THE HETEROGENEITY OF THE ANTIBODY SITE TO A GIVEN ANTIGENIC DETERMINANT *

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The heterogeneity of the reactive sites of antibodies induced by a given antigen is presumably dictated by the differences inherent in the specific determinants on that antigen (1, 2). Determinants may differ to varying degrees: they may differ completely in chemical composition; they may possess the same haptenic group yet differ in the type of linkage to adjoining structures of the molecule; or they may have identical chemical structure as well as the same type of linkage but differ in the immediate chemical environment and consequently in spatial orientation and geometry. It is expected that differences in antigenic determinants, chemical or physical of any magnitude, would be reflected in sufficient differences in the antibody site to ensure some measure of specificity. The question arises as to whether even one single physicochemically defined antigenic determinant is also capable of inducing antibodies with heterogeneously reactive sites.

It has long been believed that one given determinant induces one common reactive site. This arises from the tacit assumption that the combining site of the antigenic determinant and corresponding site on the antibody are strictly complementary. However, it has since been

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suggested that the active sites of a population of antibody molecules induced by a given determinant, may indeed be heterogeneous and possess various conformations with different degrees of complementarity to the corresponding determinant on the intact antigen (3, 4, 5). On the basis of this theory, the various species of antibodies would be expected to show correspondingly differing reactivities with the inducing antigen; and those which are not completely complementary (i.e., subcomplementary) would be expected to be most effective in forming a precipitating complex.

The experiments reported herein so indicate. More specifically, it will be shown that immunization with p-azobenzenearsonate conjugates of insulin, gelatin and casein, produces a variety of antibodies to the haptenic group. Only some are precipitable by the immunizing conjugated antigen. Those remaining in the supernatant can be separated by exhaustive precipitation performed successively with one hapten carrier protein conjugate followed by another.

EXPERIMENTAL

p-azobenzenearsonate was coupled to crystalline porcine insulin* (Eli Lilly), calf skin gelatin (Eastman), casein (Matheson), bovine serum albumin (Armour) and horse γ-globulin (Pentex) using established procedures (6). Five groups of 10 rabbits each, were immunized concurrently with the different conjugated antigens under otherwise identical conditions. Each group was injected subcutaneously

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with 10 mg of one conjugated antigen in complete adjuvant (Difco) once weekly for four weeks and serum secured two and four weeks after the last injection. Subsequently, one recall injection of 10 mg was given subcutaneously without adjuvant, at monthly intervals for three months. Serum was also secured two weeks after each injection. All these sera proved to be quite comparable in the type of reactions described below. Sera were absorbed first with the nonconjugated immunizing antigen (not shown in graph), followed by the conjugated immunizing antigen, and in turn by the non-immunizing hapten carrier proteins. Exhaustive absorptions were performed by the repeated additions of subequivalence quantities of the protein conjugate, to a mixture of 1 ml of antiserum and 1 ml of saline. In this manner, precipitable antibodies were removed by the minimum amount of the absorbing protein. This was particularly important when absorptions were done with the immunizing antigen since any large excess of antigen remaining in the supernatant caused an inhibition of subsequent precipitin formation with hapten carrier proteins. All reaction mixtures were incubated for 1 hr at 22° C followed by 23 hours at 0° C. For protein measurements (7), the precipitate was separated by centrifugation followed by three washings with 3 ml of saline. Centrifugation was performed at 3000 g for 20 minutes at 4° C. All manipulations and additions were applied equally to the experimental immune and pre-immune serum samples. The latter served as reference controls. The data shown in Fig. 1 are representative of most of the antisera obtained; a full account will appear elsewhere.

It was found that exhaustive absorption of antisera to globulin

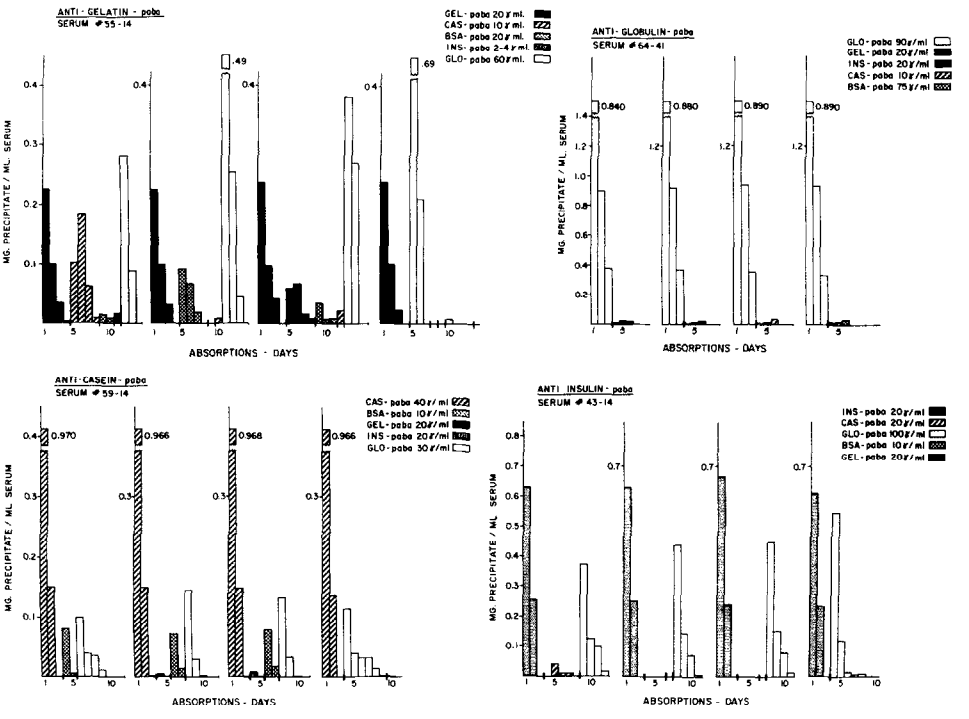

Fig. 1. Is a composite of four graphs showing successive daily absorptions of anti-p-azobenzenearsonate (paba) conjugates of insulin gelatin, casein and $\overline{\gamma}$-globulin. The first absorption was done with the immunizing protein conjugate followed by a variable order of absorptions with hapten carrier protein. Each graph represents four such variations according to the key shown for each antiserum. Where no values are recorded, no precipitate was obtained. Details in text.

(Fig. 1) and albumin conjugates (not shown) with the immunizing antigen removed practically all measurable antibodies. This was not the case with antisera to conjugates of casein, insulin and gelatin. In these instances, after absorption with the immunizing antigen, a considerable amount of antibody was left behind in the supernatant. In the case of the anti-insulin conjugate, the remaining antibody reacted almost completely with only one of the other hapten carrier protein conjugates. As to anti-casein and anti-gelatin conjugates, there were two and three other types of antibody, respectively, that remained in the supernatant, unreactive with the immunizing antigen, but precipitated out partly

by one hapten carrier protein and partly by another.

DISCUSSION

The data presented herein indicate that rabbit antisera to p-azobenzenearsonate conjugates of casein, porcine insulin or bovine gelatin, contain a variety of anti-azobenzenearsonate antibodies of which only a part is absorbed out by the immunizing antigen. The remaining antibodies readily react and precipitate with the same hapten on a different carrier protein. By contrast, almost all antibodies induced by the more highly antigenic compounds, bovine serum albumin and horse γ-globulin, are precipitable by the immunizing antigen. These results cannot be easily interpreted in terms of the prevailing classical notions. However, they do indeed lend themselves readily to simple interpretation within the framework of the new theory of subcomplementarity between the reactive sites of antigen and antibody (3, 4, 5). In fact, these results were predicted on that basis and prompted this study.

Clearly, the lymphoid system cannot be endowed with a unique anticipatory mechanism as to foresee the physicochemical structures immediately vicinal to the haptenic group on the non-immunizing carrier protein molecules. It cannot then be committed in advance to pattern a complementary antibody site to an unforeseen determinant with which it had no prior encounter. It is therefore not possible to portray the antibody site and the corresponding haptenic site on the carrier protein molecule as complementary. The alternative is that the pattern is less than complementary, i.e., subcomplementary, since a certain measure of complementarity is necessary for the

recognition of the haptenic group. A subcomplementary fit then does indeed result in the full expression of the precipitin reaction. By analogy therefore, the precipitin reaction occurring with the immunizing protein conjugate can also be regarded as the result of an interaction of two molecules possessing a subcomplementary fit. Consequently, the antibodies remaining after absorption with the immunizing conjugate, would then be considered either as complementary or noncomplementary. The latter can be dismissed from consideration since noncomplementary antibodies would not recognize the haptenic group. Therefore, antibodies not precipitable by the immunizing antigen can best be defined as complementary. It is clear from Fig. 1 that within the group defined as complementary antibodies, a certain measure of heterogeneity exists since none of the hapten carrier proteins (other than globulin) was capable of absorbing out all antibodies not precipitable by the immunizing antigen. This may be related to the limited variety of determinants available on any one of these azo-proteins.

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