TABLE I

SUBSTRATES FOR ADENOSINE KINASE

Nucleoside	K_m *(M)*	*V (nmoles monophosphate per unit of enzyme per min)*
N^6-Methyl-3'-amino-3'-deoxyadenosine	$1.2 \cdot 10^{-4}$	0.07 (0.02)
Formycin (8-aza-9-deaza-adenosine)	$2.2 \cdot 10^{-4}$	0.34
3-Isoadenosine	$8.6 \cdot 10^{-3}$	2.40 (0.80)
3'-Deoxy-3-isoadenosine	$3.0 \cdot 10^{-3}$	0.27 (0.09)

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Purification of bovine renin

The present study is the first to describe the purification of renin (EC 3.4.4.15) from beef kidneys. The methods include many used in early efforts to purify pig renin[1–5], human renin[6–8] as well as those used in later efforts[9–15]. The purified enzyme was characterized by electrophoresis on polyacrylamide gel.

An aqueous (2 l/kg) extract of kidney tissue was purified by a $(NH_4)_2SO_4$ fractionation at 4° as follows: 0.8 M $(NH_4)_2SO_4$ (pH 2.6) for 20 min; pH 3.6 for 16 h; the supernatant to 2.5 M and the resulting precipitate diluted to 1.3 M (pH 6.5); the resulting supernatant to 2.3 M and the final precipitate diluted to 0.8 M before application to the Sephadex G-25 column. Because of the easy measurement, Na^+ from the tissue was used to follow the desalting process. The batch step with CM-Sephadex (C-50), equilibrated with 0.1 M (pH 5.0), entailed centrifugation of the gel after elution and washing with 1.1 M ammonium acetate (pH 6.8). Preparative multiphasic zone electrophoresis on polyacrylamide gels was performed essentially with the apparatus and method described by JOVIN *et al.*[16]. The buffer system was slightly modified as previously

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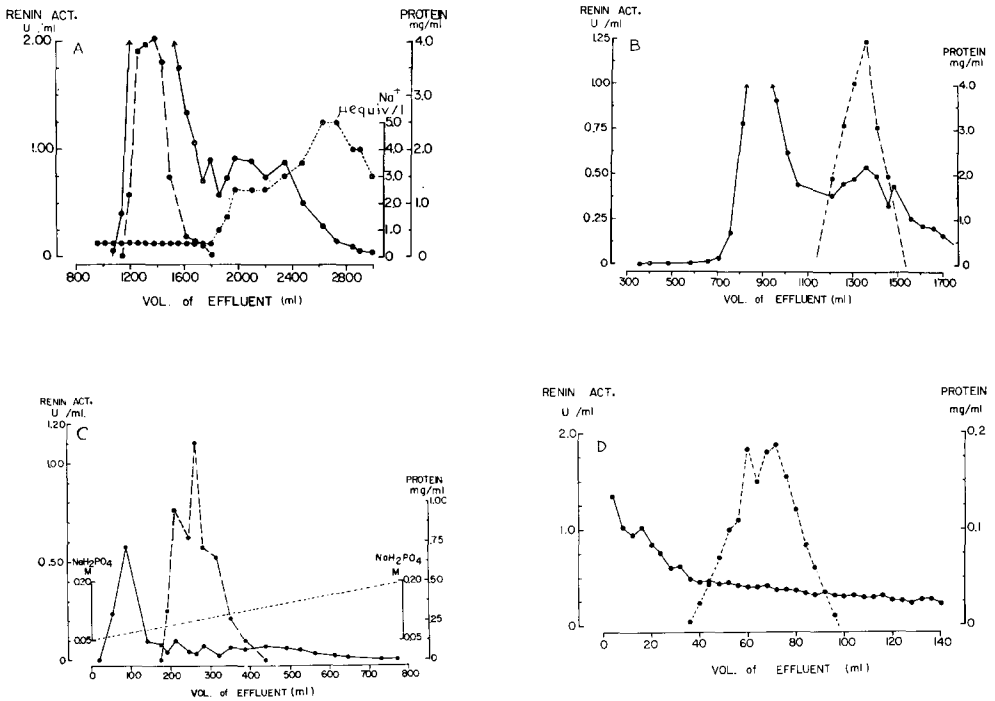

Fig. 1. Chromatographic and electrophoretic procedures. Renin activity (●— — —●) and protein concentration (●—●) are depicted in every graph. (A) Sephadex G-25 (coarse), 6 cm × 105 cm, in distilled H_2O; 10.2 g protein applied. Flow rate, 5.0 ml/min. ● · · · · ●, Na^+ concentration. (B) Sephadex G-100, 6 cm × 80 cm, 0.05 M ammonium acetate (pH 5.0). 2.18 g protein applied. Flow rate, 0.5 ml/min. (C) DEAE-cellulose (DE-32), 1 cm × 80 cm, gradient elution of 400 ml 0.05 M NaH_2PO_4 starting buffer and 400 ml 0.20 M NaH_2PO_4 limit buffer (pH 6.4) throughout. — — —, molar gradient. 80 mg protein applied. Flow rate, 0.32 ml/min. (D) Preparative scale disc electrophoresis, upper gel 2.5% acrylamide (20 ml), lower gel 7.5% acrylamide (50 ml); column 15 cm^2 × 4.5 cm. 10 mg protein applied.

Fig. 2. Analytical disc electrophoresis of material from preparative disc electrophoresis. Tube 2, a sample from combined fractions representing the renin activity from preparative disc electrophoresis. Tubes 1 and 3, samples from combined inactive fractions eluted before (1) and after (3). the active peak.

described[17]. Protein content of the fractions was estimated either by ultraviolet absorption at 280 mμ or by the method of LOWRY *et al.*[18]. Renin activity was assayed by pressor response in male Sprague–Dawley rats[19]. Pig renin (Nutritional Biochemicals) was used as a standard reference. For analytical purposes, electrophoresis on polyacrylamide gels was performed according to the discontinuous zone method of ORNSTEIN[20]. A Coomassie blue stain was employed[21]. Renal material, purified as far as the Sephadex G-100 chromatographic step, was injected weekly 4 times into Dutch-belted

TABLE I

RESULTS OF PURIFICATION PROCEDURES

Overall recovery, 4.5%. Overall purification, 1893-fold.

Procedure	*Total protein (g)*		*Total renin (activity units)*		*Recovery (%)*		*Specific activity (munits/mg protein)*	
	Batch 1	*Batch 2*	*Batch 1*	*Batch 2*	*Batch 1*	*Batch 2*	*Batch 1*	*Batch 2*
1. H_2O extract	168.0	136.5	1925	2100			11.5	15.4
2. $(NH_4)_2SO_4$, 0.8 M, pH 6.2 → 2.6 → 3.6	11.6	9.0	1348	900	70	42	162	100
3. $(NH_4)_2SO_4$, 2.5 → 1.3 M, pH 3.6 → 6.5	9.6	7.3	446	324	33	36	46.2	44.4
4. $(NH_4)_2SO_4$ fractionation	4.9	5.3	186	200	37	56	38	38
5. Sephadex G-25	2.18		204		53		93.6	
6. Sephadex G-100	0.465		172		84		320	
7. CM-Sephadex (batch)	0.80		84		49		1050	
8. DEAE-cellulose	0.0156		96		114		6154	
9. Preparative disc electrophoresis	0.00276		62		95*		22 460	

* 65 units applied.

rabbits. Precipitating antisera were developed, and immunoelectrophoresis[22] was performed on the material after various stages of purification.

Table I shows data concerning the purification steps. More detailed results of column chromatography and multiphasic zone electrophoresis are shown in Fig. 1. Better purification would result from modification of Steps 3 and 4. In Fig. 2 is shown the material from the preparative multiphasic zone electrophoresis after analytical disc electrophoresis. On immunoelectrophoresis, material after DEAE-cellulose chromatography had only one precipitin line, but measurement of renin activity in segments of the agar showed no clear change in activity across the precipitin line.

In the present study disc electrophoresis, as well as standard methods of salt fractionation and column chromatography, was used in the purification of bovine renin. Overall purification was 1900-fold. The maximum purification of 2500-fold was obtained at the peak activity from preparative disc electrophoresis. Employing chromatographic methods, PEART *et al.*[11] achieved a purification of 13 750-fold for pig renin and of LUBASH AND PEART[14] 13-fold for human renin. HAAS *et al.*[5] in 1953, using fractionation methods, achieved a remarkable 500 000-fold purification of pig renin. The present results of immunoelectrophoresis with precipitating antisera did not indicate antibody to beef renin. Preparative disc electrophoresis on polyacrylamide

gel produced a 4-fold increase in the specific activity and a 95% yield. It was determined by analytical disc electrophoresis that after the final purification procedure, renin activity was associated with at least four electrophoretic components. It is interesting to note that SKEGGS *et al.*[12] also found that activity of pig renin, under certain pH conditions, associated with four clearly distinct protein peaks.

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