Some Properties of Lactate Dehydrogenase Found in Human Urine

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1. Urinary lactate dehydrogenase was concentrated and subjected to starch-block electrophoresis. The isoenzyme pattern obtained was shown to be similar to that of the kidney cortex and medulla but different from that of the bladder and kidney pelvis. 2. The values for the relative activity and Michaelis constant of urinary lactate dehydrogenase were similar to those for kidney cortex and medulla but significantly different from those obtained for bladder and kidney pelvis. 3. The molecular weight of urinary lactate dehydrogenase was estimated by thin-layer chromatography on Sephadex G-200. The values obtained for several samples of urine ranged from 129 000 to 155 000 and were very close to that of the crystalline rabbit-muscle enzyme (140 000). 4. The question of the possible origin of urinary lactate dehydrogenase is discussed and the conclusion drawn that the kidney and not the plasma is the most likely source.

Many cellular enzymes occur in body fluids and in particular the properties of serum enzymes have been widely investigated. Comparatively little work however has been carried out on enzymes in urine, because of the low activities usually found. One such enzyme that has been detected in urine is lactate dehydrogenase (Rosalki & Wilkinson, 1959), and is the subject of this investigation. Crockson (1961) has suggested that the urinary enzyme arises from the serum and has claimed to show a correlation between urinary lactate-dehydrogenase activity and proteinuria. Other workers have shown that a raised serum enzyme concentration caused by disease (West & Zimmerman, 1958; Pojer, Mojzis & Tovarek, 1960) does not produce a correspondingly high output in the urine. Also, if the serum enzyme concentration is elevated by injection of crystalline lactate dehydrogenase, the urinary excretion is unaffected (Kemp & Laursen, 1960). In view of these observations it was decided to examine further the properties of urinary lactate dehydrogenase and to compare it with the enzyme found in bladder, kidney and serum. A preliminary account of this work has been presented at a meeting of The Biochemical Society (Plummer, 1965).

EXPERIMENTAL

Materials. Sodium pyruvate was obtained from British Drug Houses Ltd., Poole, Dorset, and sodium 2-oxobutyrates from the Sigma Chemical Co., St Louis, Mo., U.S.A. NADH₂ was purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Solutions of the above were prepared immediately before use. The marker proteins used in

the thin-layer chromatography on Sephadex G-200 were as follows: cytochrome *c* and albumin (British Drug Houses Ltd.); trypsin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex); ox-heart lactate dehydrogenase, rabbit-muscle lactate dehydrogenase and γ -globulin (Sigma Chemical Co.). All solutions were prepared in water redistilled in Pyrex apparatus (L. V. D. Scorah, Birmingham).

Tissue extracts. Human kidney and bladder tissue was obtained at autopsy within 24 hr. of death and extracts were prepared as described by Plummer, Elliott, Cooke & Wilkinson (1963).

Protein determination. The protein content of the extracts was determined by a biuret method (Wootton, 1964). The value obtained by measuring E_{210} (Tombs, Souter & MacLagan, 1959) compared favourably with the biuret method.

Determination of dehydrogenase activity. (a) With pyruvate as substrate. A spectrophotometric method based on that of Wróblewski & La Due (1955) was used. A suitable amount of the enzyme was mixed with 0.067 M-sodium phosphate buffer, pH 7.4, containing NADH₂ (0.33 μ mole) and equilibrated at 25° for 15 min. Sodium pyruvate was then added to a final concentration of 0.7 mM, bringing the final volume to 3 ml., and the oxidation of NADH₂ was followed at 340 m μ in a Uvispek spectrophotometer fitted with a Gilford Unit (Hilger and Watts Ltd., London).

(b) With 2-oxobutyrates as substrate. The method described above for pyruvate was used except that 3.3 mM-sodium 2-oxobutyrates replaced sodium pyruvate (Rosalki & Wilkinson, 1960).

All enzyme activities are expressed as μ moles of NADH₂ oxidized/min. at 25°. The 'relative activity' is defined as the ratio of dehydrogenase activities with 2-oxobutyrates and pyruvate as substrates at the concentrations indicated, which are optimum for human sera.

Concentration of urine. Overnight samples of urine were

obtained from healthy adult males and concentrated by dialysis against 30% Carbowax for 18 hr. at 4°. The concentrate was then dialysed for 4 hr. against distilled water or dilute phosphate buffer, pH 7.4, as required. When further concentration was necessary this was carried out by vacuum dialysis through a collodion membrane (Membranfiltergesellschaft G.m.b.H., Göttingen, Germany).

Starch-block electrophoresis. This was carried out at 4° by a similar technique to that described by Plummer *et al.* (1963).

Molecular-weight estimation. An estimate of the molecular weight of the urinary lactate dehydrogenase was obtained by thin-layer chromatography on Sephadex G-200 (Andrews, 1964; Morris, 1964). The solvent used was 0.067 M-phosphate buffer, pH 7.4. Samples of crystalline lactate dehydrogenase and several marker proteins were run for comparison. Lactate dehydrogenase was detected by placing Whatman no. 3 filter paper soaked in the enzyme reaction mixture over the plate. This was incubated for 30 min. at 37° and the fluorescence of the plate under ultra-violet light was then examined, and the regions containing NAD were noted.

RESULTS

Overnight excretion of lactate dehydrogenase. The variation of enzyme activity with enzyme concentration was determined by using unconcentrated urine. Most of the urines examined showed no deviation from linearity when up to 0.6 ml. was included in the reaction mixture. This suggested the absence of lactate-dehydrogenase inhibitors in most cases. Inhibitors were detected in about 4% of the samples studied but could be readily removed by dialysis. The mean lactate-dehydrogenase activity in 50 male urine samples was $3.0 \mu\text{moles/min./l.}$ with a standard deviation of $\pm 1.8 \mu\text{moles/min./l.}$ The overnight excretion of the enzyme activity was 0.88 ± 0.38 (S.D.) $\mu\text{mole/min.}$ Samples of urine collected from one person on five separate occasions gave a similar variation [0.80 ± 0.26 (S.D.) $\mu\text{mole/min.}$], showing that the enzyme excretion is not constant.

After concentration of about 100-fold the enzyme activity was again determined. The recovery was generally 80–100% with the occasional value as low as 60%. Concentration provided material, with an activity of about $0.3 \mu\text{mole/min./ml.}$, that was suitable for further investigation.

Stability of the urinary enzyme. For the unconcentrated urine storage for 24 hr. at 4° or room temperature resulted in no loss of enzyme activity. After 24 hr. at room temperature the results were variable, probably owing to bacterial contamination. Storage for up to 4 days at 4° resulted in a 25% loss of lactate-dehydrogenase activity. Similar results were obtained after storage of the concentrated urine. Freezing at -18° resulted in complete loss of enzyme activity for both untreated and concentrated urine. Whenever possible the activities were determined within an hour of obtaining

the sample, but never later than after 24 hr. storage at 4°.

Dehydrogenase activities of tissue extracts and urine. The dehydrogenase activities of human kidney and bladder extracts and urine are shown in Table 1. There is little difference in the lactate-dehydrogenase activity of the cortex and medulla but the kidney pelvis and the bladder have a much lower activity. The 'relative activity' of the urinary enzyme shows a similar value to that found in the kidney cortex and medulla. The values for the Michaelis constants shown in Table 2 confirm the similarity of the urinary and kidney enzymes. The bladder-epithelium extract was prepared by scraping the surface of the tissue and homogenizing as described above.

The epithelial cells of the bladder contain less enzyme and have a higher relative activity than the whole bladder extract.

Starch-block electrophoresis. Separation of the lactate-dehydrogenase isoenzymes by starch-block electrophoresis showed the urine contained principally peaks 1 and 2 with traces of peaks 3, 4 and 5 (Fig. 1). The urinary isoenzyme pattern resembled that obtained from kidney cortex. There is little difference between the kidney cortex and medulla

Table 1. *Dehydrogenase activities of human kidney and bladder extracts and urine*

Enzyme source	Dehydrogenase activity ($\mu\text{mole/min./mg.}$ of protein)		Rel. activities: 2-oxobutyrate/ pyruvate
	Pyruvate	2-Oxo- butyrate	
Urine	0.029	0.026	0.90
Kidney cortex	0.87	0.77	0.88
Kidney medulla	0.80	0.70	0.87
Kidney pelvis	0.17	0.13	0.77
Bladder	0.036	0.022	0.62
Bladder epithelium	0.018	0.013	0.74

Table 2. *Michaelis constants (K_m) and percentages of H and M forms of lactate dehydrogenase derived from human tissues*

Enzyme source	K_m (mm)		% H M	
	Pyruvate	2-Oxobutyrate	H	M
Urine	0.062	1.09	79	21
Kidney cortex	0.066	0.92	78	22
Kidney medulla	0.062	0.91	78	22
Kidney pelvis	0.077	1.32	65	35
Bladder	0.085	1.41	55	45

isoenzyme content but the pelvis was different from the rest of the kidney (Fig. 2). The principal peaks for the bladder tissue were peak 3 with small amounts of peaks 1 and 2 and traces of peaks 4 and 5, and thus is different from the urinary enzyme (Fig. 1). The recoveries of enzyme activity were in the range 50–80% of that applied to the block and were greater for those enzyme sources with fast-moving peaks of activity. The electrophoretic patterns of all tissues examined were related to the values obtained for the relative activities (Table 1) and Michaelis constants (Table 2). Tissues containing predominantly fast-moving peaks gave lower K_m values and higher relative activities than tissues with slow-moving isoenzymes.

The five isoenzymes of lactate dehydrogenase are considered to be tetramers of two polypeptide chains, the H and M chains. The composition of isoenzymes 1–5 is given by H_4 , H_3M , H_2M_2 , HM_3 and M_4 respectively (Appella & Markert, 1961; Cahn, Kaplan, Levine & Zwilling, 1962). The total activity of the H forms is given by $(1) + \frac{1}{2}(2) + \frac{1}{2}(3) + \frac{1}{4}(4)$ where (1), (2), (3) and (4) represent the activities of each isoenzyme obtained from Figs. 1 and 2. The total M activity was similarly obtained and the

results are expressed as a percentage of the total (Table 2).

Estimation of molecular weight. A plot of distance of migration on Sephadex G-200 against the logarithm of the molecular weight of the protein markers gave a smooth curve. The molecular weight of the urinary lactate dehydrogenase calculated from this graph varied from 129 000 to 155 000. The molecular weight of several different crystalline lactate dehydrogenases is reported to be 140 000 (Pesce, Stolzenbach, Freedberg & Kaplan, 1963).

DISCUSSION

Lactate dehydrogenase is present in human male urine in small but detectable amounts. Only a slight loss of activity occurred during concentration

Fig. 1. Lactate-dehydrogenase activity in fractions of human urine (a) and bladder extract (b) separated by starch-block electrophoresis in barbitone buffer, pH 8.6. Samples containing 1 mg. of protein were placed on the block. Alb and Hb denote the positions occupied by albumin and haemoglobin markers respectively, and the arrow shows the point of application. The numbers 1–5 show the positions of lactate-dehydrogenase isoenzymes.

Fig. 2. Lactate-dehydrogenase activity in fractions of human kidney extracts (a, pelvis; b, medulla; c, cortex) separated by starch-block electrophoresis in barbitone buffer, pH 8.6. Other details are as in Fig. 1.

and none at all during storage at 4° or room temperature for 24 hr. The presence of inhibitors of lactate dehydrogenase in urine has been reported when lactate is used as substrate (Dorfmann, Amador & Wacker, 1963; Schoenberger & Wacker, 1966). In the present study most samples of urine were shown to be free from detectable quantities of inhibitor, in agreement with other workers who have also used pyruvate as substrate (Riggins & Kiser, 1963; Güttler & Clausen, 1965).

The existence of lactate dehydrogenase in five molecular forms is now well established. The relative amounts of the five isoenzymes depend on the tissue of origin and provide a useful way of characterizing the tissue. Human plasma contains all five isoenzymes and so the urinary lactate dehydrogenase would be expected to show a similar isoenzyme pattern if it arose from the plasma. The results of starch-block electrophoresis of urine show that the principal isoenzyme is peak 1 with a moderate amount of peak 2 and traces of the other forms (Fig. 1). This pattern is similar to that of the kidney cortex and medulla but different from that of the bladder and kidney pelvis (Fig. 2).

Lactate-dehydrogenase isoenzymes differ from each other in several properties other than the electrophoretic mobility. These include the substrate affinity (Plagemann, Gregory & Wróblewski, 1960; Wilkinson & Withycombe, 1965) and the relative activities with 2-oxobutyrate and pyruvate as substrate (Rosalki & Wilkinson, 1960). The values of these parameters provide a useful indication of the isoenzyme distribution (Plummer *et al.* 1963; Plummer & Wilkinson, 1963; Wilkinson & Withycombe, 1965) and the present results confirm this observation. The Michaelis constant and relative activity of urinary lactate dehydrogenase are similar to those of kidney cortex and medulla but significantly different from those of bladder and kidney pelvis (Tables 1 and 2) and plasma. The relative activity provides a rapid means of assessing the isoenzyme distribution of a tissue without recourse to the more lengthy procedure of electrophoresis. The relative activity of all urines examined gave values very close to 0.9. This indicates that the urinary isoenzyme pattern is constant in spite of the somewhat wide range of total activity excreted. These results all suggest that the kidney and not the bladder or plasma is the most likely source of urinary lactate dehydrogenase.

In male urine some of the lactate dehydrogenase could arise from the seminal fluid, which contains an unusual isoenzyme of lactate dehydrogenase (Zinkham, Blanco & Kupchyk, 1963; Goldberg, 1963) with a high relative activity (1.17) and a low K_m (0.18 mm) for 2-oxobutyrate (Wilkinson & Withycombe, 1965). These values bear no relationship to those found for the five usual isoenzymes and

are different from the values obtained for urinary lactate dehydrogenase (Tables 1 and 2). This suggests that the seminal fluid does not significantly contribute to the lactate dehydrogenase of male urine. Further, the relative activity of five female urines examined was 0.92, which is not significantly different from that obtained for male urine.

Some urinary enzymes appear to have a molecular weight different from the enzyme found in the kidney. Alkaline phosphatase in the urine has a molecular weight half that of the same enzyme from kidney (Butterworth & Moss, 1966), and the value for urinary β -galactosidase may be the same or twice that of the kidney enzyme (Dance, Price & Robinson, 1966). In contrast with these results the molecular weight of urinary lactate dehydrogenase was essentially the same as that of the kidney and rabbit-muscle enzymes. A protein of this molecular weight would not be expected to pass across the glomerular membrane in any quantity, and so it is unlikely that the plasma contributes to any significant extent to the lactate dehydrogenase found in human urine.

The results of this present work show that urinary lactate dehydrogenase has similar properties to that of the kidney enzyme and therefore most likely arises from this organ. The urinary enzyme, however, is not identical with that found in kidney, but this may perhaps be explained by considering that some kidney cells contribute more to the urinary enzyme than others. Results show that the isoenzyme pattern of the cortex, medulla and pelvis is not the same, and such isoenzyme heterogeneity may be present within these zones. The cells of the renal tubular epithelium probably make a major contribution to the total urinary lactate dehydrogenase. Renal tubular cells occur in urine in greatly increased quantities after ingestion of a large dose of aspirin (Scott, Denman & Dorling, 1963) and preliminary experiments suggest a direct correlation between the urinary cell count and lactate-dehydrogenase activity.

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REFERENCES

- Andrews, P. (1964). *Biochem. J.* **91**, 222.
- Appella, E. & Markert, C. L. (1961). *Biochem. biophys. Res. Commun.* **6**, 171.
- Butterworth, P. J. & Moss, D. W. (1966). *Biochem. J.* **99**, 9p.
- Cahn, R. D., Kaplan, N. O., Levine, L. & Zwillling, E. (1962). *Science*, **136**, 962.
- Crockson, R. A. (1961). *Lancet*, **i**, 140.
- Dance, N., Price, R. G. & Robinson, D. (1966). *Biochem. J.* **98**, 44p.

- Dorfmann, L. E., Amador, E. & Wacker, W. E. C. (1963). *J. Amer. med. Ass.* **184**, 1.
- Goldberg, E. (1963). *Science*, **139**, 602.
- Güttler, F. & Clausen, J. (1965). *Enzymol. biol. clin.* **5**, 55.
- Kemp, E. & Laursen, T. (1960). *Scand. J. clin. lab. Invest.* **12**, 463.
- Morris, C. J. O. R. (1964). *J. Chromat.* **16**, 167.
- Pesce, A., Stolzenbach, F., Freedberg, I. & Kaplan, N. O. (1963). *Fed. Proc.* **22**, 241.
- Plagemann, P. G. W., Gregory, K. F. & Wróblewski, F. (1960). *J. biol. Chem.* **235**, 2288.
- Plummer, D. T. (1965). *Biochem. J.* **96**, 74P.
- Plummer, D. T., Elliott, B. A., Cooke, K. B. & Wilkinson, J. H. (1963). *Biochem. J.* **87**, 416.
- Plummer, D. T. & Wilkinson, J. H. (1963). *Biochem. J.* **87**, 423.
- Pojer, J., Mojzis, A. & Tovarek, J. (1960). *Rev. franç. Étud. clin. biol.* **5**, 919.
- Riggins, R. S. & Kiser, W. S. (1963). *J. Urol.* **90**, 594.
- Rosalki, S. B. & Wilkinson, J. H. (1959). *Lancet*, ii, 327.
- Rosalki, S. B. & Wilkinson, J. H. (1960). *Nature, Lond.*, **188**, 1110.
- Schoenberger, G. A. & Wacker, W. E. C. (1966). *Biochemistry*, **5**, 1375.
- Scott, J. T., Denman, A. M. & Dorling, J. (1963). *Lancet*, i, 344.
- Tombs, M. P., Souter, F. & MacLagan, N. F. (1959). *Biochem. J.* **73**, 167.
- West, M. & Zimmerman, H. J. (1958). *J. Lab. clin. Med.* **52**, 185.
- Wilkinson, J. H. & Withycombe, W. A. (1965). *Biochem. J.* **97**, 663.
- Wootton, I. D. P. (1964). *Micro-analysis in Medical Biochemistry*, 4th ed., p. 138. London: J. and A. Churchill Ltd.
- Wróblewski, F. & La Due, J. S. (1955). *Proc. Soc. exp. Biol., N. Y.*, **90**, 210.
- Zinkham, W. H., Blanco, A. & Kupchik, L. (1963). *Science*, **142**, 1303.