# The Reaction of Choline Dehydrogenase with some Electron Acceptors

By MICHAEL C. BARRETT* and ALAN P. DAWSON
*School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.*

1. The choline dehydrogenase (EC 1.1.99.1) was solubilized from acetone-dried powders of rat liver mitochondria by treatment with *Naja naja* venom. 2. The kinetics of the reaction of the enzyme with phenazine methosulphate and ubiquinone-2 as electron acceptors were investigated. 3. With both electron acceptors the reaction mechanism appears to involve a free, modified-enzyme intermediate. 4. With some electron acceptors the maximum velocity of the reaction is independent of the nature of the acceptor. With phenazine methosulphate and ubiquinone-2 as acceptors the $K_m$ value for choline is also independent of the nature of the acceptor molecule. 5. The mechanism of the Triton X-100-solubilized enzyme is apparently the same as that for the snake venom-solubilized enzyme.

The choline dehydrogenase (EC 1.1.99.1) is tightly bound to the mitochondrial inner membrane. Williams & Sreenivasan (1953) used cholate to solubilize the enzyme from rat liver mitochondria. Korzenovsky & Auda (1958) solubilized the enzyme in the non-ionic detergent *p*-iso-octyl phenoxypoly-ethoxyethanol. Rendina & Singer (1959) introduced digestion of acetone-dried powders of rat liver mitochondria with *Naja naja* venom as a means of solubilizing the membrane-bound enzyme. Various techniques have been used for the assay of choline dehydrogenase. Williams & Sreenivasan (1953) used 2,6-dichlorophenol-indophenol as the electron acceptor for the enzyme, but there were limitations in its use as the primary acceptor. Kearney & Singer (1956) have suggested that phenazine methosulphate is perhaps the best artificial electron acceptor. Although ferricyanide may be used as the terminal electron acceptor in assays of the membrane-bound choline dehydrogenase, it does not react with the solubilized enzyme. Drabikowska & Szarkowska (1965) have shown that the venom-solubilized choline dehydrogenase is capable of utilizing Q-6† as an electron acceptor. Barrett & Dawson (1975) have shown that ubiquinone is an essential requirement for choline oxidation in rat liver mitochondria.

Rendina & Singer (1959) and Kimura & Singer (1962) have reported that for the venom-solubilized enzyme preparations the $K_m$ for choline was 7mM in a manometric assay at 38°C with phenazine methosulphate as the primary acceptor and oxygen as the terminal acceptor, with optimum activity in

range pH7.6–8.2. These authors did not attempt any kinetic analyses of the enzyme-catalysed reaction.

The present paper describes a study of the kinetics of reduction of some electron acceptors by the choline dehydrogenase solubilized by treatment of acetone-dried mitochondrial powders with *Naja naja* venom. The kinetics of reduction of phenazine methosulphate by choline dehydrogenase solubilized by Triton X-100 is also given in this paper.

## Experimental

### Materials

Choline chloride was obtained from Hopkin and Williams Ltd., Romford, Essex, U.K., and was purified by recrystallization from ethanolic solution. Betaine hydrochloride was purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] and 2,6-dichlorophenol-indophenol were obtained from Hopkin and Williams Ltd. Tris, as Trizma Base, phenazine methosulphate and *Naja naja* venom were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. *p*-Benzo-quinone was purchased from BDH Chemicals Ltd. and purified by steam distillation. Q-1 and Q-2 were the gift of Dr. G. E. Boxer (Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A.). All other reagents were of A.R. or highest available grade from Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.

### Choline dehydrogenase assays

All assays were performed at 38°C in a Pye–Unicam SP.500 spectrophotometer fitted with a water-jacketed cell housing. The output from the

* Present address: Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

† Abbreviation: Q-*n*, ubiquinone, where *n* = number of intact isoprene units in side chain.

instrument was fed into a Vitatron recorder fitted with a logarithmic amplifier such that absorbance changes could be recorded. The assay temperature used was 38°C since the majority of previous studies on the choline dehydrogenase were performed at this temperature (see Kimura & Singer, 1962).

(a) *Phenazine methosulphate–2,6-dichlorophenol-indophenol assay.* The use of 2,6-dichlorophenol-indophenol as the terminal electron acceptor in the reaction of the succinate dehydrogenase with phenazine methosulphate was proposed by Redfearn & Dixon (1961). The reoxidation of reduced phenazine methosulphate by 2,6-dichlorophenol-indophenol forms the basis of a spectrophotometric assay which is more sensitive than the original manometric assay. Salach & Bednarz (1973) have used the phenazine methosulphate–2,6-dichloro-phenol-indophenol assay for the L-3-glycerophos-phate dehydrogenase solubilized from pig brain mitochondria. For choline dehydrogenase assays the assay medium contained $30mM$-KH$_2$PO$_4$–KOH buffer, pH7.6, $1mM$-KCN and $50\mu M$-2,6-dichloro-phenol-indophenol. Standard assays of enzyme activity were performed with $1mM$-phenazine metho-sulphate and the assay was started by the addition of $16mM$-choline chloride. The disappearance of oxidized 2,6-dichlorophenol-indophenol was followed as the decrease in absorbance at 600nm. The extinction coefficient for oxidized 2,6-dichlorophenol-indophenol at 600nm was taken to be $2.1 \times 10^4$litre·mol$^{-1}$·cm$^{-1}$ (Armstrong, 1964). The initial reaction velocity was proportional to enzyme concentration up to a velocity of at least $0.18\mu mol$ of 2,6-dichloro-phenol-indophenol/min and the progress curve was linear with time for the first minute throughout this range. The unit of enzyme activity is defined as being the amount of enzyme that catalyses the reduction of $1\mu mol$ of 2,6-dichlorophenol-indo-phenol/min under the standard assay conditions.

(b) *Phenazine methosulphate–O$_2$ assay.* A polaro-graphic modification of the manometric phenazine methosulphate–O$_2$ assay was adopted. The assay medium was identical with that used for phena-zine methosulphate–2,6-dichlorophenol-indophenol assays except that the latter was omitted. The oxygen-electrode chamber was covered with a thick black cloth during assays to exclude light.

(c) *2,6-Dichlorophenol-indophenol assay.* Williams & Sreenivasan (1953) used 2,6-dichlorophenol-indophenol as the primary acceptor for assay of the cholate-solubilized choline dehydrogenase. The assay conditions used in the present investigation were as for the phenazine methosulphate–2,6-dichlorophenol-indophenol assay except that the former was omitted. No standard assay conditions were adopted for this acceptor.

(d) *Quinone and ubiquinone assays.* Drabikowska & Szarkowska (1965) have shown that Q-6 can act as the acceptor for the venom-solubilized enzyme. To maintain long-chain ubiquinones in optically clear solution, high concentrations of alcohols and detergents are necessary (Dawson & Thorne, 1969a). Choline dehydrogenase preparations solubilized by venom and by Triton X-100 have been found to be inactivated and inhibited in a complex manner by ethanol and other aliphatic alcohols; the venom-solubilized enzyme is also inhibited by various detergents (M. C. Barrett & A. P. Dawson, unpublished work). Thus the use of the water-insoluble ubiquinones for assay of the choline dehydrogenase is excluded. The short-chain ubi-quinones Q-1 and Q-2 and the simple quinone, *p*-benzoquinone, were used in assays without large amounts of alcohols or detergents, since they are all sufficiently water-soluble. The assay medium com-prised $30mM$-KH$_2$PO$_4$–KOH buffer, pH7.6, $1mM$-KCN, at 38°C. Q-1 and Q-2 were added as small quantities of concentrated ethanolic solution and the ethanol concentration in these assays was kept constant at $5\mu l$ of ethanol/ml of assay medium. No standard assay procedures were adopted with Q-1, Q-2, or *p*-benzoquinone as acceptor.

### Preparation of mitochondria

Rat liver mitochondria were prepared as described by Barrett & Dawson (1975), except that both the 0.25M-sucrose isolation medium and the 0.1M-KCl final suspension medium were buffered with $10mM$-Tris–HCl buffer, pH7.6. The mitochondrial sus-pension was dried by treatment with acetone at −20°C in the manner of Dawson & Thorne (1969a). The volumes of acetone used for the two treatment steps were 5litres and 3litres respectively, for mitochondria prepared from 25–30 animals and suspended in 100ml of KCl–Tris buffer. The yield of acetone-dried mitochondrial powder was variable, but was within the range 2.5–5.0g of powder/100g of liver. The dried powder was stored desiccated at −20°C until use.

### Protein determinations

Protein was determined by the micro-biuret method of Goa (1953), with bovine serum albumin as standard. Turbid samples were clarified by the addition of Triton X-100 to a final concentration of 1.5% (w/v) before the addition of the Benedict's reagent.

### Solubilization of the enzyme with Naja naja venom

Solubilization of choline dehydrogenase by phos-pholipase A from *Naja naja* venom was performed as described by Kimura & Singer (1962) with minor

modifications. All steps were carried out at 0–4°C unless stated otherwise. A sample of mitochondrial powder (800 mg dry wt.) was washed by resuspension, with homogenization, in 50 mM-$KH_2PO_4$-KOH buffer, pH 7.6 (40 ml). The suspension was stirred for 10 min and centrifuged at $42000g_{max}$. for 15 min at 2°C in the SS34 angle rotor of a Sorvall RC-2B centrifuge. The supernatant was discarded and the pellet resuspended in twice the original volume of phosphate buffer (80 ml) and centrifuged once again. The twice-washed pellet was resuspended in 10 mM-Hepes–NaOH buffer, pH 8.0 (40 ml). A solution of Naja naja venom was prepared in the same Hepes–NaOH buffer (2 mg dry wt. of venom/ml of buffer) and added to the mitochondrial suspension to give 1 mg dry wt. of venom/100 mg of mitochondrial protein. The suspension was incubated at 30°C for 1 h in a stirred vessel in a thermostatically controlled water bath. The suspension was then centrifuged at $105000g_{av}$. for 1 h at 2°C in the $8 \times 25$ ml angle rotor of a MSE Superspeed 50 centrifuge. The pale-yellow supernatant was carefully decanted from the pellet and was stored frozen at −20°C until use. Under these storage conditions the enzyme activity was essentially constant for at least 3 weeks, but repeated freezing and thawing caused inactivation and was avoided as far as possible.

### Solubilization of the enzyme with Triton X-100

The choline dehydrogenase was solubilized by treatment of acetone-dried mitochondria with the non-ionic detergent Triton X-100. The twice-washed mitochondria were resuspended in 30 mM-$KH_2PO_4$-KOH buffer, pH 7.6, at 2°C, by using the same amount of mitochondria and volume of buffer as for the venom digestion. A solution of Triton X-100 (5%, w/v) in water was added to the mitochondrial sus-

pension such that [protein]/[detergent] (both concentrations in mg/ml) was 1.75:1. This ratio was found to give optimum conditions of yield and purification of the enzyme on solubilization. Increasing the detergent concentration was found to increase the yield but decrease the specific activity of the solubilized enzyme. The suspension was stirred at 0°C for 20 min and then centrifuged in the same manner as for the venom-treated mitochondria.

Both the venom-solubilized and Triton X-100-solubilized enzyme preparations were used without further purification. The venom-solubilized preparation was used with a specific activity of 0.28 unit/mg of protein, which represented an increase in specific activity over that of the original acetone-dried mitochondria of 6.4 times. The Triton X-100-solubilized preparation was used at a specific activity of 0.36 unit/mg of protein, which represented a purification in terms of increase of specific activity of 8.1 times over the original acetone-dried mitochondria.

### Expression of results

Initial reaction velocities were measured at various concentrations of acceptor and choline and expressed in the form of double-reciprocal plots (Lineweaver & Burk, 1934). Lines were fitted to the points by eye.

### Results

#### Phenazine methosulphate as electron acceptor

By using the phenazine methosulphate–2,6-dichlorophenol-indophenol assay, initial reaction velocities for the venom-solubilized enzyme were measured at various concentrations of choline and phenazine methosulphate and the results are shown in Fig. 1 in the form of Lineweaver–Burk plots. The slopes of the double-reciprocal plots are
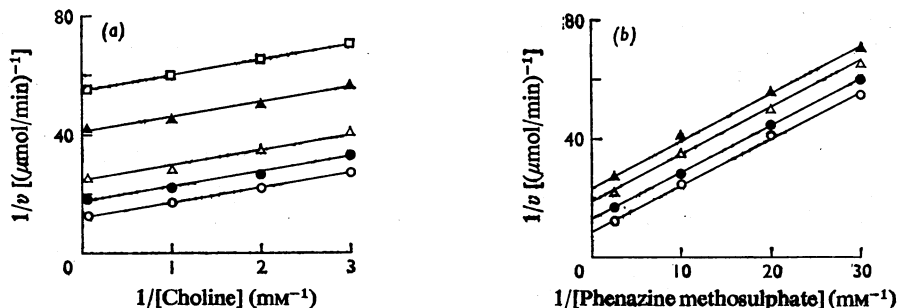


Fig. 1. Kinetics of phenazine methosulphate reduction

Initial-velocity measurements were performed at 38°C in the phenazine methosulphate–2,6-dichlorophenol-indophenol assay as described in the text with 0.12 unit of enzyme in each assay. The assay medium comprised 30 mM-$KH_2PO_4$-KOH buffer, pH 7.6, with 1 mM-KCN. (a) Phenazine methosulphate concentrations were: ○, 0.4 mM; ●, 0.2 mM; △, 0.1 mM; ▲, 50 μM; □, 33 μM. (b) Choline concentrations were: ○, 16.7 mM; ●, 1.0 mM; △, 0.5 mM; ▲, 0.33 mM.
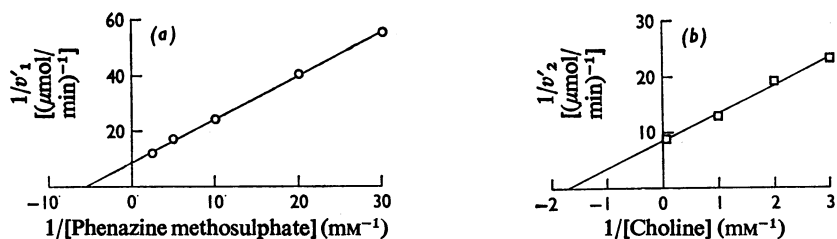
Fig. 2. *Secondary plots for the determination of* (*a*) $K_{m1}$ *and* (*b*) $K_{m2}$ *from the data of Fig.* 1
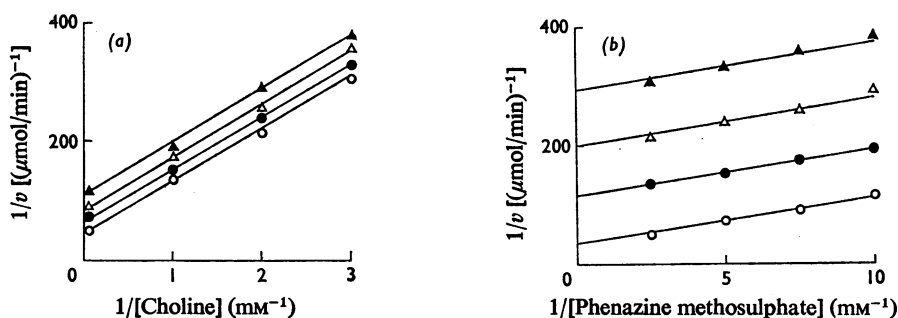


Fig. 3. *Kinetics of phenazine methosulphate reduction in the presence of betaine hydrochloride*

Assays details are same as for Fig. 1 except that 42 mM-betaine hydrochloride was included in each assay. Each assay was performed with 0.024 unit of enzyme. (*a*) Phenazine methosulphate concentrations were: ○, 0.4 mM; ●, 0.2 mM; △, 0.13 mM; ▲, 0.1 mM. (*b*) Choline concentrations were: ○, 10 mM; ●, 1 mM; △, 0.5 mM; ▲, 0.33 mM.

in both cases independent of the concentrations of the invariant substrate. These results may be explained by a reaction mechanism described as Type IV (i) by Dalziel (1957) and as a Ping Pong Bi Bi reaction mechanism by Cleland (1963):

$$E+S_1 \rightleftharpoons ES_1 \rightleftharpoons E'+P_1$$

$$E'+S_2 \rightleftharpoons E'S_2 \rightleftharpoons E+P_2$$

In this mechanism there is a free modified-enzyme intermediate E'. The mechanism gives rise to the following rate equation (1):

$$v_0 = \frac{V}{1+(K_{m1}/[S_1])+(K_{m2}/[S_2])} \quad (1)$$

where $K_{m1}$ is the Michaelis constant for choline at [acceptor] = ∞ and $K_{m2}$ is the Michaelis constant for the electron acceptor at [choline] = ∞. There is no $K_{1,2}/[S_1][S_2]$ term. A similar mechanism was proposed for the lipoamide dehydrogenase (Massey *et al.*, 1960).

The true Michaelis constants for the substrates can be determined by extrapolation of the 1/apparent $V$ values taken from the double-reciprocal plots in the manner of Florini & Vestling (1957). The secondary plots of $1/V_1'$ against 1/[phenazine methosulphate]

and $1/V_2'$ against 1/[choline] are shown in Figs. 2(*a*) and 2(*b*) respectively, in which $V_1'$ is the apparent $V$ at [phenazine methosulphate] ≠ ∞, [choline] = ∞ and $V_2'$ is the apparent $V$ at [choline] ≠ ∞, [phenazine methosulphate] = ∞. The intercepts on the abscissae give the reciprocal values of $K_{m2}$ and $K_{m1}$. $K_{m1}$ is found to be 0.59 mM-choline and $K_{m2}$ is 0.18 mM-phenazine methosulphate.

Dervartanian *et al.* (1966) have pointed out that the mechanism of the type described above as eqn. (1) cannot be readily distinguished from a mechanism yielding the more general form of the rate equation:

$$v_0 = \frac{V}{1+(K_{m1}/[S_1])+(K_{m2}/[S_2])+(K_{1,2}/[S_1][S_2])} \quad (2)$$

when the $K_{1,2}/[S_1][S_2]$ term is very small. If mechanism (2) were to apply, the double-reciprocal plots will appear as parallel lines, since the deviation from parallel will be very small. Such a deviation can be increased by performing the velocity measurements in the presence of a constant high concentration of a competitive inhibitor. This would have the effect of increasing the size of the $K_{1,2}/[S_1][S_2]$ term. Betaine is a competitive inhibitor of the choline dehydrogenase with a $K_i$ of 1.2 mM (M. C. Barrett & A. P. Dawson, unpublished work). Fig. 3 shows
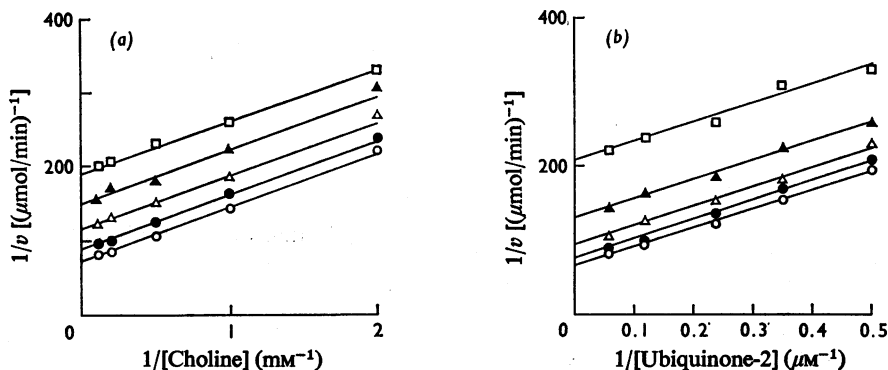
Fig. 4. *Kinetics of ubiquinone-2 reduction*

Q-2 assays were performed as described in the text with 0.015 unit of enzyme. A constant ethanol concentration of 5 $\mu$l/m was used in each assay. (*a*) Ubiquinone-2 concentrations were: O, 17 $\mu$M; ●, 8.5 $\mu$M; △, 4.25 $\mu$M; ▲, 2.83 $\mu$M; □, 1.99 $\mu$M (*b*) Choline concentrations were: O, 8 mM; ●, 5 mM; △, 2 mM; ▲, 1 mM; □, 0.5 mM.

double-reciprocal plots of $1/v_0$ versus $1/[$choline$]$ at a series of phenazine methosulphate concentrations and $1/v_0$ versus $1/[$phenazine methosulphate$]$ at a series of choline concentrations in the presence of 42 mM-betaine hydrochloride. This concentration of inhibitor should increase any deviation from parallel-lines kinetics, since the $K_{1,2}/[S_1][S_2]$ term would be increased by a factor of 36 times; the results show that in the presence of betaine the lines apparently remain parallel.

The Triton X-100-solubilized enzyme was also shown to possess parallel-lines kinetics, indicating that the enzyme prepared by treatment of mitochondria with Triton X-100 and that prepared by treatment of mitochondria with *Naja naja* venom show similar reaction pathways. For a number of different enzyme preparations the values of $K_{m1}$ were 0.9±0.32 mM-choline and 1.4±0.13 mM-choline for the venom-solubilized enzyme ($n = 6$) and the Triton X-100-solubilized enzyme ($n = 4$) respectively. The values of $K_{m2}$ were 0.18±0.03 mM-phenazine methosulphate and 0.17±0.03 mM-phenazine methosulphate for the venom-solubilized enzyme ($n = 6$) and Triton X-100-solubilized enzyme ($n = 4$) respectively. By using a Student's $t$ test the values of $K_{m1}$ for the two different enzyme preparations were found to be significantly different ($P < 0.01$). This difference may be due to the presence of inhibitory concentrations of Triton X-100 in the detergent-solubilized enzyme preparations.

By using the polarographic phenazine methosulphate-$O_2$ assay it was found that for the venom-solubilized enzyme parallel-line Lineweaver–Burk plots were also obtained. The values of $K_{m1}$ and $K_{m2}$ were found to be 0.8±0.04 mM-choline and 0.18± 0.05 mM-phenazine methosulphate respectively for four different enzyme preparations.

## Ubiquinone-2 as electron acceptor

Kinetic measurements with Q-2 as electron acceptor showed that double-reciprocal plots of $1/v_0$ versus $1/[$choline$]$ and $1/v_0$ versus $1/[$Q-2$]$ are of the parallel-lines type. The results are shown in Fig. 4. Secondary plots (not shown) produced values of $K_{m1} = 1.3$ mM-choline and $K_{m2} = 5.1$ $\mu$M-Q-2.

## V' measurements for different electron acceptors

By using phenazine methosulphate and Q-2 as primary acceptors the values of $K_{m1}$ are essentially the same. When the acceptor concentration is infinite but the choline concentration is not:

$$V' = \frac{V}{1 + (K_{m1}/[S_1])}$$

where $V'$ is the velocity under these conditions. Thus the maximum velocities for the reduction of those electron acceptors for which $K_{m1}$ is the same can be readily compared by determining $V'$ values at a single concentration of choline. The results of such a determination are shown in Fig. 5 for phenazine methosulphate, 2,6-dichlorophenol-indophenol, Q-1, Q-2 and *p*-benzoquinone as acceptors. All acceptors used gave identical values of $V'$. The constancy of $K_{m1}$ has been shown only with phenazine methosulphate and Q-2 as acceptors. It seems probable that since $V'$ is constant for 2,6-dichlorophenolindophenol, Q-1 and *p*-benzoquinone and the same as with phenazine methosulphate and Q-2 as acceptors, then $V$ and $K_{m1}$ are also constant. Table 1 shows the apparent Michaelis constants for the different electron acceptors measured at 10 mM-choline.
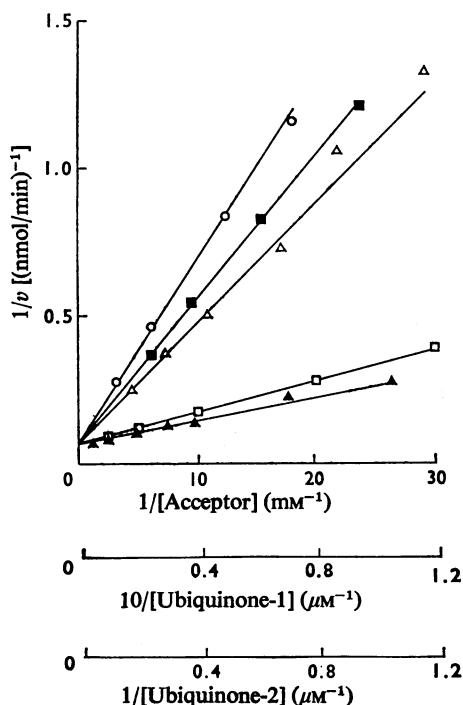
Fig. 5. *Comparison of $V'$ values for various electron acceptors*

All initial-velocity measurements were carried out in 30 mM-KH$_2$PO$_4$–KOH buffer, 1 mM-KCN, pH 7.6, at 38°C with a constant choline concentration of 10 mM, with 0.014 unit of enzyme. ○, 2,6-Dichlorophenol-indophenol; ■, *p*-benzoquinone; △, ubiquinone-1; □, phenazine methosulphate (by using the phenazine methosulphate–2,6-dichlorophenol-indophenol assay procedure); ▲, ubiquinone-2.
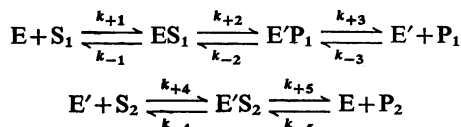
Table 1. *Apparent Michaelis constants for various electron acceptors, measured at 10 mM-choline*

| Acceptor | $K'_{m2}$ |
|---|---|
| 2,6-Dichlorophenol-indophenol | 1.0 mM |
| *p*-Benzoquinone | 0.83 mM |
| Phenazine methosulphate | 0.18 mM |
| Q-1 | 87 $\mu$M |
| Q-2 | 5 $\mu$M |

**Discussion**

With the electron acceptors phenazine methosulphate and Q-2 the venom-solubilized choline dehydrogenase appears to have a reaction mechanism

that involves a free modified-enzyme intermediate. A possible reaction scheme would be:

$$E+S_1 \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES_1 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E'P_1 \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E'+P_1$$

$$E'+S_2 \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} E'S_2 \underset{k_{-5}}{\overset{k_{+5}}{\rightleftharpoons}} E+P_2$$

Application of the steady-state treatment to this system, when $[P_1] = [P_2] = 0$, yields the rate equation:

$$v_0 = \frac{V}{1+(K_{m1}/[S_1])+(K_{m2}/[S_2])}$$

where

$$V = \frac{e \cdot k_{+2}k_{+3}k_{+5}}{k_{+2}k_{+3}+k_{+2}k_{+5}+k_{-2}k_{+5}+k_{+3}k_{+5}}$$

$$K_{m1} = \frac{k_{+5}(k_{+2}k_{+3}+k_{-1}k_{+3}+k_{-1}k_{-2})}{k_{+1}(k_{+2}k_{+3}+k_{+2}k_{+5}+k_{-2}k_{+5}+k_{+3}k_{+5})}$$

$$K_{m2} = \frac{k_{+2}k_{+3}(k_{-4}+k_{+5})}{k_{+4}(k_{+2}k_{+3}+k_{+2}k_{+5}+k_{-2}k_{+5}+k_{+3}k_{+5})}$$

Both $V$ and $K_{m1}$ involve the $k_{+5}$ term, which would be expected to vary with the nature of the electron acceptor. It has been shown that $V'$ is independent of the nature of the acceptor with five different acceptors and that $K_{m1}$ is similar for phenazine methosulphate and Q-2 as acceptors. The implication is that $k_{+5}$ does not make a significant contribution to the $V$ and $K_{m1}$ terms. Rearrangement of the $V$ and $K_{m1}$ terms gives:

$$V = \frac{e \cdot k_{+2}k_{+3}}{k_{+2}+k_{-2}+k_{+3}+[(k_{+2}k_{+3})/k_{+5}]}$$

$$K_{m1} = \frac{k_{+2}k_{+3}+k_{-1}k_{+3}+k_{-1}k_{-2}}{k_{+1}[k_{+2}+k_{-2}+k_{+3}+(k_{+2}k_{+3})/k_{+5}]}$$

Thus $k_{+5}$ will have no significant effect on the values of $V$ and $K_{m1}$ only if $(k_{+2}k_{+3}/k_{+5}) \ll (k_{+2}+k_{-2}+k_{+3})$. It appears that at least for Q-2 and phenazine methosulphate as acceptors $k_{+5}$ is sufficiently large for $V$ and $K_{m1}$ to be independent of the nature of the acceptor. That $V'$ is independent of the nature of the acceptor, with 2,6-dichlorophenol-indophenol, *p*-benzoquinone, phenazine methosulphate, Q-1 and Q-2, indicates that for all five acceptors $k_{+5}$ is large. In these cases $V$ and $K_{m1}$ may be simplified as:

$$V = \frac{e \cdot k_{+2}k_{+3}}{k_{+2}+k_{-2}+k_{+3}}$$

$$K_{m1} = \frac{k_{+2}k_{+3}+k_{-1}k_{+3}+k_{-1}k_{-2}}{k_{+1}(k_{+2}+k_{-2}+k_{+3})}$$

The reaction mechanism for choline dehydrogenase is thus similar to that of the L-3-glycerophosphate dehydrogenase (Dawson & Thorne, 1969*b*).

For the Triton X-100-solubilized choline dehydrogenase the values of $K_{m1}$ and $K_{m2}$ are similar to those for the venom-solubilized enzyme. This perhaps indicates that the different solubilization techniques produce enzyme preparations of identical reaction mechanism.

The very low values of $K'_{m2}$ for Q-1 and Q-2, and the previously demonstrated essentiality of ubiquinone for the mitochondrial choline oxidase (Barrett & Dawson, 1975), indicates that ubiquinone may be the primary electron acceptor for the choline dehydrogenase *in vivo*. However, the enzyme preparation used in this present study cannot be considered pure. The extraction procedure is similar to that for the L-3-glycerophosphate dehydrogenase (Ringler & Singer, 1958). The enzyme preparation may well contain other mitochondrial inner-membrane dehydrogenases. It is possible that in extracting dehydrogenases a component concerned with conferring ubiquinone reactivity to the preparation is also extracted. Unless a highly purified preparation of choline dehydrogenase were to be available then the question of whether ubiquinone is or is not the primary electron acceptor, *in vitro*, cannot be answered.

Practical difficulties mentioned above have made it impossible, so far, to study the kinetics of reduction of the long-chain ubiquinone homologues.

Rendina & Singer (1959) reported that for the venom-solubilized choline dehydrogenase the $K_m$ for choline was 7mM at 38°C measured in a manometric phenazine methosulphate-$O_2$ assay at pH7.6. In the present investigation it has been shown that the value of $K_m$ measured at pH7.6 and 38°C is 0.9mM-choline and 1.4mM-choline for the venom-solubilized and Triton X-100-solubilized enzyme preparations respectively. Also, the $K_m$ for choline appears to be the same for the venom-solubilized enzyme assayed either by the polarographic assay or by the spectrophotometric phenazine methosulphate-2,6-dichloro-phenol-indophenol assay. The discrepancy between the previously published value of $K_m$ for choline and those values reported here cannot be readily explained.

## References

Armstrong, J. M. (1964) *Biochim. Biophys. Acta* **86**, 194–197

Barrett, M. C. & Dawson, A. P. (1975) *Biochem. J.* **148**, 595–597

Cleland, W. W. (1963) *Biochim. Biophys. Acta* **67**, 104–137

Dalziel, K. (1957) *Acta Chem. Scand.* **11**, 1706–1723

Dawson, A. P. & Thorne, C. J. R. (1969a) *Biochem. J.* **111**, 27–34

Dawson, A. P. & Thorne, C. J. R. (1969b) *Biochem. J.* **114**, 35–40

Dervartanian, D. V., Zeylemaker, W. P. & Veeger, C. (1966) in *Flavins and Flavoproteins* (Slater, E. C., ed.), pp. 183–209, Elsevier, Amsterdam

Drabikowska, A. & Szarkowska, L. (1965) *Acta Biochim. Pol.* **12**, 387–394

Florini, J. R. & Vestling, C. S. (1957) *Biochim. Biophys. Acta* **25**, 575–578

Goa, J. (1953) *Scand. J. Clin. Lab. Invest.* **5**, 218–222

Kearney, E. B. & Singer, T. P. (1956) *J. Biol. Chem.* **219**, 963–975

Kimura, T. & Singer, T. P. (1962) *Methods Enzymol.* **5**, 562–570

Korzenovsky, M. & Auda, B. V. (1958) *Biochim. Biophys. Acta* **29**, 463–464

Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666

Massey, V., Gibson, Q. H. & Veeger, C. (1960) *Biochem. J.* **77**, 341–351

Redfearn, E. R. & Dixon, J. M. (1961) *Biochem. J.* **81**, 19 P

Rendina, G. & Singer, T. P. (1959) *J. Biol. Chem.* **234**, 1605–1610

Ringler, R. L. & Singer, T. P. (1958) *Biochim. Biophys. Acta* **29**, 661–662

Salach, J. I. & Bednarz, A. J. (1973) *Arch. Biochem. Biophys.* **157**, 133–144

Williams, J. N. & Sreenivasan, A. (1953) *J. Biol. Chem.* **203**, 899–906