Precipitation Patterns of Normal and Pathologic Blood Sera with Cationic Detergents. (18810)

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Cationic detergents of the quaternary-ammonium-salt-type form precipitating complexes with serum proteins which are soluble in an excess of either the protein or the detergent. The zone of precipitation, the so-called equivalence zone, varies with the different detergents(1-3). Similarly, the intensity of precipitation depends upon the concentration of the reacting protein. Thus characteristic peaks of precipitation are observed which broadly indicate the maximum interference, by the detergent, with the activities of certain enzymes, especially with oxidative processes(4).

Under normal physiological conditions the concentration of the proteins, as well as the other components of serum capable of reacting with detergents, remain constant within narrow limits and one would therefore expect relatively slight variations in the intensity of precipitation taking place when various samples of normal serum are treated with cationic detergents. Considerable differences in serum protein concentrations are known to exist under certain pathological conditions however, which may appreciably influence the reaction between serum and detergent. Since it seemed to us that such possible variations in the degree of precipitation could be characteristic for certain diseases, we have investigated whether physiological or pathological changes in the composition of the serum influence the rate and intensity of the serum protein precipitation when treated with a cationic detergent under standard conditions.

Experimental. To decreasing amounts of blood serum made up to 0.5 ml volumes with 0.9% NaCl solution were added 0.1 ml amounts of different concentrations of cationic detergent. The ensuing turbidities were measured at various intervals of time. *Blood serum**: Non-hemolytic blood serum from normal persons and from patients suffering from various diseases were used. *Cationic detergents*: The preliminary tests were carried out with the following compounds: Bradosol(5-7), (β-phenoxy-ethyl-dimethyl-dodecyl ammonium bromide); Zephiran(8), (alkyl-dimethyl-benzyl ammonium chloride); CTAB (9,10), (cetyl trimethyl ammonium bromide). In identical concentrations, the 3 compounds produced with serial dilutions of the same normal blood serum samples different precipitation patterns and turbidities with precipitation peaks at differing serum dilutions. With CTAB only a very slight precipitation was observed in all dilutions of serum, the turbidities obtained with Zephiran were more pronounced, while Bradosol gave a series of progressive turbidities in a characteristic and distinctive precipitation pattern (Table I).

Test procedure. In all experiments a broad range of various serum dilutions was used in order that the full range of precipitation, its pattern and the role of interfering

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TABLE I. Turbidity of Normal Human Blood Serum Measured .5, 2, 5, 10, 30 and 60 Min after Addition of 1% Bradosol.

Time in min	Ratio of serum to saline solution 10:0	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
.5	0*	0	0	0	1	2	3	4	2	1
2	0	1	1	1	2	3	4	4	2	1
5	2	2	3	3	4	4	5	5	3	2
10	4	4	5	5	5	6	6	5	4	2
30	5	5	5	5	5	6	6	6	5	2
60	5	5	5	5	5	6	6	6	5	2
120	5	5	5	5	5	6	6	6	5	2

* These values express various degrees of turbidity, 0 denoting no turbidity and 6 maximum turbidity. Relationship of these values to Lumetron readings is shown in Table II.

TABLE II. Turbidity Factors. Density of the standard serum-talcum suspensions of tubes 1 to 7. Measured in Lumetron Colorimeter, with 420 filter.

Density	Turbidity factor
<.3	0
.38	1
.64	2
.87	3
1.11	4
1.35	5
1.64	6
1.89	7

factors could be studied. For practical purposes, however, the following procedure was adopted: 0.5, 0.45, 0.35, 0.3, 0.25 ml amounts of serum were introduced into small test tubes (7 x 0.8 cm) and the volume made up to 0.5 ml with 0.9% sodium chloride solution. After shaking, 0.1 ml amounts of 1% Bradosol in 0.9% NaCl solution were added and the tubes again shaken. Thirty seconds, 2 and 5 minutes after the addition of Bradosol the turbidities were measured. All reactions were carried out at room temperature. *Measurement of turbidities.* Immediately upon the addition of the detergent, a turbidity is produced in certain tubes which progressively increases within the next few minutes. Since under these circumstances it was impossible to measure accurately the turbidities at 30″, 2′ and 5′ intervals by means of photo-electric apparatus, we have estimated by eye the turbidities occurring in the serum tubes, with turbidity standards, the standards consisting of various amounts of talcum suspended in serum so as to correspond to the photo-electric readings as shown in Table II.

Results. 30 seconds after addition of the detergents, undiluted normal serum (tube 1) and dilutions contained in tubes 2 to 4 remained clear, but considerable turbidity had formed in the last 2 tubes. Within a few minutes all tubes showed progressive turbidities and precipitations. The precipitation pattern of sera obtained from certain pathological cases, however, presents two consistent variations from the normal serum pattern described above. Thus the following turbidity (or precipitation) patterns can be distinguished:

1. *Normal turbidity pattern.* Observed with sera from normal patients and illustrated by the turbidity sequence shown in Table III, upper part. This pattern is constant.

2. *Abnormal turbidity patterns.* Certain sera samples obtained from patients with pathological processes such as nephrosis, jaundice, cancer, pulmonary infarction, gave either of the following turbidity patterns: a. *Abnormal turbidity pattern with heavy precipitation.* In this type even undiluted serum responds to the addition of Bradosol with the immediate development of a very heavy precipitate. As shown in Table III, middle section, very slight or no progressive precipitation is observed. b. *Abnormal turbidity pattern without any precipitation.* Certain sera, upon addition of 1% Bradosol, yielded no or very insignificant precipitation in all the dilutions tested. In some cases all tubes remained clear during the entire observation while in a few instances faint turbidities developed after 2 to 5 minutes. This pattern

TABLE III. Turbidity Patterns. Turbidities measured 30 sec, 2 min and 5 min after addition of 1% Bradosol. Factors according to Table I.

	Time	Tube No. 1	2	3	4	5	6
1. Normal	30 sec	0	0	0	0	1	2
	2 min	1	1	2	2	3	4
	5 ″	2	2	3	3	4	5
2a. Abnormal	30 sec	7	7	7	7	7	7
with heavy	2 min	7	7	7	7	7	7
precipitation	5 ″	7	7	7	7	7	7
2b. Abnormal	30 sec	0	0	0	0	0	0
without any	2 min	0	0	0	0	0	1
precipitation	5 ″	0	0	0	0	1	1

TABLE IV. Types of Precipitation Obtained with Various Plasma Fractions and Bradosol.

Protein, Armour & Co.	Conc., %	Reaction with 1% Bradosol
Bovine albumin	5.6	No precipitation
Fraction IV4	5.6	" "
β globulin	1.75	Strong " , equal to precipitation shown in Table III, 2a
γ globulin	1.75	= β globulin
Fibrinogen	.6	Extremely strong precipitation, stronger than precipitation shown in Table III, 2a

was characteristic for icteric sera and is shown in Table III, lower portion. Intermediate reactions between the normal and abnormal patterns were observed with certain sera and it was, especially in the earlier stages of this investigation, rather difficult to classify a particular reaction with either the normal or abnormal pattern.

Mechanism of reaction. Efforts were made to explain the reasons for these variations. 1. *Influence of the Bradosol concentrations on the precipitation of normal sera.* The addition of 0.1 ml amounts of 2% Bradosol solution to the serum dilutions immediately produced a heavy and rapid precipitation in all tubes including those containing undiluted serum whereas a 1% solution of Bradosol yielded a progressive precipitation and the distinctive pattern described above; lower concentrations (0.5% and 0.1%) yielded only faint turbidities. 2. *Influence of the serum concentration.* Immediately upon the addition of 1% Bradosol solution to undiluted serum from normal individuals, little or no turbidity developed, but as the serum became more diluted, a progressively increasing turbidity occurred reaching a maximum at a dilution of 1:2. In dilutions of 1:8 and higher, the degree of precipitation decreased. 3. *Reaction between Bradosol and the various normal serum constituents.* The following protein fractions were tested with 1% Bradosol. Each protein was dissolved in 0.9% NaCl solution to yield concentrations approximately similar to their concentrations in normal blood-serum and these solutions were diluted as described in the section on methods. From these experiments it may be inferred that the turbidity and precipitation of blood serum-detergent mixtures are associated mainly with the presence of globulins and fibrinogen. Determinations of pH values of the various serum-detergent mixtures indicated that the values remained constant within the dilution series of the individual sera and no significant differences were observed between normal and abnormal sera. The pH range found fell between 7.12 and 7.82. 4. *Abnormal precipitation patterns.* It seems probable that the pattern characterized by heavy precipitation is due either to a lowering of the serum protein concentration or disturbances in the normal albumin-globulin-fibrinogen ratio. Another factor responsible for immediate and heavy precipitation in certain pathological sera is the presence of increased amounts of lipids. Positive reactions were obtained in normal lipemic sera which, in the absence of lipids, gave the normal precipitation pattern.

Absence of any precipitation (abnormal pattern b) was observed in sera obtained from patients with jaundice. This form of reaction occurred also in icteric sera of nephrotic or carcinoma patients in which according to previous experience we would have expected a heavy precipitation. Ox-bile and sodium desoxycholate added to normal and strongly precipitating sera in concentrations of 2% to 0.5% completely prevented precipitation of blood serum proteins. Concentrations of desoxycholate below 0.1% also gave evidence of interfering activity. Like ox-bile(11, 12), Suramin(13, 14), Tamol N(15), soap, and sodium laurylsulfonate(3) strongly interfered with serum precipitation. All these substances are known to counteract the germicidal activity of cationic detergents. It

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is possible to titrate the concentration of precipitation-preventing substances in icteric sera by adding decreasing amounts of icteric serum to tubes containing dilutions of normal serum. One icteric serum (G.) prevented the Bradosol precipitation of the normal serum even when added to yield a final concentration of 1:4. Since a similar suppression was obtained in presence of 0.2% sodium desoxycholate, this particular icteric serum contained a precipitation-preventing factor corresponding to the strength of 0.8% of a sodium desoxycholate.

Comparison between the cationic detergent-precipitation and the coagulation of blood serum by heat. Variations in the precipitation pattern seem to be due to qualitative and quantitative changes in protein concentration. The "heat coagulation test" of serum as studied by Mayer(16), Rosenow(17), Glass(18), Huggins(19, 20), and others is based upon similar changes in the protein concentration. We have compared the results of the heat coagulation tests, performed as originally described by one of us (R.L.M.) with the precipitation test. In general, the correspondence between both tests was good, especially with normal sera and with certain samples of sera from various diseases which gave a normal precipitation pattern and a normal coagulation at 75 to 77°C. Many sera with abnormal precipitation patterns also had an elevated heat coagulation point. But we have encountered various sera in which the precipitation test gave an abnormal pattern while the serum coagulated at the normal temperature range and vice versa. We are not able at this time to explain many of these discrepancies. A similar lack of correlation has been observed also in icteric sera from nephrotic or cancer patients, with normal precipitation patterns but abnormally high coagulation points. We believe that here increased amounts of bile salts were responsible for these discrepancies. Bile salts do not interfere with the heat coagulation tests but, as shown above, strongly affect the precipitation test which is highly sensitive to an increase in bile salts content.

Discussion. The precipitation and the heat coagulation reactions are by their very nature "unspecific." From our earlier work(16) on heat coagulation we concluded that abnormal coagulation temperatures are indicative of certain disturbances in the serum protein balance but are not specific for any particular disease, in spite of the fact that sera from various disease processes, such as cancer or nephrosis, coagulate more frequently at temperatures well over 79°C than did sera from other pathological conditions. Nevertheless, heat coagulation tests have temporarily been advocated as a specific diagnostic procedure especially as a cancer test(18, 19).

The precipitation patterns are influenced by certain secondary factors as for example, the presence of increasing amounts of bile salts or lipids, which do not affect the heat coagulation of serum. Under these conditions the simultaneous exploration of both reactions in a particular case may constitute a more sensitive tool than either of these methods alone, and the confrontation of the results of both the heat coagulation and precipitation reactions may enable the clinician to eliminate and interprete certain "false normal" precipitation reactions(21).

Summary. Normal human blood serum forms a precipitate upon the addition of cationic detergents, particularly Bradosol, when tested in progressive serum dilutions up to 1:2 with a characteristic pattern of increasing turbidities. Sera from patients suffering from various diseases show entirely different patterns. This reaction may constitute a helpful clinical laboratory test.†

† After this paper was submitted for publication, two articles by R. F. Jacox *et al.* appeared in *J. Lab. and Clin. Med.*, vol. 37, 721 and 728, 1951, on a similar reaction with a cationic detergent, Octab.

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