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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3. C. B. Ferster and B. F. Skinner, *Schedules of Reinforcement* (Appleton, New York, 1957).
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.
5. N. Guttman and H. I. Kalish, *J. Exptl. Psychol.* **51**, 79 (1956).
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 (1957); E. R. Hilgard, L. V. Jones, S. J. Kaplan, *J. Exptl. Psychol.* **42**, 94 (1951).
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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,

or by treatment with a bifunctional organic mercurial. Details of the experiments follow.

One hundred milligrams of a purified peptic digest of rabbit antiovalbumin gamma globulin ($s_{20,w}$ = 5.2) was adjusted to *p*H 5.4, ionic strength 0.007, in acetate–chloride buffer. The solution was added to a 22 by 360 mm column of carboxymethylcellulose, containing 0.7 meq of carboxyl groups per gram, obtained from the Brown Company, Berlin, N.H. Three fractions were collected by successive elutions with acetate buffers of *p*H 5.4 and ionic strengths of 0.007, 0.1, and 0.9. The fractions contained 39, 30, and 13 percent, respectively, of the total protein.

The first two fractions were concentrated by pervaporation, carried out with the lower end of the dialysis bag immersed in constantly stirred saline–borate buffer, *p*H 8, μ = 0.16, and were dialyzed against the same, cold buffer. Approximately 90 percent of the protein in the first fraction migrated with a velocity (s_{20}) of 3.2 *S*; a small amount of somewhat faster moving material was present. The second fraction exhibited two peaks with s_{20} = 3.5 (55 percent) and 5.1 (45 percent), respectively. The effect of 2 mg of either fraction on the reaction of 1.4 mg of untreated antiovalbumin gamma globulin with an optimum concentration of antigen was tested, and essentially complete inhibition was observed in each case. A heavy precipitate was formed by the antibody in the absence of inhibitor.

The next experiments, on recombination, were done with the products obtained by treatment of the purified 5 *S* protein with 0.01*M* 2-mercaptoethylamine hydrochloride for 1 hour at 37°C in 0.05*M* acetate buffer, *p*H 5.0. After the reaction, the protein migrated as a single peak with s_{20} = 3.4 *S*. This concentration of 2-mercaptoethylamine (0.01*M*) has been found to be approximately minimal for complete breakdown into ~3.5 *S* fragments under the conditions used (*5*).

Sixty milligrams of the reduced protein in 3 ml was passed through an 8 by 150 mm column of IR-120 resin in the sodium cycle at *p*H 5.0 and room temperature; 0.05*M* acetate buffer, *p*H 5.0, was used for elution. The eluate gave a negative nitroprusside test. In the ultracentrifuge 40 percent of the protein now migrated with a velocity (s_{20}) of 5.1 *S*, suggesting that the 3.5 *S* fragments had partially recombined into the original 5 *S* units. This result has been confirmed in several similar experiments.

In another experiment with 250 mg of protein and 0.01*M* 2-mercaptoethylamine in a volume of 8 ml, the reducing agent was removed on a shorter column (8 by 75 mm) in the cold room at *p*H 6. Under these conditions there was very little recombination; the nitroprusside test on the eluate again was negative. Portions of the eluate were treated at *p*H 5 with increasing amounts of the bifunctional organic mercurial, 3,6-bis-(acetoxymercuri methyl)-dioxane (*7, 8*), added as a 0.002*M* solution in water. The molar ratios of the mercurial to 5 *S* protein originally present (molecular weight 106,000) were 0.4, 0.7, 1, and 4:1. After standing overnight in the refrigerator, a small amount of precipitate, representing less than 6 percent of the total absorbance at 280 mμ in each case, was removed by centrifugation. For the samples containing 0.4, 0.7, and 1.0 times the equivalent amount of mercurial, the extents of recombination were 45, 50, and 35 percent, respectively. The s_{20} values were 4.9, 5.0, and 4.9, respectively, for the faster peaks and 3.4, 3.3, and 3.4 for the slower peaks in the three experiments. A control, incubated overnight without the mercurial, showed no appreciable change in the sedimentation pattern (s_{20} = 3.3).

When four times the equivalent amount of mercurial was used, over 90 percent of the protein migrated with a velocity of 3.7 *S*. This failure to recombine is similar to the results observed in the dimerization of serum albumin (*7*), and can probably be attributed to the fact that excess mercurial reacts in a 1:1 rather than 1:2 ratio with the sulfhydryl (SH) groups (*7*). Actually, under the most favorable conditions quantitative recombination to 5 *S* fragments would not necessarily be expected because of the difficulty of producing univalent fragments with exactly one SH group per molecule. The stoichiometry requires further investigation. The precipitation observed may have been due to polymerization of units containing more than one SH group.

The results indicate that the univalent fragments comprising the 5 *S* protein are partially separable under the mild conditions prevailing on a column of carboxymethylcellulose at *p*H 5.4. This is probably not attributable to impurities in the adsorbent, for when fresh, untreated Whatman's coarse cellulose powder was used instead of carboxymethylcellulose, nearly half the protein was similarly degraded to 3.2–3.4 *S*. Two possible explanations are: (i) There are sufficiently strong reducing groups in the cellulose to split the S-S bond, and (ii) the large surface catalyzes a rearrangement which forces the fragments apart. Other possibilities also exist and the mechanism is at present uncertain.

The recombination on IR-120 can probably be attributed to reoxidation of sulfhydryl groups. This would be consistent with the observation that the bifunctional mercurial also brought about a large amount of recombination to units having almost exactly the same *s* value. Experiments aimed at resynthesis of precipitating antibody from blocking fragments are in progress. It should also be of interest to attempt to prepare antibody of mixed specificity (*9*).

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