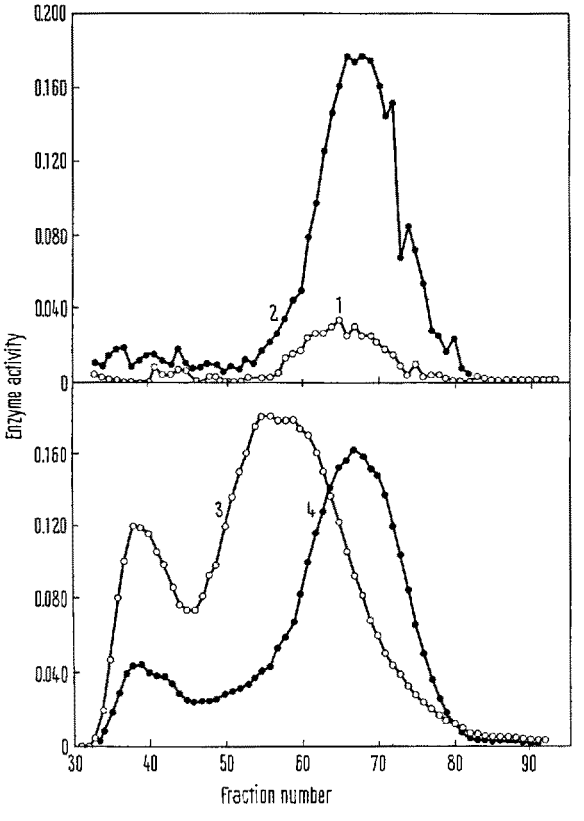
Are Erythrocytes a Source of Wound Arylaminopeptidases?

The activity and amount of arylaminopeptidases (AAP's) have been demonstrated histochemically and biochemically to increase in wound tissue, even during the very first post-operative hours[1]. Peptidase activity in wounds has been related to the removal of fragmented fibers and to the maintenance of a sufficient amino acid pool for essential protein syntheses[2]. The origin of these enzymes is thus an important question. We have previously shown that the increased amounts of wound AAP's are not derived exclusively from blood plasma[3] or from the invading leucocytes[4]. Erythrocytes have recently been shown to contain aminopeptidase B[5]. In order to elucidate the possible role of erythrocytes as a source of wound AAP's we made an experimental study on rats.

Material and method. If the increase in AAP's is due to erythrocytes, their enzyme pattern ought to be qualitatively similar to that in the wound tissue of the same individual. Erythrocyte and tissue samples from each rat were processed separately and the results were compared reciprocally. When studying the enzymic hydrolysis, the 2-naphthylamines of L-methionine and L-valine were used as substrates since they are hydrolyzed by most of the AAP's in the wounded skin tissue of the rat[6]. The preparation of the wound tissue (1-day-old wound) sample and the determination of the enzyme activity have been described earlier[7]. Rat erythrocytes were collected from the animals immediately after decapitation. The decapitation was performed 5 min after i.v. injection (to tail) of 10 U heparin per 1 g body weight. About 10 ml of whole blood was obtained in this way from rats weighing 250-300 g. Sedimentation of the red cells was accomplished by the addition of plasma of normal humans with blood groups B[8]. The optimum proportions were 2 parts of human plasma to 1 part of heparinized rat blood. The resulting red blood cells were handled as follows. About 30 min after the addition of the human plasma the mixture was centrifuged for 10 min at 1200 g. The red cells were washed 3 times with cold 0.9% sodium chloride solution followed by centrifugation at 1200 *g* for 10 min. The cells were then suspended in cold distilled water (10 ml per approx. 1 g of red cell suspension) to accomplish the disruption of the cells. The resultant mixture was centrifuged 5 min later at 42,000 *g* for 10 min at +2°C. The supernatant fluid was analyzed for its aminopeptidase activity.

Results and discussion. The Figure shows an example of the fractionation of erythrocyte and wound tissue AAP's. There are obvious qualitative differences between the wound tissue and erythrocyte AAP's. The differences are more accentuated when L-methionyl-2-naphthylamine is used as the substrate, but the dissimilarities become clearly and constantly visible by employing both of the substrates.

In order to study the substrate specificity, the enzyme preparations were tested for their ability to hydrolyze



Fractionation of arylaminopeptidases of rat wound tissue (1-day-old wound) and erythrocytes by gel permeation chromatography on Sephadex G-200. Substrates: L-methionyl-2-naphthylamine and N-L-arginyl-2-naphthylamine. Column: 15×800 mm. Elution: 0.1 *M tris*-HCl buffer, pH 7.15. Fraction volume: 1.5 ml. Hydrostatic pressure: 20 mm H_2O. Temperature: +4°C. Sample volume: 2 ml.
Curve 1: Erythrocytes, L-methionyl-2-naphthylamine.
Curve 2: Erythrocytes, N-L-arginyl-2-naphthylamine.
Curve 3: Wound tissue, L-methionyl-2-naphthylamine.
Curve 4: Wound tissue, N-L-arginyl-2-naphthylamine.

The ability of the erythrocyte and wound tissue preparations to hydrolyze some amino acid 2-naphthylamines

2-naphthylamine of	Erythrocyte preparation	Wound tissue preparation
L-arginine	100[a]	100
L-lysine	46	73
L-alanine	30	47
L-leucine	8	37
L-methionine	12	81
L-valine	0	24
L-isoleucine	0	6

[a] The numbers indicate relative hydrolytic abilities, as compared to the hydrolysis of N-L-arginyl-2-naphthylamine (= 100).

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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.

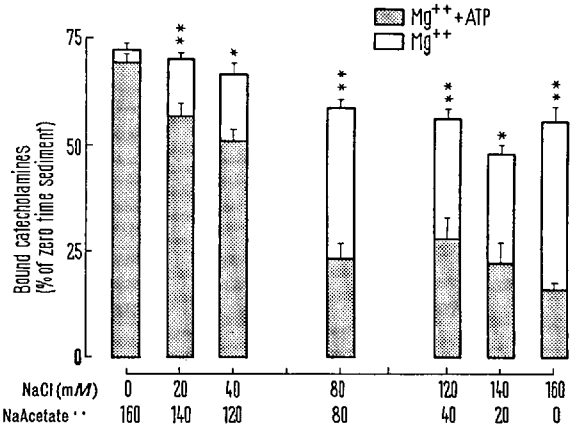

Fig. 1. Effect of chloride and acetate ions on ATP-evoked catecholamine release. Aliquots of chromaffin granules were incubated at 30 °C in media of varying Cl^- and CH_3COO^- concentrations containing 0.5 m*M* Mg^{++} in the presence or absence of 0.5 m*M* ATP. An aliquot of the granule suspension was 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).

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