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Concentration of 17 Elements in Subcellular Fractions of Beef Heart Tissue Determined by Neutron Activation Analysis

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CONCENTRATION OF 17 ELEMENTS IN SUBCELLULAR FRACTIONS OF BEEF HEART TISSUE DETERMINED BY NEUTRON ACTIVATION ANALYSIS

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Summary

Subcellular fractions of beef heart tissue are investigated, by means of neutron activation analysis, with respect to their concentration of 17 different elements. A recently developed ion-exchange technique combined with gamma spectrometry is used. The homogeneity of the subcellular fractions is examined electronmicroscopically.

The following elements are determined: As, Ba, Br, Ca, Co, Cs, Cu, Fe, Hg, La, Mo, P, Rb, Se, Sm, W and Zn. The determination of Ag, Au, Cd, Ce, Cr, Sb and Sc is omitted, in view of contamination.

Reproducible and characteristic patterns of distribution are obtained for all elements studied.

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Introduction

In addition to the bulk elements in mammalian tissues, the presence of a great number of elements in minute quantities, the so-called trace elements, is well documented (4, 5, 15, 16, 20, 21, 25, 43, 44). Only some of these trace elements are proved to be of biological significance (7, 35, 45). They function mainly as catalysts or activators in enzyme systems or other organic complexes. Some two hundred enzyme systems are known to be influenced by trace elements (35). Certain enzymes may be activated by different elements, whereas others - the so-called metalloenzymes - contain particular trace elements as a structural part. Examples of metalloenzymes are ceruloplasmin, cytochrome oxidase, ascorbic acid oxidase, buturyl CoA dehydrogenase and tyrosinase containing Cu, as well as cytochromes, catalase and DNPH cytochrome c reductase containing Fe, and xanthine oxidase containing both Fe and Mo. Several Zn metalloenzymes are known, e. g. carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase, glutamic acid dehydrogenase and lactic dehydrogenase.

Although most trace elements normally occurring in mammalian tissues have no known biological function, several authors consider that many of those now regarded as physiologically inactive will, with great probability, be found to participate in vital biological processes (9, 35, 45). Some authors suggest that one way of obtaining information which may indicate biological significance of trace elements is to gain increasing knowledge of their exact loci and associations within the cell (42, 45). A few investigations of some trace elements in subcellular fractions have also appeared (17, 42). Thus, Thiers and Vallee (42) investigated the concentration of 8 elements (among them 5 trace elements) in subcellular fractions of rat-liver tissue. They found reproducible and characteristic patterns of distribution, which they considered to provide evidence of the functional significance of these elements.

In a previous study (49), I determined the concentration in normal human heart tissue of 24 trace elements, some of which had not been determined earlier in such tissue.

The aim of the present study was to investigate the distribution of these trace elements in subcellular fractions of heart tissue.

Experimental

Cell fractionation

Three cell fractionation experiments were performed (I, II and III in Tables I-VI). Heart muscle tissue from three cows killed a few hours before starting the experiments was used. Pieces were cut from the left ventricle with a glass knife. Fat and connective tissue were removed. The heart muscle pieces were scraped with the glass knife. The muscle pulp obtained was weighed, and transferred to chilled 0.25 M sucrose. Homogenization was performed in a glass tube with a Teflon pestle, using 2 ml of sucrose per g of muscle.

The first two centrifugation steps were performed in an International Model HR-1 centrifuge with head no. 858 and 856, respectively. The homogenate was transferred to 250-ml polyethene bottles. Nuclei, cell debris and erythrocytes were sedimented at 2000 rpm ($\sim$ 500 g) for 10 min. The supernatant was decanted into 50-ml polyethene tubes and recentrifuged at 10,000 rpm ($\sim$ 10,000 g) for 20 min. to give the mitochondrial pellet. The third centrifugation step was performed in a Spinco Model L centrifuge, rotor 40. The supernatant from the second centrifugation step was decanted into 11-ml polyethene tubes, and recentrifuged at 40,000 rpm (105,000 g) for 60 min. to obtain the sarcotubular fraction.

Whole heart tissue and material from the different fractions obtained were transferred to weighed quartz ampoules. The ampoules with content were weighed, dried and sealed as described previously. They were then ready for irradiation with thermal neutrons in an atomic reactor. 3 ml of the supernatant from the third centrifugation step was decanted into a 10-ml polyethene tube, and stored at -20° C until required for neutron activation.

Great care was taken at all stages of the cell fractionation procedure to reduce the risk of contamination. The samples were not allowed to be in contact with other material than cleaned glass or plastic. The aluminium caps of the polyethene tubes used in centrifugation steps two and three were sprayed with plastic lacquer, and rinsed with 6 N HCl and demineralized water. All glass instruments and plastic tubes and bottles were also thoroughly cleaned before use with 6 N HCl and demineralized water. The 0.25 M sucrose solution used was prepared from sucrose crystals (Mallinckrodt) and demineralized water. A 5-ml sample of the sucrose solution was investigated by neutron activation analysis for its content of trace metals.

The amount of sucrose added to the different subcellular fractions was determined, in order to correct the wet weight determinations.

Protein determinations were performed with the Biuret method and sucrose determinations were performed with the primary L--cysteine-sulfuric acid reaction of hexoses (51).

Neutron activation analysis

The samples were irradiated together with standards, as previously described (49).

The sealed quartz ampoules were irradiated in the R2-reactor at Studsvik with a thermal neutron flux of $2.\ 10^{13}$ n/cm^2/sec. for

48 - 75 hr. The supernatant and sucrose solution samples were irradiated in the R1-reactor in Stockholm with a thermal flux of $1.2 \cdot 10^{12}$ n/cm^2/sec. for 95 hr. A decay interval of 2 or 3 days elapsed before the chemical processing.

Chemical separation was performed with a recently developed ion-exchange technique (31, 32, 33), and combined with subsequent γ-spectrometry.

The γ-spectrometric measurements were carried out with a transistorized 512-channel pulse-height analyzer, attached to a 3" x 3" NaJ (Tl) welltype crystal.

The elements were identified and quantitatively determined as previously described (49).

Chemical recovery corrections were made in accordance with the mean values determined earlier (49, 50).

Electron microscopy

Electron-microscopical analysis was performed of the mitochondrial and sarcotubular fractions. The pellets were fixed in a 1 per cent solution of osmium tetroxide according to Zetterqvist (52), dehydrated in a graded alcohol series, and embedded in Epon according to Luft (24). Sections were cut by a LKB Ultrotome ultramicrotome, and inspected in a Hitachi H 5-6 or a Siemens Elmiskop I electron microscope. In some cases, sections were treated with uranyl acetate to increase the contrast of the sections.

Results

A comparison between the concentration of 23 trace elements in whole heart tissue from man and from cattle is shown in Table I. The values are given in μg/g wet tissue. In column 1 are seen the

range and the median values in normal human heart tissue determined in a previous study (49). Column 2 shows the values obtained from two calves, and column 3 those from 3 adult animals. The cattle hearts were found to contain the same elements in similar concentration as the human hearts. Slight differences were, however, present with respect to some elements. Thus, the amount of Cd, Se and Zn was somewhat lower in the cattle hearts than in human heart tissue. One Ag value and one Cs value in beef were below the lower limit of the human range. Although the Ba values in calves were somewhat higher than those in adult animals, all the Ba values in cattle lay within the range of the human values.

The concentration of the trace elements in different subcellular fractions obtained from three cell fractionation experiments is presented in Tables II-IV. Table II lists the elements with known biological function, Table III the elements with suspected biological function, and Table IV those without known biological function. The amounts are given in μg/g wet tissue, the bracketed values denoting the amounts in μg/g protein. Correction has been made for the contribution of sucrose to the wet weight.

The protein content as per cent of the wet weight in the different cell fractionation experiments was for whole heart tissue 23.9, 24.6, 23.7, for nuclear and cell residue fraction 21.1, 21.9, 20.8, for mitochondrial fraction 35.7, 35.8, 34.3, for sarcotubular fraction 58.2, 57.9, 58.9, and for supernatant 1.12 and 1.27. The dry weight as per cent of the wet weight was for whole heart tissue 22.5, 20.7, 21.5, for nuclear and cell residue fraction 19.8, 18.1, 16.8, for mitochondrial fraction 23.4, 21.4, 21.7, and for sarcotubular fraction 29.0, 28.2, 27.1.

The distribution of the elements with known biological function showed some noteworthy patterns. The amount of Ca and Cu was very high in the mitochondrial fraction, Cu on a wet weight basis being about 15-20 times as high as in the whole heart tissue. The

sarcotubular fraction had a remarkably high Fe concentration. The distribution of P and Zn was uniform, whereas Co showed a slight increase in the mitochondrial and sarcotubular fractions as compared to whole heart tissue.

The supernatant was found to have an exceedingly low concentration of all the trace elements studied, with the exception of Br, Rb and Cs. On a protein basis, on the other hand, the figures for most of the elements were higher in the supernatant than in the whole heart tissue.

Among the elements with suspected biological function (Table III), Ba and Mo were obtained in high concentration in both the mitochondrial and the sarcotubular fraction, especially in the latter, whereas these fractions had a reduced content of Br and Rb compared with whole heart tissue. On a protein basis, the distribution of Se was uniform.

Some of the elements without known biological function - e. g. Hg, La and W (Table IV) - were found to occur in very high concentration in the sarcotubular fraction. The concentration of these elements in the mitochondrial fraction was also high. The concentration of Cs was, however, low in both fractions.

The percentage distribution of the elements studied in the different fractions is listed in Table V. Column 1 shows the protein content expressed in per cent. It is seen that the greater part of As, Ba, Ca, Cu, P, Sm and Zn was present in the nuclear and cell residue fraction, whereas Br, Cs, Rb and Se occurred chiefly in the supernatant. Fe, La and Mo showed a slight predominance in the nuclear and cell residue fraction. Hg and W were present to the same extent in the nuclear and cell residue fraction as in the supernatant.

The percentage distribution of some additional elements is given in Table VI, from which it is evident that some contamination had occurred. These elements were therefore omitted from the study. The problem of contamination will be discussed in the following.

Discussion

One of the great advantages of neutron activation analysis is that the risk of contamination of the samples after irradiation is practically non-existent. In this work, however, the samples were handled before irradiation, which involves serious risks of contamination. Great care was therefore taken to reduce such risks. The samples were cut and scraped with a glass knife and homogenized in a glass tube with a Teflon pestle. This implies that small particles of glass or teflon were a potential source of contamination (41). In the centrifugation steps performed, no metallic object was allowed to be in direct contact with the samples. The aluminium caps of the polyethene tubes were therefore sprayed with plastic lacquer and rinsed. All glass and plastic objects were rinsed with 6 N HCl and demineralized water. Despite the precautions taken, contamination occurred with respect to certain elements, as shown in Table VI. This contamination unquestionably derived to a great extent from the sucrose solution used. The concentration in the sucrose solution of the different elements studied is shown in Table VII.

For comparison, Table VII also shows the concentration of the elements in the whole heart tissue used for cell fractionation. The amounts are given in μg/g solution and μg/g wet tissue, respectively. Most of the elements occurred in extremely low concentration in the sucrose solution. However, the concentration of some elements i. e., Ag, Cd, Cr, Sb and Sc, was of the same order of magnitude as in the whole heart tissue, and the probability that the contamination of these elements derived from the sucrose solution

is obvious. In the case of Cr, the amount present in the supernatant was less than in the sucrose solution used, which means that Cr had been added to the different subcellular fractions.

Various sources of error in the radiochemical procedure used have been discussed in earlier papers (49, 50). Nor is it possible to disregard sources of errors in the cell fractionation procedure. The subcellular fractions obtained by differential centrifugation may have been inhomogeneous. The nuclear and cell residue fraction is known to contain erythrocytes and mitochondria, in addition to nuclei and unruptured cells. The mitochondrial and sarcotubular fractions were examined electron microscopically. The homogeneity of these fractions was considered to be satisfactory.

When cytoplasmic pellets are examined in the electron microscope, only a small portion of the entire pellet can be studied at one time. This involves a certain risk of basing the results on a statistically biased sample. In order to minimize this source of error, I attempted to make sections of the mitochondrial and sarcotubular pellet from the upper and middle, as well as the lower strata of the pellets. In no case and at no level of the pellets examined were the contaminations found to predominate over the particulates in question (i. e., mitochondria and sarcotubular fragments respectively).

The investigation of which the present communication only represents a part has primarily focused its interest to condition concerning the human heart. With the present state of knowledge successful cell fractionation seem to be dependent on a rapid access to the tissue sample. Thus, technical considerations made me use beef heart tissue.

Naturally, the trace element concentration obtained in subcellular fractions of heart tissue from cattle is not directly applicable to the conditions in human heart tissue. However, the comparison

between the trace element content of the whole heart tissue from man and from cattle revealed no qualitative differences, and the quantitative differences obtained with respect to some elements were small.

The present grouping of trace elements, i. e., into those with, with suspected and without biological function, is not definite. Some of the elements which today have no known biological function may, in the future, prove to participate in vital processes.

The concentration of the elements in the subcellular fractions is shown in Tables II-IV. Characteristic and reproducible patterns of distribution were obtained for all 17 elements studied. The concentration of each element in the same fraction from different hearts was much the same, whereas the concentration in different fractions from the same heart varied. Thiers and Vallee (42) also reported characteristic and reproducible patterns of distribution of 8 elements in subcellular fractions from rat-liver tissue.

Among the elements with known biological function (Table II), Ca and Cu were highly enriched in the mitochondrial fraction. On a protein basis, Ca was obtained in a concentration about 5 times that in the whole heart tissue, the corresponding figure for Cu being about 10 times.

A high Ca concentration in mitochondria is consistent with other investigations. Thus, Thiers and Vallee (42) found a large amount of Ca in the mitochondrial fraction isolated from rat-liver tissue. Peachey's electron-microscopical observations of rat-kidney mitochondria (30) suggest that the granules normally observed within the mitochondrial matrix have the ability to accumulate divalent cations, e. g. Ca and Ba. The mitochondrial granules of intact cells (whole cells of toad urinary bladder) also had this accumulative ability. It is well known that isolated mitochondria

from kidney, liver and heart are able to accumulate certain elements, among others Ca (1, 2, 3, 12, 14, 23, 26, 37). In the case of Ca, this accumulative ability of rat-kidney mitochondria has been shown to be extremely strong (46).

The large amount of Ca obtained in the mitochondrial fraction was not accompanied by any increase in the concentration of P in this fraction. Slater and Cleland (36) pointed out that most of the phosphate present in mitochondria from rat-heart muscle exists in the form of phospholipid. Strickland and Benson (38) found, in sheep and beef heart muscle, that the phospholipids in general are remarkably similar in different subcellular fractions. The concentration of P obtained in the particulate subcellular fractions in this work was also uniform.

The concentration of Co obtained showed a slight increase in the mitochondrial and sarcotubular fractions. Swendseid et al. (40) investigated the distribution of vitamin B_{12} in mouse liver, and found enrichment of B_{12} in the mitochondrial fraction. This enrichment was of the same order of magnitude as the increase in Co concentration in the mitochondrial fraction obtained in the present study.

Cu was obtained in the highest concentration in the mitochondrial fraction. Hermann and Kun (17) investigated the distribution of Cu in subcellular fractions of rat-liver tissue, and also found the highest concentration in the mitochondrial fraction. Thiers and Vallee (42), however, who also studied subcellular fractions of rat-liver tissue, found most of the Cu in the supernatant. A high content of Cu in the mitochondrial fraction is consistent with the fact that the Cu enzyme cytochrome oxidase (6, 10, 11, 34, 39) is found to be associated with mitochondria.

Fe was obtained in extremely high concentration in the sarcotubular fraction, with a 30-to-40-fold increase as compared to the

whole heart tissue on a wet weight basis. This high amount of Fe in the sarcotubular fraction is probably due mainly to accumulation of ferritin particles (compare 8, 22).

It has been shown that various Fe metalloenzymes are present in both mitochondrial and microsomal preparations. The iron may be present either as heme iron or non-heme iron. The DPN-cytochrome c reductase of the microsomes is an example on an iron enzyme (10, 11, 18, 19, 39). The various cytochromes (13, 39), which contain heme iron and succinic dehydrogenase (6, 11, 34, 39) e. g., containing non-heme iron, are associated with mitochondria. In the present study, a slight increase in Fe concentration was, in fact, found in the mitochondrial fraction as compared to the whole heart tissue.

No large differences were noted between the concentration of Zn in the different subcellular fractions. On a protein basis, Zn was found in the highest concentration in the supernatant, which is in agreement with the results of Thiers and Vallee (42) in rat-liver tissue. The concentration of Zn in the mitochondrial fraction was also found to be slightly increased as compared to that in the whole heart tissue. This distribution of Zn in beef-heart tissue may be compared to the localization in liver tissue from different animals of certain Zn enzymes, e. g. alcohol dehydrogenase (11, 29) and carbonic anhydrase (11) in supernatant and glutamic dehydrogenase in mitochondria (3, 11).

In the groups of elements with suspected or no known biological function, some different patterns of distribution can be discerned. Ba, Hg, La, Sm and W were found to be greatly and Mo somewhat enriched in both the mitochondrial and the sarcotubular fractions. The concentration of Br, Cs and Rb in these fractions was, on the contrary, strongly reduced as compared to that in the whole heart tissue. On a protein basis, the concentration of As and Se in the mitochondrial fraction and of Se in the sarcotubular fraction was

of the same order of magnitude as in the whole heart tissue, whereas the concentration of As was somewhat reduced in the latter fraction. On a protein basis, the concentration of the elements in these groups was higher in the supernatant than in the whole heart tissue, and as far as As, Br, Cs, Mo, Rb and Se are concerned, the highest concentration was found in the supernatant. In the case of Mo, the high concentration obtained in the supernatant may be compared to the distribution of xanthine oxidase, a Mo metalloenzyme, in rat-liver tissue (11, 47), which was found to occur only in the supernatant.

The distribution of trace elements recorded in subcellular fractions from beef heart tissue in the present study has, in the aforegoing, often been compared to that of various metalloenzymes in subcellular fractions of tissue from other animals and organs. Differences may, however, exist between different animals and organs as regards the distribution pattern of elements and metalloenzymes, respectively.

The reproducible and characteristic patterns of distribution noted for the elements which I studied may indicate that, in addition to the elements with known biological function, further trace elements may be of biological significance.

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Table I

	Human heart tissue		Heart tissue from calves		Heart tissue from adult animals		
	Range	Median			I	II	III
Ag	0.0006 - 0.025	0.0019	0.0013	0.00076	0.0005	0.0007	0.0007
As	0.00097 - 0.0124	0.00236	-	-	0.00707	0.00166	0.00304
Au	0.0000084 - 0.000113	0.0000338	0.0000224	0.0000306	0.0000854	0.0000309	0.0000438
Ba	0.0067 - 0.047	0.020	0.027	0.030	0.0070	0.0075	0.0070
Br	1.01 - 4.58	2.03	-	2.37	3.60	1.09	3.29
Ca	23.6 - 96.4	45.6	61.1	52.8	31.2	34.6	34.4
Cd	0.009 - 0.028	0.012	0.0009	0.0017	0.0044	0.0009	0.0005
Ce	0.0012 - 0.0082	0.0016	-	0.0080	0.0034	0.0025	0.0037
Co	0.0009 - 0.0183	0.0121	0.0073	0.0034	0.0085	0.0053	0.0059
Cr	0.0017 - 0.0195	0.0062	0.0060	0.0059	0.0038	0.0017	0.0019
Cs	0.0066 - 0.0216	0.0114	0.0166	0.0133	0.0073	0.0070	0.0041
Cu	1.98 - 5.22	3.75	-	-	4.12	4.43	4.84
Fe	20.9 - 52.8	35.2	46.2	39.1	37.2	35.8	33.1
Hg	0.000 - 0.0957	0.0432	0.0029	0.0091	0.0149	0.0332	0.0609
La	0.00010 - 0.0029	0.00029	0.00097	0.00084	0.00025	0.00029	0.00051
Mo	0.0257 - 0.127	0.0513	0.0592	0.0557	0.0542	0.0415	0.0565
Rb	1.70 - 5.59	2.45	3.24	2.13	3.11	2.61	1.77
Sb	0.0006 - 0.0036	0.0015	0.0021	0.0029	0.0018	0.0048	0.0017
Sc	0.000003 - 0.000108		-	-	0.000026	0.000098	0.000013
Se	0.097 - 0.245	0.177	0.089	0.066	0.059	0.047	0.058
Sm	0.0002 - 0.023	0.0025	-	-	0.0029	0.0019	0.0036
W	0.00072 - 0.0019	0.0012	-	-	0.0012	0.0012	0.0012
Zn	18.2 - 32.4	25.0	19.2	23.3	12.8	15.9	17.2

Trace element concentration in human and beef heart tissue.

Values in µg/g wet tissue.

Table II

Element	Ca			Co		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	31.2 (130)	34.6 (141)	34.4 (145)	0.00850 (0.0355)	0.00528 (0.0215)	0.00585 (0.0247)
Nuclear and cell residue fraction	20.3 (96.4)	22.9 (105)	23.9 (115)	0.00367 (0.0174)	0.00412 (0.0188)	0.00405 (0.0195)
Mitochondrial fraction	215 (602)	242 (676)	314 (915)	0.0207 (0.0579)	0.0163 (0.0456)	0.0160 (0.0468)
Sarcotubular fraction	48.6 (83.5)	66.7 (115)	80.4 (137)	0.0202 (0.0347)	0.0318 (0.0549)	0.0347 (0.0589)
Supernatant	<2 (<170)		<2 (<157)	0.00205 (0.183)		0.000072 (0.00565)

Element	Cu			Fe		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	4.12 (17.2)	4.43 (18.0)	4.84 (20.4)	39.2 (156)	35.8 (146)	33.1 (140)
Nuclear and cell residue fraction	3.43 (16.3)	3.55 (16.2)	3.63 (17.5)	18.9 (90)	17.6 (80)	16.0 (77)
Mitochondrial fraction	82.8 (232)	65.5 (183)	91.7 (267)	79.4 (222)	76.5 (214)	68.9 (201)
Sarcotubular fraction	18.9 (32.4)	17.2 (29.7)		1060 (1820)	1050 (1800)	1310 (2230)
Supernatant	0.23 (20.5)		0.26 (20.4)	6.9 (616)		7.3 (573)

Element	P			Zn		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	2210 (9250)	1920 (7790)	1930 (8140)	12.8 (53.5)	15.9 (64.7)	17.2 (72.6)
Nuclear and cell residue fraction	1310 (6210)	1280 (5850)	1200 (6060)	10.2 (48.5)	11.5 (52.5)	13.4 (64.4)
Mitochondrial fraction	2160 (6040)	2450 (6860)	3480 (10200)	27.2 (76.4)	29.9 (83.6)	35.7 (104)
Sarcotubular fraction	1790 (3070)	2570 (4430)	3150 (5350)	15.3 (26.3)	15.5 (26.8)	23.0 (39.1)
Supernatant	317 (28300)		186 (14600)	1.2 (107)		2.0 (157)

Elements with known biological function. Amounts in µg/g wet tissue.

Bracketed figures: µg/g protein.

Table III

Element	Ba			Br		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	0.0070 (0.029)	0.0075 (0.031)	0.0070 (0.030)	3.60 (15.1)	1.09 (4.43)	3.29 (13.9)
Nuclear and cell residue fraction	0.0049 (0.023)	0.0056 (0.026)	0.0045 (0.022)	1.08 (5.1)	0.24 (1.1)	1.25 (6.0)
Mitochondrial fraction	0.042 (0.12)	0.050 (0.14)	0.053 (0.16)	0.35 (0.98)	0.06 (0.17)	0.35 (1.02)
Sarcotubular fraction	0.18 (0.31)	0.13 (0.22)	0.095 (0.16)	1.01 (1.74)	0.17 (0.29)	0.64 (1.09)
Supernatant	<0.001 (<0.089)		<0.001 (<0.079)	1.01 (90.2)		0.98 (77.2)

Element	Mo			Rb		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	0.0542 (0.227)	0.0415 (0.169)	0.0565 (0.238)	3.11 (13.0)	2.61 (10.6)	1.77 (7.5)
Nuclear and cell residue fraction	0.0276 (0.131)	0.0195 (0.089)	0.0283 (0.136)	1.22 (5.80)	1.04 (4.75)	0.66 (3.17)
Mitochondrial fraction	0.123 (0.343)	0.125 (0.350)	0.179 (0.521)	0.15 (0.42)	0.05 (0.14)	0.14 (0.41)
Sarcotubular fraction	0.470 (0.808)	0.247 (0.427)		0.25 (0.43)	0.21 (0.36)	0.20 (0.34)
Supernatant	0.0099 (0.884)			0.72 (64.3)		0.47 (36.9)

Element	Se		
Cell fractionation experiment	I	II	III
Whole heart tissue	0.059 (0.247)	0.047 (0.191)	0.058 (0.245)
Nuclear and cell residue fraction	0.024 (0.114)	0.023 (0.105)	0.025 (0.105)
Mitochondrial fraction	0.084 (0.235)	0.048 (0.134)	0.085 (0.249)
Sarcotubular fraction	0.233 (0.400)	0.159 (0.274)	0.123 (0.209)
Supernatant	0.014 (1.25)		

Elements with suspected biological function. Amounts in µg/g wet tissue.
Bracketed figures: µg/g protein.

Table IV

Element	As			Cs		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue		0.00166 (0.00675)	0.00304 (0.0128)	0.0073 (0.0305)	0.0070 (0.0285)	0.0041 (0.0173)
Nuclear and cell residue fraction	0.00275 (0.0131)	0.00133 (0.00608)	0.00208 (0.0100)	0.0029 (0.0181)	0.0023 (0.0105)	0.0014 (0.0067)
Mitochondrial fraction	0.00843 (0.0236)	0.00276 (0.00771)	0.00657 (0.0192)	0.0027 (0.0076)	0.0008 (0.0022)	0.0007 (0.0020)
Sarcotubular fraction	0.00106 (0.00182)	0.00108 (0.00186)		0.0130 (0.0223)	0.0031 (0.0054)	0.0014 (0.0024)
Supernatant			0.00054 (0.042)	0.0019 (0.167)		0.0012 (0.0942)

Element	Hg			La		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	0.0149 (0.0623)	0.0332 (0.135)	0.0609 (0.257)	0.00044 (0.0018)	0.00029 (0.0012)	0.00051 (0.0022)
Nuclear and cell residue fraction	0.0071 (0.0337)		0.0294 (0.141)	0.00023 (0.0011)	0.00019 (0.00087)	0.00028 (0.0014)
Mitochondrial fraction	0.0902 (0.252)	0.206 (0.577)	0.239 (0.696)	0.0030 (0.0083)	0.0028 (0.0077)	0.0043 (0.012)
Sarcotubular fraction	0.315 (0.541)	0.548 (0.946)	0.476 (0.808)	0.011 (0.019)	0.0057 (0.0098)	0.030 (0.051)
Supernatant	0.0031 (0.277)		0.0155 (1.22)			∿0.00008 (∿0.006)

Element	Sm			W		
Cell fractionation experiment	I	II	III	I	II	III
Whole heart tissue	0.0029 (0.012)	0.0019 (0.0077)	0.0036 (0.015)	0.0018 (0.0075)	0.0015 (0.0061)	0.0022 (0.0093)
Nuclear and cell residue fraction	0.0015 (0.0070)	0.0011 (0.0051)	0.0023 (0.011)		0.0007 (0.0032)	0.0010 (0.0048)
Mitochondrial fraction	0.014 (0.039)	0.010 (0.029)	0.025 (0.072)	0.020 (0.056)	0.034 (0.095)	0.018 (0.051)
Sarcotubular fraction	0.045 (0.077)	0.020 (0.034)	0.098 (0.17)	0.20 (0.34)	0.045 (0.083)	0.34 (0.58)
Supernatant	0.00034 (0.021)					0.0005 (0.039)

Elements without known biological function. Amounts in µg/g wet weight.

Bracketed figures: µg/g protein.

Table V

Element	Protein			As			Ba			Br		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	88	89	88		80	69	70	74	65	30	26	38
Mitochondrial fraction	0.4	0.6	0.8		0.6	1.3	1.6	2.6	4	0.03	0.02	0.06
Sarcotubular fraction	0.19	0.16	0.14		0.07		1.9	1.2	0.8	0.02	0.01	0.01
Supernatant	10.9		10.9			36	<33		<28	65		60

Element	Ca			Co			Cs			Cu		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	65	67	70	43	77	69	39	33	34	83	80	75
Mitochondrial fraction	1.8	2.7	5.3	0.6	1.2	1.6	0.1	0.05	0.09	5.3	5.8	11
Sarcotubular fraction	0.1	0.1	0.1	0.2	0.4	0.3	0.1	0.03	0.02	0.4	0.3	
Supernatant	<15		<12	56		25	60		59	13		11

Element	Fe			Hg			La			Mo		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	51	49	48	48		48	53	64	55	51	47	50
Mitochondrial fraction	0.6	0.8	1.2	1.6	2.4	2.3	1.7	3.6	2.1	0.6	1.2	1.9
Sarcotubular fraction	2.2	2.0	2.3	1.6	1.2	0.5	2.0	1.6	3.3	0.7	0.4	
Supernatant	43		45	48		51			~34	42		

Element	P			Rb			Se			Sm		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	59	66	62	39	40	37	45	49	43	68	70	73
Mitochondrial fraction	0.3	0.5	1.0	0.01	0.008	0.05	0.4	0.4	0.8	0.1	0.2	0.4
Sarcotubular fraction	0.06	0.09	0.09	0.006	0.006	0.007	0.3	0.2	0.1	0.1	0.07	0.2
Supernatant	33		30	53		54	55			28		

Element	W			Zn		
Cell fractionation experiment	I	II	III	I	II	III
Nuclear and cell residue fraction		46	44	80	72	78
Mitochondrial fraction	2.9	8.6	4.8	0.6	0.7	1.2
Sarcotubular fraction	8.5	2.1	9.1	0.09	0.07	0.07
Supernatant			47	17		21

Percentage distribution of the elements.

Table VI

Element	Ag			Au			Cd			Ce		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	63	94	94	>100	>100	>100	>100	>100	>100	>100	>100	>100
Mitochondrial fraction	6	12	23	1	6	11	1.1	6	12	2.3	8	4.2
Sarcotubular fraction	6	6	10	4.3	4.3	11	0.6	3	2.8	2.7	2.5	2.4
Supernatant	23		>100	36			70		>100	>100		

Element	Cr			Sb			Sc		
Cell fractionation experiment	I	II	III	I	II	III	I	II	III
Nuclear and cell residue fraction	>100	>100	>100	90	64	>100	>100	25	>100
Mitochondrial fraction	19	64	59	11	4	15	2.7	0.6	6.4
Sarcotubular fraction	2.7	10	20	3.6	1.5	11	0.9	0.2	2
Supernatant	0		0	>100		>100	>100		>100

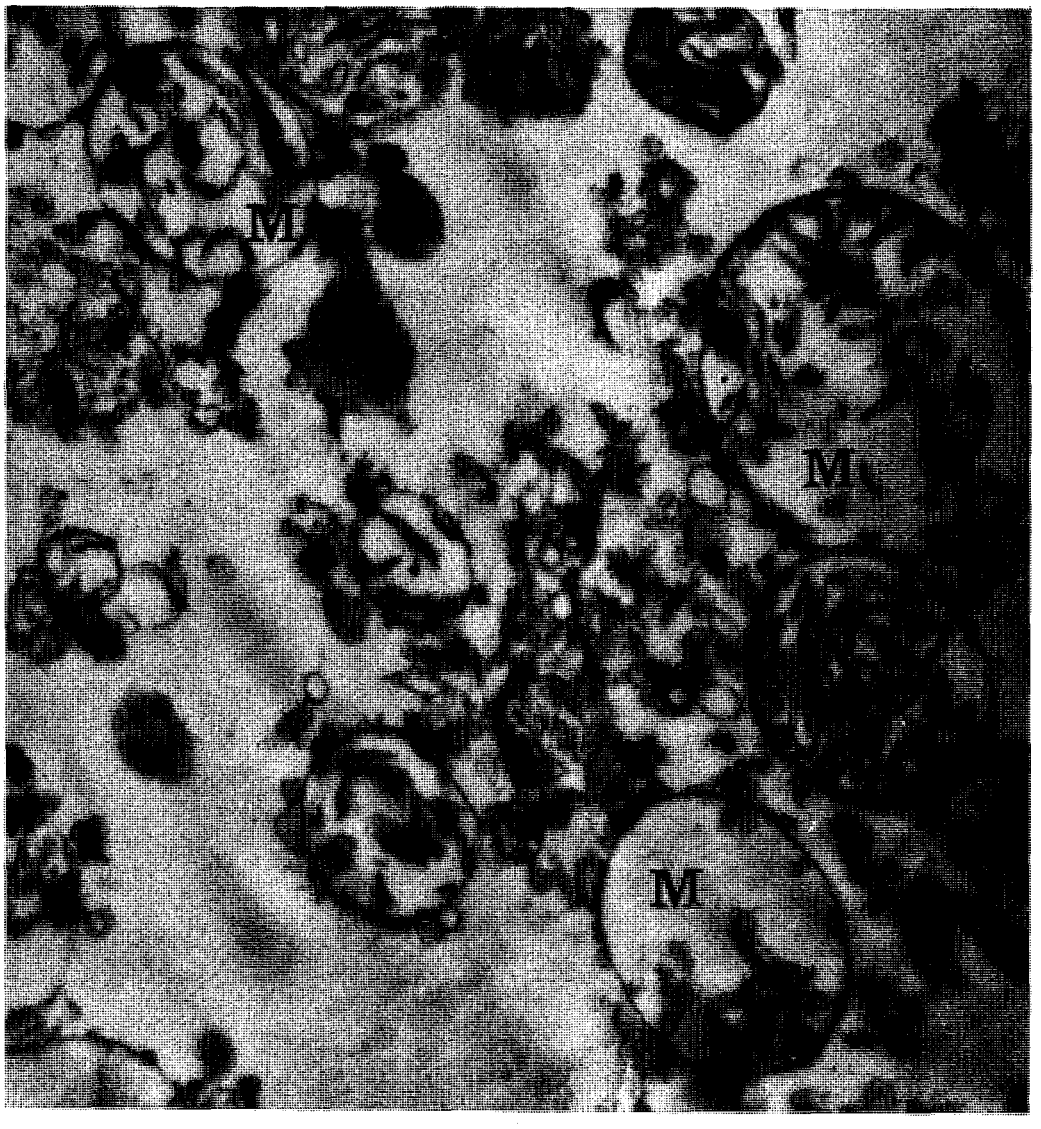
Percentage distribution of some additional elements.

Table VII

	Sucrose solution	Whole heart tissue
Ag	0.0008	0.0005 - 0.0007
As	0.00019	0.00166 - 0.00707
Au	0.0000034	0.0000309 - 0.0000854
Ba	0.0010	0.0070 - 0.0075
Br	0.0028	1.09 - 3.60
Ca	2	31.2 - 34.6
Cd	0.0009	0.0005 - 0.0044
Co	0.0002	0.0053 - 0.0085
Cr	0.0082	0.0017 - 0.0038
Cs	0.0001	0.0041 - 0.0073
Cu	0.01	4.12 - 4.84
Fe	2.2	33.1 - 37.2
Hg	0.0021	0.0149 - 0.0609
La	0.00004	0.00025 - 0.00051
Mo	0.0094	0.0415 - 0.0565
P	3.6	1920 - 2210
Rb	0.004	1.77 - 3.11
Sb	0.0007	0.0017 - 0.0048
Sc	0.000010	0.000013 - 0.000098
Se	0.001	0.047 - 0.059
W	0.00018	0.0015 - 0.0022
Zn	0.25	12.8 - 17.2

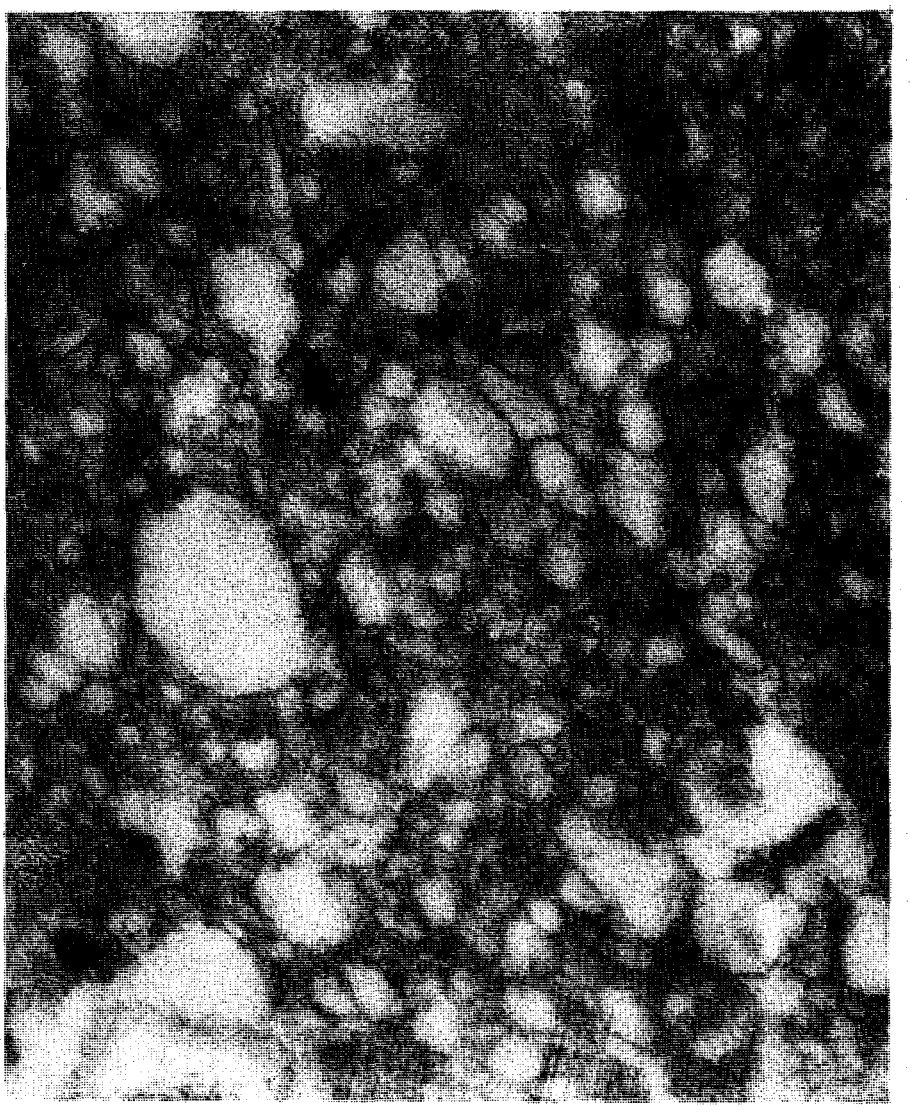
Trace element content of sucrose solution as compared to that of whole heart tissue.

Fig. 1



Electron micrograph of a section of cow heart mitochondria isolated in 0.25 M sucrose. The samples were fixed in 1 per cent osmium tetroxide at pH 7.2 - 7.4, embedded in Epon and examined under a Hitachi H 5-6 microscope. Most mitochondria (M) appear somewhat swollen and rounded-off. Surrounding the mitochondria, some debris can be seen, which may or may not have derived from broken mitochondria. x 20, 000.

Fig. 2



Electron micrograph of a section of a cow heart sarcotubular fraction. The samples were fixed and embedded as in Fig. 1, and the section examined under a Siemens Elmiskop I microscope. The predominating component in this fraction is of vesicular nature, and can be compared to the sarcotubular fraction of Muscatello et al. (27, 28). The aggregated small and very dense particles in the lower left corner probably represent ferritin particles. x 80, 000.

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