedt and Nagel (*2*) showed records from frogs and birds which indicate little more than some sort of electrical disturbance caused by x-rays; these records bear little resemblance to the electroretinogram as recorded today, in response to light, with modern equipment. Elenius and Sysimetsä (*3*) gave a brief report on low, threshold responses in human subjects suffering from cataracts.

Attempts to produce an electroretinogram in response to x-rays usually fail, due, apparently, in large part to inability to stimulate the retina with intense, quick flashes of high-energy x-rays. In the research reported here the difficulty was overcome with the experimental setup shown in Fig. 1. The high-intensity beam was built up, while the ½-in. lead shutter protected the eye of the frog from the beam. Slits of various widths in the lead shutter were passed over the eye, by remote control, much as a focal-plane shutter operates in a camera. The movement of the shutter gave exposure times proportional to the width of the slit and the speed of movement of the shutter. The duration of the exposure was monitored