STUDIES ON THE CHEMISTRY OF SEROUS EFFUSIONS.*

By ALBERT A. EPSTEIN, M.D.

(From the Laboratory of Physiological Chemistry of the Pathological Department of the Mt. Sinai Hospital, New York.)

The formation of serous effusions in different diseases and particularly in nephritis has been the subject of much study and discussion. Although the results of analyses of serous fluids recorded in the literature show striking variations in the composition of such fluids in different pathological conditions, they throw but little light on the chemical factors concerned in their formation. This failure to account for the differences in the chemical composition of such fluids is due to two causes: (1) a lack of definite knowledge of the processes by which serous fluids of different chemical composition are formed normally in different parts of the body, and (2) the fact that there are relatively few studies on the subject in which simultaneous examinations of blood serum and serous fluids were made.

Recent studies by the author on the chemical composition of blood serum show that certain changes occur in different diseases.[1] Particularly striking is the change from normal in the composition of the protein colloids and other constituents which takes place in parenchymatous nephritis, in which effusions are of common occurrence, as contrasted with other forms of renal disease, in which effusions do not occur.[2]

Without attempting to enter into a discussion at the present time of the relation of the chemical changes in the blood serum to the nature and occurrence of effusions in disease, I wish to put on record the chemical findings of forty-three puncture fluids, some of which were made simultaneously with analysis of blood serum.

* This work was done under the tenure of the Theodor Escherich Fellowship in Pathology. Received for publication, July 23, 1914.

[1] Epstein, A. A., *Jour. Exper. Med.*, 1912, xvi, 719.

[2] Epstein, *idem*, 1913, xvii, 444.

The analyses of the effusions were carried out along lines similar to those followed in the study of the blood sera.[3] The substances determined were the proteins, chlorides, and incoagulable and non-protein nitrogen. Ash and total solids were also estimated in several of the fluids. A number of the fluids which were opalescent and of a chyliform appearance were analyzed for fats and other ether-soluble substances. These, however, will not be reported in the present study.

The fluids subjected to analysis may be grouped according to the following scheme which is based on clinical data.

1. Cutaneous effusions: (*a*) nephritic and (*b*) cardionephritic.
2. Pleural effusions: (*a*) inflammatory and (*b*) non-inflammatory including (1) nephritic, (2) cardiac, (3) cardionephritic, and (4) new growth.
3. Abdominal effusions: (*a*) inflammatory, tuberculous, and (*b*) non-inflammatory, including (1) new growth, (2) cirrhosis of the liver, (3) cardiac, and (4) cardionephritic.
4. Hydrocele fluid.

CUTANEOUS EFFUSIONS.

TABLE I.

Cutaneous Effusions.

Case No.	Total protein.	Incoagulable nitrogen.	Total globulin.	Euglobulin.	Pseudoglobulin.	Albumin.	Chlorides.	Total solids.	Ash.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
	Gm. per 100 c.c. of fluid.										
Nephritis.											
4	0.098	0.004	0.080	0.048	0.032	0.018	0.406	1.230	0.980	1:0.24	81
44	0.171	0.162	0.104	0.091	0.095	.....	0.397	.....	.....	1:0.60	56.1
29	0.145	0.063	0.079	0.024	0.055	0.066	0.433	1.357	0.870	1:0.84	54
Cardionephritis.											
14	0.462	0.035	0.155	0.032	0.123	0.307	0.412	1.515	0.950	1:2.5	28.5
70	0.119	0.035	0.043	0.018	0.025	0.076	0.420	.....	.....	.....	30
201	0.100	0.053	0.018	.....	.....	0.082	0.400	.....	.....	.....	18

The six cutaneous fluids (table I), of which three are of nephritic and three of cardionephritic origin, have certain features in com-

[3] Epstein, *Jour. Exper. Med.*, 1912, xvi, 719.

mon and differ in certain others. The protein content of all these fluids is very low, ranging in the nephritic group from 0.098 of a gram to 0.171 of a gram per 100 cubic centimeters, and in the other group from 0.100 of a gram to 0.462 of a gram per 100 cubic centimeters of fluid. The two classes of fluids differ in the percentage relations of albumin and globulins. Of the three nephritic fluids the globulins constitute 81 per cent., 56.1 per cent., and 54 per cent., respectively, of the total protein. The proportion of globulins in the second group of fluids is much lower; namely, 28.5 per cent., 30 per cent., and 18 per cent., respectively.

Although the amount of protein in these fluids is far below that of blood sera, the percentage of globulin in each group of cutaneous fluids is similar to that of the corresponding group of blood sera. Fluids 4 and 201 are from cases whose blood serum was examined at the same time. Table II shows the values obtained in the analysis of the corresponding serum and edema fluids.

TABLE II.

Case 4. Nephritis.

		Edema fluid.	Blood serum.
Gm. per 100 c.c. of fluid	Total protein	0.098	5.125
	Incoagulable nitrogen	0.040	0.280
	Total globulin	0.080	4.325
	Albumin	0.018	0.800
	Chlorides	0.406	0.412
	Total solids	1.230	.....
	Ash	0.980	.....
	Per cent. of globulin	81.0	83.0
Case 201. Cardionephritis.			
Gm. per 100 c.c. of fluid	Total protein	0.100	3.330
	Incoagulable nitrogen	0.053	0.154
	Chlorides	0.400	0.435
	Globulin	0.018	2.381
	Albumin	0.082	0.949
	Per cent. of globulin	18.0	71.5

In both cases the quantity of protein in the effusions is very small as compared with that of the blood serum. In the nephritic effusion the percentile relation of the protein fractions corresponds to that of the blood serum. In the cardionephritic case this parallelism does not exist.

The chloride content of these fluids is uniformly high when compared with that of normal blood sera, but much lower than the content in the blood sera of corresponding cases (compare fluids 4 and 201 and their corresponding blood sera). The total solids are low in all the fluids analyzed. The ash, on the other hand, is practically the same as that found in normal blood sera. The incoagulable and non-protein nitrogen in all but one fluid (44) is very low when compared with the blood sera belonging to these cases. In this respect the incoagulable nitrogen is like the chlorides, except that the difference in the quantity of incoagulable nitrogen in the effusions and in the blood sera is much greater than that exhibited by the chlorides.

A comparison of the results obtained in the analyses of the subcutaneous fluids with those of other effusions shows that their composition is unique. They are of a pale watery appearance like lymph, containing very little protein (less than 0.5 per cent.) and a large amount of chlorides. The incoagulable nitrogen is relatively low, certainly far below that found in the blood sera of the cases examined.

PLEURAL FLUIDS.

Most of the pleural fluids analyzed (table III) are of inflammatory origin. Fluids 20, 21, 54, 60, 61, 80, and 204 are from cases of pleurisy of unknown etiology. Fluids 52, 53, and 206 are from cases of probable tuberculosis, and fluid 30 is from a case of empyema. The remaining effusions are presumably of non-inflammatory origin; two (205 and 56) are from cases of nephritis; of the others, 63 is from a case of intrathoracic lymph sarcoma, and 202 and 203 are also due to neoplasms of undetermined nature.

All of the above fluids are rich in protein, the amounts ranging from 1.250 grams to 6.250 grams per 100 cubic centimeters. The inflammatory fluids of unknown etiology (20, 21, 54, 60, 61, 80, and 204) show a moderate elevation of the percentage of globulin as compared with the content of globulin in normal blood serum. The incoagulable and non-protein nitrogen found in these fluids present values which are for the most part somewhat above the standard figure for blood serum. These values vary from 0.049 to 0.080 of a gram per 100 cubic centimeters of fluid. Likewise the chloride content of these fluids is higher than that of normal blood serum.

TABLE III.

Pleural Effusions.

Case No.	Total protein.	Incoagulable nitrogen.	Total globulin.	Euglobulin.	Pseudoglobulin.	Albumin.	Chlorides.	Ash.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
	Gm. per 100 c.c. of fluid.									
Inflammatory. Unknown etiology.										
20	4.400	0.046	1.780	0.470	1.310	2.620	0.362	0.650	1:1.5	40
21	3.969	0.080	1.882	0.392	1.490	2.087	0.355	0.650	1:1.1	47.6
60	3.831	0.050	1.437	0.650	0.787	2.394	0.404	.....	1:1.6	39.3
61	3.412	0.045	2.537	0.569	1.968	0.875	0.412	.....	1:0.34	74.3
54	4.781	0.067	2.056	0.881	1.175	2.715	0.397	.....	1:1.3	43
80	5.487	0.049	2.625	0.822	1.803	2.862	0.412	.....	1:1.1	47.6
204	6.775	0.022	2.187	4.588	.....	.....	0.391	.....	1:2.14	32
Inflammatory. Tuberculosis.										
52	4.275	0.022	2.387	1.181	1.206	1.888	0.372	.....	1:0.8	56
53	6.250	0.056	2.969	0.812	2.157	3.291	0.376	.....	1:1.1	47.6
206	5.231	0.039	3.506	.....	.....	1.725	0.405	.....	1:0.48	67
Inflammatory. Empyema.										
30	5.019	0.086	1.027	0.625	0.402	3.992	0.372	.....	1:3.8	20.8
Non-Inflammatory. Nephritis.										
56	3.581	0.060	1.469	0.631	0.838	2.112	0.426	.....	1:1.4	41
205	1.250	0.164	0.875	.....	.....	0.375	0.426	.....	1:0.3	70
Non-Inflammatory. Neoplasms.										
63	4.250	0.053	1.776	0.490	1.280	2.484	0.390	.....	1:1.4	41.8
202	5.023	0.038	.....	.....	.....	.....	0.440	.....	.....	.....
203	4.950	0.048	.....	.....	.....	.....	0.433	.....	.....	.....

Four fluids in this series are of special interest (20, 21, 60, and 61). They represent fluids which were obtained from two patients upon whom the paracentesis was repeated. Fluids 20 and 21 were removed from the chest of a patient at an interval of three days. Notwithstanding the short interval the two fluids show considerable differences in composition. The fluid from the second paracentesis (21) contains less protein than the first,—3.969 grams as compared with 4.400 grams per 100 cubic centimeters; but the ratio of globulin to albumin is higher by 7.6 per cent. than that present in the fluid from the first paracentesis. The incoagulable nitrogen in the second fluid is almost double that of the first. The chloride content, on the other hand, is slightly lower. The ash content is the same in

both fluids, and if we compute the chlorides in these fluids as sodium chloride, then we obtain values of 0.613 and 0.592 of a gram per 100 cubic centimeters, respectively, which leaves a balance of 0.037 of a gram and 0.058 of a gram per 100 cubic centimeters of fluid for the other salts in the ash.

The second set of effusions show different changes in composition from those observed in the fluids just described. In this instance the second paracentesis was performed twelve days after the first. The fluid from the second paracentesis (61) has a lower protein content than the first (60), *i. e.*, 3.412 grams as compared with 3.831 grams per 100 cubic centimeters. The incoagulable nitrogen falls slightly, whereas the chloride content rises considerably. The most striking change occurs in the percentage of globulin; it rises from 39.3 per cent. to 74.3 per cent. In both sets of analyses it is found that, whereas the percentage of total globulin rises in the second fluid, the percentage of euglobulin to pseudoglobulin falls. This is especially marked in the second set of fluids (60 and 61). It is likely that the cause for the change in the quantitative relations of the globulin rests on a difference in the rate of secretion or resorption of the albumin and globulin fractions of the serum. This, however, will be considered at another time.

The three fluids from the tuberculous cases are in most respects like the other effusions of inflammatory origin. They possess a high protein content. One of these (53) contains an unusually large amount of protein, practically as much as blood serum. It may be added that in this particular fluid a very unusual amount of fibrin was found.

Fluid 30 is from a case of empyema, resulting from a pneumonic infection. This fluid possesses a high protein content, a large amount of incoagulable nitrogen, a small amount of chlorides, and a very low percentage of globulin (20.8 per cent.). In this latter fact we find a contradiction to the usual statement and belief that the serum of purulent fluids is rich in globulins, because of the presence of disintegrating pus cells in the fluid.

In its general character the fluid from a case of intrathoracic lymphosarcoma resembles the fluids that are of inflammatory origin. It does not present any distinguishing features.

The last fluids in this series are from cases of chronic nephritis. In their protein content these are the lowest in the series. The incoagulable nitrogen in one of these (56) is only moderately elevated, whereas the chlorides are rather high. In the second fluid both groups of substances show very high values.

The chemical relation of these fluids to the blood sera of the corresponding cases can be seen in table IV.

TABLE IV.

Case 56.

		Pleural fluid.	Blood serum.
Gm. per 100 c.c. of fluid	Total protein	3.581	7.525
	Incoagulable nitrogen	0.060	0.120
	Total globulin	1.469	2.537
	Albumin	2.112	4.988
	Chlorides	0.426	0.480
	Per cent. of globulin	41.7	34.0
	Case 205.		
Gm. per 100 c.c. of fluid	Total protein	1.250	7.087
	Incoagulable nitrogen	0.164	0.187
	Globulin	0.875	4.775
	Albumin	0.375	2.312
	Chlorides	0.426	0.412
	Per cent. of globulin	70.0	67.0

As table IV shows, striking differences exist between the composition of the effusion fluids, and that of the corresponding blood sera. In one instance (56) the pleural fluid contains less than half of the protein found in the blood serum. In the other case (205) there is even less protein, constituting less than 18 per cent. of that in the blood serum. The incoagulable nitrogen in the first of these fluids is half of that of the corresponding blood serum, in the second it constitutes almost 90 per cent. The chloride in one fluid (56) is lower than the value found in its related blood serum. In the second fluid the chloride content exceeds that of the blood serum.

The percentage of globulin in each fluid corresponds to that of the blood serum from the same case, but in both it is higher than that of the blood serum.

The differences observed in the composition of the pleural fluids and that of the blood sera from the same cases are not easy to

explain. The difference in the amounts of incoagulable nitrogen and chlorides indicates two points: first, that the diffusion of the organic bodies constituting the incoagulable nitrogen is of a different order from that of the inorganic salts; and second, that the difference in the content of these two groups of substances in the pleural fluid and the blood cannot be accounted for by the accepted rules of diffusion and osmosis alone.

ABDOMINAL FLUIDS.

Of the abdominal effusions twenty fluids were examined (table V). Of these, one is of tuberculous origin, two are due to neoplasms of abdominal viscera, nine are due to cardiac insufficiency, four are produced by cirrhosis of the liver, and four occurred in nephritis. One of the latter was from a case of pure nephritis and the other three were from nephritis complicated by disease of the heart.

The character of these fluids is like that of the pleural fluids of the same origin. The two carcinomatous fluids contain moderate amounts of protein, of which half or more is globulin. The incoagulable nitrogen in these two fluids is comparable with the incoagulable nitrogen content of normal blood serum. The chloride content presents no unusual values. In the tuberculous fluid the protein content is moderate in amount, but very much less than that found in pleural fluids of similar origin. The percentage of globulin is equivalent to that of blood serum. The chloride content is high. The other ingredients also closely approximate the values usually obtained in the examination of the blood.

The abdominal fluids of cardiac origin appear to stand in etiologic relationship to static disturbances. The composition of these fluids, with one or two exceptions, is more or less uniform. Their protein content varies between 1.567 and 4.712 grams per 100 cubic centimeters. In seven of the fluids the protein ranges above 3 grams. In nearly all of the latter the globulin content is the same as that found in the serum of normal blood. The same is true of the incoagulable nitrogen. With but two exceptions (7 and 69), the chloride is considerably elevated, ranging from 0.362 of a gram to

0.454 of a gram per 100 cubic centimeters. In one instance (7) the ash was also determined, and was found to be equal to that of normal blood serum.

TABLE V.

Abdominal Effusions.

Case No.	Total protein.	Incoagulable nitrogen.	Total globulin.	Euglobulin.	Pseudoglobulin.	Albumin.	Chlorides.	Total solids.	Ash.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
	Gm. per 100 c.c. of fluid.										
Tuberculous and New Growths.											
105	1.725	0.045	0.281	.....	.....	1.444	0.398	.....	.....	1:5.1	16.3
209	3.725	0.040	1.838	.....	.....	1.887	0.385	.....	.....	1:1.+	49
208	3.681	0.043	1.462	.....	.....	2.219	0.418	.....	.....	1:1.5	39.7
Cardiac.											
7	2.080	0.037	0.882	0.189	0.693	1.198	0.362	3.400	0.958	1:1.36	42.3
28	1.567	0.049	0.692	0.275	0.417	0.975	0.454	.....	.....	1:0.6	62
40	3.675	0.084	1.919	0.625	1.294	1.756	0.412	.....	.....	1:0.9	52.2
69	4.694	0.055	1.787	0.750	1.037	2.907	0.376	.....	.....	1:1.6	38
81	4.712	0.055	1.656	0.456	1.200	3.056	0.404	.....	.....	1:1.9	34
32	3.230	0.053	1.062	0.372	0.690	2.168	0.412	.....	.....	1:2	33.3
66	3.702	0.076	1.462	0.500	0.962	2.240	0.418	.....	.....	1:1.5	39.9
68	3.123	0.077	1.331	0.463	0.868	1.792	0.404	.....	.....	1:1.3	42.6
207	3.381	0.040	.....	.....	.....	.....	0.440	.....	.....	.....	.....
Cirrhosis of the liver.											
15	3.332	0.090	1.625	0.525	1.100	1.707	0.428	4.815	0.950	1:1.05	49
26	3.017	0.051	1.012	0.205	0.707	2.005	0.390	.....	.....	1:2.0	33.3
67	0.521	0.039	0.325	0.125	0.200	0.193	0.326	.....	.....	1:0.6	62.3
79	0.686	0.038	0.400	0.131	0.769	0.256	0.497	.....	.....	1:0.6	62.3
Nephritis.											
1a	0.285	0.035	0.285	.....	.....	.....	0.420	1.040	0.875	.....	100
Cardionephritis.											
32	3.230	0.053	1.062	0.372	0.690	2.168	0.412	.....	.....	1:2	33.3
66	3.702	0.076	1.462	0.500	0.962	2.240	0.418	.....	.....	1:1.5	39.9
68	3.123	0.077	1.331	0.463	0.868	1.792	0.404	.....	.....	1:1.3	42.6

In one case (table VI) both the blood serum and the ascitic fluid were examined. The protein content of the ascitic fluid is less than half of that of the blood serum, but the percentage of globulin is nearly double that of the other. In other respects the two fluids are alike.

Of the fluids arising in cases of cirrhosis of the liver, two (15 and 26) resemble the abdominal fluids of cardiac origin; the others differ considerably. The first two contain over 3 grams of protein per 100 cubic centimeters of fluid, and the percentage of globulin is the same as that found in the fluids of cardiac origin. The same

TABLE VI.

Case 207.

		Ascitic fluid.	Blood serum.
Gm. per 100 c.c. of fluid	Total protein	3.381	7.575
	Incoagulable nitrogen	0.040	0.040
	Globulin	1.481	1.956
	Albumin	1.900	5.619
	Chlorides	0.440	0.433
	Per cent. of globulin	43.0	26.0

is true of the incoagulable nitrogen and chloride contents. In the other two fluids (67 and 79) the protein content is rather low,—slightly over 0.5 of a gram per 100 cubic centimeters,—but the globulin fraction is very high in both, being 62.3 per cent. The incoagulable nitrogen in these two effusions is low, or comparable to that of normal blood serum. The chloride content in one fluid (67) is very low, 0.326 of a gram per 100 cubic centimeters, in the other exceedingly high, 0.497 of a gram per 100 cubic centimeters. It would have been of interest to compare the quantities of the different ingredients with those of the blood serum of the corresponding cases, but these were not available.

Four abdominal fluids of renal origin were examined. Of these only one is from a case of pure nephritis. The others are from cases of nephritis complicated by heart disease. The latter resemble the composition of the fluids of cardiac origin described above. The protein content is over 3 grams to 100 cubic centimeters. The incoagulable nitrogen is slightly elevated; the chloride content is high, but unlike the other fluids. The globulin percentage is low, averaging 38.6 per cent. In this the fluids resemble normal blood serum. The fluid of purely nephritic origin (1a) contains a small amount of protein (0.285 of a gram to 100 cubic centimeters), but all of it is globulin. In this respect this fluid is different from all the other fluids examined excepting the subcutaneous effusions

of nephritic origin. The incoagulable nitrogen is low, but the chloride content is very high, 0.420 of a gram per 100 cubic centimeters. The value obtained in the analysis of the ash is low and considerably under that of normal blood serum. If we deduct from the quantity of ash the salt equivalent of the chlorides in it, there remains but a small balance for other salts.

The abdominal fluid just described gains in interest when the results are compared with values obtained in the analysis of the blood sera from nephritic cases.[4]

HYDROCELE FLUID.

One hydrocele fluid was also examined; the results are shown in table VII. This fluid is rich in protein, but its globulin content is

TABLE VII.

Hydrocele Fluid.

Case No.	Total protein.	Incoagulable nitrogen.	Total globulin.	Euglobulin.	Pseudoglobulin.	Albumin.	Chlorides.	Total solids.	Ash.	Ratio of globulin to albumin.	Per cent. of globulin in protein.
	Gm. per 100 c.c. of fluid.										
17	5.332	0.061	1.100	0.270	0.830	4.232	0.412	6.960	0.945	1:3.8	20.7

low. The chlorides are rather high, as is the ash. The other ingredients present nothing striking.

SUMMARY.

A comparison of the results obtained in the analysis of the different effusions shows that they vary, (1) according to location, and (2) according to the disease in which they are produced. Thus the subcutaneous effusions are totally different in their composition from the abdominal or pleuritic fluids; and again the abdominal fluid of nephritic origin is different from those of cardiac or other origin.

The cutaneous effusions are characterized by a very low protein content and a small amount of incoagulable nitrogen. Of the protein present in these fluids, the globulin constitutes the greater

[4] Epstein, *Jour. Exper. Med.*, 1912, xvi, 719.

portion. In the mixed cardionephritic fluids the ratio of globulin to albumin is lower than that in normal blood serum; but when compared with the blood sera of cardionephritic cases there appears to be a certain parallelism. The chloride content of these fluids is considerably above that found in normal blood serum, but is comparable with those of corresponding cases.

The effusions occurring in serous cavities differ from the cutaneous ones by their higher protein content. The highest values are attained in the pleural fluids, in which the protein present is almost the same as that in normal blood serum. The incoagulable nitrogen in these fluids is uniformly low. The chlorides vary in amount according to the nature of the case. In the effusions of inflammatory origin the values are lower than those found in the blood. In the pleural fluids of other than inflammatory origin the chloride content is either the same or higher than that of blood serum. The globulin-albumin ratio in all of these fluids except one[5] (table III, fluid 61) is higher than that of normal blood serum. Otherwise the highest value is presented by a pleural fluid from a case of nephritis (table III, fluid 205) and the lowest by an empyema fluid (table III, fluid 30). The latter case is of especial interest because of the theory, first propounded by Schmidt, that the leucocytes in the purulent fluid are largely responsible for high globulin content.

The abdominal fluids in general are less rich in protein than the pleural effusions. Those of cardiac origin give the highest protein values, but the globulin-albumin ratios are lower than in the pleural fluids or in the blood sera of corresponding cases. Only in two fluids does the globulin reach a percentage of 62 and 52.2. In both these cases the chloride content is also high. The incoagulable nitrogen in all of them is rather low. In one fluid of purely nephritic origin the protein content is low and all of it is globulin. This is suggestive, when compared with the values obtained in the analysis of the blood sera from nephritic cases. The results obtained in the analysis of abdominal fluids from cardionephritic cases approach very closely those obtained in purely cardiac cases.

[5] This fluid was obtained at a second tapping. The fluid obtained at the first paracentesis is shown in table III, fluid 60.