STUDIES ON ISOLATED CELL COMPONENTS

XV. THE DISTRIBUTION OF CERTAIN ENZYMES IN THE ELECTROPHORETIC PATTERN OF THE SOLUBLE PHASE OF RAT LIVER

N. G. ANDERSON, W. D. FISHER[1] and M. L. ANDERSON

Biology Division, Oak Ridge National Laboratory,[2] Oak Ridge, Tennessee, U.S.A.

Received August 1, 1960

In a previous study [5], large and characteristic differences between the electrophoretic patterns of the soluble proteins of rat liver, kidney, brain, and testis were noted. A correlation was found between the average mobility and nuclear volume such that tissues with larger nuclei tended to show lesser amounts of slow-moving proteins. However, a detailed study of such inter-organ differences awaits a more complete analysis of the electrophoretic patterns in terms of enzymatic and other biological activities. In this paper a method for simultaneously sampling both limbs of a Tiselius cell is presented, together with data on the distribution of catalase, β-glucuronidase, rhodanese, acid phosphatase, esterase, adenosine deaminase, glutathione reductase, and nucleoside phosphorylase in the pH 7.5 electrophoretic pattern of the soluble phase of rat liver.

EXPERIMENTAL

Livers from adult male Sprague–Dawley rats were perfused, homogenized, and the soluble phase was obtained as previously described [2, 3, 5]. Dialysis and electrophoresis were carried out in a 0.1 ionic strength, pH 7.5, phosphate buffer containing NaCl [17].

Electrophoresis was carried out in 11-ml quartz cells in a Spinco Model H electrophoresis apparatus for an average of 200 minutes at 20 ma. When the run was complete, both limbs were photographed and the lower section of the cell was slid across to prevent fluid movement while the protein fractions were being recovered. The compensator and breathing tube were then opened, carefully removed, and two parallel sampling needles attached to the same rack and pinion-mounted support, were slowly lowered into the cell. The vertical movement was calibrated to allow

[1] Present address: Department of Radiology, College of Medicine, State University of New York, Syracuse, N.Y.

[2] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

[3] By reducing the diameter of the tubing used, cross-contamination has recently been reduced to 10 per cent.

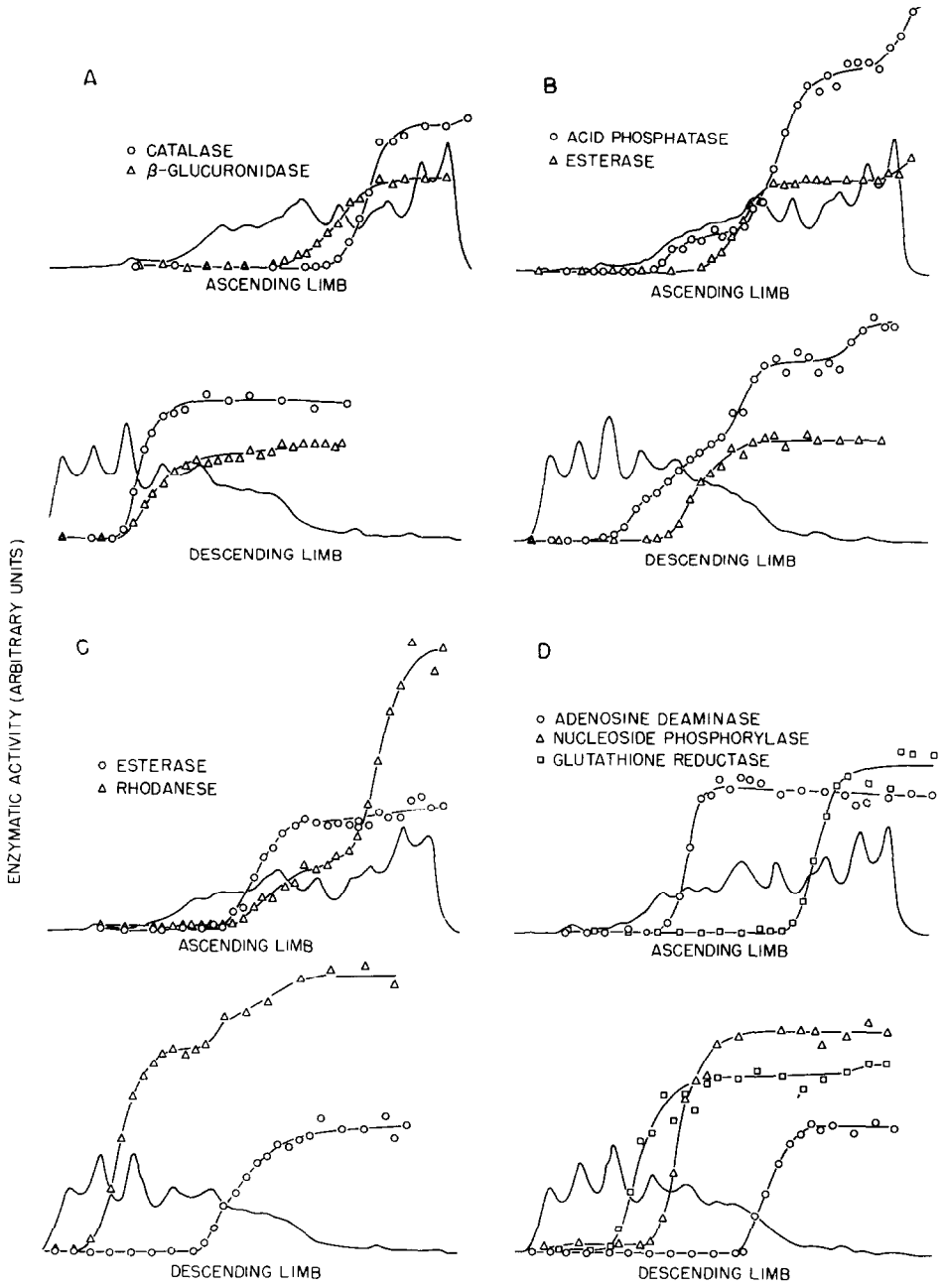
the needles to be lowered in 2-mm steps. A small, dual peristaltic pump was designed, which moved fluid slowly out through both needles simultaneously. At each of the 32 sampling levels, 0.152 ml of protein solution was withdrawn during movement of the boundary to needle tip level. Sufficient buffer was then withdrawn to give a volume of 2 ml per step, thus rinsing protein solution from the needle, pump, and associated tubing. Tests showed that each sample was contaminated with 30 per cent of the previous sample.[3] Results were therefore corrected by this amount. The method was checked by comparing the results obtained from the ascending and descending limbs. Since these are sampled in opposite directions with respect to the patterns, systematic errors would be observed as a difference in position of an activity in the two limbs. Photographs were taken after every second or third sampling to facilitate localization of the samples and associated activities in the pattern. All samples were analyzed immediately or after refrigeration overnight.

Enzymatic assays.—For catalase determination by Feinstein's method [9], samples from the electrophoresis cell were diluted 1:10 with buffer containing 0.025 per cent bovine serum albumin; 0.25 ml of this mixture was used together with one-half the volume of reagents prescribed. Adenosine deaminase and nucleoside phosphorylase were determined by Schneider and Hogeboom's method [22], in which 0.2- and 0.1-ml samples, respectively, of the electrophoretic fractions were used. Enzyme samples (0.25 ml) were adapted to a final volume of 3.0 ml by the method described by Racker [21] for assaying glutathione reductase. For the remainder of the determinations, 0.5-ml samples of the enzyme fractions were used. Rhodanese was measured in half the reagent volumes listed [6], and at an incubation temperature of 25°C. For esterase determinations, acetylsalicylic acid was the substrate [13]; *o*-carboxyphenylphosphate was used for acid phosphatase determinations [14]. β-Glucuronidase was determined by the method described by Fishman and Bernfeld [10].

RESULTS

Representative results obtained from the analysis of eight sets of patterns are shown in Fig. 1. The enzyme determinations give integral curves and the Schlieren curves for the electrophoretic pattern show the first derivative of the concentration vs. cell height. The inflection point of the enzyme activity curves therefore locate the "peak" of the activity in the Schlieren diagram. Acid phosphatase, esterase, and rhodanese assays were performed on three separate runs; two runs were analyzed for each remaining enzyme. Rhodanese exhibited two boundaries, a large and very slow one just in front of the T–0.24 peak,[1] and a smaller one in the area of the T–3.02 peak. Catalase was found in a single boundary on the leading edge of the T–1.11 peak. Glutathione reductase was found to be localized very close to catalase. β-Glucuronidase appeared in the T–2.08 peak and nucleoside phosphorylase

[1] The T unit [4] is defined as the electrophoretic mobility in cm $\times 10^5$/volt/cm/sec.


A
○ CATALASE
△ β-GLUCURONIDASE
ASCENDING LIMB
DESCENDING LIMB
B
○ ACID PHOSPHATASE
△ ESTERASE
ASCENDING LIMB
DESCENDING LIMB
C
○ ESTERASE
△ RHODANESE
ASCENDING LIMB
DESCENDING LIMB
D
○ ADENOSINE DEAMINASE
△ NUCLEOSIDE PHOSPHORYLASE
□ GLUTATHIONE REDUCTASE
ASCENDING LIMB
DESCENDING LIMB
ENZYMATIC ACTIVITY (ARBITRARY UNITS)

corresponded to the T–3.02 peak. Esterase was located between T–3.02 and 3.83; adenosine deaminase was found in the ill-defined region around T–4.6. As shown in Fig. 1, acid phosphatase is located in two, and possibly three, areas of the pattern. This enzyme shows a markedly dissimilar distribution in the ascending and descending peaks, which suggests the presence of an activator among the slower-moving proteins. The results are summarized in Fig. 2, where the first derivative of the enzyme activity curves is compared with the Schlieren diagram.

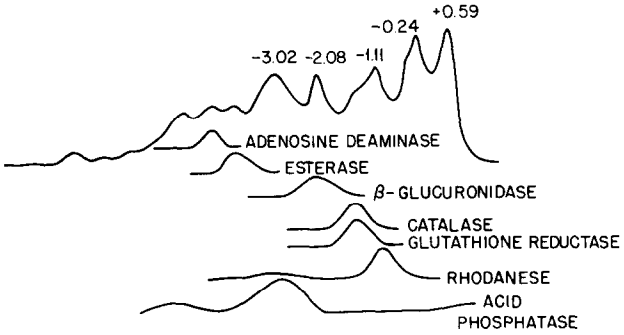


Fig. 2.—Summary of enzyme distributions and mobilities of major electrophoretic peaks in the soluble phase of rat liver at pH 7.5. Enzyme activities are plotted as the first derivatives of the activity curves shown in Fig. 1. Mobilities are expressed in T units.

DISCUSSION

With all the enzymes studied, results from the ascending and descending limbs agreed, except for acid phosphatase. With this enzyme, activity increases in the fast-moving, small peaks on the descending side only and in the slowest peak on the ascending limb only. It seems likely that boundaries appearing on only one side are caused by enzyme activators.

As previously discussed [3, 5], the presence of distinct peaks in either the ultracentrifuge or electrophoresis cell is attributable to (*a*) the presence of a few proteins in high concentration against a background of many proteins in low concentration, (*b*) the existence of distinct families of proteins having similar physical properties, (*c*) the formation of complexes, or (*d*) a combination of these factors. The results presented here, with the possible exception of acid phosphatase, suggest that extensive complexing does not occur. The

Fig. 1.—Distribution of enzymatic activities in the electrophoretic pattern of the soluble phase of rat liver at pH 7.5.

number of enzymes examined is not large enough to enable us to decide between the two other possibilities suggested.

The results obtained with esterase do not suggest a widespread spectrum of "isozymes", as has been reported after starch gel electrophoresis of a mixture of mouse liver proteins and microsomes [16]. The localization of this activity near the fifth (T–3.02) peak and a wide spread in acid phosphatase activity agree with the results obtained by paper electrophoresis [19].

Rhodanese seems to be one homogeneous enzyme in guinea pig [24] and beef liver and kidney [26]. In rat liver this enzyme is largely particle bound [7, 12, 15, 23]. Two forms of the enzyme have been described, however [8, 24], in agreement with the results obtained here.

Catalase, which seems to be largely particle bound in the cell [1, 11, 25], exhibits a fairly homogeneous electrophoretic boundary at pH 7.5. Evidence for two liver catalases [18, 20] has been reported, however, and three activity peaks have been demonstrated chromatographically in this laboratory (N. G. Anderson and H. E. Bond, unpublished).

Although, in the present study, nucleoside phosphorylase exhibited the sharpest boundary, the resolution of the technique used is not sufficient to allow us to decide whether the slightly wider boundaries observed with esterase are caused by enzyme heterogeneity or experimental variability. More refined localization of enzymatic and other activity in free electrophoresis patterns will require a more accurate device for recovering fractions from the Tiselius cell.

SUMMARY

The distribution of catalase, rhodanese, acid phosphatase, nucleoside phosphorylase, esterase, adenosine deaminase, glutathione reductase, and β-glucuronidase in the free-electrophoresis pattern of the soluble phase of rat liver at pH 7.5 has been determined.

REFERENCES

1. Adams, D. H. and Burgess, E. A., *Brit. J. Cancer* **11**, 310 (1957).
2. Anderson, N. G., *Exptl. Cell Research* **11**, 186 (1956).
3. Anderson, N. G. and Canning, R. E., *Exptl. Cell Research* **17**, 465 (1959).
4. Anderson, N. G., Canning, R. E., Anderson, M. L. and Shellhamer, R. H., *Exptl. Cell Research* **16**, 292 (1959).
5. Anderson, N. G. and Swanson, H. D., *Exptl. Cell Research*, in press.
6. Bernheim, F., *in* The Enzymes, p. 857. Ed. by J. B. Sumner and K. Myrbäck. Academic Press, New York, 1950.
7. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F., *Biochem. J.* **60**, 604 (1955).

8. De Ritis, F., Coltorti, M. and Giusti, G., *G. Clin. Med.* **37**, 285 (1956). (*Excerpta Med.*, Sect. II, 9, 928.)
9. Feinstein, R. N., *J. Biol. Chem.* **180**, 1197 (1949).
10. Fishman, W. H. and Bernfeld, P., *in* Methods in Enzymology, Vol. **I**, p. 262. Ed. by S. P. Colowick and N. O. Kaplan. Academic Press, New York, 1955.
11. Greenfield, R. E. and Price, V. E., *J. Biol. Chem.* **220**, 607 (1956).
12. Greville, G. D. and Chappell, J. B., *Biochim. et Biophys. Acta* **33**, 267 (1959).
13. Hofstee, B. H. J., *Science* **114**, 128 (1951).
14. —— *Arch. Biochem. Biophys.* **51**, 139 (1954).
15. Ludewig, S. and Chanutin, A., *Arch. Biochem.* **29**, 441 (1950).
16. Markert, C. L. and Hunter, R. L., *J. Histochem. Cytochem.* **7**, 42 (1959).
17. Miller, G. L. and Golder, R. H., *Arch. Biochem.* **29**, 421 (1950).
18. Nakatani, M., *Bull. Agr. Chem. Soc. Japan* **22**, 308 (1958).
19. Pellegrino, C. and Caravaglios, R., *Arch. sci. biol. Bologna* **41**, 233 (1957).
20. Price, V. E. and Greenfield, R. E., *J. Biol. Chem.* **209**, 363 (1954).
21. Racker, E., *in* Methods in Enzymology, Vol. **II**, p. 722. Ed. by S. P. Colowick and N. O. Kaplan. Academic Press, New York, 1955.
22. Schneider, W. C. and Hogeboom, G. H., *J. Biol. Chem.* **195**, 161 (1952).
23. Sörbo, B. H., *Acta Chem. Scand.* **5**, 724 (1951).
24. —— *ibid.* **7**, 1129 (1953).
25. Thomson, J. F. and Klipfel, F. J., *Arch. Biochem. Biophys.* **70**, 224 (1957).
26. Westley, J., *J. Biol. Chem.* **234**, 1857 (1959).