Quantitative Estimation of a Primate-Specific Esterase in Normal and Malignant Tissue[1] (37263)

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Several isoenzyme forms of esterase exist in primate tissues, providing patterns that may be tissue as well as species specific (1). A primate-specific esterase isoenzyme has been found to occur in fetal and adult tissues as well as in cultured cells (2). Electrophoretically it migrates slowly and, in agarose electrophoresis, is cathodal with respect to the origin. It is referred to, therefore, as the cathodal esterase. Urine serves as a good source of the esterase. Purification and partial characterization has shown that it is an aliesterase, inhibited by 10^{-4} *M* diisopropyl-fluorophosphate but not by eserine. It has a molecular weight of approximately 136,000 (3).

There has been much speculation concerning the source of various enzymes in the urine (7–10). This report adds to evidence that the kidney itself produces large quantities of the primate-specific esterase. Also we submit preliminary evidence that quantitative differences exist in the primate-specific esterase content of certain malignant tissues.

Materials and Methods. Human tissue source. Normal and malignant tissues from human males and females ranging in age from 23 to 88 yr were obtained at autopsy.

Tissue extraction. After extensive washing in phosphate buffered saline at pH 7.4, tissue extracts were prepared as previously described (4). Protein concentration determinations were by the Folin-Ciocalteu method.

Quantitative immunoelectrophoresis. A modification of Laurell's quantitative immunoelectrophoresis technique was used (5, 6). A rabbit antiserum prepared against the fraction of human urine precipitated at 1.6 to 2.8 *M* $(NH_4)_2SO_4$ was used as a source of standard antibody. A kidney extract with a protein concentration of 42 mg/ml was used as a standard antigen. Aliquots of the kidney standard were initially frozen at —20° and a freshly thawed sample was used for each experiment. Glass slides (80 × 100 mm) were covered with 13 ml of a 1% agarose solution prepared in a buffer solution made of equal volumes of 0.05 *M* barbital (pH 8.2) and triple distilled water. A 1% (v/v) concentration of standard antiserum was incorporated in the gel. Wells were cut in the gel (4 mm diam) with 10 mm between the centers of adjacent wells. Each well was filled with 10 μl of antigen. Dilutions of the standard antigen were used in the last three wells of each plate. Electrophoresis was carried out in 0.05 *M* barbital buffer ($\mu = 0.05$) (pH 8.2) at 5–7 V/cm for 4.5 hr. The distance of migration for each sample was measured from the center of the peak to its base. An arbitrary value of 100 "esterase units" was assigned to the standard preparation of kidney extract. The distance migrated by 1:2 and 1:4 dilutions of antigen represented 50 and 25 units, respectively. A curve plotted on a semilog scale showed the relationship between esterase units and the migration (mm) of the standard antigen (Fig. 1). The number of units for each unknown sample was determined from the standard curve. The relative esterase content of a sample was determined by dividing the number of esterase units by the protein concentration. A dose–response determination established a linear

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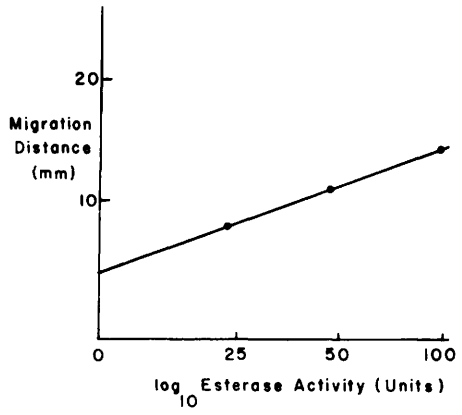

FIG. 1. Migration distance of three dilutions of a human kidney extract standard determined by quantitative immunoelectrophoresis and plotted against logarithm of esterase content expressed as arbitrary units (see text).

relationship between the logarithm of concentration of the standard antigen and its migration distance in 23 separate electrophoretic runs (Fig. 2).

Results. Figure 3 shows a typical quantitative immunoelectrophoresis plate which illustrates the relationship between enzyme content and the distance migrated. The standard kidney extract (wells 7, 8 and 9) demonstrate the progressive reduction in migration distance of the esterase as the sample is diluted. The same standard kidney extract was used on each plate submitted to electrophoresis because subtle variations within the procedure might influence the migration of the test samples. Examples of these variations would be the concentration of agarose in the gel, the thickness of the agarose gel layer on the plate, the voltage across the plate during electrophoresis, and the exact time length of electrophoresis. Therefore the kidney standard provided an internal control on each plate. In actual practice, there were negligible differences in the standard migration distance among experiments carried out on different days as shown by the small standard errors in Fig. 2.

The values of 76 normal tissues and 7 malignant tumors were compiled to determine the mean relative esterase content. Table I shows the results of the quantitative estimamation of the esterase in various tissues expressed in terms of migration distance per mg and of esterase units per mg. Kidney had the highest relative concentration of 2.65 units/mg and pancreas had the lowest of 0.36 units/mg. Kidney has twice the concentration of esterase found in the adrenals ($p < 0.01$). Liver closely follows adrenal with heart and brain occupying a middle position on the scale. Spleen, lung and pancreas have the lowest concentration of esterase. A comparison of the mean of 4 lung tumors with that of normal lung showed no significant difference. However, the mean esterase concentration of 3 liver tumors was more than twice that of normal liver; a significant difference ($p < 0.01$).

Discussion. A quantitative analysis of cathodal esterase among various tissues has shown that kidney has the highest relative concentration. This suggests that the esterase, as it exists in urine in relatively large amounts, could be derived from the kidney. The possibility that certain urinary antigens, including enzymes, may leak from tissues into the serum and then be concentrated by the kidneys has been postulated to explain the abundance of these antigens in the urine (7, 8). However, this explanation is unlikely in the case of the cathodal esterase with a molecular weight of 136,000. More likely the main source of enzymes in the urine is the renal tissue itself. De Vaux St. Cyr, Hermann and Talal (10) demonstrated 2 esterases in the urine which have their origin in

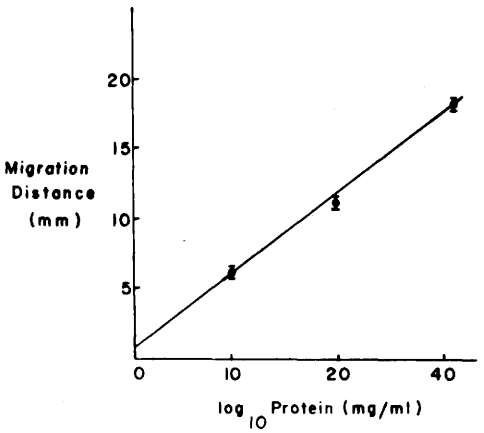

FIG. 2. Dose–response relationship of the mean migration distance (mm ± SE) to the concentration of protein with standard kidney extract; data compiled from 23 different electrophoretic runs.

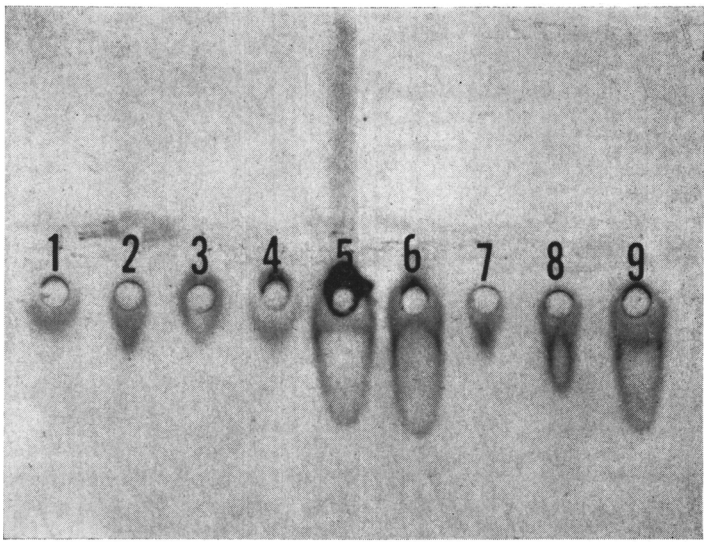

FIG. 3. Quantitative immunoelectrophoretic analysis of tissue extracts, stained for esterase activity with indoxyl acetate. Wells: (1) pancreas, 55 mg/ml; (2) lung, 35 mg/ml; (3) heart, 36 mg/ml; (4) spleen, 110 mg/ml; (5) liver, 108 mg/ml; (6) kidney, 82 mg/ml; (7) 1:4 dilution of kidney standard; (8) 1:2 dilution of kidney standard; (9) kidney standard, 42 mg/ml.

the kidney. One of these may be the equivalent of the cathodal esterase presently under study. Determination of the level of the cathodal esterase in the urine may serve as an indicator of renal injury.

The significance of the variation in esterase concentration of different tissues is difficult to assess. The possibility that the esterase is differentially inactivated in various tissues following death must be considered. All tissues were collected between 4 and 12 hr after death. The length of time which elapsed between death and tissue extraction did not significantly influence esterase activity among tissues of the same origin as may be seen by the small standard error values in Table I. To check this point further, we incubated pieces of human kidney and human liver taken at autopsy 4 hr previously and kept at 37 and 25° for 12 hr. Then the

TABLE I. Levels of Cathodal Esterase in Normal and Abnormal Human Tissues.[a]

Tissue		$\bar{X}$ distance migrated (mm)	$\bar{X}$ protein (mg/ml)	Esterase concn: mm migrated/mg ± SE	Esterase concn: units/mg ± SE
Normal					
Kidney	(12)	18.8	62.6	0.33 ± 0.02	2.65 ± 0.31
Adrenal	(5)	13.0	48.6	0.35 ± 0.05	1.33 ± 0.08
Liver	(12)	14.7	78.2	0.21 ± 0.02	1.22 ± 0.22
Heart	(9)	12.2	53.6	0.25 ± 0.02	0.92 ± 0.31
Prostate	(4)	11.0	54.7	0.27 ± 0.06	0.86 ± 0.12
Brain	(4)	7.3	25.0	0.31 ± 0.03	0.75 ± 0.11
Lung	(10)	7.8	47.4	0.21 ± 0.02	0.46 ± 0.08
Spleen	(14)	8.8	67.4	0.17 ± 0.02	0.46 ± 0.06
Pancreas	(4)	6.6	57.4	0.12 ± 0.01	0.36 ± 0.07
Malignant					
Lung	(4)	7.7	41.9	0.19 ± 0.03	0.52 ± 0.11
Liver	(3)	15.3	43.3	0.35 ± 0.03	2.51 ± 0.20

[a] Numbers in parentheses indicate the number of samples tested.

TABLE II. Testing for *in Vitro* Esterase Inactivation.

(°)	(hr)	Protein (mg/ml)	Migration (mm)	Units esterase/mg
Kidney				
4	0	51	12	1.07
25	12	52	12	1.05
37	12	52	13	1.30
Liver				
4	0	100	14	0.90
25	12	92	14	0.97
37	12	80	14	1.12

tissues were extracted and compared for esterase content (Table II). There was no discernible drop in esterase concentration, making it unlikely that the differences presented reflect a differential inactivation of enzyme activity in various tissues. Moreover, the human-specific esterase has been a very stable enzyme during *in vitro* studies. Repeated freezing and thawing of esterase preparations did not appear to reduce the activity of the esterase nor alter its migration in standard zonal electrophoretic and immunoelectrophoretic procedures.

Nonetheless, a differential effect of factors present *in situ* on esterase activity after death cannot be entirely excluded. It is known that the *in vivo* level of an enzyme in normal animal tissues represents a balance between the rate of synthesis and the rate of degradation. In the case of liver catalase, 2 distinct genetic factors affect the enzyme activity (11). One controls its catalytic activity and the other, its content. The content-controlling factor is known to be tissue-specific. It is plausible that similar tissue-specific factors control the content of other enzymes, including esterase.

Blanco and Zinkham (1) found an increase in *total* esterase activity in liver, kidney and heart with age. These data are consistent with our findings that adult liver, kidney and heart have a relatively high concentration of cathodal esterase compared to other tissues tested. This isoenzyme may then be significant in the process of aging, at least in some tissues. The same authors point out that all tissues have the same set of genes capable of controlling synthesis of the enzyme but that during development various factors modify their expression. In the mouse, the enzymes, β-galactosidase and β-glucuronidase, are controlled by separate, unlinked structural genes, yet the ratio of the 2 enzymes is constant in liver, heart and brain during development (12). Hence they have a common developmental pattern presumably controlled by the same set of regulatory genetic factors. While the ratio of the 2 enzyme activities is constant within a single organ, its range varies during aging from tissue to tissue which suggests a further regulatory mechanism of a tissue-specific time-dependent character.

Some data on the control of cathodal esterase production have been reported. Bartholomew, Bartholomew and Rose (2) have shown that a human diploid lung line, Wl-38, transformed by SV40 virus, has a markedly reduced ability to synthesize this esterase. In other studies we have also observed a marked reduction of the cathodal esterase in 2 other SV40 transformed cell lines as well as a spontaneously transformed culture of the established FL amnion line.

The importance of isoenzyme patterns in the diagnosis of malignancy is an active area of investigation. Many transplantable rat hepatomas demonstrate quantitative changes in isoenzyme content (13). The degree of alteration in rat hepatoma isoenzymes is more marked as the degree of dedifferentiation of the tumor increases. In the data presented here, esterase content is seen to be relatively low in normal lung. Accordingly, there is no increase in esterase concentration in malignant tissue of the lung. Liver has high levels of cathodal esterase and in hepatomas the esterase is greatly increased. In the change from normal to malignant growth, a cancerous cell may retain the unique isoenzyme pattern of the tissue of origin though the quantity of the isoenzyme may vary.

Summary. A relative quantitative estimation of cathodal esterase in normal and malignant tissues has been performed. The highest concentration of esterase was found in the kidney. A comparison shows that human hepatomas have a distinctly increased level of the esterase over normal

liver.

1. Blanco, A., and Zinkham, W. H., Bull. Johns Hopkins Hosp. **118**, 27 (1966).

2. Bartholomew, E., Bartholomew, W. R., and Rose, N. R., J. Immunol. **103**, 787 (1969).

3. Therrien, G. D., Rose, N. R., and Bartholomew, W. R., Prep. Biochem. **1**, 259 (1971).

4. Kite, J. H., Kornstad, L., Bartholomew, W. R., and Rose, N. R., Lab. Invest. **17**, 515 (1967).

5. Laurell, C. B., *in* "Protides of the Biological Fluids" (H. Peeters, ed.), Vol. 14, p. 499. Elsevier, Amsterdam (1966).

6. Laurell, C. B., Anal. Biochem. **15**, 45 (1966).

7. Halbert, S. P., Greene, E. L., and Pallavicini, C., *in* "Protides of the Biological Fluids" (H. Peeters, ed.), Vol. 16, p. 559. Pergamon, Bruges (1968).

8. Greene, E. L., Halbert, S. P., and Pallavicini, J. C., Int. Arch. Allergy Appl. Immunol. **40**, 861 (1971).

9. Mattenheimer, H., Symp. Modern Concepts in Renal Dis. **55**, 1493 (1971).

10. De Vaux St. Cyr, C., Hermann, G., and Talal, N., Rev. Fr. Etud. Clin. Biol. **8**, 241 (1963).

11. Ganschow, R. E., and Schimke, R. T., J. Biol. Chem. **244**, 4649 (1969).

12. Meisler, M., and Paigen, K., Science **177**, 894 (1972).

13. Weinhouse, S., Cancer Res. **31**, 1166 (1971).

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