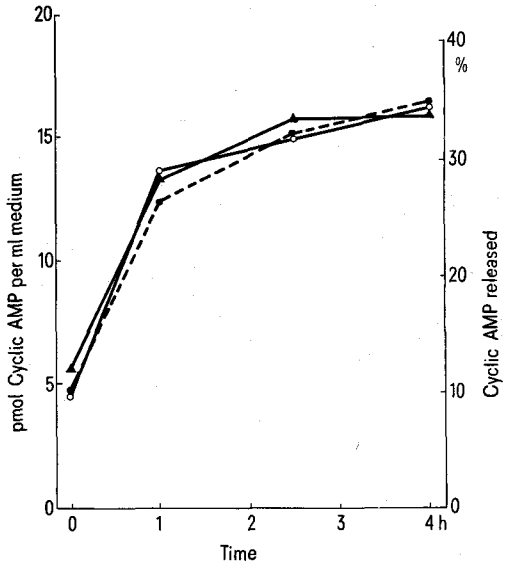

Release of [^{3}H]-cyclic AMP from re-sealed human erythrocyte ghosts incubated at 37 °C in 0.15 *M* choline chloride, unbuffered (▲); and buffered at pH 7.40 with 20 m*M* Hepes (●), or 20 m*M* $NaHCO_3$ in an atmosphere of 95% air, 5% CO_2 (○).

Leakage of material absorbing at 280 nm was very low, and could not be correlated with the efflux of cyclic AMP. This suggests that the integrity of the resealed ghosts was maintained throughout the incubation period, and that the release of cyclic AMP may be a selective process. This is supported by OLSEN and CAZORT[10] who showed that the extrusion of non-cyclic adenine nucleotides from resealed erythrocyte ghosts does not increase with time.

Our results complement those of FRANKLIN and FOSTER[11] who showed that Hepes did not increase the efflux of cyclic AMP from hormone-stimulated human embryonic fibroblasts. However, these authors relied on endogenous cyclic AMP and did not establish whether the efflux observed was caused by the high intracellular cyclic AMP concentration or as a consequence of the hormone-plasma membrane interaction. Resealed erythrocyte ghosts do not require hormones to effect an efflux of cyclic AMP, and may prove to be a model system suitable for studying such phenomena in the resting cell state.

The present study shows that following the direct incorporation of cyclic AMP into resealed erythrocyte ghosts at a concentration of 0.625 μ*M*, there is an efflux of the cyclic nucleotide which is not enhanced by the presence of Hepes. It seems unlikely therefore, that Hepes causes cell membranes to become permeable to cyclic AMP.

Zusammenfassung. Menschenerythrocyten-Schatten wurden in Gegenwart von [^{3}H]-Adenosin-3′5′-phosphat undurchlässig. Der Ausfluss der Nukleotide, in isotonischem Cholinchlorid, wurde von Hepes nicht beeinflusst.

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Esterase Activity in Renin and Kallikrein Extracts Obtained from Rat Kidneys

Esterase activity (EA) determination using substrates such as benzoyl arginine ethyl-ester (BAEE) is one of the most commonly used methods either for disclosing kallikrein-like activity or for quantitative evaluation of kallikrein in fluids and tissue extracts. Although the physiological significance of renal kallikrein is not known, it has been postulated that this enzyme might function as an antagonist of renin[1]. Therefore it seemed of importance to evaluate both enzymatic activities in kidneys under different conditions, particularly in renal hypertension. The occurrence of a kininogenase activity has been reported in renin preparations obtained from kidneys of different animal species, ascribed to a renal kallikrein present as a contaminant[2]. On the other hand, Ng[3] has assigned to renin an intrinsic capacity to generate kinins. Here are reported: a) experiments undertaken to find a method to purify both enzymes contained in rat renal tissue, and to clarify whether esterase and kininogenase activities are actually inherent to renal renin; and b) the main steps of a purification procedure which allowed a separation of kallikrein and renin in two different peaks from a single renal extract. Renal kallikrein was obtained as a protein free of renin-like activity, and renin was isolated as a purified extract with a pressor activity equivalent to 6.5 G.U. per mg, still with a conspicuous esterase activity upon BAEE but with no detectable kininogenase activity.

Methods. Two separate batches of kidneys (total 1.2 kg) from Sprague-Dawley rats were used. The procedure to dehydrate with acetone and to reduce the tissue of fine powder has been described[4]. The latter was submitted to 3 succesive extractions with a 2% NaCl solution containing 12.7 mg/100 ml of EDTA. After vigorous stirring in the cold room and centrifugation (12,000 *g*), the residue was discarded and the supernatants were pooled, dialyzed against water and liophylized. The residue was submitted to 4 succesive extractions under stirring with 50, 40, 30 and 20 ml of 0.04 *M* ammonium acetate. Supernatants which contain practically all the kallikrein and renin activities with 3.25 E.U. per mg of protein were further purified according to the steps shown in the Table. Further purification of renin using different methods was followed

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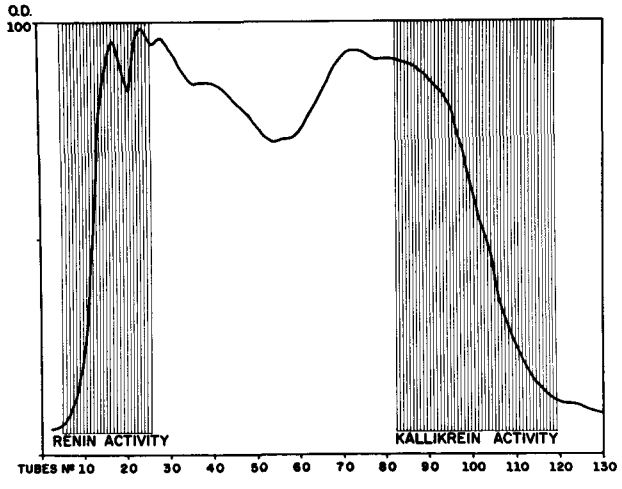
Purification procedure		Kallikrein		Renin	
Steps		Total Protein (mg)	E.U.[a] per mg	Total Protein (mg)	E.U.[a] per mg
1	Gel filtration (Sephadex G-100) equilibrated with 0.05 *M* ammonium acetate. In a single peak we found kallikrein and renin activities with a total E.A. of 15050 E.U.				
2	Chromatography in CM-32 column equilibrated with 0.01 *M* acetic acid. Kallikrein was eluted with 0.05 *M* ammonium acetate at pH 4.6; succesively renin was eluted with 0.05 *M* ammonium acetate at pH 6.8.	567	9.44	2100	0.5
3	Renin and kallikrein fractions were further chromatographed separately in DE-32 columns by ionic gradient[5].	14.17	184	244	254
4	Renin was rechromatographed in DE-32 column similar to Step 3.			170	2.1[b]

[a] E.U. corresponds to amount of kallikrein which hydrolyzes 0.05 *M* of BAEE, at 25 °C, and pH 8.1[8]. [b] This renin had 6.5 G.U. of pressor activity per mg.

by considerable loss of its specific activity, whereas kallikrein was finally obtained as a pure homogeneous protein having 1720 E.A. per mg. A detailed description of the final steps of renal kallikrein purified procedure will be published elsewhere.

Kallikrein was measured in the extracts through its direct oxytoxic effect upon uterus[2] and by its hypotensive effect on rat blood pressure; kininogenase activity was investigated using as substrate kininogen II obtained from rat plasma[6] and the formation of kinins was evaluated by bioassay, on cat jejunum immersed in Krebs solution[2]. This preparation responds to bradykinin with a contraction but it is insensitive to angiotensin[7]. E.A. in the different fractions, was determined according Porcelli procedure[8] using BAEE as substrate. Renin activity in the extracts was investigated, checking the effect upon the blood pressure in anesthetized rats. The most purified renin fraction was evaluated in Goldblatt pressor units, using as standard purified porcine renin generously supplied by MRC, and containing 5 G.U. per vial. Furthermore in the purest samples of renin and in kallikrein fraction obtained in step 4, Table, the ability to form angiotensin from angiotensinogen from plasma of nephrectomized rats was determined using the NASJLETTI and MASSON method[9].



DE-32 Chromatography of the dialyzed renin fraction collected in the step 2. Elution of the material was achieved by ionic gradient[5]. The hatched areas correspond to effluent fractions where renin and kininogenase activities were found.

Results and discussion. The Figure shows the chromatogram obtained in the effluent collected from DE-32 column step 3 (Table) showing the separation of kallikrein and renin activities.

No renin-like activity (angiotensin formation) was detected in kallikrein after the 3rd step of the purification procedure. Renin fraction at the same step showed a specific pressor activity of 6.5 G.U. per mg when compared to the Medical Research standard, and has also an E.A. equivalent to 2.54 E.U. per mg. This preparation exhibited no kininogenase activity, using cat jejunum to detect the formation of kinins in a dose up to 3.6 mg for 0.2 ml of kininogen II. Furthermore an E.A. was also found in renal fractions which have neither kallikrein nor renin activity. The experiments rule out the hypothesis that renin is able to release bradykinin-like peptides from kininogen II and provide the evidence that E.A. found in renin purified extracts is not due to kallikrein but probably to a contaminant. Measurement of E.A. is not an adequate method to evaluate kallikrein in renal tissue.

Riassunto. In questa nota viene descritto il procedimento usato per separare la renina dalla callicreina del rene di ratto. La frazione proteica della renina presenta un'attività pressoria di 6.5 G.U. per mg e contiene, fra le varie impurità, un'attività esterasica di 2.54 E.U. per mg, ma non attività chininogenasica.

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