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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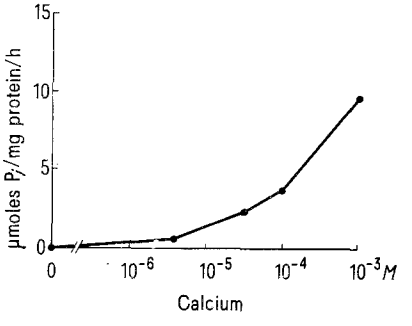


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## Activation of Succinate Dehydrogenase and the Effect of Cyanide

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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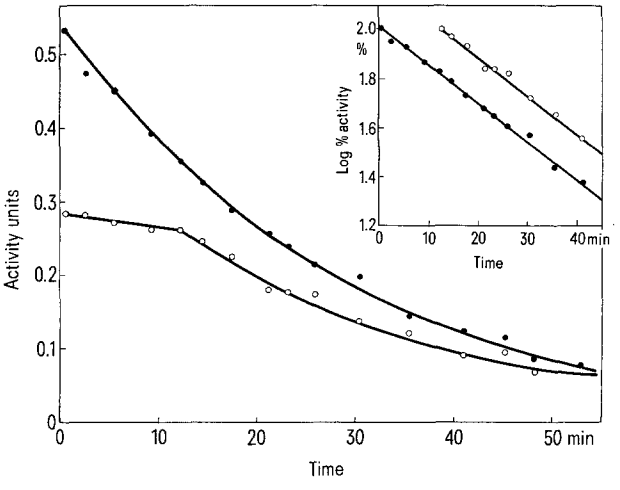
Table I. Solubilization of succinate dehydrogenase by KCN: Comparison between extracted PBF and succinate-DCIP reductase inactivation

| Incubation time (min) | Extracted PBF (%)[a] | Succinate-DCIP reductase inactivation (%) | |
|---|---|---|---|
| | | — Activation | + Activation |
| 0 | 0 | 0 | 0 |
| 10 | — | 6 | 30 |
| 15 | 42 | 16 | 42 |
| 30 | — | 52 | 66 |
| 45 | 70 | 71 | 81 |

Conditions as in the Figure. [a] Total PBF in particles (%).

Peptide-bound flavin was determined according to CERLETTI and GIORDANO[7]; the incubation mixture was cooled and centrifuged for 60 min at 32,000 × *g*. An aliquot of the supernatant was precipitated at 65% saturation of $(NH_4)_2SO_4$ to remove excess cyanide and the precipitate analyzed as usual.




Inactivation of succinate-DCIP reductase activity of Keilin-Hartree heart muscle preparations by KCN. The incubation of the particles (12.5 mg/ml$^{-1}$) was performed with 60 m*M* KCN (pH 7.4) at 30 °C, after a 15 min preequilibration time at the same temperature. Activation was performed in 40 m*M* succinate (pH 7.4) for 20 min at 25 °C. ○—○, $\Delta A_{600nm} \cdot min^{-1}/50\ \mu l^{-1}$ without activation. ●—●, $\Delta A_{600nm} \cdot min^{-1}/50\ \mu l^{-1}$ after succinate activation.

*Results and discussion.* The solubilization of membrane-bound succinate dehydrogenase from Keilin-Hartree heart muscle preparations by KCN can be followed by measuring the decrease of succinate-DCIP reductase activity of the particles[6]. The Figure shows the typical pattern of the inactivation reaction. Depending on whether the activity is measured with (upper curve) or without (lower curve) full activation by succinate, a lag period is found during which the activity remains almost constant. The half-times of both curves are the same, apart from the lag period, as depicted in the inset of the Figure. A quenching of the inactivation reaction due to activation of succinate dehydrogenase by anions[8] can be ruled out, as the ionic strenght of the medium is already high and on the basis of control experiments with KCl.

The conclusion that the lag is only apparent and the inactivation reaction actually proceeds from KCN addition, is supported primarily by the disappearance of the lag when fully activated enzyme is measured. Furthermore, peptide-bound flavin (PBF) solubilized under the same conditions, amounts at short times to approximately 40% (Table I). This value corresponds quite well to the percentage of inactivation found when the enzyme is measured after full activation.

Membrane-bound succinate dehydrogenase is considered to exist in equilibrium between an activated state and a deactivated one[3,4]. Thus, the appearance of the lag

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Table II. Effect of KCN on the activity of soluble succinate dehydrogenase (SD)

| Incubation conditions | Activity (%) KCl | | KCN | |
|---|---|---|---|---|
| | — Activation | + Activation | — Activation | + Activation |
| 50 min, $N_2$, 25 °C | 45 | 250 | 85 | 250 |
| 100 min, $N_2$, 25 °C | 30 | 245 | 62 | 210 |
| 10 min, air, 25 °C* | 50 | — | 50 | — |
| 40 min, air, 25 °C* | 15 | 75 | 35 | 75 |
| 10 min, air, 22 °C | — | 140 | 45 | 120 |
| 60 min, air, 22 °C | 25 | 100 | 40 | 100 |
| 120 min, air, 22 °C | 10 | — | 35 | — |
| 10 min, $N_2$, 30 °C | 37 | — | 75 | — |
| 30 min, air, 30 °C | 12 | 50 | 25 | 50 |

Each sample contained 5 mg/ml$^{-1}$ SD at the gel eluate stage[9], in 50 m*M* phosphate buffer (pH 7.4). KCN or KCl were 50 m*M*, except for the second experiment *, in which the concentration was 20 m*M*. Activation was performed in 40 m*M* succinate (pH 7.4) for 15 min at 25 °C under $N_2$.

Table III. Solubilization of succinate dehydrogenase by KCN in the presence of various effectors

| Conditions | Extracted PBF (%) |
|---|---|
| No addition | 100 |
| Succinate, 40 m*M* | 0 |
| NADH, 5 m*M* | 43 |
| IDP, 5 m*M* | 21 |

The Keilin-Hartree heart muscle preparations were pretreated with each effector for 15 min at 30 °C (in the samples with NADH and succinate, 1 m*M* KCN was also added). Other conditions as in the Figure.

period may be interpreted as resulting from a preferential interaction of $CN^-$ with one of the two forms of the enzyme.

In relation to these findings, the effect of KCN on the butanol-solubilized succinate dehydrogenase was investigated. The enzyme was incubated with KCN in various conditions; KCl was added to the control to yield the same ionic strength. The activity was measured at different times without and with full activation by succinate (Table II). In the latter case, no substantial difference between controls and KCN-treated samples is observed, whether incubations are carried out under nitrogen or in air. No protection against irreversible inactivation is then afforded by KCN. On the other hand, if activation by succinate is not performed, the activity in the KCN-treated samples always remains higher in the various conditions tested. These results suggest that KCN partially preserves succinate dehydrogenase against spontaneous deactivation. We shall recall that the flavoprotein solubilized by KCN[6] shows a lower degree of deactivation, as activity remains constant with time and succinate enhances this activity 1.4 times only.

These data suggest a preferential interaction of $CN^-$ with activated succinate dehydrogenase. The lag period thus describes a situation of stationary state for the activated form, being the solubilized enzyme replaced by conversion of the deactivated one. However, effectors which are well known for their activating action on the particulate enzyme[1], prevent to different extents the resolution of succinate dehydrogenase from the membrane by cyanide (Table III).

In our previous work[6] we have shown that $CN^-$ binds to the non-haem iron of succinate dehydrogenase. We may thus conclude that the conversion of the enzyme from a deactivated into an activated state results in an exposure of the non-haem iron-labile sulphide chromophore. Nevertheless, interaction of the enzyme with effectors causes a hindrance at the site of $CN^-$ binding. As to the mechanism of activation, the present data are more consistent with the scheme proposed by McDonald-Gibson and Thorn[4] which includes a free equilibrium between a deactivated and an activated form of succinate dehydrogenase, i.e. the process of activation does not necessarily occur through the binding of an activator[10].

*Riassunto.* Analizzando l'effetto del cianuro sulla Succinato Deidrogenasi, si conclude che si ha una interazione preferenziale del $CN^-$ con la forma attivata, libera dell'enzima. L'attivazione modificherebbe perciò l'accessibilità del gruppo ferro non eme.

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*via Celoria 2, I-20133 Milano (Italy),*
*14 November 1973.*

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[10] This investigation was supported by a grant from Consiglio Nazionale delle Richerche of Italy.

## Effects of Tetrahydrocannabinols on Cyclic AMP Levels in Rat Brain Areas

The role of adenosine 3′5′-monophosphate (cyclic AMP) as an intracellular mediator of hormone action has been well established[1]. Goldberg[2] had previously reported that dibutyryl cyclic AMP potentiated the end plate potential in isolated rat diaphragm by apparently facilitating the release of acetylcholine. *Trans*-(−)-$\Delta^8$-Tetrahydrocannabinol ($\Delta^8$-THC) has been shown to have an effect similar to that of anticholinergic agents in abolishing the behavioral inhibition of habituating experiences in mice[3]. In a previous paper we reported decreases in rat brain acetylcholine levels after injection of $\Delta^8$- and $\Delta^9$-THC[4]. Therefore, we thought that the action of the THC's on the cholinergic system could possibly be mediated by cyclic AMP. This report describes the effect of the THC's on the concentration of rat brain cyclic AMP.

Table I. Effects of $\Delta^8$-THC and $\Delta^9$-THC on cyclic AMP levels in rat brain areas

| Brain area | Cyclic AMP | | |
|---|---|---|---|
| | Control | $\Delta^8$-THC | $\Delta^9$-THC |
| Cortex | 0.17 ± 0.08 | 0.29 ± 0.04 | 0.14 ± 0.09 |
| Hypothalamus | 2.39 ± 0.65 | 2.19 ± 0.33 | 2.26 ± 0.27 |
| Cerebellum | 1.18 ± 0.16 | 1.08 ± 0.05 | 1.17 ± 0.21 |
| Medulla | 1.25 ± 0.30 | 1.03 ± 0.08 | 1.20 ± 0.12 |
| Midbrain | 0.84 ± 0.17 | 1.29 ± 0.17[a] | 0.88 ± 0.09 |

1 h after receiving either 10 mg/kg of the THC or 4% Tween 80-saline i.v. brain areas from rats were assayed for the level of cyclic AMP (pmole/mg wet tissue). Each value represents the mean (± S.E.) of 8 determinations. [a] Significantly different from the vehicle control, $p < 0.05$.

*Methods.* Male Sprague-Dawley rats in groups of 4 were injected i.v. with 10 mg/kg of $\Delta^9$-THC or $\Delta^8$-THC in 4% Tween-80-saline and sacrificed after 1 h. Animals used in the determination of cyclic AMP levels were sacrificed in a

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