OXIDATIVE PHOSPHORYLATION AND RESPIRATORY CONTROL IN HOUSEFLY MITOCHONDRIA*

CHARLES T. GREGG**, CHARLES R. HEISLER AND LEMAR F. REMMERT

Department of Agricultural Chemistry, Oregon Agricultural Experiment Station and Department of Chemistry, Oregon State College, Corvallis, Oreg. (U.S.A.)

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SUMMARY

Mitochondria isolated from housefly thoraces were found to resemble other animal mitochondria in their ability to oxidize pyruvate rapidly, in contrast to the reports of other investigators. High P/O ratios were also obtained without addition of serum albumin to the incubation medium and it was shown, by two procedures, that the rate of respiration in these preparations is very markedly controlled by the availability of a phosphate acceptor.

INTRODUCTION

Studies of oxidative phosphorylation and related processes in insect mitochondria were made possible in recent years by the pioneering experiments of LEWIS AND SLATER[1] and of SACKTOR[2]. Prior to this time investigations made on intact insects had revealed that certain flying insects, such as bees and flies, required an extremely rapid expenditure of energy to sustain flight. In such insects the rate of oxygen consumption, upon initiation of flight, was estimated to be 50–100 times the rate of respiration at rest[3].

Early studies by WATANABE AND WILLIAMS[4], by SACKTOR[2], and by HOSKINS, CHELDELIN AND NEWBURGH[5] had indicated the presence of the citric acid cycle in thoracic mitochondria from houseflies and from honey bees, suggesting that the reactions associated with this cycle might be of primary importance in supplying the energy for flight. However, glycolysis in insect muscle produces equimolar quantities of pyruvate and α-glycerophosphate[6,7] and flight muscle is unique in its high content of α-glycerophosphate dehydrogenase activity[8]. In recent manometric studies with mitochondria from housefly thoraces SACKTOR AND COCHRAN[9] found that, when short

Abbreviations: ADP and ATP, adenosine di- and tri-phosphate, respectively; CoA, coenzyme A; DPN and TPN, di- and tri-phosphopyridine nucleotide, respectively; EDTA, ethylenediamine tetraacetate; P/O, μmoles of phosphate esterified per μg atom of oxygen consumed; Q_{O_2}, microliters of oxygen consumed/h/mg of mitochondrial protein; TPP, thiamine pyrophosphate; Tris, tris(hydroxymethyl)aminomethane.

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** Present address: Department of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Md. (U.S.A.).

equilibration times were used, the rate of α-glycerophosphate oxidation was approximately twice that of succinate, glutamate, or α-ketoglutarate. When measurements were made over very short time intervals (up to 3 min) using the vibrating platinum electrode, CHANCE AND SACKTOR[10] found that the rate of α-glycerophosphate oxidation was ten times the rate of succinate oxidation and 50 times the rate for pyruvate. Under these conditions they obtained a Q_{O_2} of 215 for the oxidation of α-glycerophosphate in a phosphorylation medium, which would suggest a Q_{O_2} of only 4 or 5 for pyruvate oxidation. CHANCE AND SACKTOR[10] suggested, therefore, that α-glycerophosphate is the principal substrate for the activation of the respiratory chain during flight and that the citric acid cycle is not of primary importance in flight metabolism.

CHANCE, SACKTOR, COCHRAN AND KING[9-11] also found that in their insect mitochondrial preparations the rate of respiration was independent of the concentration or presence of ADP, in contrast to the situation commonly encountered in other animal tissues. This led to the proposals that the respiratory rate in these flight muscles is controlled by the concentration of α-glycerophosphate[10] or the activity of the α-glycerophosphate oxidase[12]. More recently BUCHER, KLINGENBERG AND ZEBE[13] demonstrated respiratory control, by the ADP concentration, in mitochondria from locust muscle. Earlier GONDA, TRAUB AND AVI-DOR[14] had isolated mitochondria from whole mosquitoes which showed respiratory control by the ADP concentration, though with rather low acceptor ratios. SACKTOR[15] reported similar effects with minces of housefly muscle. However, control of respiration by the ADP concentration has not been demonstrated previously in well-washed mitochondria from flies.

Our investigations have been made with housefly mitochondria isolated by a somewhat different technique than used by others. These mitochondria oxidize pyruvate at much higher rates than those reported by CHANCE AND SACKTOR[10], and require the presence of a phosphate acceptor for maximal respiration.

METHODS AND MATERIALS

Mitochondria were isolated as described previously[16], except that in some experiments only the thoraces of the adult houseflies (*Musca domestica*) were used as source material. In the procedure described previously a mortar and pestle was used for a preliminary grinding of the houseflies, followed by further blending in the Potter-Elvehjem homogenizer. This secondary blending led to high yields of mitochondria from whole flies and gave preparations with satisfactory stability. It is possible to wash such preparations twice (about 40 min required) and then to hold the concentrated suspension at 0° for at least 2 h with only a small loss of activity. When isolated thoraces were used in the present study, the secondary blending was found to be unnecessary and the comminution was done entirely with a mortar and pestle.

Oxygen consumption and esterification of inorganic phosphate were measured as described previously[16]. The amount of mitochondrial protein per flask was determined by the method of JACOBS *et al.*[17].

The Tris (Sigma 121), ATP, sodium pyruvate and hexokinase (Type II) were products of Sigma Chemical Company; sodium α-glycerophosphate, 98 % minimum purity, was obtained from Eastern Chemical Corporation; EDTA (practical grade) was obtained from Eastman Organic Chemicals, converted to the disodium salt and recrystallized twice by the method of BLAEDEL AND KNIGHT[18]; crystalline bovine

serum albumin was obtained from Armour. Other chemicals used were of reagent grade. Glass redistilled water was used in preparing all solutions.

RESULTS AND DISCUSSION

Isolation and coenzyme requirements of active housefly mitochondria

Preparation from different tissues: In the course of these studies mitochondrial preparations with qualitatively similar properties have been obtained from whole flies, from heads and thoraces, and from the isolated thoraces alone. Preparations from thoraces alone and from whole flies are compared in Table I for their activities with pyruvate and α-glycerophosphate as substrates. The activity with either substrate

TABLE I

ACTIVITIES OF MITOCHONDRIAL PREPARATIONS FROM WHOLE FLIES AND ISOLATED THORACES

Each flask contained 100 μmoles Tris, 10 μmoles ATP, 150 Kunitz-McDonald units of hexokinase, 23 μmoles $MgCl_2$, 100 μmoles glucose, 250 μmoles sucrose, 100 μmoles potassium phosphate, 3 μmoles EDTA, either 30 μmoles pyruvate, 30 μmoles pyruvate plus 1–2 μmoles fumarate, or 90 μmoles α-glycerophosphate, and either 3 to 9 mg mitochondrial protein from whole flies or 5 to 7 mg from isolated thoraces. The pH was 7.4; total fluid volume, 3.2 ml including 0.2 ml of 20% KOH in the center well. Incubated 30 min at 25°. The values given are averages, with the range in parentheses.

Starting material	*Number of experiments*	*Substrate*	$QO_2(25°)$	*P/O*
Whole flies	7	Pyruvate	100 (80–120)	2.65 (2.26–3.04)
Thoraces	2	Pyruvate	75 (72– 78)	2.80 (2.76–2.85)
Thoraces	3	Pyruvate/fumarate	114 (87–134)	2.98 (2.68–3.16)
Whole flies	6	α-glycerophosphate	78 (60–102)	1.21 (0.89–1.45)
Thoraces	4	α-glycerophosphate	82 (50–110)	1.12 (0.70–1.50)

was essentially the same, regardless of whether whole flies or the isolated thoraces were used as a starting material. These results are consistent with the idea that the isolation procedure gives a preparation consisting predominately of the so-called "giant mitochondria"[19] of the thoracic flight muscle, regardless of the other tissues present in the starting material.

Isolation media: Sucrose media, varying in concentration from 0.20 *M* to 0.36 *M* and frequently containing other constituents, have been used for isolating insect mitochondria capable of oxidative phosphorylation[1,2,9,20,21]. WATANABE AND WILLIAMS[22], in their early morphological studies, found that a "fuzzy degeneration" took place in sucrose media and recommended a medium of 2.5% serum albumin in 0.16 *M* phosphate buffer as optimal for preserving the mitochondrial structure. Although a complete survey of media for isolating mitochondria was beyond the scope of this investigation, certain comparisons were made in an attempt to improve the quality of our preparations and to reproduce the results of other investigators. The medium described previously[16] was found to be the most satisfactory of those studied. Use of this medium led routinely to preparations which catalyzed rapid oxidation of pyruvate and gave high P/O ratios without the addition of serum albumin to the incubation medium.

Particular comparison was made between our standard isolation medium and

a medium containing 0.25 *M* sucrose and 0.005 *M* EDTA (see ref. 9). Mitochondria isolated in the latter medium usually gave somewhat lower Q_{O_2} values than did our standard preparations. Five preparations obtained with the sucrose–EDTA medium were tested with α-glycerophosphate as substrate; in two of these, no phosphorylation was obtained with α-glycerophosphate unless serum albumin (2 %) was included in the incubation medium. These results are similar to those reported by SACKTOR, O'NEILL AND COCHRAN[23] who used the sucrose–EDTA medium. However, the P/O ratios which we obtained with pyruvate as substrate were similar to those obtained with our standard preparations. The preparations made in sucrose–EDTA also gave lower acceptor ratios than will be shown below for mitochondria isolated with the usual medium[16] and, in some cases, showed no evidence of respiratory control when α-glycerophosphate was used as the substrate.

The effects of including phosphate and Tris in the isolation medium were tested in early experiments. In these experiments, whole flies were homogenized in an initial medium containing 0.25 *M* sucrose, 0.003 *M* EDTA and 0.006 *M* each of α-ketoglutarate, citrate, succinate and pyruvate. The homogenate was divided immediately into two or more aliquots. Mitochondria were isolated from one of the aliquots and tested as a control preparation. Phosphate, Tris, or a combination of the two were added to other aliquots of the homogenate, following which the mitochondria were isolated and compared to the control preparation. The results of such experiments are shown in Table II. It can be seen that including either 0.05 *M* Tris or 0.1 *M* phosphate in the isolation medium gave greatly increased activity, especially with respect to the amount of phosphate esterified. The addition of both Tris and phosphate gave higher activity than was achieved with either alone.

We have not studied the effect of omitting EDTA, Mg^{++}, and substrates from our

TABLE II

EFFECT OF TRIS AND/OR PHOSPHATE IN THE MEDIUM FOR ISOLATING MITOCHONDRIA FROM WHOLE FLIES

The flask contents were as described in Table I except as follows: 175 μmoles Tris, 200 μmoles glucose, 1110 μmoles sucrose, 1.6 mg DPN, 0.78 mg cytochrome *c*, 1.88 mg TPP, 0.4 mg TPN, 0.2 mg CoA and 2.4 μmoles each of α-ketoglutarate, citrate and succinate per 3.0 ml of medium. In Expts. 1, 2 and 3 each flask also contained 22.4 μmoles of pyruvate; in Expts. 4 and 5, 12.4 μmoles of pyruvate. The initial isolation medium contained 0.25 *M* sucrose, 0.003 *M* EDTA and 0.006 *M* each of α-ketoglutarate, citrate, succinate and pyruvate and was adjusted to pH 7.4. Where added to the isolation medium the Tris was at 0.05 *M* and phosphate was at 0.1 *M*. Incubated 60 min at 25°. All values are averages from duplicate flasks.

Exp. number	*Added to initial isolation medium*	*O_2 consumed (μg atoms)*	*Phosphate esterified (μmoles)*	*P/O*
1	—	17.4	6.3	0.36
	Phosphate	25.4	38.4	1.51
2	—	18.3	10.6	0.58
	Phosphate	21.0	25.9	1.23
3	—	12.3	3.1	0.25
	Tris	25.1	25.3	1.01
	Tris, phosphate	26.0	41.0	1.58
4	Tris	25.4	16.3	0.64
	Tris, phosphate	21.5	29.4	1.37
5	Tris	10.8	8.4	0.78
	Tris, phosphate	21.6	33.8	1.56

homogenizing medium. However, ZIEGLER, LESTER AND GREEN[24] found that incubation of mitochondrial fractions in a medium containing 0.003 *M* $MgCl_2$, 0.003 *M* EDTA, 0.006 *M* malate and 0.0003 *M* DPN restored phosphorylation which had been lost during storage or extensive washing. Their report suggests that EDTA, Mg^{++}, and substrates may exert protective effects during the isolation of mitochondria. KLINGENBERG AND BUCHER[25] have found that their mitochondrial preparations showed respiratory control only when the isolation medium contained at least 0.001 *M* EDTA. If Mg^{++} was present at a higher concentration than EDTA, only uncoupled mitochondria were obtained.

Cofactor requirements: Initial studies made with preparations isolated in a medium without Tris or phosphate led to the routine inclusion of a cofactor solution (DPN, TPP, TPN, CoA, and cytochrome *c*) in the incubation medium, since the mixture greatly stimulated respiration and phosphorylation with only a small decrease in the P/O ratio. In later experiments, using mitochondria isolated in our standard medium[16], the cofactor mixture was no longer found to be stimulatory and was therefore omitted in the experiments to be described here.

The properties of housefly mitochondria

Pyruvate oxidation: Since early studies by WATANABE AND WILLIAMS[4] and by SACKTOR[2] had indicated that the citric acid cycle is operative in thoracic mitochondria from houseflies, only supplementary evidence for the complete oxidation of pyruvate was sought in this investigation. In a number of experiments it was found that succinate, α-ketoglutarate and glutamate were oxidized by the housefly mitochondria at rates which averaged 42 %, 31 % and 55 %, respectively, of the rate obtained with pyruvate. In other experiments the addition of only 3 μmoles of pyruvate to our standard phosphorylation medium, or the addition of 10 to 20 μmoles to a system containing ATP and Mg ($8 \cdot 10^{-3}$ *M*) but no hexokinase, led to oxygen uptake values corresponding to 75 to 95 % of the amounts required for complete oxidation of the added substrate. The oxidation of pyruvate (0.01 *M*) in these preparations is abolished completely by an equimolar concentration of malonate. While these results do not give absolute proof that every step of the citric acid cycle is operative, they suggest strongly that pyruvate is oxidized completely in the housefly mitochondria and that the citric acid cycle is the probable route.

Comparison of activities with pyruvate and α-glycerophosphate: In order for oxidative phosphorylation to function effectively as a source of energy for flight, respiration must take place at a rapid rate (high Q_{O_2}) and be efficiently coupled to phosphorylation (high P/O ratio). Therefore, these two ratios were used as criteria of the activity of the mitochondrial preparations from the standpoint of energy production. As shown in Table I the insect mitochondria have a high Q_{O_2}, and the P/O ratio is near the theoretical maximum, in the case of pyruvate oxidation. The Q_{O_2} values in Table I are comparable to those obtained with mammalian mitochondria[26,27] and the energy production rate ($Q_{O_2} \times$ P/O) with pyruvate is comparable to the best values obtained by HOLTON *et al.*[27] for heart sarcosomes and higher than those found by the same authors for rat-liver mitochondria.

The Q_{O_2} values in Table I are in sharp contrast to those reported by CHANCE AND SACKTOR[10]. The rates given for oxidation of α-glycerophosphate, obtained by the manometric method, are about one-third as high as CHANCE AND SACKTOR obtained

using the platinum electrode and a phosphorylation medium. It is possible that our preparations would yield higher Q_{O_2} values for α-glycerophosphate oxidation if a platinum electrode were available for short-period measurements. With pyruvate as substrate, however, the Q_{O_2} values given in Table I and obtained manometrically are 20 to 25 fold as high as the Q_{O_2} of 4 or 5 implied by CHANCE AND SACKTOR[10]. There appears, therefore, to be a significant difference between the mitochondrial preparations used by CHANCE AND SACKTOR and those used in the present study. The greatest quantitative difference is not that our preparations fail to oxidize α-glycerophosphate rapidly but that the preparations used by CHANCE AND SACKTOR, for unknown reasons, apparently oxidized pyruvate very slowly. Since the oxidation of pyruvate by our preparations yielded usable energy at a rate ($Q_{O_2} \times$ P/O) nearly three times that for α-glycerophosphate, it would appear to be premature to conclude that α-glycerophosphate is the principal substrate activating the respiratory chain during flight[10]. The possibility does exist that the live insect uses α-glycerophosphate to initiate flight and then utilizes pyruvate for energy production once flight is under way.

Respiratory control and the effect of serum albumin: The degree to which respiration in a mitochondrial preparation is controlled by the availability or the concentration of a phosphate acceptor may be measured by several methods[28–30] and obviously the extent of control indicated may depend upon the exact conditions under which this test is made. In this investigation we used, as the primary measure of respiratory control, the ratio of the rates of respiration obtained manometrically in the presence and in the absence of added phosphate acceptor, this ratio being referred to as an acceptor ratio. A high acceptor ratio (from 4 to 10), particularly when associated with a high P/O ratio, may be regarded as an indication that respiration is "tightly-coupled" with phosphorylation and controlled by the availability of a phosphate acceptor[28,29].

As shown in Table III the acceptor ratios obtained with pyruvate do indicate that respiration in insect mitochondria is dependent upon the availability of a phosphate acceptor. For these experiments, ATP and the hexokinase system were used to provide a continuously regenerated supply of ADP. Under these conditions, addition of 1 μmole of ATP per flask gave Q_{O_2} and P/O values as high as those obtained with 10 μmoles, and only moderate reductions in these values were noted with as little as 0.3 μmole of ATP per flask. Despite this, addition of ATP gave the large increases in the rate of pyruvate oxidation noted in Table III. Respiratory control was present

TABLE III

ACTIVITY AND RESPIRATORY CONTROL IN HOUSEFLY MITOCHONDRIA

Each flask contained hexokinase, either 30 μmoles of pyruvate plus 1 to 2 μmoles of fumarate or 90 μmoles of α-glycerophosphate, and 5 to 7 mg mitochondrial protein from isolated thoraces. Ten μmoles of ATP were added to obtain respiratory rates and P/O ratios in the presence of a phosphate acceptor; the ATP was omitted in other flasks to obtain respiratory rates in the absence of an acceptor. The flask contents were otherwise as described in Table I. Incubated 20–30 min at 25°. The values given are averages with the range in parentheses.

Number of experiments	*Substrate*	Q_{O_2} *(with ATP)*	*P/O*	*Acceptor ratio**
5	Pyruvate + fumarate	97 (72–134)	2.87 (2.68–3.11)	12.2 (5.9 –24.7)
3	α-glycerophosphate	72 (50– 89)	1.11 (0.70–1.48)	1.73 (1.41– 1.92)

* Ratio of respiratory rates in the presence and in the absence of added ATP.

even when α-glycerophosphate was used as substrate, though the acceptor ratios were not as impressive as those obtained with pyruvate. This difference may indicate that in our preparations electron transport from DPNH to flavoprotein is more tightly coupled to phosphorylation than is true of the other two sites in the respiratory chain at which ATP is formed. Similar differences between the acceptor ratios, obtained with pyridine nucleotide-linked substrates and with substrates such as succinate and α-glycerophosphate, have been reported by others[13,25,27,31].

The acceptor ratios given in Table III for pyruvate oxidation are substantially higher than previously reported for insect mitochondria with pyridine nucleotide-linked substrates[13,14] but even the highest ratios are only about one-half the figure obtained for intact insects[3]. The acceptor ratios given here for α-glycerophosphate oxidation are somewhat lower than those found by BUCHER *et al.*[13] for locust mitochondria. This group found that the respiratory control of pyruvate oxidation is more stable to aging than is the case for α-glycerophosphate oxidation. They also found that with prolonged aging the respiratory activity with pyruvate and citric acid cycle intermediates is lost, without impairment of the rate for α-glycerophosphate. In BUCHER's preparations the highest control ratios with α-glycerophosphate were obtained with mitochondria with a high content of endogenous substrate[13,25]. In contrast, the thoracic mitochondria used in these studies had an insignificantly low endogenous respiration (usually zero).

Recently CHANCE AND BALTSCHEFFSKY[30] have suggested that a respiratory-control ratio, defined as the respiratory rate with ADP divided by the rate after added ADP has been phosphorylated, is a more critical test for respiratory control. We do not believe that this criterion is always suitable as a test for respiratory control, because a high ratio would require not only that respiration be controlled by the availability of ADP but in addition that the ATPase activity in the preparation be minimal. If a preparation contained a highly active ATPase (which could be unrelated to the coupling mechanism) this would, in the presence of ATP, provide a continuously regenerated supply of ADP. Thus, the test suggested by CHANCE AND BALTSCHEFFSKY could result in a comparison of respiratory rates with two effectual concentrations of ADP. Their test would appear to be suitable for showing that respiration is controlled by the ADP concentration but inconclusive for proving the absence of such control.

Despite the objection mentioned above, it was considered pertinent to determine whether or not our preparations would show evidence of respiratory control even when measured as CHANCE AND BALTSCHEFFSKY[30] suggested. Since an oxygen electrode was not available for these studies a single addition of 20 μmoles of ADP was used in manometric measurements of respiratory rates before and after phosphorylation of the added ADP. The results of a typical experiment are shown in Fig. 1. Comparison of the respiratory rate in the presence of ADP (12–18 min) with the rate in the presence of ATP (28–50 min) indicates a respiratory-control ratio[30] of approx. 10. The oxygen uptake from the time of adding ADP until the terminal rate was established (28 min) corresponds to a P/O ratio of 2.8, assuming complete phosphorylation of the added ADP. It was observed in such experiments that a Mg^{++} concentration of $10^{-5}M$ permitted demonstration of high respiratory-control ratios. Omission of Mg^{++} led to low respiratory rates in the presence of ADP. With Mg^{++} at $10^{-4}M$ the respiratory rate did not decline during the experiments, presumably because ADP was regenerated continuously by the Mg^{++}-stimulated ATPase, which we have demonstrated in these

preparations. Serum albumin was added to the incubation medium in these experiments to reduce possible ATPase stimulation by even small amounts of the endogenous uncoupling agent[32] which might be formed during the incubation. Even a small amount of ATPase activity would lower the apparent respiratory-control ratio by maintaining a low concentration of ADP during the terminal part of such experiments. When serum albumin was omitted in these experiments, respiratory-control ratios of approx. 5 were obtained.

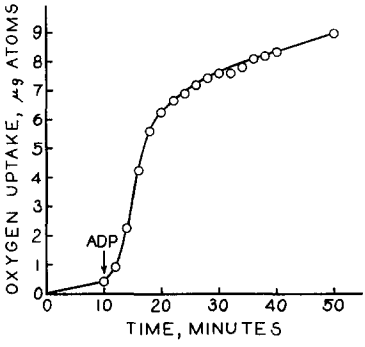

Fig. 1. Effect of ADP on respiration in mitochondria from housefly thoraces. Each flask contained 100 μmoles Tris, 1 μmoles ATP, 0.03 μmole $MgCl_2$, 250 μmoles sucrose, 100 μmoles potassium phosphate, 3 μmoles EDTA, 30 μmoles pyruvate, 2 μmoles fumarate, 45 mg serum albumin, and 7.6 mg mitochondrial protein. The pH was 7.4; total fluid volume, 3.2 ml including 0.2 ml of 20% KOH in the center well. Incubated at 25°. 20 μmoles of ADP were tipped in at the time indicated. Each point is the average of results from two flasks.

Respiratory control has thus been demonstrated in our preparations of washed housefly mitochondria through the use of two different procedures. These results are similar to those obtained by BUCHER *et al.*[13,25] who used mitochondria from locust flight muscle. It would appear that the mechanism of respiratory control in insect muscle may be the same as it is in other animals which have been studied, contrary to the proposals of CHANCE, SACKTOR AND ESTABROOK[10,12].

The reasons for the differences in oxidative activity and respiratory control between our preparations and those made by SACKTOR *et al.*[9,10,12], from housefly muscle tissue, are not understood completely. SACKTOR's mitochondrial preparations are capable of high P/O ratios but their respiration appears to have been released from dependence on a phosphate acceptor. Recently REMMERT AND LEHNINGER[28] have described a "releasing" factor, derived from mitochondria, which is capable of transforming mitochondrial respiration from the "tightly-coupled" to the "loosely-coupled" state. Many authors have shown the presence, in animal[32-34] and in insect[21] mitochondria, of an uncoupling agent or agents. The actions of both the releasing and uncoupling factor(s) are prevented by including serum albumin in the medium giving, with certain insect preparations, increased oxygen or phosphorus uptake, or both[1,21]. SACKTOR, O'NEILL AND COCHRAN[23] have reported an absolute requirement for a high level of serum albumin for phosphorylation in housefly mitochondria and suggested that this may reflect the presence of uncoupling factors in their preparations. The presence of uncoupling factors might partially explain the lack of respiratory control which was also observed with SACKTOR's preparations[9,10]. However, it should be noted that these preparations did not show respiratory control even in the presence of a high concentration of serum albumin.

The effect of serum albumin on oxidative phosphorylatin in our preparations of housefly mitochondria has been tested using both pyruvate and α-glycerophosphate as substrate. As shown in Table IV, the added protein gave slight or insignificant increases in the P/O ratio when pyruvate was used as substrate. With α-glycero-

phosphate, the added albumin always stimulated oxidation and phosphorylation and led to higher P/O ratios. However, with neither substrate was serum albumin required to obtain substantial phosphorylation and, as shown in Table III, high acceptor ratios are obtained with our preparations without the addition of serum albumin.

TABLE IV

EFFECT OF SERUM ALBUMIN ON OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA FROM HOUSEFLY THORACES

The flask contents were as described in Table I except that serum albumin was added where indicated. All values are averages from duplicate flasks.

Exp. number	*Serum albumin concentration (%)*	*O_2 consumed (μg atoms)*	*Phosphate esterified (μmoles)*	*P/O*
		With pyruvate as substrate		
141	0	21.5	59.4	2.76
	2	16.7	46.5	2.78
150	0	20.0	58.0	2.90
	2	20.4	61.5	3.01
152	0	21.0	62.0	2.95
	2	20.6	66.6	3.23
		With α-glycerophosphate as substrate		
141	0	14.8	17.2	1.16
	2	15.9	20.1	1.26
145	0	11.7	11.9	1.02
	2	14.7	16.4	1.12
147	0	8.7	8.0	0.92
	2	13.0	17.4	1.34

The most striking difference between our method of isolating flight muscle mitochondria and that used by SACKTOR is in the composition of the isolation medium; our medium being more complex, distinctly hypertonic, and well buffered. The mitochondria prepared in the two media differ markedly concerning respiratory control, as already noted. A parallel exists in investigations on *Locusta* mitochondria. REES'[20] earlier preparations of locust flight muscle mitochondria showed quite high P/O ratios but no dependence of respiration on the presence of ADP. These preparations were made in 0.20 *M* sucrose alone. The recent work of BUCHER's group[13,25] has shown that locust mitochondria do show respiratory control when isolated in a medium containing 0.30 *M* sucrose, 0.002 *M* EDTA and buffered at pH 7.2 with 0.01 *M* triethanolamine. Thus, it would appear that the medium and also the technique employed in isolating insect mitochondria may largely determine the Q_{O_2} and P/O values with different substrates, the requirement for serum albumin, and the degree of respiratory control which are observed with such preparations.

It can be seen that although it appeared only a few years ago, from the evidence then available, that insect mitochondria were "unique" in their ability to oxidize α-glycerophophate at many times the rate with pyruvate or citric acid cycle intermediates, in the mechanism of respiratory control, in their requirement for serum albumin and in their low P/O ratios compared to mammalian mitochondria, this view may no longer be tenable. Recent work has demonstrated that insect mitochondria can metabolize pyruvate and citric acid cycle intermediates at rates comparable with that

of α-glycerophosphate oxidation and with high P/O ratios; that they do not always require serum albumin and that they may utilize the same respiratory control mechanism which is found in other species. In fact, flight muscle mitochondria resemble mammalian heart mitochondria with respect to these properties as well as with respect to their ATPase pattern[35] and the composition of their respiratory chains[25,36].

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