INFLUENCE OF THE SUPPORTING MEDIUM ON THE FRACTIONATION OF PROTEINS BY ZONE ELECTROPHORESIS*

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Since various supporting media used for zone electrophoresis each offer certain advantages and limitations[1-3], an attempt was made to combine two different media[4]. During the course of this work, it became apparent that the results of the fractionation of mouse plasma were significantly different when protein was allowed to migrate in a medium of potato starch granules or in one of cornstarch gel. Therefore, the two-dimensional technique of electrophoresis[5-7] was used to obtain increased insight into the mechanism of the separation. The following supporting media were studied: (a) a slab of potato starch granules, (b) a cornstarch gel stiffened by the addition of amylose, and (c) the combination of the two. The results of these experiments will be described in the present paper.

EXPERIMENTAL

Starch electrophoresis

Experiments were carried out in a 30 × 5 × 1.3 cm plastic tray. Strips of Whatman No. 3 filter paper were used to connect the two ends of the tray to buffer vessels, which in turn were connected by paper strips to vessels containing silver–silver chloride electrodes. All experiments were carried out at an ionic strength of 0.05 in veronal citrate buffer of pH 8.6 or 7.4 or phosphate buffer of pH 7.0.

The technique of using potato starch granules was based upon the method described by KUNKEL AND SLATER[8]. For most experiments, the potato starch was extensively washed by first allowing the granules to settle from a suspension with two volumes of buffer, after which the wet granules were further washed on a funnel by dripping through them five volumes of buffer over a period of several hours. Some samples of potato starch were washed with dilute ammonium or sodium hydroxide, then with water and were finally dried in a desiccator**.

Zone electrophoresis on a supporting medium of conrstarch gel was performed according to BERNFELD AND NISSELBAUM[9]. A combination of the two media was achieved by fitting a cardboard or wooden form covered with aluminum foil in the center of the tray leaving 3.5 cm wide sections at each end. After the starch paste was poured

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** Starch washed with alkali obtained through courtesy of Dr. GAIL L. MILLER.

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into the ends and allowed to gel overnight in the cold, the form was removed, and the center section was filled with potato starch granules.

The progress of the electrophoretic separation was observed under ultraviolet light, and the electric field was maintained until the albumin fraction had moved 9–10 cm away from the γ-globulin fraction.

A 3 mm × 3 mm longitudinal segment was then removed from the center of the block and applied to a sheet of Whatman No. 1 filter paper. A filter paper electrophoresis at pH 8.6 was then carried out in an electric field perpendicular to the one in the starch medium.

Filter paper electrophoresis

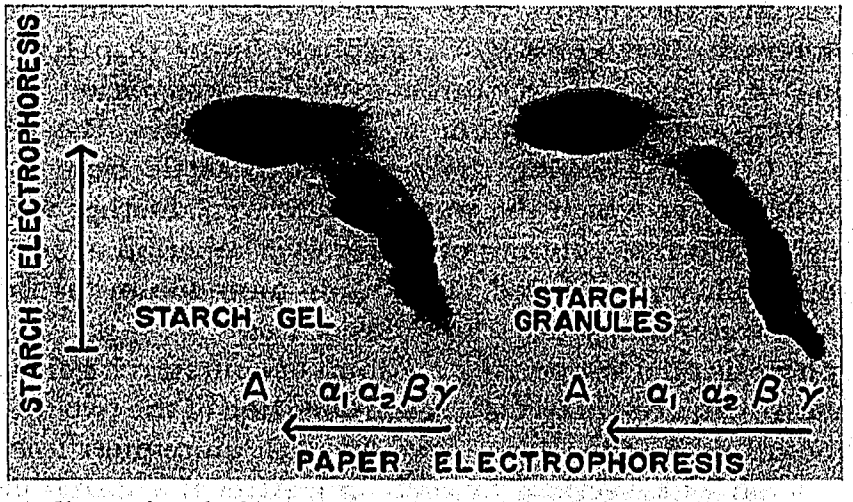
Experiments were carried out in a horizontal strip apparatus* with 0.1 ionic pH 8.6 veronal citrate buffer. After applying a current of 16–18 mA for 1–1 ½ to 2 h, the starch segment was removed from the paper. Electrophoresis on paper was then continued for 3 to 4 h at 16–18 mA, or overnight at correspondingly lower field strength. Bromphenol blue was used for staining the protein.

Plasma from normal or from tumor (Sarcoma I)-bearing mice of the A/Jax inbred strain was used. Tumor-bearing animals were bled 10 to 12 days after the implantation.

RESULTS

Influence of the medium

When the mouse plasma was separated by electrophoresis on filter paper in pH 8.6 veronal citrate buffer, the five common components were resolved; and the pattern was similar to that of a pool of human plasma. Since the mouse plasma was from an inbred strain of animals, the pattern was quite reproducible, and samples from different



Figs. 1a and b. Two-dimensional electrophoresis of mouse plasma in a starch medium (vertical direction) and on paper (horizontal direction) both in veronal citrate buffer at pH 8.6; (a) in cornstarch gel, (b) in potato starch granules.

* Purchased from Laboratory Glass Instrument Corp., New York.

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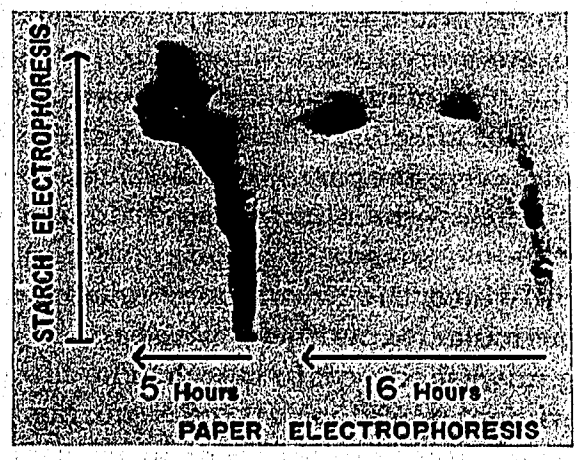
animals were not subject to the variations found with human plasma from different individuals.

The results obtained by two-dimensional electrophoresis in veronal citrate buffer pH 8.6 with plasma from tumor-bearing animals are shown in Figs. 1a and 1b. It is evident that the mobility of the α_2-globulins is significantly different in a corn-



Figs. 2a and b. Two-dimensional electrophoresis of mouse plasma on a medium of unwashed potato starch granules with sections of cornstarch gel at both ends in veronal citrate buffer; the pH of the buffer (8.6) was uncontrolled because of the lack of washing the medium (see text). Similar patterns are obtained on the same medium equilibrated with buffer of pH 7.0.

starch gel (Fig. 1a), from that in a medium of potato starch granules (Fig. 1b). In the former medium the α_2-globulins have a mobility closer to that of the β-globulin, while in potato starch granules, this protein fraction is found nearer to albumin. The mobilities of all the other protein components appeared to be identical in the two starch media. In neither of the starch media at pH 8.6 did the α_1-component separate from albumin, while these two components can easily be separated at this pH on paper electrophoresis.

The medium consisting of a combination of starch gel and starch granules gave a pattern identical with that of potato starch granules alone. However, one striking difference was noted; that is, the absence of electro-osmotic flow. In this respect, the fractionation on the combined media resembled that obtained on the cornstarch gel.

The effect of the supporting medium on pH

In both starch media at pH 7.0 in phosphate buffer a component was found to move ahead of albumin, while no such fast protein fraction was found in these media at pH 8.6 in veronal citrate buffer as was shown in Fig. 1. From the filter paper pattern of the two-dimensional experiment (like that shown in Fig. 2), this component is clearly identified as an α_1-globulin, since it appears in its normal position between albumin and the α_2-globulins on paper at pH 8.6. A similar pattern was found in the starch media in veronal citrate buffer of pH 7.4, which shows that the phenomenon was not due to the phosphate ions of the buffer.

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The reversal of the mobilities of albumin and α_1-globulin on the starch media upon lowering the pH from 8.6 to 7.0 emphazises the prime importance of the pH of the starch media. This is particularly relevant in the case of a medium consisting of potato starch granules, since this material frequently retains a considerable amount of acidity which can only be removed by repeated washings. One lot of potato starch, for instance, was found to lower the pH of distilled water to 4.39. Thus, when certain lots of potato starch were made into a block with pH 8.6 veronal citrate buffer, without previously washing the potato starch with buffer, the pattern was identical with that found at pH 7. Washing the starch with alkali, then with water and drying it was not effective in eliminating the pH effect, as observed by the fast moving α_1-component. When potato starch granules were prepared, by thoroughly washing and equilibrating them with buffer of pH 8.6, the α_1-globulin was never found to move ahead of albumin at this pH.

The type of pattern in which the α_1-component moved ahead of the albumin is shown in Fig. 2a. This particular experiment was carried out on a combination gel potato starch media using unwashed potato starch. The filter paper electrophoresis of the two-dimensional experiment was done at 400 V during 5 h. Fig. 2b was obtained from a segment from the same starch experiment after electrophoresis on filter paper for 16 h at 180 V. The latter shows more clearly than Fig. 2a that the component having the same mobility as albumin on potato starch granules appears as an α_2-globulin on filter paper. The component which moved ahead of the albumin spread on prolonged paper electrophoresis so that it does no longer show in Fig. 2b, although it was faintly visible on the original filter paper.

DISCUSSION

The results have shown a significant difference between the mobilities of the α-globulins on potato starch granules and those on a cornstarch gel. Differences between the fractionation of the hemoglobin-binding globulins on potato starch granules and a potato starch gel have also been found by BEARN AND FRANKLIN[10]. While it thus appears established that plasma fractionation is influenced by the nature of the supporting medium, our results have further shown that a combination of the two starch media retains some of the characteristics of each. The combined medium has all the advantages of starch granules with respect to easy elution without having the disadvantage of the electro-osmotic flow. The absence of electro-osmotic flow in stiff starch gels has been previously demonstrated[9], and the mechanism involved here is probably mechanical hindrance of flow. The prevention of electro-osmotic flow facilitates location of proteins according to electrophoretic mobility, and a combination of gel with other media may be quite useful, for example in the fractionation of carbohydrate on polyvinyl chloride[11] which has been observed to have an excessively high electro-osmotic flow rate[12].

In addition, our results have demonstrated that the medium itself may, in the case of potato starch granules, affect the fractionation by altering the pH of the

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buffer solution, obviously due to the presence of acidic impurities. This effect could be eliminated by thoroughly washing and equilibrating the starch granules with the buffer solution, but not by washing with dilute alkali and H_2O. This emphazises the necessity of proper preparation of the potato starch granules. KUNKEL[1] has recommended the use of warm buffer to wash potato starch.

In 1942 SEIBERT and coworkers[13,14] using the moving boundary technique, first described a component moving faster than albumin in pH 7.7 phosphate buffer and suggested that this might be the α-component which LONGSWORTH had found to move more slowly than albumin in veronal buffer at pH 8.6[15]. Since then many workers have described "prealbumins" in various types of electrophoretic separations[5,7,16-22] and some have suggested their association with plasma mucoproteins. Our results have shown quite clearly that the component moving ahead of albumin on both cornstarch gel and potato starch granules is the α_1-globulin. This behavior of α_1-globulin is no doubt a different phenomenon from some of the observations of "prealbumin" components which have been found to occur in small amounts in human serum at pH 8.6.

SUMMARY

1. The fractionation of mouse plasma by zone electrophoresis on (a) potato starch granules, (b) cornstarch gel and (c) a combination of the two media has been compared by two-dimensional electrophoresis with filter paper electrophoresis as the second stage.

2. A significant difference between the two starch media was observed in that α_2-globulins migrate in a cornstarch gel more closely to the β-globulin than they do in potato starch granules. The component which appears as α_1-globulin on filter paper electrophoresis does not separate from the albumin on either starch medium at pH 8.6 and migrates ahead of the albumin at pH 7.0.

3. A combination of potato starch granules with sections of gel at the electrode ends yields the type of fractionation identical with that of potato starch granules alone, but prevents electro-osmotic flow, thus combining the advantages of both media.

4. Potato starch granules may alter the pH in the supporting medium, and, hence, markedly influence the type of fractionation. Only thorough washing and equilibrating the potato starch with buffer resulted in reproducible results with different lots of potato starch and yielded types of fractionation compatible with the respective pH of the buffer.

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