Table 2. Plasma fibrinogen level in post partum period of eclampsia

	Fibrinogen* Value in antepartum**	Days in post partum*** 2nd day	4th day	6th day
Eclampsia	1650	1820	1467	1510
Blood pressure reading (mm Hg)	190/110		120/90	120/90

* Average value of 3 patients. ** Before and *** after confinement.

in Carl Zeiss PMQ II spectrophotometer against reagent blank.

Results and discussion. The results presented in table 1 clearly demonstrate that there was a significant difference in plasma fibrinogen level between persons suffering from eclampsia and normal pregnant women of 3rd trimester. Pre-eclamptic patients exhibited a tendency for higher plasma fibrinogen level, which usually started developing in the 2nd trimester. The plasma fibrinogen value increased by 70% in pre-eclampsia and the increase was more striking in eclampsia, where the value was as high as 145%. Table 1 further shows that the plasma fibrinogen level did not change significantly in essential hypertension. The absence of high level of fibrinogen in essential hypertension indicates that the rise in eclampsia and pre-eclampsia was not due to the sudden increase of blood pressure. Howie et al.[7] also did not find any change in coagulation and fibrinolysis in essential hypertension. The increase of fibrinogen level in eclampsia was possibly not due to general protein synthesis, as the total plasma protein value increased only by 30% in eclampsia (table 1). Data presented in table 1 were obtained by the thrombin precipitation method, but when calcium was used, almost the same results were obtained. It is also interesting to note that the plasma fibrinogen level of these patients still showed higher level after confinement when most of the clinical symptoms disappeared (table 2). In a recent paper, Dube et al.[8] reported that there was very little change in the plasma fibrinogen level in pre-eclampsia and eclampsia. By contrast, we got a consistent significant increase under these conditions.

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Multiple forms of human kidney mutarotase

J. Okuda, I. Miwa, Y. Toyoda and S. Baba[1]

Department of Clinical Biochemistry, Faculty of Pharmacy, Meijō University, Tempaku-ku, Nagoya 468 (Japan), and The Second Department of Internal Medicine, School of Medicine, Kōbe University, Kusunoki-cho, Ikuta-ku, Kōbe 650 (Japan), 9 June 1977

Summary. 4 forms of mutarotase from human kidney were demonstrated by DEAE-cellulose column chromatography. A major form of them was purified to homogeneity.

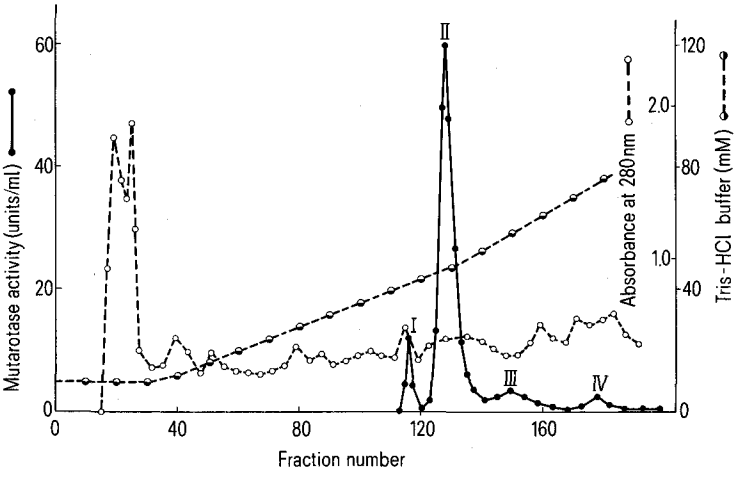
Mutarotase [aldose 1-epimerase, EC 5.1.3.3] catalyzes the anomeric interconversion of D-glucose and other sugars, and is known to be present in high activity in the kidney, liver, and small intestine of mammals[2-5]. The physiological function of mutarotase, however, is still obscure.

We reported previously that 4 different forms of mutarotase exist in rat kidney[6]. Mulhern et al.[7] have reported the purification of human kidney mutarotase, but did not describe the presence of multiple forms. Thus we attempted to examine whether the multiple forms of human kidney mutarotase occur or not. In the present paper, we describe that 4 forms of mutarotase exist in human kidney as well, and that a major form (type II) was purified to homogeneity.

Materials and methods. Mutarotase activity was determined by the method of Miwa and Okuda[8], and 1 unit of the enzyme was defined as the amount which converts 1.0 μmole of α-D-glucose to β-D-glucose per min under the assay condition used. Protein was determined by the method of Lowry et al.[9], using crystalline bovine serum albumin as a standard. The enzyme was purified by a modification of previously reported procedures[6]. The whole kidney (500 g) obtained from a total of 5 autopsies (3 women and 2 men ages 25 to 50) excluding renal diseases, at intervals ranging from 18 to 48 h postmortem, was homogenized with 500 ml of 0.1 M Tris-HCl buffer (pH 7.2) and 200 ml of chloroform. The homogenate was centrifuged and the supernatant fractionated by ammonium sulfate precipitation (between 40 and 60% saturation), followed by sequential column chromatography on Sephadex G-75 (4.5 × 70 cm) and DEAE-cellulose (Whatman, DE 52) (2.5 × 30 cm).

Results and discussion. Mutarotase in human kidney was resolved into 4 active fractions (designated type I, II, III, and IV in the order of elution) by Tris-HCl buffer (pH 7.2) gradient elution from DEAE-cellulose column (figure). This result indicates that 4 different forms of mutarotase exist in human kidney as well as in rat kidney, and the multiplicity of mutarotase appears to be widely observed in various species of mammals. Any of the Km values for α-D-glucose of crude preparations of type I, II, and IV of human kidney mutarotase was 12 mM and that of type III was 16 mM.

Crude mutarotase type II was further chromatographed on a hydroxylapatite (Bio-Rad, Bio-Gel HTP) column (2.5 × 20 cm) by elution with 1 l of a linear sodium phosphate buffer gradient (5 to 70 mM, pH 7.2). The enzyme (type II) was homogeneous when checked by polyacrylamide disc gel electrophoresis, and its specific activity was 285 units/mg of protein. The pH optimum of



DEAE-cellulose column chromatography of human kidney mutarotase. Column bed, 2.5×30 cm; flow rate, 25 ml/h; eluting buffer, 1.2 l of linear Tris-HCl buffer gradient (10 to 120 mM, pH 7.2). Fractions of 5 ml were collected.

activity was at 7.4 with 0.02 M EDTA buffer. These properties of mutarotase type II were almost the same as those of the enzyme (type II) from rat kidney. However, no cross-reaction could be observed between purified human kidney mutarotase type II and antiserum raised in rabbit against rat kidney mutarotase type II.

Bailey et al.[4] have reported that about 50% of serum samples taken from patients with known renal disease elevated mutarotase levels and that the enzyme was absent from urine samples of all individuals studied. On the other hand, we have recently found that the kidney mutarotase appears in both serum and urine of patients[10] and rats[11] with nephrotic syndrome. We are now studying the cellular localization of mutarotase in rat kidney with the antibody against mutarotase type II from rat kidney, and which form of mutarotase appears in serum and urine of patients with nephrotic syndrome in order to obtain a clue for elucidating the physiological function of mutarotase.

1 The Second Department of Internal Medicine, School of Medicine, Kōbe University, Kusunoki-cho, Ikuta-ku, Kōbe 650, Japan.
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Effect of sucrose on lipogenesis of rats chronically treated with ethanol[1]

S.N. Pennington, H.A. Barakat, G.L. Dohm and C.P. Smith

Department of Biochemistry, School of Medicine, East Carolina University, Greenville (N. C. 27834, USA),
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Summary. The effect of chronic ethanol administration with and without sucrose on the lipogenic enzymes of liver and adipose tissue of rats was studied. Ethanol markedly influenced the adipose lipogenic enzymes at 28 days. Sucrose caused a 2–10fold increase in lipogenic enzymes of both adipose and liver.

Research into the effects of ethanol is tied to the use of isocaloric control experiments. In the rat model, the control experiment often involves animals given an isocaloric carbohydrate solution. DeCarli and Leiber[2] have proposed a method using a totally liquid diet to administer ethanol with carbohydrate used as an isocaloric control. This approach has the advantages of being convenient and allowing direct measurement of caloric intake plus the ethanol-treated animals gain weight at a rate comparable to liquid diet controls. It has been our experience, however, that animals dosed in this manner do not gain weight as rapidly as those allowed free access to rat chow, especially over long experimental periods[3]. In an attempt to find a method of ethanol dosing that would allow normal weight gain, experiments were performed in which some effects of sucrose were observed that are pertinent to ethanol research.

Materials and methods. Male Holtzman rats weighing 300–320 g at the start of the experiment were used. In 1 group, 15 animals were given ethanol daily (4 g/kg) as a 50/50 (v/v) solution in normal saline by stomach tube. 15 control animals received an equal volume of normal saline.

In the 2nd dosing procedure, 15 animals were given a mixture of 25% sucrose and 25% ethanol in water (w/v/v) as their only source of fluid, and an equal number of animals received a 25% solution of sucrose only. All animals were allowed free access to chow and were weighed on alternate days and the dosage adjusted accordingly.

At the end of 28 and 56 days, 6 animals from each of the 4 groups were sacrificed by decapitation and the livers and epididymal fat pads rapidly excised and chilled in ice-cold 1.15% KCl. The livers were homogenated in a glass-teflon homogenizer using 0.05 M potassium phosphate buffer, pH 7.4 containing 1.0 mM EDTA and 1.0 mM DTT. Adipose tissue was homogenized in a glass homogenizer using the same buffer. The particle-free supernatant solution was prepared by centrifuging the homogenates at 105,000 × g for 60 min.