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Oxidative Phosphorylation in Liver of Poikilothermic Animal

(Rana pipiens) [1]

by

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In homoiothermic animals, the efficiency of the electron transport system in forming high energy phosphate bond of adenosine triphosphate (ATP) from the energy stored in the glucose molecule, is about 40 per cent as calculated from the data of HUNTER (1951). This figure as compared with those for man-made machines indicates, according to KREBS and KORNBERG (1957) a very high degree of economy. On the other hand, presence of oxidative phosphorylation was also found in a number of plants and invertebrates, including the muscle of *Ascaris lumbricoides* (CHIN and BUEDING 1954), which is of particular interest, because this parasite is presumably devoid of cytochromes. In homoiothermic animals the rest of the energy (60 per cent) dissipates in form of heat; this must be connected with the maintenance of constant body temperature. Therefore it seemed to us of interest to investigate oxidative phosphorylation in a poikilothermic animal, to establish whether there exist particular adaptations which are different than those of homoiotherms. The existence of such a possibility is supported by the fact that mitochondria from different sources

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may have not only different structures (Palade 1952; DeRobertis et *al* 1962), but also the rate of enzymatic activity can be different, as shown by Green and Hatefi (1961) for oxidations, or by Tolani and Talwar (1963) for the activity of cytochrome oxidase in various parts of the brain.

As experimental animal we chose *Rana pipiens*, caught from January-March in the southwestern United States. We hoped that in winter frogs the differences, if they exist, would be more pronounced, because of the decreased rate of metabolism.

METHODS

The frogs were decapitated and thoroughly bled, the livers immediately removed, washed and kept in ice-cold physiological solution. Then they were quickly weighed in ice-cold pans and homogenized in chilled glass homogenizer, in ice, with a 0.25 M sucrose solution to make a 10 per cent homogenate. The latter was first centrifuged 10 minutes at 700 × g in order to remove intact cells, cell debris and nuclei: the supernatant was then centrifuged 10 minutes at 7000 × g in a Spinco Model L preparative ultracentrifuge. The pellet of mitochondria was resuspended in sucrose, centrifuged 10 minutes at 20000 × g and finally diluted in such way that 0.5 ml mitochondrial suspension corresponded to approximately 500-1000 mg of original wet weight.

The measurement of oxidative phosphorylation, as ratios of Phosphorus to Oxygen (P: O), was carried out according to Hunter (1955). Oxygen uptake was measured with the Warburg technique at 30°. The digest in each cup consisted of phosphate buffer at pH 7.4, 30 μmoles of substrate, the co-factors (NAD, NADP, Cytochrome c, ATP and Mg^{++}), NaF in concentration of 0.01M (to decrease the activities of adenosine triphosphatase and phosphatase). A trapping system of hexokinase and glucose was present. To this digest, 0.5 ml of mitochondrial suspension was added. The experiments were stopped after 20-25 minutes, when the amount of oxygen used corresponded to 5-10 μatoms. After addition of trichloracetic acid to precipitate the proteins, the contents of the cups were centrifuged and the inorganic phosphate measured by the method of Lowry and Lopez (1946).

RESULTS AND DISCUSSION

The results of our experiments are summarized in Table I from which it follows that the P: O ratio for pyruvate is 2.4 ± 0.23, of isocitrate 2.5 ± 0.10, α-ketoglutarate alone 2.3 ± 0.12, α-ketoglutarate + malonate 2.6 ± 0.07, for succinate 1.7 ± 0.06 and for malate 2.3 ± 0.26. These figures correspond in the intact cell to 3 molecules of ATP formed for each atom of oxygen with the substrate used and 2 molecules for succinate. These data are equal to those obtained with mammalian mitochondria, except for the step: α-ketoglutarate-succinate, which in rat liver is 4.0 (Copenhaver and Lardy 1952; Krebs et al. 1953), and in our case yielded P: O 3.0 even in the presence of malonate (added in half the molar amount of α-ketoglutarate). Although Slater and Holton (1954) found an identical P: O ratio for heart muscle, we are hardly inclined to accept 3.0 as the correct figure in our experiments.

Table 1.

P:O *ratios of Frog Liver Mitochondria with various substrates of the Krebs Cycle.*

In parentheses number of experiments.

Substrate	P: O observed	P: O in the cell
Pyruvate	2.4 ± 0.23 * (8)	3.0
Isocitrate	2.5 ± 0.10 (6)	3.0
α-ketoglutarate	2.3 ± 0.12 (16)	3.0
α-ketoglutarate + malonate .	2.6 ± 0.07 (32)	4.0 **
Succinate	1.7 ± 0.06 (5)	2.0
Malate	2.3 ± 0.26 (6)	3.0

* Standard error of the mean.
** For justification see text.

Controls with rat liver mitochondria under identical conditions yielded P: O ratio of 4.0, which seems to us to be the correct one,

because the possibility exists that, in our case, the preparative procedure for isolation of mitochondria, such as the concentration of sucrose, may have partly inactivated this step. In view of the fact that all the other substrates gave the same figures as those found for rat liver mitochondria, we are led to assume that this step also, with great likelihood, does not differ from the one in homoiotherms. Thus from our results we conclude that oxidative phosphorylation of the frog liver is qualitatively identical with that of mammalian liver and that no special adaptations exist as far as the efficiency of this system is concerned.

A comparison with hibernating animals may be of interest here: Allee, Emerson et al. (1949) expressed the opinion that "hibernating mammals become essentially poikilotherm during hibernation." Studies on such animals did not show any qualitative changes in enzyme systems, although there is a decrease in enzymatic activity. This is evidenced by the fact that the utilization of glucose decreases in hibernating animals, as indicated by its level in the blood: according to Zajączek (1961) there is a drop of 50 per cent (see his paper for an excellent review of this problem). On the other hand, the nature of basal metabolism seems to be very characteristic: Dontcheft and Kayser (1935) measured the RQ of hibernating animals (grey squirrel, marmot and hedgehog) and found it to be rather constant: 0.7 in all three cases. Similarly, Barthélemy and Bonnet (1924) showed that tadpoles maintained at temperatures between 8° and 21°, a treatment which shortens the disappearance of gills from 30 to 8 days, show no changes in basal metabolism, the RQ being 0.75. Bělehrádek in his book on temperature and living matter (1935) concludes that "it seems that temperature plays no part in the coefficient of utilization of energy in living system, nor in the utilization of nutritive substances." With these words, he stresses that there are no qualitative differences in the metabolism (as expressed by its RQ).

The experiments by Fleischmann (1937) in which he showed that the oxygen uptake in tissues of hibernating animals does not change, as compared to non-hibernating ones, seem to support our point of view that there are no qualitative differences in the metabolism of poikilothermic and homoiothermic animals. There is, however, the great probability that—since enzymatic processes

obey the Van't Hoff law—in cooler surroundings the rate of oxidative phosphorylation, i.e., the rate at which pyruvate molecules enter the Krebs cycle, may be lower.

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SUMMARY

Oxidative phosphorylation by mitochondria of livers from winter frogs (Rana pipiens) was measured in order to determine whether there exist in poikilothermic animals particular adaptations in the efficiency of the electron transport system as compared with homoiotherms.

P: O ratios obtained with pyruvate, succinate, ketoglutarate, malate and isocitrate were essentially the same as those found using rat liver mitochondria.

It is concluded that there are no qualitative differences between poikilothermic and homoiothermic animals with respect to efficiency of energy metabolism.

ZUSAMMENFASSUNG

Zur Abklärung der Frage, ob bei poikilothermen Tieren im Vergleich zu homoiothermen besondere Anpassungen in Bezug auf die Wirksamkeit des Elektronentransportsystems vorkommen, wurde die oxydative Phosphorylierung von isolierten Lebermitochondrien von Winterfröschen *(Rana pipiens)* bestimmt.

Die für Pyruvat, Succinat, Ketoglutarat, Malat und Isocitrat erhaltenen P: O-quotienten entsprachen im wesentlichen denjenigen von Mitochondrien aus Rattenleber.

Daraus wird geschlossen, dass zwischen poikilothermen und homoiothermen Tieren hinsichtlich der Wirksamkeit des Energiestoffwechsels kein Unterschied besteht.

RÉSUMÉ

La phosphorylation de mitochondries isolées du foie de *Rana pipiens* a été étudiée, pour déterminer si les animaux poïkilothermes diffèrent des homéothermes par l'efficacité du système de transfert d'électrons.

Les quotients P: O obtenus pour le pyruvate, le succinate, le kétoglutarate, le malate et l'isocitrate correspondent à ceux des mitochondries du foie de rats.

On en conclut qu'il n'y a point de différence entre l'efficacité du métabolisme énergétique des animaux poikilothermes et celui des homéothermes.

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