[Chem. Pharm. Bull. 22(10)2337—2340(1974)]

UDC 615.36.076.9

# Mutarotase Activity in Serum and Urine of Rat with Experimental Nephrotic Syndrome

Ichitomo Miwa, Keiko Ikeda (née Ina), Yoshio Suzuki, Jun Okuda,[1a] and Kazuo Maeda[1b]

*Faculty of Pharmaceutical Science, Meijo University[1a] and Daini Red Cross Hospital of Nagoya[1b]*

(Received April 2, 1974)

The mutarotase activity in the kidney, liver, serum, and urine of rats with experimental nephrotic syndrome induced by the intravenous injection of rabbit anti-rat kidney serum, was measured by our sensitive assay. It became clear that the mutarotase activity in the liver was almost constant, that in the kidney markedly decreased, and a considerable amount of mutarotase appeared in the serum and urine. It was therefore concluded that some amount of mutarotase in the kidney of rat was surely released into the serum and urine when the animal was suffered from nephrosis.

Mutarotase (aldose 1-epimerase, EC 5.1.3.3) which catalyzes the mutarotation of D-glucose and its configurationally related sugars is known to be present in high activity in the kidney, liver, and small intestine of mammals, and it is reported that, among these organs, the kidney contains the highest specific activity of mutarotase.[2,3] These results were recently confirmed by one of the authors, Miwa.[4] Therefore, the release of mutarotase of the kidney into the serum or urine is expected when the mammal is suffered from the renal disease. Bailey, *et al.*[3] reported the significant elevation of mutarotase activity in the serum of some patients with renal disease, but they could not detect any mutarotase activity in urine. Hill, *et al.*[5] reported that rats with glycosuria induced by nephrotoxic agents (potassium dichromate, mercuric chloride, and uranyl nitrate), release considerable quantity of mutarotase into the urine from the kidney. In the present study, it was found that some amount of mutarotase in the kidney of rats with experimental nephrotic syndrome induced by the intravenous injection of rabbit anti-rat kidney serum was released into the serum and urine. The result is described in detail.

## Experimental

**Reagents**——Each solution of β-D-glucose oxidase (EC 1.1.3.4) and α-D-glucose was prepared as described in the previous paper.[6] Phlorizin (a potent mutarotase inhibitor) purchased from Nakarai Chemicals, Ltd., Kyoto was dissolved in ethylene glycol and kept in warm water to diminish the viscosity of the solution.

**Apparatus**——A polarographic oxygen analyzer (model 777, Beckman Instruments Inc., Fullerton, Calif.) connected with a recorder (EPR-2TC, Toa Electronics Ltd., Tokyo) was used for measurement of oxygen consumption. Ten μl-microsyringes (Jintan Terumo Co., Ltd., Tokyo) were employed to measure the volume of the reagent solutions.

**Serum Total Cholesterol Determination**——Serum total cholesterol was determined by the method of Zurkowski.[7]

---

1) Location: *a) Yagoto, Tenpaku-cho, Showa-ku, Nagoya; b) Myoken-cho, Showa-ku, Nagoya.*
2) J.M. Bailey, P.G. Pentchev, and J. Woo, *Biochim. Biophys. Acta*, **94**, 124 (1965).
3) J.M. Bailey, P.H. Fishman, S. Mulhern, and R. Murray, *Clin. Biochem.*, **3**, 11 (1970).
4) I. Miwa, *Anal. Biochem.*, **45**, 441 (1972).
5) J.B. Hill, T.H.L. Bier, and W.D. Huffines, *Biochem. Med.*, **1**, 62 (1967).
6) I. Miwa and J. Okuda, *J. Biochem.*, **75**, 1177 (1974).
7) P. Zurkowski, *Clin. Chem.*, **10**, 451 (1964).

NII-Electronic Library Service

**Protein Determination**——Protein in whole kidney homogenates was determined by the method of Lowry, *et al.*[8] with crystalline bovine serum albumin as the standard. Protein in urine was determined by nephelometry using 3% sulfosalicylic acid solution.

**Production of Nephrotic Syndrome in Rats**——The nephrotic syndrome in rats was induced by the intravenous injection of anti-rat kidney serum obtained from rabbits according to the method of Heymann and Lund.[9] One third of the dose was given by intravenous injection on each of 3 consecutive days. The third day when the last injection of serum was carried out was chosen as the starting day (zero day) of every experiments. The rats injected with normal rabbit serum were used as the control rats.

**Collection of Urine Sample**——The 24 hr-urine sample was collected by placing rats in a metabolism cage in which food spillage and urine contamination were minimized by a series of baffles.

**Assay of Mutarotase Activity**——Mutarotase activity in the kidney and liver was assayed according to our improved method[6] using a polarographic oxygen electrode, $\beta$-D-glucose oxidase, and phlorizin. For the assay of mutarotase activity in serum and urine, these materials were dialyzed against 0.02M EDTA buffer (pH 7.0) for 15 hr at 4° to eliminate a certain mutarotase inhibitor (unpublished result) and were used as samples for mutarotase assay.

## Results

The determination of the serum cholesterol levels and the protein contents in the urine and kidney of rats injected with rabbit normal serum and rabbit anti-rat kidney serum was performed to ascertain the production of nephrotic syndrome by the method of Heymann and Lund.[9]

The serum cholesterol levels of rats (experimental rats) injected with anti-rat kidney serum changed in the almost same manner as described in the previous paper,[10] that is, the serum cholesterol levels of experimental rats were distinctly higher than those of control rats from the first day to the 14th day, and lowered nearly to the normal level after the 21st day. The urinary protein, which was scarcely detected in the urine of control rats, was found in the urine of experimental rats at a level between 2 and 6.5 g/dl through the experimental period (Fig. 1). The protein content in the kidney of experimental rats was significantly smaller than that of control rats from the first to the 14th day as well as in the case of the serum cholesterol level, and became almost the same as the normal level after the 21st day (Fig. 2). The fall of the kidney protein level would reflect the occurrence of edema in the kidney. Although the urinary protein did not disappear even after the 29th day, from these determinations it was supposed that the nephrotic syndrome is produced in rats almost just

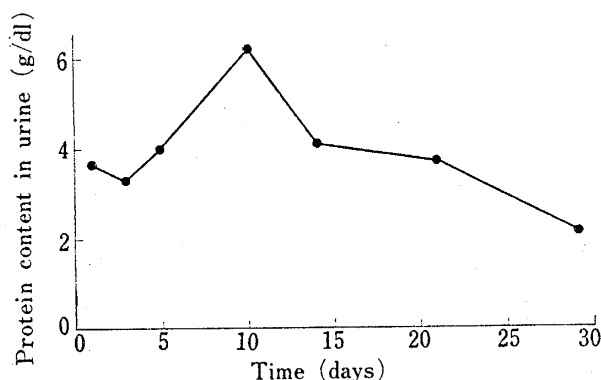


Fig. 1. Changes of Protein Content in the Rat Urine after Intravenous Injection of Anti-Rat Kidney Serum obtained from Rabbit

Values are expressed as mean for five determinations.

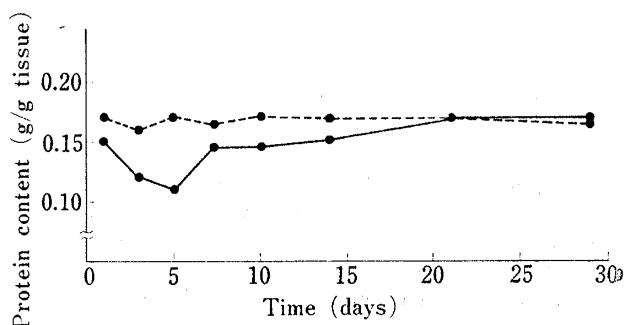


Fig. 2. Changes of Protein Content in the Kidney of Control Rats (---•---) and Nephrotic Rats (—•—)

Values are expressed as mean for five determinations.

8) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
9) W. Heymann and H.Z. Lund, *Pediatrics*, **7**, 691 (1951).
10) Y. Suzuki, K. Ina, M. Harada, and I. Yamagami, *Folia Pharmacol. Japon.*, **68**, 572 (1972).

NII-Electronic Library Service

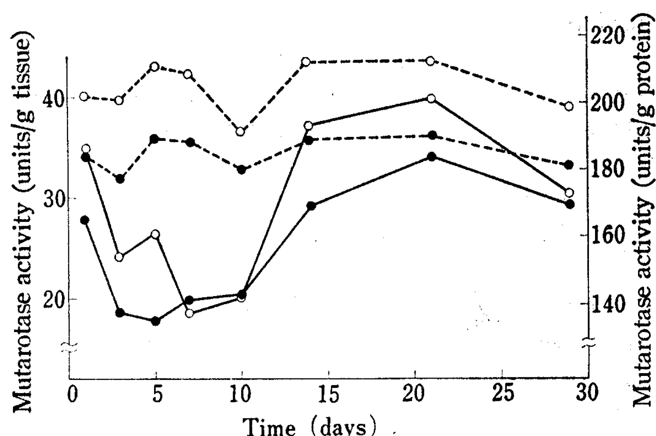


Fig. 3. Changes of Mutarotase Activity in the Kidney of Control Rats (Dotted Lines) and Nephrotic Rats (Solid Lines)

Open circles, in terms of units/g protein; closed circles, in terms of units/g tissue. Values are expressed as mean for five determinations.

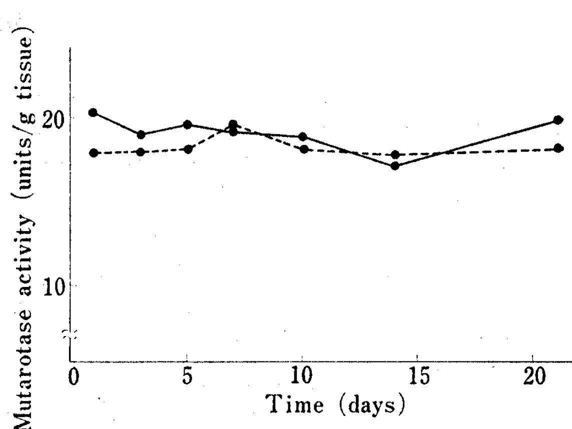


Fig. 4. Changes of Mutarotase Activity in the Liver of Control Rats (---•---) and Nephrotic Rats (—•—)

Values are expressed as mean for five determinations.

after the final injection of the rabbit anti-rat kidney serum and fades practically away 3 weeks after the injection.

The mutarotase activity in the kidney was determined to know whether the release of mutarotase from the kidney can be observed or not by the injection of the rabbit anti-rat kidney serum. Fig. 3 shows that the total mutarotase activity of the kidney of nephrotic rats is clearly lower than that of control rats from the first to 14th day and returns toward the control level 3 weeks after the injection of the anti-serum, so the muratotase would be lost from the kidney of nephrotic rats throughout 2 weeks after the injection of the anti-serum. On the other hand, the mutarotase of the liver was not lost at all through the experimental period (Fig. 4).

The mutarotase activity was found both in the serum and urine of nephrotic rats, though not so much, through the concordant period with the fall of the kidney protein content and the rise of the serum cholesterol, while any mutarotase activity was not found in the serum and urine of the control rats (Fig. 5 and 6). The mutarotase activity was distinctly higher in the urine than in the serum through all of the time.

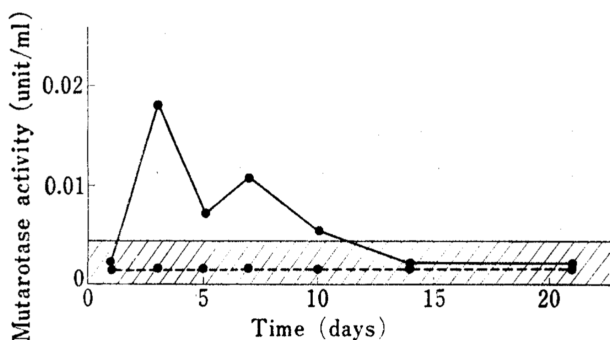


Fig. 5. Changes of Mutarotase Activity in the Serum of Control Rats (---•---) and Nephrotic Rats (—•—)

Hatched area: The activity less than 0.003 unit/ml cannot be determined precisely by our method.[8] Values of pool samples obtained from five animals are expressed.

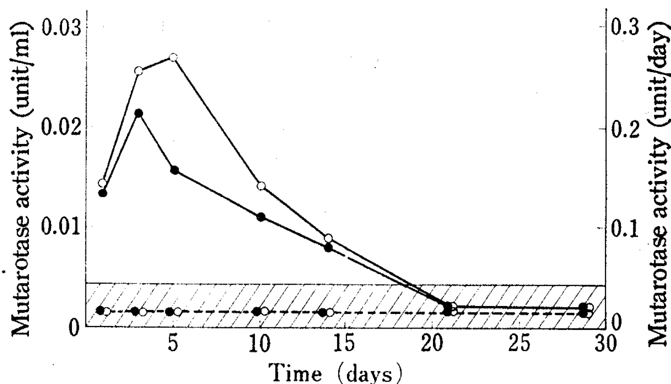


Fig. 6. Changes of Mutarotase Activity in the Urine of Control Rats (Dotted Lines) and Nephrotic Rats (Solid Lines)

Open circles, in terms of unit/ml; closed circles, in terms of unit/day. Hatched area: The activity less than 0.003 unit/ml cannot be determined precisely by our method.[8] Values are expressed as mean for five determinations.

NII-Electronic Library Service

## Discussion

For the experimental investigation of nephrotic syndrome, the rats have been most frequently used, because the enzyme distribution in the kidney of this species corresponds largely to the enzyme distribution in the different parts of the human kidney, and also the method of the preparation of nephrotic syndrome in rats by the intravenous injection of rabbit anti-rat kidney serum is known to be excellent in reproducibility of syndrome and in its similarity to the human nephrosis. Since, actually, the high urinary protein (Fig. 1) and serum cholesterol levels, and lower kidney protein levels (Fig. 2) were observed in all rats injected with rabbit anti-rat kidney serum, the clear nephrotic syndrome was thought to be induced in each rat injected with the anti-serum.

Bailey, *et al.*[3] reported that about 50% of the serum sample taken from patients with known renal disease had elevated mutarotase levels and that any urine sample had not elevated. On the other hand, we found that the considerable quantities of mutarotase appeared in both the serum and urine of rats with experimental nephrotic syndrome (Fig. 5 and 6), and the appearance was well correlated with the loss of mutarotase from the kidney of nephrotic rats (Fig. 3). Since the mutarotase activity in the liver of nephrotic rats did not change throughout experimental period (Fig. 4) and other tissues contain only much smaller amount of mutarotase compared with kidney and liver,[2,4] the mutarotase activity in the serum and urine of nephrotic rats would be originated from the kidney, but not from the liver and other tissues. Our findings do not conflict with the results of Hill, *et al.*[5] that the loss of mutarotase from the rat kidney by treating with nephrotoxic agents is accompanied by the appearance of mutarotase in urine.

Although we already reported the changes of some enzyme activities (alkaline phosphatase, lactate dehydrogenase, and leucine aminopeptidase) in the kidney, serum, and urine of rats with the same experimental nephrotic syndrome as described here, any enzyme was not proved to be lost from the kidney into the serum and urine.[10]

Moreover, it was found that mutarotase in serum and urine samples was stable for at least 2 weeks when they were stored frozen after dialyzing against EDTA buffer (0.02 M, pH 7.0).

From all the facts mentioned above and the preliminary observation of markedly elevated urinary mutarotase levels of patients with nephrotic syndrome, it will be expected that the measurement of mutarotase activity in the serum or urine of patients with renal disease may be useful as a clinical guide to the nature and progress of the renal disease.

It was supposed according to rough calculation that only 5% of mutarotase released from the kidney of experimental rats through the first 3 days appeared in serum and urine during that period, *i.e.* about 95% of released mutarotase was digested or inactivated through various processes.

Since the mutarotase in rat kidney is suggested from our histochemical studies[11] and other reports[5,12] to be localized in the renal tubules, it appears that not only glomeruli but also renal tubules are fairly damaged in rats with nephrotic syndrome induced by the present method.

11) To be published.
12) J.M. Bailey, P.H. Fishman, and P.G. Pentchev, *J. Biol. Chem.*, **245**, 559 (1970).

NII-Electronic Library Service