late hematopoiesis, previously found in the urine of patients with diseases of the leucemia group,[1,2] have been shown to occur in the acid fraction of beef liver lipids.

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Electrophoretic Patterns of Seminal Plasma from Some "Abnormal" Human Semens.*

VICTOR ROSS, EDGAR G. MILLER, JR., DAN H. MOORE, AND HELEN SIKORSKI.

From the Department of Biochemistry and the Electrophoresis Laboratory, College of Physicians and Surgeons, Columbia University, N. Y.

We have previously published[1] some chemical and electrophoretic data on the proteins of normal human seminal plasma. We have since had an opportunity of obtaining semen specimens which were considered "abnormal" in one respect or another. These have been examined electrophoretically and, to a limited extent, chemically as well, to learn whether any differences between the proteins of such specimens and normal ones could be detected. The patterns are recorded here together with several additional ones of normal seminal plasmas.

The earlier work had demonstrated the presence in human seminal plasma of the following electrophoretic components: P1, the proteose fraction ($\mu = -.6 \times 10^{-5}$ cm^2 volt^{-1} sec^{-1}); P2 and P3, globulins,† each with a water soluble and a water insoluble fraction ($\mu = -2.9$ and -4.6 respectively), and probably a third globulin (P2a) with an intermediate mobility. A glycoprotein P4 ($\mu = -5.7$) is also present as well as a still faster moving component P5 ($\mu = -6.3$), which was considered related to P4 and possibly derived from it.‡ P4 and P5 are not always present together in the same specimen.

Methods and Results. The methods employed were as previously described.[1] All electrophoretic examinations were done on cell free, centrifuged specimens in phosphate buffer containing 0.055 M NaCl (pH 7.85, ionic strength 0.1) at ca. 2°C.

The electrophoretic patterns 1, 2, and 3 (Fig. 1) are of 3 normal specimens and show the presence of P1 (proteoses), P2 and P3 (globulins), and either P4 or P5 or both (glycoprotein). P2a was present in one of the 3 specimens and in the pooled mixture of 5 normal specimens, as is shown in pattern 4.

The usual peaks P1, P2, P3, and P5 were seen in the patterns, shown in illustrations 5 and 6 in the figure, of 2 abnormally viscous

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1 Ross, V., Moore, D. H., and Miller, E. G., Jr., *J. Biol. Chem.*, 1942, **144**, 667.

† We refer to P2, P2a, and P3 as globulins because there are water soluble and water insoluble fractions of each of these in fresh seminal plasma. If, however, seminal plasma is allowed to stand for several days before dialysis against water, no insoluble fraction separates. The term "globulin" may, therefore, not be accurate.

‡ When acetic acid is added to the water soluble fraction following dialysis of a fresh specimen against water, the precipitate is stringy and is soluble in 0.1% acetic acid containing 1% sodium chloride. If the acetic acid is added to the fresh, undialyzed plasma, the precipitate is not stringy and does not dissolve in this solution. When these operations were carried out on separate portions of the 5 pooled specimens (pattern 4, Fig. 1), the mobilities of the precipitates, following purification by dissolving in sodium hydroxide and reprecipitation with acetic acid, were 6.0 and 7.0 respectively (ascending, 1 hour). The two kinds of acetic acid precipitate, on analysis, yield similar values for nitrogen and reducing substance following hydrolysis.[1] Absorption in the ultraviolet[2] by the two forms is similar also.

2 Ross, V., and Ross, L., in press.

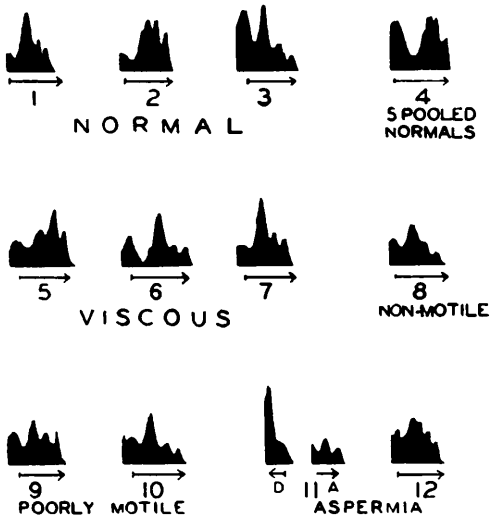

FIG. 1.

Electrophoretic patterns of seminal plasma from normal and "abnormal" human semens. Numbers under the individual patterns refer to the specimens listed in Table I. All pictures are of the ascending limb except in Pattern 11 where, because the light was filtered out in the region between components P2 and P3, portions of the ascending and descending limbs are shown.

specimens which failed to liquefy in several hours. One of these specimens had, in addition, component P2a. A sample from the same individual at another time also contained P2a, but this time lacked P2 (pattern 7). P4 was not observed in any of the 3 plasmas. This may possibly be related to the failure to liquefy normally. The addition of acetic acid to a portion of the specimen illustrated in pattern 6, yielded a precipitate which on resolution had an electrophoretic mobility of 6.2, while the pattern of the supernatant fluid contained no peak with a similar mobility. This happens in normal seminal plasmas also, and rules out the possibility that the peak with this mobility represents albumin.

The plasmas from the group of semens in which the sperm were either poorly motile or non-motile, or in which no sperm were present, contained the proteins P1, P2, P3, and P5 (patterns 8, 9, 10, 11, and 12). P2a was also present in one of the 2 aspermic specimens and possibly in the other, since there was in the specimen of plasma shown in Fig. 11, some component which filtered out visible and infra-red light and had a mobility between those of components P2 and P3. The descending limb of the electrophoresis cell gave evidence of the presence of P4 in the specimen represented by pattern 8.

Protein concentrations of several of the specimens were determined from the electro-

TABLE I.

Electrophoretic Mobilities of Proteins in Seminal Plasma from Normal Semen Specimens and from Some "Abnormal" Semen Specimens.

Phosphate buffer containing 0.055 M NaCl, pH 7.85, ionic strength = 0.1, Potential = 6.3 volts per cm.

Pattern No.	Kind of specimen	Mobilities × 10⁵					
		P1	P2	P2a	P3	P4	P5
1	Normal	0.7	3.2		4.8	5.8	
2	" *	0.2	2.9	3.8	4.4		6.4
3	"	0.0	2.4		4.5	5.4	6.1
4	5 pooled normals	1.1	2.6	4.2	4.9	5.6	
5	Viscous	†	3.1		5.0		6.3
6‡	"	0.3	2.6	3.6	5.2		6.4
7‡	"	0.2		3.4	5.1		6.5
8	Non-motile sperm*	0.2	2.8		4.8		6.7
9	Consistent poorly motile sperm	0.1	2.9		4.8		6.4
10	Poor motility, low count	0.4	2.9		5.1		6.6
11	No sperm present§	0.8	2.6		4.8		6.0
12	" " "	0.9	3.1	4.1	4.9		6.3

Mobilities were calculated from 1½-hour ascending patterns except in Pattern 11 in which both ascending and descending patterns were used and Pattern 6 in which the 2-hour pattern was used.

* P4 peak was present in descending limb pattern.

† P1 had a slight positive charge in this experiment.

‡ Two specimens from the same individual.

§ P2a might have been present also. The light was filtered out by the solution in the cell in the region where P2a would be seen if present.

phoretic patterns. The values were: for pattern 1, 0.7%; pattern 7, 0.7%; pattern 8, 0.7%; pattern 9, 0.8%; pattern 10, 0.8%. Thus there was no significant difference, in this respect, between the plasmas of several kinds of abnormal semens and that of a normal specimen. The refractive indices of the several protein components were assumed to be the same and equal to that of serum globulin.

Summary. Electrophoretic examination of the plasmas from specimens of human semen which were either (1) abnormally viscous or in which (2) the sperm were either poorly motile or non-motile, or which (3) contained no sperm revealed no definitely significant deviation in protein components from normal specimens. Components P1 (proteose), P2 and P3 (globulins) were present in all these specimens as they are in normal ones. Component P2a (globulin), described in an earlier report as being present in occasional normal specimens, was found in some of the abnormal ones also. Normal specimens contain either P4 or P5 (glycoprotein) or both. Component P5 was present in all the abnormal specimens. The absence of P4 from all but one of these is noteworthy, but the varied nature of the abnormality makes interpretation difficult.§

§ We wish to record our appreciation to Dr. John MacLeod and Dr. Robert S. Hotchkiss of Cornell University Medical College for the semen specimens.

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The Cold Agglutination Test in the Diagnosis of Primary Atypical Pneumonia.*

GORDON MEIKLEJOHN. (Introduced by Monroe D. Eaton.)

From the Research Laboratory, California State Department of Public Health, Berkeley.

Since February, 1943, when Peterson, Ham, and Finland[1] reported on cold autohemagglutination in primary atypical pneumonia and suggested that this procedure might differentiate the primary atypical pneumonias from other types of pneumonia, all serum specimens coming to this laboratory from patients with respiratory diseases have been tested for cold agglutinins. The chief purposes of this investigation have been (1) to determine how frequently significant titers of cold agglutinins are found in primary atypical pneumonia and (2) to determine how often similar titers are encountered in other respiratory diseases.

Materials and Method. 155 serum specimens from 74 patients diagnosed as primary atypical pneumonia were tested. These came from 3 hospitals where the disease is common. Diagnosis was made on the basis of the clinical picture, sputum examination, and X-ray findings. Three or more specimens were obtained from each of 20 patients, 2 specimens from 34 patients, and one specimen from 20 patients.

The control group consisted of 133 serum specimens from individuals with the following diagnoses: Bacterial pneumonias (mostly pneumococcal) 22, pulmonary tuberculosis 23, febrile upper respiratory infections 17, influenza (type A), 7, coccidioidomycosis 6,† pneumonias of uncertain etiology 8, lymphogranuloma venereum 20, and normal individuals 36. All specimens in this group were taken later than the 10th day of disease and were tested while fresh.

The technic employed was as follows: Twofold serial serum dilutions commencing at

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation in cooperation with the California State Department of Public Health.

1 Peterson, O. L., Ham, T. H., and Finland, M., *Science*, 1943, **97**, 167.

† These specimens were supplied by Dr. C. E. Smith, Stanford Medical School.