Pour les constituants de haut poids moléculaire isolés sur colonne de Sepharose, les mêmes remarques concernant leur composition chimique, nous autorisent à distinguer des éléments macromoléculaires de type collagène (fraction Ic) ainsi que des fractions glycoprotéiques possédant une copule glycannique constituée de chaînes disaccharidiques et, ou hétérosaccharidiques (Ib, Ia et Id).

Cette notion d'hétérogénéité, le pourcentage variable de ces différentes formes moléculaires plus ou moins sensibles à l'action de la collagénase soulèvent l'aspect du rôle des éléments de structure hétéropolysaccharidique dans la microphysiologie de la filtration ainsi que dans la pathologie glomérulaire[12].

Zusammenfassung. Beitrag zur chemischen Zusammensetzung der Glykoproteine aus der glomerulären Basalmembran der Menschenniere.

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Ca^{2+}-ATPase Activity in Isolated Secretory Granule Membranes

It is generally believed that the function of secretory (zymogen) granules is to store digestive enzymes within the gland cell and subsequently release these enzymes for secretion. DOUGLAS and POISNER[1] have demonstrated that calcium is a vital requirement for induction of enzyme secretion, and ISHIDA et al.[2] have shown that release of amylase from secretory granules requires calcium. Although rat parotid glands contain large concentrations of calcium segregated within the zymogen granule[3], it is uncertain how calcium ions accumulate in the granules and how calcium is involved in amylase secretion. In this study we have demonstrated the existence of a calcium-activated ATPase (Ca^{2+}-ATPase) in isolated zymogen granule membranes, and we speculate as to the function of this enzyme activity.

Materials and methods. Isolated zymogen granule membranes were prepared from rat parotid glands according to the methods of AMSTERDAM et al.[4], and also by the method of KIRSHNER et al.[5]. ATPase activities, i.e., Ca^{2+}-ATPase, Mg^{2+}-ATPase and ($Na^+ + K^+$)-ATPase were determined as follows: In each flask zymogen granule membranes (approximately 0.04 mg) were incubated with 4 m*M* ethyleneglycol-*bis*-(-aminoethyl ether), N, N'-tetraacetic acid (EGTA), and 30 m*M* *tris*-HCl at pH 7.1. In addition, each incubation flask that was used for Ca^{2+}-ATPase determinations contained 95 m*M* NaCl plus 0 to 1 m*M* free CaCl; each incubation that was used for ($Na^+ + K^+$)-ATPase determinations contained 3 m*M* $MgCl_2$, 80 m*M* NaCl and 15 m*M* KCl; and each incubation flask that was used for Mg^{2+}-ATPase contained 0.1 m*M* ouabain, 3 m*M* $MgCl_2$, 80 m*M* NaCl and 15 m*M* KCl. The final volume was 2 ml and all incubations were performed at 37 °C. ($Na^+ + K^+$)-ATPase was calculated as the difference between activities in the presence and absence of ouabain. Ca^{2+}-ATPase was calculated as the difference produced by the addition of calcium when potassium was replaced by sodium (WATSON, et al.[6]) *Tris*-ATP (3 m*M*) was added to start the reaction and the mixture was incubated for 60 min. The reaction was terminated by the addition of 1 ml of 15% trichloroacetic acid. The inorganic phosphate produced during incubation was determined using the method of FISKE and SUBBAROW[7]. The membrane preparations were assayed for protein content according to the method of LOWRY et al.[8], and succinate dehydrogenase (SDH) activity was measured using the method of GREEN et al.[9]. The total SDH activity of isolated zymogen granule membranes was less than 1% of the $250 \times g$ supernatant fraction.

Results and discussion. Isolated zymogen granule membranes contain Ca^{2+}-ATPase, Mg^{2+}-ATPase and ($Na^+ + K^+$)-ATPase activities. The mean values and standard errors for these activities are given in the Table. Activities are expressed as μmoles of inorganic phosphate liberated per mg protein in 60 min. As shown in the Figure, minimal activation of the Ca^{2+}-ATPase was noted at a calcium concentration of 3×10^{-6} *M*. As the calcium concentration was increased to 1 m*M* the Ca^{2+}-ATPase activity increased. In 2 of the experiments the calcium concentration was increased to 3×10^{-2} *M*. This resulted in a further increase in Ca^{2+}-ATPase activity of about 30%. Higher, more unphysiologic concentrations were not tested. All ATPase activities were similar when

ATPase activities of isolated zymogen granule membranes

ATPase	μ*M* P_i/mg protein/h
Ca^{2+}-ATPase	9.6 ± 3.2
Mg^{2+}-ATPase	14.1 ± 3.0
($Na^+ + K^+$)-ATPase	5.0 ± 2.7

[Ca] = 1 m*M*, *N* = 5.

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membranes were prepared either according to AMSTERDAM et al.[4] or KIRSCHNER et al.[5]. It is unlikely that these activities are the result of the non-mitochondrial parotid gland Ca^{2+} pump described by SELINGER[10] because our preparation is derived from a low speed ($1000 \times g$) centrifugation for 10 min, whereas SELINGER's preparation was obtained by a $100{,}000 \times g$ centrifugation of a postmitochondrial ($10{,}000 \times g$) supernatant.

SCHRAMM and DANON[11] previously described a Mg^{2+}-ATPase activity associated with zymogen granules and more recently ISHIDA et al.[2] reported a similar finding. However, ISHIDA et al.[2] were unable to demonstrate ($Na^+ + K^+$)-ATPase activity. Previous workers utilizing other systems have suggested that Mg^{2+}-ATPase participates in the structural change of synaptic vesicles[12] and chromaffin granules[13], but ISHIDA et al.[14] have demonstrated that there is no correlation between Mg^{2+}-ATPase of zymogen granules and a structural change causing release of amylase.

($Na^+ + K^+$)-ATPase has been correlated with active Na^+ and K^+ transport in various tissues[15] and may be involved with the active transport of sodium and potassium across zymogen granules.

ISHIDA et al.[2] have shown a requirement for calcium in the release of amylase from granules, and WALLACH and SCHRAMM[3] have demonstrated that calcium is secreted in conjunction with exportable protein. Although WALLACH and SCHRAMM[3] hypothesize that exportable protein may be joined to calcium on its way from ribosomes to granules during synthesis of secretory granules, there is no evidence concerning the possibility that calcium may be directly taken up into the secretory granules. WALLACH and SCHRAMM[3] did not find any significant ^{45}Ca binding to the secretory granules when ^{45}Ca was added to the homogenizing medium, but their experiments were carried out under conditions that were not optimum to demonstrate active transport processes.

Our results suggest the presence of ATPase activity in zymogen granule membranes. Calcium-ATPase has been linked to active calcium transport in various tissues[16,17], and we postulate that the Ca^{2+}-ATPase described may be involved in the uptake or perhaps release of calcium in secretory granules.

Zusammenfassung. Membranen sekretorischer Granula wurden von der Ohrspeicheldrüse (Parotis) der Ratte isoliert und auf Kalzium-ATPase-Aktivität (Ca^{2+}-ATPase) untersucht und postuliert, dass die gefundene Ca^{2+}-ATPase eine Rolle in der Aufnahme und Abgabe von Kalzium in sekretorischen Granula spielt.

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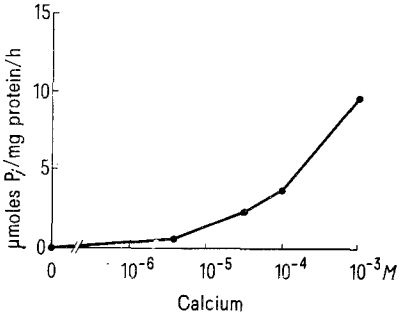

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Activation of Succinate Dehydrogenase and the Effect of Cyanide

Several agents (substrate, competitive inhibitors, nucleotides, anions etc.)[1] increase the catalytic centre activity of membrane-bound and soluble succinate dehydrogenase (succinate: [acceptor] oxidoreductase, EC 1.3. 99.1). A conformational change in the protein has been suggested by KEARNEY[2] to occur in the transition to the activated state. Various schemes for this activation process have been proposed[3–5], which postulate a reversible cycle among different forms of the enzyme.

In our studies on the interactions of succinate dehydrogenase with cyanide[6], we made the following observations, which are related to the activation phenomenon. During solubilization of membrane-bound succinate dehydrogenase by KCN, a discrepancy was found between the amount of peptide-bound flavin extracted and inactivation of succinate:2,6-dichlorophenolindophenol (DCIP) reductase activity. Furthermore, either the enzyme solubilized by cyanide or by butanol and treated with KCN show a lower degree of deactivation.

In this paper we interpret these data as a consequence of a preferential interaction of cyanide anion with a free, activated form of succinate dehydrogenase. Activation is thus suggested to modify the accessibility of the non-haem iron group.

Materials and methods. All chemicals were analytical grade from Merck or Sigma Chemical Co. The conditions for activity measurements were as described elsewhere[6].

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