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Flavin and cytochrome contents in the mitochondria of the heart and liver

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Abstract

With a certain fixed methods of analyses, we carried out the determination of flavins and cytochromes in the mitochondria (Mt) and electron transfer particles (ETP) of the heart and liver of rats and cows, and made a comparison of the data with one another. Our findings may briefly be summarized as follows. 1. The concentration of each component of the beef heart mitochondria proved to be 0.47 for acid extractable flavins; 0.22 for acid nonextractable flavin; O. 75 for cytochrome (cyt.) a; 0.58 for cyt. b; and O. 51 for cyt. C + Cl, all units being mμ mole per mg of protein. 2. In the beef liver mitochondria it was 0.46 for acid extractable flavins; 0.18 for acid non-extractable flavin; 0.092 for cyt. a; 0.089 for cyt. b; and 0.122 for cyt. C+Cll likewise all units in term of mμ mole per mg of protein. 3. In the case of rat heart mitochondria, it was found to be O. 42 for acid extractable flavins; 0.22 for acid non-extractable flavin; 0.88 for cyt. a; 0.41 for cyt. b; and 0.62 for cyt. C + Cll all in mμ mole per mg of protein. 4. In the rat liver mitochondria it was 0.56 for acid extractable flavins; 0.19 for acid non-extractable flavin; 0.20 for cyt. a; 0.14 for cyt. b; and 0.19 for cyt. C+Cl. 5. The concentration ratios of Fs, cyt. a and cyt. b of the mitochondria, what are considered to be intrinsic and fixed components of the mitochondrion. to those of the electron transfer particles were 1. 3 in both the beef heart and the rat heart, while 2.2 in the beef liver and 2.1 in the rat liver. 6. These findings were compared with the data reported by other workers, and also a discussion was made on the molecular organization of the mitochondrial inner membrane.

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FLAVIN AND CYTOCHROME CONTENTS IN THE MITOCHONDRIA OF THE HEART AND LIVER*

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The most fundamental function common to all mitochondria is energy transduction by respiration, but depending on the kind of cells mitochondria show a considerable difference in the structure and function. For the elucidation of this fundamental function of mitochondria as well as for a better understanding of mutual relationship of the structure to the function, it is necessary to accurately measure the respiratory enzyme contents of mitochondria in various tissues. We find several reports[1~5] dealing with the measurements of respiratory enzyme contents in various tissues, but as the methods of measurements and calculations differ by individual investigators, it is not so easy to compare these data with one another. In addition, there is hardly any report giving comparative values of such enzyme contents in various tissues by systematic assays conducted with the same method. Using mitochondria isolated in a fairly pure form from the heart and liver tissues of cow and rats we conducted quantitative assays of the repiratory enzymes contained in these mitochondria with special emphasis on the contents of flavins and cytochromes *a*, *b* and $c+c_1$, and present the results of our comparative study of these data in this paper.

MATERIALS AND METHODS

Mitochondria were isolated from the fresh heart and liver of cow and rats by the respective method mentioned in the following, and all the isolations were done at the temperature of 0~4°C.

Isolation of beef heart mitochondria : The isolation was conducted by a modified method of CRANE *et al*[6]. Namely, with the fresh beef heart obtained directly at a slaughter house the heart muscle (about 700g) obtained after removing atrium, epicardium, fatty tissues under the epicardium and endo-

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cardium, was chopped fine and washed with 0.25M sucrose solution (containing 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.6) previously cooled to 0°C, and the chopped pieces were minced, and blended 1～2 minutes in a homoblender (Nippon Seiki Co., Ltd.) at top speed. In blending the medium was added in such amount as to make its final volume 2 to 3 liters. The homogenate so obtained was subjected to the step-wise filtration through a double layer of gauze and Tetron cloth to remove muscle fibers, and the pH of the filtrate was adjusted to 7.4 by adding 5N KOH to it. This filtrate was fractionated in Kubota refrigerated centrifuge KR-6P, Rotor No. 8; Spinco Model L-2, Rotor 19; or Kubota continuous flow centrifuge KCF-62. The centrifugation was done at 700 x g for 10 minutes (in the case of continuous flow centrifugation, at 900x g, flow speed 140 ml/10 min) to remove nuclei, unbroken muscle and red cells. The supernatant was further centrifuged at 7, 000 x g for 10 minutes (in the continuous flow centrifugation at 9, 000 x g, flow speed 140 ml/10 min) and the mitochondrial fraction was obtained as residue. Since the sediment after 700 x g centrifugation contained a considerable amount of mitochondria, it was resuspended in the isolation medium by gentle homogenization in a Teflon homogenizer and centrifuged at 700 x g for 10 min. The supernatant was then centriguged at 7, 000 x g for 10 min. and the residue thus obtained was combined with the mitochondrial fraction described above. The mitochondrial fraction harvested in this manner is suspended in about 700 ml of 0.25 M sucrose solution containing 0.05% bovine serum albumin by gentle homogenization with a loosely fitted Teflon homogenizer moving it up and down once. This suspension is superimposed on approximately an equal volume of 0.34 M sucrose solution, centrifuged at 500 x g for 10 min, and the suppernatant as far as the oblique surface of the sediment is decanted by aspiration, and is further centrifuged at 7, 000 x g for 10 min. By this procedure it is possible to obtain a mitochondrial fraction practically free of red cells. This fraction is again suspended in a 0.25 M sucrose solution neutralized with KOH. The mitochondrial fraction was washed 1～2 times with neutralized 0.25 M sucrose solution in Kubota refrigerated centrifuge KR-6P, Rotor No. 3, resuspended in a 0.25 M sucrose solution, and stored at −20°C to be used for the estimation of components by thawing wherever needed. In the case where the first mitochondrial fraction is collected by the use of Kubota refrigerated centrifuge KR-6P, Rotor No. 8 or Spinco Model L-2, Rotor 19, if red cells, nuclei and unbroken cells that gather at the bottom layer are carefully removed by several washings, an equally good mitochondrial preparation can be obtained even omitting the superimposing procedure.

The isolation of rat heart mitochondria : The isolation was carried out by a modification of Hagihara's method[7]. With 5～10 fresh rat hearts after

removing the atrium and epicardium as far as possible these are chopped into fine pieces in a small amount of 0.25 M sucrose solution containing 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4, and 2 mg of crystalline bacterial proteinase (Nagarse product) per g wet weight of these pieces are added and incubated at 0°C for 20 min. Then proteinase is removed as well as possible by washing with 0.25 M sucrose solution several times. The enzyme-treated heart muscle is added with about 10-fold volume of the isolation medium as used in the isolation with beef heart, and is homogenized in a glass homogenizer by moving up and down 3~5 times, again placed in a Teflon homogenizer and homogenized gently up and down 3 times. The homogenate so obtained is centrifuged in a Kubota refrigerated centrifuge at 60 x g for 10 minutes, and the supernatant is superimposed on a 0.34 M sucrose solution and centrifuged at 700 x g for 10 min, and nuclei, unbroken muscle and red cells are removed as residue. From the supernatant the mitochondria were obtained and washed just as in the case of beef heart mitochondria.

Isolation of liver mitochondria : For the isolation of liver mitochondria both from beef and rat livers a slightly modified method of HOGEBOOM[8] is employed.

Preparation of the electron transfer particles (ETP): ETP were prepared by a slightly modified form of the method of GREEN *et al*[9]. Namely, the mitochondria suspended in 0.25 M sucrose solution is sonicated with an ultrasonic oscillator of Kaijo Dempa Co., Ltd, (7ψ tip, 20 Kc, 1 min/ml of mitochondrial suspension), then centrifuged at 26, 000 x g for 10 minutes to remove unbroken mitochondria, further centrifuged at 140,000 x g for 30 minutes. The ETP fraction thus obtained is suspended in a 0.25 M sucrose solution, and stored at −20°C to be used for the estimation of the respiratory enzymes, which is carried out as in the case with mitochondria.

Estimation of flavins : For this purpose a slight modification of the method of BLAIR *et al*[4]. is used. Unless otherwise mentioned, all the procedures are carried at 0~4°C under dim light. One sample is used for the assays of acid extractable flavins and acid non-extractable flavin, and another sample serves for the assay of total flavins.

After adding 2 ml distilled water to 1 ml mitochondrial suspension (about 40mg protein), the test tube containing the mixture is dipped in a boiling water bath for 3 minutes, immediately cooled to 0°C, to which 0.5 ml of 50% TCA is added, mixed for 10 minutes, and after leaving it standing for 15 minutes, it is centrifuged at 70, 000 x g for 10 min. The supernatant containing the acid extractable flavin is decanted into a 10 ml-brown color test tube. Two ml of this are mixed with 0.16 ml of 6 N KOH and about 0.4 ml of 1 N Na_2HPO_4,

final pH near 7.4, and distilled water is added to this mixture to make its final volume 3.0 ml. The sediment remaining after the extraction is washed with 6 ml absolute alcohol, and then with 6 ml of water twice. The washed residue is suspended in 1 ml of 0.1 M phosphate buffer (pH 7.4) by homogenization and protein estimation of the suspension is done. To this 0.1 M phsophate buffer, pH 7.4, is added until its final volume is 2.0 ml, and by adding 20 mg trypsin (1—250) to it, is digested at 38°C for 30 min, further an equal volume of trypsin is added and digested again at 38°C for 30 min, and then after cooling it to 0°C the extraction is conducted with TCA in the identical manner as described previously. The extraction of total flavins is conducted directly with mitochondria by the same method as employed in the extraction with the washed residue mentioned above. Flavins so extracted are estimated by a Shimazu spectrophotometer QR-50. Sodium dithionite serves as the reducing agent. The absorbance was determined in the oxidized, reduced and reoxided states at the wave length of 450 mμ and 530 mμ, respectively. The computation of flavin concentration from these values is done with the same formula as described by BLAIR *et al*[4].

$$\text{Corrected } \Delta A = [(A_{450\,m\mu} \text{ reoxidized} - A_{450\,m\mu} \text{ reduced}) - (A_{530\,m\mu} \text{ reoxidized} - A_{530\,m\mu} \text{ reduced})]$$

For convenience the result of acid extractable flavins is represented by F_D, and acid non-extractable flavin by F_s.

Determination of cytochromes : For the quantitative analyses of cytochromes we referred to the methods of CHANCE[10] and GREEN *et al*[3]. After recording the difference of spectra between the oxidized form and the reduced form on a Cary recording spectrophotomer, Moded 14, the calculation is done with respective α-absorption bands. First we divide 9 ml of the test solution, containing an appropriate concentration of mitochondira (20～40 mg protein for heart mitochondria, 100—200 mg protein for liver mitochondria), 50 mM Tris-HCl buffer, pH 8.0, 0.45 ml of neutralized 10% cholate, 0.9 ml of 10% deoxycholate, pH 8.0), into three equal portions. To one portion of this test solution a bit of solid ferricyanide (a half on an earpick) is added, to another 0.02 ml neutralized 1 M KCl and 0.02 ml neutralized 1 M ascorbate, and to the last one a bit of sodium dithionite. The difference spectrum of each of ascorbate reduced form versus ferricyanide-oxidized form, hydrosulfite reduced form versus ascorbate reduced form and hydrosulfite reduced form versus ferricyanide oxidized form, is estimated and the quantities of cyt. $c+c_1$, cyt. *b*. and cyt. *a* are computed from respective values. The absorbance indices used in the computation are as follows; [10]

$\varepsilon = 19.1\,mM^{-1}$, $cm^{-1}(\Delta 553-\Delta 540)$ for cyt. $c+c_1$;
$\varepsilon = 22\,mM^{-1}$, $cm^{-1}(\Delta 562-\Delta 575)$ for cyt. *b*; and
$\varepsilon = 16\,mM^{-1}$, $cm^{-1}(\Delta 605-630)$ for cyt. *a* (heme a).

Since cholate (final concentration, 0.5%) and deoxycholate (final concentration, 1%) added to the test solution for the purpose of lessening the turbidity decrease the absorbancy by about ten per cent in average, the values in the results are corrected accordingly.

Determination of protein contents : This was done by the Biuret method of GORNALL *et al*[11].

RESULTS

The concentrations of flavins and cytochromes of beef heart mitochondria (BHM) and electron transfer particles (ETP) isolated from them are listed in Table 1. The estimated values represent the average of 3 batches of BHM in

Table 1 Concentration of Flavins and Cytochromes of Beef Heart Mitochondria and Electron Transfer Particles

Components	Mitochondria		Electron transfer particles		ETP/BHM
	Mean*	Relative concent.	Mean*	Relative concent.	
Flavins					
Total	0.69	0.92	0.71	0.73	1.03
F_S	0.22	0.29	0.29	0.30	1.32
F_D	0.47	0.63	0.42	0.43	0.89
Cytochromes					
a (heme)	0.75	1.00	0.98	1.00	1.30
b	0.58	0.77	0.76	0.78	1.31
c + c_1	0.51	0.68	0.51	0.52	1.00

* μmole of pigment per gram of protein.

every case. As regard to the relative concentration, cytochrome *a* was taken as 1, and the ratio of each component to it is given. The ratio of the concentration of each respiratory enzyme of ETP to that of BHM is represented as ETP/BHM. F_S, cytochromes *b* and *a*, which are not solubilized and considered to be the fixed components of mitochondria, all show this ETP/BHM ratio to be 1.3, whereas F_D and cytochromes $c+c_1$ of ETP give a value lower than BHM or about an equal value. This seems to be due to the fact that cytochrome *c* and some of the flavoproteins, whose flavin is acid extractable, have a weaker binding with the membrane and they contain water-soluble parts. The water soluble flavoproteins are of flavoproteins other than NADH dehydrogenase. The comparison of the numerical values obtained in this experiment with those reported by other investigators will be taken up in the discussion.

The concentration of the components of beef liver mitochondria (BLM) and of ETP isolated from BLM is illustrated in Table 2. In this instance, all the

Table 2 Concentration of Flavins and Cytochromes of Beef Liver Mitochondria and Electron Transfer Particles

Component	Mitochondria		Electron transfer particles		ETP/BLM
	Mean*	Relative concent.	Mean*	Relative concent.	
Flavins					
Total	0.64	6.96	0.84	4.00	1.3
F_S	0.18	1.96	0.32	1.53	1.8
F_D	0.46	5.00	0.52	2.48	1.1
Cytochromes					
a	0.092	1.00	0.21	1.00	2.3
b	0.089	0.97	0.23	1.10	2.6
$c + c_1$	0.122	1.33	0.17	0.81	1.4
c**	0.081	0.88			
c_1**	0.041	0.45			

* μmole of pigment per gram of protein.

** These values are calculated assuming that two-thirds of the absorption band with a maximum at 553 mμ are contributed by cytochrome c and one-third by cytochrome c_1 [13].

values are also the average of three batches of BLM. The computation of the concentration of cytochromes *c* and c_1 is made on the assumption that 2/3 of $c+c_1$ depend on c and 1/3 of it on c_1[13]. The molecular ratio of each component on the basis of cytochrome c_1 as 1, is F_s: F_D: cyt. b: cyt. c_1: cyt. c: cyt. a = 4: 11: 2: 1: 2: 2. The concentration ratio of the respiratory enzymes of ETP to that of BLM is approximately similar with respect to F_s, cyt. b and cyt. a, being about 2.2 in average as shown in Table 2.

The average contents of pigments in rat heart mitochondria (RHM) are given in Table 3. These are relatively similar to the values of respiratory pigments of

Table 3 Concentration of Flavins and Cytochromes of Rat Heart Mitochondria and Electron Transfer Particles

Component	Mitochondria		Electron transfer particles		ETP/BLM
	Mean*	Relative concent.	Mean*	Relative concent.	
Flavins					
Total	0.64	0.73	0.79	0.75	1.2
F_S	0.22	0.25	0.31	0.30	1.4
F_D	0.42	0.48	0.48	0.46	1.1
Cytochromes					
a	0.88	1.00	1.05	1.00	1.2
b	0.41	0.47	0.55	0.52	1.3
$c + c_1$	0.62	0.71	0.72	0.69	1.2

* μmole of pigment per gram of protein.

BHM. The concentration ratio of the components of ETP to those of RHM is as shown in the Table 3. With respect to the fixed components, such as F_s, cyt. *a* and cyt. *b*, the ratio gives similar values, the average of which is 1.3.

The concentration of respiratory components of rat liver mitochondria (RLM) is shown in Table 4. The molar ratio on the basis of cytochrome c_1 as 1

Table 4 Concentration of Flavins and Cytochromes of Rat Liver Mitochondria and Electron Transport Particles

Component	Mitochondria		Electron transfer particles		ETP/RLM
	Mean*	Relative concent.	Mean*	Relative concent.	
Flavins					
Total	0.75	3.75	0.87	2.02	1.2
F_s	0.19	0.95	0.35	0.81	1.8
F_D	0.56	2.80	0.52	1.21	0.9
Cytochromes					
a	0.20	1.00	0.43	1.00	2.2
b	0.14	0.70	0.32	0.74	2.3
c + c_1	0.19	0.95	0.40	0.93	2.1
c**	0.13	0.65			
c_1**	0.063	0.32			

* μmole of pigment per gram of protein.

** These values are calculated assuming that two-thirds of the absorption band with a maximum at 553 mμ are contributed by cytochrome c and one-third by cytochrome c_1 [18].

is F_s: F_D: cyt. c_1: cyt. c: cyt. a = 3: 9: 2: 1: 2: 3, and the ratio of each component of ETP to that of RLM is similar as far as the fixed components such as F_s, cyt. a and b are concerned, the average being 2. 1.

Next, it is possible to calculate the 'ratios of the soluble protein to in soluble protein of mitochondria from the extent of the condensation of the fixed components in ETP, the results of which are given in Table 5. Both BHM

Table 5 Concentration (per cent) of Soluble and Insoluble Proteins in Heart and Liver Mitochondria Calculated from the Ratio of Concentrations of Fixed Components, Such as Cytochromes *a*, *b* and Acid-Nonextractable Flavin, Between Mitochondria and Electron Transfer Particles.

	Total protein	Soluble protein	Insoluble protein
Beef heart mitochondria	100	23	77
Beef liver mitochondria	100	54	46
Rat heart mitochondria	100	23	77
Rat liver mitochondria	100	52	48

and RHM show about 20% of soluble protein and the remaining 80% corresponds to insoluble protein. BLM and RLM both show a little more than 50% corresponds to insoluble protein. The results of BHM coincide well with the data of LINNANE and ZIEGLER[14]. According to HOGEBOOM and SCHNEIDER[15] when rat liver mitochondria are sonicated, 51% of the total mitochondrial proteins is solublized, and this value is also very close to the value we obtained. It is clear from these results that such procedures enable us to approximate the insoluble protein/soluble protein ratio of mitochondria irrespective of the extent of sonication.

DISCUSSION

Up to date the study of structure and function of mitochondria has been carried out chiefly with beef heart mitochondria as the material, and in the case where its main purpose is to study the mitochondrial function, rat liver mitochondria or beef heart mitochondria has been used. However, it has been shown that the structure and function of mitochondria differ considerably according to the kind of cell. Therefore, even in the study of energy conversion (transfer) by respiration, which is the most fundamental and common function to all mitochondria, not only the investigation of beef heart mitochondria but also a comparison with mitochondria of various cells would make it easier to elucidate this problem and to yield more reliable results. As a step towards this direction it seems necessary to measure accurately the respiratory enzyme content in mitochondria of various kinds of cells, but we do not have any uniform method of the measurement nor any infallible extinction coefficients for the study. As such methods of the study differ with individual investigators and the comparison of data from one another is not so easy. Thus, it would seem desirable to carry out systematic analyses of various cells with one and the same method to compare the data, but such a study is rare. In view of this, we made a comparative study of the contents of respiratory enzymes of mitochondria from several sources. The summarized data of our study are presented in Table 6. As can be seen in this table, the concentration of flavins does not differ materially by the source of mitochondria but cytochrome concentration differs considerably by the mitochondrial source, especially so by organs, for example, it is much lower in liver mitochondria than in heart mitochondria. Even with the same organ it is clear that, while there is not any appreciable difference between the beef heart and rat heart, a distinct difference in the values can be observed between the beef liver and rat liver. Especially noteworthy is the fact that the concentration of cytochrome *a* in the beef liver mitochondria is extremely low, being about 46% that of rat liver mitochondria, and only about 13% and 10% respectively of beef heart and rat heart. According to ODA[16] there is a certain correlation

Table 6 Ratio of Cytochrome and Flavin Concentrations Between Liver- and Heart-mitochondria, and Rat and Beef-mitochondria

Component	BLM/BHM	RLM/RHM	RHM/BHM	RLM/BLM
Flavins				
Total	0.93	1.2	0.93	1.2
F_S	0.82	0.86	1.0	1.1
F_D	0.98	1.3	0.89	1.2
Cytochromes				
a	0.13	0.23	1.2	2.2
b	0.16	0.34	0.71	1.6
c + c_1	0.24	0.31	1.2	1.6

Abbreviations: BLM; beef liver mitochondria, BHM; beef heart mitochondria, RHM; rat heart mitochondria, RLM; rat liver mitochondria.

between the cytochrome *a* concentration in mitochondria of various cells and the number as well as the lengths of cristae of mitochondria, and he states that the longer the total length of mitochondrial cristae corresponds to the higher the concentration of cytochrome *a*. However, it is obvious from the preceding results that this relationship is not appreciable to F_S which is a fixed component of mitochondrion same as cytochrome *a*. The concentration of respiratory enzymes in mitochondria not only differs by the kind of the originating cell but also the concentration ratios among these enzymes differ according to the orignating cell. This fact, along with the findings of the molecular structure of the inner mitochondrial membrane now being clarified in our own laboratory[17~23] as well as in the laboratory of GREEN[24,25], offers a warning, so to speak, to the generally accepted concept that "these respiratory enzyme proteins form complexes in a certain fixed ratio and they are arranged within the mitochondrial inner membrane as a complex enzyme entity"[4,26], and it indicates rather a possiblity that the arrangement of enzyme protein molecules on the mitochondrial membrane is of a more flexible combination. It seems only natural that the concentration raitos of these enzyme proteins need not necessarily be simple integral ratios. In view of the flexibility in the molecular arrangement of enzyme proteins just mentioned, the role played by mobile components (coenzyme Q, cytochrome c) in the electron transport system will be taken notice of.

As has been already stated, systematic information on the contents of mitochondrial respiratory enzymes is rare and we can find only a few data in literatures. Table 7 shows just such data and though they are rather complex, the outstanding items essenital for comparing these data, wave length pair and millimolar extinction coeffecient, are also presented. As is obvious from this table, the millimolar extinction coefficient used varies considerable with different laboratories. The data obtained by KING *et al.*[5,27] with beef heart mito-

Tbble 7 Concentrations of Flavins and Cytochromes of Mitochondria, Reported from Other Laboratories

Reported by	*	Flavins**			Cytochromes**			
		Total	Acid extract-able	Acid non-extract-able	a_3	a	b	$c + c_1$
Chance *et al*[1,12].	Wave length used	465 ; 500 (D)			445 ; 455	605 ; 630	564 ; 575	550 ; 540 553 ; 540
	ε ($mM^{-1}cm^{-1}$)	11			91	16	20	19
	Rat liver	(2.2			1.2	1.0	0.42	1.09)
	Rat liver	0.72			0.22	0.20	0.18	0.34
		(3.6			1.1	1.0	0.9	1.7)
Estabrook *et al*[2].	Wave length used	460 ; 500 (D)			444 ; 455	605 ; 625	562 ; 575	551 ; 540
	ε ($mM^{-1}cm^{-1}$)	11.5			90	16	20	19.1
	Rat liver	0.69			0.20	0.21	0.10	0.31
		(3.3			1.0	1.0	0.5	1.5)
Green *et al*[3]. (Blair *et al*.[4])	Wave length used	(reox − red. at 450 mμ) − (reox − red. at 530 mμ)				605 ; 630 (D)	563 ; 577	550 (D) 554 (D)
	ε ($mM^{-1}cm^{-1}$)	10.3				16.5	28.5	18.5 17.1
	Beef heart	0.66	0.46	0.20		1.31	0.68	0.66
		(3	2	1		6—7	3	3
	ETPH (beef heart)	0.66	0.38	0.28		1.62	0.85	0.63
		(2	1	1		6	3	2)
King *et al*[5,27].	Wave length used	(ox − red. at 450 mμ) − (ox − red. at 530 mμ)			445 ; 465	605 ; 630	562 ; 575	553 ; 540
	ε ($mM^{-1}cm^{-1}$)		10.7	10.3	91	16	22	19.1
	Beef heart	0.45	0.34	0.11	1.13	0.80	0.60	0.65
		(0.56	0.43	0.14	1.4	1.0	0.75	0.81)
Williams[28]	Main wave length used					605 ; 630	563 ; 577	550 ; 535 554 ; 540
	ε ($mM^{-1}cm^{-1}$)					12.0	14.3	21.0 18.8
	Rat liver					0.295	0.245	0.432
Vanneste[29]	Main wave length used					605 ; 630	563 ; 577	550 ; 535 554 ; 540
	ε ($mM^{-1}cm^{-1}$)					13.1	28.5	25.1 18.8
	Beef heart				(1	1	1	1.5)

* In the column, wave length used, millimolar extinction coefficient, and the origin of mitochondria are described in the order.

** Pigment concentrations are expressed as μmoles per mg of protein. Parentheses illustrate pigment concentrations relative to cytochrome a.

*** D means that concentrations of components are determined by direct spectrum.

chondria and by ESTABROOK *et al.*[2] with rat liver mitochondria, both of whom use practically the same millimolar extinction coefficients as we do, are quite close to our own data. In contrast, GREEN *et al.*[3] give generally higher values of cytochromes, especially high concentration of cytochrome *a*. This discrepancy seems to be due to the difference in millimolar extinction coefficient they used and also in the point that they made a direct analysis of not sufficiently transparent samples. Considerings the difference in the millimolar extinction coeffecient the data of WILLIAMS[28], with exception of cytochrome $c+c_1$. are relatively close to our own.

SUMMARY

With a certain fixed methods of analyses, we carried out the determination of flavins and cytochromes in the mitochondria (Mt) and electron transfer particles (ETP) of the heart and liver of rats and cows, and made a comparison of the data with one another. Our findings may briefly be summarized as follows.

1. The concentration of each component of the beef heart mitochondria proved to be 0.47 for acid extractable flavins; 0.22 for acid nonextractable flavin; 0.75 for cytochrome (cyt.) *a*; 0.58 for cyt. *b*; and 0.51 for cyt. $c+c_1$, all units being mμ mole per mg of protein.

2. In the beef liver mitochondria it was 0.46 for acid extractable flavins; 0.18 for acid non-extractable flavin; 0.092 for cyt. *a*; 0.089 for cyt. *b*; and 0.122 for cyt. $c+c_1$, likewise all units in term of mμ mole per mg of protein.

3. In the case of rat heart mitochondria, it was found to be 0.42 for acid extractable flavins; 0.22 for acid non-extractable flavin; 0.88 for cyt. *a*; 0.41 for cyt. *b*; and 0.62 for cyt. $c+c_1$, all in mμ mole per mg of protein.

4. In the rat liver mitochondria it was 0.56 for acid extractable flavins; 0.19 for acid non-extractable flavin; 0.20 for cyt. *a*; 0.14 for cyt. *b*; and 0.19 for cyt. $c+c_1$.

5. The concentration ratios of F_s, cyt. *a* and cyt. *b* of the mitochondria, what are considered to be intrinsic and fixed components of the mitochondrion, to those of the electron transfer particles were 1.3 in both the beef heart and the rat heart, while 2.2 in the beef liver and 2.1 in the rat liver.

6. These findings were compared with the data reported by other workers, and also a discussion was made on the molecular organization of the mitochondrial inner membrane.

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