IDIOTYPY OF RABBIT ANTIBODIES

II. Comparison of Idiotypy of Various Kinds of Antibodies Formed in the Same Rabbits against *Salmonella Typhi**

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In the preceding paper (1), an answer has been given to a few of the questions raised by the idiotypy of rabbit immunoglobulins (2) with the following conclusions: that each idiotypic pattern detected in antisera against *Salmonella typhi* was carried by antibodies; that the antibodies of one rabbit against antigenic materials other than *S. typhi* did not carry the idiotypic patterns of the anti-*S. typhi* antibodies, or even carried definitely different idiotypic patterns; that the idiotypic specificities of the antibodies of different individuals against *S. typhi* were definitely different without any sign of hereditary transmission that could be comparable to that of allotypic specificities.

In this paper, the comparison of the idiotypic specificities will be restricted to antibodies elaborated against *S. typhi* by the same individuals. The antibodies to be compared will differ from each other: (*a*) by the stage of the anti-*S. typhi* immunization at which they had been collected; (*b*) by the immunoglobulin class to which they belong in one given serum sample; (*c*) by their ability or inability to be precipitated by the polysaccharide of *S. typhi*.

The discussion of the results of the preceding paper, on the basis of fairly simple postulates, led us to consider comparatively the antibody heterogeneity involving differences in antibody functions or idiotypic specificities, and the cellular heterogeneity to which the antibody heterogeneity may be logically assumed to be correlated. This discussion will be resumed in the present paper and applied to the results obtained as answers to the questions mentioned above.

Materials and Methods

The antibacterial and anti-idiotypic immunizations have been described in the preceding paper (1) in which the immunizing and anti-idiotypic rabbits are listed in Table I. The techniques of immunochemical analysis (double diffusion in cells or in tubes) and of immunoelectrophoretic analysis in agarose were used as described in references (3 and 4).

Serum Protein Fractionations and Antibody Preparations.—Fractionations by exclusion

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chromatography were made on Bio-Gel P-300 packed in 12.5 mm × 90 cm columns. IgG fractions were purified by chromatography on DEAE-cellulose (5). Ultracentrifugations in sucrose gradients were performed by the method described in reference (6). Total globulin fractions were prepared by precipitating anti-*S. typhi* sera by an equal volume of saturated ammonium sulfate, centrifuging, dissolving the precipitate, dialyzing it against normal saline, and concentrating it in a dialysis bag to reduce the volume of the fraction to about $\frac{1}{5}$ the volume of the serum precipitated.

Antibodies against the polysaccharide of *S. typhi* were prepared from the specific precipitate of one anti-*S. typhi* serum (rabbit 3-24) and of a macroglobulin fraction of exclusion chromatography (rabbit 8-03) by dissolution of the precipitate (formed in substantial antigen excess) in glycine buffer 0.5 M, pH 2.0, followed by exclusion chromatography on Bio-Gel P-300 in much the same way as had been done for antibodies against lysozyme (7). This procedure succeeded in the separation of IgM antibodies, but not of IgG antibodies which left the column in the same fractions as the polysaccharide.

The serum of rabbit 3-24 used for the antibody preparation was a mixture of the 39th and 40th bleedings (S39-40). In the precipitating mixtures of this serum with increasing amounts of *S. typhi* polysaccharide, there was an appreciable overlapping of the reactions of the supernatants with antigen and antibody; 9 μg of polysaccharide mixed with 1 ml of serum still left some precipitating antibody which had disappeared at 13 μg, while the mixture with 5 μg already contained some excess of precipitating hapten. 23 ml of serum were precipitated by 0.375 mg of polysaccharide. The precipitate, centrifuged the next day and washed three times with normal saline in the cold, was dissolved in 2 ml of cooled glycine buffer (pH 2.0) and the solution was passed on a column of 12.5 mm diameter and 90 cm length containing Bio-Gel P-300, placed in the cold room (4°C). The effluents were collected in separate fractions of 1 ml in tubes containing 1.8 ml of borate buffer 1 M pH 8.0. Several peaks were observed on the curve of optical densities. Three mixtures were made with the fractions which were dialyzed against normal saline and concentrated under suitable pressure. The first mixture was that of the fractions of the first peak, excluded on Bio-Gel P-300 and expected to contain the IgM. This mixture, concentrated in dialysis bags (optical density 3.6), was the only one that gave a precipitation reaction with the somatic antigen of *S. typhi*; it was used as the solution of purified antibodies. The others contained polysaccharide detectable by precipitation reactions with antisera against *S. typhi*, so that it was not possible to study the IgG antibodies precipitated by the polysaccharide.

The supernatant of the same precipitation of S39-40 with the polysaccharide was used as the preparation containing the antibodies not precipitable by the polysaccharide. In order to remove the excess polysaccharide: (*a*) this supernatant was precipitated by half saturated ammonium sulfate; (*b*) a small amount of anti-*S. typhi* serum globulins of another rabbit was added to the dialyzed solution of this precipitate.

In view of the preparation of anti-polysaccharide antibodies of rabbit 8-03, the macroglobulin fraction prepared from the serum of the second bleeding (equivalence of 1 ml with the polysaccharide between 40 and 50 μg) was precipitated by 70 μg of polysaccharide to 1 ml of fraction, and the precipitate treated as above. The solution of IgM antibodies, separated from the precipitate and concentrated, had an optical density of 1.1. The preparation containing antibodies not precipitable by the polysaccharide was the supernatant of the precipitation of the IgG fraction of the same serum sample (equivalence of 1 ml with the polysaccharide between 10 and 15 μg) by 25 μg of polysaccharide to 1 ml of fraction. This preparation was concentrated (optical density 12.7).

Anti-IgG and Anti-Macroglobulin Sera.—The anti-IgG sera used in sections 2 and 3 in looking for IgG in the IgM-containing macroglobulin or IgM antibody preparations were: (*a*) the serum of a goat hyperimmunized against human IgG (purified by DEAE-cellulose chromatography) with complete Freund's adjuvant; this serum gave a fairly strong cross-

reaction with rabbit serum and with purified rabbit IgG (the amount of nitrogen precipitated by rabbit IgG from 1 ml of goat serum was 600 μg); (*b*) the serum of the first bleeding of a sheep injected with rabbit IgG (purified by DEAE-cellulose chromatography) and complete Freund's adjuvant (the amount of nitrogen precipitated by rabbit IgG from 1 ml of sheep serum was 55 μg).

The anti-macroglobulin serum used for detecting the presence of IgM (or other macroglobulin antigens) in the IgG preparations was the serum of a rooster. The immunizing material injected into this rooster with complete Freund's adjuvant was the fraction of the first peak of exclusion chromatography of rabbit serum on Sephadex G-200, deprived of β-lipoprotein by dextran sulfate precipitation (8). This serum had been absorbed with rabbit IgG which had been purified by DEAE-cellulose chromatography, and with a purified preparation of α-macroglobulin. The reaction of this rooster serum, in gels (simple or double diffusion in tubes or cells) with rabbit serum, gave rise to several precipitation zones, one of which had been identified as due to the IgM.

RESULTS

1. Comparison of the Idiotypic Specificities of Antibodies against the Same Antigenic Material Obtained at Different Times in One Individual

The observations relevant to this question were performed with different serum samples of several rabbits; the comparison was made on serum samples which were collected within a short interval of time in several rabbits, and within a longer interval in one other rabbit.

Bleedings Made with a Short Interval between Them, Close to the Beginning of the Immunization

Rabbit 8-03 was immunized in the usual way against *S. typhi*, injected and bled as stated in the Materials and Methods of the preceding paper (1).

The interval between the first two bleedings giving the sera S1 and S2 of rabbit 8-03 whose reactions with anti-idiotypic sera will be compared was only 17–25 days, and just a single injection of *S. typhi* was made during this interval. The anti-*S. typhi* serum used for agglutinating the bacteria injected for the anti-idiotypic immunizations was that of S2. Six rabbits were injected with these agglutinates. Out of the four rabbits whose sera precipitated S2 of rabbit 8-03, only three also precipitated (more faintly) S1.

It is seen in Fig. 1 that the anti-idiotypic serum 8-62 gives three precipitation zones in its reaction with S2, and that all three idiotypes so demonstrated are absent from S1, just as they are absent from the serum taken from rabbit 8-03 before its immunization. Accordingly, the anti-idiotypic serum of rabbit 8-62 does not give any visible reaction at the interface in liquid medium with the serum of bleeding S1 of rabbit 8-03 diluted 1:2 in normal saline. However, the serum of this S1 bleeding of rabbit 8-03 gives a fairly strong precipitation reaction with the somatic antigen and with the polysaccharide of *S. typhi* (1), see Table I. In addition, the same S1 bleeding of rabbit 8-03 gives a fairly strong reaction of precipitation, in liquid media, and one or several precipitation zones in gels with other anti-idiotypic sera.

The reaction of the same serum samples of rabbit 8-03 with another anti-

idiotypic serum is shown on Fig. 2A. Two precipitation zones are visible in front of S1, and two or possibly three in front of S2. The second (the farther from the antibody layer) of the two zones in front of S1 is hardly visible in front of S2 where it is apparently much fainter and closer to the antigen layer than in front of S1; this indicates that the concentration of the idiotype which is responsible for this zone is definitely smaller in S2 than in S1.

A striking feature of the reaction in Fig. 2A is that the two most readily visible precipitation zones in front of S2 are both definitely continuous with the first zone in front of S1. A similar observation of a precipitation zone which is single in front of S1 and bifurcated in front of S2 was made with the anti-*S. typhi* sera of two other rabbits, as may be seen on Fig. 2B and 2C. It should be noticed that the reaction, under the same conditions, of the anti-*S. typhi* serum samples

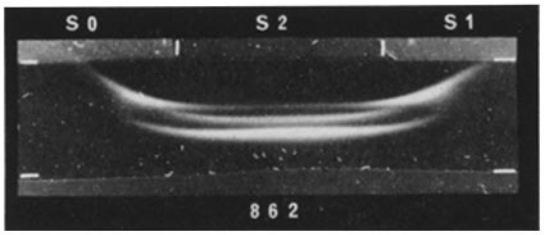

FIG. 1. Reaction in a cell (double diffusion in agar) of three serum samples of rabbit 8-03 with the anti-idiotypic serum of rabbit 8-62. SO is the serum of a bleeding of rabbit 8-03, made before any immunization. S2 and S1 are serum samples, both strongly precipitating against the somatic antigen of *S. typhi*, taken 34–38 days and 13–17 days after the beginning of the immunization of rabbit 8-03 against *S. typhi*. Rabbit 8-62 had been injected with *S typhi* bacteria agglutinated by S2. The white dashes indicate the interface between the various gel layers.

S1 and S2 of the latter rabbit (2-63) with an anti-idiotypic serum different from that in Fig. 2C (that of rabbit 2-89 instead of 2-88) had a different appearance (Fig. 2D). The reaction of serum 2-89 with S2 appears, at least at the first sight, to give rise to a single precipitation zone which seems to be bifurcated into a zone visible in front of the whole width of the S1 layer and a zone which ends long before reaching the right limit of the cell toward S1. Even if this were actually a bifurcation of a zone which would be single in front of S2, the latter feature would make this bifurcation quite different from those which are visible on the above figures. A more thorough examination of the figure strongly suggests that this appearance is due to a superimposition of two zones in front of S2, one of them being hidden by the other, except at its right hand end. If this interpretation is correct, all the bifurcated zones observed in the reactions of the anti-*S. typhi* sera of three rabbits were single in front of S1 and double in front of S2. It will be seen below (Results, 3) that a somewhat similar bifurcation (or even trifurcation) of a precipitation zone was observed in the reaction

of serum samples of two late bleedings of rabbit 3-24. To try to state these results in more general terms, it might be said that the bifurcation is such that a

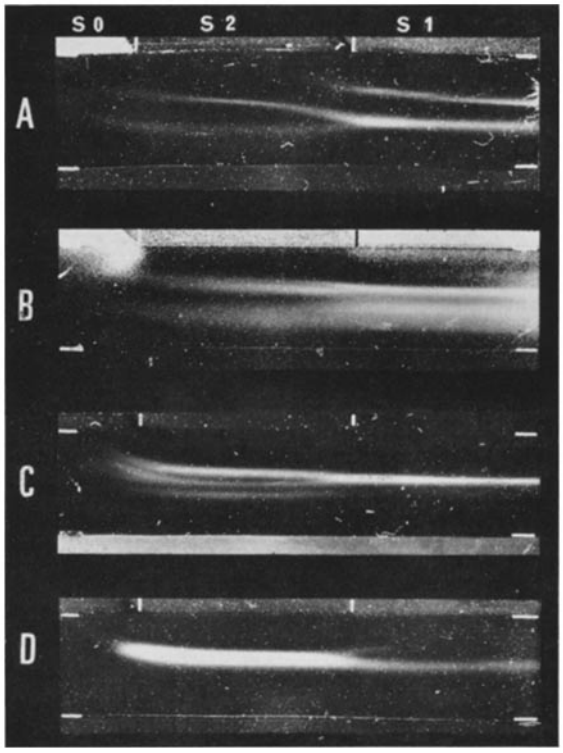

FIG. 2. In each of the four cells, reaction (double diffusion in agar) of an anti-idiotypic serum with three samples of serum of the corresponding immunizing rabbit: SO, serum taken before any immunization; S2 and S1, samples taken 34–38 days and 13–17 days after the beginning of the immunization against *S. typhi*. The immunizing serum sample was S2. A: S0, S2, and S1 of rabbit 8-03 reacting with the anti-idiotypic serum of the third bleeding of rabbit 8-55. B: S0, S2, and S1 of rabbit 7-99 reacting with the anti-idiotypic serum of rabbit 8-54. C: S0, S2, and S1 of rabbit 2-63 reacting with the anti-idiotypic serum of rabbit 2-88. D: S0, S2, and S1 of rabbit 2-63 reacting with the anti-idiotypic serum of rabbit 2-89. In A, B, and C, two precipitation zones, distinct in front of S2, are continuous with a single zone in front of S1. An appearance of a bifurcated zone, turned the other way round but, even otherwise, different from the above pictures, is visible in D and is likely to be due to the superposition of the precipitation zones of two idiotypes, one of which would be present in S2 and S1, and the other only in S2.

precipitation zone, single in front of a bleeding of rank n, becomes double in front of bleeding $n + 1$. The possible meaning of these bifurcated precipitation zones will be considered in the chapter of discussion.

Serum Samples Taken with a Long Interval between Them, Several Months after the Beginning of Immunization

The conditions required for studying antibodies produced in one rabbit at long intervals against the same antigenic material are not easy to achieve; this is particularly true if it is desirable that, after a first immunization, a second immunization not be started until the serum is no longer precipitable by the anti-idiotypic sera prepared against the immune sera obtained after the first immunization. Such were the conditions realized with rabbit 3-24. Among several rabbits in which the same immunizations had been undertaken, rabbit 3-24 was the only one which survived a sufficiently long time for this experiment. The course of the successive immunizations, injections, and bleedings of rabbit 3-24, has been stated in the Materials and Methods of the preceding paper (1).

Two series of rabbits were injected with *S. typhi* bacteria agglutinated by two samples of serum from rabbit 3-24:S7 and S38; they will be termed anti-id S7 and anti-id S38. Only one rabbit of the first series (3-76) gave a sufficiently strong anti-idiotypic serum. Two rabbits of the second series giving sufficiently strong anti-idiotypic sera (6-52 and 6-53) were chosen. The sera of the best bleedings of these three rabbits were used in the following reactions in agar gels.

Fig. 3A shows the neighboring reactions of the serum samples S37 and S5 with the anti-id S7 serum of rabbit 3-76. The S5 serum gave rise to several precipitation zones, one of which, very dense, will be designated as *r* and is continuous in front of S5 and of S37. Several less dense zones appeared later than the *r* zone. Closer to the layer of anti-idiotypic serum, one zone, designated as *m*, is denser and more easily visible in front of S37 than in front of S5. This difference of density may be explained in terms of antigen concentration. This zone is farther from the antibody source in front of S5 than of S37 because the antigen (an idiotypically distinct kind of antibodies of the serum samples of rabbit 3-24) is less concentrated in the former layer than in the latter; this is a sufficient reason for a smaller density in double diffusion. Between the densest zone, *r*, and the antigen layers, one finds first, on the S37 side, a zone, *s*, which is apparently parallel to the sources of diffusion, so that it is difficult to decide whether, in front of S5, this zone is hidden by the denser *r* zone (or else if it becomes continuous with the latter, which seems less likely). Finally, a less dense zone, called *t*, is parallel to the sources of diffusion, showing that this zone is due to an antigen, of which the concentrations in S5 and in S37 are very similar. These four precipitation zones show that, among the antibodies of the sera of rabbit 3-24, there are four antigens, or four idiotypes, two of which are more concentrated in S37 than in S5, while one (and perhaps two) seems to have similar concentrations in both samples.

Fig. 3B shows the neighboring reactions of the serum samples S5 and S37 with the anti-idiotypic serum of rabbit 6-52 against S38. There is no visible difference between these two reactions which give rise essentially to two precipitation zones; a third, much fainter one, is seen closer to the antigen source. Such a third zone is not clearly visible in all other reactions, so that its correspondence with precipitation zones given by the reactions of the other anti-idiotypic sera has not been established. When the S37 serum is reacted with the anti-idiotypic

sera of rabbits 3-76 anti-S7 and 6-52 anti-S38 side by side (Fig. 3C), a definite continuity is observed between zones *m* and *r* in front of 3-76 and the two densest zones in front of 6-52.

The anti-idiotypic serum of rabbit 6-53 against the antibodies of S38 of rabbit 3-24 was reacted in a cell with three serum samples of rabbit 3-24; Fig. 4 left to right: that of the first bleeding, made before any immunization of this

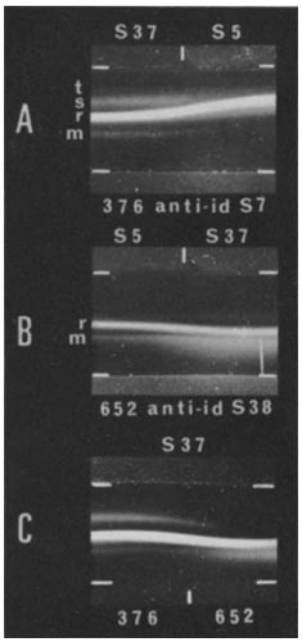

FIG. 3. A and B: reaction, in two cells (double diffusion in agar), of the sera of the 37th and 5th bleedings (S37 and S5) of rabbit 3-24 with the anti-idiotypic sera of rabbit 3-76 (injected with bacteria agglutinated with the serum of the seventh bleeding of rabbit 3-24:A), and of rabbit 6-52 (injected with bacteria agglutinated with the serum of the 38th bleeding of rabbit 3-24:B). The symbols used for designating the precipitation zones are written on the left, in front of these zones. C: reaction of the anti-idiotypic sera 3-76 and 6-52 with S37.

rabbit; S37; and S5. In front of S37, five precipitation zones are visible on the photograph taken with a wide angle of incidence of the light, the second one (counted, as usual, from the antibody layer to the antigen layer) being partially hidden by the first, from which it is distinct only at its ends (this zone is not visible in other reactions of S37 with serum 6-53). Only one zone is seen in front of S5, and this zone is continuous with the first zone in front of S37. The appearance of the other four zones, seen in front of S37, is similar at their ends; this shows that the four idiotypes (among the anti-*S. typhi* antibodies of S37), which manifest a reaction with the anti-idiotypic serum 6-53, are lacking in S5,

in the same way as they are lacking in the serum taken from rabbit 3-24 before its immunization. A reaction of the S37 serum with the two anti-idiotypic sera 6-52 and 6-53 shows a continuity of the first zone of 6-53 with zone *m* of 6-52. The fate of the four other zones of 6-53 in these reactions is more difficult to establish because of the very dense *r* zone of 6-52 which apparently does not correspond to anything in 6-53, but prevents us from observing how most of the zones of this serum end in front of 6-52. The problem of the correspondence of the various precipitation zones observed in the reactions of the serum samples of rabbit 3-24 with the three anti-idiotypic sera is not of crucial importance, and it has been solved at least for two of these zones, *m* and *r*, which will have to be considered in the following results.

The reactions of the 3-76 and 6-52 anti-idiotypic sera with S5 and S37 show at least three precipitation zones of three idiotypes common to these two serum

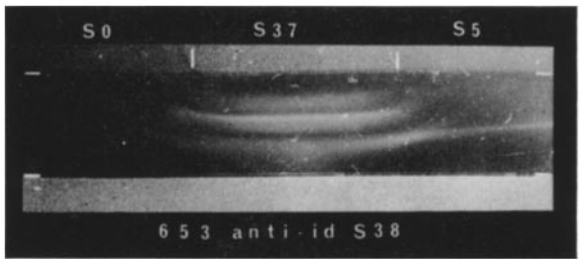

FIG. 4. Reaction in a cell (double diffusion in agar) of three serum samples of rabbit 3-24 (S0, taken before any immunization, S37, and S5), with the anti-idiotypic serum of rabbit 6-53, injected with bacteria agglutinated with the serum of the 38th bleeding of rabbit 3-24. Among the various idiotypes which manifest themselves by precipitation zones in the reaction of the anti-idiotypic serum with S37, only one is apparently present in S5.

samples. The reactions of serum 6-53 show that at least four antigens are present in S37 and absent from S5, and that one antigen, already counted in the above reactions, is common to the two serum samples (zone *m*). Therefore, among the anti-*S. typhi* antibodies of S37, at least seven idiotypes, i.e., seven antigens with distinct idiotypic specificities, can be enumerated.

In this comparative analysis of two serum samples of rabbit 3-24, a long interval (29 months) had elapsed between two successive immunizations against *S. typhi*, and 11 months between the second immunization against *S. typhi* and the preceding immunization, which was against *Salmonella typhimurium*. The purpose of this long interval had been to realize experimental conditions as close as possible to the impossible ones in which two immunizations would be performed in the same individual and could be considered independent from each other. In spite of its length, this interval proved far from restoring the immunological virginity of rabbit 3-24, as regards the antigenic material of *S. typhi*. In the conditions realized, a manifestation of so-called "immunological memory" was that at least three idiotypes were found in both serum samples

in spite of the 29 months between the two bleedings. None of the idiotypes distinguished in the first sample was lacking in the second. On the contrary, four idiotypes were found in the second serum sample and not in the first one.

It should be noticed, however, that the idiotypes which are distinguished in a serum sample depend on the rabbit which gave the anti-idiotypic serum. This dependence could already be noticed about rabbit 8-03 (comparison of Fig. 1 and 2A) and about rabbit 2-63 (comparison of Fig. 2C and 2D). It is particularly visible in the unexplained differences between the reactions of the two anti-idiotypic sera of rabbits 6-52 and 6-53 against the antibodies of S38 of rabbit 3-24, although the material and route of immunization of rabbit 6-52 and 6-53 were exactly the same. If by chance we had only rabbit 6-52 and not rabbit 6-53, no difference would have been detected between the sera of S5 and S37. This may have led us to imagine that with some other rabbit, there might have been a chance that idiotypes would have been observed in the S5 serum and not in the S37 serum. To try and express as rigorously and objectively as possible the results of this experiment, it may be concluded (*a*) that, with the anti-idiotypic sera available, no idiotype was found present in S5 and absent from S37, three idiotypes at least being present in both; and (*b*) that four idiotypes were present in S37 and absent from S5. Although obtained with one given anti-idiotypic serum and not with the other, and in serum samples of only one rabbit, the latter conclusion is a definite one, and is apparently not liable to be contradicted by the results obtained with another anti-idiotypic serum.

With the reservations expressed, the observations made in the comparison of successive bleedings of a given rabbit in the two experimental conditions considered, may be summarized as follows.

(*a*) In the comparison of the first two bleedings, it was found that an anti-idiotypic serum against S2 did not precipitate S1, although this first bleeding contained an appreciable concentration of anti-*S. typhi* antibodies.

(*b*) In the comparison of the first two bleedings by neighboring reactions in gels, a bifurcation of an apparently single zone in S1 into two zones in S2 has been observed several times.

(*c*) In comparing the sera of two late bleedings (the 5th and the 37th) of one rabbit, separated by an interval of 29 months, no idiotype was detected in S5 which was not detected in S37 in the reaction of one anti-idiotypic serum against S7 and of two anti-idiotypic sera against S38. On the contrary, among the seven (at least) idiotypes observed in S37, four idiotypes (all four detected by the same anti-idiotypic serum) were absent from S5.

2. *Comparison of the Idiotypic Specificities of Antibodies Belonging, in one Serum Sample of One Individual, to Two Different Immunoglobulin classes, IgG and IgM*

Preparations of IgG and of macroglobulin fractions containing the IgM were made, starting from the serum of the 5th bleeding (S5) and from that of the 37th bleeding (S37) of rabbit

3-24. For this purpose, serum samples of each of the two bleedings were fractionated on sucrose gradients; then the fractions containing the IgM with some IgG were submitted to exclusion chromatography on columns of Bio-Gel P-300. The fractions containing the IgG in these fractionations were combined, concentrated in dialysis bags under a suitable pressure, submitted to chromatography on DEAE-cellulose columns, and the effluents containing the IgG were collected. Interfacial reactions in liquid media were carried out in order to look for macroglobulins in the IgG preparations and for IgG in the macroglobulin preparations. The reagents used for this purpose, described in Materials and Methods, were: a rooster serum anti-rabbit macroglobulin, a goat serum anti-human IgG, which gives a very strong cross-reaction with rabbit IgG, and a sheep serum anti-rabbit IgG.

TABLE I

Comparative Interfacial Precipitation Reactions of Macroglobulin and IgG Fractions of Two Anti-S. typhi Serum Samples with Anti-IgG, Anti-Macroglobulin and Anti-Idiotypic Sera‡*

Rabbit number and anti-*S. typhi* serum sample		324 "S5"						324 "S37"					
Macroglobulin (Mg) or IgG preparation		Mg			IgG			Mg			IgG		
Optical density at 280 mμ		3.0			20.0			3.3			5.8		
		+	±	0	+	±	0	+	±	0	+	±	0
Anti-IgG sera	Goat			1	1280	2560	5120		1	2	160	320	640
	Sheep			1	320	640	1280			1	40	80	160
Anti-macroglobulin serum	Rooster	80	160	320			1	80	160	320			1
Anti-idiotypic sera	Rabbit 3-76	20	40	80	20	40	80	20	40	80	10	20	40
	" 6-52	20	40	80	20	40	80	20	40	80	10	20	40

* In the columns below the signs +, ±, and 0, each number means the dilution (i.e., *n* for a concentration *1/n*) which gives, for each preparation considered a reaction, the intensity of which may be expressed by the sign above the column.

‡ For information concerning these immune sera, see Materials and Methods of this paper or the preceding one (1).

The results of these reactions and of those in which the same fractions were reacted with anti-idiotypic sera are shown in Table I. It seems from these reactions apparently that there are in the serum samples studied, idiotypes of anti-*S. typhi* antibodies in immunoglobulins of the IgM class as well as of the IgG.

It was of interest to look for possible similarities or relationships between the idiotypes of these two immunoglobulin classes. For this purpose, the macroglobulin and IgG fractions purified from S37 were reacted side by side in double diffusion cells with the anti-idiotypic sera against the anti-*S. typhi* antibodies

of rabbit 3-24. The most obvious result in the reaction of the antisera 3-76 and 6-52 was that the densest precipitation zone, designated above as *r*, is perfectly continuous in front of the macroglobulin and IgG layers. This is visible in the reaction of Fig. 5A. In Fig. 5B where the IgG preparation has been diluted 1:5,

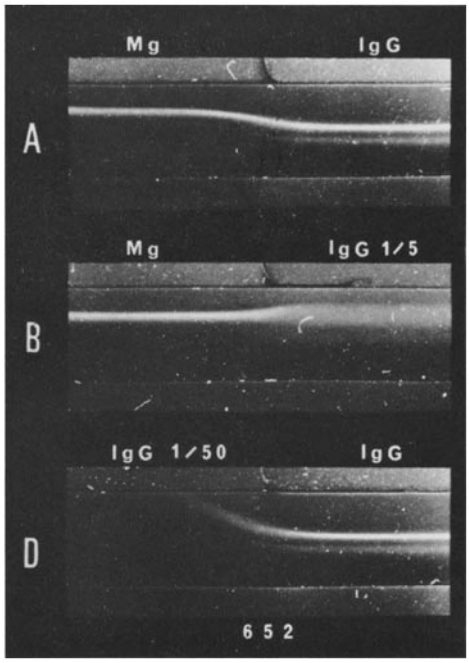

FIG. 5. Reaction in three cells (double diffusion in agar) with the anti-idiotypic serum of rabbit 6-52: A, of a preparation of macroglobulin (Mg) and of IgG of S37 of rabbit 3-24; B, of the same macroglobulin preparation and a 1:5 dilution of the same IgG preparation as in A; D, of a 1:50 dilution of the same IgG preparation as in A and this IgG preparation, undiluted. It may be noticed: (*a*) that the *r* precipitation zone is perfectly continuous in front of the macroglobulin (containing IgM) and IgG layers in A; (*b*) that, when the IgG preparation is diluted 1:5, the position of the *r* precipitation zone in front of it is the same as in front of the macroglobulin layer, but that its appearance is definitely different; (*c*) that, when the IgG layer is diluted 1:50, there is definitely no *r* precipitation zone in front of it, although the IgG content is still definitely larger in this dilution than in the macroglobulin layer, as is shown by the results in Table I.

the precipitation zone in front of this IgG dilution is continuous with that in front of the IgM, with a very similar position but with a quite different appearance. This difference shows that the two parts of this precipitation zone are due to the same anti-idiotypic antibodies reacting with two antigens which these antibodies do not distinguish, although their properties are not exactly the same. In Fig. 5D, the macroglobulin preparation has been replaced by a

1:50 dilution of the IgG preparation, so that, as shown by the results in Table I, the IgG content of this layer is definitely larger than the IgG content in the macroglobulin layer shown in Fig. 5A and 5B. Under these conditions, there is no precipitation zone in front of the layer of diluted IgG, thus supplying a further demonstration that the continuous zones in Fig. 5A and 5B could by no

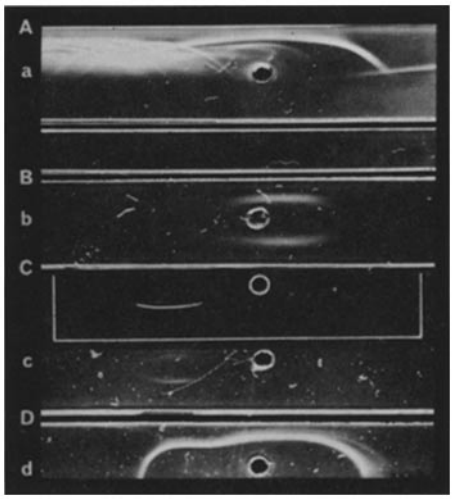

FIG. 6. Immunoelectrophoretic analysis, in agarose, of three fractions of the anti-*S. Typhi* serum of bleeding S37 of rabbit 3-24: IgG fraction (well *b*), macroglobulin fraction, the same as in Table I and Fig. 5 (well *c*); fraction salted out by half saturated ammonium sulfate (well *d*). The same anti-idiotypic serum (of rabbit 6-52) has been placed in troughs *B*, *C*, *D*. Total rabbit serum has been placed in well *a* and an anti-rabbit serum sheep serum in trough *A*. The IgM precipitation zone (between *c* and *d*), which is fairly faint in the photograph may be seen more clearly in a drawing inserted in the middle of the figure. This precipitation zone, compared with those between *b* and *B* or *C*, and between *d* and *D*, shows that there are IgG and IgM antibodies, reacting separately in *b* and *c*, and together in *d*, which carry the same idiotypic pattern.

means be accounted for by a contamination of the macroglobulin fraction by a sufficient amount of IgG.

The electrophoresis, in agarose, of the same macroglobulin and IgG fractions, and of a total globulin fraction precipitated by half saturated ammonium sulfate, and their subsequent reaction with an anti-idiotypic serum (Fig. 6) shows in addition that there is an idiotype with the IgM mobility, which is not distinguished by the anti-idiotypic serum used, from an idiotype having the IgG mobility.

Another reaction in which IgM and IgG idiotypes of rabbit 8-03 were not distinguished by anti-idiotypic antibodies will be reported in the next section.

It may be concluded from the reactions reported that there are anti-*S. typhi*

antibodies of the IgM and IgG classes which carry the same idiotypic patterns, since there are anti-idiotypic antibodies which do not distinguish between them.

3. Comparison of Idiotypic Specificities of Antibodies against the Somatic Antigen of S. typhi Which, in a Given Serum Sample of One Individual, are Precipitated or Not Precipitated by the Polysaccharide

The Two Kinds of Antibodies to be Compared and the Effect of the Absorption of Anti-Salmonella Sera by the Polysaccharide on Their Reaction with the Homologous Anti-Idiotypic Sera.—It was shown by Boivin and Mesrobeanu that the somatic antigen of *S. typhi* could be split by acid hydrolysis into two parts: a polysaccharide, which remains in solution, and the rest of the antigenic complex, which becomes definitively insoluble under these conditions (9).

It was observed that among the antibodies which are obtained in the rabbit by an immunization either against the whole bacteria or against the somatic antigen, and are precipitable by this antigen, only a part is precipitated by the polysaccharide (10). Even though the meaning of the differences between these two kinds of antibodies, which will be considered in the Discussion, is not elucidated, the question of the comparison of their idiotypic specificities had to be raised.

A clear-cut effect of this absorption was the disappearance of one or several precipitation zones in the reactions of the observed antisera with the corresponding anti-idiotypic sera. This effect was observed for example in the reactions: of the serum of the 37th bleeding (S37) of rabbit 3-24 with the anti-idiotypic sera 3-76 (Fig. 7A) and 6-53 (Fig. 7B), of the anti-*S. typhi* serum of rabbit 8-03 with the anti-idiotypic serum 8-55 (Fig. 7C), and of the anti-*Salmonella tranoroa* serum of rabbit 1-70 with the anti-idiotypic serum of rabbit 3-48 (Fig. 7D).

A more thorough examination of these figures reveals that the position of certain of the precipitation zones which were not canceled by the absorption is not exactly the same in the reactions of the anti-*Salmonella* sera absorbed and unabsorbed. These zones are slightly closer to the antigen source in the reaction of the absorbed sera than in the reaction of the unabsorbed. This is visible for example in Fig. 7A for the densest zone *r*, in Fig. 7C and in Fig. 7D.

The explanation of this difference is that the antigens which are reacting in the precipitation zones whose positions have been moved closer to the antigen source are less concentrated in the absorbed than in the unabsorbed sera, and therefore the absorption has decreased their concentration without eliminating them completely. This would mean that the antigens with which we are concerned are present, with the same idiotypic specificity, in antibodies which are precipitated by the polysaccharide and in antibodies which are not. This consequence needed to be checked by appropriate experiments.

Comparison of the Idiotypic Specificities of Antibodies Recovered from the Specific Precipitate of an Anti-S. typhi Serum with the Polysaccharide and of the Anti-

bodies Which are not Precipitated by the Polysaccharide.—The following experiments were undertaken in order to compare the reactions of two preparations in such a way that (*a*) the only antibodies contained in one preparation were among those which are precipitated by the polysaccharide of *S. typhi*, and (*b*) that the antibodies against *S. typhi* of the same serum sample contained in the other preparation were not precipitable by the polysaccharide.

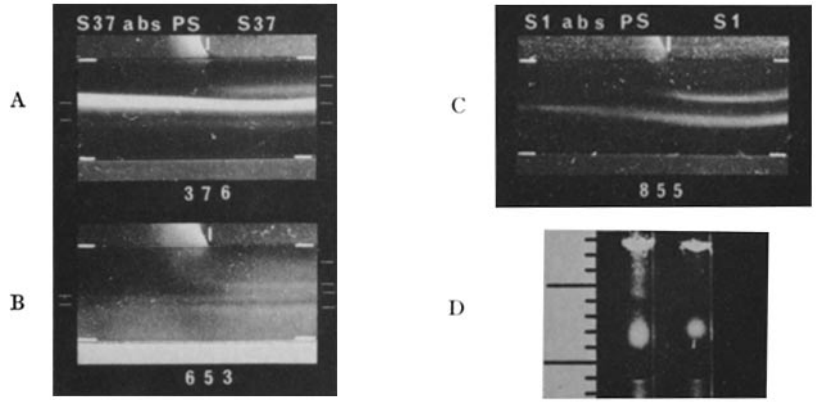

FIG. 7 A and B. Compared reaction in two cells (double diffusion in agar) of the serum of the 37th bleeding of rabbit 3-24 (S37) and of the same serum sample absorbed with the *S. typhi* polysaccharide (S37 abs PS) with the anti-idiotypic sera of rabbit 3-76 (A) and 6-53 (B). White dashes have been placed in front of the precipitation zones in order to draw attention to those which are lacking in the reaction of the absorbed S37 serum.

C. Compared reaction in a cell (double diffusion in agar) of the serum of the first bleeding (S1) of rabbit 8-03, and of the same serum sample absorbed with the polysaccharide of *S. typhi* (S1 abs PS), with the anti-idiotypic serum of the third bleeding of rabbit 8-55.

D. Compared reaction in two tubes (double diffusion in agar) of the anti *S. tranoroa* serum of rabbit 1-70 (24th bleeding) unabsorbed and absorbed with the polysaccharide of *S. tranoroa* (upper layers of the left hand and right hand tubes) with the anti-idiotypic serum of rabbit 3-48 (lower layer of both tubes).

The serum against *S. typhi* used in the first experiment was a mixture of the 39th and 40th bleedings of rabbit 3-24 (S39-40). Fig. 8 shows the reaction of the anti-idiotypic serum of rabbit 3-76 with the two preparations made from S39-40: the fraction of IgM antibodies separated from the precipitate with the polysaccharide (optical density 3.6), and the supernatant of this precipitation treated as described in Materials and Methods. The best visible precipitation zone in this reaction is the most dense one designated above as *r*. It is seen that this zone is continuous in front of all antigen layers, although it is subdivided into two (or even three) zones in front of S39-40 and in front of its supernatant which is not precipitated by the polysaccharide.

It is not possible to be certain whether (*a*) the three idiotypes whose presence

in the supernatant of the precipitation is manifested by distinct zones are also separated from each other in the IgM recovered from the precipitate, their proportions and those of the anti-idiotypic antibodies being such that the three zones are very close to each other and cannot be distinguished, or (*b*) if the idiotypic patterns which are carried by three distinct molecules in the former antigenic preparations are carried by a single molecule in the latter. In any case, there was no sign in the reactions reported above

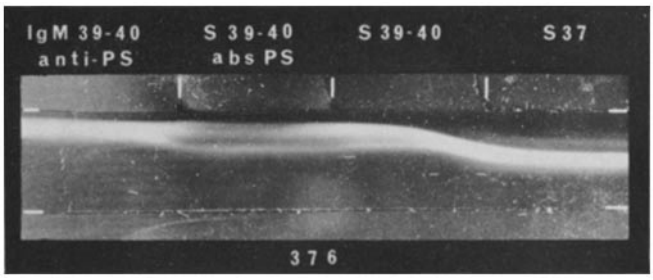

FIG. 8. Reaction in a cell (double diffusion in agar) of the anti-idiotypic serum of rabbit 3-76 with two serum samples and two fractions of a serum sample of rabbit 3-24; from right to left: the serum of the 37th bleeding of rabbit 3-24 (S37), a mixture of sera of the 39th and 40th bleedings of the same rabbit (S39–40), the same serum mixture absorbed with the *S. typhi* polysaccharide (S39–40 abs PS), and an IgM solution recovered from the precipitate of the same serum mixture with the *S. typhi* polysaccharide (IgM 39–40 anti-PS). The densest precipitation zone, which is the only one visible in front of the left hand antigen layer, is continuous in front of all four layers, showing that the anti-idiotypic antibodies which are reacting in this zone (*r* zone, Fig. 3) do not distinguish between the *r* idiotypic pattern carried by the IgM antibodies precipitated by the polysaccharide, and the *r* idiotypic pattern carried by the anti-*S. typhi* antibodies which are not precipitated by the polysaccharide. Another important detail is to be observed on this figure. The *r* precipitation zone, which is single in front of S37 (and has been observed to be a single zone in all other reactions of S5 and S37 and of IgM and IgG antibodies of S37 with the anti-idiotypic sera 3-76 and 6-52) is triple in front of S39-40 abs PS and at least double in front of S39-40.

nor in the present reactions that these three idiotypic patterns are carried by distinct molecules in S37, nor in S5.

A similar experiment was performed with the serum of the second bleeding (S2) of rabbit 8-03, except that the anti-polysaccharide antibody was recovered from the precipitate of an IgM fraction, and that the antibody not precipitable by the polysaccharide was the supernatant of the precipitation of an IgG fraction with the polysaccharide (see Materials and Methods). In the cell of Fig. 9, the solution of recovered IgM antibody had an optical density of 1.1, and the solution of nonprecipitable IgG had an optical density of 12.7. In the reaction of these two fractions side by side in the same gel with the anti-idiotypic serum 8-55, a precipitation zone of moderate density in front of the recovered IgM antibody is continuous with one of the two precipitation zones in front

of the supernatant of the IgG precipitate. It must be pointed out that no IgG was detected in the IgM antibody preparation, although the IgG nonanti-polysaccharide, even diluted 1:640 or 1:1280, still precipitated the anti-IgG sera, so that the precipitation zone which, in the reaction of Fig. 9, is continuous in front of the two antigen layers, could not be attributed to an IgG contamination. Therefore, this reaction supplies a supplementary evidence to the conclusion of the preceding section.

From this comparison between the behavior of the antibodies precipitated by the polysaccharide and of the other antibodies in the sera studied, it may be concluded (*a*) that there are antibodies precipitable by the polysaccharide,

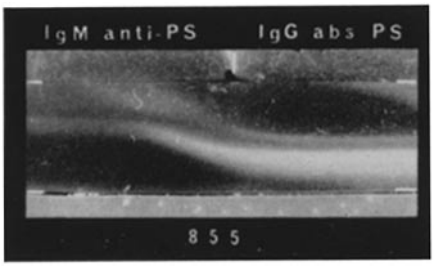

FIG. 9. Compared reaction in a cell (double diffusion in agar) of the anti-idiotypic serum of the fifth bleeding of rabbit 8-55 with two fractions of the anti-*S. typhi* serum of the second bleeding of rabbit 8-03: a solution of IgM antibodies recovered from the specific precipitate of a macroglobulin preparation with the polysaccharide of *S. typhi* (IgM anti-PS), and a solution of IgG absorbed with the polysaccharide (IgG abs PS). It may be seen that a precipitation zone is continuous in front of these two antigen layers; this shows that the anti-idiotypic antibodies which react in this precipitation zone with a given idiotypic pattern do not distinguish between the IgM antibodies precipitated by the polysaccharide and the IgG not precipitable by the polysaccharide, which both carry the idiotypic pattern with which we are concerned.

whose idiotypic patterns are not found on the nonprecipitable antibodies, and (*b*) that there are also antibodies precipitable by the polysaccharide which carry the same idiotypic patterns as other antibodies nonprecipitable by the polysaccharide.

DISCUSSION

In view of the observation of idiotypic patterns which are, in one serum sample, peculiar to antibodies precipitable by the polysaccharide and, even more, of idiotypic patterns found indistinctly both in precipitable and nonprecipitable antibodies (Results, 3), the interpretation of this difference in precipitating properties needs to be discussed. When these two kinds of antibodies were distinguished (10), it seemed reasonable that, among the total antibodies against the somatic antigen, a part was directed against antigenic determinants

carried by the polysaccharide, while another part, which was not precipitated by the polysaccharide but by the unsplit somatic antigen, was directed against antigenic determinants carried by the rest of this antigen (11). The question became somewhat less simple when it was noticed that the precipitation, with the somatic antigen, of the antibodies which are not precipitated by the polysaccharide, may be inhibited by a sufficiently high concentration of this polysaccharide (12). An explanation of the two kinds of antibodies, different from that outlined above, might then have been considered possible. It is conceivable that these two kinds of antibodies might be directed against the same antigenic determinants present on the polysaccharide and the unsplit antigen, but that a part of these antibodies would not be precipitated unless the precipitating antigen would fulfill certain conditions realized in the somatic antigen and not in the polysaccharide. These conditions might, for example, have pertained to the solubility of the antigenic molecule, to its molecular size, or to the presence of a large number of repeating units of the same specific groups.

The possibility of an explanation which assumes that the specific groups against which the two kinds of antibodies are directed are the same does not necessarily follow from the inhibitory power of the polysaccharide. This is suggested by the observations made by Landsteiner and Van der Scheer that, in the precipitation reactions of hen ovalbumin with anti–hen ovalbumin rabbit sera, a similar inhibition may be exerted by cross-reacting ovalbumins of other species (13).

Even if the antibodies which are distinguished by the polysaccharide precipitation were directed against the same antigenic determinants, it is perhaps not very likely that they would have also the same affinity for these determinants or that their different behavior would be correlated only to their belonging to different immunoglobulin classes. In other words, it is not unlikely that these two kinds of antibodies which differ by their precipitating properties toward the polysaccharide, even those which carry the same idiotypic patterns, would differ by their function and consequently by some part of their specific structure. This possibility would be in agreement with a remark stated in the discussion of the preceding paper (1): that, in a given serum sample, the idiotypic heterogeneity is apparently more restricted than the heterogeneity of the antibody functions.[1] This remark led to thinking that the same idiotypic patterns might be shared by antibodies with different functions.[2]

[1] An indication in keeping with this remark might be supplied by the reaction of the S37 serum sample of rabbit 3-24, absorbed with the *S. typhi* polysaccharide, with the anti-idiotypic serum of rabbit 3-76. In agar, this reaction gives rise to two precipitation zones of extremely different densities (Fig. 7A), so that most of the precipitate appearing in the mixture of the same reagents may be very likely considered to be made of one idiotype of S37 and of the antibodies of serum 3-76 combined with it. Now the nitrogen precipitated in the reaction of 2 ml of S37 absorbed with the polysaccharide and 1 ml of anti-idiotypic serum 3-76 (also absorbed with the polysaccharide) amounts to 184 μg. It would seem therefore that the

It has been seen (Results, 1) that, among the anti-*S. typhi* antibodies of one given individual (rabbit 3-24), the number of idiotypes seems to increase as the immunization is longer and stronger. It may be useful to look for indications of the possible relationships between idiotypes that are individualized at successive stages of the immunization of a given individual. An indication may probably be sought in the observation of certain precipitation zones which are bifurcated when two successive samples of anti-*S. typhi* serum of one rabbit react side by side in the same gel with the same anti-idiotypic serum. Bifurcated zones of that kind were observed in the reaction of the anti-*S. typhi* sera of several rabbits (Figs. 2 and 8). In all cases in which this bifurcation was clearly observed, that is in three cases (in addition to the case of rabbit 3-24, discussed below), the single zone was observed in front of the anti-*S. typhi* serum of the earlier bleeding, of rank n, (serum "S(n)") and the double zone in front of the serum of the next bleeding, of rank $n+1$ (serum "S($n+1$)").

It is convenient to use symbols to designate the families of antigenic (or idiotypic) determinants which are involved in the single or double precipitation zones that constitute a bifurcation. In the serum S($n+1$), the antigen molecules which are precipitated in the second precipitation zone (that closer to the antigen source) carry a family or population y of determinants which are not present on the antigen molecules responsible for the first precipitation zone. If G_x is the antigen responsible for the first zone, whose determinants constitute a family or population x, the antigen responsible for the second zone is either G_y (y being a family or population of determinants different from x) or G_{xy} carrying, in addition to the determinants y, those which are also on G_x. In the latter case, we are concerned with a complex precipitating system (15). The antigen responsible for the single precipitation zone in front of S(n), or at least most of it, is G_{xy}, and carries the determinants of both families or populations x and y. To be rigorous in the interpretation of such reactions, the possibility must be mentioned that G_x exists also in S(n), but that its concentration, considered as a function of the concentrations of G_{xy} in the same serum sample, and of the anti-x and anti-y antibodies in the anti-idiotypic serum, is too small to give a precipitation zone distinct from that of G_{xy} (15). However, it is simpler and perhaps more plausible to assume that all the idiotype of S(n) involved in this reaction is G_{xy}.

Thus, it appears as if two families or populations of idiotypic determinants were carried by distinct molecules in S($n+1$) (or at least as if the determinants

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amount of the protein precipitated from 1 ml of S37 serum as a single idiotype would be in the range of 100 μg. It cannot be excluded that in the same amount of immunoglobulin more than one idiotype would be distinguished by other anti-idiotypic sera, but the reaction with serum 3-76 strongly suggests some common structure responsible for a common idiotypic specificity. The question is raised whether an amount of immunoglobulin in this range may be likely considered homogeneous from the standpoint of the structures which are related to antibody function.

[2] However great the variety of antigenic determinants of polysaccharides may be (14), the variety of determinants in protein antigens is perhaps even greater. It is expected that the discussion of the possible relationships between idiotypic specificities and antibody specificities against different antigenic determinants will take advantage of the studies, undertaken in this laboratory, of idiotypy of anti-protein antibodies.

of one of the two families were present on one idiotypic molecule without the others), while both families of idiotypic determinants would be carried by the same molecules in S(n).

If it is assumed that different idiotypes are synthesized by different cells, and that the cells which synthesize antibody molecules with common idiotypic determinants have a common origin, one is led to the further assumption that the cells which synthesize the idiotypes responsible for the two zones in the reaction of S(n+1) have a common origin.

It might be imagined that these two families or populations x and y of idiotypic determinants are carried, in the G_{xy} idiotype, by the two different polypeptide chains of the same immunoglobulin molecule and that, for unknown reasons, cells derived from the cell line which synthesized the G_{xy} idiotype synthesize only one of the same two polypeptide chains, the other chain being different in the new immunoglobulin synthesized. If such an explanation were to be general, the number of precipitation zones into which a single zone in the reaction of S(n) subdivides in the reaction of S (n+1) should not be larger than two. This would not be consistent with another reaction in which three precipitation zones in S(n+2) instead of two were apparently continuous with a single zone in front of S(n) (Fig. 8). It should be added that, in the former three cases of a bifurcated zone, S(n) was the serum of the first bleeding, made 13–17 days after the beginning of the immunization, while in the case of a trifurcated zone, the bleeding n, which was the 37th one of rabbit 3-24, had been collected 30 months after the first injection of *S. typhi*.

The compared idiotypic analysis of the sera of the 5th and 37th bleedings (S5 and S37) of rabbit 3-24 shows that the immunization continued for a long time, and resumed after a long interval, may provoke the appearance of new idiotypes. The long interruption of the immunization did not cause the irrevocable disappearance of any detected idiotype, since all those detected in S5 were also detected in S37. The possibility that other idiotypes of S5, which would be absent from S37, escaped detection, has been discussed in the Results. Since these observations were made on a single rabbit, it would probably not be wise to attribute general significance to them until they have been extended to other rabbits.

It will be supposed again that a distinct idiotype is a product of synthesis of a given cell line different from those which synthesize the other idiotypes. Thus, once a cell line has been committed to the synthesis of a given idiotype, it appears as if, in the case of rabbit 3-24, even after a long interval of rest, at the end of which the concerned idiotype would be no longer detectable, the same cell line would have survived and would have preserved its potential to synthesize this idiotype. Studies on allotypy have shown that the concentration of allotypically different immunoglobulins in rabbit serum seemed to depend on the number of cells committed to the synthesis of each distinct kind of immuno-

globulin, rather than on differences in average rates of synthesis or of turnover.[3] If it might be assumed that these conclusions apply also to the cellular synthesis of idiotypes, then the observations discussed would lead to the conclusion that, after the interruption of immunization, the number of cells which synthesized the idiotypes detected had decreased until the concentration of these idiotypes in the serum had become negligible. However, representatives of each of the cell lines committed to the synthesis of the various idiotypes would have survived in the organism. When the immunization was resumed, the surviving representatives of each of these cell lines would have multiplied actively, causing the reappearance of the corresponding idiotype in serum (S37) at a concentration similar to, or larger than, their concentration in S5 at the end of the previous immunization. An alternative possibility, different from the case which is apparently that of allotype synthesis, might be that the surviving cells would be perhaps numerous but would not synthesize antibody until the immunization is resumed. Then the appearance of antibody in the serum after the resumption of the immunization might be not only the result of the division of cells whose antibody synthesis had not been stopped, but also (and perhaps only) the result of synthesis by the same, previously inactive, cells.

The hypothetical conclusions of this somewhat speculative reasoning have to be compared to the conclusions of cellular studies. Experiments of transfer of cells from rats immunized against bacteriophage ΦX 174 "1½ to 15 months after primary immunization at a time when the level of circulating antibody had reached a plateau or was declining" to irradiated rats subsequently challenged by an injection of antigen have shown (19) that there is strong evidence that immunological memory is carried by small lymphocytes.

The appearance of "new" idiotypes, found in S37 and not in S5 would indicate that cell lines other than before would be committed to their synthesis. It is natural to wonder whether there is a relationship, or a common origin, between these new cell lines and the former ones, or whether they arose from a new stimulation of cells not belonging to the same population. It may be tempting to look for an answer to this question in the observation of the bifurcated or trifurcated precipitation zones discussed above. But this argument should probably not be extended to the new precipitation zones which show no continuity with the former ones under the form of a bifurcation or of a trifurcation.

A contrast should be noted between certain of the observations made in the comparison of S5 and S37 of rabbit 3-24 and certain observations in the comparison of S1 and S2 of rabbit 8-03. An anti-idiotypic serum (8-62) against S2

[3] This conclusion resulted from the comparison of the proportions of allotypically different molecules in serum and of the cells which synthesized them; these proportions were in remarkable agreement (16–18).

of rabbit 8-03 definitely precipitates S2, giving three distinct precipitation zones (Fig. 1) and does not at all precipitate S1, in gelled or in liquid media. However, S1 strongly precipitates the somatic antigen and polysaccharide of *S. typhi* (1), see Table I. These observations indicate that the anti-*S. typhi* antibodies of S1 of rabbit 8-03 are devoid of the idiotypic patterns detected by the anti-idiotypic serum 8-62 on the antibodies against *S. typhi* present in S2. It is not yet possible to know whether this contrast between observations in both cases on a single rabbit, is to be correlated with time between the beginning of the immunization and the first of the two bleedings.

On the basis of the above premises admitted by hypothesis (that the cells which synthesize antibody molecules with common idiotypic determinants have a common origin), it follows from the observation of the same idiotypic pattern in IgG and in IgM antibodies that the antibody molecules which carry the same idiotypic pattern in these two immunoglobulin classes were supposedly synthesized by the same cells or at least by cells belonging to the same cell line.

The results of cellular studies by other authors had already indicated that immunoglobulins belonging to these two classes were sometimes contained in, or secreted by, the same cells. In man, double staining by fluorescent anti-IgG and anti-IgM antibodies showed that immunoglobulins of both classes were present in certain cells (20). In rats immunized against *Salmonella adelaide* flagella, the antibodies able to immobilize the flagella, released by separated cells, were found by Nossal et al. to belong to both IgG and IgM classes in an appreciable proportion of cells (21). These double producer cells were observed "only at times when the switch from IgM to IgG antibody was occurring". This simultaneous double production by a few cells was therefore considered to be the sign of a more general successive double production such that the same cells that would have first synthesized IgM antibodies would thereafter synthesize IgG antibodies, after a period of transition, during which antibodies of both classes would have been synthesized (21).

A further analysis of findings, similar to those reported above, of the similar idiotypic patterns in IgM and IgG antibodies might supply an important clue to the understanding of what is common in the structure of IgM and IgG, especially if this analysis were to show that idiotypic determinants common to both classes of immunoglobulins are carried by the heavy polypeptide chains. It is not necessary a priori that the idiotypic determinants which are common to antibodies of both classes be carried by the heavy chains, since it is known that the same kinds of light chains enter the constitution of IgM and IgG.

It has been generally believed for a number of years that the heavy chains of the different immunoglobulin classes are quite different, because of the distinct isotypic specificity of the Fc fragments of papain digestion from immunoglobulins of different classes. This opinion had to be revised when the allotypic

patterns of the *a* group in the rabbit, which were known to be located in the Fab fragments of the IgG molecule, were, in this laboratory, detected also in the IgM (22); this observation led to the assumption that the part of the heavy chains which carried the said allotypic determinants is common to both γ and μ chains. A more elaborate hypothesis was to assume that the variable region of the immunoglobulin heavy chains of the various classes would be controlled by the same structural gene, the variability of structure of these regions being the result of somatic mutations (23). More recently, an overlapping was observed between the location, in the rabbit IgG heavy chains, of the amino acid residues that vary with antibody specificity and those which are different in $Aa1^+$ and $Aa3^+$ rabbits (24); variations in composition of nitrogen terminal peptides of rabbit heavy chains were also found to be related to allotypy (25). These findings imply that allotypic *a* determinants are located on the variable region of the heavy chain.

An important piece of evidence in the discussion of what is common to IgM and IgG, in addition to the light chain, would be obtained if it were found that the heavy chains are involved in the similarity of idiotypic specificities of IgM and IgG antibodies. The presence of the same allotypic *a* markers in IgM and IgG already suggested the control by the same structural gene of the structures which carry these allotypic markers in both immunoglobulin classes and which apparently involve the variable region of their heavy chains. The presence of the same idiotypic markers on the heavy chains (likely on the variable regions of the heavy chains) of IgM and IgG antibodies supposedly synthesized by the same cells would imply, in addition, a common structure of at least a part of their variable regions, and therefore a community of the events which led to the variability of the concerned part.

SUMMARY

The idiotypic patterns detected in the anti-*Salmonella typhi* serum of one given bleeding have been sought in other bleedings of the same rabbit. Idiotypic patterns carried by antibodies of the second bleeding are not always found in those of the first bleeding. Bifurcated precipitation zones in gels (double diffusion in cells) have been observed in the reaction of several anti-idiotypic sera with the sera of the first and second bleedings of three rabbits, and apparently indicate that idiotypic patterns which were carried by the same molecule in the first bleeding were carried by two separate molecules in the second bleeding. In a comparison of idiotypy of two anti-*S. typhi* serum samples of one rabbit, the collection of which had been separated by a very long interruption of the immunization, all the idiotypes detected in the early bleeding were also detected in the late bleeding; several idiotypes were detected in the serum of the late bleeding and not in that of the early bleeding.

Idiotypy has been observed and compared in IgM and in IgG antibodies.

There are, in each of these two classes, antibodies that are not distinguished from antibodies of the other class by anti-idiotypic sera, and consequently that carry the same idiotypic patterns.

A comparison has been made between the idiotypy of the two kinds of antibodies which, in anti-*S. typhi* sera, are precipitated and not precipitated by the polysaccharide. Certain idiotypic patterns are carried only by the antibodies of the first kind. There are also idiotypic patterns which are carried by antibodies of these two kinds, among which certain anti-idiotypic antibodies do not discriminate.

Possible cellular implications of observations made on idiotypy at different stages of the immunization of one individual have been discussed.

BIBLIOGRAPHY

1. Oudin, J., and M. Michel. 1969. 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. *J. Exp. Med.* **130:**595.
2. Oudin, J., and M. Michel. 1969. Sur les spécificités idiotypiques des anticorps de lapin anti-*S. typhi*. *C. R. Hebd. Séances Acad. Sci. Paris.* **268:**230.
3. Oudin, J. 1960. Allotypy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of seven main allotypes. *J. Exp. Med.* **112:**107.
4. Scheidegger, J. J. 1955. Micro-méthode d'immuno-électrophorèse. *Int. Arch. Allergy Appl. Immunol.* **7:**103.
5. Levy, H. B., and H. A. Sober. 1960. A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. Exp. Biol. Med.* **103:**250.
6. Kunkel, H. G. 1960. Macroglobulins and high molecular weight antibodies. *In* The Plasma Proteins. F. W. Putnam, editor, Academic Press, Inc., New York. **1:** 294.
7. Givol, D., S. Fuchs, and M. Sela. 1962. Isolation of antibodies to antigens of low molecular weight. *Biochim. Biophys. Acta.* **63:**222.
8. Burstein, M. 1956. Sur l'isolement d'une fraction des lipoprotéines sériques après précipitation par l'héparine ou par les héparinoïdes de synthèse. *C. R. Hebd. Séances Acad. Sci. Paris.* **243:**527.
9. Boivin, A., I. Mesrobeanu, and L. Mesrobeanu. 1933. Extraction d'un complexe toxique et antigénique à partir du bacille d'Aertrycke. *C. R. Séances Soc. Biol. Filiales.* **114:**307.
10. Hornus, G. J. P., and P. Grabar. 1941. Relations quantitatives entre l'antigène glucido-lipidique 0 et le haptène glucidique du bacille typhique et les immunsérums de lapin. *Ann. Inst. Pasteur (Paris).* **66:**136.
11. Staub, A.-M. 1961. Constitution chimique de quelques sites présents à la surface des enterobacteriaceae responsables de leur spécificité vis à vis des anticorps et des bactériophages. *Pathol. Microbiol.* **24:**890.
12. Grabar, P., and J. Oudin. 1947. Etude sur le polyoside O du bacille typhique et préparation d'un sérum de lapin spécifique de ce polyoside. *Ann. Inst. Pasteur (Paris).* **73:**627.

13. Landsteiner, K., and J. Van Der Scheer. 1940. On cross-reactions of egg albumin sera. *J. Exp. Med.* **71**:445.
14. Staub, A.-M. 1968. Bases chimiques de la spécificité des polyosides bactériens et des groupes sanguins. *Bull. Inst. Pasteur.* **66**:1111.
15. Oudin, J. 1948. L'analyse immunochimique qualitative; méthode par diffusion des antigènes au sein de l'immunsérum précipitant gélosé; deuxième partie. *Ann. Inst. Pasteur (Paris).* **75**:109.
16. Pernis, B., G. Chiappino, A. S. Kelus, and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. *J. Exp. Med.* **122**:853.
17. Oudin, J., and P. Bornstein. 1964. L'utilisation d'immunsérums d'animaux d'autres espèces que le lapin en vue de l'étude de l'allotypie des globulines γ du lapin. *C. R. Hebd. Séances Acad. Sci. Paris.* **259**:945.
18. Bornstein, P., and J. Oudin. 1964. A study of rabbit γ-globulin allotypy by means of hetero-immunizations. *J. Exp. Med.* **120**:655.
19. Gowans, J. L., and J. W. Uhr. 1966. The carriage of immunological memory by small lymphocytes in the rat. *J. Exp. Med.* **124**:1017.
20. Mellors, R. C., and L. Korngold. 1963. The cellular origin of human immunoglobulins (γ2, γ1M, γ1A). *J. Exp. Med.* **118**:387.
21. Nossal, G. J. V., A. Szenberg, G. L. Ada, and C. M. Austin. 1964. Single cell studies on 19S antibody production. *J. Exp. Med.* **119**:485.
22. Todd, C. W. 1963. Allotypy in rabbit 19S protein. *Biochem. Biophys. Res. Commun.* **11**:170.
23. Lennox, E. S., and M. Cohn. 1967. Immunoglobulins. *Annu. Rev. Biochem.* **36**:365.
24. Koshland, M. E. 1967. Location of specificity and allotypic amino acid residues in antibody Fd fragments. *Cold Spring Harbor Symp. Quant. Biol.* **32**:119.
25. Prahl, J. W., and R. R. Porter. 1968. Allotype-related sequence variation of the heavy chain of rabbit immunoglobulin G. *Biochem. J.* **107**:753.