

IDIOTYPY OF RABBIT ANTIBODIES

I. COMPARISON OF IDIOTYPY OF ANTIBODIES AGAINST *SALMONELLA* TYPHI WITH THAT OF ANTIBODIES AGAINST OTHER BACTERIA IN THE SAME RABBITS, OR OF ANTIBODIES AGAINST *SALMONELLA* TYPHI IN VARIOUS RABBITS*

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Idiotypy shares with allotypy the characteristic that antigenic specificities proper to it in one category of proteins, as one given class of immunoglobulins, are not carried by the protein molecules of this category in all the individuals of the same animal species. But, in contrast to allotypy which has been observed in several other kinds of proteins after its discovery among immunoglobulins, idiotypy is by definition restricted to antibodies.

One of the most two important features of idiotypy—also the most striking difference between idiotypy and immunoglobulin allotypy—is that an idiotypic specificity found in antibodies against one given antigen has been found neither in the serum of the nonimmunized animal, nor in antibodies against another, noncrossreacting antigen. It was this striking difference that first made the authors conscious that what they observed in rabbits was definitely different from conventional allotypy (1).

A second important feature of idiotypy, which contrasts also with conventional allotypy, is that each idiotypic specificity, carried by antibodies of one given rabbit against *Salmonella typhi*, has not been found yet (at least not exactly under the same form) in antisera of other rabbits against the same antigenic material.¹

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¹ The reluctance of the authors to propose a new term for the new phenomenon before being quite sure that this was necessary made them present their first results as a peculiar kind of allotypy in which each allotypic specificity would be restricted (*a*) to a single antibody, and (*b*) to a single individual. Actually, the newly observed phenomenon was fundamentally different from allotypy, and the use of the same term for both would have been extremely confusing in the necessarily elaborate discussions which involve the three different kinds of antigenic specificities carried by the same immunoglobulin molecule (i.e. isotypic, allotypic, and idiotypic specificities). Consequently, it was found necessary to coin a new word of the same family as those proposed in 1956 (2) and in 1960 (3, 4, 5). In the same way as in "allotypic" or "isotypic," where "typic" (from τυπος, print or type) stands for antigenic specificity, "allo" (from ἄλλος, other) stands for different, and "iso" (from ἴσος, the same) stands for similar, "idiotypic" was proposed (6, 7). The first part of the word (idio, from ἴδιος, peculiar) is justified by the extreme peculiarity of the antigenic specificities in question. An idiotype is a peculiar kind of protein antigen defined by its idiotypic specificity. In this paper, we will

These two features which characterize idiotype have been described in the rabbit at about the same time as the observation made in man by Kunkel, Mannik, and Williams, who used suitably absorbed rabbit immune sera prepared against purified human antibodies. These antibodies possess "individual antigenic specificities" proper to antibodies against one given antigen in one individual (8).²

The reason why no observation of idiotype in the rabbit had been made during many years of experiments on allotypy is the apparent dependence of these observations on the procedure of immunization used. The immunizing material that had constantly been used in this laboratory over many years for the preparation of anti-allotypic immune sera was antigen-antibody precipitate with complete Freund's adjuvant. The immunizing antigen which elicited the formation of anti-allotypic antibodies was the antibody of the precipitated complex. Our first observations of rabbit idiotype were the result of the use we made of a somewhat different material which had been previously used by others in different fields (e.g., 11, 12), namely an agglutinate of whole bacteria and antibodies against them. Observations similar to ours (1) were also reported by Gell and Kelus (13). The main difference between the procedures used and the results obtained by these authors and by us is that, instead of the agglutinated *Salmonella* we had used with an appreciable proportion of failures in anti-idiotypic immunizations, they used the same procedure of immunizations by agglutinated *Proteus* as in former papers of one of them on allotypy (e.g., 12), and that this material was 100% successful in the production of anti-idiotypic antibodies (14).

In the present paper, we will have to consider on an experimental basis a number of aspects of the problems raised by idiotype (15), some of which have already been treated (1). We will start with (a) the antibody nature of the antigens which are precipitated by the anti-idiotypic immune sera. Then we will compare the idiotypic specificities of antibodies in the cases (b) where these antibodies are those of one individual against different antigens; (c) where these antibodies are those of different individuals against the same antigenic material. Other questions pertaining to the comparison of idiotypic specificities of antibodies obtained in the same individuals against *S. typhi* will be considered in the next paper (16).

designate as anti-idiotypic immune sera or antibodies those which recognize the idiotypic specificities. The terms "immunizing antibody" or "immunizing serum" or even "immunizing rabbit" will designate the antibodies used as the immunizing material in a given anti-idiotypic immunization, or the immune serum which contained these antibodies, or the rabbit from which this immune serum was taken.

² The term "individual antigenic specificity" used by these authors is the same as that used to designate those antigenic specificities each of which is peculiar to a given myeloma protein (9, 10). The idiotypic specificities in the present study in the rabbit, and the individual antigenic specificities observed in human antibodies by Kunkel, Mannik, and Williams have apparently the same characteristics in two different species. The comparison of the characteristics of the idiotypic specificities with the individual antigenic specificities of myeloma proteins is less obvious, and will be considered in the Discussion. The use of the same term "individual antigenic specificities" for antibodies and for myeloma proteins seems to indicate that its comprehension is wider than that of the term "idiotypic specificities".

Materials and Methods

Immunizations.—Anti-*Salmonella* immunizations were carried out in rabbits usually randomly chosen in a population of various origins. These rabbits were immunized as stated in reference (1). In addition to *Salmonella typhi* 0-901³ (living bacteria), three other immunizing materials were injected into rabbits which had been already injected with *S. typhi* bacteria, namely: *Salmonella tranoroa*³ (living bacteria), *Salmonella typhimurium*³ (heat-killed bacteria), and *Pneumococcus* type II (formalin-killed bacteria). In the following paragraph, designed to describe the details of the injection and bleeding protocols, information will be given in the following order for each event (injection or bleeding): the number of days elapsed since the first injection of *Salmonella* bacteria (*S. typhi* or *S. tranoroa*), the day of this first injection being counted as 0; the dose of bacteria, expressed, for *Salmonella*, as the approximate number of bacterial cells per injection, in billions (e.g., 10^9 , or $2 \times 10^9 \dots$) and, for *Pneumococcus*, as the approximate number of μg of bacterial nitrogen per injection; the nature of the injected bacteria, when these bacteria were not *S. typhi* (*S. tra*, *S. tranoroa*; *S. TM*, *S. typhimurium*; *Pn*, *Pneumococcus* type II); the route of injection (*iv* for intravenous, *sc* for subcutaneous). The sera of several successive bleedings made at short intervals without intervening injections were often mixed; the symbol S1, S2 \dots is attributed to the serum of a bleeding or mixture of bleedings at the time or times indicated before this symbol.

The immunization was, during the first 5 wk or so, conducted approximately in the following way in all rabbits.

0, 10^9 *iv*; 3, 2×10^9 *iv*; 7, 4×10^9 *iv*; 13, 15, 17, S1; 28, 4×10^9 *iv*; 34, 36, 38, S2 \dots

After bleedings S2, the immunization of rabbits 3-5 and 1-70 was continued in the following way: 129, 4×10^9 *iv*; 136, S3; 463, S4.

484, *Pn* 20 μg *sc*; 485, 486, 487, *Pn* 20 μg *iv*; 491, *Pn* 40 μg *sc*; 492, 493, 494, *Pn* 40 μg *iv*; 497, *Pn* 80 μg *sc*; 498, 499, 500, *Pn* 80 μg *iv*; 505, 507, S5; 511, S6; 518, *Pn* 80 μg *sc*; 519, 520, *Pn* 80 μg *iv*; 526, 528, S7; 532, S8.

After the S8 bleeding of rabbit 1-70, the immunization of this rabbit was continued as follows: 540, 6×10^9 *iv*; 542, S9; 544, S10; 546, S11; 549, S12; 553, S13; 556, S14; 563, S15; 570, S16; 577, S17; 584, S18; 592, S19; 618, S20; 639, S21.

714, *S. tra* 10^9 *iv*; 717, *S. tra* 2×10^9 *iv*; 722, *S. tra* 4×10^9 *iv*; 728, 730, 732, S22; 746, 749, S23; 750, *S. tra* 4×10^9 *iv*; 755, 757, 759, S24.

In two rabbits (3-22 and 3-24), the immunization was started against *S. tranoroa*. Rabbit 3-24 was immunized as follows: O, *S. tra* 10^9 *iv*; 3, *S. tra* 2×10^9 *iv*; 7, *S. tra* 4×10^9 *iv*; 12, 15, 18, S1; 28, *S. tra* 4×10^9 *iv*; 34, 36, 38, S2; 56, S3; 70, S4.

70, 10^9 *iv*; 73, 2×10^9 *iv*; 77, 4×10^9 *iv*; 83, 85, 87, S5; 97, S6; 98, 4×10^9 *iv*; 104, 106, 108, S7; 126, S8; 133, S9; 140, S10; 147, S11; 162, S12; 175, S13; 216, S14; 223, S15; 421, S16.

430, *S. TM* 10^9 *iv*; 434, *S. TM* 2×10^9 *iv*; 438, *S. TM* 4×10^9 *iv*; 444, 447, 449, S17; 456, S18; 463, *S. TM* 4×10^9 *iv*; 468, 470, 472, S19; 483, S20; 497, S21; 504, S22; 511, S23; 525, S24; 532, S25; 546, S26; 553, S27; 560, S28; 581, S29; 595, S30; 668, S31; 735, S32; 778, S33; 826, S34; 946, S35.

958, 10^9 *iv*; 962, S36; 2×10^9 *iv*; 966, 4×10^9 *iv*; 972, 974, S37; 982, 4×10^9 *iv*; 987, 988, S38; 1001, S39; 1011, S40.

Rabbit 3-22 received the same course of immunization as 3-24 until the end of the anti-*S. typhi* immunization (421st day, S16), but no anti-idiotypic serum of sufficient strength was obtained against it.

Anti-idiotypic immunizations were made in rabbits which, in the available population of various origins, were randomly chosen, except for their phenotypes which had to include all

³ The antigenic formulas of the *Salmonellae* which were used in these immunizations are: for *S. typhi* 0-901, 9,12:-:-; for *S. tranoroa*, 55:k:z39; for *S. typhimurium*, 4,12:-:- (17).

the allotypic specificities of groups *a* and *b* of the immunizing rabbit. This precaution was designed, among other reasons, in order to avoid the complication of formation of antibodies against allotypic determinants which might have rendered interpretation of gel diffusion pattern difficult. An error in recording the phenotypes resulted in the formation of anti-Aa1 antibodies in rabbit 2-40. These immunizations were carried out as previously described (1). Certain of the anti-idiotypic immunizations undertaken or continued since 1963 were longer than described in reference (1), the total number of injections of agglutinated bacteria being sometimes as large as 40, over approximately 9 months.

Antigen-Antibody Reactions and Antigen Preparations.—Interfacial reactions were made in narrow tubes (internal diameter 1-2 mm); the serum placed at the upper layer was diluted 1:1 in normal saline.

The techniques of immunochemical analysis (double diffusion in cells or in tubes) were used as described in reference (4). More or less precise details could sometimes be seen on photographs according to the smaller or larger angle of incidence of light (18).

The somatic antigen of *S. typhi* and that of *S. tranoroa*, and the corresponding polysaccharides obtained by acetic hydrolysis of the former, were prepared according to Boivin et al. (19).

Equivalence proportions between anti-*Salmonella* sera (either immunizing or anti-idiotypic sera) and *Salmonella* antigens were routinely determined.

Determinations of nitrogen in the washed antigen-antibody precipitates were made according to the technique of Folin-Ciocalteu as described in reference (20).

RESULTS

The immune sera, either immunizing or anti-idiotypic, used in the experiments related to one or other of the questions below and in those described in the next paper (16) are listed in Table I.

1. *The Antibody Nature of the Antigens which are Precipitated by the Anti-Idiotypic Sera*

The first of the features which were found peculiar to rabbit idiotypy and lacking in allotypy was that the anti-idiotypic immune sera which precipitated the immune sera against *S. typhi* used for their preparation did not precipitate the sera taken in the same rabbits before their immunization against *S. typhi* (1). As may be seen in Table I, the phenotype of the rabbits chosen for the production of the immunizing and of the anti-idiotypic immune sera were such that the attempts of anti-idiotypic immunization could not lead to the appearance of antibodies against known allotypic patterns of the *a* or *b* groups.⁴ In one case, however, an error in recording the allotypic phenotypes resulted in the immunization of an Aa1⁻ rabbit (2-40) with bacteria agglutinated by the antiserum of an Aa1⁺ rabbit (1-70). This immunization resulted in the appearance of anti-Aa1 antibodies together with anti-idiotypic antibodies,

⁴ At the time of most of the experiments reported in this paper, the Ab9 specificity described by Dubiski and Muller (21) was not yet known. But Ab9 has not been found here in several hundred rabbits tested since Dubiski and Muller's paper with an anti-Ab9 serum kindly supplied by Doctor Dubiski. Ab9 seems therefore to be extremely rare in the rabbit population available to us.

The serum of the immunized rabbit 2-40 precipitated the immunizing anti-*S. tranoroa* serum of rabbit 1-70, but precipitated also the serum taken in rabbit 1-70 before its anti-*S. tranoroa* immunization, and the other Aa1⁺ rabbit sera as well. The latter precipitations were obviously due to anti-allotypic (anti-Aa1) antibodies. The presence of these antibodies, and that of other precipitating antibodies, directed against idiotypic patterns of the anti-*S. tranoroa* antibodies was demonstrated by neighboring reactions shown in Fig. 1A and 1B. In these reactions, the precipitation zones due to anti-allotypic and anti-idiotypic antibodies are clearly distinct, the latter being visible only in the reaction of the anti-idiotypic serum with the homologous immunizing anti-*S. tranoroa* serum. In addition, the absorption of the immune serum 2-40, both anti-allotypic and anti-idiotypic, with the serum of a non-immunized Aa1⁺ rabbit, removed the anti-Aa1 antibodies. The absorbed immune serum no longer precipitated any nonimmune serum but still precipitated the immunizing anti-*S. tranoroa* immune serum, thus confirming that its anti-allotypic and anti-idiotypic properties were quite distinct.

The fact that the anti-idiotypic immune sera, either after a suitable absorption in an exceptional case, or without any previous absorption in all other cases, precipitated the anti-*Salmonella* immune serum used for the anti-idiotypic immunization, but never the serum of the same rabbit taken before its anti-*Salmonella* immunization, strongly suggested that the antigens which these anti-idiotypic sera precipitated were the anti-*Salmonella* antibodies.

This suggestion was confirmed by the result of absorption of the immunizing anti-*S. typhi* sera with the Boivin antigen (or somatic O antigen) of *S. typhi*.

In such absorptions carried out by mixing suitable amounts of immune serum and of antigen, it is nearly always impossible to remove all the precipitating antibodies without adding some excess antigen and, on the other hand, all the anti-idiotypic immune sera of Table I are also anti-*Salmonella*. For these reasons, it was necessary to absorb also the anti-idiotypic sera with the somatic antigen of the *Salmonella* concerned (in most cases, *S. typhi*). Otherwise the anti-*Salmonella* antibodies of the anti-idiotypic sera would have precipitated the bacterial antigen present in the absorbed immunizing antiserum. Once this precaution was taken, the precipitation of the immunizing serum with the anti-idiotypic serum disappeared (Table I).⁵

It might be added that when the rabbit which supplied the immunizing anti-*S. typhi* serum was allowed to rest for a sufficiently long time without injec-

⁵ In 1963 (1) we stated that the precipitation of the immunizing anti-*Salmonella typhi* serum with the anti-agglutinate (anti-idiotypic) sera was considerably diminished or nearly suppressed when the former serum was absorbed by the somatic antigen of *Salmonella enteritidis* whose specificity is very similar to that of *S. typhi* 0-901. In the experiments performed since then, the somatic antigen of *S. typhi* 0-901 itself, instead of that of *S. enteritidis*, was used for such absorptions, which resulted in the complete suppression of the precipitation of the immunizing sera with the anti-idiotypic sera.

TABLE I
Anti-Idiotypic Immunizations

| 1 | 2 | 3 | 4 | | 7 | 8 | 9 | 10 | 11 | | 13 | 14 |
|--------------------|--|---|--|---------------------------------------|---|---|---|---|--|------------------------------------|---|-----------------|
| | | | No. | By poly saccharide | | | | | Reaction with immunizing antibacterial serum | Intensity of inter-facial reaction | | |
| Rabbit no. and sex | Phenotype; bleedings used for anti-idiotypic immunizations | Immunizing material | Nitrogen precipitated in serum, bleeding | | Rabbits injected with bacteria sensitized by antiserum of rabbit in column 1; No. and sex | Phenotype | Bleeding of rabbit in column 7 used | Reaction with serum of rabbit in column 1 taken before antibacterial immunization | Reaction with immunizing antibacterial serum | Amount of N precipitated | Reaction with immunizing antibacterial serum previously absorbed with homologous <i>Salmonella</i> antigens | |
| | | | $\mu\text{g./ml.}$ | By somatic antigen $\mu\text{g./ml.}$ | | | | | | $\mu\text{g./ml.}$ | Polysaccharide | Somatic antigen |
| 3-5 ♂ | Aa(1+2-3+)b(4-5+6-) S2 | <i>S. typhi</i> | 1 | 146 | 1-8 ♂ 1-71 ♀ 1-75 ♀ 2-25 ♂ | Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) | 5 1 5 5 | 0 0 0 0 | 0 0 ++ 0 | 62 | + | 0 |
| 1-70 ♀ | Aa(1+2-3-)b(4-5+6-) S1, S2 | <i>Pneumococcus type II</i> <i>S. typhi</i> <i>S. typhi</i> | | | 9-2 ♀ 1-68 ♀ 1-69 ♀ 2-32 ♀ | Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) | 5 5 5 5 | 0 0 0 0 | 0 L* 0 ++ | | ++ | |
| 2-63 ♀ | S24 Aa(1-2-3+)b(4+5-6-) S2 | <i>Pneumococcus type II</i> <i>S. typhi</i> <i>S. tranoroa</i> <i>S. typhi</i> | 1 | 83 | 2-40 ♀ 2-41 ♀ 3-48 ♂ 3-49 ♂ 2-68 ♀ 2-88 ♀ 2-89 ♂ 2-97 ♀ 3-00 ♂ | Aa(1-2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6-) Aa(1+2-3+)b(4-5+6+) Aa(1+2-3+)b(4-5+6+) Aa(1-2+3+)b(4+5-6-) Aa(1-2+3+)b(4+5-6-) Aa(1-2+3+)b(4+5-6-) Aa(1-2+3+)b(4+5-6-) Aa(1-2+3+)b(4+5-6-) | 6 3 6 3 4 6 2 4 3 | ++ 0 ++ 0 ++ ++ ++ ± L | ++ 0 ++ 0 ++ ++ ++ ± L | | + | 0 0 0 |
| 2-65 ♂ | Aa(1+2-3+)b(4+5-6-) S2 | <i>S. typhi</i> | | | 2-75 ♂ 2-86 ♀ 2-87 ♀ 2-95 ♀ | Aa(1+2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) | 4 2 5 5 | 0 0 0 0 | ++ ± ++ ± | | 0 | 0 0 0 |

TABLE I (Continued)

| | | | | | | | | | | |
|----------|---------------------------|--|--|--|--|--|-------------------------------|-------------------------------|------|---|
| 3-22 ♂ | Aa(1-2-3+)b(4+5-6-) S7 | <i>S. typhi</i> <i>S. typhi</i> | 2-98 3-37 ♂ 3-43 ♂ 3-54 ♀ 3-71 ♀ 3-75 ♀ | Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) | 2 3 3 4 4 7 | 0 0 0 0 0 0 | + | + | 0 | 0 |
| 3-24 ♂ | Aa(1-2-3+)b(4+5-6-) S7 | <i>S. typhi</i> | 3-52 ♀ 3-53 ♀ 3-65 ♀ 3-70 ♂ 3-76 ♀ | Aa(1+2-3+)b(4+5-6+) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) | 5 3 5 3 7 | 0 0 0 0 0 | L 0 0 0 ++ | L 0 0 0 ++ | 184† | 0 |
| 6-73 § ♂ | Aa(1-2-3+)b(4+5+6-) S2 | <i>S. typhimurium</i> <i>S. typhi</i> | 5-47 ♀ 6-40 ♀ 6-43 ♂ 6-52 ♂ 6-53 ♂ 6-58 ♀ | Aa(1+2-3+)b(4+5+6-) Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6+) Aa(1-2-3+)b(4+5-6-) | 2 2 2 7 4 2 | 0 0 0 0 0 0 | ++ + 0 ++ ++ 0 | ++ + 0 ++ ++ 0 | 0 | 0 |
| 7-99 § ♀ | Aa(1-2-3+)b(4+5-6-) S2 | <i>S. typhi</i> | 6-82 ♀ 6-87 ♂ | Aa(1-2+3+)b(4+5+6-) Aa(1-2-3+)b(4+5+6-) | 2 7 | 0 0 | 0 ++ | 0 ++ | + | 0 |
| 8-03 § ♂ | Aa(1-2-3+)b(4+5-6-) S2 | <i>S. typhi</i> | 8-50 ♂ 8-52 ♂ 8-53 ♀ 8-54 ♀ 8-60 ♀ 8-61 ♀ | Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) | 1 4 2 6 1 1 | 0 0 0 0 0 0 | 0 L 0 ++ 0 0 | 0 L 0 ++ 0 0 | + | 0 |
| 8-04 ♂ | Aa(1+2-3+)b(4+5+6-) S2 | <i>S. typhi</i> | 8-55 ♀ 8-62 ♀ 8-69 ♂ 8-71 ♂ 8-72 ♀ 8-73 ♀ 8-88 ♀ 9-13 ♀ 9-14 ♂ 9-15 ♀ | Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5-6-) Aa(1-2-3+)b(4+5-6-) Aa(1+2-3+)b(4+5+6-) Aa(1+2-3+)b(4+5+6-) Aa(1-2-3+)b(4+5+6-) Aa(1-2-3+)b(4+5+6-) | 6 6 1 3 3 4 5 5 5 2 | 0 0 0 0 0 0 0 0 0 0 | ++ ++ 0 = | ++ ++ 0 = | + | 0 |

* Interfacial reactions noted as L were extremely faint.

† The anti *S. typhi* serum of rabbit 3-24 used in this reaction was that of S37 absorbed by the polysaccharide.

§ Rabbits 6-73, 7-99 and 8-03 were born in the animal room of the laboratory from known parents (see Results, 3).

tions of *S. typhi*, its serum became no longer precipitable by the homologous anti-idiotypic sera. This observation had been previously reported (1). Other similar observations will be reported below. But, in these cases, it was noticed that ability of the serum to faintly precipitate the bacterial antigens survived

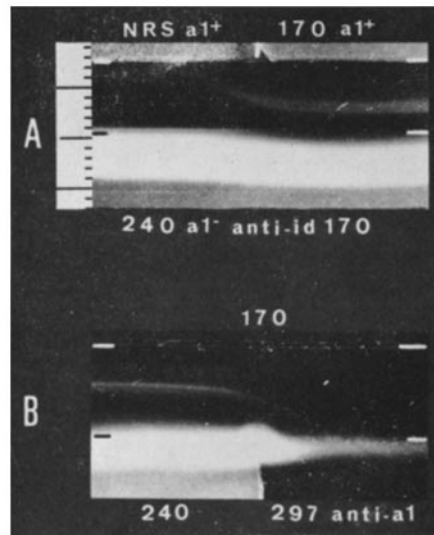


FIG. 1. Reactions in agar (double diffusion in cells). A. Reaction of the anti-idiotypic serum of an Aa1⁻ rabbit (2-40), injected with *S. tranoroa* bacteria agglutinated by the serum of rabbit 1-70 (Aa1⁺), with the anti-*S. tranoroa* serum of this rabbit and with the serum of another, nonimmunized (or "normal", NRS), Aa1⁺ rabbit. A precipitation zone is visible in front of the anti-*S. tranoroa* serum of rabbit 1-70 and is attributable to the reaction of anti-idiotypic antibodies. A much wider and denser zone is visible in front of both antigen layers and is attributable to the reaction of anti-Aa1 antibodies, which is confirmed by the reaction in B. B. Reaction of the anti-idiotypic serum of rabbit 2-40 and of an anti-Aa1 serum (that of rabbit I-2-97 which was different from rabbit B-2-97 mentioned in Table I) with the anti-*S. tranoroa* serum of rabbit 1-70, showing the unique and continuous Aa1 precipitation zone in front of the two antisera. On these two photographs, the precipitation zone due to anti-allotypic and to anti-idiotypic antibodies are clearly distinct and easily identified. The white (or black) dashes indicate the levels of the interfaces between the various gel layers. A millimetric scale (left of the A photograph) has been photographed at the same enlargement as the cells.

the ability of the same serum to be precipitated by the anti-idiotypic sera. This would suggest that, even if all the antigens in the immunizing antiserum seem to be anti-*S. typhi* antibodies, all the anti-*S. typhi* antibodies of the same rabbit, which may be present in a serum sample other than the immunizing one, do not necessarily act as antigens in the precipitation reaction with the available anti-idiotypic sera. Some aspects of this particular problem will be considered in the next paper (16). In addition, even in the anti-*S. typhi*

serum sample used for the anti-idiotypic immunization, a part of the anti-*S. typhi* antibodies may not be precipitated by the anti-idiotypic sera. This is shown by certain features of the reaction of these sera with the immunizing serum absorbed by the somatic antigen. It is not always necessary that this absorption be complete for the reaction with the anti-idiotypic sera to disappear. This reaction has sometimes disappeared while some amount of antitoxin able to precipitate the somatic antigen remains in the supernatant liquid of the absorption.

From these experiments, it may therefore be concluded that the antigens (or idiotypes) of anti-*S. typhi* sera which are precipitated by the anti-idiotypic sera are antibodies precipitable by the somatic antigen of *S. typhi*. But a part of the antibodies precipitable by the somatic antigen in the immunizing sera may sometimes not be precipitated by the available anti-idiotypic sera.

2. Comparison of the Idiotypic Specificities of the Antibodies of One Given Individual against Different Immunizing Materials

Once it had been shown that the antigenic patterns against which the anti-idiotypic sera are directed are carried by antibodies, it remained to be determined whether or not these idiotypic patterns are more or less similar in antibodies elicited in one given rabbit by immunizations against distinct antigens. It might have been conceivable that some feature would be (a) common to precipitating antibodies appearing in one rabbit after a hyperimmunization, whatever the immunizing antigen, and (b) absent from the immunoglobulins of the nonhyperimmunized animals. A first experiment designed to test this possibility has been previously reported (1). Two rabbits (3-5 and 1-70) were hyperimmunized against *S. typhi*, and their hyperimmune sera were used respectively for the agglutination of bacteria to be injected into two series of rabbits. Each of these anti-agglutinate immunizations produced respectively one fairly strong precipitating anti-idiotypic serum against the respective two immunizing anti-*S. typhi* sera (1-75 against 3-5, and 2-32 against 1-70). The two rabbits whose anti-*S. typhi* sera had been used for the preparation of the anti-idiotypic immunizing material were allowed to rest 11 months. At the end of this period, their sera were no longer precipitated by the anti-idiotypic sera (Fig. 2A). Then these rabbits were immunized against *Pneumococcus* type II, so that their sera, collected after this immunization, precipitated the pneumococcal polysaccharide type II. There was no precipitation of these antipneumococcal sera by the previously prepared anti-idiotypic immune sera.

It might have been imagined that this lack of common detectable idiotypic patterns in antibodies of one given rabbit against *S. typhi* and against *Pneumococcus* could be related to the wide taxonomic distance between the two bacteria, or to the long time elapsed between the two immunizations. Another experiment was therefore undertaken, in which the two successive immuniza-

IDIOTYPY OF RABBIT ANTIBODIES I.

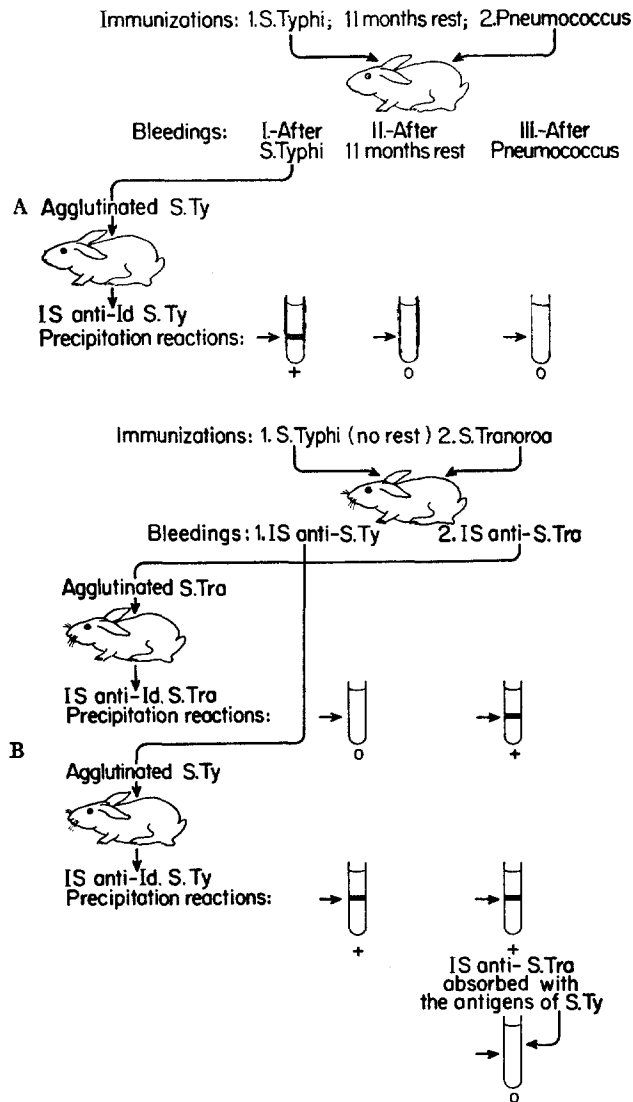


FIG. 2. Diagrams of two experiments designed to compare the idiotypic specificities of antibodies appearing in the same rabbits against the antigens of different bacteria by comparing the reactions of the anti-idiotypic sera with different samples of serum of the immunizing rabbits. IS anti-id *S. Ty* and IS anti-id *S. Tra* stand for immune serum against the idiotypic patterns of anti-*S. typhi* and of anti-*S. tranoroa* antibodies. A. The two successive immunizations (separated by an interval of 11 months) of rabbits 3-5 and 1-70 were against *S. typhi* and against *Pneumococcus* type II. B. The two successive immunizations (following each other without interval) of rabbit 1-70 were against *S. typhi* and against *S. tranoroa*. The anti-*S. tranoroa* serum sample still contained some antibodies against *S. typhi*, which had been absorbed before the last reaction figured on the right.

tions, closely following each other, were directed against two kinds of *Salmonellae*—*S. typhi* and *S. tranoroa*⁶—showing no cross-reactivity when tested by agglutination.

The experiment was carried out in the following way so that no long interval of time between the successive immunizations of the same rabbit was needed (Fig. 2B). Rabbit 1-70 was immunized against *S. typhi*, as described in Materials and Methods, and bled. Its immune serum was used for the agglutination of killed *S. typhi* bacteria, which were then injected into four rabbits in order to obtain anti-idiotypic sera; only one gave an anti-idiotypic immune serum of sufficient strength (anti-id-*S. typhi*). A second course of immunization, this time against *S. tranoroa*, was then administered to rabbit 1-70, previously immunized against *S. typhi*. The sera collected (three bleedings) at the end of this second immunization were used for agglutinating *S. tranoroa* bacteria which were injected into four rabbits in order to obtain anti-idiotypic immune sera; two of them gave sufficiently strong anti-idiotypic immune sera (anti-id-*S. tranoroa*).

The anti-*S. typhi* immune serum obviously did not contain any amount of anti-*S. tranoroa* antibodies, since it had been collected before the anti-*S. tranoroa* immunization. Accordingly, this anti-*S. typhi* serum was precipitated by the anti-id-*S. typhi* serum and not by the anti-id-*S. tranoroa* sera. On the contrary, the anti-*S. tranoroa* serum contained some amount of anti-*S. typhi* antibodies and precipitated the somatic antigen of *S. typhi* because of the preceding immunization of the rabbit against *S. typhi*. Accordingly, the anti-*S. tranoroa* serum was precipitated not only by the anti-id-*S. tranoroa* sera, but also by the anti-id-*S. typhi* serum. But the latter reaction disappeared when the anti-*S. tranoroa* serum was absorbed by the somatic antigen of *S. typhi*. No common idiotypic specificity was therefore detected in the antibodies of the same rabbit against the two *Salmonellae*.⁷

In both the experiments reported, the absence of common idiotypic specificity in antibodies of one given individual against two different bacteria is logically correlated with the absence of appreciable cross-reactivity between the two bacteria successively injected into the same rabbit. This correlation is in agreement with an observation made in rabbit 3-24 which will be considered from another standpoint in the next paper (16). Rabbit 3-24, a long time after a first immunization against *S. typhi*, was injected with *S. typhimurium* (a *Salmonella* which cross-reacts with anti-*S. typhi* sera). Its serum, taken before the latter immunization, was no longer precipitated by the anti-idiotypic serum against its anti-*S. typhi* antibodies. The serum samples taken after the immunization against *S. typhimurium* were precipitated by the same

⁶ We want to thank Doctor L. Le Minor, chef du Service des Enterobactériacées de l'Institut Pasteur, to whom is due the choice and supply of a *Salmonella* fulfilling these requirements: *S. tranoroa* (22).

⁷ Another rabbit was first immunized against *S. tranoroa* and then against *S. typhi* (rabbit 3-24). Among the best anti-idiotypic sera of the rabbits which had been injected with *S. typhi* bacteria agglutinated by the serum of one or the other bleeding of rabbit 3-24 (3-76, 6-52, 6-53), not one precipitated the anti-*S. tranoroa* serum previously prepared in rabbit 3-24.

anti-idiotypic serum, but much less strongly than the anti-*S. typhi* samples of serum of the same rabbit 3-24. It seems therefore that when two samples of serum of the same rabbit cross-react with two different antigenic materials, there may be something common between the idiotypic patterns carried by the antibodies against these two antigenic materials.

From these experiments, it seems reasonable to conclude, in spite of the small number of rabbits involved, that the idiotypic specificities carried by antibodies against one given bacterium in one given rabbit are not merely related to hyperimmunization, but that these idiotypic specificities are distinct in antibodies of one rabbit against distinct noncross-reacting bacteria.

3. Comparison of the Idiotypic Specificities of the Antibodies of Various Rabbits against the same Antigenic Material

The Reaction of Anti-Idiotypic Sera with the Anti-S. typhi Sera of the Immunizing Rabbits and of other Randomly Chosen Rabbits.—In our first paper on these problems, we mentioned that each of the anti-idiotypic sera we had prepared possessed precipitating properties only toward the anti-*S. typhi* serum used in its preparation, and that none of them precipitated any of the 17 anti-*S. typhi* sera of other rabbits with which the reaction had been attempted (1). A confirmation of the limitation of the precipitating power of the anti-idiotypic sera to the anti-*S. typhi* serum of the immunizing rabbit has been looked for in the experiments which have been undertaken since then. In these experiments, 21 anti-idiotypic sera have been reacted with 27 anti-*S. typhi* sera. It has already been mentioned that the anti-idiotypic sera used in these experiments are also anti-*S. typhi* sera, although they are usually less strongly precipitating toward the somatic antigen and toward the polysaccharide of *S. typhi* than the anti-*S. typhi* sera obtained according to the route of immunization used for preparing the immunizing antisera. Antisera of these rabbits have also been used as anti-*S. typhi* sera in tentative reactions with anti-idiotypic sera; however, the antisera used for this purpose were usually not those of the same bleedings which gave the anti-idiotypic sera, but rather those of bleedings which had been made earlier in the immunization course, because these earlier bleedings were more strongly anti-*S. typhi*.

All these reactions were carried out in narrow glass tubes, at the interface between the two liquid layers of a pure anti-idiotypic serum and of a diluted anti-*S. typhi* serum. In the interfacial reactions previously reported (1), the anti-*S. typhi* sera were used at the same dilution as in the search for the allotypic specificities of the *a* and *b* groups, that is 1:10 in normal saline. Since then, a higher concentration (1:4, and later 1:2) of the anti-*S. typhi* sera placed at the upper layer of the tubes was used, these concentrations being still sufficiently low to supply a difference of density with the lower layer preventing the two from mixing together. Under these conditions, the definitely positive interfacial reactions of each anti-idiotypic sera with the respective immunizing anti-*S. typhi* serum were not the only precipitation reactions which were observed, but the others were extremely faint. Most, but not all of these reactions

of an anti-idiotypic serum with the anti-*S. typhi* serum of a rabbit other than the immunizing one occurred also when one of the two sera was replaced by a sample of serum taken in the same rabbit before its immunization, so that they have definitely no relevance to idiotypy. As regards those reactions which were observed only between two immune sera, it is very difficult to appreciate how significant they are, and what is their meaning, when their intensity, so faint that they are hardly visible, bears no comparison with that of the precipitation reactions which unambiguously pertain to idiotypy.

The Reaction of Anti-Idiotypic Sera with the Anti-S. typhi Sera of the Parents of the Immunizing Rabbits.—If the extremely faint reactions just reported are not taken into account, the results seem to show that an anti-idiotypic pattern of antibodies against *S. typhi* apparently does not exist in two different individuals without any family relationship, unless this event is very exceptional. This led to the assumption that the idiotypic patterns of the anti-*S. typhi* antibodies of one individual would not be found in the anti-*S. typhi* antibodies of its parents. Three matings were carried out, all six parents being homozygotes for Aa3, and four parents homozygotes for Ab4, the father and mother of rabbit 6-73 being respectively A_b^5/A_b^5 and A_b^5/A_b^5 . The parents and one individual of each litter were immunized against *S. typhi*, and a series of rabbits were injected in the usual way with *S. typhi* bacteria agglutinated with the immune serum of the latter cub. The immunizing rabbits of the three families were 6-73, 7-99 and 8-03. In all three cases, one or several anti-idiotypic sera were prepared which precipitated the immunizing anti-*S. typhi* serum. In none of the three cases did any of these anti-idiotypic sera precipitate the anti-*S. typhi* sera of the parents of the rabbit which gave the immunizing antiserum.

We can conclude from these experiments (a) that, even if the very faint reactions mentioned above were to be proven significant, the idiotypic specificities of the antibodies against *S. typhi* are widely different in different individuals, and (b) that the idiotypic specificities of these antibodies found in one given individual appear not to be inherited from its parents.

DISCUSSION

Similarities and Differences between Idiotypy and Other Phenomena

The observations from which the notion of idiotypy in the rabbit originated (1) left no doubt that the antigens which were endowed with idiotypic specificities were immunoglobulins. Caution made us examine briefly the possibility that the antigenic material which was precipitated by the immune sera which we have since then termed "anti-idiotypic" might be some bacterial product that would have persisted in the blood stream of the rabbits. The effect of the absorption of rabbit antibodies by bacterial antigens provided strong evidence against this view. Other objections against this explanation were also mentioned, e.g., the small total weight of the bacteria injected, and also the fact that each anti-idiotypic serum precipitated only the homologous immunizing

serum. A somewhat more elaborate discussion on the same subject has been published since then by Kelus and Gell, with a similar conclusion (14).

It may be useful, in the beginning of the present discussion, to place idiotypy among the other kinds of phenomena that may be considered as having some similarity with it.

Idiotypy and "Anti-Antibodies".—It would be difficult to undertake a satisfactory discussion of the relationships between idiotypy and what numerous authors have called "anti-antibodies". The history outlined by Sevag (23) of the experiments undertaken since the last century with the aim of obtaining anti-antibodies would give an idea of the complexity of such a discussion, and this idea would not be contradicted by the work undertaken since then.

The word anti-antibody may be understood in a number of various ways, all equally justified in spite of their different meanings. According to the definition adopted (always more or less arbitrarily), it seems that either the anti-idiotypic antibodies, or the anti-allotypic antibodies, or both, or even neither, would have to be considered anti-antibodies. In a recent review under this heading, which necessarily includes various subjects, Gell and Kelus have proposed to restrict the meaning of the word "anti-antibody" to "an antibody which will react as such with an Ig molecule *because* that molecule is an antibody, not just because it is a γ -globulin" (24). But this definition does not allow us safely to include anti-idiotypic antibodies in the defined notion, nor to exclude them from this notion. It seems therefore that a discussion of the relationships between anti-idiotypic antibodies and anti-antibodies would amount essentially to a question of words, and would therefore hardly deserve a longer digression.

Idiotypy and Allotypy.—It would seem at first glance that idiotypy resembles allotypy. One might be tempted to consider idiotypy as a limiting case of quantitative differences, such that a given allotypic specificity would be the prerogative of antibodies of one individual (or of a restricted group of individuals, of which a single representative is known thus far) against a given antigen. This would seem difficult to admit in view of the extremely large number of allotypic variants of immunoglobulins that would have to be assumed: a number which is larger than that of the individuals tested so far, and so large that it would make hereditary transmission most unlikely. It has been reported above (Results, 3) that the idiotypic specificities carried by anti-*S. typhi* antibodies of three rabbits born from three different matings were in each case not detected on the anti-*S. typhi* antibodies of their parents. This absence of sign of hereditary transmission constitutes a definite difference with allotypy and confirms that this difference is not only a matter of degree.⁸

The Nonreproducibility of the Observations on Antibody Idiotypy: Similarities

⁸ In the anti-*Proteus* sera of 30 rabbits which were the offspring of two bucks, Kelus and Gell looked for the idiotypic specificities of the anti-*Proteus* antibodies of these two bucks and did not find them (14).

and Differences with Certain Features of Myeloma Proteins.—One might still try to characterize an aspect of the difference between allotypy and idiotypy by saying that the experimental facts that pertain to idiotypy of rabbit anti-*S. typhi* antibodies are not reproducible. Since it is however usual to demand that a scientific fact be reproducible, and since it would be difficult to exclude idiotypy from experimental science, one might try to reconcile these two apparently contradictory remarks by further noticing that a certain form of non-reproducibility is a feature which is common and peculiar to the experimental facts that pertain to idiotypy. Thus one might claim that their reproducibility consists in this peculiar feature.

The individual antigenic specificities of myeloma proteins (10) also evoke the idea of nonreproducibility. But, in that case, we are no longer concerned with experimental facts, since the proteins under study appeared as the result of a disease which was not purposely provoked by the experimenter, in man, at any rate. Even in the mouse, in which the study of myelomas was largely enriched by the possibility of artificially provoking the disease (25), the experimental character of the observations made are incomparably more limited than the experimental character of the observations on rabbit antibody idiotypy. The two kinds of observations are only partially comparable in their meaning and in the consequences that may be drawn from them. It is not yet quite obvious, in spite of several compelling arguments, that the homogeneity of the myeloma proteins is the only feature (besides their tumoral origin) that distinguishes them from the normal immunoglobulins. The ascription of an antibody function to myeloma proteins, which, for a long time, was only a likely hypothesis, was confirmed in a number of cases (26), so that its generalization seems justified. However, the antigen against which these antibodies are directed is still unknown in most cases. It is still impossible in all cases to choose this antigen, and to provoke in one given individual the formation of antibodies of that kind against two different antigens. These reasons make it impossible to try to distinguish, in the antigenic specificity peculiar to each myeloma protein, what is correlated with the antibody specificity against various antigens, and what is correlated with the origin of these antibodies in different individuals, so that the following discussion does not apply to myeloma proteins.

The Apparent Role of Random Chance in the Determination of Idiotypic Specificities

We will first attempt to see whether things seem to happen as though the choice of the idiotypic specificities of the antibodies against a given antigen were, in each individual, the result of "lot-drawing" among a large number of possibilities, all of which would be common to all individuals of the same species, or at least to all individuals of the same genotype in terms of immunoglobulin allotypy.

According to this model, it is sufficient that the number of individuals studied be small enough, as compared to that of the possible idiotypic specificities, for the probability of observing the same idiotypic specificity in two randomly chosen individuals to be small or even negligible (without being zero). This model does not exclude that the same idiotypic specificity might be observed by random chance in two different individuals, but it excludes a role played by heredity, which indeed was not observed in our experiments. A model that would be opposed to the above would be to presume that the different idiotypic specificities arise by precise laws, e.g., those of heredity or of differentiation. This idea is difficult to reconcile with the observation that the antibodies against *S. typhi* of all rabbits examined so far bear different idiotypic specificities. It would then be difficult to escape the internal contradiction arising from the fact that the role of heredity (which was not observed) is hard to dissociate from the notion of individuality and is, however, hard to reconcile with a variety of idiotypic specificities so great that this variety seems so far unlimited. Preference will therefore be given to the model of lot-drawing among a very large set of idiotypic specificities which might potentially be the same for all individuals.

One ought to consider whether the idiotypic specificities of the antibodies of one given individual against different antigens may be regarded in the same way as the idiotypic specificities of various individuals against the same antigen. These idiotypic specificities of antibodies of one given individual against different antigens were found to be different. However, the number of effective immunogens which it is practicable to employ in a given individual is necessarily much smaller than the number of individuals in which the antibodies against one given antigen may be studied. We will assume the general rule that nothing common is found in the idiotypic specificities of the antibodies of one given individual against distinct noncross-reacting antigens. Therefore, the present discussion leads us to wonder if things seem to happen (*a*) as though the choice of the idiotypic specificity of antibodies of one given individual against one given antigen were, for each antigen, the result of a lot-drawing among a set of a large number of possible idiotypic specificities; (*b*) as though this set were the same for antibodies against all antigens that would be synthesized by a given individual; (*c*) according to the above model, as though this set were also the same for all individuals of the species, or at least for those of the same genotype. This model would apparently have the advantage of economy, since the number of possibilities implied by it would not necessarily be greater than that implied by the above model which concerns only individuals.

This speculative reasoning would perhaps be even more justified (but perhaps not simpler) if, instead of the idiotypic specificities, or of the idiotypic patterns, each of which is made of an assortment of a number of idiotypic determinants, it would bear on these separate determinants themselves.

In practice, as long as each of the anti-idiotypic sera, which are available

against antibodies specific for one antigen, precipitates these antibodies in only one individual, it is difficult or impossible to look for experimental presumptions favorable or unfavorable to the model just outlined. This search might become possible if it were to occur that the antibodies against one given antigenic material had idiotypic specificities that would be at least partly similar in different individuals. If, in different individuals, this similarity would not extend to idiotypic specificities of antibodies against other antigens, this might be considered an unfavorable presumption. If, in different individuals, this similarity were to extend to idiotypic specificities of antibodies against distinct, non-cross-reacting antigens, this would be a favorable presumption. The precipitation reactions which are given by certain anti-idiotypic sera against anti-*S. typhi* antibodies with certain antisera, other than the homologous immunizing antisera, against *S. typhi* are so faint and questionable that this material is not suitable for the examination of such a possibility. It is expected from the work undertaken in this laboratory that idiotypic systems, other than those in which the antigenic material is *S. typhi*, will be more suitable. Heterologous reactions between anti-idiotypic sera and antisera of rabbits other than the homologous immunizing ones have been observed in this laboratory (27)⁹ and extensively studied in the case where the immunizing material was *Salmonella abortusequi* (27). In human antibodies, antigenic similarity designated by the term of "cross specificity" has been observed among a group of macroglobulins with cold agglutinin activity and "individual antigenic specificities" (28).

These considerations on the apparent role of random chance in the determination of idiotypic specificities may be summarized by saying that things seem to happen as if the idiotypic specificities or determinants of the antibodies of one given individual against one given antigen were lot-drawn among a set of a very large number of possibilities which are common to all individuals of the same genotype (in terms of immunoglobulin allotypy).

Speculation on the Relationship of Idiotype to the Primary Structure of Antibodies and to Some Aspects of their Cellular Origin

Antibody Heterogeneity and Idiotypic Heterogeneity Considered in All Individuals of the Same Animal Species.—There is no necessity to stress the fact that the immunoglobulins can assume an extremely large variety of antibody specificities according to the antigenic determinants carried by a very large number of antigens. This variety is still increased by that of the avidities that different antibodies may have toward one given antigenic determinant, and also by the presence of diverse antibody classes and subclasses. In the following, the term antibody function will refer to the two properties of specificity and avidity which together constitute the functional basis of antibody diversity. The structural

⁹ Oudin, J. Unpublished material.

basis of this diversity apparently lies in the primary structure of the so-called variable regions of the immunoglobulin polypeptide chains.

It might be tempting to assume that there is a one to one relationship between a given primary structure of the polypeptide chains and a given antibody function, so that the antibody molecules endowed with a given function would have necessarily the same structure.

Among the antigenic specificities of several kinds that are distinguished in immunoglobulins, the isotypic and allotypic specificities apparently reflect differences in primary structures (6, 7). It seems reasonable to assume that the same is true for idiotypic specificities, and in addition, that the structural differences concerned in the latter case are located in the variable regions of the polypeptide chains. The assumption of a one to one relationship between a given precise antibody function and a given precise structure does not seem to agree with the observations on idiotype. It is difficult to assume that antibodies directed against the same determinants of the somatic antigen of *S. typhi* do not exist in any of the individuals among which the same idiotypic specificity was not found. It seems even difficult to assume that, among these immunoglobulin molecules supposed to have the same antibody specificity, there are none (in several different individuals) which have a similar affinity for the antigen. Thus, it seems likely that, in different individuals, distinct idiotypic specificities are carried by immunoglobulin molecules with similar antibody functions. A further order of magnitude seems therefore to be added by idiotype to the already great heterogeneity of immunoglobulins necessary for antibody functions.

Antibody Heterogeneity and Idiotypic Heterogeneity Considered in One Given Immune Serum Sample of One Individual.—The multiplicity of the antibodies that may be formed in the immunization against a single polysaccharide has been emphasized (29). The very great variety of antibody functions even among antibodies of a purified preparation agrees with the apparently very great heterogeneity of their variable sequence (30). However, idiotypic heterogeneity, such as can be visualized from the number of idiotypes that can be individualized by distinct precipitation zones in the reaction of one given sample of anti-*S. typhi* serum with a corresponding anti-idiotypic serum, appears to be fairly limited. It seems to be so, even though it has been seen that a certain amount of antibodies which are precipitable by the somatic antigen of *S. typhi* may be nonprecipitable by the available anti-idiotypic sera. It might still be said that the idea that one usually has of great antibody heterogeneity leads to the assumption that, among the antibody molecules which are precipitated by the same anti-idiotypic antibodies in a single distinct precipitation zone, all are not likely to have exactly the same antibody function and consequently the same structure. This seems to be even more justified when a denser precipitation zone is considered; an example will be given in the next paper (16).

These considerations seem to suggest that, in a given serum sample of one

individual, the immunoglobulin molecules which have a common idiotypic pattern include a certain variety of molecules with somewhat different antibody functions and therefore with different amino acid sequences of the variable part of their polypeptide chains.

The Possible Cellular Basis of the Heterogeneity of Antibody Function and Idiotypic Heterogeneity.—It is tempting to look for the possible implications of the preceding in the cellular field. Since no relevant direct observations of cellular nature are available, the premise will be the generally accepted opinion that one given cell synthesizes a single kind or variant of light chain and a single one of heavy chain immunoglobulin.¹⁰ In addition to the homogeneity of myeloma proteins, the observations which support this rule derive from certain properties of immunoglobulins: their antigenic specificities—*isotypic* or *allotypic*—and their antibody specificities against given antigenic materials, all properties reasonably considered to reflect differences in the primary structure of the constant or variable regions of the polypeptide chains. It seems that the immunoglobulin synthesized by each cell is homogeneous from the standpoint of these three properties, with certain cited exceptions. There are certain observations which definitely suggest that the specialization of each cell in the synthesis of the product of a single allele is transmitted by each cell to its progeny (31). It is not as clear as in the case of the *isotypic* specificities or of the antibody specificities that the region—constant or variable—of the polypeptide chains which is concerned in *allotypic* specificities is always the same. Certain *allotypic* patterns have been located in the constant regions (Fc fragments of papain digestion in mice, and in man for most of the Gm factors). On the other hand, evidence has been supplied that certain *allotypic* determinants are located in the variable region of rabbit heavy chains, since the amino acid residues which are involved in *allotypic* determinants overlap with those which vary with the antibody specificity (32), and *allotypically* related variations in composition were detected in the N-terminal cyanogen bromide cleavage peptide of heavy chains (33).

The question of the cellular specialization in antibody synthesis might be posed from another standpoint. Knowing that several idiotypes may be represented among the antibodies of a given individual against a given antigen, is a single cell able to synthesize more than one idio^{type}? Until an experimental answer can be obtained, it will be assumed in the following discussion that only one idio^{type} can be synthesized by a single cell. It will be assumed also that the same is true for the cells from the division of which each of these cells derives; the question of the synthetic capacity of the progeny of a given cell is one of the objects of the following discussion.

The Possible Relationships between the Two Levels of Molecular Variability,

¹⁰ There may, however, be certain exceptions of systematic character to this rule and these will be considered in the next paper (16).

Those of Antibody Function and of Idiotypic Specificity.—It has been tentatively concluded above that, of the heterogeneities at these two levels, among antibodies of one individual, the more restricted one was that revealed by idiotypic differences, and the more extended one was that of antibody functions. The simplest way to imagine the relation between the two levels of heterogeneity is probably to assume that, in one given individual, all the molecules which can be defined by the structure responsible for a given antibody function would possess the same idiotypic specificity, but that the reverse is not the case since they would share this idiotypic specificity with molecules endowed with other antibody functions. Each group of molecules with a given structure responsible for a given antibody function would be a subdivision of a group of molecules with a common idiotypic specificity. This is compatible with the idea that the number of antibody functions is apparently larger than that of idiotypic specificities.

It would be also conceivable that, among the molecules endowed with a given antibody function determined by a given kind of structure, several idiotypic specificities would be represented. Since the various groups of molecules with a given idiotypic specificity common to all molecules inside each group are supposed to be less numerous than the groups of molecules with a given antibody function common to all molecules inside each group, this would imply an overlapping between the two kinds of groups. The simplest way to visualize this possibility is to assume that the idiotypic specificities and the antibody functions are distributed independently of each other among the antibodies of one individual against a given antigen.

The Possible Relationships between the Two Levels of Cellular Specialization Corresponding to the Two Considered Levels of Molecular Variability.—It is generally admitted that each cell synthesizes a single kind of each polypeptide chain, homogeneous from the standpoint of its primary structure. Thus, the simplest way of transposing to the cellular field the first kind of molecular heterogeneity at the two levels discussed in the preceding paragraph, is to imagine that the cells which synthesize the molecules with the same idiotypic pattern belong to the same cell line or to the same clone, from which the cells which synthesize the molecules with different antibody functions have been derived and have differentiated, whatever the mechanism of this differentiation may be.

A cellular correspondence to the second kind of molecular heterogeneity considered in the preceding paragraph is less easy to imagine. The hypothesis that the structures concerned in one of the two levels of heterogeneity are not part of the primary structure of the polypeptide chains, but, for example, that they belong to prosthetic groups, does not seem to merit much attention. Another possibility to be considered might be that certain elements of primary structure responsible for heterogeneity at one of the two levels would be coded by DNA that entered the cell or parent or ancestor cell by a mechanism other than cell

division. This possibility would have to be seriously considered if cells known not to be derived from a common ancestor were experimentally found to synthesize related idiotypes.

SUMMARY

Sera of rabbits immunized against *Salmonella typhi* have been studied for the idiotypic nature of certain of their components, i.e., the property of these components to possess an antigenic specificity which is different in individual rabbits, and which varies with the antigens against which these rabbits have been immunized. The reagent used (precipitating anti-idiotypic sera) have been prepared by injecting rabbits with bacteria agglutinated by anti-*S. typhi* sera (immunizing sera) as was done in the first observations by the authors of the phenomenon in the rabbit. These first observations have been confirmed and extended.

In contrast to allotypy, the anti-idiotypic sera precipitate the corresponding immunizing sera, but not the sera taken in the immunizing rabbits prior to their immunization against *S. typhi*, nor the immunizing sera absorbed with the somatic antigen of *S. typhi*, demonstrating that idiotypes are antibodies.

The idiotypic specificities of the antibodies of one rabbit against *S. typhi* are not detected in the antibodies of the same rabbit against another noncross-reacting *Salmonella* (*S. tranoroa*) and vice versa; nor are they detected in the anti-pneumococcal antibodies of the same rabbit.

Each anti-idiotypic serum fails to precipitate anti-*S. typhi* sera of rabbits other than the immunizing one except for certain extremely faint reactions, the significance of which has not been established. The idiotypic specificities of anti-*S. typhi* antibodies of three rabbits were not found in anti-*S. typhi* antibodies of their parents. This lack of a sign of hereditary transmission of idiotypic specificities contrasts with allotypy. The apparent role of random chance in the determinism of the idiotypic patterns or of the idiotypic determinants has been discussed.

Unless it were admitted that antibodies with similar functions do not exist in different individuals, idiotypy apparently adds an order of magnitude to the antibody variability which had been previously envisaged. In one given individual, the heterogeneity of the idiotypic specificities seems to be less extended than that of the antibody functions. The possible relationships between these two levels of molecular variability and between the corresponding levels of cellular variability have been discussed.

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