STUDIES ON THE CONTENT AND ORGANIZATION OF THE RESPIRATORY ENZYMES OF MITOCHONDRIA

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ABSTRACT

(1) The mathematical calculations relating spectrophotometric data with the data of Allard *et al.* (4, 5) on mitochondrial counts, is presented. Such a calculation indicates that an "average mitochondrion" from rat liver would contain about 17,000 molecules of each cytochrome pigment. (2) Hematocrit determinations relating respiratory pigment content for mitochondria isolated from a variety of tissues have been presented, showing a fivefold variability depending upon the source of the mitochondria. (3) Speculations on the organization of the respiratory enzymes associated with the membrane structure of the mitochondria are discussed.

Keilin (1), during his early studies on biological oxidation using heart muscle extracts, recognized that the cytochrome pigments were associated with the particulate material of the cell. Likewise, histologists, employing dyes of various types, such as Janus Green B, indicated the localization of the enzymes of oxidative metabolism with the particulates of the cell. Recently, the development of techniques for the fractionation of cell homogenates, coupled with the determination of various enzymatic activities, has substantiated these earlier observations. Thus, today it is generally accepted that the enzymes involved in biological oxidations, *i.e.* the cytochrome pigments a, a_3, b, c, and c_1, are associated with the membrane structure of the mitochondrion (see Ball and Barrnett (2) and Siekevitz and Watson (22) for summary). Nevertheless, few data have been available to relate the hypotheses of the biochemist, with specific regard to the organization of these particulate-bound enzymes, to the observations and measurements of the electron microscopists.

Two approaches to this problem have been carried out. One is the recent attempt to determine experimentally the localization of dehydrogenase enzymes within the mitochondria as represented by the studies of Barrnett and Palade (27). They observed, with the electron microscope, extremely dense particles presumed to be reduced tellurite resulting from the action of mitochondrial dehydrogenase systems. The other is the speculative approach first presented by Claude (28) and more recently applied by Green (30) to studies on heart muscle particles and by Lehninger (31) to studies on rat liver mitochondria and fragments derived from liver mitochondria by treatment with digitonin. The present paper is an extension of this latter approach.

During the course of experimentation designed to investigate the factors influencing the relationships of the phosphorylation process to the respiratory enzymes, data were obtained (3) which permit an *estimate* of the respiratory pigment concentration of mitochondria isolated from the livers of rats. These results taken together with the data of Allard *et al.* (4, 5) on the content of mitochondria in rat liver cells, can be used to

give an *estimate* of the respiratory enzyme content in an "average" liver mitochondrion. This paper presents the result as well as the assumptions implicit for these calculations. In addition, the relationship between pigment content and volume of packed mitochondria has been determined for mitochondria isolated from a variety of tissues These results, considered together with the electron microscopists' proposed representation of an "average" mitochondrion, permit one to speculate on the molecular organization of the respiratory pigments associated with the mitochondrial membranous structure.

PREPARATIONS AND METHODS

Mitochondria from liver were generally prepared in 0.25 M sucrose as described by Schneider (6). The mitochondria (sarcosomes) of heart were prepared in 0.32 M sucrose containing 1 mM versene, pH 7.4, following the procedure recommended by Slater and Cleland (7) while those from the flight muscle of the house-fly, *Musca domestica*, were prepared by the method described by Sacktor (8). Mitochondria from kidney were prepared by the method devised by Hollunger (24).

Protein concentrations were determined using biuret reagent previously standardized *versus* dialyzed serum albumin.

The pigment concentrations were determined from the difference spectra as described by Chance (9). This was accomplished using the wavelength scanning recording spectrophotometer designed and developed by Chance and his colleagues (10–12). All spectral and enzymatic studies were carried out at room temperature using an isotonic buffer containing 0.026 M Na^+, 0.099 M K^+, 0.006 M Mg^{++}, 0.108 M Cl^-, 0.013 M HPO_4^{--}, and 0.003 M $H_2PO_4^-$. Neutralized sodium glutamate, sodium succinate, and sodium adenosine diphosphate (ADP) were employed as indicated.

Volume measurements of the centrifuged pellets of mitochondria were made using Bauer-Schenck hematocrit tubes with samples of material being centrifuged for 1 to 1½ hours at 2900 R.P.M. (1900 *g*) in a refrigerated International centrifuge.

RESULTS AND DISCUSSION

Pigment Concentration: Fig. 1 shows the difference spectrum of the reduced minus the oxidized pigments of a rat liver mitochondrial preparation.

The methods of obtaining such spectra and the interpretation of these spectra in terms of individual cytochrome,[1] flavoprotein or pyridine nucleotide absorption bands have been described in detail by Chance and Williams (3). The wavelengths selected as representative of an individual pigment and used for the determination of the optical density difference between an absorption band maximum and a minimum or isosbestic point as well as the extinction coefficients employed are summarized in Table I.

Applying these conditions, the relative concentration of the pigments or the concentration in terms of milligram of protein may then be directly determined. The relative contribution of cytochromes *c* and c_1 (13) to such spectra has been discussed in a previous communication (14) where it was shown that the absorption band at 551 mμ is a composite, two-thirds of which is contributed by cytochrome *c* and one-third by cytochrome c_1. The concentration of the various pigments reduced in the presence of substrate upon attaining an anaerobic condition is presented in Table I and is expressed as mols per milligram of protein in Table II. These results represent the average for the data obtained for six different preparations of rat liver mitochondria; the pigment concentration was generally determined at four different dilutions for each preparation. The reproducibility of such results, as shown by the extreme maximal and minimal values which were obtained, indicates that the determination of the concentration of the cytochrome pigments varies about 20 per cent while the spectrophotometric determination of the concentrations of flavoprotein and pyridine nucleotide were more variable.

Mitochondrial Counts: The data of Allard *et al.* (4, 5) on the studies of mitochondrial population of rat liver cells are summarized in Table III. In order to relate these results to those presented above one must assume that the total nitrogen content (TNC) employed by Allard *et al.* may be converted to the biuret protein determinations employed here, by the factor of 6.25. With this assumption one may then calculate the concentration of any pigment in a single "average"-

[1] It has been estimated, from the influence of carbon monoxide on the spectrum of the reduced pigments, that cytochrome *a* contributes about 80 per cent of the absorption band at 605 mμ while cytochrome a_3 contributes about 20 per cent. The inverse applies for the absorption band at 444 mμ. For simplicity, the optical density change at 605 mμ will be considered as the contribution of cytochrome *a* while that at 444 mμ will be considered that of cytochrome a_3.

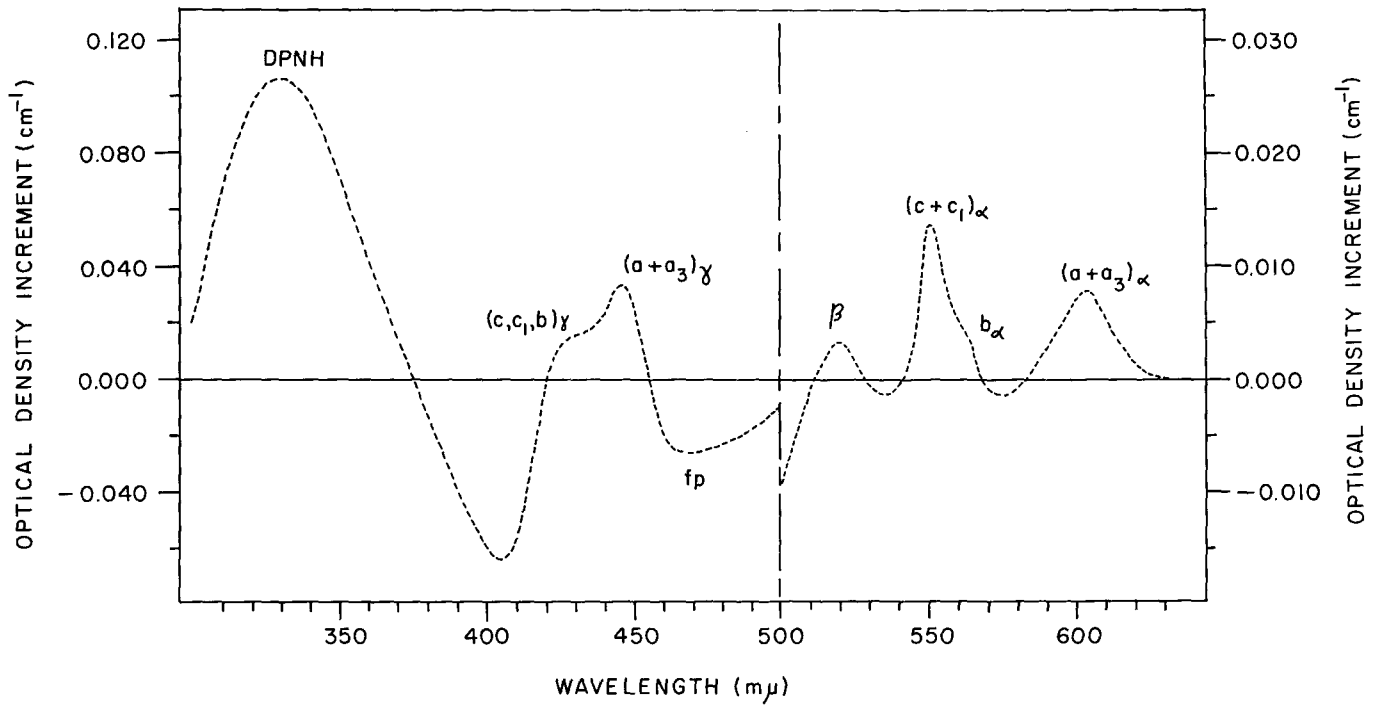

FIGURE 1

The difference spectrum of the reduced minus the oxidized pigments of a rat liver mitochondria suspension; 0.2 ml. of a suspension of mitochondria (47 mg. of protein/ml.) was diluted to 4.0 ml. with isotonic buffer and divided into two cuvettes; 0.03 ml. of a 0.1 M solution of ADP was then added to each cuvette and the base-line of equal light absorption was recorded. 0.02 ml. of a 0.5 M solution of sodium glutamate was then added to one cuvette. After utilization of the oxygen in this cuvette, the difference in light absorption of the reduced minus the oxidized pigments was recorded. Temperature, 23°; half-effective band width, 1 to 1.5 mμ.

TABLE I

Optical Density Changes of the Respiratory Pigments of Rat Liver Mitochondria

Pigment	Measuring wavelength*	Optical density change	ϵ mM/liter	Concentration	
				mM/liter of sample	M/liter of mitochondrial‡ suspension
Cytochrome *a*	605–625	0.008	16.0	5.0×10^{-4}	10.0×10^{-9}
Cytochrome *b*	562–575	0.005	20.0	2.5×10^{-4}	5.0×10^{-9}
Cytochrome $c + c_1$	551–540	0.015	19.1	7.8×10^{-4}	15.6×10^{-9}
Cytochrome a_3	444–455	0.048	90.0	5.3×10^{-4}	10.6×10^{-9}
Flavoprotein	460–500	0.032	11.5	28.0×10^{-4}	56.0×10^{-9}
Pyridine nucleotide	340–375	0.120	6.2	193.0×10^{-4}	386.0×10^{-9}

0.2 ml. of mitochondrial suspension was diluted to 4.0 ml. with isotonic buffer and placed in two cuvettes of a light path of 10 mm. All conditions are as described for Fig. 1.

* The convention employed in this paper is that the first mentioned wavelength represents an absorption band maximum for a pigment. The second mentioned wavelength represents an absorption band minimum or an isosbestic point. The optical density measurement recorded represents the difference in optical density change at the first mentioned wavelength minus that occurring at the second mentioned wavelength.

‡ Converted to mols per liter of original suspension of undiluted mitochondria.

mitochondrion. For the case of cytochrome *a* the calculation is as follows:

(1) The concentration of cytochrome *a* has been determined to be 2.1×10^{-10} mols/mg. of protein (Table II);

(2) The estimated number of mitochondria per milligram of protein, using the data of Allard *et al.* (Table III), is 7.2×10^{9};

(3) Therefore, there are 2.9×10^{-20} mols of cytochrome *a* per mitochondrion;

(4) Introducing Avogadro's number one calculates that there are about 17,000 molecules of cytochrome *a* per "average" mitochondrion.

Since the cytochrome pigments are generally considered (*cf.* Lehninger *et al.* (25)) to be of about equal concentrations this would indicate there are therefore about 17,000 respiratory chains or sequences in each mitochondrion (the low concentrations of cytochromes c_1 and *b*, as represented by the data presented in Table II, relative to cytochromes *a*, a_3, or *c*, may invalidate this general hypothesis). Since the sum of the concentrations of respiratory pigments is about seven to eight times the concentration of cytochrome *a* one may calculate that there would be about 130,000 molecules of respiratory pigments enzymatically reducible in each mitochondrion. From this data one may also estimate that there is an average of about 500,000 molecules of intramitochondrial pyridine nucleotide in each mitochondrion.

In the process of estimating the above values the following assumptions have been made:

(1) That the data of Allard *et al.* (4, 5) may be applied to the data obtained during the spectrophotometric studies presented here;

(2) That the total nitrogen content values of Allard *et al.* are related to the protein concentration determined with biuret reagent by the factor 6.25;

(3) That the extinction coefficients employed are correct.

The only comparative figures for those calculated here are presented by Claude (28) who estimated an average volume of $0.4\mu^3$ and a density of 1.2 for mitochondria. Claude calculated, using an average molecular weight for each protein of 35,000, the possible localization of 2000 sequences of the type represented by the cytochrome chain. Lehninger (31) has also indicated in a recent review that there may be several thousand such respiratory assemblies in a single rat liver mitochondria. For such calculations, such as those performed by Claude (28) or as presented here, it must be stressed that they apply only for the nebulous term "average mitochondrion." No consideration of variation in mitochondria has been included in this calculation.

Volume of Mitochondria: One means of assessing the validity of some of the above assumptions is to determine the volume occupied by mitochondria in a given suspension and relate this to the number

TABLE II

Concentration of Pigments of Rat Liver Mitochondria

Pigment	Average concentration *mols/mg. of protein*	Minimal and maximal values *mols/mg. of protein*	Relative concentration*
Cytochrome *a*	2.1×10^{-10}	1.8 to 2.3 $\times 10^{-10}$	1.0
Cytochrome *b*	1.0×10^{-10}	0.8 to 1.3 $\times 10^{-10}$	0.5
Cytochrome *c*‡	2.0×10^{-10}	1.8 to 2.3 $\times 10^{-10}$	1.0
Cytochrome c_1‡	1.1×10^{-10}	0.9 to 1.2 $\times 10^{-10}$	0.5
Cytochrome a_3	2.0×10^{-10}	1.7 to 2.3 $\times 10^{-10}$	1.0
Flavoprotein	6.9×10^{-10}	5.3 to 10.0 $\times 10^{-10}$	3.3
Pyridine nucleotide	63.0×10^{-10}	34 to 88 $\times 10^{-10}$	30.0

* The concentration of cytochrome *a* has been taken to be 1 and all other concentrations are therefore expressed in relation to cytochrome *a*.

‡ These values are calculated assuming that two-thirds of the absorption band with a maximum at 551 mμ is contributed by cytochrome *c* and one-third by cytochrome c_1 (13).

of mitochondria estimated from the protein determination. An example of such a calculation is as follows:

(1) A 0.5 ml. suspension of rat liver mitochondria (23.5 mg. of protein) suspended in 0.25 M sucrose, when centrifuged for a hematocrit determination, showed an average pellet volume of 0.15 cm.^{3};

(2) From Table III one can calculate that there would be 1.1×10^{12} mitochondria/cm.^{3} in this packed pellet;

(3) The "average" volume of a mitochondrion in this sample, therefore, is 9.1×10^{-13} cm.^{3}.

The electron microscopists' picture (15) presently considered as representative of a rat liver mitochondria, shows a cylindrical form measuring about 1 to 4 μ in length and 0.3 to 0.7 μ in diameter. If the radius of such a mitochondrion is considered to be about one-tenth that of its length, and if one applies the formula for the volume of a cylinder ($V = \pi r^2 h$) the size of the "average mitochondrion" from the calculation above of 9.1×10^{-13} cm.^{3} shows *r* equal to 0.31 μ and *h* equal to 3.1 μ. These values agree very well with those proposed above from the observations of the electron microscopists.[2] Similar results were obtained when the experiment was repeated twice more with other preparations of rat liver mitochondria. This calculation assumes a tight packing of the mitochondria in the pellet and does not consider the volumes occupied by the fluid trapped between each mitochondrion.[2] An attempt to minimize this correction was carried out by centrifugation of the samples for about 1 hour more to insure that packing of mitochondria was optimal.

Pigment Concentration per Unit Volume: The hematocrit technique described in the preceding section may also be used to obtain a value of pigment concentration per unit volume of mitochondria and may thus serve as a means of correlating the pigment concentration of mitochondria isolated from a variety of tissues. A summary of the results of a series of such experiments is presented in Table IV. In addition, protein concentration, expressed as grams of protein per 100 ml. of packed mitochondrial pellet, are included

TABLE III

The Mitochondrial Population of Liver (from Allard et al. (4, 5))

No. of mitochondria/cell	2554
No. of cells/gm. of fresh liver	133.0×10^6
No. of mitochondria/gm. of fresh liver	33.0×10^{10}
Mg. (TNC)/mitochondrion	22.3×10^{-12}

The mg. of protein/mitochondrion is obtained by multiplying 6.25 times the mg. (TNC)/mitochondrion giving a value equal to 1.39×10^{-10} mg. By inverting this value one obtains the number of mitochondria/mg. of protein as 7.2×10^9.

[2] Under the hypotonic conditions of these experiments it is more probable that the mitochondria are not rod shaped but rather spherical. The calculation of the volume occupied by a sphere shows that the average diameter of the mitochondrion in this instance would be 1.2 μ. Consideration of the trapped fluid between mitochondria would introduce, in this case, a correction of about 20 per cent in the calculated diameter.

TABLE IV

Pigment Concentration of Mitochondria from Various Sources

Source	Cytochrome *a* concentration *mols/liter of mitochondrial pellet*	Protein concentration *gm./100 ml. of mitochondrial pellet*	Cytochrome *a* concentration *mols/mg. of protein*
Flight muscle	8.3×10^{-5}	13.7	6.1×10^{-10}
Rat heart	7.8×10^{-5}	14.0	5.6×10^{-10}
Rat liver	2.8×10^{-5}	15.2	1.9×10^{-10}
Guinea pig liver	1.6×10^{-5}	19.8	0.8×10^{-10}
Mouse liver	3.4×10^{-5}	17.0	2.0×10^{-10}
Guinea pig kidney	3.6×10^{-5}	15.3	2.4×10^{-10}

in Table IV. The data on pigment concentration is presented only[1] for cytochrome *a*, as measured by the magnitude of the absorption band at 605 mμ in the difference spectrum (*cf.* Fig. 1). Since the relative concentration of the other hemoproteins to cytochrome *a* has been given, a similar correlation can generally be obtained for any pigment chosen.

Since no correction for fluid trapped between mitochondria has been included in the calculation of the molar concentration of pigments in the mitochondrial pellet, these values may be as much as 40 to 50 per cent higher than calculated. In a similar manner a like correction would apply to the determined protein concentrations.[3]

It is apparent from the data presented in Table IV that there is considerable variation in pigment concentration per unit volume of mitochondria isolated form a variety of tissues. The highest values are obtained with mitochondria isolated from the hearts of rats or insect flight-muscle sarcosomes while the lowest value is obtained with mitochondria isolated from the liver of guinea pigs. Of interest is the variation observed for mitochondria isolated from the same organ (the liver) but from different animal species (rat, mouse, and guinea pig). This variation agrees with that observed by Drabkin (16) on the content of cytochrome *c* as isolated from various tissues of animals of different species. Also of interest is the narrow range of variation of the protein concentrations. One may conclude from this series of experiments that the protein concentration, when expressed as grams of protein per 100 ml. of mitochondrial pellet, is remarkably constant, varying from 14 to 20 per cent. The cytochrome content, however, when expressed per unit volume of mitochondria varies by a factor of about 5, ranging from 1.6×10^{-5} mols per liter of mitochondrial pellet to 8.3×10^{-5} mols per liter. Although one might predict from these results on the variation in pigment content that there might be observable, by the electron microscope technique, rather large variations in the internal membrane structure of the mitochondria of the various tissues, the appearance of such a small variation in the protein concentrations would tend to speak against such a conclusion. The observations that have been made by Palade (17), who speculated on the variability observed in terms of different enzyme concentrations per unit volume of mitochondria, may be explained by the hypothesis that the cytochrome content is an index of insoluble enzymes associated with the mitochondrial membrane. Since the protein concentration determined here is the sum of both soluble and insoluble protein one would conclude that those mitochondria which have a high concentration of cytochromes per milligrams of protein (flight muscle and heart muscle) contain much lower concentrations of soluble protein than those mitochondria containing low cytochrome concentrations (liver).

Although there is a marked difference in respiratory enzyme content in mitochondria isolated from a variety of sources, measurements on the activity of these enzymes (using mitochondria isolated from the livers of rat and guinea pig as well as those of kidney and heart) show a remarkable similarity (3). Studies of oxygen uptake during optimal conditions of oxidative

[3] It is interesting that Shelton *et al.* (23) have determined, using different assumptions and means of measurement, that the concentration of cytochrome *c* in mouse liver mitochondria is 2×10^{-5} M/liter. When a correction factor of 40 per cent for intermitochondrial fluid is applied to the data presented in Table IV the values agree very well.

phosphorylation have shown however that the turnover numbers for the cytochromes, using a substrate such as sodium succinate, are very nearly constant varying for cytochrome *c* from about 16 to 20 seconds^{-1} for those tissue sources mentioned above. This raises a problem of interest to those concerned with the influence of increasing the spatial proximity of the enzymes by closer packing of the molecules to their respective enzymatic activities.

Speculation on the Organization of Respiratory Enzymes: The proposed size of an "average mitochondrion" of rat liver has been mentioned above, *i.e.*, a cylindrical form measuring 1 to 4 μ in length and 0.3 to 0.7 μ in diameter. Again taking the mean of these values and assuming that a mitochondria may be considered a cylinder, one can calculate that the surface area exposed by the external membrane of the mitochondrion is about 5.5×10^8 A^2. In addition to an external membrane the electron micrographs have shown (15, 18) that mitochondria contain a double membrane structure. There are also present what Palade (15) has termed "cristae mitochondriales" increasing the effective surface area available for those enzymes associated with the membrane of the mitochondrion. If, however, one considers these cristae as lamellae, perpendicularly bisecting the cylinder, the area increase per lamella would be about 0.8×10^8 A^2. These data, taken together with measurements on the thickness of the membranes of the mitochondria, indicating that the double membrane is about 160 A thick (15, 18) with the thickness of each of the osmium dense layers being about 45 A with a less dense area of about 70 A between these osmiophilic dense areas, may be applied to a speculation on the organization of the oxidative enzymes.

A point which bears upon this subject is the series of observations indicating that the respiratory enzymes, *i.e.* the cytochromes and flavoproteins, are associated (22) with the mitochondrial membranous structure, a question which has been firmly resolved by the studies of Ball and Barrnett

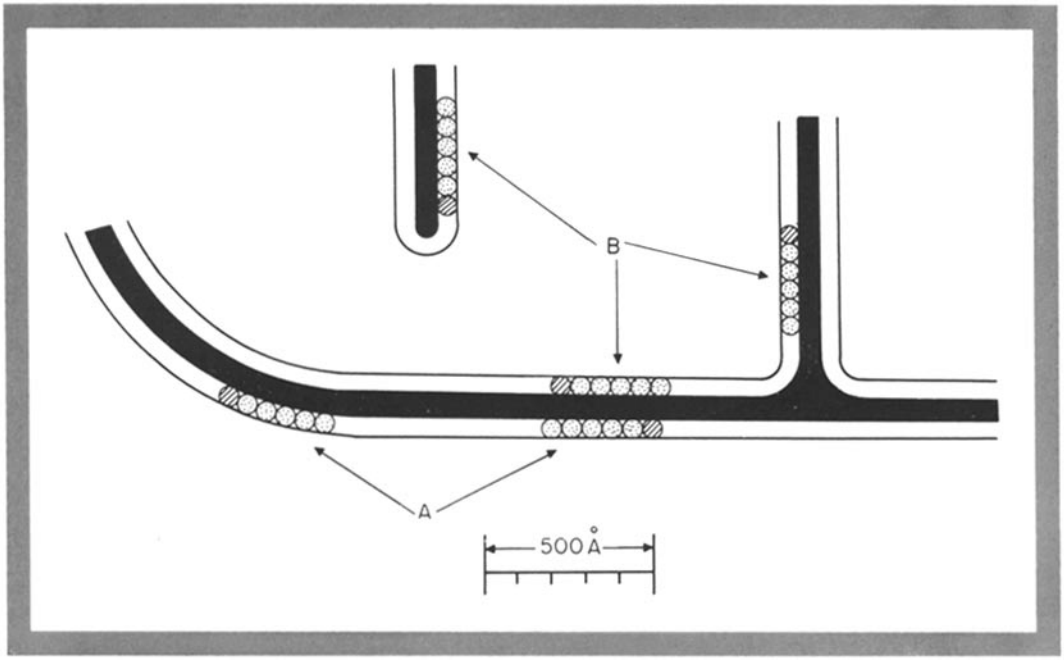

FIGURE 2

A schematic proposal for the respiratory pigment distribution in the mitochondrial membrane structure. The diagramatic representation, proposed by Sjöstrand and Rhodin (18), has been employed but modified so that the black areas represent areas of high lipid concentration while the circles, of about 60 A diameter, represent cytochrome or flavoprotein molecules. The details of this figure are discussed in the text.

(2) and Siekevitz and Watson (22). These pigments are presumed to be bound with lipids (30) and are, according to present speculation, of rather high molecular weight (cytochrome *c*, the only member of the respiratory chain to be isolated in relatively pure form is, however, of rather low molecular weight, *i.e.*, 12,000).

In order to correlate the data on the number of molecules per mitochondrion with the area available one must speculate on the size of each respiratory enzyme. The most closely related compound to a cytochrome which has been fully characterized is that of crystalline hemoglobin. X-ray crystallographic studies have shown (19) that crystalline hemoglobin with a molecular weight of 64,000 has the dimensions 55 A × 55 A × 70 A. Taking the smaller dimensions for the hemoglobin molecule, it would present a surface area 2.4×10^3 A². The third dimension of 70 A would correspond to the thickness of one of the osmium dense layers of the mitochondrial membrane. Applying this assumption that each oxidative pigment is similar in size to a hemoglobin molecule, the calculation shows that the respiratory pigments would occupy an area of about 3.3×10^8 A². This value corresponds very closely to the surface area of the external membrane of the mitochondria, *i.e.*, 5.5×10^8 A².

The following points, however, must be considered. Are the respiratory enzymes associated with the external membrane of the mitochondrion? Since there is but a very slow reaction of the endogenous respiratory chain with exogenous cytochrome *c* (20) and since the respiratory chain of liver mitochondria is relatively unreactive with externally added diphosphopyridine nucleotide (DPNH) (3, 20, 21) one would conclude that the reactive sites of the cytochrome pigments and their associated flavoproteins could not be a part *per se* of the external membrane of the mitochondrion. Second is the question of the interpretation of the electron micrographs. The locali-

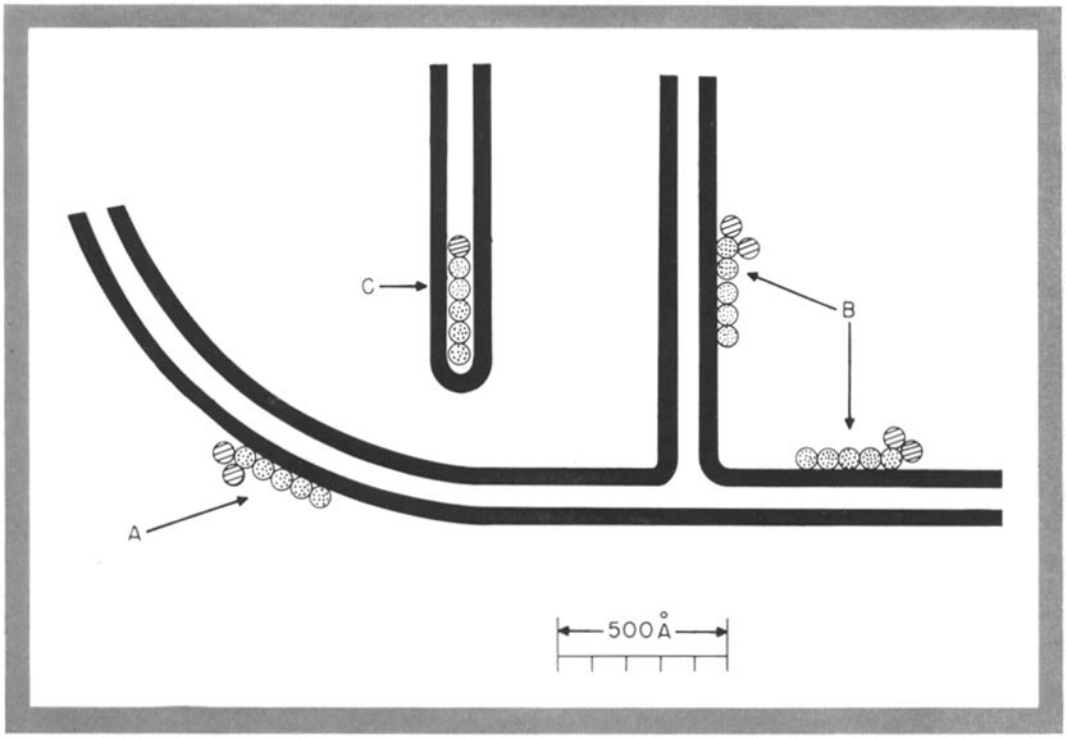

FIGURE 3

An alternative schematic proposal for respiratory pigment distribution in the membrane structure of the mitochondrion. As in Fig. 2 the blackened areas represent areas of high lipid concentration while the circles represent either molecules of cytochrome or flavoprotein.

zation of lipid and protein and their association with osmium dense areas as seen in the electron micrographs is still controversial (2, 26, 29). Therefore, any speculation involving the exact location of enzymes must be tendered with reservations. If one considers that the double membrane observed in mitochondria results from a lipid layer embedded within two layers or protein, one internal and the other external to this lipid layer, such as Sjöstrand and Rhodin (18) have proposed, then the organization of the respiratory chains may be visualized as shown in Fig. 2. The other alternative is that the osmium dense areas represent areas of high lipid concentration. In this latter case one may picture the organization of the respiratory pigments as shown in Fig. 3. In both Figs. 2 and 3 the schematic representation proposed by Sjöstrand and Rhodin (18) has been employed with the modification that the blackened areas represent high concentrations of lipids rather than the usual areas of high concentration of osmium deposition. In these figures the circles represent protein molecules of about 60 A diameter; those with dots within them representing cytochromes while those cross-hatched represent flavoproteins. In either of the situations presented in Figs. 2 and 3 one may exclude Case *A*, *i.e.* the choice where the respiratory pigments are associated with the external membrane of the mitochondria, based on the low reactivity of the respiratory carriers with either exogenous cytochrome *c* or DPNH as discussed above. Likewise it is unlikely that reactive sites of the respiratory carriers are embedded within two layers of lipid (Fig. 3, case *C*) for there would be a very limited reactivity of the respiratory chain with the endogenous pyridine nucleotide presumed to be distributed in the matrix of the mitochondria. Thus one is left with the possibility that the respiratory pigments are associated with the internal of the double mitochondrial membrane structure as shown in either Fig. 2 or 3 as case B. This conclusion is in agreement with the observations of Barrnett and Palade (27) who localized areas of deposition of reduced tellurite resulting from the action of mitochondrial dehydrogenases and concluded that the small dense particles, presumably of reduced tellurite and thus indicative of dehydrogenases, "lie in the matrix in close relationship to the membrane outlining the cristae." This would also conform with the representation presented by Green (30) who considers many of the electron carriers as lipoproteins, possibly employing the lipid layer as the common cohesive substance for the cytochrome sequence.

The above hypothesis presents many interesting questions which, although they are relevant, are not answered by these speculations. One is the question of the relationship of the enzymes of the oxidative phosphorylation process which one tacitly assumes must be intimately associated with the respiratory enzymes. In addition, one must account for the multitude of enzymes of the citric acid cycle as well as those for fatty acid oxidation, many of which are also insoluble enzymes and presumed to be associated with the mitochondrial membrane. All these increase the demand on the postulated surface area available in each mitochondrion. Indeed, if there were

TABLE V

Percentage Contribution of Pigment Protein to Total Protein

Source	Total pigment concentration* *mols/liter of mitochondrial pellet*	Calculated‡ grams pigment *protein/liter of mitochondrial pellet*	Per cent of total mitochondrial protein
Flight muscle	5.8×10^{-4}	37	27.0
Rat heart	5.5×10^{-4}	35	25.0
Rat liver	2.0×10^{-4}	13	8.5
Guinea pig liver	1.1×10^{-4}	7	3.5
Mouse liver	2.4×10^{-4}	15	8.8
Guinea pig kidney	2.5×10^{-4}	16	10.5

* The total pigment concentration is determined as seven times the concentration of cytochrome *a* presented in Table IV.

‡ Based upon the assumption that the average molecular weight of each pigment protein is 64,000.

twenty lamellae in an "average mitochondrion," the respiratory pigments alone, assuming one is justified in selecting hemoglobin as a representative size, would occupy about 9 per cent of the available surface area presented by the membrane in contact with the matrix fluid.

It is of interest that a calculation of the per cent protein of the mitochondria, using the molecular weight of hemoglobin (64,000) as an average for each pigment, shows that the respiratory pigments make up a considerable proportion of the protein concentration of the mitochondria (Table V). Lehninger in a recent review (31) has stated that he has carried out a similar calculation assuming 100,000 as the molecular weight and concludes that the respiratory pigments may represent nearly 20 per cent of the protein of a rat liver mitochondrion.

It must be emphasized that one should consider the present paper as purely speculative and representative only of a static picture of the mitochondrion. It is hoped that some of the data presented here may stimulate further investigations and lead eventually to an understanding of the complex arrangement of protein molecules within the mitochondrial membrane. Subsequent studies on the relationship of structures to function of the respiratory enzymes may assist in resolving some of these questions.

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