Scanning Microscopy

Volume 2 | Number 3 Article 48

5-1-1988

Intracellular Water Distribution and Aging as Examined by X-Ray Microanalysis

Thomas von Zglinicki
Humboldt University

Scanning Microscopy, Vol. 2, No. 3, 1988 (Pages 1791-1804) 0891-7035/88$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

INTRACELLULAR WATER DISTRIBUTION AND AGING AS EXAMINED BY X-RAY MICROANALYSIS

Thomas von Zglinicki*

Department of Ultrastructural Pathology and Electron Microscopy,
Institute of Pathology, Charité, Humboldt University,
Schumannstrasse 20/21, 1040 Berlin,
German Democratic Republic

(Received for publication February 08, 1988, and in revised form May 01, 1988)

Abstract

The results reviewed here demonstrate that 1. the distribution of dry mass as observed in frozen-dried cryosections might be used as an unbiased measure of intracellular dry mass resp. water distributions in the tissue in vivo and 2. the well-known loss of water from cells during aging is solely due to a water loss from mitochondria without changes in the water content of all other components of the cell in the case of rat liver and heart muscle.

The reason for the water loss might be increased counter ion binding by membrane-bound enzymes due to decreased fluidity of the inner mitochondrial membrane with aging rather than changes of the permeability of the membrane or chemical modifications of mitochondrial proteins or DNA. It is assumed that the observed changes lead to decreased intramitochondrial diffusion of substrates and to conformational changes of enzymes. This would decrease both the velocity and the binding constants of certain energy-supplying reactions and could therefore play an important role in the aging process.

KEY WORDS: X-ray microanalysis, frozen-dried cryosections, heart muscle, liver, mitochondria, water content, aging.

*Address for correspondence: Thomas von Zglinicki, Dept. of Ultrastructural Pathology and Electron Microscopy, Institute of Pathology, Charité, Humboldt University, Schumannstrasse 20/21, 1040 Berlin, German Democratic Republic. Phone no: 2862883.

Introduction

Aging is one of the most common and, simultaneously, most complex phenomena of the biological world. To improve our understanding of this process we should look for its determinants at the most fundamental level of life, i.e., at the energetic level, and with respect to the regulation of biological processes at the level of electrostatic forces, because all biological processes are reactions of polyelectrolytes in an ionic medium (Douzou and Maurel, 1977). In searching for age-related changes in the system responsible for electrostatic control of bioenergetics we should have a good chance to find out something about the principles of aging phenomena.

Electrostatic control of cellular processes is exerted via the interplay of local ionic concentrations and water content. Water loss is a common feature of tissues during senescence (Solomon et al., 1976) and has been attributed to decreased cellular water content (Lustyik and Zs.-Nagy,1985; von Zglinicki and Lustyik, 1986). Taking into account the enormous degree of morphological and functional specialization within any cell, examination of ionic and water distribution on a subcellular scale is necessary. X-ray microanalysis of cryosections is the method of choice. However, while frozen-hydrated cryosections would be ideally suited for the direct measurement of ionic and water distributions, true ultrastructural resolution in microanalytical research has not yet been obtained by using frozen-hydrated cryosections due to technical problems including mainly sensitivity to radiation damage, low intrinsic contrast and difficulties in retaining full hydration (for review, see Steinbrecht and Zierold, 1987).

On the other hand, all necessary information also remains in the frozen-dried cryosection. Once it has been experimentally tested that the local dry

mass in the frozen-dried cryosection is an unbiased measure of the dry mass distribution in the living cell, local dry mass resp. water fractions might be measured by either background intensity of paired X-ray microanalytical measurements (Somlyo et al., 1979), by evaluation of contrast of the electron micrographs (Zeitler, 1971, Linders et al., 1981) or by measuring dark field current in the scanning transmission electron microscope (STEM) (Zierold, 1986). Water fractions in relative terms as obtained by these methods might be converted into local water content by using appropriate internal standards (Rick et al., 1979) or by adding independent information about, for instance, mean cellular water content (von Zglinicki and Bimmler, 1987a).

Necessary conditions to obtain native values by any of these methods are: 1. Constant section thickness, at least between adjacent compartments, in the hydrated state. 2. No net water movement between compartments in the course of preparation, especially during freezing and 3. No differential shrinkage of different compartments during freeze-drying.

Results of a test of these assumptions by using isolated mitochondria in a concentrated albumin solution as a model for organelles within the cytoplasm (von Zglinicki et al,. 1987) are reviewed here.

The methods have been adopted to examine the age-dependency of organelle water content and molar ionic concentrations in rat liver and heart muscle (von Zglinicki and Bimmler, 1987a,b). As it will be shown here, profound changes in the water content of mitochondria from both tissues have been found which indeed suggest age-dependent changes in the ionic regulation of mitochondrial function leading most probably to decreased energy availability in old cells. Possible mechanisms and consequences will be discussed here as a contribution to a hypothesis of aging.

Materials and Methods

Proof of the unbiasedness of water fraction measurements from frozen-dried cryosections.

This was done by a comparison of dry mass measurements of isolated mitochondria in vitro by interference microscopy with results obtained from frozen-dried cryosections of the same sample of isolated mitochondria (von Zglinicki et al., 1987). Briefly, mitochondria were isolated from livers of rats (Wistar strain) aged 3 weeks (young group) and 14 months (adult group) according to Butler and Judah (1970) in 0.21 M mannitol, 0.07 M sucrose, 10 mM EDTA, 2 mM Tris, pH 7.4. After isolation, mitochondria were resuspended in a solution containing 10 mM EDTA, 2 mM Tris, and varying concentrations of bovine serum albumin (BSA, fraction V, Biomed Krakow, see Barer and Joseph, 1955a). The concentration of BSA was monitored by using an Abbé-type refractometer. The albumin solution was used in excess to avoid dilution by contaminating amounts of the isolation medium (Barer and Joseph, 1955b). Osmolarity of the albumin solutions was measured by using a vapor pressure osmometer (KNAUER). By interference microscopy the optical path difference between mitochondria and surrounding medium was measured. In every suspension 30 paired measurements were performed. The regression line between the path difference $\Delta ø$ and the refractive index of the medium n was calculated by a least square fit using measurements performed in suspensions with BSA concentrations between 0.11 and 0.31 g/ml. From this the caliper diameter t_m and the refractive index n_m of mitochondria were calculated using the formula $\Delta ø = (n_m - n)t_m$ (Barer and Dick, 1957) and ordinary correlation statistics.

From the refractive index the dry mass concentration of mitochondria was computed according to the formula $C_m = (n_m - n_{H2O})/\tilde{a}$ with $\tilde{a} = 0.18$ ml/g (Barer and Joseph, 1954).

In parallel, drops of the mitochondrial suspensions with 0.26 (young) and 0.28 (adults) g/ml BSA were deposited on silver pins (Sevéus, 1978) and frozen by fast dipping into liquid propane. The cold gas layer directly above the liquid propane was blown away directly before dipping and the specimen was moved as fast as possible through the air above the chilling medium and at least 6 cm through the liquid propane.

Preparation and measurements of sections was done exactly in the same way as was described for tissue sections (see below).

Measurement of the age-dependency of local water content and elemental concentrations

Measurements were performed on male Wistar rats between 1 week and 24 months of age. Three to five rats were used for each organ and age group. Age groups examined were 1,2,4 and 15 weeks and 6, 12 and 24 months. Animals were sacrificed by cervical dislocation, and specimens of heart or liver tissue were obtained by a cryobioptical method while the heart was still beating, avoiding both traumatic and ischemic injuries to the cells studied (von Zglinicki et al., 1986). Briefly, small tissue cylinders were simultaneously excised and frozen by using a liquid propane chilled, hollow needle.

Ultrathin sections, about 100-200 nm

Table 1

Mitochondrial dry mass as measured by different techniques

technique	young		adult		t	p
	DC	n	DC	n		
IF	45.9±3.2	60	52.5±2.4	96	1.650	10 %
XRMA	39.1±2.8	19	55.7±3.4	23	3.769	0.1%
SMD	44.8±0.8	72	50.1±1.0	53	4.139	0.1%
F	1.189		0.724			
p	NS		NS			

Dry mass concentration DC (in weight %, mean ± s.e.m., n, number of mitochondria measured in the young and adult group, respectively) of isolated rat liver mitochondria as measured by various techniques. Means have been compared between the age groups by a t-test and within the age groups by an analysis of variance. The t- or F-value, respectively, and the corresponding error probability p, are given. IF, interference microscopy, XRMA, X-ray microanalysis, and SMD, scanning microdensitometry of micrographs.

thick, were cut in an LKB ultrotome III with Cryokit at temperatures of 170-190 K and mounted on formvar-covered carbon-coated Al grids. At liquid nitrogen temperature the grids were transferred to a vacuum evaporator and freeze-dried at a pressure of less than 10^{-4} torr. The copper block surrounding the grids rewarms to 190 K in about 2 ½ hour. After overnight rewarming to ambient temperature the grids were carbon-coated without breaking the vacuum and transferred within about 1 min through the air into the microscope column. Sections were examined in a SIEMENS Elmiskop 102 equipped with a KEVEX 7000 microanalyser in transmission mode at 80 kV. Vacuum pressure was 1.10^{-5} torr and an anticontamination device was used. Before starting X-ray microanalysis, a micrograph from the area of interest was taken at a magnification of 3000 to 5000 with a total electron dose of less than 2.10^{-4} C/cm^2. Beam current was monitored in the plane of the final image by using the small isolated screen of the Elmiskop 102.

Spectra were obtained from cytoplasm, rough endoplasmic reticulum (RER), mitochondria, and regions of condensed and decondensed chromatin within the nuclei in the liver cells and from A-band, I-band including the Z-line, myofibrils (in cross-sections of cells), and mitochondria in heart muscle cells. Some of the measurements were performed as paired analysis. Spot diameter was 0.3 µm. Under these conditions of analysis no mass loss was observable, but a weak contamination is built up over the analysis period of 100 secs life time. Spectra were corrected for contamination and extraneous radiation and quantification was done according to Hall et al. (1973) by using aluminium-carbon foils as standards, as previously described (von Zglinicki, 1983). The background window used was between 5.1 and 5.45 keV. Per compartment and age group between 15 and 64 measurements were taken.

The optical transmission of all identified compartments was measured on the micrographs by scanning microdensitometry using an automatic image analysis system (Simon et al., 1980) to evaluate the dry mass of these compartments (Zeitler, 1971, Linders et al., 1981). Transmission values of the compartments were related to those of the cytoplasm in liver cells and of the myofibrils or the I-band in myocytes, respectively.

The results from this and from measurements of background intensity by paired X-ray microanalysis were pooled together to give relative estimates of dry mass in the cellular compartments. Measurements were performed only in those sections in which the size of the ice crystal remnants was smaller than about 100 nm. From the relative dry masses the absolute water resp. dry mass content of the compartments was obtained by a combination of these data with the mean cellular dry mass of hepatocytes (Lustyik and Zs.-Nagy, 1985) and myocytes (von Zglinicki and Lustyik, 1986) obtained by X-ray microanalysis of frozen hydrated and frozen-dried bulk specimens and with volume fractions of the cellular compartments obtained by morphometrical measurements (David et al., 1981; David, 1985). Details of the calculations have been given (von Zglinicki and Bimmler, 1987a).

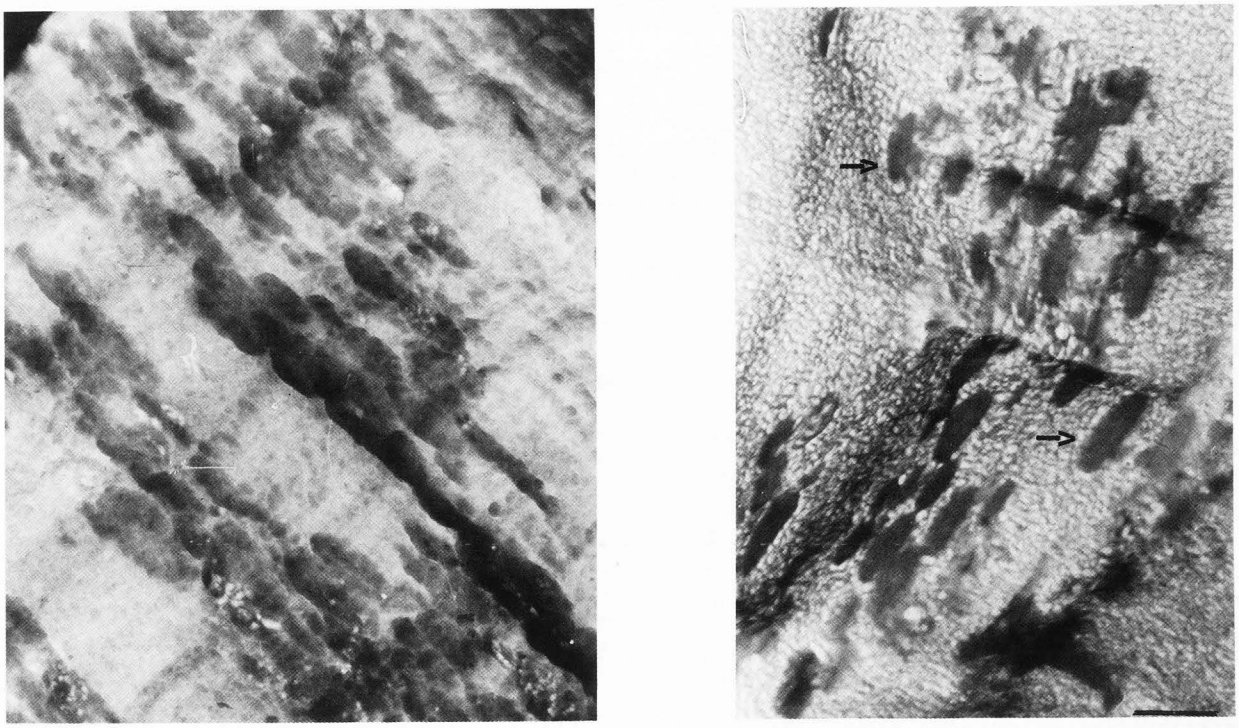

Fig. 1: Isolated rat liver mitochondria in BSA solution. Left: Well frozen section. Right: Freezing rate was comparatively low, therefore the mean size of ice crystal remnants is in the order of about 100 nm. Arrows mark the first weak haloes around mitochondria. The bar corresponds to 1 µm.

Results

Isolated mitochondria

Isolated rat liver mitochondria are suspended in a solution containing 10 mM EDTA, 2 mM Tris and bovine serum albumin (BSA) in concentrations between 0.11 and 0.31 g/ml as a model for organelles within the cellular matrix. The BSA matrix did not contain significant amounts of sodium, phosphorus, or potassium. 132 ± 18 mmol/kg dry weight of Cl and 69 ± 10 mmol/kg dry weight of Ca were found by X-ray microanalysis. The osmolarity O_{BSA} follows the regression $O_{BSA} = 199C_{BSA} + 8.6$ with a correlation coefficient of $r = 0.983$. C_{BSA} is in g/ml solution and O_{BSA} is obtained in mOsm. This regression line corresponds to a molecular weight about one order of magnitude smaller than that of pure BSA and indicates contamination of the BSA used by small molecules. However, the osmolarity even of the most concentrated BSA solution used is only 70 mOsm.

Table 2

Ionic concentrations in isolated rat liver mitochondria

Age group	P	Cl	K	Ca	n	Σ	O_{BSA}
young	232 ± 31	157 ± 26	47 ± 9	83 ± 13	23	519 ± 43	60.3
adult	277 ± 32	118 ± 13	92 ± 12	121 ± 19	26	608 ± 41	64.3
t	1.101	1 .342	3.000	1.651		1.498	
df	47	25	47	25		47	
p	NS	NS	1%	NS		NS	

Concentrations are given in mM/l H_2O (mean ± s.e.m.). Concentration sums Σ have been calculated (see text) with the s.e.m. obtained by the Gaussian law of error propagation. For comparison, osmolarities O_{BSA} (in mOsm) of the BSA matrices used are given. n is the number of mitochondria probed. Means have been compared by a t- or Welch-test, respectively. The t value, the degree of freedom df, and the error probability p are given.

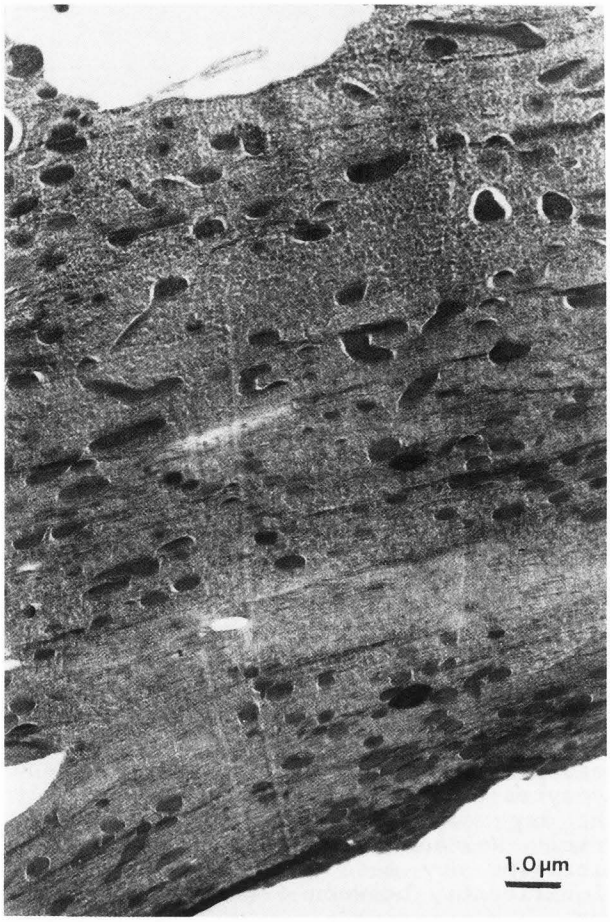

Fig. 2: Frozen-dried cryosection of rat liver showing increasing size of ice crystal remnants and increasing frequency of mitochondrial haloes from bottom to top. The bar denotes 1 µm.

In Table 1 dry mass concentrations of isolated rat liver mitochondria in vitro as measured by interference microscopy are compared with those measured on frozen-dried cryosections of mitochondrial suspensions by X-ray microanalysis and scanning microdensitometry of the micrographs. There is a significant increase in mitochondrial dry mass with age of animals. In each of the age groups there are no significant differences between the results of separate techniques. This holds when freezing is fast enough to prevent the efflux of water from mitochondria.

This condition seems to be fulfilled if the size of the ice crystal remnants in the medium is below about 100 nm (Fig. 1). If the freezing rate decreases, there will be time enough for subcooled water to flow out from the mitochondria. It crystallizes in the form of haloes around them (see Fig. 2).

Ionic concentrations of isolated rat liver mitochondria are given in Table 2. Na and Mg are below the detection limit in these measurements. In calculating the concentration sums Σ every P atom measured by X-ray microanalysis is treated as one ionic entity, neglecting the comparatively small contributions of higher phosphorylated nucleotides and of the P bound in nucleic acids and lipids. Moreover, there is equilibrium between monophosphate and diphosphate which depends on the unknown pH of isolated mitochondria. There are a lot of uncertainties, therefore, which do not allow a reliable computation. Although the concentration sums might be overestimated by as much as 100 to 200 mmol/l, the comparison with the osmolarity of the BSA matrix demonstrates clearly that the vast majority of the ions in isolated mitochondria is osmotically inactive.

Dry mass concentrations of mitochondria isolated from rat heart muscle and brain have been measured in vitro by interference microscopy, too. Data are given in Table 3. For comparison with the water content of mitochondria within the respective tissues (see below) results are expressed as water content in weight per cent. Table 3 demonstrates a significant decrease with age of the water content of isolated mitochondria from all three tissues studied.

In Table 4 the mean diameters of isolated mitochondria are compared with those of mitochondria in the tissues of rats being 3 weeks and 27 months old, respectively. In the case of rat liver the sizes of isolated and tissue mitochondria are found to be in good agreement. This might be arbitrary, taking into account the dependence of stereological estimations of mean size on numerous assumptions (Weibel, 1979), including changes of the absolute and relative volume of compartments during conventional fixation, dehydration, and embedding procedures used here. However, both methods measure a decrease of the size of mitochondria with aging in agreement with morphometrical estimations of the age dependency of the mean mitochondrial volume (David, 1985). On the other hand, diameters of isolated and non-isolated mitochondria from heart muscle of young rats differ widely. Therefore, a decrease in size of isolated heart muscle mitochondria with age is found, while both morphometric (David et al., 1981) and stereological measurements indicate an increase in size of mitochondria within the aging myocytes.

Age dependency of the water distribution in rat heart muscle and liver cells

Local elemental concentrations and water content have been measured in those parts of the sections only, where ice

Table 3

Age dependence of mitochondrial water content

Age group	liver WC	n	heart muscle WC	n	brain WC	n
young (3 weeks)	54.1±2.9	60	53.3±0.6	120	49.6±0.7	120
senescent (27 months)	25.4±2.0	200	45.4±0.7	120	37.5±0.8	120
t	8.147		8.569		11.383	
p	<0.1%		<0.1%		<0.1%	

The water content of mitochondria isolated from different tissues of young and senescent rats as measured by interference microscopy (Water content WC in weight %, mean ± s.e.m.) and the numbers of measured mitochondria n are given. The age groups are compared by t- or Welch-test, respectively. The t value and the error probability p are given.

Table 4

Age dependence of rat liver mitochondrial diameters

Age group	Method	Liver	Heart
young	IFM	678±107	1221±38
	STE	734± 21	504±23
senescent	IFM	472± 32	744±23
	STE	578± 14	617±30
F		9.163	115.787
CDM		121	86

Mean diameter (in nm, mean ± s.e.m.) of isolated mitochondria as measured by interference microscopy (IFM) and of mitochondria within the respective tissues as measured stereologically (STE) according to Lord and Willis (see Weibel, 1979) on conventionally processed tissue sections from young (3 weeks) and senescent (27 months) rats. Numbers of mitochondria measured are those given in Table 3 for IFM and 100 each for STE measurements. Means have been compared by an analysis of variance and the F value and the Critical Difference between Means (CDM) for the 5% significance level are given.

crystal remnants are small and no haloes around mitochondria could be found (Fig. 2).

The age dependencies of the local water content of whole cells, mitochondria and cytoplasm or myofibrils, respectively, are given for the case of liver cells in Fig. 3a and for heart muscle in Fig. 3b. In liver cells, no significant age dependency of the water content in nuclei and RER regions is found. Mean values are 61.4 ± 1.1 % in regions of condensed chromatin, 79.3 ± 0.8 % in regions of decondensed chromatin and 62.2 ± 1.2 % in the RER. Whereas both cytoplasmic, respectively myofibrillar, and mitochondrial dry mass increase during early development, it is evident from Fig. 3 that the increase in cellular dry mass during aging both in hepatocytes and myocytes is solely due to an increase in the dry mass concentration in mitochondria. Neither cytoplasmic nor myofibrillar dry mass concentration change significantly between 6 and 24 months of age.

Fig. 3a further demonstrates a close fit with the water content of isolated mitochondria. This is not so in the case of heart muscle (compare Fig. 3b and Table 3) and might be accidental. However, the same tendency has also been proven by both independent techniques in heart muscle.

Elemental concentrations on a dry mass basis do not change appreciably with age neither in the mitochondria nor in the cytoplasm resp. myofibrils (von Zglinicki, 1987a). However, if expressed as concentrations per compartment water, ionic concentrations in mitochondria increase significantly with age mostly due to the decreased water content (von Zglinicki and Bimmler, 1987b) This is evident from Fig. 4 also, where the sums of the molar ionic concentrations in mitochondria, liver cytoplasm and myofibrils are given. No significant change of concentration sums with age is seen in the cytoplasm or in the myofibrils. However, the sums of ionic concentrations in mitochondria increase significantly with age both in liver and heart muscle cells.

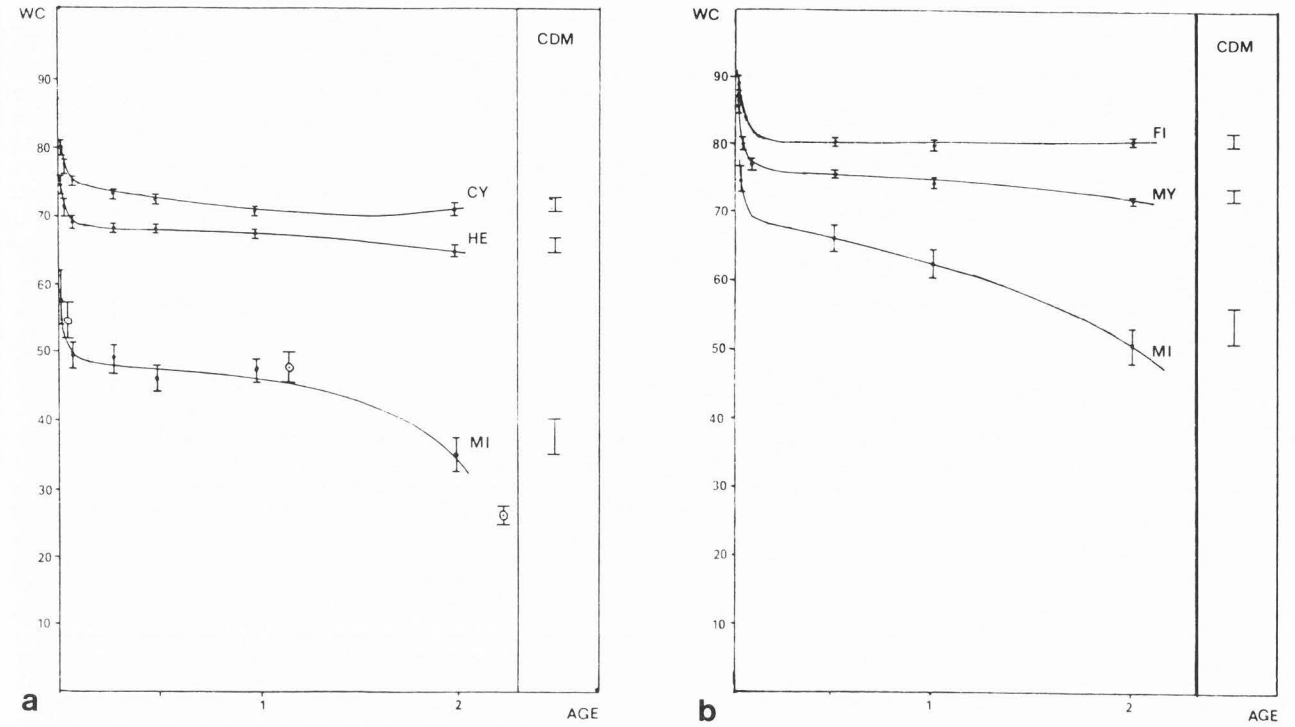

Fig. 3: Water content (WC, in % of wet weight, mean ± s.e.m.) vs. age in years in rat hepatocytes (a) and myocytes (b). Differences between means larger than the indicated Critical Difference between Means (CDM) are significant at the 5 % level. (a) Hepatocytes: HE, whole hepatocytes; MI, mitochondria; CY, cytoplasm. Points indicate results obtained on frozen bulk specimens (HE) respectively on frozen-dried cryosections of tissue (MI, CY) and circles indicate results obtained by interference microscopy of isolated mitochondria. (b) Myocytes: MY, whole myocytes; FI, myofibrils; MI, mitochondria.

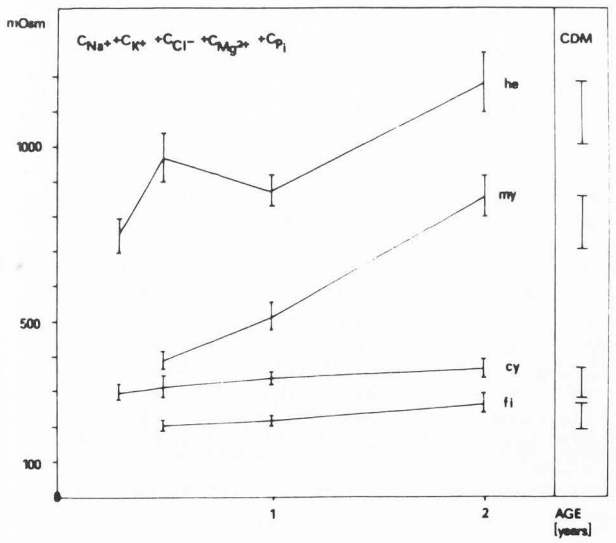

Fig. 4: Sums of local ionic concentrations per compartment water as indicated in the figure vs. age. cy, rat liver cytoplasm; fi, myofibrils; he, liver cell mitochondria; my, myocytic mitochondria. The Critical Difference between Means (CDM) for the 5 % level is indicated.

Discussion

Methodological aspects

The cryobioptical technique for preparation of tissue used in this study has been demonstrated to be capable of avoiding traumatic and ischemic injury to the cells studied (von Zglinicki et al., 1986). This preparation technique rests on the same basic ideas as the principle of the Wollenberger clamp (Hagler and Buja, 1984; Somlyo et al., 1985) and some recent cryoballistic methods (for review, see Robards and Sleytr, 1985). Mean concentrations of Na, K, and Cl measured in heart muscle and liver cells by this way have been shown to be in good accordance with recent results of independent techniques (von Zglinicki et al., 1986). However, the technique used here involves transfer of the frozen-dried cryosections through the moist room air into the electron microscope and X-ray microanalysis at ambient temperature. The question remains, therefore, to what extent cellular concentrations of elements other than Na, K, and Cl and the cellular distribution of all elements are

affected by possible rehydration artifacts and radiation damage at ambient temperature. A comparison of data measured in parallel on rat liver sections prepared as described here and by using a closed cryotransfer system plus microscope cold stage reveals only minor changes in local ionic and P concentrations but significantly decreased S concentrations in some of the organelles of hepatocytes measured at room temperature (von Zglinicki and Uhrik, 1988). Transfer artifacts could be ruled out as a reason for these changes (von Zglinicki and Zierold, manuscript in preparation). Therefore, irradiation at room temperature seems to affect S concentration measurements. This corresponds with earlier observations (Rick et al., 1982). However, the validity of concentrations obtained for the other elements or ions seems to be well verified.

Radiation damage does not influence the results of water content measurements done by scanning microdensitometry of electron micrographs, because the micrographs were taken at a total dose of less than 2.10^{-4} C/cm^2, which is low enough not to cause any significant mass loss even at ambient temperature (Bahr et al., 1965; Cosslett, 1978). As indicated by the agreement of the concentrations of nearly all elements except S measured at ambient and liquid nitrogen temperature (von Zglinicki and Uhrik, 1988) and by the agreement of dry mass concentrations measured by X-ray microanalysis and scanning microdensitometry, radiation damage at ambient temperatures does not seem to change the local mass thickness in frozen-dried cryosections under our conditions of analysis. However, in addition to extraneous radiation effects, it might contribute to the larger coefficient of variation of dry mass measurements by X-ray microanalysis as compared to scanning microdensitometry we found (see Table 1) in agreement with Linders et al. (1981).

The use of densitometry or of dark field current measurements in the STEM (Zierold, 1986) is preferred, therefore, over X-ray microanalysis for the evaluation of relative local water fractions.

The agreement between mitochondrial dry mass concentrations measured in vitro by interference microscopy and on frozen-dried cryosections of isolated mitochondria (Table 1) demonstrates that the freezing regimen is capable of avoiding significant water fluxes over submicrometer distances. Although the water within mitochondria supercools to deeper temperatures than that in the cytoplasm or in the BSA matrix (Mazur, 1984) as shown by the presence of ice crystal remnants in the matrix but not in mitochondria (Figs. 1 and 2) there is not time enough for the water to flow over distances much larger than about 100 nm under optimal freezing conditions. Mitochondrial haloes seem to be good indicators for 'bad' freezing, that means for freezing too slow for X-ray microanalysis at organelle resolution.

Prerequisites for the use of frozen-dried cryosections to measure the local water content in cells are, in addition, the absence of local variations of section thickness due to the sectioning process itself or to differential shrinkage during freeze-drying. Both effects could, in principle, compensate for each other to give results concurring with in vitro measurements. Variation of section thickness was excluded by examining the same mitochondria on serial sections which should lead to a negative correlation of results obtained on adjacent sections, if the section thickness varies significantly between mitochondria and medium. This was found not to be the case (von Zglinicki et al., 1987).

The data obtained for the water content of isolated mitochondria by using freeze-dried cryosections do not only concur with the results of interference microscopical measurements, but are in line with results of a number of independent techniques. The low water content of mitochondria has been recognized long ago by phase contrast microscopical investigations of native cells. It is well known, that isolated mitochondria are banded in the analytical ultracentrifuge at a density of about 1.2 which corresponds to a water content of about 50 %. According to all known X-ray microanalytical data, this density cannot be due to the presence of heavier elements in concentrations large enough to be remarkable. By tracer techniques the matrix water space of isolated mitochondria has been repeatedly measured to be in the order of 50 % (for review, see Lund and Wiggins, 1987).

The model system examined here should resemble the freezing and freeze-drying properties of cells sufficiently well. The concentrations of albumin used are in the order of the dry mass concentration of the hepatocyte cytoplasm. Additional organelles in the cytoplasm would, if at all, most probably act as additional diffusional barriers for water and ions. Moreover, from all important cellular organelles mitochondria differ most from the cytoplasm in terms of dry mass. Differences in freezing and/or drying behavior should therefore be greatest between mitochondria and cytoplasmic matrix. No such differences have been found in the model system studied.

The facts presented here provide a justification for using frozen-dried

cryosections to evaluate local dry mass or water fractions of cellular compartments. Moreover, since the diffusion constants of water and small ions in the cell are practically equal, it might be assumed that there are also no ionic shifts during freezing and freeze-drying on the organelle scale. The conversion of local water fractions to the water content of single organelles in absolute terms as used here is quite indirect, requiring independent estimations of mean cellular dry mass and of volume fractions of the main cellular compartments (von Zglinicki and Bimmler, 1987a). A direct estimation of the water content of one of the cellular compartments would be preferable. This can easily be done in isolated cells by interference microscopy of the cytoplasm (Barer and Joseph, 1954) but cannot be done for cells within the tissue. In some cells it is possible to measure the water content of e.g., the nucleus directly by X-ray microanalysis of frozen-hydrated and frozen-dried tissue sections (Saubermann and Scheid, 1985). However, in many cases the contrast of frozen hydrated sections will be too low to ensure the presence of homogeneity of the compartments studied. Mean cellular water content might be obtained by using frozen-hydrated cryosections or bulk specimens (Zs.-Nagy et al., 1982). In some favorable cases it might be possible to evaluate the mean cellular dry mass simply from the dry mass of the tissue. However, this is restricted to those tissues which are composed of only one sort of cells as, e.g., muscle (von Zglinicki and Lustyik, 1986).

An elegant idea is that of Rick and al. (1979) to supply the specimen with its own dry mass standard consisting of an albumin layer surrounding the sample. However, this has its own limitations: embedding of a tissue sample into any medium prior to freezing might change its ionic composition and/or its water content. Moreover, constancy of the section thickness over much larger distances as necessary for the technique used here is required, a demand which seems to be hard to fulfill.

Mitochondrial water loss and aging

There are large differences between the diameters of isolated and non-isolated mitochondria from hearts of young rats (Table 4) indicating that by the methods used a certain fraction of large mitochondria, possibly subsarcolemmal ones, has been preferentially isolated. This might also explain the large differences between the water content of isolated (Table 3) and non-isolated (Fig. 3b) heart muscle mitochondria found in the young group.

However, the loss of water from mitochondria as the sole reason for the decreasing cellular and tissue water content in rat heart muscle and liver during aging seems to be well verified (see above). Why mitochondria lose water during aging of the animals is as yet a matter of speculation. From the constancy of the concentrations of osmotically active substances in the extracellular space (Frolkis, 1975) and in the cytoplasm (Fig. 4) it is clear that the water loss cannot be due to a passive efflux driven by osmotic changes outside the mitochondria. As indicated by the opposite tendencies found for the age dependency of mitochondrial sizes at least in non-isolated mitochondria (Table 4, and David et al., 1981; David, 1985; Hansford, 1983) there must be different growth phenomena of mitochondria in different tissues in addition to osmotic changes. Nevertheless, osmotic equilibrium remains the sole determinant of mitochondrial water content.

Our results obtained from isolated mitochondria in hypoosmotic media (Table 2) suggest that the majority of intramitochondrial ions is osmotically ineffective, that means bound, e.g., as counter ions to mitochondrial polyelectrolytes. This is in line with X-ray microanalytical investigations of ionic distributions in intracellular compartments not separated by membranes, i.e., the condensed/decondensed chromatin in rat liver nuclei (von Zglinicki and Bimmler, 1987a) and the A-band/I-band in rat heart muscle (von Zglinicki, 1988). These results indicate severe deviations from the behavior of homogeneous Donnan compartments which have been attributed to counter ion binding. An increase of the bound fraction of mitochondrial ions with age would decrease the osmotic pressure and would lead to an immediate water loss until the osmotic equilibrium is reached again.

Increased counter ion binding could be due to chemical modifications of the mitochondrial proteins and/or the mtDNA with age leading to an increased charge density on the surface of these macromolecules. This would be in line with the 'mitochondrial clock hypothesis' (Harman, 1986; Miquel and Fleming, 1986). However, no changes in the mtDNA (Bunn and White, 1985) and no or at best quantitative changes in the expression of mitochondrial proteins (Fleming et al., 1986) with age could be detected up to now. On the other hand, decreasing fluidity, decreasing ratio of unsaturated/saturated fatty acids and increasing cholesterol content of the inner mitochondrial membrane with advancing age have been well documented (Daum, 1985; for review see also Hansford, 1983).

Interestingly, these changes, like those of the water content, have been

found to depend on age of the animals, not on age of the mitochondria, which have half-lives in the order of some days to one month. However, it is clear now that mitochondrial proteins are selectively degraded (Duque-Magalhaes and Gualberto, 1987) and that mitochondrial proliferation takes place essentially by division of existing mitochondria (Kleinig and Sitte, 1986). Therefore, membrane alterations may persist and accumulate during the life span of animals. These peroxidative changes have shown to be due to the production of oxygen radicals within the electron transport chain of mitochondria, mostly at the site of the cytochrome b 566 (Nohl and Jordan, 1986). A constant fraction of about 20 % of all oxygen radicals produced escapes from quenching by mitochondrial superoxide dismutase and might be responsible for the peroxidation of membrane lipids (Nohl and Hegner, 1978).

The majority of mitochondrial enzymes is bound to the inner membrane (Schwerzmann et al., 1986). The activity of the mitochondrial ATPase, for example, has been shown to depend on the membrane fluidity and the degree of saturation of membrane lipids (Zsigmond and Clandinin, 1986). It would be expected, therefore, that changes in the membrane fluidity should affect the amount of surface charges of membrane-bound proteins exposed to the intramitochondrial space. Increased rigidity of the membrane with advancing age most probably will increase this charge fraction seen by the intramitochondrial ions. Counter ion binding would increase in proportion to the exposed charge fraction (Katchalsky, 1971).

There is, in principle, one other explanation for a decreased osmotic efficiency of intramitochondrial ions with age. It would occur, if the reflexion coefficient of the membrane for these ions and especially for K as the main intracellular cation decreases (von Zglinicki, 1987b). Such a decrease could be caused by the age-dependent alterations of the membranes described. However, a decrease of the reflexion coefficient would imply a significant increase of the membrane permeability for the ion(s) in question. This would lead to increased ionic recycling at the expense of the membrane potential and/or the $\Delta\mu_{H+}$ (Jung et al., 1977) This driving force of the oxidative phosphorylation, however, has been found to remain fairly constant during aging (Hansford, 1983; Victorica et al., 1985). The reflexion coefficient hypothesis (von Zglinicki, 1987b) is not favored, therefore.

The expected primary consequences of the mitochondrial water loss are twofold: First, diffusion becomes limited. This is very clear if one keeps in mind that the value measured in old rat liver mitochondria is in the order of water content of bone. A number of mitochondrial transport systems may have first order kinetics with their V_{max} dependent on the concentration of the respective metabolites at the inner membrane site of the transporter (Hansford, 1983). Diffusion of metabolites within the mitochondria to this site might therefore be well rate-limiting.

The second consequence of water loss comes into play via the increased ionic strength which alters the electrostatic interaction between polarized molecules and leads not only to increased counter ion binding but to condensation of polyelectrolytic macromolecules as well. This in turn results in sterical hindrance of enzymic functions.

Obviously, different enzyme systems differ in their sensitivity to changes in their ionic environment. Most probably, the enzymes involved in glutamate + malate transport and/or utilization are among the most susceptible. State 3 respiration with glutamate + malate as substrate has been found to be impaired at higher age by a number of investigators (Hansford, 1983; Victorica et al., 1985). The NADH CoQ reductase, which is responsible for the transfer of electrons from, among other substrates, malate, has been shown to be extremely sensitive to the combined action of free radicals and Ca^{2+} (Malis and Bonventre, 1986).

In addition to the aging process the action of free radicals and Ca^{2+} ions plays an important role in ischemia and post-ischemic reperfusion injury. It is interesting in this respect that decreased water content has been found also in mitochondria from the ischemic heart (Michael et al., 1980).

Moreover, Malis and Bonventre (1986) found the mitochondrial ATPase activity to be sensitive even to the action of free radicals alone. As already mentioned, mitochondrial ATPase activity has been demonstrated to depend on the fluidity and chemical composition of the inner membrane (Zsigmond and Clandinin, 1986). Changes in the ionic environment of the enzyme due to loss of water might easily be the common cause.

Changes in mitochondrial energy output would have serious effects on all aspects of cellular life. The expression of genes, macromolecular synthesis and the production of regulatory small molecules like cAMP are under energetic control (Snoeij et al., 1986), either directly or via ionic changes (Douzou and Maurel, 1977; Cahn and Lubin, 1978) due to the energy dependence of cellular ionic pumps. The expression of originally silent genes (Goldstein and Reis, 1984) and changes in the protein synthesizing

capacity (Makrides, 1983) of cells certainly play a role in the aging process, but might be secondary to a decrease in the energy status. However, although the correlation between free radical production and aging seems to be well established (for review, see Sohal and Allan, 1986), the experimental evidence for a decrease of the energetic potential of cells during aging is not yet fully satisfactory (Hansford, 1983) and, in part, even contradictory (Manzelmann and Harmon, 1987). Further work remains to be done.

References

Bahr GF, Johnson FB, Zeitler E (1965) The elementary composition of organic objects after electron irradiation. Lab Invest 14, 1115-1123.

Barer R, Dick DAT (1957) Interferometry and refractometry of cells in tissue culture. Exp Cell Res Suppl 4, 103-126.

Barer R, Joseph S (1954) Refractometry of living cells. I. Basic principles. Quart J Microsc Sci 95, 399-423.

Barer R, Joseph S (1955a) Refractometry of living cells. II. The immersion medium. Quart J Microsc Sci 96, 1-26.

Barer R, Joseph S (1955b) Refractometry of living cells. III. Technical and optical methods. Quart J Microsc Sci 96, 423-447.

Bunn CL, White FA (1985) Mitochondrial DNA in aging human cells. In: Molecular Biology of Aging: Gene Stability and Gene Expression (RS Sohal, ed). Raven Press, New York, pp 77-92.

Butler WH, Judah JD (1970) Preparation of isolated rat liver mitochondria for electron microscopy. J Cell Biol 44, 278-284.

Cahn F, Lubin M (1978) Inhibition of elongation steps of protein synthesis at reduced potassium concentrations in reticulocytes and reticulocyte lysate. J Biol Chem 253, 7798-7803.

Cosslett VE (1978) Radiation damage in the high resolution electron microscopy of biological materials: A review. J Microsc 113, 113-147.

Daum G (1985) Lipids of mitochondria. Biochem Biophys Acta 822, 1-42.

David H (1985) The hepatocyte-development, differentiation and ageing. Exp Pathol, suppl 11,44-63.

David H, Bozner A, Meyer R, Wassilew G (1981) Pre- and postnatal development and ageing of the heart. Exp Pathol, suppl 7, 96-144.

Douzou P, Maurel P (1977) Ionic control of biochemical reactions. Trends Biochem Sci 2, 14-17.

Duque-Magalhaes MC, Gualberto JM (1987) Regulation of mitochondrial proteolysis. Selective degradation of inner membrane polypeptides. FEBS Letters 210, 142-146.

Fleming JE, Melnikoff PS, Latter GI, Chandra D, Bensch KG (1986) Age dependent changes in the expression of Drosophila mitochondrial proteins. Mech Ageing Dev 34, 63-72.

Frolkis WW (1975) Mechanismen des Alterns. Berlin, Akademie-Verlag, p 60.

Goldstein S, Reis RJS (1984) Genetic Modification during cellular aging. Mol Cell Biochem 64, 15-30.

Hagler HK, Buja LM (1984) New techniques for the preparation of thin freeze-dried cryosections for X-ray microanalysis. In: Science of Biological Specimen Preparation. M Müller, RP Becker, A Boyde, JJ Wolosewick (eds). Scanning Electron Microsc, Inc, AMF O'Hare, IL, pp 161-166.

Hall TA, Clarke-Anderson H, Appleton T (1973) The use of thin specimens for X-ray microanalysis in biology. J Microsc 99, 177-182.

Hansford RG (1983) Bioenergetics in aging. Biochem Biophys Acta, 41-80.

Harman D (1986) Free radical theory of aging: Role of free radicals in the origination and evolution of life, aging and disease processes. In: Free Radicals, Aging, and Degenerative Processes. Alan R. Liss, Inc, New York, NY, pp 3-49.

Jung DW, Chave E, Brierley GP (1977) Energy-dependent exchange of K^+ in heart mitochondria. K^+ efflux. Arch Biochem Biophys, 460-470.

Katchalsky A (1971) Polyelectrolytes. Pure Appl Chem 26, 327-374.

Kleinig H, Sitte P (1986) Zellbiologie. 2nd ed. Gustav Fischer, Stuttgart, New York, 299-301.

Linders PWJ, Stols ALH, Stadhouders AM (1981) Quantitative electron probe X-ray microanalysis of thin objects using an independent mass determination method. Micron 12, 1-4.

Lund P, Wiggins D (1987) The matrix water space of mitochondria in situ in isolated hepatocytes. Biosci Rep 7, 59-66.

Lustyik G, Zs-Nagy, I (1985) Alterations of the intracellular water and ion concentrations in brain and liver cells during aging as revealed by energy-dispersive X-ray microanalysis of bulk specimens. Scanning Electron Microsc 1985;I, 323-337.

Makrides SC (1983) Protein synthesis and degradation during aging and senescence. Biol Rev Cambridge Philos Soc 58, 343-422.

Malis CD, Bonventre JV (1986) Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. J Biol Chem 261, 14201-14208.

Manzelmann MS, Harmon HJ (1987) Lack of age-dependent changes in rat heart mitochondria. Mech Ageing Dev 39, 281-288.

Mazur P (1984) Freezing of living cells: mechanisms and implications. Amer J Physiol 247, C125-C142.

Michael LH, Seitz P, McMillin-Wood J, Chang D, Hazlewood C, Entman ML (1980) Mitochondrial water in myocardial ischemia: Investigation with nuclear magnetic resonance. Science 208, 1267-1268.

Miquel J, Fleming J (1986) Theoretical and experimental support for an 'oxygen-radical - mitochondrial injury' hypothesis of cell aging. In: Free Radicals, Aging, and Degenerative Diseases. Alan R Liss, Inc, New York, NY, pp 51-74.

Nohl H, Hegner D (1978) Do mitochondria produce oxygen radicals in vivo? Eur J Biochem 82, 563-567.

Nohl H, Jordan W (1986) The mitochondrial site of superoxide formation. Biochem Biophys Res Comm 138, 533-539.

Rick R, Dörge A, Bauer R, Gehring K, Thurau K (1979) Quantification of electrolytes in freeze-dried cryosections by electron microprobe analysis. Scanning Electron Microsc 1979;II, 619-625.

Rick R, Dörge A, Thurau K (1982) Quantitative analysis of electrolytes in frozen-dried sections. J Microsc 125, 239-247.

Robards AW, Sleytr UB (1985) Low temperature methods in electron microscopy. (Practical methods in electron microscopy, Vol 10, AM Glauert, ed). Elsevier, Amsterdam , 88-91.

Saubermann AJ, Scheid VL (1985) Elemental composition and water content of neuron and glial cells in the central nervous system of the North American medicinal leech (Macrobdella decora). J Neurochem 44, 825-834.

Schwerzmann K, Cruz-Orive LM, Eggman R, Sänger A, Weibel ER (1986) Molecular architecture of the inner membrane of mitochondria from rat liver: A combined biomedical and stereological study. J Cell Biol 102, 97-103.

Sevéus L (1978) Preparation of biological material for X-ray microanalysis of diffusible elements: I. Rapid freezing of biological tissue in nitrogen slush and preparation of ultrathin frozen sections with absence of trough liquid. J Microsc 112, 269-279.

Simon H, Krantz D, Voss K, Wenzelides K (1980) Automated data sampling in sections from selected liver diseases. Pathol Res Pact 170, 388-401.

Snoeij NJ, van Rooijen HJM, Penninks AH, Seinen W (1986) Effects of various inhibitors of oxidative phosphorylation on energy metabolism, macromolecular synthesis and cyclic AMP production in isolated rat thymocytes. A regulating role for the cellular energy state in macromolecular synthesis and cyclic AMP production. Biochem Biophys Acta 852, 244-253.

Sohal RS, Allan RG (1986) Relationship between oxygen metabolism, aging and development. Advances in free radical Biology and Medicine, vol 2, Pergamon Press, 117-160.

Solomon S, Wise P, Ratner A (1976) Postnatal changes of water and electrolytes in rat tissues. Proc Soc Exp Biol Med 153, 359-362.

Somlyo AP, Somlyo AV, Shuman H (1979) Electron probe analysis of vascular smooth muscle. Composition of mitochondria, nuclei and cytoplasm. J Cell Biol 81, 316-335.

Somlyo AP, Bond M, Somlyo AV (1985) Calcium content in mitochondria and endoplasmic reticulum in liver frozen rapidly in vivo. Nature 314, 622-625.

Steinbrecht RA, Zierold K (1987) Cryotechniques in biological electron microscopy. Springer, Berlin, Heidelberg, New York.

Victorica J, Clark A, Machado A, Satrustegui J (1985) Impairment of glutamate uptake and absence of alterations in the energy-transducing ability of old rat brain mitochondria. Mech Ageing Dev 29, 255-266.

Weibel ER (1979) Stereological methods. I. practical methods. Academic Press, London, New York , 43-45.

Zeitler E (1971) Trockengewichtsbestimmung von Teilchen mit dem Elektronenmikroskop. In: Methodensammlung der Elektronenmikroskopie (G. Schimmel and W Vogell eds). Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Sect. 4.1.5.

Zglinicki T von (1983) Quantitative Röntgenmikroanalyse biologischer Ultradünnschnitte mit Aluminium-Kohle-Aufdampfschichten als Standards. Acta Histochem 72, 195-201.

Zglinicki T von (1987a) Die Bedeutung der Röntgenmikroanalyse für die experimentelle Medizin und Diagnostik. Doctor of Sciences Thesis. Humboldt University, Berlin.

Zglinicki T von (1987b) A mitochondrial membrane hypothesis of aging. J Theor Biol 127, 127-132.

Zglinicki T von, Uhrik B (1988) X-ray microanalysis with a closed cooling chain: Is it necessary? J Microsc, in press.

Zglinicki T von, Bimmler M (1987a) The intracellular distribution of ions and water in rat liver and heart muscle. J Microsc 146, 77-86.

Zglinicki T von, Bimmler M (1987b) Intracellular water and ionic shifts during growth and ageing of rats. Mech Ageing Dev 38, 179-187.

Zglinicki T von, Bimmler M, Purz H-J (1986) Fast cryofixation technique for X-ray microanalysis. J Microsc 141, 79-90.

Zglinicki T von, Bimler M, Krause W (1987) Estimation of organelle water fractions from frozen-dried cryosections. J Microsc 146, 67-75.

Zglinicki T von, Lustyik G (1986) Loss of water from heart muscle cells during aging of rats as measured by X-ray microanalysis. Arch Gerontol Geriatr 5, 283-289.

Zglinicki T von (1988) Monovalent ions are spatially bond within the sarcomere. J Gen Physiol Biophys, in press.

Zierold K (1986) The determination of wet weight concentrations of elements in freeze-dried cryosections from biological cells. Scanning Electron Microsc 1986;II, 713-724.

Zsigmond E, Clandinin MT (1986) Modulation of mitochondrial ATPase sensitivity to inhibitors and stimulators by diet-induced changes in membrane lipid. Int J Biochem 18, 505-511.

Zs.-Nagy I, Lustyik G, Bertoni-Freddari C (1982) Intracellular water and dry mass content as measured in bulk specimens by energy dispersive X-ray microanalysis. Tissue Cell 14, 47-60.

Discussion with Reviewers

M.F. Wendt-Gallitelli: Most of the data presented in the paper (Tab.1,2,3) stem from isolated mitochondria, which represent a strong selection of the whole population of mitochondria in situ. I am not convinced that data concerning water content in isolated organelles can be compared directly with the water content of the same organelles in situ. The author should give the necessary information for justifying this statement and discuss this point.

Author: As stated in the paper, the agreement between water content of isolated rat liver mitochondria and that of mitochondria within the tissue is surely accidentally. The degree of hydration depends on the amount of ions lost during isolation and on the concentration of osmotically active substances in the medium. Isolation conditions, and especially the osmolarity of the medium, were identical for young, adult and senescent rats. It is interesting that despite of this and despite of the fact that there might be some preference for a certain fraction during isolation from heart muscle water loss is found in isolated mitochondria. However, whilst the main conclusion of the paper, namely that mitochondrial water loss occurs in vivo during aging at least in heart muscle and liver cells stems from the measurements of tissues in situ, the results obtained on isolated mitochondria prove the second conclusion of the water loss as an intrinsic property of mitochondria, withstanding even dramatic changes in the extramitochondrial osmolarity.

M.F. Wendt-Gallitelli: The author presents results showing significant differences between the diameter of mitochondria isolated from tissues and that measured stereologically in sections of conventionally fixed and embedded tissue, in which shrinking is unavoidable. I do not see the sense of such information about diameters measured in two systems (isolated mitochondria and mitochondria of chemically fixed tissue) in which strong artifacts (changes of volume) during preparation are unavoidable. How can the author justify his statement and his extrapolation to in situ mitochondria?

Author: There are of course volumetric changes of tissue compartments both on an absolute and relative scale during conventional processing for electron microscopy. Such changes might also occur during, e.g., freeze-drying. In my opinion, the best we can do is to compare the results of different techniques. Morphometry of conventionally prepared tissue samples has been proven to be a reliable technique capable of giving at least the direction of changes occuring in vivo (see Weibel (1979) for review). Morphometrical estimates of the age dependence of mitochondria measured by different researchers coincide qualitatively if the same tissue is concerned, despite inter-laboratory variations in the processing techniques.

K. Zierold: You attribute the haloes seen in Fig.2 around the mitochondria to ice crystal damage. In my opinion the haloes arise by different rates of shrinkage of the cytoplasm and the mitochondria as caused by freeze-drying. The rate of shrinkage is related to the water content of the particular compartment. Please comment!

Author: As far as I know it cannot be decided at present whether mitochondrial haloes arise during freezing or during freeze-drying. If freeze-drying is involved - and our recent results showing increased frequency of mitochondrial haloes after exposure of sections to moist air and repeated freeze-drying give some support to this idea - the rate of lateral shrinkage seems to depend rather on mean ice crystal size than directly on the water content of the compartments. Otherwise, haloes should be found also in well-frozen sections bearing no or very small ice crystals, which was never the case. Moreover, the coincidence of data given in Table 1 argues against a significant dependence of the amount of shrinkage on water content of compartments.

K. Zierold: The data of water content you give in Table 1 indicate that scanning microdensitometry is more precise than

X-ray microanalysis or interference microscopy. Could you comment on the accuracy of the various methods?
Author: As compared to densitometry, X-ray microanalysis suffers from the higher electron dose needed. The difference is about 4 orders of magnitude for structures of mitochondrial size. Taking this into account, frozen-dried sections can be regarded as quite insensitive against radiation damage. In my opinion, the main reason for the decreased precision of X-ray microanalytical measurements is the need for subtracting the external background, which in our system generally amounts to between 50 and 75% of the total. According to the Gaussian law of error propagation this increases the error by a factor between 1.4 and 2.6 (cf. Table 1). On the contrary, in densitometry the contribution of the background intensity in the image is almost negligible. The problem with interference microscopy of mitochondria is, of course, that one works at the resolution limit of the light microscope.

K. Zierold: Have you found differences in the morphology of mitochondria in cryosections as depending on the tissue (liver or heart muscle), age and on preparation conditions (in vivo and isolated)? Have you found cristae in cryosectioned mitochondria?
Author: I have never seen any ultrastructure within mitochondria in frozen-dried cryosections due to their highly condensed appearance. I did not measure e.g., the mean size of mitochondria on cryosections, because the magnification of these images is not sufficiently reproducible due to widely differing objective lens settings necessary for X-ray microanalysis.

G.M. Roomans: Could you speculate on strategies to counteract water loss by mitochondria during aging?
Author: The matter seems to be extremely complicated. Till now, all antioxidants tested failed to increase the maximum life span in mammals, although some did so in Drosophila (Miquel et al. 1982). However, the average life span in rodents could be increased, partly due to beneficial effects on disease. Although this seems to be encouraging at first glance, these effects seem to be mostly due to a reduction of the basal metabolic rate which overweights the true antioxidant properties (Harman 1986). So actually the potent antioxidants have deleterious effects on mitochondrial coupled respiration (Horrum et al. 1987), which renders them unsuitable as life-prolonging drugs in man. Moreover, feeding of animals with one antioxidant seems to suppress the intracellular concentrations of others. We are currently thinking about possibilities of stabilizing the mitochondrial function in old animals in order to bypass the effects of water loss. There are certain drugs capable of restoring mitochondrial energy metabolism which can be tested in the aging process, too.

Additional references

Horrum MA, Harman D, Tobin RB (1987) Free radical theory of aging: Effects of antioxidants on mitochondrial function. Age 10, 58-61.

Miquel J, Fleming JE, Economos AC (1982) Antioxidants, mitochondrial respiration and aging in Drosophila. Arch. Gerontol. Geriatr. 1, 349-363.