DIFFERENTIAL EFFECTS OF MERCURIAL REAGENTS ON MITOCHONDRIAL THIOL GROUPS AND MITOCHONDRIAL PERMEABILITY

V. A. Knight, C. T. Settlemire, and G. P. Brierley*
Department of Physiological Chemistry, College of Medicine
Ohio State University, Columbus, Ohio 43210

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Thiol groups have been implicated in mitochondrial swelling by a number of investigators (see Lehninger, 1962, for a review) and it has been known for some time that mercurial reagents affect the turnover and retention of K^+ by mitochondria (Gamble, 1957; Scott and Gamble, 1961). We have recently reported that mercurial reagents such as p-chloromercuriphenyl sulfonate (CMS), p-hydroxymercuribenzoate (CMB), and mersalyl activate the energy-linked accumulation of monovalent cations and a closely related osmotic swelling by isolated heart mitochondria (Brierley et al 1967; 1968a). In media containing a permeant anion (such as acetate) these reagents are approximately equivalent in effectiveness and activate only the energy-linked uptake of cation without a corresponding induction of passive permeability. An extension of these studies has revealed, however, that the extent of reaction of these mercurial reagents with the mitochondrial membrane is dependent upon the anionic composition of the suspending medium and the effects of these reagents on energy-linked transport of cations, passive permeability to cations and anions, uncoupling, and ATPase activity depend on the degree to which the membrane thiols are reacted with the mercurial. Important differences between CMS and CMB have come to light in these

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* Established Investigator of the American Heart Association.

studies and the present communication compares the effect of these reagents on mitochondrial swelling and contraction reactions.

Table I

UPTAKE OF ^{203}Hg-LABELED CMS AND CMB BY ISOLATED BEEF HEART MITOCHONDRIA

Suspending Medium	^{203}Hg-CMS (mumoles/mg)	^{203}Hg-CMB
Sucrose (0.25 M)	20.6	40.0
KCl (0.12 M)	15.5	41.0
K+ Acetate (0.12 M)	17.5	21.5

Beef heart mitochondria prepared with Nagarse and EGTA (Brierley et al, 1968b) (5 mg of protein) were reacted with the indicated ^{203}Hg-labeled mercurial (The Radiochemical Centre, Amersham) at a concentration of 100 uM in 8 ml of the indicated medium. The mitochondria were removed by filtration (Millipore, 0.45 u filters with glass fiber prefilter) after 3 min at 25° and the uptake of mercurial calculated from the decrease in radioactivity in the filtrate.

Results - The dependence of the uptake of labeled CMS or CMB by intact heart mitochondria on the composition of the suspending medium is shown in Table I. CMS reacts with between 15 and 20 mumoles of SH regardless of the suspending medium. The less polar CMB also reacts with about 20 mumoles of SH per mg in acetate, but in isotonic sucrose or in a Cl^- medium this reagent reacts with about twice as many thiol groups. CMB also reacts with the higher number of thiols when mitochondria are reacted in salts of other non-permeant anions such as bromide and N-2-hydroxyethylpiperazine-N'-2 ethane sulfonate (HEPES). The lower level of reactivity of CMB is found in salts of permeant anions such as formate, phosphate, and fluoride. Reactivity of a membrane with an organic mercurial under these conditions is a complex function of the polarity of the acid groups (carboxylate vs sulfonate) and the ligands available to the Hg atom of the mercurial, as well as the orientation of protein SH groups and other factors. It is tempting to speculate, however, that

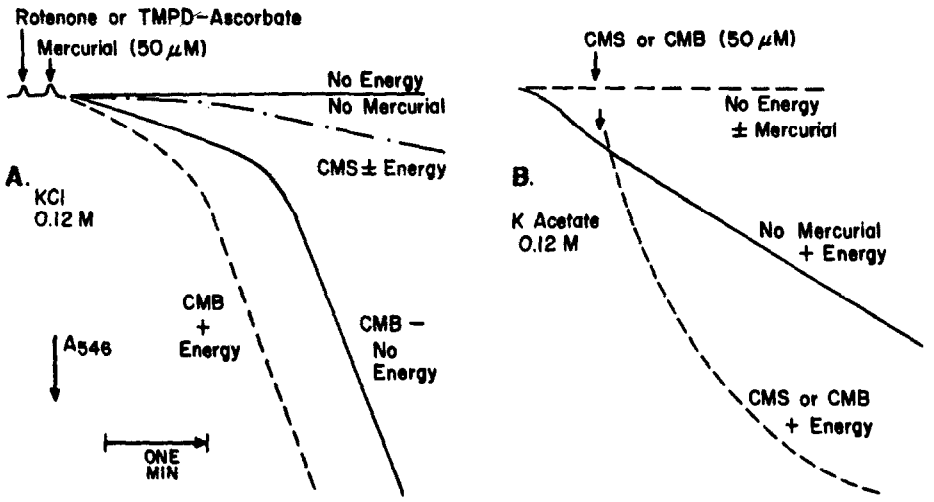

Fig. 1 - The effect of CMS and CMB on mitochondrial swelling in isotonic KCl and K+ acetate. Isolated heart mitochondria prepared with Nagarse and EGTA (Brierley et al 1968b) (5 mg of protein) were added to 8 ml of KCl (0.12M) containing 5 mM Tris Cl(pH 7.0) in Part A and the corresponding amounts of K+ acetate and Tris acetate in Part B. Traces designated "no energy" contained rotenone (5 ug/mg) to suppress endogenous respiration; those designated "energy" contained Tris ascorbate (2 mM) and tetramethyl-phenylenediamine (TMPD) (0.5 mM) as the respiratory substrate. Either CMB or CMS (50 uM) was added at the indicated point and the reaction was followed by recording the absorbance at 546 mu in a stirred plexiglass cuvette with the Eppendorf photometer.

the additional reactivity of CMB in Cl^- and other non-permeant anion salts might result from a change in orientation of the membrane in non-permeant, as opposed to permeant anion media. This point is presently being pursued further in our laboratory.

Regardless of the mechanism by which the modification of additional SH-groups occurs when CMB reacts with the mitochondrion in isotonic KCl, this modification is closely related to a major alteration in membrane permeability (Fig. 1). Under these conditions a passive uptake of K+ and osmotic swelling of the mitochondria occur after a lag of about two minutes when the particles are treated with CMB. Passive permeability to K+ and Cl^- does not develop as a result of CMS treatment in KCl (low level of SH-reacted; Table I) and passive permeability of K+ does not result from interacting the membrane with either CMS or CMB in isotonic

K+ acetate (both conditions give the low level of SH reactivity; Table I). Addition of energy in the form of ascorbate-tetramethylphenylenediamine (TMPD) oxidation produces a spontaneous uptake of K+ in the presence of the permeant acetate anion (Brierley, et al 1968b). When CMS or CMB are added under these conditions the accumulation of K+ and swelling are activated (Brierley, et al 1968a). In the Cl^- medium the presence of energy accelerates the opening which results from CMB treatment, but has no effect in the presence of CMS (Fig. 1). These results indicate that reaction of the low level (ie. about 20 mumoles/mg) of mitochondrial thiol groups is sufficient to activate the energy-linked uptake of cations, but that passive permeability to K+ and Cl^- results only when the higher level (ca. 40 mumoles/mg) of SH groups has been modified.

An additional feature of the modification of mitochondrial thiol

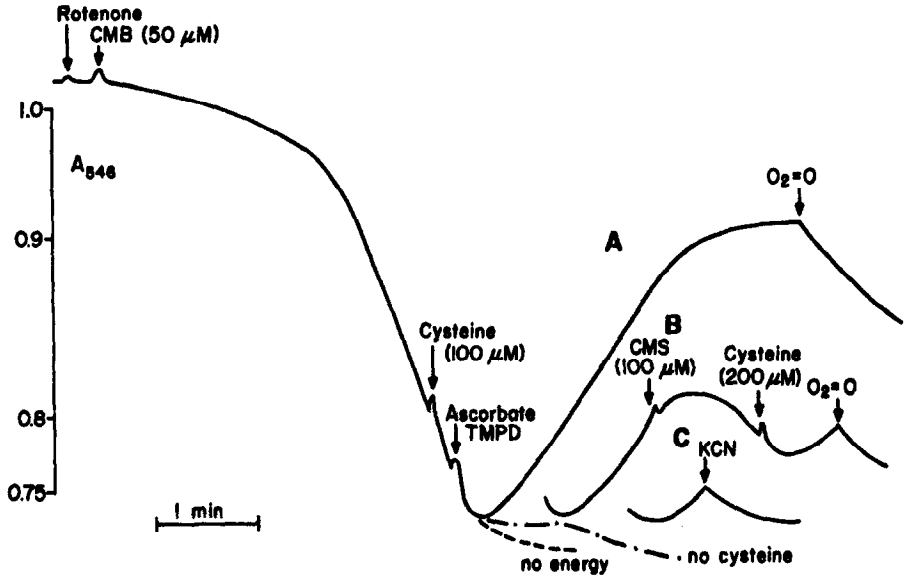

Fig. 2 - Energy-linked reversal of CMB-dependent swelling in isotonic KCl. Swelling was induced by CMB in the absence of energy as described in the legend for Fig. 1. At the indicated points a molar excess of cysteine and ascorbate - TMPD were added to support the contraction. Part A shows the effect of omitting either cysteine or ascorbate - TMPD as well as the trace obtained in the absence of further additions. Part B shows the effect of adding sufficient CMS to remove the molar excess cysteine and the effect of a second addition of excess cysteine. Part C shows the effect of the addition of KCN to inhibit respiration.

groups by CMB in a Cl^- medium is seen in Fig. 2. In this experiment the mitochondria are treated with 50 uM CMB in isotonic KCl. A large-amplitude passive swelling results in a few minutes which can be reversed osmotically by sucrose but not by addition of a source of energy. Addition of cysteine, however, causes an extensive, energy-linked contraction to occur. Under these conditions the cysteine removes only about 15 mumoles of mercurial per mg from the membrane. It is apparent therefore that about 60% of the thiol groups which originally reacted with the CMB remain modified under these conditions. The contraction is strictly dependent on respiration, but is ratherinsensitive to uncouplers of oxidative phosphorylation. When respiration is inhibited by KCN or by anaerobiosis the contraction ceases and passive swelling occurs, indicating that the membrane in this condition retains a passive permeability to both K^+ and Cl^-. The contraction can also be reversed by the addition of either CMS or CMB in molar excess over the cysteine, and the swelling which results under these conditions does not appear to be energy-linked. Several cycles of energy-linked contraction and passive swelling can be demonstrated by alternate addition of cysteine and either CMS or CMB (cf. Fig. 2).

Discussion - A scheme which appears consistent with the observed results is shown in Fig. 3. In the absence of mercurial the membrane is impermeable to Cl^- (Chappell and Crofts, 1966) and accumulates K^+ only by the energy-linked pathway. Net accumulation of K^+ and the resulting osmotic swelling are limited by inability of Cl^- to enter the mitochondrion. In the presence of the permeant acetate anion, energy-linked K^+ accumulation and osmotic swelling occur. Modification of what may be the external thiol groups of the membrane (the 20 mumoles/mg level of SH) by addition of CMS or CMB in acetate results in accelerated energy-linked influx of K^+ but no passive permeability to cation is induced. A similar situation may be presumed to exist for CMS interaction in Cl^- media, but in this case

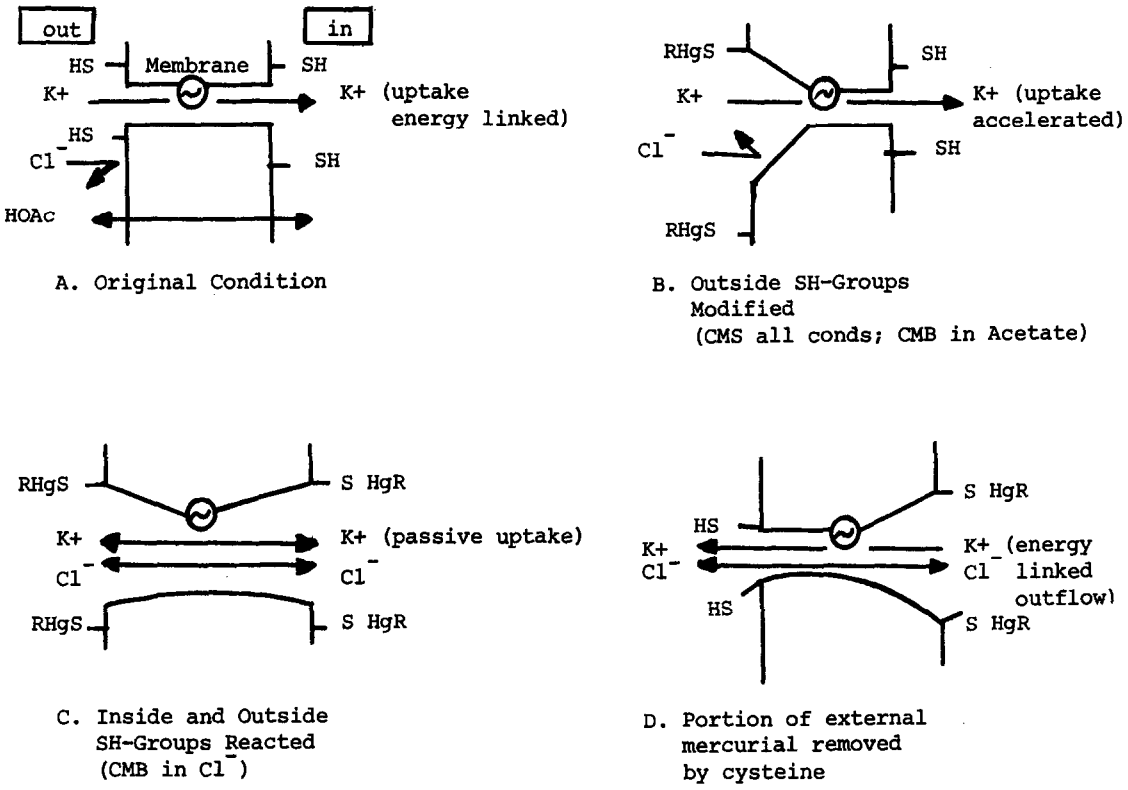

Fig. 3 - Postulated relationships between membrane thiol-groups and the effects of CMB and CMS on mitochondrial swelling and contraction.

Cl^- impermeability again prevents extensive accumulation. When deeper SH groups (perhaps on the inner surface) are reacted by CMB in a Cl^- medium it is suggested that an extensive modification of the membrane results which now permits passive uptake of both K+ and Cl^- in the direction of the concentration gradient. The presence of a source of energy accelerates this process although it is debatable whether any energy-linked ion uptake occurs in the later stages of the opening. Addition of cysteine under these conditions removes a portion of the bound CMB (and almost) all bound CMS). We suggest that this condition may partially reverse the modification of the exterior of the membrane. Under these conditions (large amounts of K+ and Cl^- inside the particle and the interior

modified by mercurials to a greater extent than the exterior), addition of a source of energy results in an energy-linked extrusion of KCl and contraction. Passive permeability is largely retained in this case since interuption of the energy supply results in resumption of the passive swelling process (Fig. 2).

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