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Antibody Combining Site: The B Polypeptide Chain

Abstract. *The antibody molecule consists of several polypeptide chains. Peptides, which appear to have been derived from the binding region of the rabbit-antibody molecule directed against* p*-azobenzenearsonate, have been isolated. The particular polypeptide chain from which these peptides are derived has now been identified as the B chain described by Fleischman, Pain, and Porter.*

Gamma-globulin molecules of several animal species consist of several polypeptide chains linked by disulfide bonds. A question of interest has been the relation of the combining sites of the antibody molecule to these chains. Edelman and Benacerraf (*1*) separated what they called H and L chains from the antibody molecule and indicated that the L chain seems to be important in the antibody-combining site. Fleischman, Pain, and Porter (*2*), by a different method, separated the molecule into chains A and B which appear to be, respectively, the H and L chains described by Edelman and Benacerraf and they suggested that antibody activity resides in the A chains. Our findings are that a peptide apparently from the binding region of rabbit antibody against *p*-azobenzenearsonate (anti-R_p) comes from that part of the antibody molecule which falls into fraction B of Fleischman, Pain, and Porter (*2*). This was shown through use of the "paired-label" technique (*3*). In applying this technique, one portion of antibody is iodinated with I^{131}-labeled iodine in the presence of hapten and another portion is iodinated (to the same extent) with I^{125}-labeled iodine in the absence of hapten. The preparations are mixed and digested and the peptides are isolated. Any peptide for which the ratio of the two isotopes differs from that in the original mixture is derived from a portion of the molecule in which the antibody-hapten complex has affected the iodination.

A solution of 30.1 mg of specifically purified rabbit anti-R_p (*4*) antibody in 1.9 ml of borate buffer of *p*H 8 was added to 1.5 ml of 1*M* glycine buffer (*p*H 9) and iodinated at 0°C by the dropwise addition of 2.80 ml of the glycine buffer containing 6.5 μmoles of I^{125}-labeled hypoiodite (1.4×10^8 count/min). Another portion of the same antibody solution containing in addition 60 μmoles of sodium benzenearsonate was treated similarily except that the hypoiodate was labeled with I^{131} (4.9×10^8 count/min). After 2 hours at 0°C, both preparations were individually transferred to dialysis bags. Dialysis was carried out at 5°C against 2 liters of saline buffered with borate; the dialyzate was changed twice daily for 3 days. During the second dialysis, 0.25 g of potassium iodide was added to the outer solution. The I^{125}-labeled preparation contained 25.9 iodine atoms per molecule and the I^{131} preparation, 26.1 atoms per molecule.

Portions of each solution equivalent to 19 mg of protein were mixed, concentrated by pervaporation, and then dialyzed against 0.55*M* tris-HCl buffer of *p*H 8.2. The protein concentration was adjusted to 20 mg per ml. The reduction (in 0.75*M* mercaptoethanol), alkylation, and fractionation by chromatography (1*M* propionic acid and a 51 × 2.8 cm column of Sephadex G-75) were all carried out according to Fleischman *et al.* (2). The results are shown in Fig. 1 where the radioactivity of I^{131} in counts per minute per tube is shown as a function of the tube number. Seventy-four percent of the I^{131} was in tubes 21 to 28 and 26 percent in tubes 29 to 45. Tubes 22 and 23 were pooled as fraction A_1, tubes 24 to 26 as fraction A_2, and tubes 30 to 40 as fraction B. The fractions were each dialyzed against 9 volumes of distilled water. Essentially no radioactivity appeared in the outer solutions during dialysis. The solutions were lyophilized and the residues were taken up in a volume of 0.5*M* formic acid containing 0.33 mg of pepsin per milliliter, resulting in a calculated concentration of 10 mg of iodinated protein per milliliter in each case. The solutions were incubated at 37°C for 16 hours. Dialyzed and lyophilized portions of the protein before and after reduction and alkylation were also digested.

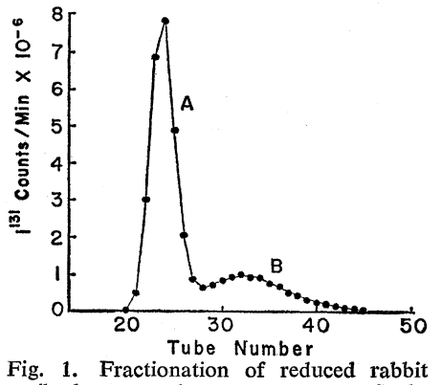

Fig. 1. Fractionation of reduced rabbit antibody to *p*-azobenzenearsonate on Sephadex G-75 in 1*M* propionic acid. Four ml fractions were collected. The antibody had been "pair-iodinated" in the presence and absence of hapten before reduction.

The digests were lyophilized and the peptides were separated by paper chromatography and high-voltage paper electrophoresis (*5*). Radioautographs were prepared and areas on the papers corresponding to spots on the radioautographs were cut out. The concentration of I^{125} and I^{131} (in counts per minute) on each piece of paper were determined and the ratio of the amount

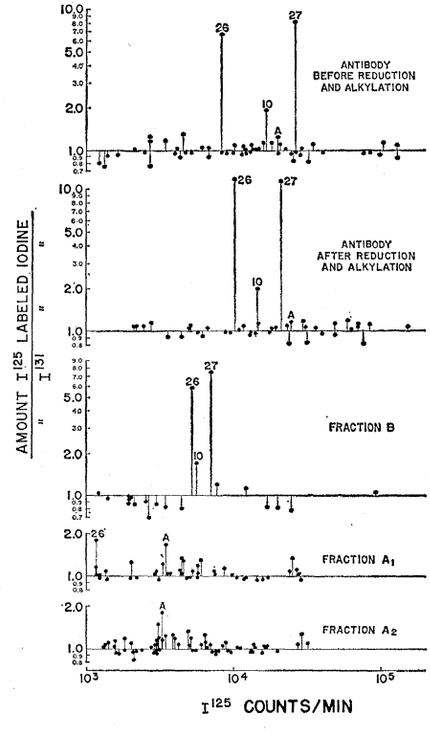

Fig. 2. Iodinated peptides derived from "pair-iodinated" rabbit antibody to *p*-azobenzenearsonate. Each dot represents a spot on the radioautograph.

of I^{125} to I^{131} for each spot was calculated. The patterns for the digest of the mixture of the two iodinated proteins or the digest of the reduced and alkylated, but unfractionated, mixture were essentially the same. The results for both digests are shown at the top of Fig. 2. For most of the spots the ratio of the amount of I^{125}- to I^{131}-labeled iodine was essentially unity, that is, it was the same as in the original mixture. However, the ratio for three of the spots was high, indicating that more of the iodinated peptides represented by these spots came from the antibody iodinated (I^{125}) in the absence of hapten than from the antibody combined with hapten during iodination (I^{131}) (*6*).

The amino acid sequences from which these latter iodinated peptides were obtained are apparently closely associated with the combining site since their rate of iodination was greatly decreased by the presence of hapten blocking the site; however, peptides with a high ratio not from the site could come about by a conformational change taking place on combination of antibody with hapten, if such a conformation change caused a decreased rate of iodination of residues elsewhere than in the site.

Peptic digests of fraction B from the same iodinated preparation showed these same three high-ratio spots (Fig. 2). They were absent in the digests of fractions A_1 and A_2 except for a very slight amount of radioactivity in a position corresponding to one of the spots (No. 26) which showed a slightly increased ratio in the digest of fraction A_1 (*7*). Moreover, the digests of fractions A_1 and A_2 gave no spots showing a particularly large deviation from unity in the ratio (*8*).

These results show that the high-ratio peptides are from B chains rather than from A chains and that the binding of hapten by antibody affects primarily the iodination of the B chain. If the high-ratio peptides are indeed from the binding site, the B chain forms at least part of the site. If the high ratios are due to a conformational change, the binding site can be in either chain with resultant decreased reactivity of the B chain towards iodination (*9*).

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23 AUGUST 1963

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6. The iodinated residue from these spots has been isolated and identified as diiodotyrosine. [O. Roholt and D. Pressman, *Federation Proc.* **22**, 556 (1963).]
7. This was probably due to a trace of residual unsplit antibody. The fact that this spot was not found in fraction A_2 gives some indication of the completeness of separation of B from A_2.
8. The largest deviation was attributable to spot A (Fig. 2) found in both digests with a ratio only one-fifth that of No. 26 or No. 27. Nothing was found in the corresponding position of the peptide pattern from fraction B. The corresponding spot in the pattern from whole antibody did not show elevated ratios (spot A, Fig. 2).
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Maturation of Performance with Space-Displaced Vision

Abstract. *This study tests two specific hypotheses of neurogeometric theory: that a critical period in maturation of space-displaced visual feedback in behavior occurs in childhood, and that a differential organization of inverted, reversed, and inverted-reversed visual feedback in motion will be found at the time when children are first capable of giving compensatory response to the spatial disorientation of vision. In keeping with theoretical expectations the results showed that when the different inverted and reversed feedback conditions could be performed, the response to the inverted feedback condition was the poorest, while that to the reversed condition was the most effective.*

The apparatus used consisted of an industrial RCA vidicon camera (TV-Eye) unit and a 21-inch portable television monitor, which together comprised the closed-circuit television system, and an electronic hand-writing analyzer (*1*). The camera was mounted directly over the work area. A dove prism affixed to its lens was used to displace the visual field. The feedback image was of normal size. The electronic handwriting analyzer was used to time the duration of the manipulative and travel components of writing, drawing, and dotting motions.

In this controlled situation, a cloth curtain hid the subject's movements from his direct vision, so he had to control them by watching the television monitor.

Each subject performed three tasks under four different visual feedback conditions, namely, with normal vision and with righ-left reversed, inverted, and inverted-reversed feedback. The task was performed in a compensatory way. This means that the subject had to reorient the direction of his drawing, writing, and dotting motions so that the patterns made always appeared normally oriented on the television monitor. Each subject began with the normally oriented feedback and was then given the other three conditions in random fashion. The subjects made rows of dots, wrote *a*'s, and drew right triangles with the right angle in the lower left-hand corner (*2*). Five characters were made in a row and four rows were written or drawn under all four visual-feedback conditions. The electronic motion analyzer timed the contact and travel movements separately in each trial to 1/100 of a second.

The subjects were 36 parochial-school boys ranging in age from 9 years 6 months to 13 years 6 months. All were of normal intelligence and behavior for their grade level.

Of the 36 boys who started the experiment, only 12 were able to complete the tasks under all four conditions of visual displacement. In keeping with our assumptions, there was a definite age factor in the ability to respond to the different conditions of displacement. That is, there appeared to be a fairly definite break at about age 12 between those who could do the tasks and those who could not.

Of the 15 boys aged 9, 10, and 11, there was only one who could perform under all four conditions of feedback. Of the 21 boys aged 12 and 13 years, there were 11 who could perform under all four conditions successfully and ten who could not. The difference between these two age groups was significant at the 5 percent level, using a corrected chi-square test for small frequencies.

The age-break in performance just described appears even clearer when the following results are considered. Of the ten boys aged 12 to 13 years who failed, five of them were able to do all but the inverted condition. This contrasts with only four of the 14 younger failures who were able to do all but the inverted condition. Of these five failures aged