Experiments with purified choline acetylase[4] prepared from Squid head ganglia with a specific activity of about 50 μM acetylcholine formed per mg protein per hour indicate that SH groups are necessary for the action of this enzyme. In these experiments acetyl CoA (prepared by acetylating CoA (Pabst) with acetic anhydride)[5] and choline were used as substrates. The enzyme activity was found to be depressed by all sulfhydryl inhibitors tested. The results are summarized in the Table.

Addition of ethylene diamine tetraacetate (EDTA) to the reaction mixture in phosphate buffer increased the CoA liberated up to 40%; with tris(hydroxymethyl)aminomethane buffer recrystallized twice from alcohol the activity was increased 450%. The activity was the same with either buffer in the presence of EDTA. Apparently traces of metal inhibit the enzyme and are removed by complex formation with EDTA. In these experiments the enzyme activity was assayed by a modified nitroprusside test for liberated CoA[6].

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OXIDATIVE PHOSPHORYLATION BY HEART MUSCLE MITOCHONDRIA

by

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The yields of oxidative phosphorylation by heart muscle mitochondria for a number of substrates of the KREBS' cycle were reported in a recent communication[1]. The one-step oxidation of α-ketoglutarate to succinate was examined in particular detail in this investigation. On the basis of this study it was concluded that the P/O ratio for the oxidation of α-ketoglutarate to succinate was more than 3. This result was in agreement with that obtained by other investigators using tissue preparations different from heart[2-7]. At about the same time a report on the same subject appeared by SLATER AND HOLTON[8]. These authors used a method for the preparation of heart particles somewhat different than that employed by us[9], and obtained a P/O of below 3 for the oxidation of α-ketoglutarate in the presence of malonate. SLATER AND HOLTON[8] expressed the belief that the P/O values of above 3 for this step obtained with liver mitochondria by COPENHAVER AND LARDY[3] were in error because preincubation of the Warburg flasks for 5 minutes was inadequate to insure thermal equilibration, resulting in underestimation of the oxygen consumption during the test period. (It should be emphasized that COPENHAVER AND LARDY specifically stated that the 5 minute preincubation period was adequate for thermal equilibration under their conditions.) Since a 5 minute preincubation period was also used in our experiments with heart mitochondria, the same criticism might be applied to our results. It was demonstrated with heart muscle mitochondria that the rates of oxygen consumption, α-ketoglutarate disappearance, and phosphate uptake were approximately linear when measured at 5 minute intervals during an incubation period of 20 minutes (*cf.* Fig. 1[1]). This suggested that thermal equilibration was adequate in our experimental procedure. Nevertheless,

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in view of the differences in reported results it might be helpful to define certain of our experimental conditions in greater detail and to present additional data.

Thin-walled one-sidearm Warburg vessels of 13 ml volume (3 ml V_f, 10 ml V_g) were employed. After removal from the ice bath these vessels were incubated with shaking in a circular Warburg apparatus[10] at 30°. The stopcocks were closed after 4 minutes, initial readings were taken after 5 minutes and the hexokinase-glucose mixture was added at the same time. Flasks were added to the water bath at 30 second intervals and initial and final readings of the manometers were staggered accordingly. Incubation periods of from 10 to 20 minutes were employed.

Although a 5 minute preincubation period was adequate, in the experiments reported here flasks were preincubated for 10 minutes, and oxygen consumption and phosphate disappearance were observed at various time intervals up to 30 minutes. With α-ketoglutarate as substrate an average P/O value of 3.22 was thus obtained, Table I. In a similar experiment an incubation temperature of 15° instead of 30° was used. At the lower temperature only 90–120 seconds were needed to reach thermal equilibrium; an average P/O of 3.36 was observed, Table I. Some additional data on phosphate uptake during the oxidation of various substrates in the absence of malonate was obtained, Table I. The data obtained here are in substantial agreement with those obtained previously[1], except that an average value of P/O = 2.72 for fumarate (6 trials) was found. It is quite clear that the discrepancy between the results of SLATER AND HOLTON[8] and ours cannot be explained on the basis of the manometric manipulations.

TABLE I

Substrate	*Malonate*	*Temperature of incubation*	*P/O*
	M	°C	
α-Ketoglutarate	0.01	30	3.22 (3)
α-Ketoglutarate	0.01	15	3.36 (8)
α-Ketoglutarate		30	2.93 (6)
Fumarate		30	2.72 (6)
Succinate		30	1.64 (6)

All flasks contained 20 μMoles of substrate per 3 ml of fluid volume. Numbers in parentheses refer to the number of trials.

The P/O value of over 3 which was obtained for the one-step oxidation of α-ketoglutarate would imply that there might be a phosphorylation on the oxidation level of oxygen to cytochrome *c*, a fact which has already been pointed out by KREBS *et al.*[6]. JUDAH[5] demonstrated that the oxidation of cytochrome *c* reduced by ascorbic acid in rat liver mitochondria is accompanied by a phosphorylation. If the assumption is correct that ascorbic acid in this system merely serves to reduce cytochrome *c* directly it would give support to the presence of a phosphorylation at the cytochrome oxidase level.

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