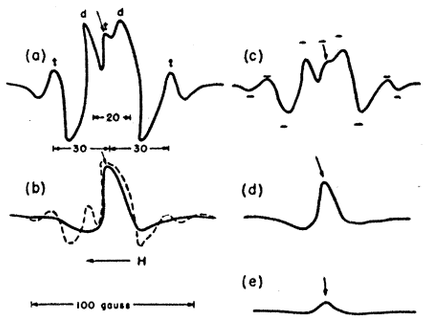

Fig. 2. Paramagnetic resonance measured at −195°C in dry bacterial spores previously irradiated anaerobically at 25°C. (*a*) Hyperfine patterns composed of doublet *d* and triplet *t* spectra. (*b*) Spectra produced by reaction of these radicals with O_2 at 25°C (see text). (*c*) Anaerobic annealment of radicals in (*a*) at 100°C for 10 minutes; signal heights for this sample before annealment are given by the horizontal lines. (*d*) Reaction with oxygen of radicals remaining after annealment. (*e*) Reaction of radicals in (*a*) with nitric oxide.

in helium) applied after irradiation (Fig. 2*e*). Similar biological and physical results with nitric oxide have been reported independently by Sparrman *et al.* (*6*) in another system.

While the close correlation shown between the biological and physical measures of radiation damage does not require any causal relationship of events, the results do support with physical evidence derived from the same system the proposed mechanism (*1*) of the latent oxygen effect (*7*).

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7. We are pleased to acknowledge valuable discussions with Dr. M. L. Randolph of Oak Ridge National Laboratory. This work was performed under the auspices of the U.S. Atomic Energy Commission. These results were announced on 26 February 1960 at the fourth annual meeting of the Biophysical Society in Philadelphia. An abstract appeared in the *Proceedings of the Biophysical Society* (1960), p. 36.

27 June 1960

Simultaneous Generalization Gradients for Appetitive and Aversive Behavior

Abstract. In the presence of a bright light five monkeys were trained to press a lever to avoid shock and to pull a chain for food reward. When tested with a series of lights dimmer than the conditioning stimulus, the monkeys showed a sharp gradient of effect for the rewarded response, in contrast to a very flat gradient for the avoidance response.

When an organism is trained to make a response in the presence of a particular stimulus, this response will also normally occur in the presence of other stimuli that are physically similar to the original conditioning stimulus. The result of this phenomenon is often a "gradient of stimulus generalization"—an orderly decline in the probability of response, which takes place as the physical difference between the original stimulus and various test stimuli is increased. Stimulus generalization is a major explanatory concept in such areas as learning theory, psychotherapy, and abnormal psychology (*1*).

The study reported here was designed to determine whether there is any difference between generalization gradients for reward-controlled and punishment-controlled behavior. The technique used to investigate this problem permitted a comparison of the two gradients for individual subjects and may be applicable to other problems in experimental psychology and psychopharmacology.

Five young male rhesus monkeys were the subjects. The experimental test chamber was a commercially produced model (Foringer) which provided an automatic mechanism for reward delivery, an electrifiable grid, implementation for two possible responses (pulling a chain which hung in the center of the chamber and pressing a lever mounted on one wall), and a 110 v a-c, 60-watt house light mounted above a circular screen of milk glass in the top of the chamber. During generalization testing the intensity of this house light was varied in discrete (though unequal) steps by means of a group of fixed resistors in series with the house light. The 11 possible test-light intensities were calibrated on several occasions with a General Electric foot-candle light intensity meter placed approximately 1 foot below the glass screen on which the house light was projected.

All the subjects were first trained to press the lever, which postponed shock for 10 seconds; by responding at least once every 10 seconds the subjects could avoid shock entirely (*2*). During this training period, and all subsequent training periods prior to the generalization test, the house light was on continuously at its maximum intensity (28.1 ft-ca).

After the animals became proficient at avoiding shocks, the chain was introduced into the apparatus, and each pull of the chain was rewarded with a pellet of food. The avoidance schedule was still in effect, so that lever-pressing continued even during the learning of the chain-pulling response. Eventually all the subjects learned to press the lever to avoid punishment and to pull the chain to produce food reward on a 2-minute variable-interval schedule (*3*).

After ten additional sessions of exposure to the concurrent schedules of reward and avoidance in the presence of the brightest light intensity (*4*), a generalization test session was programmed. During this test, light of 11 different intensities was presented, 12 times at each intensity, in a mixed order. Each stimulus presentation lasted 30 seconds. No rewards or punishments were obtainable during this test. The test procedure was quite similar to that of Guttman and Kalish (*5*).

For several experimental sessions after this generalization test the subjects were put on concurrent reward-avoidance schedules as before; they were then given a second generalization test (*6*).

Chain-pulls and lever-presses in response to each of the 11 light intensities were recorded. Generalization gradients, relating response strength (the ratio of total number of responses to each intensity to total number of responses to all intensities) to log intensity of the test light are shown in Fig. 1. These data are group means for the two generalization tests combined. Considered separately, the data for individual subjects are very similar to the group results.

Figure 1 displays a clear difference

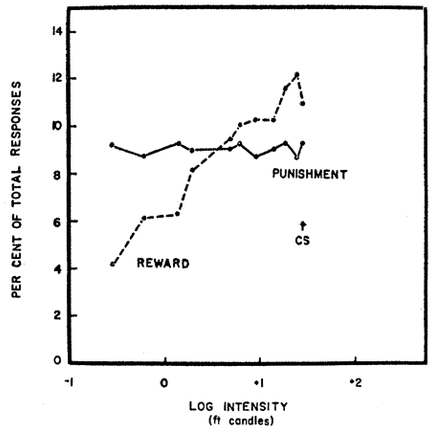

Fig. 1. Generalization gradients for reward-controlled and punishment-controlled behavior.

between avoidance and reward gradients. The reward gradient is much steeper; it was found that the subjects were all much more likely to respond to stimuli of a high intensity (close to that of the conditioned stimulus) than to stimuli of much lower intensity than the conditioned stimulus. In contrast, the avoidance gradient is almost completely flat; subjects were just as likely to respond to the dimmest as to the brightest test light (*7*).

Since the rate of avoidance responding was much higher than the rate of responding for food reward, the differences in shape of the generalization gradients might be attributable to differential response rates rather than to motivational or reinforcement factors (reward versus punishment). However, at least one similar study (*5, 8*) has shown that lowered response rate leads to a flattening of generalization gradients, a finding which would imply the opposite effect from that obtained in the experiment discussed here.

The finding of virtually indiscriminate avoidance response, in contrast to the well-discriminated rewarded response, may have relevance to clinical descriptions of hypersensitivity and seeming irrationality under conditions of strong anxiety; an "anxious" patient may respond strongly to stimuli which are only remotely similar to an original anxiety-provoking stimulus. There are experimental data from studies of human beings which also show a greater than normal amount of stimulus generalization in subjects who are highly anxious (*9*) or even schizophrenic (*10*), or who are made anxious experimentally (*11*).

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4. Note that the animals were not given any discrimination training—that is, they were not exposed to any other house-light intensities during training in the experimental chamber. One subject, however, usually had to be left in the apparatus overnight, since his daily session began at 5 P.M.; at the conclusion of the session the house light went out, and the animal remained in complete darkness until the next morning. The generalization data for this subject did not differ in any obvious way from those for the other subjects.
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6. One subject contracted a digestive-tract infection and died before a second test was possible.
7. After the conclusion of this experiment subjects were given discrimination training, so that they learned to press the lever and pull the chain only during light of one intensity (the brightest or dimmest, depending on the subject) and to cease responding when the light was of a different intensity (at the other end of the intensity continuum). Here, too, preliminary results showed avoidance gradients to be flatter than reward gradients. Both gradients were much steeper than before discrimination training, however.
8. With regard to "response rate" it might be added that D. R. Thomas and R. A. King [*J. Exptl. Psychol.* **57**, 323 (1959)] and M. Sidman (Eastern Psychological Association meetings, 1960) found no effect of response strength on generalization. However, W. O. Jenkins, G. R. Pascal, and R. W. Walker [*J. Exptl. Psychol.* **56**, 274 (1958)] found significantly flatter gradients in their more active subjects.
9. See, for example, S. A. Mednick, *J. Consult. Psychol.* **21**, 491 (19[illegible] E. R. Hilgard, L. V. Jones, S. J. Kaplan, *J. Exptl. Psychol.* **42**, 94 (1951).
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19 September 1960

Properties of the Major Component of a Peptic Digest of Rabbit Antibody

Abstract. The molecular weight of the active, major component isolated from a peptic digest of rabbit antibody was found to be 106,000. After treatment with a disulfide-splitting reagent, the molecular weight was 56,000, and the products migrated as a single peak in the ultracentrifuge. The univalent fragments thus formed can be partially recombined by passage through IR-120 cation-exchange resin at room temperature or by treatment with a difunctional organic mercurial. Some splitting of the pepsin-treated antibody molecule occurs on carboxymethylcellulose at *p*H 5.4.

Porter (*1*) has shown that papain hydrolyzes rabbit antibody into three chromatographically separable fractions, two of which block precipitation of the homologous, untreated antibody with antigen. The third is inactive but crystallizable. Fragments of rabbit antihapten antibody were found to have nearly all their specific binding sites intact (*2*) and were shown to be univalent (*3*). Peptic digestion of the antibody results in a decrease in sedimentation coefficient, for the bulk of the protein, from about 6.5 to 5 *S* (*4*). The fragments are still bivalent, as is indicated by their capacity to precipitate specifically. Subsequent treatment with one of several disulfide-splitting reagents splits the 5 *S* residue into 3.5 *S*, univalent fragments (*4*). This is accomplished by the reduction of a single, highly reactive disulfide bond (*5*). Since papain is a sulfhydryl enzyme, and is therefore used in conjunction with a disulfide-splitting reagent as activator, it was proposed (*4*) that the two enzymes may act by similar mechanisms. This suggestion was supported by the close similarity in several properties of the final products obtained by the action of either enzyme with a reducing agent present.

The method used (*5*) for isolation of the 5 *S* fragments of antibody resulting from peptic digestion consists, first, in precipitation with sodium sulfate added to a final concentration of 12.5 percent (w/v). After centrifugation, sodium sulfate is added to the supernatant to a final concentration of 19 percent. The precipitated protein thus obtained, in several preparations, migrated as a single peak with $s_{20,w} = 5.0 \pm 0.2$ *S*. The yields were 40 to 60 percent of the weight of gamma globulin used.

The molecular weight of this purified component of a peptic digest (of rabbit antiovalbumin gamma globulin) was determined. The diffusion constant was measured in a synthetic boundary cell in a Spinco model E ultracentrifuge at a protein concentration of 10 mg/ml, and sedimentation coefficients were determined at concentrations of 2.5, 4.0, 7.0, and 10 mg/ml. Both procedures were carried out at 20°C in saline–borate buffer, *p*H 8, ionic strength 0.16. The sedimentation coefficient, s_0, obtained by extrapolation to zero concentration, was 5.25 *S* and the diffusion constant was 4.7×10^{-7} cm²/sec. The partial specific volume was taken as that of untreated antibody, 0.745 (*6*), giving a molecular weight of 106,000.

After treatment of the above preparation with 0.01*M* 2-mercaptoethylamine and dialysis against saline-borate buffer, the value of s_0 at 20°C was 3.6 *S*, and the diffusion constant was 6.1×10^{-7} cm²/sec, corresponding to a molecular weight of 56,000. Symmetrical single peaks were observed for both preparations. Since the 3.6 *S* fragments migrated as a single peak, the results suggest that the reducing agent splits the molecule into two subunits approximately equal in molecular weight. This is consistent with the possibility (*4*) that the 5 *S* molecule consists of Porter's Fractions I and II, linked through a disulfide bond.

In other experiments, described below, it was found that chromatography of the purified 5 *S* material on carboxymethylcellulose at *p*H 5.4 causes partial degradation into fragments with $s \cong 3.5$, having the capacity to block the homologous precipitin reaction of untreated antibody with antigen. The results are similar to those obtained on treatment with a reducing agent.

We have also found that the 3.5 *S* fragments can be recombined to give fairly good yields of 5 *S* protein. This has been done either by passage through the ion-exchange resin, IR-120, at *p*H 5,