THE RELATION OF CEPHALIN FLOCCULATION AND COLLOIDAL GOLD REACTIONS TO THE SERUM PROTEINS [1]

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Precipitating agents of many varieties are employed to study the proteins of the blood in health and disease, giving rise to such reactions as the Takata-Ara, the Weltmann, the formol gel, and the albumin-globulin partitions by salting-out methods. More recently, the stability of certain colloidal suspensions in dilute serum has been used for a similar purpose. For example, it has been shown that emulsions of cephalin and cholesterol or colloidal gold sols are readily precipitated when mixed with the serum of patients suffering from hepatitis and allied disorders of the liver (1, 2). These substances may therefore be used in tests for the recognition and study of certain hepatic diseases.

The present report deals with the reaction of cephalin-cholesterol emulsions and colloidal gold suspension with various fractions of normal and pathological sera as obtained by electrophoretic separation. Positive colloidal gold reactions have been attributed for many years to the serum globulins (3) and more recently the gamma globulin fraction of cerebrospinal fluid has been shown to give positive reactions (4). This work was undertaken to determine whether both positive cephalin-cholesterol and positive colloidal gold reactions which show clinical similarities depend upon related factors in the serum proteins.

EXPERIMENTAL

In this study, sera were selected on the basis of the cephalin flocculation test. Among those giving positive reactions were sera from 4 cases of cirrhosis; 2 of hepatitis; 2 of secondary syphilis, 1 of which was suffering from arsenical encephalitis; and 2 of sulfonamide intoxication. Controls giving negative reactions were sera from 4 patients with non-hepatic disorders and 4 healthy volunteers. Data on 2 normal sera, examined after 5 and 6 months respectively in the ice-box, are also included, as well as data on normal rabbit serum.

The electrophoretic analyses and separations were carried out in the Tiselius apparatus (5). A short description of the technique used is given in (4). For determination of the percentage composition, the sera were diluted 1:4; but for separating various fractions, undiluted sera were frequently used. All sera were dialyzed against 0.15 M NaCl + 0.02 M phosphate buffer at pH 7.3 to 7.4, using several changes of buffer. Photographs of the migrating boundaries were taken, using the Longsworth scanning technique (6). The mobility and percentage of each electrophoretic component were calculated in the usual manner, the former from the distance moved between exposures, and the latter from the area of each component.

The cephalin flocculation tests were performed as described by Hanger (1), 0.2 ml. of whole serum (or an amount of a separated fraction present in 0.2 ml. of whole serum) being added to 4 ml. of saline and 1 ml. of cephalin emulsion. The tubes were shaken and allowed to stand at room temperature for 48 hours. Readings were made and the degree of flocculation of the cephalin emulsion recorded as 0 to ++++.

The colloidal gold tests were carried out in the usual manner, by addition of 2.5 ml. of colloidal gold solution to 0.5 ml. of serum dilutions or dilutions, in 0.4 per cent NaCl, of separated fractions of 1:10, 1:20, 1:40 . . . 1:20,480. The tubes were allowed to stand at room temperature and read after 24 hours. The color changes were expressed by numbers as follows: 0 = no change, 1 = reddish blue, 2 = lilac, 3 = blue, 4 = pale blue, 5 = colorless. The results are given by values for each of the 12 tubes, beginning with the highest concentration of serum.

RESULTS

The percentage composition of the sera studied by electrophoresis, together with the colloidal gold activity and cephalin flocculation reaction of the 21 original sera and of electrophoretically separated fractions are shown in Table I. It is apparent that positive colloidal gold and positive cephalin flocculation reactions were always obtained with the gamma globulin fraction, whether

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[2] Dr. Landow died March 27, 1942.

TABLE I

Protein composition, colloidal gold curve, and cephalin flocculation reaction of sera

Case number	Diagnosis	Electrophoretic composition (per cent)				A/G	Colloidal gold curve			Cephalin flocculation		
		Albumin	α	β	γ		Whole serum	Albumin or (Albumin+α+β)	γ	Whole serum	Albumin or (Albumin+α+β)	γ
1	Cirrhosis	37.9	3.0	31.6	27.6	0.6	332211111122	000000000000	55554433211		0	+++
2	Cirrhosis	50.1	9.3	10.9 8.4	21.4	1.0	5555554333	000000000000	555554443332	++	0	++++
3	Cirrhosis	45.4	7.4	14.3	32.2	0.8	5555554433	000000000000	555544332221	+	0	+++
4	Cirrhosis	41.6	5.5	17.2	35.7	0.7	5555543322	000000000000	555555443321	+	0	+++
5	Cirrhosis						001111111122	0000000000	555555543321	++++	0	++++
6	Hepatitis	54.5	6.7	12.8	26.0	1.2	444443311222	000000000000	5555544321	++++	0	+++
7	Hepatitis	58.3	7.8	5.5 11.6	16.8	1.4			5555554433	++++		++++
8	Lues	56.0	8.6	4.6 8.9	21.9	1.3	333333211122	000000000000	555555443311	++++	0	++++
9	Lues; arsenical encephalitis	45.2	9.1	13.8	31.9	0.8	000000000000	000000000000	555555433311	++	0	++++
10	Ulcerative colitis Reaction to sulfonamide drug	51.4	9.1	15.3	24.2	1.1	555554322111	000000000000	555544331100	++++	0	++++
11	Post sulfonamide reaction	37.2	8.9	7.5§ 8.6	29.2	0.6	233332211122	000000000000	555555443211	++++	0	++++
12	Idiopathic grand mal	60.7	6.5	12.6	20.2	1.5	111111111222	000000000000	555555432100	0	0	++++
13	Anxiety state	61.0	7.9	6.1 7.8	17.3	1.6	000000000111	000000000000	555555433200	0	0	+++
14	Anxiety state	61.0	8.5	2.9 13.5	14.1	1.6	000000000111	000000000000	555555443210	0	0	+++
15	Head injury	62.5	6.3	4.4 7.1	19.7	1.7	000000001111	000000000000	555555432110	0	0	+++
16	Normal						000000000111	000000000000	555533110000*	0	0	++++
17	Normal						00000111122	000000000000	555544321000	0	0	+++
18	Normal						112222221111	000000000000	555555554321	0	0	++++
19	Normal	61.1	3.8	8.4 7.2	19.5	1.6			5555554443	0		++++
20	Normal†	64.2		21.0	14.8	1.8	000000000000	000000000000	555554332111	++++	0	++++
21	Normal‡	65.6	12.7	6.5	15.1	1.9	333332111122	000000000000	555454322100	++++	0	+++
22	Normal rabbit serum**	64.3		18.0	17.0	1.8	000000000111	00000000000	1111113331	++++	0	++++

* 1st tube 1 : 50.
† 5 months old.
‡ 6 months old.
** Average of 7 rabbits for percentage composition.
§ Also showed 8.9 per cent of a component between β and γ, mobility 2.1×10^{-5}.

its source was normal or abnormal serum. On the other hand, in no instances did the albumin fraction or mixtures containing albumin, alpha, and beta globulins give a positive reaction in either test. In general, studies on the whole serum indicate quite a close parallelism between the colloidal gold and the cephalin flocculation tests, but the following several features appearing in the data indicate that conditions underlying the two tests are not identical:

(*1*) Strongly positive colloidal gold tests were observed in a number of instances of cirrhosis (cases 2, 3, and 4) in which the cephalin reaction was but weakly positive. On the other hand, 3 normal sera which had been kept in the ice-box for 5, 6, and 8 months, respectively, gave a ++++ cephalin reaction, although the colloidal gold reaction remained unchanged. Furthermore, several sera which showed negative cephalin flocculation tests could be made to give strongly positive reactions by inactivation at 56° C. for 30 minutes; the colloidal gold reaction was unchanged by this treatment.

(*2*) It has been demonstrated that the serum of all the usual normal laboratory animals (except the monkey) gave positive cephalin flocculation reactions (*7*). In 3 observations on serum from healthy rabbits, it is noted that a ++++ reaction was obtained although the colloidal gold test was negative.

Quantitative studies indicate lowering of the albumin component in the sera of patients with chronic hepatic disease and a relative increase of the gamma globulin fraction. It is impossible, however, to correlate with assurance the colloidal gold or cephalin flocculation reaction with these quantitative differences, although the positive tests were generally obtained in cases with low A/G ratios (Table I).

Since the gamma globulin fraction of serum was shown to carry the cephalin flocculation and colloidal gold activity, it was of importance to de-

termine whether gamma globulins from normal and pathological sera showed any quantitative difference in these properties. This was done by analyzing gamma globulin solutions for nitrogen by the micro-Kjeldahl method and determining the minimum amount of gamma globulin nitrogen which gave a positive cephalin flocculation test or which gave a 5 reading in the colloidal gold test. The sensitivity of the cephalin flocculation as a test for gamma globulin is shown in Table II, and that of the colloidal gold reaction in Table III. It will be noted that a definite cephalin flocculation was obtained when about 0.05 mgm. gamma globulin nitrogen was used and that a 5 reading was obtained in the colloidal gold test with from 0.0002 to 0.001 mgm. of gamma globulin nitrogen.

TABLE II

Sensitivity of the cephalin flocculation reaction as a test for gamma globulin

Case number	Diagnosis	Gamma globulin. Amount N added	Cephalin flocculation
		mgm.	
5	Cirrhosis	0.11	++++
		0.055	+++
		0.028	0
		0.014	0
7	Hepatitis	0.14	++++
		0.07	++++
		0.047	+++
		0.035	±
		0.028	±
16	Normal	0.096	++++
		0.019	0
18	Normal	0.116	++++
		0.058	+++
		0.029	0
		0.015	0
19	Normal	0.13	++++
		0.066	0
		0.043	0
		0.033	0
		0.026	0

Within the experimental error involved, it may be seen that the gamma globulins isolated from normal sera and from sera of cases of cirrhosis and hepatitis were the same in colloidal gold and cephalin flocculation reactivity. The cephalin flocculation test required 50 to 100 times as much gamma globulin for a +++ reaction as was needed for a 5 reaction in the colloidal gold test. In one instance, however, the gamma globulin of

TABLE III

Sensitivity of the colloidal gold reaction as a test for gamma globulin

Case number	Diagnosis	Gamma globulin N	Colloidal gold reaction	Minimum amount of gamma globulin to give a 5 reading
		mgm. per ml.		*mgm. N*
1	Cirrhosis	0.12	555554321000	0.0004
5	Cirrhosis	0.55	555555543321	0.0005
7	Hepatitis	0.70	5555554433	0.0011
16	Normal	0.048	555533110000	0.0006
17	Normal	0.05	555332100000	0.0006
18	Normal	0.58	555555554321	0.0002
18	Normal	0.145	555543311000	0.0009
18	Normal	0.058	55554321100	0.0004
19	Normal	0.66	5555554443	0.0010

Values in last column obtained by dividing the nitrogen content by the highest dilution giving a 5 reading and calculated to the 0.5 ml. used in the test.

case 19, a normal, showed considerably less flocculating power than any of the other gamma globulins tested.

By adding electrophoretically separated albumin to gamma globulin in proper proportions, it was found that the colloidal gold reaction could be completely inhibited in most instances. Similar observations have been made with chemically fractionated spinal fluid proteins (2, 3). The cephalin flocculation reaction, however, did not seem to be correspondingly inhibited. Table IV shows the result obtained with both the colloidal gold and cephalin flocculation tests when increasing amounts of electrophoretically separated albumin fraction were added to definite amounts of gamma globulins. Insufficiency of material made it impossible to carry inhibition tests to completion in all instances. Albumin and gamma globulin fractions were obtained from both normal and pathological cases. Controls indicating the activities of the gamma globulin alone are also included. For the colloidal gold test, 0.1 ml. of the mixtures indicated was used and 0.4 ml. was used for the cephalin flocculation tests. It will be seen that albumins vary in their power of inhibiting the colloidal gold reaction. For example, the albumin of case 18, a normal, had much less inhibiting power than did any of the other normal and pathological albumins using the same amounts of albumin nitrogen. It is of possible significance that this original serum, unlike most normal sera, showed a weakly positive colloidal gold curve of

112222221111. The fact that the albumin fractions do not inhibit in a uniform manner suggests that the inhibition of the colloidal gold reaction may not be due to the main component migrating as albumin but to one of the lesser components present in the electrophoretically separated albumin fractions. It is also possible that the albumins of different individuals may vary in their physical and chemical properties. For example, in one instance (case 19), using a normal gamma globulin which did not itself give a positive cephalin flocculation test in the amount used, a definite positive flocculation was obtained when mixed with albumin fraction from a hepatitis patient (case 7), but not with normal albumin (case 19). Also, addition of 1.50 mgm. of albumin N of case 19 failed to inhibit the colloidal gold reaction of 0.14 mgm. gamma globulin N of case 7, but did partially inhibit the colloidal gold reaction of 0.13 mgm. gamma globulin N of case 19. The possible significance of these two discrepancies requires further investigation.

TABLE IV

Colloidal gold and cephalin flocculation reactions of mixtures of gamma globulins and serum albumins

Gamma globulin N used	Albumin N used	Total volume of mixture	Colloidal gold (0.1 ml. of mixture)	Cephalin flocculation (0.4 ml. of mixture)
mgm.	*mgm.*	*ml.*		
Normal gamma globulin (case 18)+normal albumin (case 18)				
0.145	0.00	0.8	555543311000	+++
0.145	0.40	0.8	555553211000	+++
0.145	0.80	0.8	555543211000	+++
0.145	1.20	0.8	555543210000	+++
0.145	1.60	0.8	555543210000	++
0.058	0.00	1.0	555543211000	0*
0.058	2.00	1.0	44333221100	0
0.058	3.60	1.0	111111111000	0
Normal gamma globulin (case 18)+cirrhotic albumin (case 5)				
0.12	0.00	0.8	555543311000	+++
0.12	0.40	0.8	55433321000	+++
0.12	0.80	0.8	111112221100	++
0.12	1.20	0.8	000001111100	++
Cirrhotic gamma globulin (case 5)+normal albumin (case 18)				
0.12	0.00	0.8	555444310000	+++
0.12	0.40	0.8	555532100000	+++
0.12	0.80	0.8	555532100000	+++
0.12	1.20	0.8	555542110000	+++
0.12	1.60	0.8	555553210000	+++
Cirrhotic gamma globulin (case 5)+cirrhotic albumin (case 5)				
0.12	0.00	0.8	555444310000	+++
0.12	0.40	0.8	44433321000	+++
0.12	0.80	0.8	111122210000	+++
0.12	1.20	0.8	111100000000	+++
Normal gamma (case 16)+normal albumin (case 16)				
0.048	0.00	0.5	555533110000	
0.048	2.10	0.5	000000000000	
Normal gamma (case 17)+normal albumin (case 17)				
0.026	0.00	0.5	555332100000	
0.026	0.53	0.5	000000000000	
Hepatitis gamma globulin (case 7)+hepatitis albumin (case 7)				
0.14	0.00	0.8	5555443321	++++
0.14	0.50	0.8	5555543332	++++
0.14	1.50	0.8	5555543321	++++
Hepatitis gamma globulin (case 7)+normal albumin (case 19)				
0.14	0.50	0.8	5555544332	++++
0.14	1.50	0.8	5555533321	++++
Normal gamma (case 19)+normal albumin (case 19)				
0.13	0.00	0.8	5555444331	0
0.13	0.50	0.8	344444321	+
0.13	1.50	0.8	2223333332	0
Normal gamma (case 19)+hepatitis albumin (case 7)				
0.13	0.50	0.8	5555443221	+++
0.13	1.50	0.8	3444333211	+++

* Below sensitivity of cephalin flocculation test (cf. Table II).

DISCUSSION

In the original description of the cephalin-cholesterol reaction (1), the author suggested that a positive reaction probably depends upon changes in the fibrinogen fraction produced by a diseased liver. The experimental data here presented make

this hypothesis unlikely, since both colloidal gold and cephalin flocculating activity have been found to be associated with the gamma globulin fraction, whether derived from normal or pathological sera (Table I). Within the experimental error of the measurement, gamma globulin fractions of the same nitrogen content, obtained from normal individuals with negative colloidal gold and cephalin flocculation tests or from certain cases of liver disease giving positive tests, showed the same activity in both tests (Tables II and III).

Although the colloidal gold reactivity of the gamma globulin could be inhibited by mixing with electrophoretically separated albumin, the differences shown in inhibiting power of different albumin preparations strongly suggests that the inhibition may not be due to the major component but to some minor component migrating in the albumin fraction, or possibly to physical or chemical differences in albumins of different individuals. Further studies to determine the nature of the substances responsible for inhibition of the colloidal gold reaction are desirable and might make possible more precise understanding of the causes of colloidal gold changes in pathological sera (2).

The nature of the factors in normal serum which prevent positive cephalin flocculation reactions as yet remain obscure, as are the changes which occur in pathological sera to produce positive cephalin flocculation, since the cephalin flocculating activity of gamma globulin is unaffected by addition of the separated fractions.

The possibility must be considered that the effects of the gamma globulin fraction in causing colloidal gold and cephalin flocculation reactions may be due to the fact that under the usual conditions of fairly acid pH employed in carrying out these tests, the gamma globulin is on the acid side of its isoelectric point and is therefore positively charged, whereas the colloidal gold sols and cephalin emulsions are negatively charged.

The fact that several sera after heating to 56° C. for 30 minutes or standing for several months at ice-box temperatures developed positive cephalin flocculations suggested a similarity in properties between the substances in serum responsible for inhibition of cephalin flocculation and those of complement. However, a positive cephalin flocculation did not develop after the complement was removed (8) from 2 normal human sera by means of the precipitate formed by addition of 0.11 mgm. purified rabbit Type III antipneumococcus antibody nitrogen [3] and 0.08 mgm. Type III polysaccharide. This indicated that complement itself is not involved in inhibition of the cephalin flocculation reaction.

In general, both positive cephalin flocculation and colloidal gold tests are obtained in sera with lowered A/G ratios, as observed electrophoretically (Table I). However, the species differences observed in the cephalin flocculation tests in normal rabbits, cannot be explained on the basis of altered A/G ratio. In view of the complexity of both the colloidal gold and cephalin flocculation reactions, it is probable that the changes responsible for the development of positive tests in disease are probably due in part to variation in different sera of some factor associated with the albumin fraction (9).

SUMMARY

1. Electrophoretically separated gamma globulins from normal and pathological human sera show marked colloidal gold and cephalin flocculating activity. As little as 0.0002 to 0.001 mgm. gamma globulin nitrogen gives a 5 reading in the colloidal gold test and 0.05 mgm. gamma globulin nitrogen gives a definite cephalin flocculation.

2. Addition of electrophoretically separated albumin to gamma globulin inhibits the colloidal gold reaction, but does not significantly inhibit the cephalin flocculation. Different preparations of albumin vary in inhibiting power.

3. Sera with low electrophoretic A/G ratios show positive cephalin flocculation and colloidal gold reactions more frequently than those with normal A/G ratios, but other factors are also of importance.[4]

Miss Helen Sikorski assisted in the electrophoretic studies. The colloidal gold tests were performed by Miss Ruth Shivitz and the cephalin flocculation tests by Mr. Michael Bauer.

[3] Kindly supplied by Dr. Michael Heidelberger.

[4] After this paper had been submitted Gray (10) reported that electrophoretically separated gamma globulin would produce a positive colloidal gold test when added to normal human serum or spinal fluid and that the reaction could be inhibited by addition of albumin, confirming the previous findings in spinal fluid (4) that colloidal gold activity was associated with the gamma globulin fraction.

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