role of the renin–angiotensin system during the process of adaptation to sea water. Our results indicate, however, that the renin–angiotensin system can also act as an aldosterone stimulating factor in the Japanese eel, and that the system may participate, at least in part, in the mechanism of euryhalinity.

To sum up, the decrease of the renin content of the kidney in Japanese eels kept in sea water for 3–11 weeks showed that the renin–angiotensin system plays a part in the process of adaptation from fresh to sea water.

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HIROFUMI SOKABE
SUSUMU MIZOGAMI
TOSHIO MURASE
FUMINORI SAKAI

Department of Pharmacology,
Faculty of Medicine,
University of Tokyo.

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Renal Enzyme Excretion following Anaphylactic Shock

THE urine of man and of several laboratory animals exhibits amino-acid-arylamidase (so-called leucine-aminopeptidase or LAP) and alkaline phosphatase (AP) activities[1–3]. Histochemical investigations revealed the presence of large amounts of these enzymes in the epithelial cells of the proximal tubule[2]. Under normal conditions LAP and AP enter the urine as a result of the turnover of epithelial cells.

Experimental damage to the renal tubules causes increased excretion of LAP and AP. Clinical observations of renal diseases[1–6] and also histochemical observations[7] confirmed biochemical observations. In rats, an increase in urinary LAP and AP has been reported to follow haemorrhagic shock, severe burns and sodium tetrathionate treatment[2]. We have confirmed these findings and found, in addition, an increase in urinary AP after renal damage caused by uranium or mercury salts[8]. X-ray treatment of rat kidney[9] and renal ischaemia[10] produce the same effect.

The participation of the kidney in allergic reactions is of interest, and for this reason we have investigated whether anaphylactic shock can cause sufficient renal damage to produce an increase in urinary LAP and AP. In our investigation male albino rats were given 10 g of water daily. Twenty-four hour urine specimens were collected in metabolic cages, and normal enzyme activities were determined in a hundred animals. Fifty animals were sensitized with human albumin; after two weeks, anaphylactic shock was induced by intraperitoneal injection of 0·5–2·5 ml. of a 10 per cent solution of human albumin. For determination of LAP 0·25 mg of L-leucyl-*p*-nitroaniline in 3·0 ml. of 0·05 molar phosphate buffer (*p*H 7·2) were mixed with 0·1 ml. of freshly centrifuged urine. The extinction at 405 nm was measured immediately, and the sample was incubated at 25° C for 30 min. After incubation a second reading was taken. According to the specific absorption of nitroaniline which is the product of LAP activity, the difference between both readings multiplied by 0·108 gives the amount of mU. LAP /ml. of urine.

For the determination of AP, 5×10^{-3} molar sodium-*p*-nitrophenyl phosphate in 0·05 molar glycine buffer (*p*H 10·5) with 0·005 molar magnesium chloride were incubated at 37° C for 30 min with 0·1 ml. of centrifuged urine which had been dialysed for 3 h against tap water. The enzyme reaction is stopped by adding 10 ml. of 0·01 molar sodium hydroxide. The amount of enzymatically formed nitrophenol is determined photometrically at 405 nm. A blank, with urine added after sodium hydroxide, served for reference. One litre of urine contains 1 mmol-unit of AP and liberates, in the conditions described here, 1 mmole of nitrophenol.

Table 1

Animals	LAP (mU.)		AP (mMU)	
	Per ml. of urine	24 h urine sample	Per ml. of urine	24 h urine sample
Normal rats (a hundred animals)	6·1 ± 3·1	86 ± 42	2·0 ± 0·4	33 ± 15
24 h after anaphylactic shock (fifty animals)	35·2 ± 18·3	540 ± 111	8·4 ± 3·1	101 ± 39
	$P < 0.0001$			$P < 0.0001$

Our results are shown in Table 1. Twenty-four hours after anaphylactic shock a statistically significant increase of urinary LAP and AP is seen. It was evident that the rate of increase depends on the amount of injected antigen. Less shock and less enzyme excretion were produced by 0·5 ml. of antigen than by 2·5 ml. For several days after anaphylactic shock a further injection of antigen produced neither shock nor an increase in LAP and AP excretion.

Large values for LAP and AP in serum might lead to increased renal excretion of these enzymes, and therefore additional investigations of serum values were carried out at various intervals after antigen administration. An increase in either LAP or AP could not be detected in serum. These findings rule out the possibility that increased enzyme activities in urine depend on extrarenal factors.

Our experiments suggest that the increase in urinary LAP and AP is caused by damage to the tubules. In anaphylaxis, several mediators[11] liberated from mast cells or formed from plasma proteins produce severe vascular shock. Decreased blood pressure and reflectory vasoconstriction cause hypoxia which damages the highly sensitive tubular cells. Enzymes of the degenerated epithelium can be detected in urine. This mechanism explains, at least partially, the increased proteolytic activity of urine following allergic reactions[12–14]. Concerning the observed increase in urinary LAP, another factor could be involved. Allergic reactions activate plasmin[11] which can activate renal peptidases[15].

WOLFGANG P. RAAB

Universitätsinstitut für Medizinische Chemie,
Währingerstrasse 10,
Vienna 9, Austria.

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