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#### Transport of 2-oxoglutarate in rat-heart mitochondria.

The transport of 2-oxoglutarate ions through the inner membrane of rat-liver mitochondria is brought about by a specific translocator mediating an exchange-diffusion between 2-oxoglutarate and a dicarboxylate ion like malate or malonate (DE HAAN and TAGER, 1968). The stoichiometry of this reaction is 1 : 1 (PAPA *et al.*, 1969). Three methods are commonly used to study anion transport through the mitochondrial membrane. These are based on measure-



ment of (a) swelling of the mitochondria in solutions of the ammonium salts of the anions, (b) intramitochondrial reactions in which the anions take part, and (c) influx and efflux of the anions. In the present study the second and third methods have been used to investigate the transport of 2-oxoglutarate ions in rat-heart mitochondria.

The uptake and intramitochondrial utilization of 2-oxoglutarate are not stimulated by added dicarboxylate ions. However, in mitochondria preloaded with malonate, the exit of the latter is strongly stimulated by 2-oxoglutarate. The apparent  $K_m$  for 2-oxoglutarate is very low.

As in the case of rat-liver mitochondria (MEYER and TAGER, 1969), 2-oxoglutarate does not stimulate the exit of orthophosphate from phosphate-loaded mitochondria and the malonate—2-oxoglutarate exchange is Mersalyl insensitive. These results indicate that rat-heart mitochondria contain a 2-oxoglutarate translocator with substrate specificity similar to those of the translocator from rat-liver mitochondria.

The kinetics of the malonate—2-oxoglutarate exchange have been determined by measuring the influx of 2-oxo-[5- $^{14}$ C]glutarate as a function of time at 4° C.

The initial velocity has been measured at various concentrations of extramitochondrial 2-oxoglutarate and intramitochondrial malonate.

The kinetic constants have been calculated by a graphical method devised by FLORINI and VESTLING (1957) and show that the exchange reaction may be formulated by an equation in which the relative velocity ( $v/V$ ) is equal to a product of two functions, one for each substrate.

According to DIXON and WEBB (1964), this may indicate either that one of the two substrates reacts so much more rapidly than the other that there is an obligatory order of combination (steady-state hypothesis), or that each substrate combines with its specific site only and does not affect the affinity for the other if the breakdown of the ternary complex in the forward direction is sufficiently slow to ensure that it remains in equilibrium with the substrates (equilibrium hypothesis).

The dissociation constant (equilibrium hypothesis) for 2-oxoglutarate was found to be in the micromolar range, whereas that for malonate was found to be at least 10 mM.

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