

A CONTRIBUTION TO THE STUDY OF THE CHEMISTRY OF BLOOD SERUM.*

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Many studies of blood serum in various diseased conditions are on record, but in summarizing the results of this work Morawitz¹ concludes that but little of importance to pathology and physiology has been gained. This is an extreme view which is not justified by a comprehensive analysis and adequate interpretation of the material at hand.

Normally blood serum varies but little in its chemical composition. Its chief ingredients, the proteins (euglobulin, pseudoglobulin, and albumin), sodium chloride, urea, and the allied nitrogenous waste products, maintain definite proportions, and may be regarded as physiological constants. Variations from the normal are known to occur in many diseases. The purpose of this work is to put on record the results of an analysis of eighty sera from different sources, and to discuss the facts elicited in conjunction with the observations made by other investigators in similar conditions.

The substances principally studied were the proteins, chlorides, and incoagulable and non-protein nitrogen.² Ash and total solids were also estimated in a number of sera.³ Of the eighty sera examined, thirty-nine were from blood and forty-one from effusions of different sorts. The two groups of sera will be considered

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¹ Morawitz, P., Oppenheimer's Handbuch der Biochemie des Menschen und der Tiere, Jena, 1909, ii, 79.

² The term incoagulable nitrogen will be used collectively in place of incoagulable and non-protein nitrogen.

³ The sugar content of blood serum will not be discussed in this study. The nature of its variations is well understood and comprises a separate chapter in the chemistry of the blood.

separately. The present communication will deal only with the blood sera.

The sera examined were obtained from the following sources:

- Normal—1 case (transfusion donor)
- Diabetes insipidus—1 case
- Cardiac disease—7 cases
- Cardiorenal disease—5 cases
- Renal disease—12 cases
- Diabetes mellitus—3 cases (2 with coma and lobar pneumonia)
- Pneumonia—7 cases {
 - influenzal or tuberculous, 1
 - diabetic (lobar pneumonia), 2
 - lobar pneumonia, 4
- Emphysema of the lungs—1 case
- Polycythemia—1 case
- Achylia gastrica—1 case

This classification is made according to the clinical diagnosis of the cases, and the diagnoses are, of course, not final. Closer pathological study with post-mortem examinations might lead to somewhat different classifications, especially of the cases of cardiac and renal diseases. To those familiar with the nature of these diseases, it is known that, as a rule, they are not essential forms. Usually cardiac diseases of the chronic type, with insufficiency, etc., are accompanied by renal disturbances or by hepatic and vascular changes. A classification of these cases into distinct pathological groups is therefore not possible. With but few exceptions the blood was obtained by phlebotomy done in the hospital routine for therapeutic purposes. This is of importance because it shows that most of the cases studied were clinically severe types, some, in fact, extreme.

According to Hammarsten and others, human blood sera show normally the following values per 100 cubic centimeters of serum:

Total protein.	Globulin.	Albumin	Incoagulable and non-protein nitrogen.	Chlorides.
5.5–8.4 gm.	37–40 %	60–63 %		0.357–0.366 gm.
Mean	38.5 %	4.2–4.4 gm.	0.020–0.050 gm.	0.361 gm.
7.0 gm.	2.7 gm.	4.3 gm.	0.035 gm.	
Ratio of globulin to albumin.				
1 : 1.6				
Total solids.			Ash.	
8.20–9.20 gm.			0.850–0.912 gm.	

One normal serum analyzed by the author gave the results shown in table I, per 100 cubic centimeters of serum. On comparing the values obtained in this analysis with the standard values given above, we find that this serum probably represents the maximum normal type. It differs from the standard in two particulars; namely, the chlorides and the incoagulable and non-protein nitrogen, both of which are high. This is probably due to the fact that the person (a transfusion donor) from whom the serum was taken partook of food shortly before the serum was removed.

TABLE I.⁴
Normal Blood Serum.

Serum No.	Total protein in gm.	Incoagulable and non-protein nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudoglobulin in gm.	Albumin in gm.	Chloride in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
38	8.300	0.062	3.071	1.059	2.012	5.09	0.397	1:1.7	37

Cardiac Cases.—In the analysis of the sera from the cardiac and cardiorenal cases (table II), we find considerable individual variations, some of which are quite remarkable. Four of the twelve cases (Nos. 33, 19, 23, and 36) show values for the different substances which are within the normal range of variation. Though the total protein in these cases is nearer the minimum normal amount, as determined by Hammarsten, Abderhalden, etc., the ratio of the different protein fractions to each other and to the total protein content is quite normal. A moderate state of dilution, or hydremia, would account fully for the protein values obtained. The increase observed in the amount of incoagulable nitrogen does not contradict the possibility of an existing hydremia in these cases, especially in view of the normal amount of chloride present in the sera. The increased incoagulable nitrogen might be due to a functional disturbance of the kidneys, resulting from a passive congestion.

The remaining eight cases present some interesting and instruct-

⁴In the tables the values are given in grams per 100 c.c. of serum.

ive features. With but one exception (case 51) they all show values for the total protein which are equal to or less than the normal minimum. Sera 34 and 35 are from a case of chronic myocarditis associated with chronic pulmonary edema and anasarca. The uniformity of the results of the two determinations is striking.⁵ They both show a protein content which is only half the normal amount. In view of the fact that this case was associated with an anasarca, it might at once be concluded that the low protein content is the result of a severe hydremia. On analysis of all the data in this case it seems probable that in addition to the hydremia other factors are concerned in the peculiar results obtained.

TABLE II.
Blood Serum in Cardiac and Cardiorenal Cases.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
33	6.525	0.090	2.544	1.028	1.516	4.981	0.397			1:2.0	33.0
19	6.019	0.197	2.153	0.544	1.609	3.866	0.404			1:1.8	35.7
23	5.475	0.386	1.990	0.305	1.685	3.485	0.362	8.680	0.700	1:1.75	35.0
36	7.612	0.154	2.275	0.850	1.425	5.337				1:2.3	32.0
34	3.732	0.116	1.925	0.595	1.370	1.807	0.390	8.420	1.069	1:0.95	53.0
35	3.611	0.130	2.038	0.525	1.513	1.373	0.404	8.826	1.460	1:0.7	58.5
31	5.012	0.063	3.819	1.734	2.085	1.193	0.405			1:0.31	76.3
37	5.050	0.096	2.337	1.137	1.200	2.713	0.432			1:1.16	46.0
42	5.625	0.133	2.731	0.962	1.769	2.894	0.390			1:1.0	50.0
51	7.281	0.084	3.141	0.787	2.354	4.140	0.404			1:1.3	43.5
43	5.951	0.259	2.970	0.781	2.189	2.987	0.420			1:1.0	50.0
58	5.681	0.106	2.780	1.712	1.069	3.100	0.450			1:1.15	46.0

The term hydremia usually signifies a dilution of the blood; but when a simple dilution occurs, the different protein elements of the serum and plasma, which normally show definite quantitative relations, must in the diluted state retain their proportions. This, however, is not the case as the above figures for the serum fractions show. The globulins are much less reduced in amount than the albumin. In fact, the relation of the globulins to the albumin ap-

⁵ An interval of three days elapsed between the removal of the two specimens of blood.

pears reversed as compared with the normal; for the globulins constitute the major part of the total protein (table II, cases 34 and 35). This shows that a simple dilution of the blood cannot have taken place, and that some other factor has contributed to this result. Just what this factor is will be explained later.

Two other features in the sera under consideration are of interest. One is that the incoagulable nitrogen as well as the chloride is practically normal in amount in the first serum (No. 34); in the second specimen, however, both values are considerably above the first, and hence, above normal. The second sample of serum shows a much higher ash content than the first. The increase noticed in the amount of total solids of the second specimen, as compared with the first, is entirely in the form of ash (Nos. 34 and 35). Although the chloride content of the second serum specimen is higher than that of the first, it does not account for the rise in the ash. A large amount of inorganic material other than chloride has therefore accumulated in the blood within three days that elapsed between the removal of the two specimens of blood. The cause of the increase in the ash content may lie in a disturbed elimination of these materials through the kidneys. The point which is of greatest importance, however, in this case, is the fact that a large quantity of inorganic material (including chlorides) evidently can accumulate in the blood, without causing a corresponding retention of water; for as the other data (i. e., the protein) of the sera show, a further dilution of the blood has not taken place, and the degree of hydremia has not increased.

The other is that the sera from the other cardiac cases (Nos. 31, 37, 42, 43, 51, and 58) also show points of interest in relation to the protein and chloride content. The protein values are again below the minimum normal. If we assume here that a dilution of the blood has taken place as in the preceding cases (Nos. 19, 23, 33, and 36) we are again confronted with the fact that the globulins bear a higher proportion to the albumin than is observed in normal sera. The chloride content of the serum is also somewhat higher than that observed normally. These two facts indicate that a simple dilution of the blood cannot have taken place in these cases.

From the above findings it is seen that the sera from cardiac cases

show striking variations from the normal. From the analysis of the blood sera, the cardiac cases are divisible into two groups: (1) One group in which a moderate dilution of the blood (a true hydremia) takes place. In the sera of these cases the proteins retain their normal proportions. The hydremia may be due to a retention of water, possibly the result of a simple functional derangement of the kidneys. (2) The other in which the proteins of the serum are moderately or markedly reduced in amount, the reduction affecting principally the albumin. The proportions of the serum proteins may be so changed that the globulins preponderate. In this group of cases there is an accompanying edema or some serous effusion. However, the change in the quantitative relations of the serum proteins is not due to a simple dilution of the blood, or hydremia.

Respiratory Cases.—These include cases of polycythemia, emphysema, and pneumonia. They are grouped together because they have one feature in common, namely, respiratory embarrassment, which, as will be shown, is of importance in the production of chemical changes in the blood.

As the figures in table III indicate, the results are of very much the same character as those obtained in the cardiac cases. The polycythemia and emphysema sera show a protein content which approaches the upper normal. In all of them, however, the globulin fraction shows a marked increase. The other data in the three sera analyzed show little of importance with the exception of the increase in the salt or chloride content.

In the four sera from the pneumonia cases, the total protein content is within the normal range. In one case (serum 41) in which the total protein falls below five grams, the decrease may be accounted for by the large amount of fibrin that was present in the blood; for, after separating the serum from the blood clot, a second clot formed. This clot was very firm, and the serum separated from the thick fibrinous mass with difficulty.

In these sera, too, we observe that the globulins give a higher value than the albumin. The sera from the two cases of diabetic pneumonia (sera 9 and 73) show a relative increase in the globulins as well, although not to the same extent as in the sera of the uncomplicated pneumonia cases.

TABLE III.
Blood Serum in Respiratory Cases.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
Polycythemia.											
6	7.050	0.300	4.550	0.700	3.850	2.500				1:0.55	64.0
59	7.212	0.106	4.062	1.537	2.525	3.150	0.445			1:0.77	56.3
Emphysema.											
76	6.719	0.089	3.281	1.262	2.019	3.438	0.376			1:1.0	50.0
Pneumonia.											
22	5.275	0.248	2.825	0.575	2.300	2.450	0.355	8.490	0.880	1:0.86	54.0
27	6.588	0.118	2.370	0.700	1.670	4.218	0.376			1:1.8	35.7
41	4.696	0.152	2.704	0.513	2.191	1.992	0.397			1:0.7	57.6
50	6.844	0.077	3.562	1.513	2.049	3.282	0.359	9.212	0.834	1:0.9	52.6
77	6.437	0.120	3.393	1.025	2.268	3.044	0.390			1:0.9	52.6
9	6.241	0.087	2.940	0.240	2.700	3.301	0.426	9.368	0.870	1:1.13	47.0
73	5.969	0.116	2.812	0.729	2.083	3.157	0.384			1:1.15	46.0

TABLE IV.
Blood Serum in Diabetes Mellitus.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Euglobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
9	6.241	0.087	2.940	0.240	2.700	3.301	0.426	9.368	0.870	1:1.13	47.0
25	5.625	0.116	2.537	0.635	1.902	2.688	0.460			1:0.82	54.0
73	5.969	0.116	2.812	0.729	2.083	3.157	0.384			1:1.15	46.0

The values for the incoagulable nitrogen fluctuate considerably. The amount of chloride in all the sera except one is lower than that observed in the cardiac sera. In one instance (serum 9, from a case of pneumonia in a diabetic) the chloride content is unusually high. The ash content in all the sera is uniform and practically normal in amount.

Serum 27, from a case of a typical pneumonia of undetermined etiology (tuberculosis or grippe), does not present values like those obtained in the sera of the lobar cases. The proportion of the total globulin in this serum to the albumin is below that found in normal serum. This is in conformity with the results obtained by Erben⁶ in the analysis of sera from tuberculous cases.

In reviewing the results of this group of sera, we find once more that the globulin-albumin ratios are very much disturbed. The globulins in all but one case (serum 27) show a marked relative increase.

Cases of Diabetes Mellitus.—Of the three sera examined from this disease, two have already been presented in the preceding group. The serum from the third case (table IV, serum 25) gave results closely resembling those of the other two. An increase in the globulin content is again seen, which is somewhat greater than that observed in the other instances. In this case also, as in one of the other sera (case 9), there is a marked increase in the chloride content, reaching a value of 460 milligrams per 100 cubic centimeters of serum. The incoagulable nitrogen of these three sera is increased considerably over the normal amount.

Nephritic Cases.—Of this important group, twelve sera were examined. One serum from a case of diabetes insipidus is classed under the head of nephritic cases, inasmuch as it represents a type of renal disturbance. The twelve cases of clinical nephritis can be divided into three groups: (1) chronic parenchymatous, (2) chronic interstitial, (3) chronic mixed forms. Cases of acute nephritis unfortunately have not come under observation. All but two sera examined were from very severe forms of the disease, most of the cases being accompanied by symptoms of uremia, so that

⁶ Erben, F., *Ztschr. f. klin. Med.*, 1903, 1, 450.

the changes observed in the composition of the sera may be regarded as representative of the different types of nephritis.

The classification given above is based on clinical data and not on pathological findings. The sense which the terms parenchymatous and interstitial are intended to convey are sufficiently well understood not to require explanation. By the "mixed form" of nephritis is meant that clinical group of cases in which both the parenchymatous and the interstitial types of nephritis are, to a certain extent, represented. A contracted kidney with an intercurrent or superimposed parenchymatous inflammation may give the clinical picture of the mixed form of nephritis. On the other hand, the development of interstitial changes in kidneys affected with the parenchymatous form of the disease may lead to the same clinical result.

TABLE V.
Blood Serum in Nephritis.

Serum No.	Total protein in gm.	Incoagulable nitrogen in gm.	Total globulin in gm.	Englobulin in gm.	Pseudo-globulin in gm.	Albumin in gm.	Chloride in gm.	Total solids in gm.	Ash in gm.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
Parenchymatous.											
5	5.125	0.280	4.325	0.877	3.448	0.800				1:0.2	83.3
47	2.731	0.235	2.598	0.376	2.222	0.133	0.390		0.925	1:0.05	95.0
Interstitial.											
16	7.150	0.256	2.327	0.550	1.777	4.833	0.340	10.040	0.855	1:2.0	33.0
49	5.925	0.158	2.344	0.962	1.382	3.581	0.362	8.580	0.724	1:1.6	38.0
57	7.525	0.120	2.537	1.000	1.537	4.988	0.480			1:1.95	34.0
64	6.215	0.092	2.375	0.962	1.413	3.840	0.420			1:1.6	38.0
Mixed.											
24	5.656	0.073	2.375	0.415	1.960	3.281	0.532			1:1.1	41.7
39	5.300	0.133	2.187	0.450	1.737	3.113	0.397			1:1.4	41.2
48	6.800	0.151	2.837	0.956	1.881	3.963	0.426	9.920	0.870	1:1.4	41.7
55	5.644	0.085	2.800	1.181	1.619	2.844	0.362			1:1.0	50.0
62	5.202	0.085	2.371	1.234	1.137	2.831	0.412			1:1.2	45.6
65	6.775	0.116	2.875	0.912	1.963	3.900	0.390			1:1.3	42.4
Diabetes insipidus.											
78	7.919	0.011	2.645	0.700	1.945	5.274	0.302			1:2.0	33.3

In the sera from the cases of parenchymatous nephritis, we find amounts of protein which fall below the normal minimum value. In one instance (case 47) the reduction in the total protein is ex-

treme, reaching a value of about one third of the normal amount. In both cases, however, there is a marked relative increase in the globulin content, and a decrease in the albumin fraction. The increase of the globulins in one case (serum 5) is both absolute and relative. This case and the one mentioned above (No. 47) present such remarkable variations from normal that they deserve special consideration. In both instances the sera were of a greenish yellow hue and opalescent. The opalescence gave to both sera a chyloform appearance, not due to free fat, but to an increase in the lipoid content present in combination with the globulins. In serum 5 the total globulin not only constitutes the major part of the serum protein (83.3 per cent.), but is actually greater in amount than that found at any time in normal serum. The albumin of this serum, on the other hand, is diminished far below the normal, both in relative and absolute amount.

The incoagulable nitrogen in this case is also very high (0.280 of a gram in 100 cubic centimeters of serum).⁷ Unfortunately an estimation of the chloride content in this instance could not be made, because of an insufficient amount of serum.

In the other case (serum 47) the total globulin is practically normal in amount, but it comprises nearly the entire quantity (95 per cent.) of protein in the serum. The amount of albumin present is so small (0.133 of a gram per 100 c.c. of serum), that it is practically negligible. As in the preceding case (serum 5) the incoagulable nitrogen is very much increased (0.235 of a gram per 100 cubic centimeters of serum), but the chloride shows absolutely no variation from the normal.

The ash in this serum is unusually high. This is of interest, especially when compared with the value obtained in the chloride estimation. This shows, as already pointed out (page 723), that the mineral content of the serum can become very much concentrated, although the relative amount of chloride remains normal.

Four blood sera were analyzed from the interstitial type of nephritis. The results in this series are fairly uniform and present

⁷Part of the incoagulable nitrogen was derived from the lipoid nitrogen present in the filtrate after coagulation of the proteins. This filtrate retained some of the opalescence observed in the original serum.

a totally different picture from the one obtained in the analysis of the sera of the cases of parenchymatous nephritis. The total amount of protein in these ranges within the normal limits; and the relation which the globulins bear to the albumin and total protein is also within the normal. There is no evidence whatever of increase of the globulin fraction; if anything, there is a tendency in these cases for the globulins to fall below normal in amount.

The incoagulable nitrogen in these sera is increased considerably above normal, but it does not attain so high a value as that found in either of the two cases of parenchymatous nephritis described above. The maximum value of the incoagulable nitrogen found in this series of cases is 0.256 of a gram, and the minimum is 0.092 of a gram per 100 cubic centimeters of serum.

The chloride content in these sera varies to a marked degree. We find here two sera with very high values for chloride, 0.420 and 0.480 of a gram, and two with moderately low figures, namely 0.340 and 0.362 of a gram per 100 cubic centimeters of serum.

The incoagulable nitrogen and chloride contents do not run parallel. In one case (serum 16) in which the incoagulable nitrogen is extremely high, 0.256 of a gram, the chloride content is very low, 0.340 of a gram per 100 cubic centimeters. On the other hand, sera 48 and 57, which do not show so great an increase in incoagulable nitrogen as case 16, show a very marked increase in the chlorides, 0.426 and 0.480 of a gram per 100 cubic centimeters, respectively.

The amount of ash in the two sera examined (Nos. 16 and 49) is below that found in any of the other cases. In both these sera the chloride content is also low. The significance of this, however, will be discussed in connection with the influence of the chemical composition of serum upon the production of nephritis.

To the group of mixed forms of nephritis belong the remaining six sera in the series given in table V. The changes which can be observed in these sera resemble in part those of the cases of parenchymatous nephritis, and in part those of interstitial forms. Of the six sera, two present normal values for the total protein; in the others, the proteins are somewhat diminished in amount. But unlike the sera of the interstitial type of cases, the ratio of the globulins to the albumin is considerably higher. This ratio, however, does

not attain the value obtained in the series of parenchymatous cases. In respect to the protein content, and the relation of the fractions to each other, the sera of these cases stand between those of the parenchymatous and the interstitial forms of nephritis.

The other ingredients of the serum also show variations which belong to one or the other group of nephritic sera. These sera (cases 65, 48, and 39) show values of 0.116, 0.151, and 0.133 of a gram for incoagulable nitrogen, whereas the other three sera (cases 55, 62, and 24) yield amounts of 0.085, 0.085, and 0.073 of a gram, respectively. Similarly the chloride content in these sera shows variations in one or the other direction, but there is no parallelism between the chloride values and those for the incoagulable nitrogen.

In some respects the results obtained in the analyses of the sera from the nephritic cases are distinctive. This is especially true of the quantitative changes in the proteins of the sera from the cases of parenchymatous nephritis. The striking variations that these sera present in this respect, as compared with those of the sera from the interstitial and mixed forms of nephritis, are of great significance for a proper understanding of the chemical pathology of kidney disease. The chlorides and incoagulable nitrogen also show marked fluctuations, but they are not as distinctive of the different sera as the changes that occur in the protein quota.

The serum from the case of diabetes insipidus presents some very interesting features. The protein content is normal or upper normal. The ratio which the globulin bears to the albumin, however, is relatively low. The globulin constitutes only 33 per cent. of the protein, a percentage like that found in the sera from the interstitial forms of nephritis. The chloride and incoagulable nitrogen show very low values, much lower than the other sera examined. The peculiar results presented by this serum are in keeping with the character of the disease itself. This will appear more clearly from the deductions to be drawn later concerning the relation which the composition of the serum bears to the clinical manifestation of the disease.

The serum from a case of achylia gastrica presents values which are normal in every respect. Clinically this case showed very little abnormality apart from the gastric disturbance.

SUMMARY.

The most striking feature observed in this study is the extensive variation of the serum in relation to its proteins. It was found that in certain diseases the globulin fraction is markedly increased, whereas the total protein of the serum may be normal, or may fall far below normal in amount. The diseases in which an increase in the globulins takes place may be grouped as follows: (1) cardiac diseases associated with decompensation and serous effusions, (2) pulmonary or respiratory affections of inflammatory or non-inflammatory origin (pneumonia, emphysema, polycythemia), (3) diabetes mellitus, and (4) parenchymatous nephritis.

In the serum of chronic parenchymatous nephritis the increase in the globulin content is most pronounced and may constitute nearly all the protein, or as much as 95 per cent.

The globulin content of serum is normal or diminished in the following diseases: (1) simple achylia gastrica (short duration), (2) tuberculosis, (3) diabetes insipidus, and (4) chronic interstitial nephritis.

Other ingredients of the sera analyzed showed variations which cannot be definitely classified; but in a general way it appears upon careful analysis that an accumulation of water and salt occurs in those diseases in which the globulin fraction of the blood serum is increased.