Comme les fractions tissulaires[9] les fractions LAP 1 et LAP 2 sont inhibées par les alcools en fonction du nombre de carbone de la chaîne linéaire[3], alors que les alcools à chaînes ramifiées n'ont aucune action inhibitrice.

Les fractions LAP fongiques ne sont pas inhibées par certains peptides tels que: But H–Thr–Phe–OH à l'inverse des fractions tissulaires[10]. Ce phénomène avait déjà été mentionné par RONCARI et ZUBER[11] dans le cas de LAP isolée de *Bacillus stearothermophilus*. Certains caractères d'activité[1,3,4] permettent de rapprocher les fractions fongiques que nous avons isolées, de certaines fractions tissulaires. D'autres propriétés: non activité envers la leucine amide, non inhibition par certains peptides[10], activité moins spécifique envers des peptides synthétiques, activation par la température, sont caractéristiques des LAP extraites de micro-organismes.

Nos résultats concernant l'action de LAP 1 vis à vis de peptides synthétiques sont analogues à ceux obtenus par NAKADAI et al.[8] à partir de la fraction 1 (souche 460) dont la taille moléculaire est très différente de LAP 1. La fraction LAP 2, quoique moins active, est plus spécifique de la liaison leucyl que la fraction 1.

Summary. By use of di- or tripeptides as substrates, LAP 1 and LAP 2, 2 fractions from *Aspergillus oryzae* hydrolyze oligopeptides that possess leucine as N-terminal amino acid. LAP 1 fraction also hydrolyzes the histidyl bond. Both fractions have no activity towards peptides as glutathion, gly-pro-ala; they have low or no activity towards tyrosyl and tryptophanyl bond.

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Efflux of Cyclic AMP from Resealed Erythrocyte Ghosts is not Enhanced by Hepes

It is generally accepted that intracellular levels of cyclic AMP are controlled by the relative activities of the enzymes responsible for its synthesis and degradation: adenylate cyclase and phosphodiesterase respectively. The possibility exists however, that efflux and influx of the cyclic nucleotide may be additional factors affecting its concentration within the cell. The findings of cyclic nucleotides in several body fluids[1,2] and the hormone-stimulated release of cyclic AMP from the liver[3] and kidney[4] suggest that efflux at least may be of biological importance.

Working with WI–38 human embryonic fibroblasts, d'ARMIENTO et al.[5] observed a significant efflux of intracellular cyclic AMP when the culture medium contained organic zwitterionic buffers such as Hepes[6]. (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid). Bicarbonate-buffered media did not produce this response; hence it was postulated that cell membranes might become permeable to cyclic AMP in the presence of organic buffers.

We have tested this hypothesis directly by incorporating [^{3}H]-cyclic AMP into resealed human erythrocyte ghosts and following the efflux of the tritiated nucleotide into unbuffered, bicarbonate-buffered and Hepes-buffered isotonic choline chloride. There are many advantages in using erythrocytes for this type of study: they constitute a convenient system for incorporating exogenous compounds and following their subsequent efflux[7]; they contain only low levels of adenylate cyclase and phosphodiesterase activities, and so the transport of incorporated cyclic AMP can be unambiguously observed; and in general, erythrocyte membrane transport is similar to that of other mammalian cells.

Materials and methods. Tritiated cyclic AMP was incorporated into re-sealed ghosts by a modification of the method of WHITTAM[7]. The cells were collected and washed as described by DUFFY and SCHWARZ[8], and squirted into 7 volumes of double distilled water containing 0.625 μM cyclic AMP (sodium salt; Sigma) and [8–^{3}H]adenosine 3′,5′-cyclic phosphate (Ammonium salt; The Radiochemical Centre, Amersham) at a final specific activity of 250 μCi/μmole. After 15 min at room temperature sufficient 3 M KCl was added to restore physiological tonicity. After being washed 6 times with 0.15 M choline chloride, replicate 0.2 ml aliquots of packed ghosts were suspended in 1.8 ml each of the following 3 media: unbuffered choline chloride (0.15 M), and choline chloride (0.15 M) buffered at pH 7.40 with 20 mM Hepes or with 20 mM $NaHCO_3$. The efflux of cyclic AMP (measured as dpm of the radioactive nucleotide and converted into pmoles) and protein leakage into the various media (determined by optical density measurements at 280 nm) were monitored during a 4 h incubation at 37 °C with gentle agitation. Analysis of the efflux media by paper chromatography according to SHEPPARD and BURGHARDT[9] established that 97% of the tritium label had an Rf value identical to that of authentic cyclic AMP (sodium salt; Sigma).

Results and discussion. There was an efflux of cyclic AMP from the resealed erythrocyte ghosts into each of the 3 media studied, and in each case the efflux followed a similar pattern and was of a similar magnitude. The Figure illustrates the results from a typical experiment in which each 0.2 ml aliquot of resealed ghosts contained 83 pmoles of cyclic AMP before the incubation period. After 4 h incubation 35% of the cyclic nucleotide (i.e. 29 pmoles) had been released into the medium.

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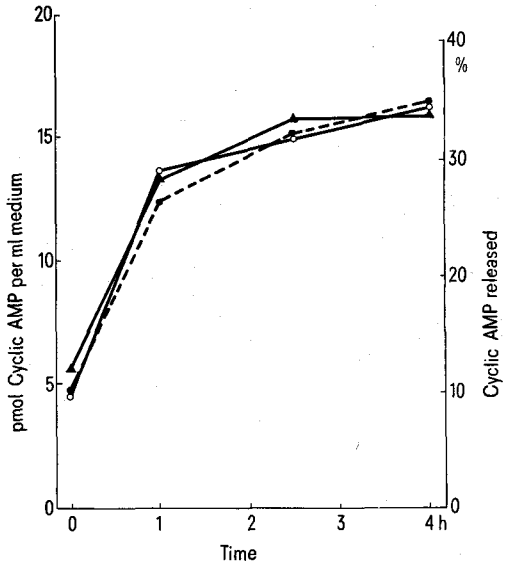

Release of [^{3}H]-cyclic AMP from re-sealed human erythrocyte ghosts incubated at 37 °C in 0.15 *M* choline chloride, unbuffered (▲); and buffered at pH 7.40 with 20 m*M* Hepes (●), or 20 m*M* $NaHCO_3$ in an atmosphere of 95% air, 5% CO_2 (○).

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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