samples. Pectinesterase activity was detected by the print technique[8] and proteins were stained with bromophenol blue on a paper replica.

Iodine resublimated (Merck, Federal Republic of Germany) dissolved in sodium iodide in concentrations of 10^{-2} to 10^{-5} M I_2 was used for inhibition. Products of carrot pectinesterase were preincubated with iodine at room temperature and inhibitory effect was stopped by addition of an excess of 0.1 M $Na_2S_2O_3$.

Results and discussion. The crude carrot pectinesterase exhibited a specific activity of 1.1 moles sec^{-1} kg^{-1} and the electrophoretic pattern of pectinesterase was similar to that obtained from the first peak of the DEAE-Sephadex A 50 column - 3 enzyme bands, all moving to the cathode (figure 3, sample B). This peak had a specific activity of 2.8 moles sec^{-1} kg^{-1} and on starch gel electrophoresis showed 8 protein and 3 pectinesterase bands (figure 3, sample B).

The purified product from the Sephadex G 75 column had a specific activity of 5.2 moles/sec^{-1} kg^{-1} and was separated by electrophoresis into 4 protein and 3 pectinesterase bands (figure 3, sample C).

All 3 pectinesterase forms from carrot moved to the cathode with the same rate as those of the tomato pectinesterase (figure 3, sample A). On TLC on Sephadex G 150 superfine, both purified products from carrot showed only one spot of pectinesterase activity and moved with a mobility identical to that of the tomato pectinesterase standard; their mol. wt was found to be near 27,000.

The pH-optimum of the purified carrot pectinesterase was estimated as being 7.8–8.0, the K_m-value 1.5×10^{-6} M methyl D-galactopyranuranosyl residues and this enzyme was activated by sodium chloride (optimum around 200 mM NaCl).

The heat stability of carrot pectinesterase (total inactivation at 70 °C) was similar to that of the tomato pectinesterase[9]. Iodine showed an inhibitory effect on carrot pectinesterase, which increased in relation to the purity of the product, similarly as found with the tomato pectinesterase[10].

From the results obtained, the carrot pectinesterase can be characterized as an enzyme very close to the tomato pectinesterase with regard to molecular and enzyme properties.

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Studies on plasma fibrinogen level in pre-eclampsia and eclampsia

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Summary. The plasma fibrinogen level of maternal blood has been estimated in 30 cases of pre-eclampsia, 60 cases of eclampsia and 35 cases of normal pregnancy of 3rd trimester. The plasma fibrinogen value increased by about 70% and 145% in pre-eclampsia and eclampsia, respectively. In essential hypertension, the fibrinogen level remains more or less the same as in normal pregnancy.

Post-mortem examination of patients who died due to eclampsia, revealed the presence of thrombi in small blood vessels[1,2]. Previous investigators[3,4] detected a material in the kidney glomeruli of normal pregnant women which was immunologically identical with fibrinogen/fibrin, and suggested that the deposition of the same may lead to the development of the endothelial cytoplasmic changes, hypertension and proteinuria which are the generalized features of eclampsia or pre-eclampsia[4]. Examination of 19 out of 21 eclamptic patients indicated the presence of blood in the cerebrospinal fluid and fibrin thrombi in the periportal areas of the liver, adrenal cortex and spleen[5]. All these findings indicated a possible change in the plasma fibrinogen level in eclampsia and pre-eclampsia. With this idea, we measured the plasma fibrinogen level of eclamptic patients and normal pregnant women, and the results are reported in this communication.

Materials and methods. Pre-eclamptic patients were selected from a group of pregnant women of blood pressure reading of 120/90 mm Hg (approximately) with generalized oedema. Eclampsia was characterized by blood pressure reading of 180/100 mm Hg and above, generalized oedema, proteinuria and convulsions in the 3rd trimester. Plasma fibrinogen was measured by clotting the same with thrombin or calcium as described by Raymond and Wilkinson[6]. The incubation mixture consisted of 0.1 ml plasma, 2 NIH units of thrombin (Sigma) or 0.5 ml calcium chloride (0.25%) and 0.5 ml sodium chloride solution (0.154 N) in a final volume of 1.1 ml. The solutions were mixed properly and incubated at 37 °C for 30 min. The clot formed was removed carefully and washed with normal saline; 0.5 ml of sodium hydroxide (3%) was added to the washed clot and kept at room temperature till the clot was completely dissolved. To the above solution, biuret reagent (0.5 ml) was added and allowed to stand for 30 min at room temperature followed by 5 min at 37 °C. The absorbance was measured at 560 nm

Table 1. Plasma fibrinogen in normal pregnancy, pre-eclampsia and eclampsia

Group	Number of cases	Plasma protein g/100 ml**	Fibrinogen* mg/100 ml	Increase (%)
Normal	35	6.25	605 ± 15.6	-
Pre-eclampsia	30	-	1030 ± 30.3	70
Eclampsia	60	8.23	1473 ± 107	143
Essential hypertension	10	-	670 ± 80	-

* Mean value ± SE. ** Average value of 5 patients.

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

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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