# Brief Note

**The Effect of Temperature on Oxidative Phosphorylation with Insect Flight Muscle Mitochondria.** By Bertram Sacktor and Richard Sanborn. (*From the Medical Laboratories, Army Chemical Center, Maryland, and the Department of Biology, Massachusetts Institute of Technology, Cambridge.*)*

The effect of different temperatures on the biochemical activity and morphology of mitochondria is examined in this paper. Previously, it was shown that alterations in mitochondrial character could be induced by fluctuations in the tonicity of the isolation and reaction media, in concentration of inhibitory cations and in supplementation with stabilizing proteins (Potter and Recknagel, 1951; Harman and Feigelson, 1952; Dianzani, 1953; Cleland and Slater, 1953; Watanabe and Williams, 1953; Sacktor, 1954). In these earlier studies it was recognized that the mitochondrial suspensions should be prepared at low temperatures in order to attain maximum stability. Furthermore, Hunter and Hixon (1949) reported that higher P/O values were obtained at 15°C. than at 30°C. There is, however, little quantitative evidence available describing the relationship between temperature and mitochondrial response. This question was considered here by determining the effect of different temperatures on both oxidative phosphorylation and gross appearance of insect flight muscle mitochondria (sarcosomes).

## *Experimental*

Houseflies, *Musca domestica*, of mixed sexes were used. These were reared in the procedure described previously (Sacktor, 1950). Their sarcosomes were isolated by the techniques already detailed (Sacktor, 1953; 1954).

Measurements for oxygen consumption and inorganic phosphate uptake were made by the methods reported in an earlier paper (Sacktor, 1954). The reaction mixtures contained 20 $\mu$ moles inorganic phosphate, pH 7.4; 15 $\mu$ moles $MgCl_2$; 20 $\mu$ moles $\alpha$-ketoglutarate; 5 $\mu$ moles ATP, 50 $\mu$ moles glucose; 0.2 ml. hexokinase; 2 per cent bovine serum albumin; 0.5 ml. mitochondrial suspension; and 0.9 per cent KCl to a final volume of 1.5 ml.

Protein, in sarcosomal preparations, was determined by the method of Lowry *et al.* (1951).

## RESULTS

The effects of various temperatures on respiration and phosphate uptake with isolated mitochondria are shown in Table I.

The data demonstrate that the rate of oxygen consumption was enhanced as the temperature increased from 0°C. to 36.8°C. A similar response was observed for the disappearance of inorganic phosphate when measured from 0°C. to 25°C. At 31.4°C., however, the rate of phosphorylation was only slightly greater than at 25°C., and at 36.8°C. even less phosphate was esterified than at 25°C.

These relationships between temperature and respiration or phosphorylation

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are characterized more precisely by Arrhenius transformations of the data,

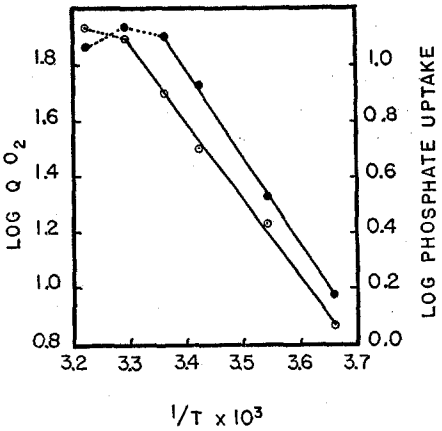


FIG. 1. Arrhenius transformations characterizing the relationship between temperature and oxygen or phosphate uptakes. $1/T$ denotes the reciprocal of the absolute temperature. Open and closed circles represent, respectively, oxidation and phosphorylation. The broken lines indicate changes from the simple activity-temperature response.

TABLE I

*Effects of Temperature on Respiration and Phosphate Uptake with Isolated Mitochondria*

| Temperature | No. of tests | $QO_2$ | | P uptake | |
|---|---|---|---|---|---|
| | | Average | Range | Average | Range |
| °C. | | *mm.³ $O_2$/hr./mg. protein* | | *μ mole P uptake/ 30 min.* | |
| 36.8 | 9 | 85 | 66–105 | 11.7 | 2.1–16.3 |
| 31.4 | 8 | 78 | 67– 94 | 13.7 | 9.5–16.6 |
| 25.0 | 8 | 51 | 42– 59 | 12.9 | 8.2–17.6 |
| 20.0 | 7 | 32 | 29– 38 | 8.5 | 6.9–10.9 |
| 10.0 | 4 | 17 | 15– 22 | 3.4 | 2.2– 5.3 |
| 0.0 | 2 | 7.5 | 7– 8 | 1.5 | 1.2– 1.8 |

shown in Fig. 1. As determined by the method of least squares, the equations that describe these lines up to 31.4°C. and 25°C., respectively, are:

(1) $\log Y_{QO_2} = 10.702 - 2.684x$

(2) $\log Y_{PO_4} = 9.975 - 2.687x$

It is evident that the slopes of the two lines are almost identical and the $\mu$ values for both oxygen and phosphate uptakes were calculated to be approximately 12,300 calories.

The parallelism of the regression lines describing the response of respiration and phosphorylation demonstrates the similar thermal behavior of the two processes. But Fig. 1 also shows that the oxygen and phosphate uptake mechanisms can be dissociated by elevated temperatures. At 31.4°C. phosphorylation was curtailed and equation (2) no longer characterized this activity. Furthermore, at 36.8°C., the rate of phosphate uptake actually decreased while the respiratory rate still increased. At this higher temperature, evidence for thermal inactivation of respiration also was noted. These changes from the simple activity-temperature response are illustrated in Fig. 1 by broken lines.

The uncoupling of phosphorylation from respiration is evident, additionally, from determinations of the ratio of phosphate uptake to oxygen uptake (P/O) at the different temperatures. At the lower temperatures the P/O values were approximately 1.2 whereas at 31.4°C. and at 36.8°C. they decreased to 0.8 and 0.6, respectively.

Concurrently with the observations described above, the sarcosomes were examined by phase microscopy. Although various morphological species were observed during the course of all experiments, it was apparent that at temperatures above 25°C. the mitochondrial population underwent a more rapid deterioration, characterized by swelling and paleness not unlike that induced by hypotonic media.

## DISCUSSION

The present study has furnished data on the effects of temperature on oxidative phosphorylation. It is apparent that the energy of activation is the same for both respiration and disappearance of inorganic phosphate. It is also clear that the phosphorylative mechanism is more labile at elevated temperatures and that temperature can be employed effectively to uncouple these two systems. Furthermore, with these flight muscle mitochondria the optimal temperature for studying oxidative phosphorylation is approximately 25°C.

The data also provide the basis for a comparison of the activation energy of a sarcosomal enzyme system with that of respiration in the living organism. Previously, Edwards (1946) determined the influence of temperature on the oxygen consumption of houseflies. From his data we derived the equation which describes this relationship. It is

$$(3) \quad \log Y_{Q_{O_2}} = 8.627 - 2.936x$$

The slope of this line is parallel to that of our respiratory data (19/20 level of confidence). The $\mu$ value for the intact fly is approximately 13,000 calories, and is not significantly different from the $\mu$ value of 12,300 calories which was obtained here for the oxygen uptake of isolated mitochondria. The present results are thus consistent with the hypothesis that the electron transfer mechanism concerned with this mitochondrial oxidation is of prime importance in the respiration of the intact insect.

## SUMMARY

The effect of different temperatures on the biochemical activity and morphology of insect flight muscle mitochondria was examined. It was found that respiration and phosphorylation have the same thermal response at temperatures of 25°C. and below. The energy of activation for both systems is approximately 12,300 calories. Oxidation and phosphorylation can be uncoupled effectively by temperature, for at temperatures above 25°C. there is more rapid heat inactivation of phosphorylation. This is evident from reduced P/O values as well as from morphological deterioration in the mitochondrial population. The thermal response of both this sarcosomal enzyme system and the respiration in the living fly are quantitatively similar.

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