THE SPECIFICITY OF SEROLOGICAL PRECIPITATION

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The process of serological precipitation is customarily described as a sequence of two phases: a rapid primary phase involving combination of antigen and antibody molecules and a slower secondary phase in which the initial molecular complexes aggregate. The progress of the second phase is typically manifested in the development of opalescence and in the formation and sedimentation of macroscopic particles of precipitate.

It is generally agreed that the primary phase is characterized by a high degree of serological specificity; indeed, the specificity of this phase may be considered the fundamental theorem of modern immunochemistry.

There is, however, no universal agreement regarding the character of the forces which operate in the secondary phase, most immunologists adhering to one or the other of two prevailing views. The first of these, which had its origins in the classical agglutination experiments of Bordet (1), holds that the primary antigen-antibody complexes aggregate with one another non-specifically, in the presence of electrolyte (2–4). The mechanism of aggregation is accordingly described in terms of a modification of electrokinetic (3) and solubility (5) properties of the suspended antigen and antibody as a consequence of combination. Recently, an alternative description has been proposed (6–8), according to which the entire course of serological precipitation and agglutination reactions is formulated in terms of specific acts of combination of antigen and antibody, rendering arbitrary the historical separation of these reactions into two phases. The ingenious arguments which have been marshalled in support of these views by their adherents have been adequately reviewed (6, 9, 10) and need not be reiterated here.

Prominent among the many experiments which have been devised to permit a decision between the opposing theories is one which may be referred to as the "mixing" experiment, in which two or more unrelated serological systems are combined and a comparison is made between the "mixed" system and the isolated systems with respect to kinetic and morphological features of the serological reaction in question.

In the case of cellular antigens, it has been possible to compare the rates of agglutination and, with optically distinguishable antigens, to investigate

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microscopically the constitution of the cellular aggregates (11). Acceleration of agglutination and cohabitation of serologically distinct antigens in a single clump are accepted as criteria of non-specific interaction.

At the present time there are available only two detailed publications of the application of the mixing experiment to precipitative systems. Hooker and Boyd (12) observed a definite acceleration of particulation in mixtures involving pairs of hemocyanins and their antisera. They interpreted this acceleration as an indication of interaction and assigned to non-specific forces a prominent rôle in the secondary phase of serological precipitation. Duncan (13), working with mixtures of crystalline hen ovalbumin and crystalline human serum albumin, confirmed and extended the observations of Hooker and Boyd. He found that mixed systems particulated *faster* than isolated systems containing the individual reagents in the same concentrations as in the mixture, but *slower* than isolated systems containing the individual reagents in double this concentration. Moreover, he carried out a mixing experiment with serologically pure precipitates prepared in bulk, the dispersed precipitates here replacing the antigen and antibody reagents, and, allowing the suspensions to reflocculate, observed an initial period in which interaction (acceleration) was absent, followed by a terminal period in which a definite interaction was apparent. On the basis of these results, Duncan contended that a specific mechanism was the primary aggregative mechanism in serological precipitation.

The present report is concerned chiefly with the description of mixed precipitation experiments carried out with the aid of a turbidimeter and a darkfield microscope. The results of these experiments, correlated with macroscopic observations of the terminal features of the precipitation reactions, fully confirm the contention of Duncan that a specific mechanism constitutes the major mechanism operative through the greater part of the historical second phase; and that non-specific mechanisms may be assigned a relatively minor terminal rôle. In addition, attention is directed to the usefulness of describing serological precipitation in terms of the formation and aggregation of elementary microscopic particles of specific precipitate, called *seromicrons*, the rôle of which has not previously been emphasized.

EXPERIMENTAL

Materials

Crystalline hen ovalbumin (EA) and the type-specific pneumococcal polysaccharide, SII,[1] were selected as antigens for study because of their lack of color and because the different morphological character of their specific precipitates permits macroscopic observations of a

[1] This material was kindly made available by Professor Linus Pauling from a supply generously provided by the Lederle Laboratories, Pearl River, New York.

sort which would not otherwise be possible. The anti-ovalbumin was a reconstituted lyophillized rabbit serum pool which was decolorized by extraction of the dried material with 2.0 M $(NH_4)_2SO_4$, followed by solution in water and dialysis against phosphate buffer (see below) in the cold. The antipolysaccharide was a negligibly colored refined concentrate of pooled rabbit antisera.[1] All stock solutions and dilutions were made with a 0.1 M phosphate buffer (PB), pH = 7.0, μ = 0.15, containing 0.01 per cent merthiolate (Lilly), and were filtered or centrifuged prior to use.

Methods

On the basis of constant-antibody optimal-proportions titrations by the particulation time method described below, three antibody-antigen ratios were selected for study in each system, one at optimal proportions and one in each of the excess regions. When mixtures of the two systems were involved, each system was in a comparable region of the titration zone. All experiments were carried out at room temperature, which was typically constant within 1°C. during the course of a single experiment. Additional technical details are given under the appropriate headings.

Turbidimetric Study of the Preflocculative Period

Mixing experiments were carried out with the aid of the Klett-Summerson photoelectric colorimeter, employed as a turbidimeter. The instrument was equipped with the No. 42 blue filter, which, according to the manufacturer, transmits between 400 and 465 mμ. Reaction mixtures, prepared as described below, were placed in cylindrical glass tubes designed for use with the instrument; the volumes of the reaction mixtures were 8.0 cc. Approximately 40 tubes were selected and calibrated over the desired range of turbidities with suitable barium sulfate[2] suspensions, with reference to one of the tubes selected as the standard. In general, the tube corrections were less than 12 scale units; in some of the experiments the tubes were selected so that the overall tube variation was approximately 2 scale units. The EA, SII, and anti-SII reagent blanks were typically 1.5 scale units or less; the anti-EA reagent blank, which was shown to be unaffected by precipitation, was approximately 12 scale units.

Preliminary tests of diluted suspensions of stable specific precipitates showed that the scale reading was not proportional to suspension concentration at high concentrations. In order to permit the arithmetic addition of the "turbidities" of two isolated suspensions for comparison with the turbidity of a third suspension, it was therefore necessary to prepare an empirical correction curve, which was established as follows:—

Mature EA specific precipitates, prepared over widely different antibody-antigen ratios, were diluted twofold and fourfold volumetrically. The scale readings for each of the three suspensions of a dilution set, corrected for tube and reagent blanks, were plotted on a log-log basis against the calculated relative suspension concentrations. These plots were experimentally parallel and linear in the range of study. One of the plots was chosen as reference and relative concentration values of 200, 100, and 50 were assigned to its three points. Concentration values for the remaining dilution sets were now found by shifting their plots along the concentration axis so as to superimpose them on the reference plot and reading off the concentration values on the arbitrary reference scale. These concentration values, which will henceforth be called *turbidities*, were plotted on ordinary paper against the corrected scale readings and the points were joined by a smooth curve to provide the desired empirical correction curve (Fig. 1). This curve was shown by experimental test to be satisfactory for numerous dilution experiments with mature EA precipitates, mature SII precipitates prepared in antibody excess, mixtures of mature EA and SII antibody-excess precipitates, and mature co-formed EA and SII precipitates in antibody excess and at optimal proportions.

[2] Shown to resemble the specific precipitates in its turbidimetric properties.

SII precipitates, prepared at optimal proportions and in antigen excess, could not be dispersed sufficiently to allow a test of the suitability of the empirical curve with these precipitates; and immature precipitates of this sort are not sufficiently stable for this purpose. In view of the demonstrated wide applicability of the empirical curve, these few technical shortcomings are not considered serious.

The use of the empirical curve in the preparation of a typical turbidity-time curve will be illustrated in detail. With volumetric pipettes, the required quantities of antigen solutions and of diluent are delivered into a lipped 6 inch test tube and the antibody solutions and diluent are delivered into the reaction tube. To start the reaction, the antibody solution is poured into the antigen solution, and the mixture is poured back into the reaction tube, which is

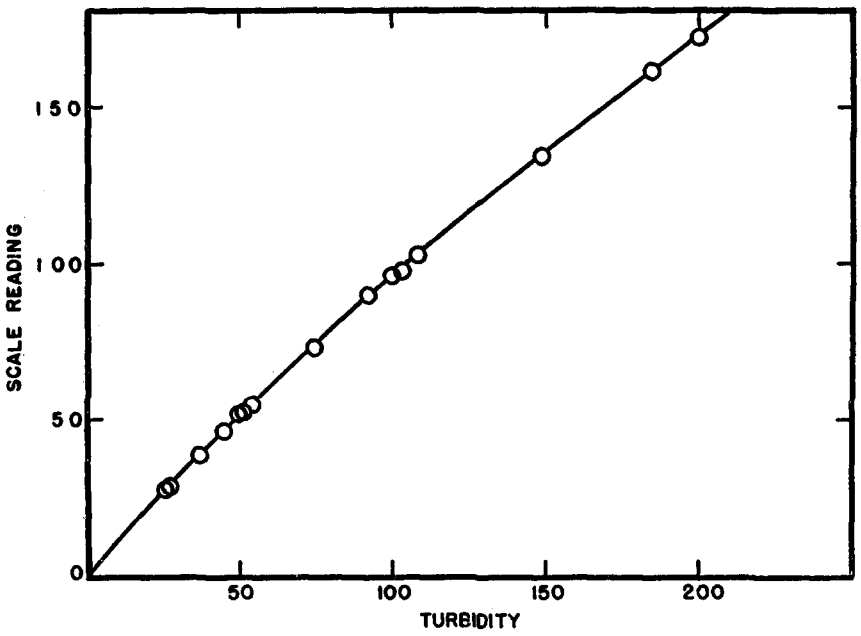

FIG. 1. Empirical correction curve for converting *scale reading*, corrected for tube and reagent blanks, to *turbidity*.

stoppered with a clean dry rubber stopper, inverted twice, wiped clean, and inserted in the turbidimeter; these operations require about 15 seconds. Readings, which refer to the standard tube set at zero with PB, are made every 30 seconds for 5 minutes, then every minute for about 10 minutes; then the tubes are gently set aside and read again at about 30 minutes. Occasionally, additional readings are made after several hours and after 24 hours. The scale readings are corrected for tube and reagent blanks and converted to turbidities by reference to the empirical curve (Fig. 1). The turbidity is then plotted against time on ordinary paper. In triplicate experiments conducted in this manner the average error is approximately ± 2 per cent during the first few minutes, when the scale readings are changing most rapidly, and approximately ± 1 per cent during the later minutes of the reaction.

It should be pointed out that, in view of the changing character of the developing precipitates, these turbidity-time curves are not amenable to simple interpretation in terms of

quantities of precipitate present during the course of a reaction; but are useful in making quantitative comparisons in the "vertical" direction; *i.e.*, at constant time. Indeed, it is this proposed use of the turbidity-time curves which gives the empirical curve (Fig. 1) its justification. All that is required to permit a comparison of the sum of the turbidities of two unrelated precipitating systems and the turbidity of a precipitating mixture of the two systems is that each of the precipitates is a member of the family to which the empirical curve is applicable. It has not been shown that the initially forming precipitates are members of this family, but it will be noted in Fig. 1 that the scale reading is approximately proportional to turbidity in dilute suspensions.

Table I shows a typical protocol for the mixing experiments. In numerous control experiments it was shown that neither of the heterologous reagents had a measurable effect on the turbidity-time curves in any region of the titration zone. This result was confirmed by ring tests and determinations of particulation time. The control data were therefore averaged

TABLE I

Type Protocol for Precipitative Mixing Experiments

Mixture	Addenda, 2.0 cc. each					
	In 6 inch test tube			In reaction tube		
1A	EA		PB	Anti-EA		PB
1B*	EA	SII		Anti-EA		PB
1C*	EA		PB	Anti-EA	Anti-SII	
2A		SII	PB		Anti-SII	PB
2B*	EA	SII			Anti-SII	PB
2C*		SII	PB	Anti-EA	Anti-SII	
3‡	EA	SII		Anti-EA	Anti-SII	

* Controls for heterologous addenda.

‡ Prepared in triplicate.

with the corresponding test data. In some experiments mixtures 1A and 2A were prepared in triplicate and the controls were omitted. Final reagent concentrations are given with the figures.

Six turbidimetric mixing experiments were carried out, two in each of the three characteristic regions of the antibody-antigen titration zone. In each experiment the two serological systems were in comparable regions of the zone. In most cases the greater part of turbidity production was complete within 15 minutes, and this time was approximately the minimum time in which particulation was visible with the naked eye in any of the systems (see below).

The results of these experiments,[3] shown graphically in Figs. 2 to 5, provide strong evidence that the preflocculative phase of precipitation in these systems is characterized by a strikingly high degree of specificity. It is well known that the turbidimeter is sensitive both to the concentration and the sizes of particles in a suspension (14). It might be argued, therefore, that a single turbidimetric identity is the fortuitous resultant of cancelling differences in these

[3] The data of two of the experiments provided no new information and are not shown.

quantities in different suspensions. When, however, the identity is observed in several systems and over a considerable period of time in each of these sys-

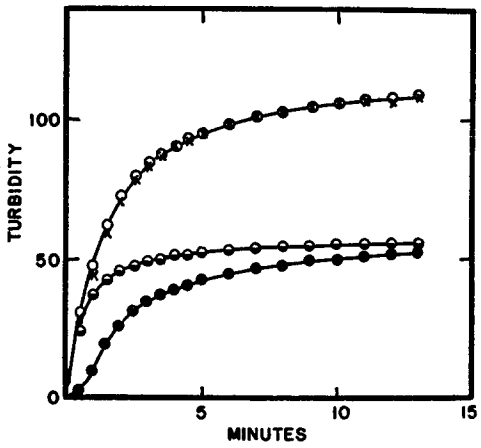

FIG. 2. Mixing experiment in antibody excess. Final reagent concentrations: ●, 1:80,000 EA; 1:12 anti-EA; ◒, 1:480,000 SII; 1:200 anti-SII; ×, 1:80,000 EA; 1:480,000 SII; 1:12 anti-EA; 1:200 anti-SII; ○, sum of EA and SII ordinates.

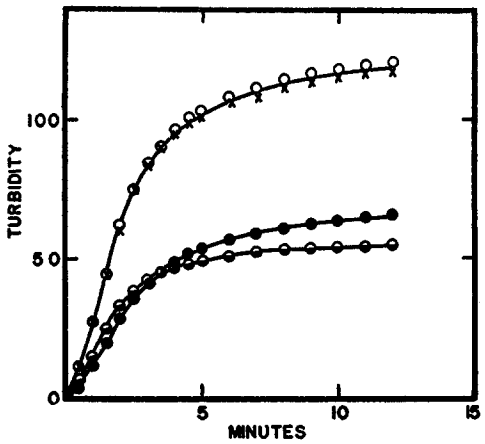

FIG. 3. Mixing experiment at optimal proportions. Final reagent concentrations: ●, 1:48,000 EA; 1:12 anti-EA; ◒, 1:320,000 SII; 1:400 anti-SII; ×, 1:48,000 EA; 1:320,000 SII; 1:12 anti-EA; 1:400 anti-SII; ○, sum of EA and SII ordinates.

tems, it cannot be justifiably dismissed as a coincidence. The simplest explanation of the present turbidimetric identities is that the serological integrities of the component systems are preserved in the mixtures, the particles of one system exerting no appreciable influence on the sizes and numbers of the particles of the other.

That the turbidimeter is indeed capable of detecting interaction in mixed precipitative systems is indicated by an experiment carried out with the EA system. Mixtures of EA and anti-EA were prepared at three characteristic

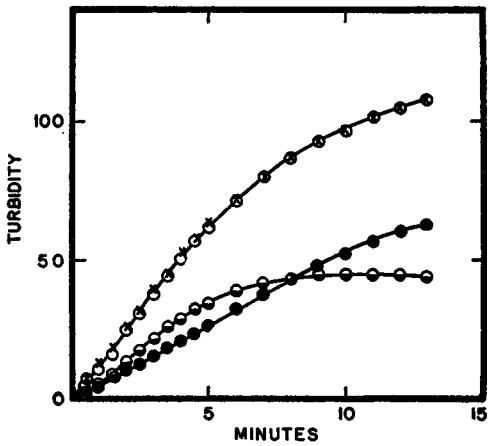

FIG. 4. Mixing experiment in antigen excess. Final reagent concentrations: ●, 1:24,000 EA; 1:12 anti-EA; ◒, 1:80,000 SII; 1:400 anti-SII; ×, 1:24,000 EA; 1:80,000 SII; 1:12 anti-EA; 1:400 anti-SII; ○, sum of EA and SII ordinates.

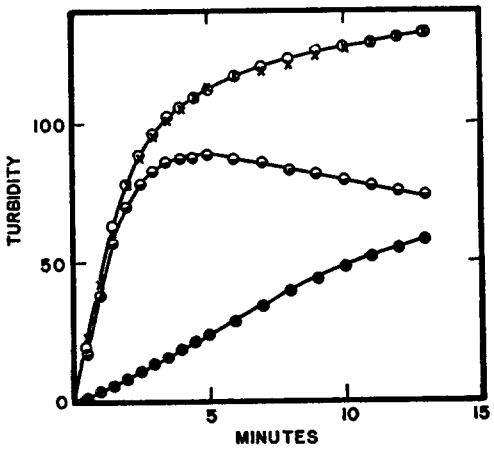

FIG. 5. Mixing experiment in antigen excess. Final reagent concentrations: ●, 1:24,000 EA; 1:12 anti-EA; ◒, 1:40,000 SII; 1:200 anti-SII; ×, 1:24,000 EA; 1:40,000 SII; 1:12 anti-EA; 1:200 anti-SII; ○, sum of EA and SII ordinates.

ratios of antibody to antigen and at three absolute concentration levels, in the ratio 1:2:4, at each antibody-antigen ratio. Turbidity-time curves were prepared for these mixtures in the usual manner. To facilitate comparison, the recorded turbidities were multiplied by the appropriate dilution factor, 4, 2, or 1, and the results for the set at optimal proportions taken as an example

are presented in this compensated form in Fig. 6. The other systems behaved in a similar manner.

Referring to this figure, it is apparent that the rate of development of turbidity is far from being proportional to the concentration of the reaction mixture (absence of superposition); *i.e.*, the three mixtures of the set do not behave as if they were simply dilutions of the same sort of suspension.

The experiment may be regarded in the following way. Let us suppose that the system at highest concentration consists of two equal portions of non-

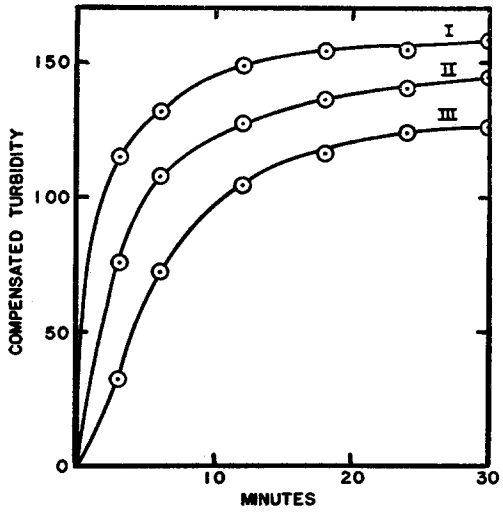

FIG. 6. Effect of absolute concentration on the development of turbidity in EA: anti-EA mixtures at optimal proportions. Final reagent concentrations in reaction mixtures: I, 1:24,000 EA, 1:5 anti-EA; II, 1:48,000 EA, 1:10 anti-EA; III, 1:96,000 EA, 1:20 anti-EA. For meaning of *compensated turbidity*, see text. (This experiment was carried out with a preparation of decolorized anti-EA which was slightly weaker than the preparation used in the previous experiments.)

crossreacting but otherwise equivalent antigens, EA_1 and EA_2, and two equal portions of non-crossreacting but equivalent antibodies, anti-EA_1 and anti-EA_2, the subscripts denoting serological type. In the hypothetical absence of interaction, system$_1$ and system$_2$ precipitate independently, but equivalently, and the mixed system is turbidimetrically identical with the sum of the two postulated component systems; *i.e.*, its curve is the intermediate curve (II) of Fig. 6. Admitting now the true crossreacting potential of system$_1$ and system$_2$, the upper curve (I) of Fig. 6 is found. The difference between the upper and intermediate curves may therefore be regarded as an index of interaction. In mixtures of systems which do not have the high crossreacting potential of the systems postulated here for the sake of argument, the inter-

action would become smaller and smaller as the crossreacting potential approached zero. If a mixing experiment is conducted with two systems known to be serologically unrelated and a turbidimetric identity is found between the mixture and the sum of the components, it must be concluded that experimentally there has been no interaction. The foregoing argument is independent of a distinction between specific and non-specific mechanisms of interaction.

Particulation Time in Mixed Precipitative Systems

The determination of the time required for particulation, as judged by the naked eye, offers a convenient means of comparison of the rates of precipitative reactions. Mixtures comparable to those studied in the previous section were prepared by adding 0.9 cc. antibody solution to 0.9 cc. antigen solution in a Wassermann tube; the contents were mixed thoroughly by tapping and were poured into a 75 mm. × 8 mm. (I.D.) tube. The time of first discernment of particles with the naked eye, with the aid of indirect illumination, was noted. Controls for the effect of heterologous reagents were negative.

The results, which are presented in Table II, indicate a strong terminal interaction in antibody excess, a weaker interaction at optimal proportions, and no appreciable interaction in antigen excess. The interaction in antibody excess and at optimal proportions was more striking in the period following the earliest visible particulation, being manifested as a more rapid growth of particles and a more rapid sedimentation. Occasionally, a slight interaction was observed in antigen excess in the postparticulation period. It is urged that more reliance be placed on comparative descriptive statements than on recorded particulation times. It was found difficult to compare two systems of different turbidities during the threshhold period of visible particulation; and it is not certain that the mixed precipitative systems showed an acceleration in this early period.

Macroscopic Observations on the Resuspension Characteristics of Mature Precipitates

The precipitates which formed in the experiments described in the preceding sections were allowed to stand at room temperature for 1 or 2 days. After this time the tubes were inverted carefully once or twice and the precipitates were examined with the naked eye. The results of these observations are summarized as follows:–

1. EA precipitates, in all proportions of antibody to antigen, suspended readily and evenly, dispersing into subvisible particles.

2. SII precipitates in antibody excess suspended readily, showing little tendency to adhere to the glass, giving large particles which dispersed into smaller, barely visible particles with moderate shaking. The medium around these particles was decidedly turbid.

At optimal proportions and in antigen excess these SII precipitates adhered firmly to the glass. With vigorous shaking they suspended as large persistent particles, distributed in a moderately clear medium. In antigen excess the precipitates dispersed as extremely large disks and membranes.

3. In antibody excess and at optimal proportions co-formed EA-SII precipitates suspended readily and evenly as subvisible particles.

TABLE II

Particulation Time in Mixed Precipitative Systems

Region	Final reagent concentrations				Approximate particulation time*
	EA	SII	Anti-EA	Anti-SII	
					min.
	1:80,000		1:12		35
Antibody excess		1:480,000		1:200	35
	1:80,000	1:480,000	1:12	1:200	23
	1:48,000		1:12		18
Optimal proportions		1:320,000		1:400	23
	1:48,000	1:320,000	1:12	1:400	15
	1:48,000		1:12		17
Optimal proportions		1:160,000		1:200	15
	1:48,000	1:160,000	1:12	1,200	13
	1:24,000		1:12		60
Antigen excess		1:80,000		1:400	60
	1:24,000	1:80,000	1:12	1:400	60
	1:24,000		1:12		60
Antigen excess		1:40,000		1:200	45
	1:24,000	1:40,000	1:12	1:200	45

* Duplicate and triplicate determinations. Recorded values are not reliable but the three values of a given mixing experiment may be compared with one another (see text).

In antigen excess, however, the mixed precipitates suspended in two forms, one portion which dispersed readily as subvisible particles and another which suspended readily as large particles, or, indeed, as a single coherent disk.

These results indicate a definite terminal interaction in mixed precipitative systems in antibody excess and at optimal proportions. In antigen excess the interaction is apparently minimal, the mixed precipitate segregating into two morphologically distinguishable types, which bear decided resemblances to the precipitates developing in the corresponding isolated component systems.

Microscopic Study of the Preflocculative Period in Mixed Precipitative Systems

The foregoing experimental results suggested the notion that the historical second phase of serological precipitation could itself be described as a combina-

tion of two distinct processes: the formation of elementary microscopic particles of specific precipitate, and the aggregation of these particles. Such a distinction appeared necessary in order to account for the high degree of specificity which appeared to prevail during the initial period of visible precipitation and the lower degree of specificity which was observed in the terminal period of this process.

Darkfield microscope studies of the precipitative systems already described were therefore carried out. It is not the purpose of the present report to give a detailed kinetic and morphological description of the early stages of specific precipitation, but merely to outline a few salient features which are pertinent to the present question.

Fresh antigen-antibody mixtures, comparable to those already described (Table II), were transferred to a glass microcataphoresis cell, with optically plane surfaces, which was placed on the stage of a Spencer binocular research microscope fitted with a substage darkfield diaphragm. In some experiments a Bausch and Lomb darkfield microscope equipped with a paraboloid darkfield illuminator was used. Observations were made at 100, 210, and 440 magnification.

As soon as the preparations could be placed under observation (approximately 20 seconds), the field appeared homogeneously turbid. Within a minute or two, depending on the system, the background assumed a definitely granular appearance and presently numerous minute particles, all in Brownian movement, could be distinguished. The particles grew in size, as judged by the increase in intensity of the illumination scattered from them and later by their distinguishable outlines; it is not possible at present to say how much this growth was compensated by a decrease in the original numbers. Aggregation of these particles became apparent within 5 minutes in most of the systems; in antigen excess all of the phenomena were considerably slowed. The component particles of the aggregates could be readily distinguished at all times and appeared to be definitely spherical, with diameters, when the reactions were approximately complete, in the range 0.1 to 1μ. This range approximately covers the particle sizes in all systems and is not intended to indicate the distribution of sizes in any given system.

Since the present evidence strongly supports the thesis that these elementary particles are elaborated through the agency of specific immunochemical forces and since they must henceforth be considered in any complete description of the process of serological precipitation, it is felt desirable to give them a name, *seromicrons*, to imply "microscopic particles of specific precipitate."

Preliminary experiments showed that under given conditions of observation the time of first discernment of these particles in darkfield, the *seromicron formation time*, was a readily measured constant for a given precipitative system. Mixing experiments involving the usual materials were therefore conducted and the seromicron formation time was used as a criterion of inter-

action. The results of one of these experiments, carried out in antibody excess, are given in Table III. Heterologous controls were negative. The table also shows results of macroscopic particulation time determinations made with the same mixtures in the manner previously described in this report.

The results strongly suggest that interaction is minimal in the early period of precipitation and that the wave of seromicrons which appeared at 107 seconds in the mixed precipitative system was a wave of SII seromicrons. Observation of a second wave at approximately 200 seconds, assuming that such a wave did occur, was not possible under the conditions of the present experiment, because of the presence of large numbers of particles at this time. On the other hand, the results point to a definite interaction in the flocculative period, the interaction becoming more and more pronounced (increased sedimentation rate, etc.) as the reaction approached termination.

TABLE III

Comparative Seromicron Formation Times and Particulation Times in a Mixing Experiment in Antibody Excess

Final reagent concentrations				Seromicron formation time*	Approximate particulation time‡
EA	SII	Anti-EA	Anti-SII		
				sec.	*min.*
1:80,000		1:12		197	38
	1:960,000		1:400	103	53
1:80,000	1:960,000	1:12	1:400	107	26

* Triplicate determinations with average error of approximately ±4 per cent.

‡ Triplicate determinations. Recorded values are not reliable but may be compared with one another (see above).

Following the experimental line laid down by Duncan (13), a mixing experiment was carried out with well flocculated EA and SII precipitates, formed in mixtures equivalent to those employed in the "simultaneous" determination of seromicron formation time and particulation time. The precipitates were dispersed homogeneously by shaking and equal-part mixtures of each with buffer and with each other were allowed to reparticulate. The results indicated a definite interaction, becoming more and more pronounced, in these mixtures of well formed serologically unrelated precipitates.

DISCUSSION

The results presented in the foregoing experimental section support the thesis that the historical second phase of serological precipitation can no longer be regarded as a unit serological process; indeed, it is likely that the customary partition of the precipitation process into primary and secondary phases has ceased to be of utility and must be abandoned. Likewise it appears that the

traditional analogy of the cellular antigen with the molecular antigen must be discarded in favor of a new analogy with the seromicron.

No attempt will be made to explain why supporters both of the non-specific theory and of the framework (lattice, alternation, etc.) theory have persisted in regarding the so called second phase of precipitation as a single process and inquiring of it, as a whole, whether it is specific or non-specific; but it seems abundantly clear that this procedure has served to perpetuate a needless controversy, for both answers are to be found.

The advantages of recognizing the multiple morphological character of specific precipitation are evident, for on this basis a simple description of serological precipitation, which accounts for all of the pertinent facts, may be proposed.

When homologous antigen and antibody molecules are mixed, the primary molecular complexes which form combine specifically with each other and with free antigen and antibody molecules. By a repetition of specific acts of combination, larger and larger molecular complexes are elaborated. Presently, when the complexes are of such a size as to provide serious optical discontinuities in the reaction system, they are detected in dark field as minute light-scattering particles, *seromicrons*. The seromicrons[4] continue to grow specifically, presumably both by the addition of small molecular complexes and free reagent molecules to their surfaces and by specific aggregation with particles of comparable size. There is no evidence of discontinuity in the primary growth process.

Aggregation of seromicrons presumably plays a minor rôle in the development of the precipitate throughout the short initial growth period. The accretion process becomes progressively slower as the supply of small complexes and free molecules is reduced and presently the aggregation of seromicrons assumes a dominant rôle. To the extent that the aggregative process results in measurable turbidimetric change, the results suggest that the initial aggregation of seromicrons is mediated by a highly specific mechanism, for aggregates are discernible in dark field during the period of turbidimetric identity. It is possible that non-specific forces intrude seriously only when the aggregates are relatively large and are able to cling to one another whenever they make contact in the proper situations (16). Further microscopic and turbidimetric experiments should be brought to bear on the question of the incidence of non-specific forces in the precipitation process, but for the moment they may provisionally be relegated to a relatively minor rôle in the terminal period.

The foregoing description does not imply that antigen and antibody will always precipitate in an arbitrary milieu. While the possibility of framework formation must be given, the specific mechanism need not be sufficient to bring

[4] For an example of the possible appearance of seromicrons in electron micrography, see reference 15.

precipitation to completion under unfavorable circumstances such as might be caused by excess of antigen or antibody, univalence of either reagent, specific hapten, complexes of antibody with non-specific serum proteins, inordinate temperatures, or inadequacy of the lipid, H ion, or electrolyte concentration (6, 7, 9, 10, 17).

SUMMARY

Precipitative mixing experiments have been conducted with the aid of a turbidimeter and a darkfield microscope. The results of these experiments, correlated with observations of macroscopic features, indicate that serological precipitation is largely governed by a highly specific mechanism except in the terminal period, when the operation of non-specific forces becomes apparent. The findings are used as the basis for a new description of precipitation in terms of the formation and aggregation of elementary particles of specific precipitate, called seromicrons.

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