Seromucoid Fraction Patterns of Individuals with Pneumonia or Leukemia

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ABSTRACT Chromatographic separations on DEAE cellulose have been carried out on the seromucoid fraction from forty-one normal individuals and twenty-three patients horpitalized with unilateral pneumococcal pneumonia. During the acute stages of their illness, all twenty-three patients showed a very marked difference in their seromucoid pattern as contrasted to the control group, both groups being comparable in age, sex, and race. As the patients recovered, their seromucoid patterns returned to that of the control group. Three patients with bilateral pneumococcal pneumonia showed seromucoid patterns similar to those of the twenty-three patients with unilateral pneumococcal pneumonia. Five individuals with viral pneumonia showed a seromucoid pattern similar to those observed in patients with pneumococcal pneumonia. The data suggested that the seromucoid pattern observed in pneumonia depended upon the stage of the disease of the patient, different patterns being observed during the acute, convalescent, and complete recovery stages. Each stage showed a characteristic pattern. The seromucoid pattern of seven patients with acute leukemia differed from that of the control groups and also differed from that observed in patients with pneumonia. Five individuals with chronic lymphocytic leukemia showed seromucoid patterns which could *not* be distinguished from those of the control group in spite of the fact that all showed clinical signs of disease at the time the blood sample was drawn for fractionation.

A great deal of evidence has accumulated that individuals suffering from many diverse diseases show elevated levels of seromucoid in their blood (1). Although there have been a number of theories to account for these elevated levels there is no definitive data available at the present time to account for this phenomenon (1). An important question which remains to be answered is whether the seromucoids found in diseased individuals are different from normal; indeed, whether there may be different seromucoids appearing in the blood depending upon the disease studied. Experiments described in this paper were designed to explore the above problems.

Harshman's (2) chromatographic method for separating seromucoids of normal human serum was improved (3) to make it more reproducible. It has been further modified to permit examination of the possible differences between the seromucoid fractions of normal individuals and those ill with one of several diseases. Using this modified method, investigations were undertaken to compare the seromucoid fractionation patterns of normal individuals with those of patients with pneumococcal pneumonia. Preliminary results had shown that the seromucoid pattern in various diseases depended upon the time at which the blood sample was taken in relation to the clinical course of the disease. Pneumococcal pneumonia was selected because (*a*) the diagnosis of this disease can be accurately determined, (*b*) the onset of the disease can be fairly accurately determined, (*c*) the disease can be controlled by antibiotics so that it would be possible to study the seromucoid fractions during the acute and convalescent stages and relate them fairly accurately to the course of the illness, (*d*) the time period for this latter study would not be of long duration, and (*e*) there would be enough cases so that statistically significant results could be obtained in a short period of time.

In this paper it will be shown that the seromucoid pattern of all twenty-three patients with unilateral pneumococcal pneumonia early in the course of hospitalization was different from that observed in normal individuals, both groups being matched for age, sex, and race. All patients showed patterns that were in general similar. Further studies showed that as the patients recovered clinically, their abnormal patterns returned to normal in 2½ to 4½ weeks. Three patients with bilateral pneumonia showed similar patterns. Other data will be presented to show that five individuals with viral pneumonia showed a pattern similar to the patients with pneumococcal pneumonia. However, the abnormal patterns in individuals with viral pneumonia took a longer time to return to normal than did those in the bacterial disease. Seven patients with acute leukemia showed a seromucoid pattern that differed from that observed in patients with pneumonia and from that seen in the control group. Five individuals with chronic lymphocytic leukemia showed seromucoid patterns which could not be distinguished from the control group.

MATERIALS AND METHODS

Preparation of Cellulose Columns DEAE cellulose was prepared and used as described previously (3). A slurry of this cellulose must stand for 2 months at 6 to 8°C and then be given another complete wash in order for fractionations to be reproducible. Following this, after standing another week at 6 to 8°C it is ready for use. The columns were prepared in 10.0 ml graduated pyrex pipettes. The bottoms of the pipettes were filled with glass wool and a bed of 9.5 cm of washed DEAE cellu-

lose was added. The columns were equilibrated for 2 hours with 0.001 M NaCl at room temperature and then left at 6 to 8°C for 14 hours. They were removed from the cold room the next morning and equilibrated for 30 minutes at room temperature. Approximately 1 ml of 0.001 M NaCl per minute dripped through the columns during the equilibration period. A total of approximately 150 ml of 0.001 M NaCl was used for the equilibration.

The reproducibility with various lots of cellulose was satisfactory. The only difficulty was that with some lots, the fifth fraction had to be eluted with 0.098 M NaCl, in other lots with either 0.10 M or 0.11 M NaCl, in order to show the differences between the normal and pathological sera in this fraction. However, by testing each lot of cellulose against standard normal and pathological sera, it was quickly possible to decide which salt concentration would be most suitable for eluting the fifth fraction with each particular lot of cellulose.

Preparation of Seromucoid Fraction The seromucoid preparation was made as described earlier (3). The determination of the concentration of seromucoid in the preparation and in each fraction was carried out as described previously using the Beckman spectrophotometer.

Elution of Seromucoid Preparations After the column had been equilibrated with a solvent as described above, the seromucoid preparation was dripped onto the column prepared as described previously. Six tubes of 3.5 ml of 0.001 M NaCl were then collected using a flow rate of 0.35 ml per minute. The seromucoid fraction was then eluted by various NaCl concentrations as described previously but using a flow rate of 0.35 ml per minute. The collection time for each tube was for 10 minutes in all instances. Between 12.0 and 12.3 total seromucoid units were used for all fractionations on DEAE cellulose.

The fractions eluted by the various salt concentrations were collected as described previously. However, the fifth fraction was collected in the first 10 tubes after the concentration was changed to the fifth NaCl concentration and then the eluent was switched to 0.135 M NaCl and 10 tubes were collected for the sixth fraction.

A standard of serum albumin was read every 25 tubes during the measurement of optical density, to check the constancy of the instrument.

Chromatographic Separation of the Seromucoid Fractions Using the Modified Method Fig. 1 shows a typical chromatographic separation of the seromucoid fractions from a normal individual using the modified method. Rechromatographic experiments carried out as described previously showed that all fractions kept the distinctive elution characteristics in the various NaCl concentrations that were used for the initial elution. This method was used because it gave more reproducible fractionations with pathological sera than did the earlier method (3).

It should be emphasized that a great many variables have been found to influence the fractionation of seromucoid on DEAE cellulose in addition to the solvents employed in eluting the various fractions of the seromucoid preparations. These include the pH and dialysis time in making the seromucoid preparations (3), the pH at which the seromucoid preparation is adsorbed to the cellulose, the length and diameter of

the column, the way in which the cellulose is washed and stored, the amount of cellulose in the column, the method used to equilibrate the column, the amount of seromucoid added to the column, and the flow rate used to elute the various fractions.

Although it is possible to fractionate seromucoid into various fractions using a number of techniques, the only method we have found which gives reproducible differences between the seromucoid patterns of normal and sick individuals is that

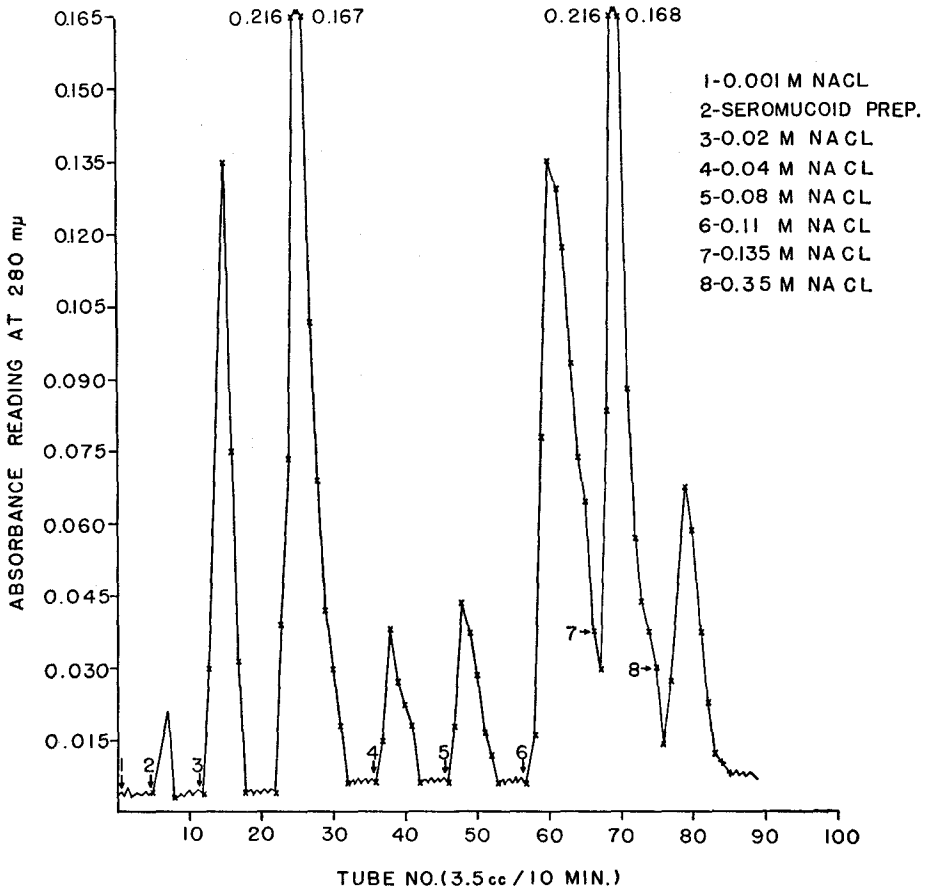

FIGURE 1. Fractionation of seromucoid on DEAE at pH 7.2.

described in this paper. The method described in this paper is not necessarily the best one to employ if one is interested in the chromatographic separation of the seromucoid fraction from other standpoints. However, as will be shown, the method does satisfy the objective of this study.

The results in Table I show that the reproducibility of the method is satisfactory for the objective of this investigation. The reproducibility of the method was tested by fractionating an aliquot of the same serum every week for 8 weeks. Similar tests with two other sera gave standard deviations approximately 1½ to 2 times those shown in Table I.

Sources of Human Sera The sick individuals included twenty-six cases of pneumococcal pneumonia, five of primary atypical viral pneumonia, seven of acute leukemia, and five of chronic lymphocytic leukemia. All were adult white males, hospitalized at the Johns Hopkins Hospital, the Baltimore City Hospitals, and the University Hospital in Baltimore. They were diagnosed by the medical staffs of these institutions, whose contribution is gratefully acknowledged. Dr. T. H. Woodward supplied the blood specimens from most of the leukemia cases.

The pneumococcal pneumonia cases were aged 28 to 62. Their diagnoses were supported by bacterial cultures and x-rays. None of the patients had received anti-

TABLE I

REPRODUCIBILITY OF FRACTIONATION METHOD IN EIGHT FRACTIONATIONS OF THE SAME SERUM*

	Fractions (eluted by various M NaCl)						
Experiment No.	0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.11 (5)	0.135 (6)	0.35 (7)
1	1.02	2.51	0.41	0.60	2.31	2.19	1.31
2	0.89	1.97	0.36	0.49	2.12	2.01	1.52
3	0.93	2.21	0.45	0.58	2.32	2.06	0.91
4	1.11	2.61	0.38	0.51	2.08	1.91	1.23
5	0.82	1.91	0.42	0.62	2.20	1.86	1.18
6	0.97	2.46	0.46	0.58	1.92	1.99	1.44
7	1.12	2.43	0.39	0.49	1.99	2.03	1.28
8	0.92	2.28	0.35	0.62	2.12	1.89	1.17
Mean	0.973	2.298	0.403	0.560	2.133	1.992	1.255
SD	0.098	0.238	0.037	0.052	0.118	0.100	0.173

* 12.0 units were always used for the chromatographic separations. Values in columns refer to total units recovered in each fraction. The number of units of seromucoid was calculated by the method described under Methods.

biotic therapy prior to admission; all were placed on penicillin treatment within 48 hours after admission.

The time of initial bleeding as related to the beginning of pathologic changes in pneumococcal pneumonia is not precisely known. The incubation period is poorly determined but is believed to be from 1 to several days. Shaking chills and fever were chosen as representing the first symptoms of the disease, as they are characteristic and easy to delineate. In Table IV, the interval from onset to hospital admission, and the interval from admission to bleeding are given for each case. Each individual was bled several times during the acute and convalescent stages of his illness.

Dramatic improvement began within 48 hours after the institution of penicillin therapy in these patients, as is usually the case. In all the cases, there were few clinical signs of illness persisting more than 3 days after initiation of penicillin therapy, and all were released from hospital within a period of 14 days.

Of the five individuals with viral pneumonia, four were young adults and one was aged 39. Cases were selected whose diagnoses were not in doubt and who had no other known diseases or superimposed bacterial infections. They had dry hacking coughs

unproductive of much sputum, and x-rays showed extensive pulmonary involvement. Their fever or other clinical complaints persisted for 3 to 6 weeks after admission. Two of the patients had dyspnea, cyanosis, and severe occipital headaches, and were hospitalized 36 and 42 days respectively.

The patients with acute leukemia were aged 43 to 59. Their peripheral white blood counts at the time of study ranged from 220,000 to 800,000. Four were classified as of the myelogenous type, and three as lymphocytic.

TABLE II

CONCENTRATIONS OF SEROMUCOID IN VARIOUS FRACTIONS FROM SERA OF FIFTEEN NORMAL WHITE MALES*

Individual's No.	Age	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
	yrs.						
1-N	28	0.80	1.92	0.21	0.27	0.52	4.86
2-N	41	1.12	2.86	0.33	0.26	0.61	5.12
3-N	53	0.94	2.77	0.43	0.54	0.32	5.04
4-N	58	1.20	3.11	0.61	0.44	0.28	4.59
5-N	53	0.81	2.83	0.26	0.47	0.24	5.16
6-N	49	1.01	2.49	0.63	0.42	0.27	4.62
7-N	52	0.87	2.67	0.45	0.65	0.31	5.13
8-N	59	1.37	2.73	0.49	0.42	0.78	5.07
9-N	33	0.71	1.61	0.24	0.62	0.58	4.93
10-N	49	0.80	2.72	0.28	0.52	0.39	5.06
11-N	54	0.79	1.82	0.31	0.76	0.27	4.72
12-N	57	0.82	2.11	0.36	0.52	0.47	4.98
13-N	49	0.74	1.84	0.43	0.57	0.69	5.10
14-N	29	0.91	2.09	0.35	0.64	0.38	4.69
15-N	37	0.89	2.91	0.30	0.60	0.28	4.98

* For fractionating all sera samples, 12.0 to 12.3 units were added to all columns. Numbers in each column refer to number of units found in each fraction as determined by procedure described under Methods.

The controls were also white males and were matched with the patients for age. They had no known disease. There were forty-one controls.

All specimens were coded by number and the personnel who carried out the fractionations did not know the source of the sera. Several bleedings from the same individual were kept frozen at −20°C and all samples fractionated on the same day. The serum of a control individual as well as a standard serum, was included in every fractionation test.

RESULTS

Seromucoid Pattern of Normal Individuals. The results in Table II show the concentration of the various seromucoid fractions in the sera of the first fifteen normal individuals that were studied. All seven fractions were present

in all sera and there is a fairly consistent over-all pattern. It should be noted that the quantity of seromucoid in the second fraction is always at least twice as much as in the first fraction. The importance of this finding will be discussed later. Twenty-six other controls gave results similar to those shown in Table II.

Since the patients with pneumonia were bled several times during the

TABLE III

SEROMUCOID PATTERNS OF FOUR NORMAL INDIVIDUALS EACH BLED ON FOUR OCCASIONS*

Individual's No.	Age	Time of bleeding	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
	yrs.							
16-N	38	Initial	1.12	2.61	0.61	0.42	0.31	4.61
		2 wks.	0.92	2.49	0.53	0.32	0.21	4.19
		3 wks.	0.98	2.29	0.49	0.38	0.44	4.28
		1 mo.	1.18	2.56	0.54	0.49	0.28	4.38
17-N	59	Initial	0.82	2.32	0.54	0.64	0.46	4.82
		2 wks.	0.98	2.41	0.39	0.81	0.32	4.13
		3 wks.	0.79	1.92	0.48	0.49	0.28	4.28
		1 mo.	0.88	2.05	0.57	0.52	0.46	4.68
18-N	45	Initial	0.91	2.42	0.49	0.46	0.51	5.12
		2 wks.	0.98	2.61	0.39	0.41	0.62	4.97
		3 wks.	0.81	2.32	0.41	0.31	0.58	5.38
		1 mo.	1.05	2.51	0.38	0.42	0.41	5.12
19-N	57	Initial	1.34	2.86	0.42	0.72	0.42	5.03
		2 wks.	1.17	2.49	0.32	0.61	0.29	5.24
		3 wks.	0.98	2.46	0.46	0.59	0.46	4.91
		1 mo.	1.22	2.76	0.36	0.68	0.34	5.28

* Twelve to 12.3 units were added to all columns. The numbers under the fractions refer to the total number of units found in each fraction.

first 2 weeks that they were being studied, as well as bled twice during their late convalescent stages, four normal individuals were bled in a similar manner. The data in Table III show that the seromucoid pattern of the several bleedings from the same individuals remained approximately the same during the month they were under study. The four individuals complained of no illness during this month. These results give further support for the reproducibility of the method.

Seromucoid Pattern of Individuals with Pneumococcal Pneumonia. Investigations were carried out on twenty-three individuals hospitalized with unilateral pneumococcal pneumonia. Modern therapy with antibiotics has changed the clinical course of pneumococcal pneumonia drastically. The classical crisis, the sudden fall in temperature, and general improvement formerly seen in

patients are no longer evident. Instead, dramatic improvement usually begins within 48 hours after the onset of penicillin therapy. All twenty-three were released from the hospital within a period of 14 days and had few clinical signs of illness from 2 to 3 days after the initiation of penicillin therapy.

Each individual was usually bled several times during the acute and convalescent stages of his illness. The seromucoid patterns of the first ten patients studied are presented in Table IV. The patients were between the ages of 28 and 62 and their age did not seem to influence significantly their seromucoid patterns. All patients shown in Table IV had pneumonia in only one lobe of the lung. The site of the lobar pneumonia in these patients had no apparent effect on their seromucoid patterns. It will be noted that during the acute stages of the illness, the second fraction is markedly lower, the third fraction is usually lower, and the fifth and sixth fractions higher than the corresponding fractions observed in the normal individuals. It should be emphasized that the normal group were matched for age, sex, and race with the patients. Although no significant difference in seromucoid patterns had been noted in normal white males between the ages of 25 to 50, the control ages were picked so that the normal population was within 2 years of the individuals with pneumococcal pneumonia. Of importance was the fact that as the patients responded to antibiotic treatment and recovered, their seromucoid patterns returned to normal within 3½ to 4½ weeks as illustrated in Table IV. The other nine patients with pneumococcal pneumonia gave results very similar to those shown in Table IV.

One patient, case 14-PN, showed a normal pattern at the end of 5 weeks. Nine days after this last bleeding he was readmitted to the hospital with another attack of pneumococcal pneumonia.[1] His first blood sample during his second attack was taken on the 1st day of admission before any treatment had been started. The data in Table V show that this person again, during the second attack, showed the seromucoid pattern seen in other patients with this disease.

In four cases of pneumococcal pneumonia, the maximum optical density readings of the seromucoid preparations varied between 0.452 to 0.486. These values are lower than those found in the nineteen patients but consistently higher than normal controls. Although these four cases showed seromucoid patterns very similar to the nineteen described above during the early stages of their disease, their patterns reached a recovery phase in 1 week that the above nineteen cases attained only after 2 weeks. There was little if any change in the total seromucoid levels during the 1st week in these four cases in spite of the reversal in the seromucoid pattern. At this point in the study it

[1] This patient was an alcoholic. In his first attack he was released from the hospital 11 days after his admission.

TABLE IV

SERIAL DETERMINATIONS OF SEROMUCOID FRACTIONS OF SERA OF TEN INDIVIDUALS WITH PNEUMOCOCCAL PNEUMONIA*

Case No.	Interval between onset‡ and admission, *hrs.* (approximately)	Interval between admission and bleeding	OD§	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
1-N	6	2 hrs.	0.54	0.64	0.63	0.32	0.68	1.03	5.21
		3 days	0.65	0.83	0.51	0.19	0.61	1.41	5.87
		7 days	0.84	0.79	0.91	0.21	0.73	1.27	5.42
		2 wks.	0.61	0.81	1.51	0.36	0.72	1.24	5.11
		3 wks.	0.49	1.04	2.41	0.47	0.59	1.06	4.59
		4 wks.	0.41	0.96	2.52	0.49	0.67	0.53	4.12
2-PN	48	3 days	0.82	0.71	0.63	0.09	0.63	1.26	5.25
		7 days	1.01	1.02	0.42	0.11	0.53	1.58	5.88
		2 wks.	0.89	0.89	1.38	0.36	0.59	1.62	5.54
		3 wks.	0.71	0.82	1.87	0.38	0.67	1.14	5.16
		4 wks.	0.53	0.91	2.44	0.41	0.52	0.92	4.27
		5 wks.	0.42	0.83	2.31	0.52	0.59	0.66	3.96
3-PN	12	2 days	0.47	0.71	1.29	0.22	0.42	1.32	5.71
		6 days	0.59	1.06	0.89	0.12	0.38	1.62	6.04
		3 wks.	0.66	0.86	1.97	0.32	0.42	0.92	5.13
		4 wks.	0.42	1.23	2.44	0.57	0.41	0.86	4.62
		5 wks.	0.33	1.16	2.29	0.59	0.42	0.86	4.12
4-PN	9	4 days	0.65	1.61	0.86	0.10	0.38	1.07	5.84
		7 days	0.72	1.38	1.01	0.05	0.38	1.47	5.31
		2 wks.	0.61	0.70	1.55	0.12	0.34	1.32	5.25
		3 wks.	0.50	0.82	1.93	0.36	0.41	0.92	4.93
		4 wks.	0.43	0.91	2.11	0.31	0.47	0.81	4.17
		6 wks.	0.38	0.96	2.43	0.33	0.39	0.52	3.44
5-PN	18	3 days	0.56	0.97	1.00	0.04	0.17	1.58	6.49
		6 days	0.61	0.82	1.12	0.12	0.24	1.49	6.28
		2 wks.	0.46	0.89	1.59	0.25	0.31	1.12	3.28
		5 wks.	0.32	1.24	2.80	0.65	0.71	0.31	3.66
6-PN	2	4 hrs.	0.54	0.64	0.63	0.13	0.08	1.28	5.42
		3 days	0.63	0.82	0.61	0.06	0.19	1.58	6.41
		7 days	0.66	1.09	1.01	0.21	0.24	1.21	5.28
		2 wks.	0.51	0.91	1.44	0.28	0.52	0.86	5.18
		5 wks.	0.42	0.81	1.92	0.29	0.56	0.36	4.23
7-PN	15	3 days	0.61	1.03	1.11	0.21	0.73	2.16	5.21
		8 days	0.53	0.82	1.55	0.361	0.66	1.72	5.04
		3 wks.	0.49	1.02	2.97	0.57	0.63	0.79	4.49
		5 wks.	0.35	0.92	2.71	0.56	0.67	0.66	3.88

* All patients were released from the hospital within 14 days after the penicillin therapy was initiated.

‡ Based on interval when patient first experienced shaking chills and fever before patient was admitted to hospital.

§ In this table and in all other tables in which such data are given, all volumes for the optical density at 280 mμ were corrected to a standard volume so that the readings could be directly compared with each other. These values show the optical density of the seromucoid fraction before fractionation.

TABLE IV—*Concluded*

Case No.	Interval between onset‡ and admission, *hrs.* (approximately)	Interval between admission and bleeding	OD§	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
8-PN	4	3 days	0.51	0.97	1.00	0.08	0.81	1.39	5.91
		7 days	0.89	0.81	0.53	0.16	0.72	1.14	5.89
		2 wks.	0.71	0.71	1.42	0.24	0.69	1.36	5.52
		3 wks.	0.64	0.81	1.82	0.31	0.71	0.91	4.98
		4 wks.	0.52	0.81	2.72	0.36	0.59	0.72	4.06
		5 wks.	0.44	1.02	2.67	0.41	0.59	0.63	3.92
9-PN	18	3 days	0.58	0.62	0.61	0.09	0.20	1.35	5.92
		7 days	0.72	1.03	0.59	0.04	0.27	1.45	5.83
		2 wks.	0.66	0.91	0.89	0.12	0.31	1.25	5.53
		3 wks.	0.58	0.81	1.23	0.36	0.28	1.11	5.27
		5 wks.	0.42	1.06	2.35	0.41	0.36	0.62	3.81
10-PN	21	3 days	1.14	0.64	0.28	0.09	0.61	1.69	5.68
		7 days	0.91	0.98	0.69	0.19	0.63	1.32	6.15
		2 wks.	0.74	1.04	1.16	0.26	0.57	1.21	5.83
		3 wks.	0.59	0.86	1.74	0.31	0.49	1.02	5.27
		5 wks.	0.48	0.93	2.57	0.34	0.51	0.58	4.34

is difficult to observe any clear relationship between the total level of seromucoid and the various changes in the seromucoid patterns.

Figs. 2 and 3 show the seromucoid content of fractions 2 and 5 plotted against the per cent of total number of normal individuals or patients. From

TABLE V

SERIAL DETERMINATIONS OF SEROMUCOID FRACTIONS FROM AN INDIVIDUAL SUFFERING A RELAPSE OF PNEUMOCOCCAL PNEUMONIA

Interval between admission and bleeding	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
2 days	0.95	0.84	0.21	0.47	1.63	5.82
7 days	1.03	1.05	0.16	0.51	1.46	5.71
2 wks.	0.89	1.43	0.31	0.53	1.23	5.41
3 wks.	0.91	1.89	0.37	0.58	1.12	5.21
5 wks.	0.81	2.12	0.33	0.57	0.89	4.7
Time after readmission*						
1 day	0.62	0.63	0.18	0.49	2.43	6.31
6 days	1.02	0.47	0.24	0.51	1.92	6.16
3 wks.	1.12	1.16	0.29	0.56	1.77	5.98
5 wks.	0.91	1.83	0.35	0.61	1.05	5.47

* Readmitted to hospital 9 days after the first 5 week blood sample was taken. He was released from the hospital 11 days after admission in the first attack and after 17 days in the second attack.

these figures, which take into account all the twenty-six patients with pneumococcal pneumonia[2] and the forty-one normal controls, it is apparent that there is less seromucoid in fraction 2 and more seromucoid in fraction 5 in the patients than in the control group. These values are all based on the initial bleeding. In the two instances in which the content of seromucoid in fraction 2 of the patients approached the values observed in the normal people, the next subsequent bleedings from both patients showed lower values within the

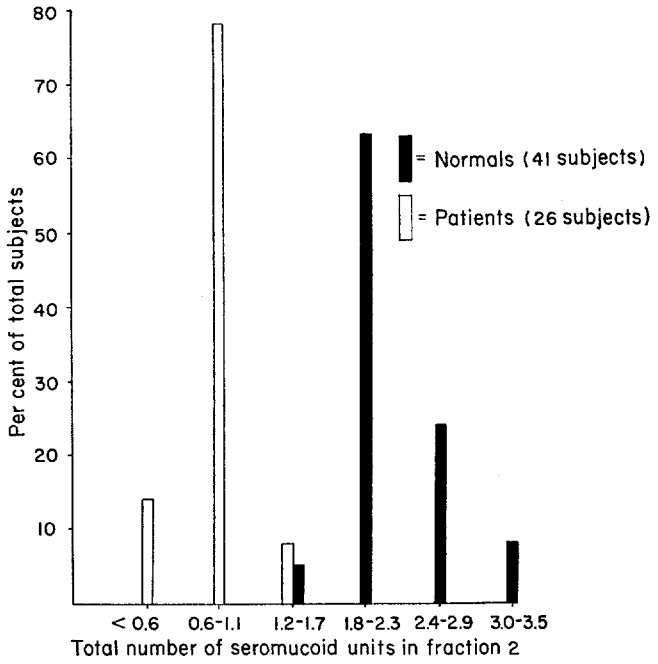

FIGURE 2. Frequency distributions of seromucoid units in fraction 2 for forty-one normal individuals and twenty-six cases of pneumococcal pneumonia on initial bleeding.

range of the other twenty-four cases. This point is emphasized because there is obviously going to be some variation from individual to individual depending upon the interval between the onset of disease and when the patient was first bled as well as other factors which determine the seromucoid response in a patient with pneumococcal pneumonia.

The seromucoid patterns for fractions 2 and 5 taken at various time intervals for three cases of pneumococcal pneumonia and two normal individuals are presented in Figs. 4A and 4B. These two fractions were chosen as they show the most consistent and marked changes in pneumonia. The patterns of the individuals shown in these graphs were picked at random from Table IV.

[2] The patients include three cases of bilateral pneumococcal pneumonia discussed on page 216.

In this study it was usually only possible to obtain three bleedings during the first 2 weeks of the disease. While the patterns observed were quite consistent for all twenty-three patients, it would be desirable to obtain more frequent bleedings during this period. These would be helpful in relating more definitively the abnormal changes in the seromucoid patterns to the pathogenesis of the disease.

Seromucoid Patterns of Individuals with Viral Pneumonia. Five individuals were studied who had viral pneumonia. The seromucoid patterns of these

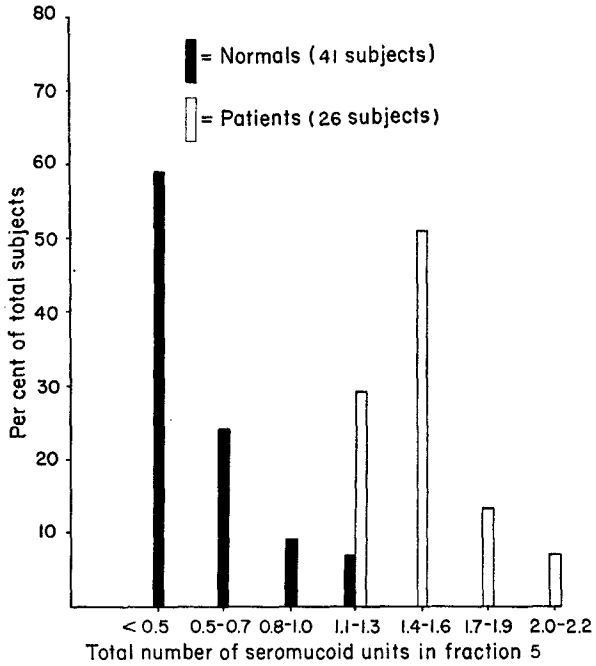

FIGURE 3. Frequency distributions of seromucoid units in fraction 5 for forty-one normal individuals and twenty-six cases of pneumococcal pneumonia on initial bleeding.

patients were investigated during the acute and convalescent stages of their illnesses. The pattern was similar to that observed for pneumococcal pneumonia described above with the following differences: all the first fractions were lower during the acute stages of the viral pneumonia infections than were usually observed in patients with pneumococcal pneumonia (Table VI). These low first fractions were similar to those observed in the three cases of bilateral pneumococcal pneumonia that were studied (Table VII). Another difference between the viral pneumonia and the pneumococcal pneumonia cases described earlier was that the seromucoid patterns of the viral pneumonia patients took a longer period to return to normal. It is of interest that the seromucoid patterns of the two patients with viral pneumonia who had

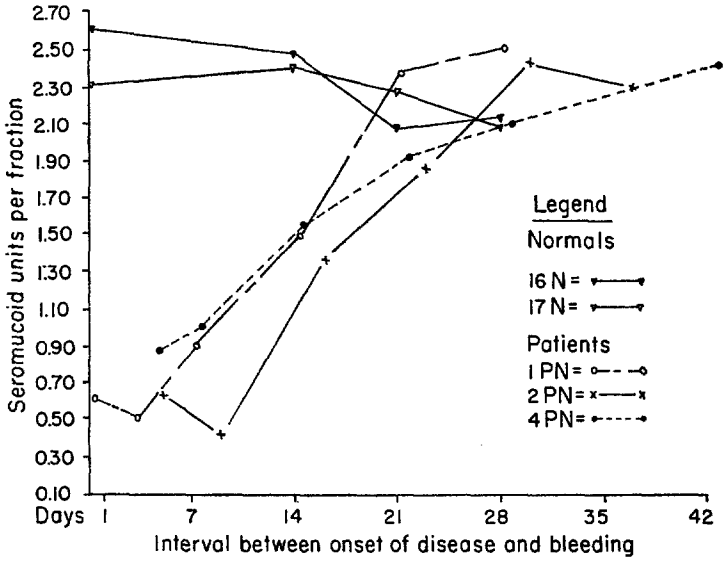

FIGURE 4A. Seromucoid content of fraction 2 during course of pneumococcal pneumonia.

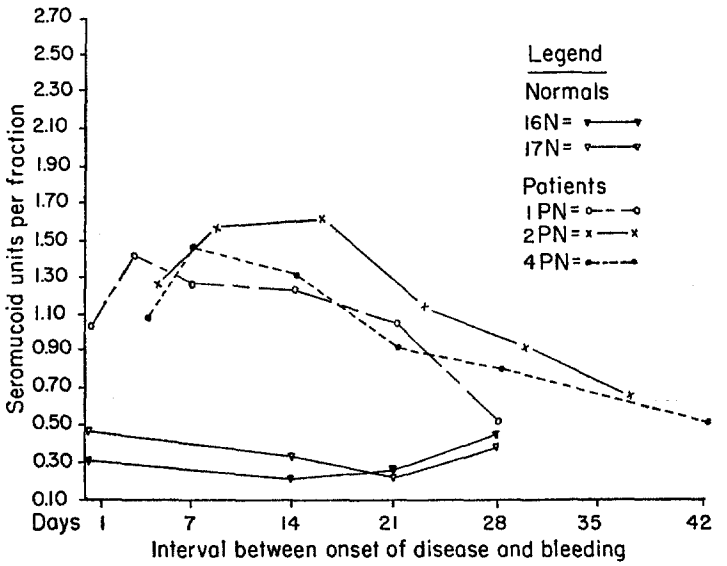

FIGURE 4B. Seromucoid content of fraction 5 during course of pneumococcal pneumonia.

dyspnea and cyanosis, and were the sickest of this group, took the longest to return to normal.

Determinations of Seromucoid Fractions in Leukemia. The seromucoid fractions in seven cases of acute leukemia were investigated. The total seromucoid

levels of the seromucoid preparations before fractionation had optical density readings which varied from 0.76 to 0.93. As can be seen from Table VIII, all patients showed first and second fractions which would have to be considered within the normal range. It should be noted, however, that the fifth and sixth fractions of individuals with acute leukemia were respectively 2½ to 4 times and 1½ times the concentration of seromucoid observed in normal individuals.

TABLE VI

SERIAL DETERMINATIONS OF SEROMUCOID FRACTIONS OF SERA OF THREE PATIENTS WITH VIRAL PNEUMONIA*

Case No.	Age	Interval between admission and bleeding	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
	yrs.							
1-VP	28	5 days	0.52	0.71	0.08	0.09	2.43	6.21
		2 wks.	0.62	0.83	0.16	0.21	2.29	6.08
		3 wks.	0.63	0.91	0.21	0.32	1.98	5.98
		5 wks.	0.82	1.02	0.26	0.42	1.52	5.79
		7 wks.	0.79	1.52	0.31	0.57	1.46	5.69
		11 wks.	0.81	1.83	0.32	0.55	1.21	5.03
		15 wks.	0.89	2.16	0.36	0.49	0.95	4.82
2-VP	32	6 days	0.59	0.71	0.21	0.19	1.68	5.92
		2 wks.	0.68	0.92	0.24	0.23	1.46	5.81
		4 wks.	1.23	0.86	0.26	0.21	1.38	5.62
		8 wks.	0.82	1.01	0.31	0.24	1.32	5.64
		12 wks.	0.87	1.52	0.36	0.27	1.18	5.41
		16 wks.	0.79	1.89	0.33	0.23	0.93	4.91
3-VP	37	4 days	0.63	0.57	0.16	0.18	1.72	6.42
		2 wks.	0.72	0.68	0.24	0.29	1.52	6.22
		6 wks.	0.89	0.53	0.28	0.36	1.42	5.94
		10 wks.	0.76	0.92	0.34	0.47	1.05	5.36
		14 wks.	0.83	1.36	0.36	0.43	1.12	5.21
		18 wks.	0.81	1.82	0.32	0.49	0.83	4.92

* Twelve to 12.2 units were added for all chromatographic separations.

The five cases of chronic lymphocytic leukemia that were investigated all showed a normal seromucoid pattern in spite of the fact that clinically they all had symptoms of chronic leukemia manifested by elevated white count, abnormal circulating immature forms, and derangement in the differential count.

Because of the anemia that accompanied all the above cases, it was possible to obtain only a single blood specimen.

DISCUSSION

Seromucoid patterns of serial bleedings from all twenty-three individuals hospitalized with unilateral pneumococcal pneumonia showed patterns

TABLE VII

SERIAL DETERMINATIONS OF THE SEROMUCOID FRACTIONS OF SERA OF THREE PATIENTS WITH BILATERAL LOBAR PNEUMONIA

Case No.	Age	Interval between admission and bleeding	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)
	yrs.							
1-BPN	42	2 days	0.31	0.52	0.00	0.22	2.12	6.85
		6 days	0.72	0.41	0.09	0.31	1.92	6.72
		2 wks.	0.82	0.81	0.19	0.24	1.62	6.17
		4 wks.	0.91	1.36	0.21	0.36	1.42	6.09
		6 wks.	0.83	1.95	0.26	0.40	0.93	5.41
2-BPN	57	1 day	0.42	0.63	0.19	0.81	1.21	5.91
		7 days	1.03	0.71	0.20	0.63	1.87	6.42
		3 wks.	0.91	1.25	0.31	0.71	1.43	5.77
		5 wks.	1.16	2.13	0.41	0.79	0.93	5.21
3-BPN	56	2 days	0.58	0.71	0.10	0.58	2.42	6.33
		10 days	0.78	0.84	0.22	0.59	2.07	6.07
		3 wks.	0.81	1.15	0.30	0.61	1.49	5.75
		5 wks.	0.91	1.62	0.32	0.55	1.15	5.03
		7 wks.	1.2	2.34	0.33	0.62	0.98	4.92

different from those of the matched control group. In all the pneumonia cases the seromucoid content of the first fraction was approximately equal to or greater than that of the second fraction. In normal individuals, the first fraction is less than half the second fraction. The abnormal pattern during the early stages of illness was characterized chiefly by a markedly lower second fraction and usually a much lower third fraction, and higher concentrations of seromucoid in the fifth and sixth fractions than those found in the control

TABLE VIII

SEROMUCOID FRACTIONS OF SERA OF SEVEN INDIVIDUALS WITH ACUTE LEUKEMIA*

Case No.	Age	Fractions (eluted by various M NaCl) 0.02 (1)	0.02 (2)	0.04 (3)	0.08 (4)	0.098 (5)	0.135 (6)	OD*
	yrs.							
1-AL	47	0.97	2.26	0.43	0.61	1.52	6.03	0.84
2-AL	43	0.91	1.92	0.51	0.68	1.87	5.72	0.79
3-AL	54	0.82	2.16	0.49	0.71	1.26	5.31	0.91
4-AL	58	0.83	1.89	0.61	0.81	1.17	6.16	0.82
5-AL	49	0.81	2.06	0.31	0.47	1.52	5.92	0.76
6-AL	41	0.84	2.21	0.51	0.62	2.14	5.89	0.91
7-AL	52	0.76	1.89	0.37	0.51	1.67	5.36	0.93

* Optical density readings of seromucoid preparations before fractionation determined as described under Methods and in Table III.

group. During convalescence, the second fraction which was markedly reduced by the disease, slowly began to increase so that by 2 weeks time it was almost back to normal; *i.e.*, twice that of the first fraction, and the fifth and sixth fractions had begun to decrease in amount. By 2½ to 4½ weeks all individuals showed the pattern that was observed in the control group.

It is of interest from the standpoint of the pathogenesis of pneumonia, that in two instances patients were admitted to the hospital only 2 to 4 hours after the onset of shaking chills and fever. Blood specimens, taken within 2 hours after they were admitted to the hospital and before any treatment had been given, showed abnormal seromucoid patterns. These results indicate that the changes in seromucoids are (*a*) initiated at an early stage in the pathogenesis of pneumonia and (*b*) are not due to treatment.

Three individuals with bilateral pneumococcal pneumonia showed seromucoid patterns similar to those described for the twenty-three patients with unilateral pneumococcal pneumonia.

Five individuals with viral pneumonia showed a pattern similar to that described above for pneumococcal pneumonia except that the seromucoid pattern of these individuals took from 3 to 5 months to return to a normal pattern. This result is not surprising, since there is no antibiotic therapy available for treating viral pneumonia and the patients were ill and hospitalized for a much longer time than were the patients with pneumococcal pneumonia. The data from all cases of pneumonia discussed above would indicate that there is a relationship between the stage of the disease and the seromucoid pattern.[3]

Seven cases of acute leukemia were studied. In spite of the fact that these individuals all showed very high total seromucoid levels, the first and second fractions were all within normal range. However, the seromucoid pattern could be distinguished from the normal pattern because the fifth fraction had 2½ to 4 times and the sixth fraction approximately 1½ times as much seromucoid as was observed in the control group. Five cases of chronic lymphocytic leukemia which showed various forms of the clinical disease, showed seromucoid patterns that could not be distinguished from the chromatographic separations carried out with sera from normal individuals.

The different patterns described in this paper are based on quantitative differences in seromucoid concentration and not on any qualitative differences. However, until detailed chemical and immunological studies are carried out it should not be taken for granted that the fractions in various diseases only differ in quantitative relationships from those observed in normal individuals. As pointed out in the previous paper (3) none of the fractions are homogeneous since they all can be subfractionated. This procedure

[3] It would be of interest to know whether the seromucoid pattern is still abnormal at a time when all other clinical and laboratory tests indicate the patient is completely recovered.

naturally leads to a great many more fractions than are described in this report, with the result that there is a great deal of variation found in the seromucoid patterns of normal individuals of similar groups. Such definitive studies would greatly help in elucidating seromucoid patterns in health and disease.

It is not clear what mechanisms account for the changes in the abnormal seromucoid patterns described above. Further studies of the seromucoid fraction patterns for other diseases, the site of synthesis of seromucoids in health and disease, and the chemical analysis of the seromucoid fractions found in various disease states along the lines already carried out for the fractions from normal individuals (2, 3) should provide an insight into the mechanisms responsible for the deranged seromucoid patterns described in this paper. The relationship between the maximum total seromucoid level and the severity of the disease together with studies relating the clinical course of the disease to the abnormal seromucoid patterns would also be of value. Such studies are under investigation in this laboratory. Until such data are collected, further speculation does not appear warranted at this time.

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