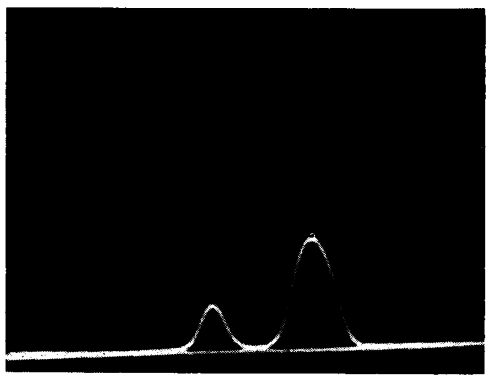

FIG. 1. The electrophoretic pattern of crystalline lactic dehydrogenase. Descending pattern in phosphate buffer pH 5.7, ionic strength 0.1. The salt boundary is just visible as a thickening of the base line on the extreme left.

identical with those in the unresolved enzyme or the crystals shown by Straub (*3*). This material migrated as a single component on electrophoresis. So far it has not been possible to crystallize the minor component.

The salting-out diagram, optical density at 280 mμ vs percentage saturation with ammonium sulfate, also shows a two-component system. The breaks in the salting-out curve are found at 40 and 43% ammonium sulfate saturation.

The activity of the enzyme was determined by measuring the rate of appearance of the absorption band of DPNH at 340 mμ. The tests were carried out in glycine-NaOH buffer, pH 9.6, with excess of DPN (*4*) and lactate. Both components were found to oxidize L(+) sodium lactate. The major component was usually somewhat more active on the basis of optical density at 280 mμ. D(−) sodium lactate was not attacked.

It seems to be an interesting fact that following the application of a rigorous test "pure" enzymes, like crystalline proteins, can often be shown to be heterogeneous. Different workers, using a variety of techniques, have provided evidence for different forms of pepsin (*5*), cytochrome *c* (*6*), ribonuclease (*7, 8*) and lysozyme (*9*). The study of such substances should aid in the elucidation of enzymatic reaction mechanisms, regardless of whether the different forms are present in nature.

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# The Application of Electrophoresis-Convection in the Purification of Enzymes: Purification of the Alkaline Phosphatase of Swine Kidneys

James C. Mathies [1]

*Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Michigan*

The separation of protein mixtures by electrophoresis-convection (*1*) has been quite successful, indicating that the application of this technique to the purification of enzymes should be explored. This note presents initial results of such a study in which a partially purified alkaline phosphatase preparation was the starting material.

The preparation of the alkaline phosphatase from swine kidneys and the determination of the enzyme activity were carried out as previously described (*2*). It was necessary to activate the enzyme before it was assayed. To do this, the final enzyme dilution was prepared in 0.01 *M* DL-alanine which was also 0.05 *M* in $NaHCO_3$-$Na_2CO_3$ buffer of a pH of 9.1. The diluted enzyme solution was incubated for 3 hr at 25° C to activate it and then assayed for enzyme activity. Total nitrogen determinations were made by the micro-Kjeldahl procedure.

Separations by electrophoresis-convection were carried out at 5° C, using a 0.1 ionic strength phosphate buffer at pH 7.2. The duration of the runs was 6 hr, and the field strength employed was 1.6 v/cm. When necessary, the protein solutions were concentrated by precipitation with $(NH_4)_2SO_4$ or acetone or by lyophilization, followed by dialysis of the enzyme against the phosphate buffer. This same phosphate buffer was used in the electrophoretic analyses.[2]

Table 1 presents the results obtained for a single-stage separation and in addition the final results for

TABLE 1

PURIFICATION OF SWINE KIDNEY ALKALINE PHOSPHATASE BY MEANS OF ELECTROPHORESIS-CONVECTION

| Experiment | TPKU* | PU/mg TN† |
|---|---|---|
| 1. Initial material | 226 | 146 |
| *a.* Fourth stage | | |
| Top cut | 12 | 392 |
| Bottom cut | 29 | 85 |
| 2. Initial material | 250 | 191 |
| *a.* First stage | | |
| Top cut | 95 | 270 |
| Bottom cut | 138 | 159 |
| *b.* Fifth stage | | |
| Top cut | 15 | 588 |
| Bottom cut | 17 | 85 |

* TPKU = total phosphatase units × $10^{-3}$.
† PU/mg TN = phosphatase units/mg total nitrogen.

[1] With the technical assistance of Eleanore D. Goodman.
[2] The electrophoretic analyses and mobility calculations were carried out by J. M. Vandenbelt and R. B. Scott, of Parke, Davis & Co. Their generous assistance is gratefully acknowledged.

a 4- and a 5-stage separation. The scheme used in these fractionations is essentially that suggested by Cann and Kirkwood (*3*) for the separation of the components of ovalbumin. A fifth-stage top cut contains the enzyme isolated in the top cell after 5 consecutive refractionations of the collected top cuts. The fifth-stage bottom cut represents 5 similar consecutive refractionations of collected bottom cuts. The enzyme not accounted for in Table 1 is contained in several fractions of intermediate purity. The purest preparation of renal alkaline phosphatase obtained (588 PU/mg TN) has three times the specific activity of the starting material, and appears to be about twice as active as the best preparations in the literature (*4*).

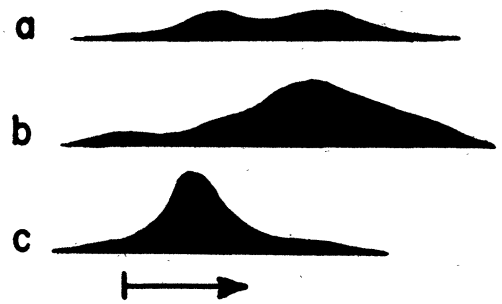


FIG. 1. Electrophoretic patterns (descending boundary). *a*, Expt 2, initial material; *b*, fifth-stage bottom cut; *c*, fifth-stage top cut.

Fig. 1 presents electrophoretic patterns for 3 of the fractions of the second experiment. Pattern *a*, the starting material for the fractionation, exhibits 2 peaks with respective mobilities of 2.2 and $5.0 \times 10^{-5}$ $cm^2/v$ sec. The broad nature of these peaks indicates the presence of more than 2 components. Pattern *b*, the fifth-stage bottom cut, is complex, but gives indications of components with mobilities of 2.2 (small amount), 4.0, 5.0, and $6.2 \times 10^{-5}$ $cm^2/v$ sec. The concentration of the more mobile components in this fraction is quite obvious from a comparison of Patterns *a* and *b*. Pattern *c*, the fifth-stage top cut, contains about 50% or more of what appears to be one component with a mobility of $1.7 \times 10^{-5}$ $cm^2/v$ sec. The presence of slower- and faster-moving components is also indicated. The use of different conditions in the electrophoretic analysis might show this fraction to be more complex than is indicated in the present pattern. However, the enrichment of this fraction in less mobile components is quite clear. The data do not permit conclusions as to the portion of the electrophoretic pattern which represents enzyme, except the obvious inference that it is contained in the slower-moving components of the starting material (*5*); hence no statements as to the absolute purity of the enzyme preparation obtained can be made.

It should be mentioned that the enzyme solutions employed in these fractionations were brown. The pigment, or pigments, responsible moved rapidly in the electrical field and were therefore concentrated quite efficiently in the bottom fractions. Consequently, the use of the Kirkwood apparatus for the removal of certain types of pigments from protein solutions is indicated.

A point of interest is that the present example is perhaps one of the least favorable that could have been chosen, since it was not possible to work at pH values near the isoelectric point of the enzyme. Much greater efficiency should be possible when enzymes that are stable in the region of their isoelectric points are employed.

The following points with respect to the use of electrophoresis-convection in enzyme purification are emphasized: (1) The yields of enzyme are practically quantitative. If the enzyme is sufficiently stable, the only losses that occur are mechanical. (2) The apparatus is much less expensive, and is better adapted for mass separations, than the Tiselius apparatus. (3) Although analytical electrophoresis equipment is of great value in orienting the course of fractionations in the Kirkwood apparatus, it is not essential when materials possessing measurable biological activity are being purified. Specific activity estimations of the fractions obtained are sufficient to guide the purification.

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## Effect of Heparin on the Growth of a Transplantable Lymphosarcoma in Mice[1]

Leonard Kreisler

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine,* and *Waldemar Medical Research Foundation, Brooklyn, New York*

It has been postulated that the gelation that occurs in mitosis prior to the formation of the spindle may have the same physical basis as the mechanism of blood clotting. Heilbrunn and Wilson (*1, 2*) have reported that heparin, in dilute solution, prevents premitotic gelation, thus inhibiting cell division. It was thought of interest to see whether this action of heparin would influence the growth of a rapidly dividing tissue, such as a tumor graft.

Mice of the inbred strain C3H/Jax and the lymphosarcoma 6C3HED were the test materials. The lymphosarcoma grows in 100% of the C3H/Jax mice, forming a discrete mass when transplanted subcutaneously. Fifteen male mice, 2 months old at the start of the experiment and of equivalent weight (approximately 25 g), were divided into three groups of 5 each. Groups 1 and 2 received intravenous injections of

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