Mucoproteins of Human Plasma. IV. Electrophoretic Demonstration of Mucoproteins in Serum at pH 4.5.* (17348)

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Mucoproteins in human plasma have been isolated and characterized chemically,[1] their increase in cancer has been discussed,[2] and the material isolated from plasma has been studied electrophoretically.[3] It was shown that in the usual electrophoresis pattern at pH 8.4 much of the mucoprotein should migrate with the α_1-globulin, and would thus be included with any other material which contributes to the α_1-globulin fraction. It was found, however, that the isoelectric points of the fractions contributing to the isolated mucoprotein were quite low, and that they would all remain negatively charged at pH 4.5. Such a pH would, then, appear more suitable for the electrophoretic demonstration of these mucoproteins in serum. It has been shown by Petermann *et al.*[4] that there is an acid component in human serum which may be demonstrated by electrophoresis at pH 4.0, and which increases in amount in those conditions in which the mucoprotein has been shown to be increased. They have suggested that this electrophoretic component may be the same mucoprotein as that with which we have been concerned, and the present studies were intended primarily to determine the extent to which such an identification can be made.

Methods. The methods employed in the electrophoresis studies were the same as those previously described.[3] All electrophoresis measurements were made at 2°C at an ionic strength of 0.1. At pH 8.4, the buffer was diethyl barbiturate, and at pH 4.5 the buffer was acetate. In both cases, the ion and undissociated acid of the buffer had a total concentration of 0.02 M. The pH is that determined at room temperature. Serum samples were generally diluted to 6 times their original volume, and thus had a total protein concentration in the neighborhood of one per cent. The patterns were photographed after 3 hours.

At pH 4.5, some precipitate forms during dialysis, and is removed before electrophoresis. The nature of this precipitate and its effect upon the electrophoresis pattern has not been thoroughly investigated, but the amounts of protein involved are small. Where protein concentrations are reported subsequently, they have been calculated from the total protein concentration of the serum, and the relative

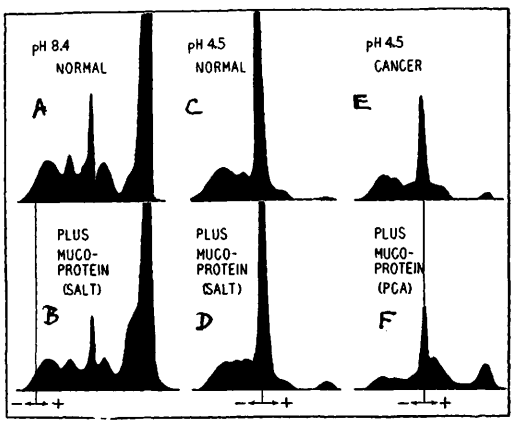

FIG. 1.

Electrophoresis patterns at pH 8.4 and pH 4.5. Boundaries descending to anode at pH 8.4, descending to cathode at pH 4.5.

A. Normal plasma at pH 8.4. B. Same normal plasma at pH 8.4 with mucoprotein (MP-1) added. C. Same normal plasma at pH 4.5. D. Same normal plasma at pH 4.5 with mucoprotein (MP-1) added. E. Serum from cancer patient at pH 4.5. F. Same cancer serum at pH 4.5, with mucoprotein added (MP-1, MP-2, and MP-3).

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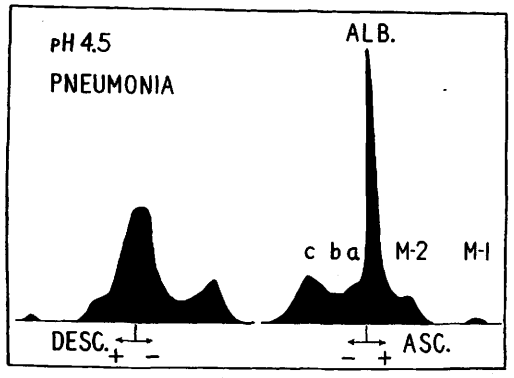

FIG. 2.
Electrophoresis patterns, ascending and descending, at pH 4.5, of serum from a patient with pneumonia. The designation of the components is shown.

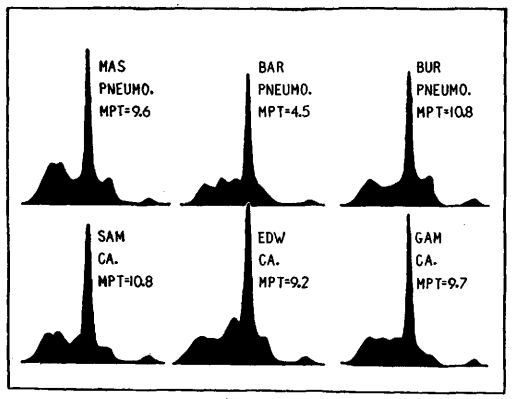

FIG. 3.
Electrophoresis patterns, descending to the cathode, at pH 4.5. The 3 upper patterns are from patients with pneumonia, the 3 lower patterns from patients with cancer. The value for MPT is that for the mucoprotein-tyrosine in mg%.

areas of boundaries in the electrophoresis pattern.

Experimental results. Electrophoresis at pH 8.4. In order to show that the mucoprotein does contribute to the α_1-globulin fraction, a sample of normal plasma was subjected to electrophoresis with and without added mucoprotein. The mucoprotein fraction used for this study was one isolated by ammonium sulfate fractionation and consisted only of the fraction designated as MP-1,[3] contaminated with some albumin. The electrophoresis patterns obtained in this experiment are shown in Fig. 1-A and 1-B, and it is apparent that the mucoprotein MP-1 does indeed increase the α_1-globulin fraction at pH 8.4.

Electrophoresis at pH 4.5. On the basis of the results obtained with the isolated mucoprotein, a pH of 4.5 was selected as being suitable for the demonstration of the mucoprotein in serum. In Fig. 2 are reproduced the ascending and descending boundaries obtained in the electrophoresis of serum from a patient (HUM) with pneumonia, indicating the division into components which we have employed, and their designations. We have designated the 2 acid components as M-1 and M-2 and the globulins as a, b, and c. The separation of the peaks is generally better in the boundary in which the globulins are descending and mucoprotein is ascending and only this boundary is given in the electrophoresis patterns of Fig. 1 and 3. Although the example in Fig. 2 is not the most satisfactory for illustrating the presence of components a, b, and c, an inspection of the patterns in Fig. 3 will indicate that such a division is justified. The acid components M-1 and M-2 are clearly evident in Fig. 2. The amounts of M-1 and M-2 are considerably elevated over the normal levels (compare Fig. 1-C). This has been true of all cases studied in which the chemical determination showed an elevation of the mucoprotein.

The electrophoresis pattern of the same normal serum which was used at pH 8.4 is shown at pH 4.5 in Fig. 1-C. As with the pathological serum of Fig. 2, there are two components corresponding to M-1 and M-2 but in considerably smaller quantities. In Fig. 1-D is shown the electrophoresis pattern at pH 4.5 of this same normal serum with the addition of the same mucoprotein preparation employed at pH 8.4. M-1 is increased, and M-2 is unchanged, suggesting that the MP-1 fraction of isolated mucoprotein may be identified with the M-1 fraction in serum. The mobility of -4.2×10^{-5} (in the ascending mucoprotein boundary) is in good agreement with that observed in the isolated mucoprotein.

In Fig. 1-E the electrophoresis pattern of serum from a patient with cancer is presented, and in Fig. 1-F the same serum with mucoprotein added. In this case, the mucoprotein was isolated from a perchloric acid filtrate of plasma by the method previously de-

TABLE I.

Analytical Values Obtained on Perchloric Acid Filtrates from the Sera of 3 Patients, GAM, BUR, and HUM. Concentrations are expressed as mg per 100 cc of original serum, and were obtained by the methods previously described.[1] Average values of the ratios of carbohydrate: tyrosine (CHO/T) and carbohydrate:glucosamine (CHO/G) obtained on mucoprotein isolated from normal human plasma[1] are given for comparison.

Sample	Tyrosine, mg%	CHO, mg%	CHO/T	N, mg%	Protein (Biuret) mg%	Glucosamine, mg%	CHO/G
GAM carcinoma	9.7	52.0	5.3	23.5	240.0	33.5	1.55
BUR (pneumonia)	10.8	55.0	5.1	27.5	270.0	33.5	1.64
HUM (pneumonia)	5.7	27.5	4.8	14.5	—	21.0	1.31
Normal	—	—	3.6	—	—	—	1.27

scribed[1] and contained in addition to MP-1 the slower components (MP-2 and MP-3) seen in these preparations.[3] The addition of such material to serum is seen to result in an increase in both the M-1 and M-2 fractions of the resulting mixture. The mobility of the M-1 fraction in serum corresponds closely with that of the MP-1 component of isolated mucoprotein. The mobility of the M-2 fraction of serum, however, (-1.4 to -2.0×10^{-5}) does not agree with the mobility of either the MP-2 (-2.4 to -2.8×10^{-5}) or MP-3 (-0.5 to -1.2×10^{-5}) of the isolated preparations. When such preparations are added to serum, we have been able to find satisfactory correspondence only between the mobilities of the M-1 and MP-1 fractions.

The relation of the electrophoretic components M-1 and M-2 to chemically determined mucoprotein. Electrophoresis patterns of a number of pathological sera at pH 4.5 are shown in Fig. 3. It will be seen that an increase in M-2 is found to accompany an increase in M-1 in either carcinoma or pneumonia. In each instance, the level of mucoprotein-tyrosine (MPT) in mg percent in the original plasma[2] is also indicated. The mucoprotein-tyrosine is between 9 and 11 mg percent in all cases except that of BAR, where it is 4.5. Inspection of the electrophoresis patterns will make it evident that the area of the M-1 peak is about the same in all except that of BAR, and that there is at least a rough correspondence between the amount of M-1 and the MPT. The amount of M-2, however, does not seem to bear any quantitative relationship to the amount of MPT.

Serum samples from three patients were studied in somewhat greater detail. Sufficient serum was obtained in each case to carry out the electrophoresis at pH 8.4 and pH 4.5; to prepare perchloric acid filtrates for the determination of the mucoprotein-tyrosine, as well as the carbohydrate, nitrogen, glucosamine, and the value of the biuret reaction.[1] The results of the chemical analyses of the filtrates are given in Table I, together with the ratios of carbohydrate:tyrosine and of carbohydrate:glucosamine for these filtrates and for the mucoprotein isolated from normal plasma. In Table II the amount of each electrophoretic component is calculated and compared with the amount of mucoprotein indicated by the chemical determination. This calculation is based upon a nitrogen content of 7.9%, determined upon the isolated mucoprotein. It must be recognized, of course, that the accuracy with which the areas of the small electrophoretic peaks for M-1 can be determined is not great, and that the translation of these areas into concentrations involves the assumption that the mucoprotein would have the same refractive index increment as that of the other serum proteins. It seems reasonable to conclude, however, that the agreement between the electrophoretic and chemical values indicates that the chemical determination is primarily a measure of M-1. Certainly, the sum of M-1 and M-2 is too great to be accounted for by the material in the perchloric acid filtrates.

In comparing the results of the electrophoresis experiments at pH 8.4 and 4.5, it is seen that M-1 does not exceed α_1-globulin in any of these cases, but may be considerably smaller (as in the case of HUM). On the other hand, the amount of M-1 plus M-2 may be considerably larger than the amount of α_1-globulin.

TABLE II.

Electrophoretic Analysis of the Sera of 3 Patients, GAM, BUR, and HUM. The concentrations are calculated from the total serum protein, assuming that the refractive index increment is the same for all components. The mucoprotein nitrogen represents the difference between that in perchloric acid and tungstic acid filtrates.*

pH	Component	Concentration g/100 cc GAM	HUM	BUR
8.4	Albumin	3.3	2.9	2.2
	α_1–globulin	0.3	0.6	0.4
	α_x– '' *	—	—	0.8
	α_2– ''	1.0	1.0	1.1
	β– ''	0.6	0.6	0.7
	γ– ''	0.9	1.2	1.3
4.5	M–1	0.3	0.1	0.2
	M–2	0.4	0.5	0.8
	Albumin	2.9	3.4	3.0
	a	0.7	0.5	0.6
	b	0.9	0.5	0.6
	c	1.1	1.2	1.1
Mucoprotein —N × 12.5		0.29	0.18	0.34

* The material designated α_x–globulin is an abnormal component migrating between the α_1– and α_2–globulin.

Electrophoretic Isolation of M-1. Since the M-1 component becomes well separated from other components of serum at pH 4.5, it is well suited to electrophoretic separation. An experiment was carried out in which serum was undiluted, except for the dilution taking place during dialysis, and in which electrophoresis was carried out in an 11 ml analytical cell with 2 center sections. Electrophoresis was carried out, with compensation, until the ascending boundary of the M-1 component had nearly reached the top of the cell, and the ascending M-2 boundary was below the middle junction of the cells. One half of one channel of the cell could then be isolated, containing M-1 alone. This material was subjected to chemical analysis, with the results given in Table III. There can be no doubt that the material represented by the M-1 peak at pH 4.5 has the same general chemical characteristics as the mucoprotein isolated from perchloric acid filtrates of serum or plasma.

TABLE III.

Analytical Values for Electrophoretically Isolated M–1.

Tyrosine	7.6 mg%*
Carbohydrate	34.4 ''
CHO/T	4.5
Nitrogen	16.2 mg%
Glucosamine	28.5 ''
CHO/G	1.2

* These refer to concentration in the solution removed from the cell.

Discussion. The identity of the M-1 component of the electrophoresis pattern of serum at pH 4.5 and the fraction of the isolated mucoprotein which we have designated as MP-1 seems to be adequately established by the experiments presented. The addition of the isolated material to serum has confirmed the identity of the electrophoretic mobilities, and the material isolated by electrophoresis has been shown to have chemical properties similar to the material isolated by fractional precipitation.

The nature of the M-2 component of the electrophoresis pattern of serum at pH 4.5 is not established. The mobility does not correspond entirely with that of any component of the isolated mucoprotein. The increase in the M-2 component resulting from the addition of isolated mucoprotein containing fractions other than MP-1 would suggest that M-2 is also a mucoprotein. The association of increases in M-1 and M-2, and the rather low isoelectric point of M-2 would point in the same direction, but certainly without being conclusive. The solution of the problem will require the isolation of a homogeneous preparation which has the electrophoretic properties of M-2 by itself or on addition to serum. The isolation of such material is now being investigated.

Summary. When serum is subjected to electrophoresis at pH 4.5, two components are seen which have isoelectric points more acid than those of albumin. One of these has been identified with a mucoprotein which has been shown to increase in amount in cancer, pneumonia, and certain other diseases. The second component, with a less acid isoelectric point, may also be a mucoprotein, but this point has not been definitely established.

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Effect of Adrenergic Blocking Agents on the Cutaneous Action of Epinephrine. (17349)

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Adrenergic blocking drugs are of clinical interest in the treatment of conditions characterized by the element of vasospasm, such as early Buerger's disease, Raynaud's disease and causalgic states. It was felt that by the direct diffusion or by ion transfer through the skin, it would be possible to avoid the generalized toxic manifestations encountered by way of the parenteral route.[1] The adrenergic blocking agents selected for this study were N-(2-chloroethyl) dibenzylamine hydrochloride (Dibenamine) introduced by Nickerson and Goodman[2] and N-(2-bromoethyl)-N-l-naphthalenemethylamine hydrobromide (SY-28) introduced by Loew and Micetich.[3] Prior to application in the human, data were obtained in a group of albino rabbits.

Methods. Albino rabbits weighing 2.0 to 4.5 kg were used. The abdomen and thorax of the animals were depilated with a special sulfide-detergent mixture[4] 24 hours prior to use. Solutions of Dibenamine and SY-28 in propylene glycol were freshly prepared prior to each experiment. Four-tenths ml of these solutions or of solvent controls were applied to rectangular strips of asbestos paper 1.25 by 12.5 cm in size. This amount of solution was sufficient to saturate the pads. The strips were then applied to the abdomen in 4 parallel sites in the following order: solvent without current, solution containing the drug without current, solution containing the drug plus current, solvent plus current. Two to four rabbits were used at each of the various concentrations of blocking agents studied. Since the active portions of both Dibenamine and SY-28 are cationic, the anode of a suitable direct current source* was used to transfer the agent into the skin. A current density of 0.33 ma/cm^2 or a total current of 5.2 ma was employed. This dosage of current was arbitrarily selected as being just below the pain threshold for human skin and was well tolerated by the rabbits. The solutions were placed in contact with the skin for exactly 5 minutes with or without current. Immediately following the 5 minute application, the asbestos pads were removed and the blocking agent wiped off with propylene glycol.

To measure the adrenergic blocking activity of the treated sites, 0.1 ml of 1:1,000, 1:10,000 and 1:40,000 epinephrine solutions were injected intracutaneously 30 minutes following application of the blocking agents. An equal amount of physiological saline served as a control. The injection sites were randomized. Vasoconstriction reached a max-

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* A McIntosh Wall Plate was used. This machine is manufactured by the McIntosh Electrical Corp., Chicago, Ill.