TABLE VI. Effect of Dinitrophenol on Fatty Acid Oxidation.

	μatoms O_2 60 min
Complete system	
+ 3 μM succinate	8.3
+ " " + 5 μM caproate	79.9
+ " " + " " + 6.5×10^{-6} M 2,4-dinitrophenol	8.3

Complete system as in Table IV.

TABLE VII.
Coupling of Oxidation and Phosphorylation.

Time, min	Inorganic phosphate utilized, μM	O_2 uptake, μatoms	P/O
0- 5	12.9	4.3	3
5-10	12.2	4.3	2.8
10-15	12.2	4.3	2.8

The complete system contained 1 ml enzyme preparation, .2 ml .1 M $MgCl_2$, .3 ml of .01 adenosine-5-phosphate, .4 ml of .1 M phosphate buffer, pH 7.4, .1 ml of .3 M NaF, .1 ml of M glucose, .05 ml of yeast hexokinase and 30 μM pyruvate. 25°C.

The higher level of magnesium requirement is the only unusual feature of the heart preparation. As prepared, the system contains the full complement of enzymes and coenzymes necessary to implement the complete oxidation of members of the citric acid cycle, fatty acid oxidation and oxidative phosphorylation. The rabbit heart system is not as stable under working conditions as the CM system of rabbit kidney. A marked decline in activity at 38°C is usually observed after some 30 minutes. The instability of the working system may be referable either to impurities in the preparation, or to the instability of the mitochondrial unit itself.

Summary. The preparation and some properties of the cyclophorase-mitochondrial system of rabbit muscle are described.

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Cyclophorase System XXIII. Correlation of Cyclophorase Activity and Mitochondrial Density in Striated Muscle.* (19375)

MILTON H. PAUL† AND E. SPERLING. (Introduced by P. W. Wilson.)
From the Institute for Enzyme Research, University of Wisconsin, Madison, Wisc.

Investigations in several laboratories(1-4) have established that mitochondria contain the complete enzymatic apparatus for implementing the reactions of the citric acid cycle and for generating oxidatively high energy phosphate bonds. The functional aspects of mitochondria are covered by the term "cyclophorase activity". Thus, some relation might be anticipated between the physiological function, and either the mitochondrial density or cyclophorase activity of that same tissue.

In this study, crude enzyme preparations were made from muscles of different physio-

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† Present address: Army Medical Service Graduate School, Army Medical Center, Washington, D.C.

logical activity. The oxidative activity was measured manometrically; the mitochondrial density of the same preparations was estimated with aid of the phase microscope.‡ The ability of the enzyme preparation to oxidize more than one substrate of the citric acid cycle was taken as a diagnostic test for cyclophorase activity. It was necessary in this study to test each enzyme preparation against several substrates simultaneously, since none of the tissues (with the exception of the myocardium(5) and pigeon breast muscle), yielded preparations active enough to oxidize these substrates to completion. The assumption was made that if the same enzyme preparation oxidizes more than one member of the citric acid cycle, the observed oxygen uptake in presence of a given substrate cannot be attributed exclusively to a one-step oxidation.

Experimental. Preparation of Enzymes. The tissue is blendorized for one minute in a small glass Waring blendor with 10 volumes of cold 0.9% potassium chloride. The suspension during this time is maintained neutral to bromthymol blue by addition of N sodium hydroxide. The tissue suspension is then passed through a double thickness of gauze to remove fibrous tissue, and centrifuged at 0°C for 8 minutes at 2500 x g. The supernatant is discarded, and the residue made up to a thick suspension with the addition of 0.9 percent potassium chloride. This suspension is used directly in the experiments. *Determination of Enzyme Activity.* The Warburg manometric technic was used for estimation of the enzymatic activity. Each manometer flask contained 1 ml of enzyme preparation, 0.2 ml of 0.1 M phosphate buffer of pH 7.4, 0.2 ml of 0.1 M magnesium chloride, 0.3 ml of 0.01 M adenosine-5-phosphate and water to a final volume of 3 ml, alkali in center well and oxygen in gas space. The reaction was carried out at 38°C. *Cyclophorase Quotient.* The standard of comparison in this study is an arbitrary quotient called the "cyclophorase quotient" which provides a basis for comparing the cyclophorase activities of different tissues.

$$\text{Cyclophorase quotient} = \frac{\text{dry wt total cyclophorase preparation (mg)} \times Qo_2\S}{\text{initial wet wt of tissue (g)}}$$

The dry weight of the cyclophorase preparation is calculated from the dry weight of 1 ml. Dry weight determinations (overnight at 110°) were carried out in duplicate. A correction is applied for the salt content of the preparation. The Qo_2 is determined on the basis of the rate for the first seven minutes following the initial equilibration period (7 minutes). *Estimation of Mitochondrial Density.* An aliquot of each tissue preparation is diluted 50 or 200 times with isotonic sucrose and examined under oil immersion lens with the phase microscope. Mitochondrial densities are rated as 0 (few or none observed), to ++++ (maximum number observed). The preparations contain, in addition to mitochondria, both nuclei and nuclear fragments and occasionally a few intact cells.

Results. Table I illustrates the correlation between the physiological activity of a muscle and both the cyclophorase activity and mitochondrial density of the tissue preparation.¶ The rabbit heart and diaphragm, and the pigeon breast muscle are called upon for strenuous rhythmic and prolonged performance, and accordingly require a vigorous, aerobic, oxidative mechanism. These tissues have been shown to have high cyclophorase quotients and abundant mitochondria. The

‡ Since the technic of mitochondrial counting has yet to be perfected our evaluation of mitochondrial density was only qualitative.

§ The cyclophorase quotient can serve only as the basis of a qualitative comparison of different tissues. The contribution of non-mitochondrial particulate elements to the dry weight of the residue is not constant from one muscle to another. However, since myosin constitutes the bulk of the residue in all the muscles compared even extreme variations in the level of other particulate elements would not change seriously the order of magnitude of the quotient. Another assumption underlying the quotient is that the activity per mitochondrion is fairly constant regardless of source. Studies in this and other laboratories provide strong support for this assumption. Finally, the conclusions drawn from differences in the values of the quotients are based on variations which are of a different order of magnitude than those which correction factors could account for.

¶ With exception of experiments on muscles of bat and mallard, all other experiments have been repeated using 3-5 individuals of the animals tested.

TABLE I. Cyclophorase Activity and Mitochondrial Density of Various Muscles.

Tissue	Cyclophorase quotient α-keto-glutarate	Cyclophorase quotient Succinate	Qo_2* α-keto-glutarate	Qo_2* Succinate	Mitochondrial density
Back muscle (white) rabbit	35	75	.5	1.1	0
Breast " " chicken	110	110	1.5	1.5	0
Gastrocnemius " rabbit	100	100	2.7	2.7	0
Soleus (reddish) "	85	120	3.4	4.7	+
Leg muscles (reddish) rat	85	220	2.5	6.7	+
Forearm muscles (red) bat	185	470	10.2	30.6	++
Diaphragm " rabbit	545	595	23	25	++
Breast muscle " mallard	875	1660	11.6	21.9	+++
Heart muscle " rabbit	1110	1820	34.5	56.7	++++
Breast muscle " pigeon	1830	2660	24	33	++++
Kidney, rabbit	2150	3080	28	40.3	++++

* Oxygen uptake in μl/hr/mg dry wt of suspension after correction for the salt content.

TABLE II.
Reproducibility of Cyclophorase Quotient.

Preparation	Cyclophorase quotient α-ketoglutarate
1	1110
2	1350
3	1230

muscles which are active only sporadically, such as skeletal muscles, generally have low cyclophorase activity and few mitochondria.

The breast muscle of birds provides a striking illustration of this thesis. In the pigeon (capable of sustained flight), the breast muscle has a cyclophorase activity as high as that of heart muscle and an abundance of mitochondria. In the chicken (limited and occasional flight), the cyclophorase activity of the breast muscle is very low, and there are few or no mitochondrial units which can be recognized by microscopic examination.

The reproducibility of the cyclophorase quotient is indicated in Table II by the results of determinations on three successive heart preparations.

Discussion. The correlation between the physiological activity of various types of striated muscle and their gross and microscopic characteristics(6) have long been emphasized. However, only more recently have the biochemical implications of these differences been considered.

It is remarkable that as far back as 1889, Knoll(7) made the following observations. "The abundance of interstitial granules" (undoubtedly referring to mitochondria in present terminology) "in the active muscles of the pigeon and the heart muscles of all animals points to a correlation between these interstitial granules and the function of the muscle." Kisch(8) has recently presented elegant electron microscope pictures of mitochondria in cardiac tissue.

Summary. The skeletal muscles which are used only sporadically generally have relatively low cyclophorase activity and a low mitochondrial count; whereas muscles which have to function either continuously or for prolonged periods (cardiac muscle, diaphragm, flying muscles), have a high cyclophorase activity and an abundance of mitochondrial units.

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