ON THE ASSOCIATED STATE OF RABBIT ALLOTYPES, THE EXISTENCE OF RABBIT ANTIBODY MOLECULES AGAINST TWO ALLOTYPES, AND THE DISSOCIATION OF HUMAN γ-GLOBULIN ANTIGENS INTO SMALLER MOLECULES.

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In previous papers (Oudin 1960 B and C) six main allotypes (i.e. proteins formerly considered uniform within an animal species, but distinguishable by immunochemical reactions) of γ-globulin were described in rabbit sera. At least five, and probably all six, possessed the same isotypic specificity (i.e. the specificity which is uniform within the species). These allotypes comprise two groups of three, b c d and a f g, and each group appears to be controlled by three allelic genes. Each time it was possible to react a serum containing two allotypes with their respective antiallotype rabbit sera, evidence was obtained indicating the presence of protein molecules bearing each allotypic specificity without the other.

An exception to the superimposition involved in a procedure of identification previously described (Oudin 1955) and extensively applied to the immunochemical analysis of human serum led us to assume that, in a b g serum, the b and g allotypes were partly associated in a mixed molecule. Generalizing this assumption in the simplest case, two allotypic molecules from different groups would be present in a mixed molecule. This hypothesis would predict the association of the allotypes of different groups 2 by 2 in 9 forms, and it would exclude the 6 forms of association of 2 allotypes belonging to the same group. As expec-

ted from the frequencies of the allotypes, only a few of the immune and nonimmune sera required to check this hypothesis in the above 15 cases of association and nonassociation were available. However, the following data were consistent with it. Three mixtures of one or two immune sera with agar were used as the lower layer in simple diffusion tubes (Fig. 1). The first contained anti-b antibody ; the second, anti-b and anti-f ; and the third anti-f. The concentrations of anti-b and anti-f antibodies in tube II were the same as in tubes I and III, respectively. Precautions were taken to avoid differences in penetration due to nonspecific effects. When the upper layer consisted of a mixture of a b+ fo serum and a bo f+ serum, the penetration of the b zone was the same in both tubes I and II, and the penetration of the f zone was the same in tubes II and III. In striking contrast, when the upper layer contained a serum of phenotype b f, the penetration of the f zone was much less in tube II than in tube III, and the penetration of the b zone slightly less in tube II than in tube I, the b and f zones being still distinct from each other in tube II. Similar, though smaller, differences in penetration of the f zones were found when the upper layer contained sera a b f, b d f, or a b d f. On the other hand, no similar evidence for the association of allelic allotypes b and d was obtained in analogous reactions with sera a b d, b d f, b d g, or a b d f.

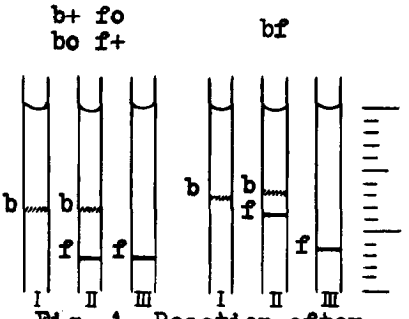

Fig. 1. Reaction after seven days.

The existence of molecules which carry two allotypic specificities makes it easier to imagine that an antibody molecule

against two different allotypes may be formed. Such antibody molecules seem to provide the most satisfactory explanation of the behavior of an immune serum prepared against the allotypes of a rabbit of phenotype a b c. This antiserum gave no visible precipitation zone, in simple diffusion, with ao bo c+ sera. After moderate absorption by an a+ bo co serum, there was no visible zone when the supernatant was reacted with a+ bo co sera. However this supernatant gave a zone with a+ bo c+ sera, but not with a mixture of an a+ bo co and an ao bo c+ sera.

These results may be explained by the presence, in the immune serum, of a substantial amount of antibody molecules both anti a and anti c and by the hypothesis 1/ that such molecules are functionally univalent, and therefore nonprecipitating, when they react with a without c or with c without a ; 2/ that for the same reason, a substantial part of them remained after absorption, provided that no excess of a+ bo co serum was added ; 3/ that they are functionally bivalent when reacted with mixed ac molecules ; with a mixture of a and c separate molecules, the complex formed is of too small a size to give a precipitation zone. Analogous observations are accounted for by the existence of antibody molecules both anti a and anti b.

It was previously mentioned that certain protein antigens of human serum behaved either as distinct antigens or a single antigen depending on the materiel being analyzed (Oudin 1960 C). Antigens G_1 and G_2 (Oudin 1960 A), although quite distinct in a number of antigen solutions, behaved as a single γ-globulin antigen when other material was examined. This dual behavior is visible in reactions of the type described above (using of course antisera against the isotypic specificities of γ-globulins) or in neighboring reactions in cells with parallel walls (Oudin 1955,

Bussard 1958). The degree of dissociation of γ-globulin in human serum varies with the duration and conditions of storage. When naturally occurring mixtures of dissociated and nondissociated γ-globulins are reacted with suitable immune sera, complex precipitating systems (Oudin 1948 and 1952) are realized, which give rise either to several precipitation zones or to only one zone according to the relative concentrations of the various reagents.

Other experiments are being made to shed more light on the interrelationships of the mixed allotypic specificities, the mixed antibody activities, the associated state of allotypes, and how the human γ-globulin dissociates, to the general problem of antibody formation.

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