various amino acid 2-naphthylamines. Erythrocyte and wound tissue preparations were processed separately and the results, as given in the Table, were compared reciprocally. The differences are most obvious concerning the ability to hydrolyze 2-naphthylamines of L-valine and L-isoleucine. Toward these substrates the wound tissue preparations show enzyme activity, but the erythrocytes are devoid of it. There are great differences in substrate specificity by the other substrates as well, especially when using 2-naphthylamines of L-methionine and L-leucine.

Fractionation by gel permeation chromatography and the study of the substrate specificity have thus shown that wound tissue AAP's differ qualitatively from the erythrocyte enzymes. Accordingly, erythrocytes cannot be an essential source of wound AAP's. The initial increase in the wound enzymes is derived neither from serum[3] nor from the immigrating leucocytes[4] in any significant measure. Our present data on erythrocytes further support the view that the augmented enzymes in wounds originate in the injured tissue itself[9].

Zusammenfassung. Mit Hilfe von Fraktionierung und bei der Untersuchung ihrer Substratspezifizität zeigte sich, dass die Arylaminopeptidasen im Wundgewebe sich qualitativ von denen in den Erythrozyten unterscheiden. Die Befunde sprechen gegen eine erythrozytäre Herkunft der Wundarylaminopeptidasen.

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Chromaffin Granules: Effects of Ions and ATP on Catecholamine Content, ATPase Activity, and Membrane Phosphorylation[1]

It has been reported that adenosine triphosphate (ATP) in the presence of Mg^{++} stimulates either the uptake[2] or release[3] of catecholamines from chromaffin granules. This difference in the effects of ATP on chromaffin granules has been interpreted as due to the presence or absence of chloride ions in the incubation media[4]. It was therefore decided to study the effect of ATP on catecholamine release, ATPase activity and membrane phosphorylation[5] of chromaffin granules incubated in media of different ionic composition.

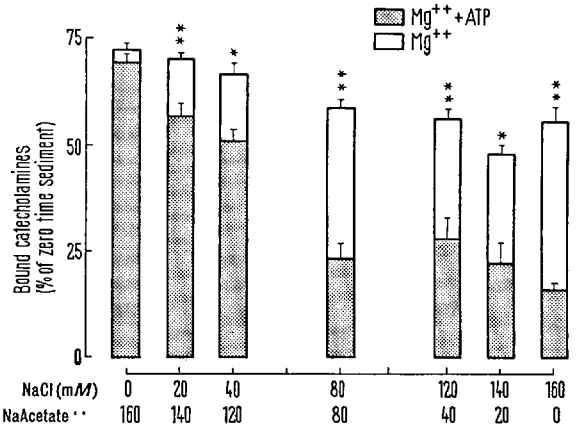

Fig. 1. Effect of chloride and acetate ions on ATP-evoked catecholamine release. Aliquots of chromaffin granules were incubated at 30 °C in media of varying Cl^- and CH_3COO^- concentrations containing 0.5 m*M* Mg^{++} in the presence or absence of 0.5 m*M* ATP. An aliquot of the granule suspension was centrifuged at the beginning (zero time) of the experiment and the catecholamine content in the resulting pellet was taken as a control value. The incubations were terminated as indicated in methods. Each bar represents the mean ± S.E. of 4 separate experiments, and indicates the amount of amines (as % of control) remaining in the granules at the end of the 30 min incubation period. * $P < 0.005$. ** $P < 0.001$.

Methods. Bovine adrenal glands were obtained from a local abattoir and chromaffin granules were obtained by means of the isotonic density gradient technique described previously[6]. The granules were incubated at 30 °C in a standard incubation medium containing (m*M*): NaCl or KCl, 160; *N-Tris* (Hydroxymethyl)-methyl-2 aminoethane sulfonic acid (TES) buffer (pH 7.0), 10; and $MgCl_2$, 0.5 or 1.0. In some experiments NaCl was partially or totally replaced by sodium bromide, fluoride, formate, acetate or propionate and KCl by postassium phosphate. Catecholamines, ATPase activity and membrane phosphorylation were determined as previously described[5].

Results. Figure 1 shows the effect upon catecholamine content, of replacing all or part of the NaCl in the incubation medium by sodium acetate. Chromaffin granules were incubated with or without ATP for 30 min and the amount of catecholamines remaining in the granules at that time was compared to control (unincubated) values. In the absence of ATP, it is clear that granules incubated in high Cl^- concentrations released more amines than granules in low Cl^- medium. When ATP (0.5 m*M*) in the presence of Mg^{++} (0.5 m*M*) was added, there was no significant ATP-induced catecholamine release during incubation in a medium containing 160 m*M* sodium acetate (0.0 m*M* NaCl) (Figure 1), but when the concentration of NaCl in the medium was 20 m*M* or greater, ATP produced a significant decrease in the catecholamine content of chromaffin granules (Figure 1). The ATP-evoked catecholamine release effect increased with graded substitution of sodium acetate by sodium chloride in the incubation medium (Figure 1).

[1] Supported by the Medical Research Council of Canada.

[2] N. KIRSHNER, J. biol. Chem. *237*, 2311 (1962). – A. CARLSSON, N-Å. HILLARP and B. WALDECK, Acta physiol. scand. *59*, suppl. 215 (1963).

[3] A. M. POISNER and J. M. TRIFARÓ, Molec. Pharmac. *3*, 561 (1967); *3*, 572 (1967): *4*, 196 (1968): *5*, 294 (1969).

[4] F. LISHAJKO, Acta physiol. scand. *75*, 255 (1969).

[5] J. M. TRIFARÓ and J. DWORKIND, Molec. Pharmac. *7*, 52 (1971).

[6] J. M. TRIFARÓ and J. DWORKIND, Analyt. Biochem. *34*, 403 (1970).

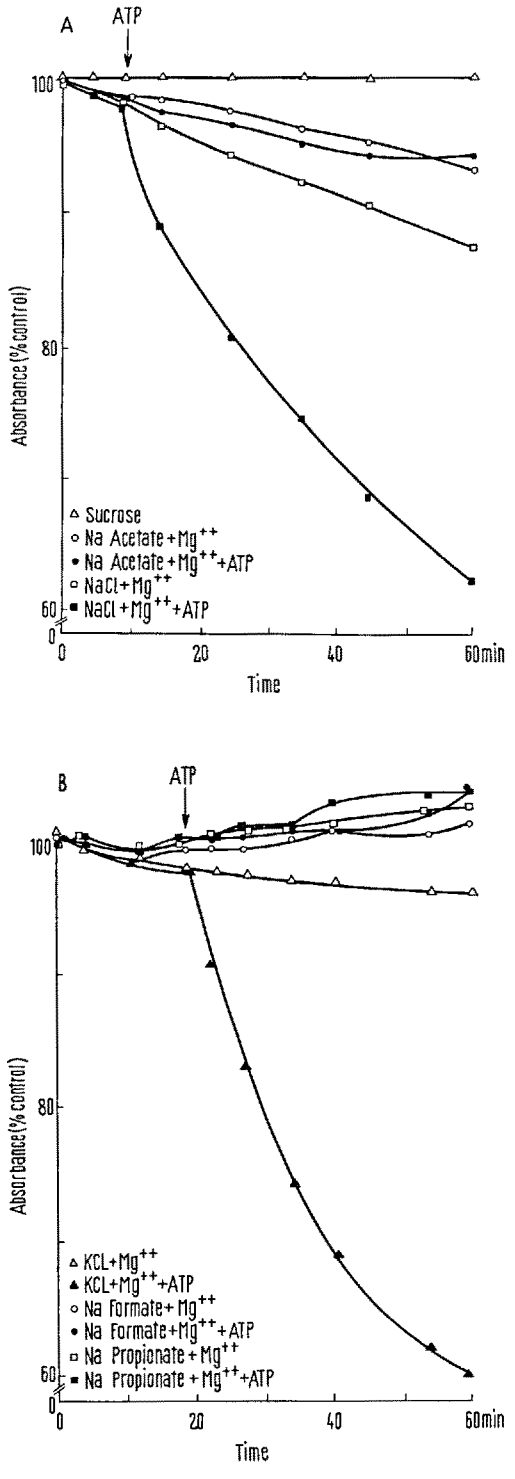

Fig. 2. Effect of the ionic composition of the incubation medium on the ATP-evoked changes in the light-scattering of chromaffin granule preparations. Aliquots of chromaffin granules were suspended at zero time in media of different ionic composition each containing: 10 m*M* TES buffer (pH 7.0), 1.0 m*M* Mg^{++}, and 160 m*M* of one of the different ions indicated on the graphs. An aliquot of chromaffin granules was also suspended in 0.3 *M* sucrose containing 10 m*M* TES buffer. The preparations were incubated at room temperature (22 °C) for 60 min. At the time indicated by the arrows, ATP at the final concentration of 1.0 m*M* was added to the preparations indicated on the graphs by closed symbols. The optical density of the preparations measured (540 nm) at different intervals of time was expressed as a percent absorbance of that found at the beginning of the incubation period. Similar results were obtained in 3 other sets of experiments.

We have previously demonstrated that ATP evokes light-scattering changes of the granule suspension during catecholamine release[3]. These changes were not observed when chromaffin granules were incubated in either 0.3 *M* sucrose or 160 m*M* sodium acetate medium, sodium formate, sodium propionate, sodium phosphate or potassium phosphate (Figure 2). However, chromaffin granules incubated in 20 m*M* or greater concentrations of NaCl or KCl responded to ATP with a fall in the optical density of the suspensions (Figures 2A and B), which paralleled catecholamine release; Br^- could substitute for Cl^- in these effects, but F^- could not. The latter effect was probably due to the inhibition of granule nucleotidases[7].

ATPase activity and phosphorylation of membrane components of chromaffin granules was also studied under conditions similar to those indicated in Figure 1. The results (Figure 3) showed that ATP hydrolysis and transphosphorylation of inorganic phosphorus (Pi) from ATP to chromaffin granule membranes were the same when tested in media containing 3 different concentrations of sodium acetate and sodium chloride. In addition, in the absence of Mg^{++} (presence of EDTA), or in the presence of N-ethyl maleimide (NEM), the 2 ATP-dependent processes were inhibited, and these effects of EDTA and NEM were observed in each of the three incubation media (Figures 3A and B). N-ethyl-maleimide was a more effective inhibitor of the ATPase activity of chromaffin granules incubated in the medium with the greatest concentration of sodium acetate (Figure 3A).

Discussion. The present experiments show that the presence of at least 20 m*M* Cl^- in the incubation medium is necessary for ATP to evoke catecholamine release. When the medium's NaCl was replaced by sodium acetate, propionate, formate or potassium phosphate, there was no change in the light-scattering or in the catecholamine content of chromaffin granules in response to ATP, even though ATP was hydrolyzed and the chromaffin granule membrane was phosphorylated. This dissociation of ATPase activity, membrane phosphorylation and catecholamine release does not necessarily mean that the 3 processes are not related[3]. Chromaffin granule integrity is not essential for ATPase activity or chromaffin granule membrane phosphorylation[5], for isolated chromaffin granule membranes hydrolyze ATP and become phosphorylated at the same rate as intact chromaffin granules[5]. It is possible that a series of events may follow the hydrolysis of ATP and membrane phosphorylation that lead to the extrusion of amines[8]. Therefore the ATP-induced release phenomenon could be inhibited at early, e.g. inhibition of ATPase by AMP, NEM, etc., or at subsequent steps, e.g. conformational changes in membrame and other chromaffin granule structures. When chromaffin granules are incubated in acetate, formate, propionate or phosphate media, the release reaction may be blocked by these ions at steps subsequent to the hydrolysis of ATP and membrane phosphorylation. This suggestion is supported by the demonstration that other subcellular organelles, the mitochondrion for example, behaves quite differently in NaCl or in sodium acetate, propionate, formate or phosphate solutions. In these Cl^- free solutions, mitochondria undergo

[7] N-Å. HILLARP, Acta physiol. scand. *42*, 144 (1958).
[8] J.M. TRIFARÓ, Endocrin. Exp. *4*, 225 (1970).
[9] G. A. BLONDIN, W. J. VAIL and D. E. GREEN, Arch. Biochem. Biophys. *129*, 158 (1969). – J. A. CHAPPELL and K. N. HAARHOFF, in *Biochemistry of Mitochondria* (Eds. E. C. SLATER, Z. KANIUGA and L. WOJTCZAK; Academic Press, New York 1966), p. 75.

different volumetric and light-scattering changes that lead to the suggestion that Cl^- can penetrate through the membranes whereas acetate, propionate, formate and phosphate, not only penetrate through the membranes but also penetrate 'into' the membranes, triggering conformational changes in this structure[9]. Therefore it is possible that acetate and other releated ions act in a similar manner upon chromaffin granule membranes and modify the final response to ATP.

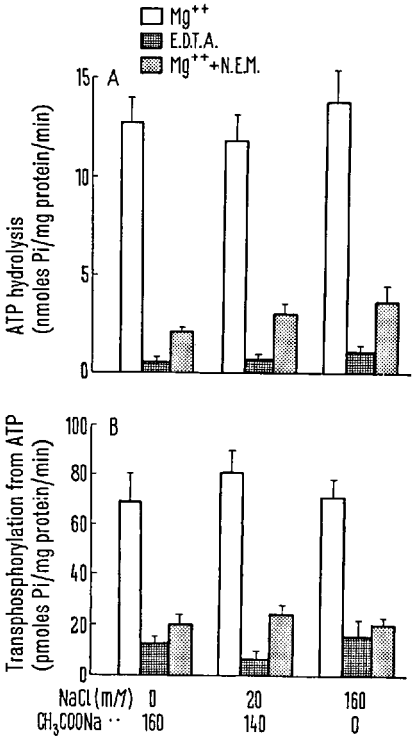

Fig. 3. Effect of chloride and acetate ions on Mg^{++}-dependent ATPase activity and transphosphorylation of Pi from ATP to chromaffin granule membranes. Chromaffin granules were incubated for 20 min at 30 °C in media containing 3 different concentrations of Cl^- and CH_3COO^-. The incubation media contained 0.5 m*M* Mg^{++} and 0.5 m*M* [γ-^{32}P] ATP (specific activity, 0.54 μCi/μmole). When EDTA was present, Mg^{++} was omitted from the incubation medium. EDTA (2.0 m*M*) or N-ethyl-maleimide (0.2 m*M*) was added to the medium 5 min prior to the addition of [γ-^{32}P]ATP. Addition of 10% trichloroacetic acid terminated the reactions. ATPase activity (A) and transphosphorylation of Pi from ATP (B) were determined as indicated in methods. Each bar represents the mean $\pm$ S.E. of 4 separate experiments.

Under our experimental conditions, 20 m*M* of Cl^- was necessary for the ATP-evoked release reaction. The intracellular concentrations of Cl^- has not yet been determined in chromaffin cells, but if we assume a passive distribution of Cl^- according with the transmembrane potential[10], and using the known experimental values for the resting potential (–24 to –30 mV) of chromaffin cells[11], the calculated (theoretical) intracellular concentration of Cl^- should be between 38 and 47 m*M*. This concentration is greater than that necessary to support ATP-evoked catecholamine release in vitro. However, preliminary experiments with bovine adrenal glands perfused with Locke's solution containing either sodium chloride, sodium acetate or sodium sulfate showed no differences when catecholamine release was evoked by 56 m*M* of KCl or CH_3COOK in the presence of 2.2 m*M* Ca^{++}, but at the end of the 2 h perfusion period the tissue level of Cl^- in the medullae was 19 m*M* (14–24, $n = 5$) as determined by the method of MATUK et al.[12]. Further studies using radioactive traces will be necessary before definitive conclusions about the role of Cl^- in the secretory process can be made.

Résumé. L'ATP induit la libération de catécholamines des granules chromaffines médullaires. Nous discutons le rôle du Cl^- dans ce processus vis-à-vis l'activité de l'ATPase et la phosphorylation des membranes. Nos résultats indiquent que la réaction aboutissant à la liberation de catécholamines est un phenomène comportant plusieurs étapes échelonnées après l'hydrolyse de l'ATP.

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[10] J. A. WILLIAMS, J. theor. Biol. *28*, 287 (1970).
[11] W. W. DOUGLAS, T. KANNO and S. SAMPSON, J. Physiol., Lond. *191*, 107 (1967). – E. K. MATTHEWS, J. Physiol., Lond. *189*, 139 (1967).
[12] Y. MATUK, J. F. MANERY and E. E. DRYDEN, Can. J. Physiol. Pharmac. *47*, 853 (1969).

The Activation of Rat Stomach Histidine Decarboxylase is Independent of the Histamine Level

Vagal excitation (insulin hypoglycemia), feeding or injection of gastrin are known to mobilize gastric mucosal histamine and to activate gastric histidine decarboxylase in the fasted rat[1,2]. Recently, it was suggested that the histamine-mobilizing and enzyme-activating effects of vagal excitation and feeding are mediated by endogenous gastrin[3–5]. KAHLSON et al.[1,2] have proposed that mobilized histamine stimulates the parietal cell to secrete and that histamine is the final common mediator of the acid-stimulating effect of both gastrin and vagal excitation. From the observations that the gastrin-induced reduction of gastric histamine preceded the activation of the histamine-forming enzyme, and that exogenous histamine reduced the enzyme activity, it was concluded that the histidine decarboxylase activity is dependent upon the mucosal histamine content via feed-back mechanism: a low level of gastric histamine induces enzyme synthesis to replenish the mucosal histamine stores, a high level of gastric histamine has a repressive effect on enzyme synthesis[1,2]. However, certain experimental observations are at variance with the above concept. At a certain dose level, insulin acti-

[1] G. KAHLSON, E. ROSENGREN, D. SVAHN and R. THUNBERG, J. Physiol., Lond. *174*, 400 (1964).
[2] G. KAHLSON, E. ROSENGREN and R. THUNBERG, J. Physiol., Lond. *190*, 455 (1967).
[3] L. R. JOHNSON, R. S. JONES, D. AURES and R. HÅKANSON, Am. J. Physiol. *216*, 105 (1969).
[4] R. HÅKANSON and G. LIEDBERG, Europ. J. Pharmac. *12*, 94 (1970).
[5] D. AURES, L. R. JOHNSON and L. W. WAY, Am. J. Physiol. *219*, 214 (1970).