The detailed data on which these considerations are based will be published in the near future in connection with a review of Hammett's equation.


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## Electrophoretic Behavior of Acid Phosphatase in Human Prostatic Extracts[1]


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In attempting the electrophoretic isolation of acid phosphatase from partially purified extracts of human prostatic tissue we noted that the electrophoretic patterns consistently showed two major protein components in addition to the enzyme. Since scarcely anything is known regarding the properties of the proteins of the prostate, we thought it advisable to present our findings at this time.

Strips of prostatic tissue were extracted overnight at 7° C with 6 ml of 0.85% NaCl per gram (wet weight) of tissue. The extract was dialyzed against distilled water until free of salt, and the insoluble material that appeared with the removal of salt was spun down and discarded. The supernate was lyophilized. A 1–1.5% aqueous solution of the dried powder was fractionated in two steps with $(NH_4)_2SO_4$ at 0° C as follows: The precipitate salted out at half saturation was discarded. The concentration of $(NH_4)_2SO_4$ was increased to two-thirds saturation, and the resulting precipitate was dissolved in a volume of water about two-thirds that of the original solution of powder and dialyzed against running tap water until free of $(NH_4)_2SO_4$. Additional protein was then removed by adjusting the dialyzed solution to pH 4.9 with *N*/10 acetic acid and storing at 0° C for 6 hr. The resulting precipitate was spun down and discarded. After a final dialysis against running tap water the supernate was dried from the frozen state. A white powder resulted.

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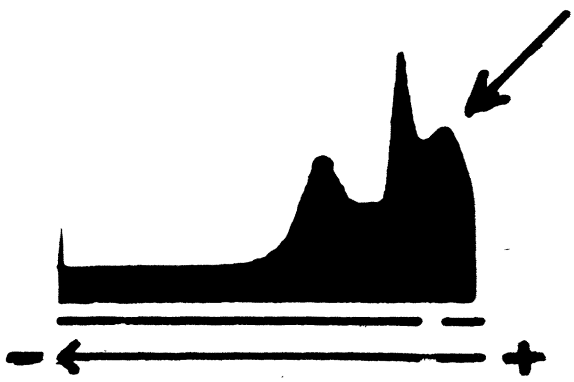
Electrophoretic analysis was carried out in the 2-ml cell of a Perkin-Elmer-Tiselius apparatus, Model 38. Patterns were obtained by both the Philpot-Svensson technique and the schlieren scanning technique, the latter being used for measurements of mobilities.

Mobilities were determined at intervals from pH 4.05 to pH 7.65 at an ionic strength of 0.05 and protein concentrations of approximately 0.75 and 1.5%. Acetate buffers, as reported by Boyd (*1*), were used for the acid range. The Gomori tris(hydroxymethyl)-aminomethane buffer (*2*) was used at pH 7.65, ionic strength 0.05. To make this buffer, 50 ml of 1 *N* HCl were added to 65.4 ml of 0.98 *M* tris(hydroxymethyl)-aminomethane solution, and the mixture made up to 1 liter with distilled water.

At the termination of each electrophoretic run, appropriate fractions were removed from the resolved mixture by means of a needle and syringe and analyzed for phosphatase activity and nitrogen content.

Eight electrophoretic analyses, using five separate extracts, were carried out. Partially purified extracts resolved over a pH range of 4.05–7.65 exhibited three major components. Only one of these seemed to represent prostatic phosphomonoesterase as judged by the activity-nitrogen ratios of the various fractions of the electrophoretically resolved solution withdrawn from the cell. The enzyme was estimated, by measurement of areas under corresponding peaks in the electrophoretic diagrams, to be approximately 10% of the total protein content.

Figure 1 is a representative pattern. The purified extract was obtained from a surgical specimen showing benign hyperplasia. The arrow denotes the component that showed the highest activity-nitrogen ratio; it is therefore assumed to be associated with prostatic phosphomonoesterase. This component has




Acetate buffer; pH 4.60
$\Gamma/2 = 0.1$; conc. = 2.5 mg N/ml; 113 min

FIG. 1. A cylindrical lens pattern (ascending limb) of partially purified prostatic acid phosphomonoesterase after electrophoresis. (Activity/nitrogen solution before electrophoresis = 86 units/mg nitrogen. Activity/nitrogen of component denoted by arrow = 113 units/mg nitrogen.) (A unit is 1 mg of phosphorus hydrolyzed in 30 min at 37° C from *M*/60 sodium β-glycerophosphate [Eastman Kodak Co.] in acetate buffer at pH 5.5, ionic strength 0.2.)

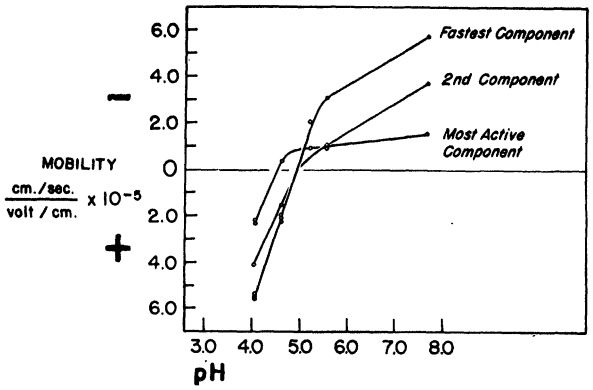



FIG. 2. Variation of electrophoretic mobility (descending limb) with pH of the major components of partially purified prostatic extracts at ionic strength 0.05.

moved toward the anode while the other two components have migrated toward the cathode.

Because of the limited supply of adenocarcinoma of the prostate only one partially purified preparation was analyzed. At pH 5.2 the pattern appeared to be similar to those of preparations from noncancerous prostates.

Figure 2 is a plot of mobility (descending limb) versus pH for the three major components. By interpolation the isoelectric points are as follows: fastest component, between pH 4.9 and pH 5.0; second component, between pH 4.9 and pH 5.0; most active component (acid phosphatase), pH 4.5.

It is of interest that our estimation of pH 4.5 for the isoelectric point of prostatic acid phosphatase is consistent with the value of Kutscher and Pany (*3*), who by the Theorell method of cataphoresis calculated the isoelectric point of purified ejaculate phosphatase to be pH 4.4.

It is apparent that the three proteins share many of the properties usually attributed to albumins. They are soluble in salt-free water and are not salted out by half saturation with $(NH_4)_2SO_4$. In addition two of the proteins remained in solution at their isoelectric points, as evidenced by the fact that they were not precipitated when the pH was adjusted to 4.9.

The electrophoretic mobilities, however, differ sufficiently to permit complete resolution of the components at pH 4.0, a point within 1 pH unit of the isoelectric point of each protein. Since Cohn *et al.* (*4*) emphasize the danger in completely characterizing proteins in terms either of their solubilities in concentrated salt solutions or of their mobilities, without regard to their other properties, no conclusion regarding the chemical nature of the three components is warranted as yet.

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## Adrenal Gland Response to Circulatory Distress in Fetal Lambs

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The fetal heart rate is generally stated to be under the control of the autonomic system (*1*). The present report shows that humoral factors are also operating. During a study of reflex control of heart rate in fetal lambs (110–149 days gestation age), marked cardiac acceleration occurred following asphyxial slowing in 11 instances in which the heart was completely denervated. Experiments were carried out to ascertain the physiological basis of such acceleration.

Grade ewes, made available from the flock of C. W. Williams, Stadacona Farms, Glyndon, Md., were anesthetized with Dial urethane, 0.5 cc/kg given intravenously. The fetus was delivered by Caesarean section. Breathing was prevented by drawing a rubber bag containing amniotic fluid over the nose at the moment of delivery. The placental circulation was maintained intact. The fetus was dried and kept warm by an electric pad. Loose ligatures were placed around the 2 umbilical arteries and veins, to permit occlusion of the vessels at will by applying traction upon the ligatures. Carotid blood pressure was recorded oscillographically by means of a strain gage transducer.

After initial observations on the state of the circulation, the 2 vagi were sectioned in the midcervical region, and the 2 stellate ganglia exposed, dissected free, their branches cut, and the ganglia removed. In addition, the postganglionic fibers from $T_3$ and $T_4$ were cut.

Upon occlusion of either the umbilical arteries or veins, bradycardia developed, rapidly when the vagus nerves were intact, and slowly after those nerves were cut. Since about two-thirds of the blood passing down the aorta goes to the placenta (*2*), substantial embarrassment of the fetal heart can be induced by occlusion of the umbilical blood vessels. In 10 of 11 instances in which the heart has been denervated, as described above, restoration of blood flow in the umbilical vessel was associated with a marked tachycardia. In these 10 cases the heart rate increased to 240–276 beats/min. Such high heart rates often were sustained for some minutes.

In order to establish the physiological basis of the cardio-acceleration occurring after circulatory distress in fetuses having denervated hearts, 3 fetuses were subjected to bilateral adrenalectomy. Two experiments were unsuccessful, due to excessive hemorrhage. One was completely successful. Repeated observations were made in this fetus. Some of the data as obtained are summarized in Figs. 1 and 2.

In Fig. 1, occlusion, first of the 2 umbilical arteries and then of the 2 veins, was maintained for 44 and 36 sec respectively (see arrows). Within 12 sec after release of the arteries and 20 sec after release of the