Passive Transport of 5,5-Dimethyl-2,4-Oxazolidinedione into Beef Heart Mitochondria

Abstract. *5,5-Dimethyl-2,4-oxazolidinedione is used to estimate intracellular pH. The mechanism of transport of this compound into beef heart mitochondria was investigated to assess the validity of its use for the measurement of intramitochondrial pH. This compound and ^{14}C-labeled carboxy dextran were simultaneously used to determine the intra- and extramitochondrial compartments and the distribution of the compound, which was passively transported into resting, respiring, and simultaneously respiring and phosphorylating mitochondria. The transport of the compound was neither associated with electron transport nor with oxidative phosphorylation.*

The controversy on the transport mechanism of 5,5-dimethyl-2,4-oxazolidinedione (DMO) across biological membranes (*1*) has shown the need for further investigation. Our study gives evidence for the passive transport of DMO into resting, respiring, and simultaneously respiring and phosphorylating mitochondria.

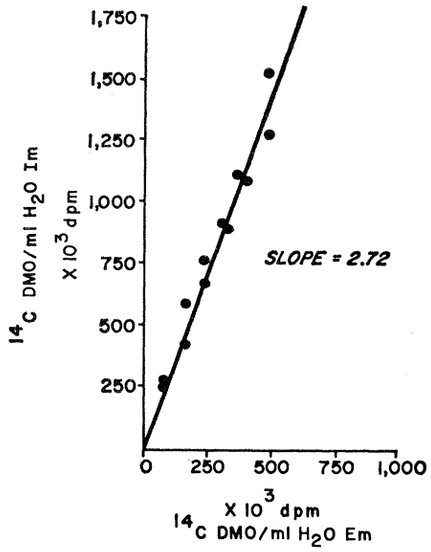

Fig. 1. Incorporation of DMO into resting beef heart mitochondria. Mitochondria were isolated (*2*) at 4°C in 0.33*M* sucrose containing tris chloride (0.05*M*, *p*H 7.5 at 4°C). Protein (32 mg) was incubated with antimycin A (0.2 μg per milligram of protein), six different concentrations of ^{14}C-DMO (10.8 × 10^5 to 1.7 × 10^5 disintegrations per minute) and one concentration of ^{14}C-labeled carboxy dextran (3.8 × 10^5 disintegrations per minute) at 4°C for 5 minutes. (DMO was equilibrated into mitochondrial compartments within 0.5 minute and remained the same for at least 10 minutes of incubation.) The total volume of the reaction mixture was 2.5 ml (in 0.33*M* sucrose containing 0.05*M* tris chloride, *p*H 7.5 at 4°C). At the end of incubation the reaction mixtures were centrifuged at 17,000 rev/min in a Sorvall refrigerated centrifuge for 20 minutes, and the radioactivity of ^{14}C-DMO and ^{14}C-labeled carboxy dextran of the medium and pellet were measured.

Intact "heavyweight" beef heart mitochondria into which several compounds are actively transported were used (*2*). The mitochondria were incubated at 4°, 25°, or 30°C. The reaction medium contained both ^{14}C-labeled carboxy dextran (400 μc/g) and DMO-2-^{14}C (7.42 mc/mmole) (*3*). The buffer composition, specific substrates, and incubation time varied for different functional states of mitochondria. The reaction mixtures were centrifuged at the end of incubation (*4*). The total amount of water in the pellet (3.97 ± 0.11 μl per milligram of protein) was measured by the gravimetric method (*5*) and by the method of dilution by ^3H_2O. The extramitochondrial water (1.72 ± 0.15 μl per milligram of protein) was determined from the content of ^{14}C-labeled carboxy dextran (*5*). Intramitochondrial water (2.25 ± 0.11 μl per milligram of protein) was calculated from the difference between total water in the pellet and extramitochondrial water (*5*). The total radioactivity in the medium and the pellet were measured in a liquid scintillation medium (*6*). The radioactivity of ^{14}C-DMO was measured by the extraction procedures of Poole *et al.* (*7*).

The radioactivity attributable to the labeled dextran was estimated from the difference. Quenching of radioactivity was detected with an automatic external standard. Quenching, when present, was quantitated by extrapolation methods (*8*). The concentrations of DMO in extra- and intramitochondrial water and their ratio were then calculated (*5*). The *p*H of the incubating medium was measured in a Radiometer *p*H meter (model 27) equipped with a temperature-controlled water bath. Protein was measured by the method of Gornal *et al.* (*9*).

To investigate the mechanism of transport of DMO in resting, respiring, and simultaneously respiring and phosphorylating mitochondria incubated with different concentrations of DMO, we studied the distribution of DMO between the intra- and extramitochondrial compartments. For the resting mitochondria, the ratio of intra- to extramitochondrial DMO for six different extramitochondrial concentrations remained at 2.7, equivalent to a *p*H gradient (intra- to extramitochondrial) of 0.18 (Fig. 1). These observations indicate the reproducibility of the method and are consistent with passive transport, as expected from the experimental conditions.

To test whether or not the transport of DMO is associated with electron transport (active transport), respiring mitochondria were incubated with different concentrations of ^{14}C-DMO. The ratio of intra- to extramitochondrial DMO for different extramitochondrial concentrations would be expected to vary if active transport were present. This ratio for five different extramitochondrial concentrations remained at 3.3, equivalent to a *p*H gradient (intra-

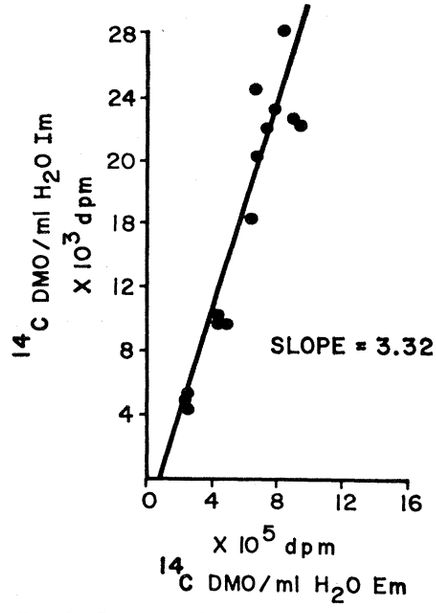

Fig. 2. Incorporation of DMO into respiring beef heart mitochondria. Mitochondria were isolated (*2*) at 4°C in 0.3*M* sucrose. (The specific activity of the mitochondria was 0.033 μatom O_2 per minute per milligram of protein.) Protein (34 mg) was incubated at 25°C, *p*H 7.5 for 1 minute. The reaction medium contained 1 to 5 μc of ^{14}C-DMO, 1 μc of ^{14}C-labeled carboxy dextran, 0.3*M* sucrose, and 0.026*M* succinate. This reaction medium contains only cations and anions of the substrate (sodium succinate) to minimize interference from other cations and anions. Sucrose (0.3*M*) supplies appropriate osmotic support for the mitochondria. The total volume of the reaction medium was 10 ml. At the end of incubation, the reaction mixtures were treated as in Fig. 1.

to extramitochondrial) of 0.44 (Fig. 2), indicating a passive transport.

To detect the saturation of active transport of DMO, if present, we incubated respiring and phosphorylating mitochondria with large concentrations of unlabeled DMO. So that quantitation and distribution could be based on radioactive analysis, ^{14}C-DMO was added. The ratio of intra- to extramitochondrial DMO for six different extramitochondrial concentrations (0.9 to 29 $\times$ $10^{-2}M$) remained at 1.4, equivalent to a *p*H gradient (intra- to extramitochondrial) of 0.20 (Fig. 3), indicating absence of active transport.

During respiratory jump activated by Ca^{++} (0.6237 $\times$ $10^{-2}M$) (Fig. 2), the ratio of intra- to extramitochondrial

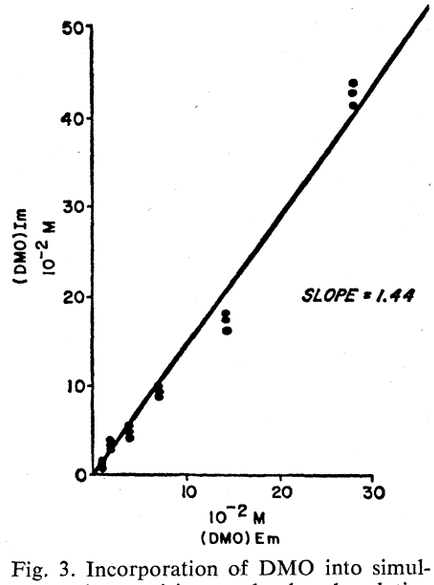

Fig. 3. Incorporation of DMO into simultaneously respiring and phosphorylating mitochondria. Beef heart mitochondria were isolated at 4°C in 0.33*M* sucrose containing tris chloride (0.05*M*, *p*H 7.5 at 4°C), and 28 mg of protein was incubated at 30°C for 1 minute. When examined in an Oxygraph (GME, Middleton, Wisconsin), these mitochondria had the following characteristics; respiratory control 2.2, P/O ratio (μmoles of adenosine triphosphate produced per μatom oxygen consumed) 3.2, and specific activity 0.082 μatom O_2 per minute per milligram of protein. The reaction medium contained: 1 μc of ^{14}C-DMO; 1 μc of ^{14}C-labeled carboxy dextran; 0.9 $\times$ $10^{-2}M$ to 29 $\times$ $10^{-2}M$ cold DMO; tris phosphate 0.002*M*; sodium pyruvate 0.004*M*; tris malate 0.004*M*; adenosine diphosphate 0.004*M*; $MgCl_2$ 0.020*M*; sucrose 0.208*M*; and tris chloride 0.05*M*, *p*H 7.5 in a total volume of 10 ml. In view of the fact that phosphate acceptor (ADP) is added to the reaction medium which contains in addition Mg^{++}, phosphate ions, and substrates, oxidative phosphorylation will predominate with about 20 percent maximum accumulation of phosphate and Mg^{++} (*10*). At the end of incubation the reaction mixtures were treated as in Fig. 1.

DMO ratio was found to be 30, equivalent to a *p*H gradient (intra- to extramitochondrial) of 1.56. This is an expected change, because a release of H^+ ions from the mitochondria and an alkalinity of the mitochondrial pellet have been shown under similar experimental conditions (*11*). Also, the *p*H gradient of 1.56 *p*H units in our studies is not far from that reported for rat liver mitochondria by Chance and Mela (*12*) who, using bromthymol blue, measured a *p*H gradient of about 1.00 *p*H unit under conditions somewhat similar to ours. Furthermore, the significant alkalinization during the Ca^{++} activation was neutralized with concomitant significant decreases in the ratio of intra- to extramitochondrial DMO (from 30 to 3.4) and in the *p*H gradient (from 1.56 to 0.3) when either 8.3 m*M* acetate or 1.7 m*M* phosphate were added, respectively, to mitochondria activated by Ca^{++} (*4*). These findings were also in agreement with those reported by Chance and Mela (*13*). In addition, these observations demonstrate that DMO anions are not carried along with the cations (Ca^{++}) into the mitochondria and that distribution of intra- and extramitochondrial DMO is a function of intramitochondrial *p*H.

Our data indicate that DMO is passively transported into resting, respiring, and simultaneously respiring and phosphorylating mitochondria. Also, the similar ratio of intra- to extramitochondrial DMO for each functional state (with different concentrations of DMO in the medium) suggest no binding of DMO to mitochondrial constituents. Therefore, the DMO method should be valid for the measurement of intramitochondrial *p*H. In view of the fact that the mitochondrial membranes possess many of the structural and functional characteristics typical of cellular membranes (*14*), it is reasonable to assume that a similar behavior of cellular membranes exists with respect to DMO.

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Luteinizing Hormone Activity in Plasma during the Menstrual Cycle

Abstract. *Daily determinations of luteinizing hormone activity in plasma throughout a menstrual cycle in ten young women showed a sharp peak of activity lasting less than 48 hours around midcycle and higher mean values during the follicular phase than during the luteal phase in nine instances.*

Elevation in urinary excretion of substances with biologic (*1*) and immunologic (*2*) characteristics of luteinizing hormone (LH) occurs about the time of the rise in basal body temperatures in women with ovulatory menstrual cycles. When we used a sensitive, precise radioimmunoassay method for luteinizing hormone in plasma (*3*), we found a corresponding elevation in samples taken daily throughout a single menstrual cycle in ten young women. Mean values during the follicular phase significantly exceeded those during the luteal phase in nine instances.

Daily samples (5 ml) of venous blood were drawn before noon on the 1st to 3rd day of menses and every day thereafter until the 1st day of the next menses. The subjects were ten healthy young women from 19 to 27