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Mutarotase Activity in Serum and Urine of Patients with Renal Disease

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Mutarotase activity in the serum and urine of normal subjects and patients with known renal disease was determined with our mutarotase assay using an oxygen electrode and β-D-glucose oxidase. The activities of alkaline phosphatase and lactate dehydrogenase in serum and urine were also determined. Mutarotase activity was found to be present in the human urine. Elevated urinary mutarotase levels were observed in 7 of the 8 patients with nephrotic syndrome. Urinary levels of two other enzymes were also raised in the same case. Any marked elevation of serum mutarotase, alkaline phosphatase, and lactate dehydrogenase levels was not observed. Urinary mutarotase activity was related to the clinical state of patients with nephrotic syndrome.

Keywords——mutarotase; renal disease; nephrotic syndrome; kidney; lactate dehydrogenase; alkaline phosphatase; human serum; human urine

Mutarotase (aldose 1-epimerase, EC 5.1.3.3) which catalyzes the mutarotation of D-glucose and its configurationally related sugars has been found in the highest concentration in the kidney among mammalian tissues.[2,3] This observation was also obtained on human beings by Bailey, *et al.*[4] Therefore, the release of the kidney mutarotase into serum or urine is expected when suffered from renal disease.

Hill, *et al.*[5] have reported that rats with glycosuria induced by nephrotoxic agents (potassium dichromate, mercuric chloride, and uranyl nitrate) release considerable quantities of mutarotase into urine from the kidney. Bailey, *et al.*[4] have reported that about 50% of serum samples taken from patients with known renal disease elevate mutarotase levels and that the enzyme was absent from urine samples of all individuals studied. Recently, the authors[6] have found that a mutarotase activity is observed in serum and urine of rats with experimental nephrotic syndrome induced by the intravenous injection of rabbit anti-rat kidney serum when the serum and urine were dialyzed against 0.02 M, pH 7.0 ethylenediaminetetraacetic acid (EDTA) buffer.

Since it was presumed from our studies[6] that the mutarotase would appear not only in the serum but also in the urine of patients with nephrotic syndrome, the enzyme activity in the serum and urine was measured of normal subjects and patients with nephrotic syndrome and other known renal diseases with our mutarotase assay[7] using an oxygen electrode and β-D-glucose oxidase (β-D-glucose: oxygen oxidoreductase, EC 1.1.3.4).

Alkaline phosphatase (Al-P; orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) and lactate dehydrogenase (LDH; L-lactate: nicotinamide adenine dinucleotide (NAD)

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2) J.M. Bailey, P.G. Pentchev, and J. Woo, *Biochim. Biophys. Acta*, **94**, 124 (1965).
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5) J.B. Hill, T.U.L. Biber, and W.D. Huffines, *Biochem. Med.*, **1**, 62 (1967).
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oxidoreductase, EC 1.1.1.27) levels in serum and urine of normal subjects and patients with renal disease were also measured to compare with the changes of mutarotase levels in serum and urine, because the former two enzymes are known to distribute in high concentrations in the kidney. This paper also describes the relation between the clinical state and the urinary mutarotase, Al-P, and LDH levels of patients with nephrotic syndrome.

Experimental

Patients and Normal Subjects——The 42 patients with renal disease who gave written informed consent and in whom percutaneous renal biopsy was performed were divided into 5 groups.

Group I: Patients with Chronic Glomerulonephritis: Microscopic hematuria and slight proteinuria were observed in all patients. Mild or moderate proliferative changes were histologically shown in the glomeruli of all patients.

Group II: Patients with Nephrotic Syndrome: High concentration of urinary protein (above 3.5 g per day), low concentration of serum total protein (below 6.0 g per dl), hyperlipemia, and edema were observed in all patients.

Group III: Patients in remission in nephrotic syndrome by treatment with steroids and/or cyclophosphamide.

Group IV: Patients with uremia.

Group V: Chronic Hemodialyzed Patients: The patients were treated with an artificial kidney for above 3 months. Urine samples could not be obtained from the patients because of their anuria.

All patients of 5 groups were free of signs and symptoms of hepatic disease.

Normal subjects. Twenty three normal subjects (12 healthy women and 11 healthy men ages 20 to 48 years) who gave written informed consent were chosen as a control group.

Reagents——Each solution of β-D-glucose oxidase, α-D-glucose, and phlorizin (a potent mutarotase inhibitor) was prepared as described in the previous papers.[6,7] Crude β-D-glucose oxidase (from *Penicillium amagasakiense*) was kindly supplied from Nagase and Co., Ltd., Osaka, Japan, and the partially purified enzyme free from mutarotase and catalase was prepared by a two-steps purification procedure using diethylaminoethyl (DEAE)-cellulose column chromatography[8] and Sephadex G-75 column chromatography.[3] α-D-Glucose, phlorizin, and EDTA were commercial samples.

Apparatus——A polarographic oxygen analyzer and microsyrings were the same as reported previously.[7]

Assays of Enzyme Activities——Urinary mutarotase, Al-P, and LDH activities were measured on 8-hours overnight urine specimens which were centrifuged at $800 \times g$ for 10 min. Serum and urinary mutarotase activities were assayed with our method[6] using an oxygen electrode and β-D-glucose oxidase after dialyzing serum and urine against 0.02 M, pH 7.0 EDTA buffer at 4° for 15 hr. After the centrifuged urine specimens were dialyzed against distilled water at 4° for 3 hr, the Al-P activity was determined according to the method of Babson, *et al*[9] and the LDH activity was determined by the method of Cabaud, *et al.*[10] Serum Al-P activity was assayed by the automated method of Morgensten, *et al.*[11] and expressed as International Units (*i.e.*, μmoles of substrate converted per min) as well as for the urinary Al-P activity. Serum LDH activity was determined by the method of Wróblewski and LaDue.[12] The serum and urinary LDH activities were expressed as Wróblewski Units.[12] All urinary enzyme activities measured were expressed as units per 8-hours urine volume and the serum LDH, mutarotase, and AL-P activities were expressed as units per ml, per dl, and per liter, respectively.

Serum Cholesterol Determination——Serum total cholesterol was determined by the method of Zlatkis and Zak.[13]

Serum and Urinary Protein Determination——Serum and urinary proteins were determined by the biuret method of Gornall, *et al.*[14]

Results

Al-P and LDH activities in the serum and urine of Group I (Table I) were very close to those of a control group (Table VI) or only slightly elevated. Serum and urinary mutarotase activities of Group I were rather lower than control levels.

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14) A.G. Gornall, C.J. Bardwawill, and M.M. David, *J. Biol. Chem.*, **177**, 751 (1949).

TABLE I. Mutarotase, Al-P, and LDH Activities in Serum and Urine of Patients (Group I) with Chronic Glomerulonephritis[a)]

Patient No.	Age Sex	Serum			Urine		
		Mutarotase	Al-P	LDH	Mutarotase	Al-P	LDH
1.	20, f	neg.[b)]	23	286	neg.	0.12	2970
			(1>)	(1>)		(1>)	(1>)
2.	41, m	neg.	30	315	neg.	0.18	620
			(1>)	(1>)		(1>)	(1>)
3.	35, f	neg.	32	315	neg.	0.47	4300
			(1>)	(1>)		(1.5)	(1.2)
4.	32, f	0.06	20	315	neg.	0.05	3260
		(1>)	(1>)	(1>)		(1>)	(1>)
5.	23, m	0.09	28	246	neg.	0.11	5860
		(1>)	(1>)	(1>)		(1>)	(1.6)
6.	24, m	neg.	33	219	neg.	0.21	2400
			(1>)	(1>)		(1>)	(1>)
7.	18, f	0.15	36	316	neg.	0.15	830
		(1>)	(1>)	(1>)		(1>)	(1>)
8.	35, f	neg.	18	230	neg.	0.59	5930
			(1>)	(1>)		(1.8)	(1.7)
9.	22, f	0.06	9	315	neg.	0.25	8230
		(1>)	(1>)	(1>)		(1>)	(2.3)

a) Values in parentheses are ratios of values found to the upper limits (mean+2 S.D.) of normal values.
b) negligible

TABLE II. Mutarotase, Al-P, and LDH Activities in Serum and Urine of Patients (Group II) with Nephrotic Syndrome[a)]

Patient No.	Age Sex	Serum			Urine		
		Mutarotase	Al-P	LDH	Mutarotase	Al-P	LDH
1.	15, m	0.21	64	—[b)]	3.10	4.13	15800
		(1>)	(1.7)		(3.5)	(12.9)	(4.4)
2.	23, m	—	32	—	3.46	1.25	15200
			(1>)		(3.9)	(3.9)	(4.3)
3.	53, m	0.44	28	325	1.40	0.63	9360
		(1.3)	(1>)	(1>)	(1.6)	(2.0)	(2.6)
4.	38, f	0.59	18	420	1.51	1.16	15100
		(1.7)	(1>)	(1.1)	(1.7)	(3.6)	(4.2)
5.	27, f	0.15	20	511	3.19	2.52	30000
		(1>)	(1>)	(1.3)	(3.6)	(7.9)	(8.4)
6.	27, m	0.12	32	357	8.59	4.55	83900
		(1>)	(1>)	(1>)	(9.8)	(14.2)	(23.5)
7.	23, m	0.53	25	—	19.10	2.91	140000
		(1.5)	(1>)		(21.7)	(9.1)	(39.2)
8.	54, m	neg.[c)]	33	365	0.05	0.08	240
			(1>)	(1>)	(1>)	(1>)	(1>)

a) Values in parentheses are ratios of values found to the upper limits (mean+2 S.D.) of normal values.
b) data not obtained
c) negligible

Urinary mutarotase, Al-P, and LDH activities of Group II (Table II) were clearly elevated in 7 of the 8 patients with nephrotic syndrome, *i.e.*, the activities were higher than the upper limits (mean +2 S.D.) of the control group. These 7 patients have suffered from nephrotic syndrome for at least one year, while the remaining patient (No. 8) who gave a control level has been attacked by the same syndrome for only two months. The serum mutarotase, Al-P, and LDH levels of Group II were slightly elevated.

The activities of each enzyme in the serum of Group III (Table III) were close to the control levels. The urinary mutarotase activities of all patients of this group were within the upper limits of normal values, while the urinary Al-P and LDH levels were elevated, though not so much. The activities of each enzyme in the serum and urine of Group IV (Table IV) were close to control levels or slightly elevated. The serum Al-P levels of Group V (Table V) were not elevated, but the serum mutarotase activity was elevated in 6 of the 10 patients.

Table III. Mutarotase, Al-P, and LDH Activities in Serum and Urine of Patients (Group III) recovered from Nephrotic Syndrome[a]

Patient No.	Age Sex	Serum			Urine		
		Mutarotase	Al-P	LDH	Mutarotase	Al-P	LDH
1.	37, f	0.32	15	—[b]	0.35	0.33	4500
		(1>)	(1>)		(1>)	(1.1)	(1.3)
2.	50, m	0.21	26	—	0.19	0.08	16750
		(1>)	(1>)		(1>)	(1>)	(4.7)
3.	28, m	0.12	12	—	neg.[c]	0.03	4150
		(1>)	(1>)			(1>)	(1.2)
4.	44, f	0.18	18	374	neg.	0.30	2500
		(1>)	(1>)	(1>)		(1>)	(1>)
5.	23, f	0.38	14	299	neg.	0.32	4300
		(1.1)	(1>)	(1>)		(1.0)	(1.2)
6.	17, f	0.32	21	318	neg.	0.12	1670
		(1>)	(1>)	(1>)		(1>)	(1>)
7.	27, m	neg.	27	344	0.34	0.40	6400
			(1>)	(1>)	(1>)	(1.3)	(1.8)
8.	25, f	0.29	26	424	0.38	0.32	4360
		(1>)	(1>)	(1.1)	(1>)	(1.0)	(1.2)
9.	19, m	0.03	24	308	0.26	0.69	5210
		(1>)	(1>)	(1>)	(1>)	(2.2)	(1.5)

a) Values in parentheses are ratios of values found to the upper limits (mean+2 S.D.) of normal vaules.
b) data not obtained
c) negligible

Table IV. Mutarotase, Al-P, and LDH Activities in Serum and Urine of Patients (Group IV) with Uremia[a]

Patient No.	Age Sex	Serum			Urine		
		Mutarotase	Al-P	LDH	Mutarotase	Al-P	LDH
1.	56, f	0.15	23	273	neg.[b]	0.27	5490
		(1>)	(1>)	(1>)		(1>)	(1.5)
2.	38, m	neg.	23	260	neg.	1.18	neg.
			(1>)	(1>)		(3.7)	
3.	41, m	neg.	34	565	0.49	0.34	6400
			(1>)	(1.4)	(1>)	(1.1)	(1.8)
4.	48, m	0.26	28	256	1.13	0.62	5970
		(1>)	(1>)	(1>)	(1.3)	(1.9)	(1.7)
5.	33, m	—[c]	24	700	5.98	0.07	1550
			(1>)	(1.8)	(6.8)	(1>)	(1>)
6.	49, m	—	23	348	0.30	0.49	1370
			(1>)	(1>)	(1>)	(1.5)	(1>)

a) Values in parentheses are ratios of values found to the upper limits (mean+2 S.D.) of normal values.
b) negligible
c) data not obtained

Table V. Mutarotase and Al-P Activities in Serum of Chronic Hemodialyzed Patients (Group V)[a]

Patient No.	Age, Sex	Mutarotase	Al-P
1.	58, f	0.06 (1>)	71 (1.9)
2.	59, m	0.15 (1>)	12 (1>)
3.	37, f	1.11 (3.2)	47 (1.2)
4.	32, f	0.50 (1.4)	—[b]
5.	25, m	0.09 (1>)	12 (1>)
6.	28, m	0.76 (2.2)	24 (1>)
7.	52, f	0.59 (1.7)	32 (1>)
8.	31, m	0.32 (1>)	13 (1>)
9.	30, m	0.79 (2.3)	30 (1>)
10.	62, f	0.44 (1.3)	30 (1>)

a) Values in parentheses are ratios of values found to the upper limits (mean+2 S.D.) of normal values.
b) data not obtained

Table VI. Mutarotase, Al-P, and LDH Activities in Serum and Urine of Normal Subjects

Serum			Urine		
Mutarotase	Al-P	LDH	Mutarotase	Al-P	LDH
0.21±0.07[a] (16)[b]	26±6 (23)	284±57 (15)	0.28±0.30 (16)	0.18±0.07 (16)	1370±1100 (16)

a) mean and standard deviation
b) number of normal subjects tested

Since it was strongly suggested from the data in Table II that the nephrotic syndrome is associated with the elevation of the urinary mutarotase, Al-P, and LDH activities, the enzyme activities, urinary protein, serum total protein, and serum total cholesterol of representative two patients with nephrotic syndrome were determined in course of time to ascertain the suggestion.

In a patient who recovered gradually from the nephrotic syndrome by treatment with prednisolone and cyclophosphamide, the initially elevated activities of the urinary mutarotase, Al-P, and LDH declined to control levels roughly in parallel with the improvement of the clinical state which was diagnosed from the increase of serum total protein levels and the decrease of serum total cholesterol and urinary protein levels (Fig. 1).

In another patient who was not treated with any drug and was not improved in nephrotic syndrome because of its resistance to steroid therapy, the urinary mutarotase, Al-P, and LDH activities were continuously and clearly higher than the control levels of each enzyme (Fig. 2).

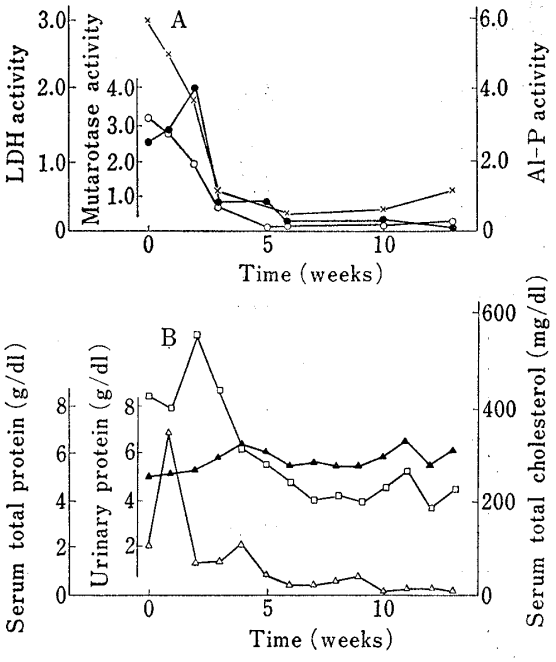

Fig. 1. A; Changes of Urinary Mutarotase (○), Al-P (●), and LDH (×) Activities of a Patient (27 years, female) with Nephrotic Syndrome
B; Changes of Serum Total Protein (▲), Urinary Protein (△), and Serum Total Cholesterol (□) Levels of the Same Patient

The patient was administered *per os* with 40 mg of prednisolone on each day of the first week and the administration was continued decreasing gradually the dosage of prednisolone to 10 mg per day on the last week. Cyclophosphamide was also administered *per os* with 100 mg on each of the first four days of every week after the fourth week. Each enzyme activity is expressed as total units per 8-hour urine volume. Only LDH activity is multiplied by 1×10^{-4}.

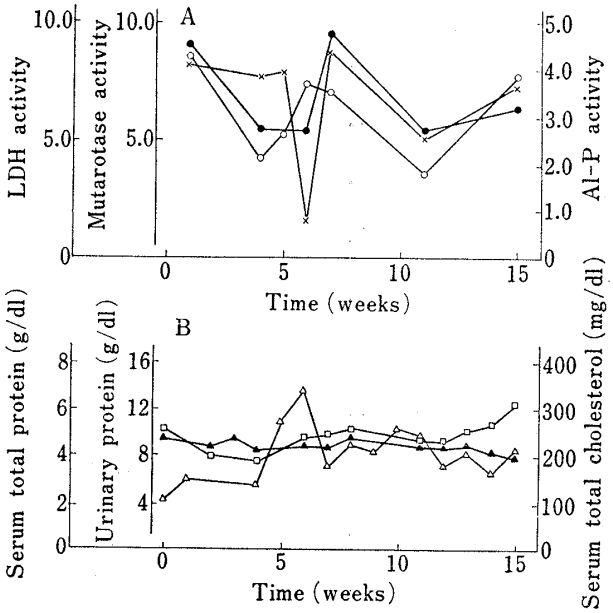

Fig. 2. A; Changes of Urinary Mutarotase (○), Al-P (●), and LDH (×) Activities of a Patient (27 years, male) with Nephrotic Syndrome
B; Changes of Serum Total Protein (▲), Urinary Protein (△), and Serum Total Cholesterol (□) Levels of the Same Patient

The patient was not treated with any steroid and other drugs. Each enzyme activity is expressed as total units per 8-hour urine volume. Only LDH activity is multiplied by 1×10^{-4}.

Discussion

While Bailey, *et al.*[4] reported that the mutarotase activity was not detected in any urine sample from normal subjects and subjects (with known renal disease) whose serum mutarotase levels were elevated, the authors found that even some of healthy adults had a detectable urinary mutarotase activity and that 7 of the 8 patients with nephrotic syndrome and 2 of the 6 patients with uremia had the urinary mutarotase activity significantly above control levels.

Bailey, *et al.* also reported that any mutarotase inhibitor was not present in both serum and urine. On the other hand, the authors suppose there is some mutarotase inhibitor, which can be eliminated by dialysis, in both serum and urine because we could not detect the mutarotase activity in both serum and urine without dialyzing samples for 15 hr at 4° as briefly described previously.[6]

Inferring from the reports[5,15] that the mutarotase in the kidney is suggested to be localized in the renal tubules and from the fact that there were clear pathological changes of the renal tubule cells in the nephrotic syndrome by light microscopy, the urinary mutarotase of patients with nephrotic syndrome would be released from the renal tubule cells. The major part of

15) J.M. Bailey, P.H. Fishman, and P.G. Pentchev, *J. Biol. Chem.*, **245**, 559 (1970).

the urinary Al-P and LDH of patients with nephrotic syndrome would also probably be released from the damaged renal tubule cells, since these enzymes are reported to be distributed mainly in the renal tubules.[16] However, the fact that the urinary mutarotase levels of Group III were not elevated at all, but the Al-P and LDH levels were somewhat elevated may imply the different localization in the renal tubule cells between the former enzyme and the latter ones. The only patient who had the nephrotic syndrome of a short period of history, but had not an elevated urinary mutarotase level would be different from the other patients of the same group in the nature of the renal tubular damage because of the short history of the nephrotic syndrome.

The reason why serum mutarotase levels were elevated in 6 of the 10 patients of Group V but Al-P levels were elevated in only 2 of the 9 patients may be due to the difference of the stability of these two enzymes in body fluids which will be primarily a function of their net turnover rates.

Urinary mutarotase levels were found to change in concordance with the changes of serum total protein, urinary protein, and serum cholesterol levels which have been usually used as a diagnostic guide for nephrotic syndrome (Fig. 1) and also to be continuously and significantly high when being in nephrotic syndrome (Fig. 2). The urinary mutarotase activity was thus related to the clinical state of patients with nephrotic syndrome. Changes of urinary Al-P and LDH levels were almost the same as those of mutarotase levels.

Although the change of urinary mutarotase activity was well parallel to those of Al-P and LDH activities in nearly every case, mutarotase is a more useful enzyme for studying urinary enzymes in renal disease than Al-P and LDH, because mutarotase in dialyzed urine is stable for at least a week either when stored frozen at $-20°$ or refrigerated at $4°$, while Al-P and LDH can not be stored stable more than a day at the same conditions.

After all, mutarotase is an interesting enzyme from the view of the applicability to diagnosis of renal disease, especially of nephrotic syndrome, since it is present in high concentration in the kidney cortex, is thought to play some role in reabsorption of D-glucose,[15,17] and is also fairly stable for storage as described above.

16) H. Mattenheimer, "Current Problems in Clinical Biochemistry," Vol. 2, ed. by U.C. Dubach, Hans Huber Publishers, Berne, 1968, pp. 119—156.
17) A.S. Keston, *Science*, **120**, 355 (1954).