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Effect of ageing and malnutrition on rat myocardium

I. The myocyte

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Summary. The effects of ageing and starvation on the rat myocardium were studied by morphometric methods. Since cardiac muscle is a tissue with a high level of anisotropy, methods based on the concept of vertical planes were used to describe quantitative alterations in the rat myocyte both at the cellular and ultrastructural level. During starvation rapid and important changes were noted, particularly in the transverse dimension of cells and organelles. The most striking change, however, was the immediate dilatation of the myocyte T-system, reflecting an adaptive interaction between the intra- and extracellular environment. At the same time exocytosis of intracellular components into the extracellular space of the T-system was observed. The ratio of mitochondria to myofibrils decreased progressively during starvation. Such a decrease, in general, may reach a point when cellular energy supply becomes compromised. A comparison between different regions of the heart showed no differences and it can be concluded that the morphological changes during starvation are the same, and equally distributed, in both ventricles. The changes described in the aged rat heart point in the direction of a hypertrophy of the aged myocyte. This leads to a lower ratio between surface and volume which finds its representation at the subcellular level in a more spherical shape of nuclei and mitochondria. Unlike what is seen in malnutrition, the mitochondrial/myofibril ratio is higher in the older rat. From the morphological point of view, the atrophy of malnutrition and the hypertrophy of ageing are opposed, but in both there is a change in the relationship of the myocyte to its environment which directly influences the substrate exchange capacity. This tends to protect the myocyte in starvation but jeopardizes the older cell.

Key words: Ageing – Malnutrition – Morphometry – Myocardium

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Introduction

Nutrition is a modulating environmental influence that strongly interferes with normal growth and development of ageing organisms (Merry 1986). Malnutrition may have clearly defined effects on specific organs, with cell shrinkage or cell loss as one of the basic mechanisms of regression. This may ultimately have a detrimental effect on organ function. Comparison of the effects of ageing and malnutrition at the level of ultrastructure and cell morphology could be of value in understanding these changes during regression. Since the rat is the most widely used experimental animal in the field of clinical nutrition, it is an appropriate model for comparing and evaluating the effects of malnutrition and ageing using morphological tools.

Materials and methods

The study design and protocol were approved by the ethics committee of the University of Antwerp. All rats for the experiments were purchased from Janssen Pharmaceutica (Beerse, Belgium). To avoid sex differences, only female Wistar rats were chosen. Three basic groups were finally studied: neonatal rats ($n=30$), adult rats ($n=50$), and senescent rats ($n=6$). The neonatal rats were studied on days 0, 1, 5, 10, 15, and 20 to evaluate major changes during the neonatal growth period. Adult rats, weighing 320 ± 22 g, 16 weeks of age, were housed individually in a controlled environment and a total caloric deprivation was instituted. Accessibility to fluids was ad libitum throughout the study, and no specific vitamin or electrolyte supplements were given. This situation gives an acute and global protein-calorie malnutrition stress that led in this study group to a malnutrition lethal time in 50% of the animals (LT_{50}) of 10 days. Analytical studies were performed on the study groups on fasting days 0, 1, 2, 4, 6, 8 and 10. Every studied subgroup consisted of 5–7 rats. Control groups of 5 rats were housed in the same conditions but received standard nourishment. They were studied on days 0 and 10. To study the effects of ageing on the ultrastructure of the myocyte a group of 6 older rats (aged 2 years) was studied. The older rats were from the same strain and the same sex as the younger rats. Since the myocardium was the target tissue under study the strain chosen was free of cardiovascular

disease (hypertension) and did not have a genetic propensity to obesity.

In the morphometric analysis of the ultrastructure of a tissue sample it is imperative that alterations during fixation and processing are kept to an absolute minimum. Fixation was performed after ether anaesthesia. The abdominal aorta was cannulated retrogradely with a microtube connected to a perfusion apparatus (Loud et al. 1978; Anversa et al. 1980b, 1986). After heparinization (500 IU IV), the heart was arrested in diastole by IV injection of potassium chloride (1 mmol/ml). The vena cava was sectioned to allow drainage of blood and perfusate, and the myocardium was perfused for 3 min with 0.1 M phosphate buffer (pH 7.2). Subsequently, the heart was perfused for 10–15 min with a mixture of 2% glutaraldehyde, 0.1 M cacodylate and 1% sucrose. The perfusion was done at a pressure of 120–150 cm of water, equal to the average diastolic pressure in adult anaesthetized rats (Lais et al. 1977). After perfusion-fixation, the heart was excised rapidly and separated carefully from the major blood vessels. Weights were recorded of the total heart, the removed right ventricle, and the remnant left ventricle with the interventricular septum.

For light microscopy, the midzones of the left and right free ventricular walls were sliced transversely and longitudinally into thin arcs. Four blocks of tissue were cut from each one extending from the endocardial to the epicardial surface. The blocks were fixed in a mixture of acetic acid (5%) – formaldehyde 40% (10%) – ethanol 100% (85%) for 4 h. The fixed samples were kept in 70% ethyl alcohol until embedding. After dehydration with isopropyl alcohol (70%) for 2 h and then in 100% isopropyl alcohol changed every 2 h for 12 h, they were immersed in toluene for 4 h and embedded in paraffin (56° C). Sections (4 μm) were stained with haematoxylin-eosin and sirius-haematoxylin for light microscopic examination.

For electron microscopy, the following fixative composition was used in the perfusion-fixation: glutaraldehyde 2%, sodium cacodylate 0.1 M, sucrose 1%. This has a pH of 7.35, total osmotic pressure of 428 mosmol/l and an effective osmotic pressure of 330 mosmol/l, which fulfils the criteria for tonicity of fixatives used for preparations of samples for ultrastructural work (Paulus 1984). After perfusion fixation, ten blocks (measuring each 1 mm^3) from either free ventricular wall were immersed for an additional 4 h in the perfusate and then washed overnight in cacodylate buffer (0.1 M) with 4% sucrose (pH 7.2). They were then post-fixed in 2% osmium tetroxide in veronal acetate buffer for 2 h and after rinsing with veronal acetate buffer, block-stained in a solution of 2% uranyl acetate in the same buffer. After dehydration with progressively increasing concentrations of acetone (70%, 90%, 100%), tissue blocks were transferred successively in Epon/propylene-oxide mixtures with increasing ratio (100% propylene, Epon/propylene 1/2, 1/1, 2/1, Epon 100%) and embedded in 100% Epon, to be polymerized at 50° C for 2 days and 60° C for 1 day. When polymerization was completed, semi-thin sections of 0.35 μm were cut with glass knives using a Reichert Ultracut ultramicrotome and stained with toluidine blue 1%. In the semi-thin sections adequate areas of longitudinally and transversely sectioned zones were selected and cut with diamond knives to get ultra-thin (0.07 μm) sections that were picked up on a copper grid of 300 mesh to be contrasted following the method of Reynolds (1963). The grids were placed in uranyl acetate (1.5% in 50% ethanol) for 15 min and then thoroughly rinsed in ethanol (50%) and carbon dioxide-free doubly-distilled water. Finally, the grids were floated on lead citrate in water for 4.5 min and rinsed with carbon dioxide-free doubly-distilled water to clear deposits. The sections were studied in a Zeiss EM 109 electron microscope at a voltage of 80 kV.

A standard set of photographs was taken to ensure a random sampling of tissue zones. From each animal, longitudinally and transversely cut non-damaged blocks were selected from both the left and right ventricular wall. Serial micrographs were taken on consecutive grid mesh squares. Using higher magnifications the left upper zone of the square was photographed. Magnifications of 1,100×, 3,000×, 7,000× and 12,000× were collected and, after exact calibration with a diffraction grating replica magnification standard, finally printed at 3,340×, 9,570×, 23,100× and 40,590×. Eight micrographs from each block were chosen for morphometric analysis.

Since the estimation of volume fraction by point counting is based on the determination of proportions, basic binomial statistics can be used to determine a minimum number of points that should be counted to have an appropriate sample size. To evaluate subcellular compartments it is best to have reasonable precision regarding determination of small proportions. Indeed, the smaller the proportion, the larger the sample needed for a given degree of precision. We can calculate that we must count 9,508 points when we require our estimate P of the smallest proportion (1%) to be within an error $e = \pm 0.002$ with a probability of $2\alpha = 0.05$ (Vandewoude 1990). Grids have been constructed with a square geometric array and with a total number of points of 121 and 963 to assure that for every component an adequate number of points was counted (more than 10,000 for the T-system and the sarcoplasmic reticulum).

Light microscopic evaluation was used to assess volume fraction changes at the tissue level. In order to obtain the volume fraction of the subcellular compartments high-power electron micrographs (9,570×, 23,100×, 40,590×) were analysed and an adequate number of test points was counted. The relationship between surface area and volume at organ, cellular and subcellular levels is of paramount importance in cellular metabolism, substrate and oxygen supplies. The determination of surface area and surface density requires, unlike the estimation of volume fraction, a high degree of isotropy in the studied structure. This requirement is not fulfilled in muscle. Muscle tissue is an example of a tissue with a very high degree of anisotropy and therefore an error will be induced systematically in the estimation of S_v using the basic relation. This can be overcome by an approach that deals with the orientation distribution of the surface under study (Cruz-Orive et al. 1985). In practice, a numerically weighted estimate of the intersection number with parallel test lines at various angles of θ (angle away from the vertical direction) should be made proportional to $\sin\theta$. To avoid this elaborate procedure it is easier to construct a test system containing line segments with various lengths and orientations, such that the test lines at an angle θ to the vertical have a total length proportional to $\sin\theta$. In this case no numerical weighing factor is needed and the total intersection number with such a test system gives directly the estimates of S_v because the curved test-lines of the grid automatically correct for the systematic error induced by the anisotropy of the tissue. All measurements dealt with were done with these adapted grids constructed for highly anisotropic tissue (Vandewoude 1990). The reader may find the conceptual development of the test grids in the Appendix.

Statistical analysis was done by Student t-test or, when the conditions for normality were not fulfilled, by the U-test after control for differences in shape or distribution of the sample by a Kolmogoroff-Smirnow analysis, pre-adjusted to a 90% confidence level.

Results

Tables 1, 2 and Fig. 1 show the effects of ageing and growth on the absolute and relative weights of the rat heart.

It is clear that both body weight and heart weight increase significantly during growth and ageing. In the neonatal period there is a rapid growth period, characterized by allometric growth, as expressed by weight. There was also no significant change in the heart fraction made up by the left ventricle (Fig. 1).

In adult and even more in aged rats, the relative weight of the heart is lower than in young rats. Looking at the ratio between left ventricular and right ventricular

Table 1. Absolute and relative weight of the rat heart according to age (mean ± SD)

	Age groups			
	Newborn	20 days	16 weeks	24 months
Body Weight (g)	1.4 ±0.1	42 ±2	297 ±9	388 ±33**
Heart Weight (g)	8.8 ±0.5 (10^{-3})	225 ±7 (10^{-3})	1.03 ±0.03	1.26 ±0.07***
Heart/Body Weight (10^{-3})	6.38 ±0.54	5.42 ±0.41	3.46 ±0.10	3.33 ±0.27*

* $P<0.01$: comparison to newborn group
** $P<0.01$, *** $P<0.005$: comparison between older and adult groups

Table 2. Right and left (ventricular) heart mass according to age in the rat heart (mean ± SD)

	Age groups		
	Young (20 days)	Adult (16 weeks)	Old (24 months)
LV (g)	0.188 ±0.004	0.822 ±0.033**	1.046 ±0.051**,***
(% total heart)	77.5 ±2.0	81.9 ±1.0*	83.0 ±1.6*
RV (g)	0.055 ±0.006	0.181 ±0.011	0.218 ±0.026
(% total heart)	22.5 ±2.0	18.1 ±1.0*	17.0 ±1.6*

LV, Left ventricular and interventricular septal mass; RV, right ventricular mass

* $P<0.05$, ** $P<0.001$: comparison to young group
*** $P<0.001$: comparison to adult group

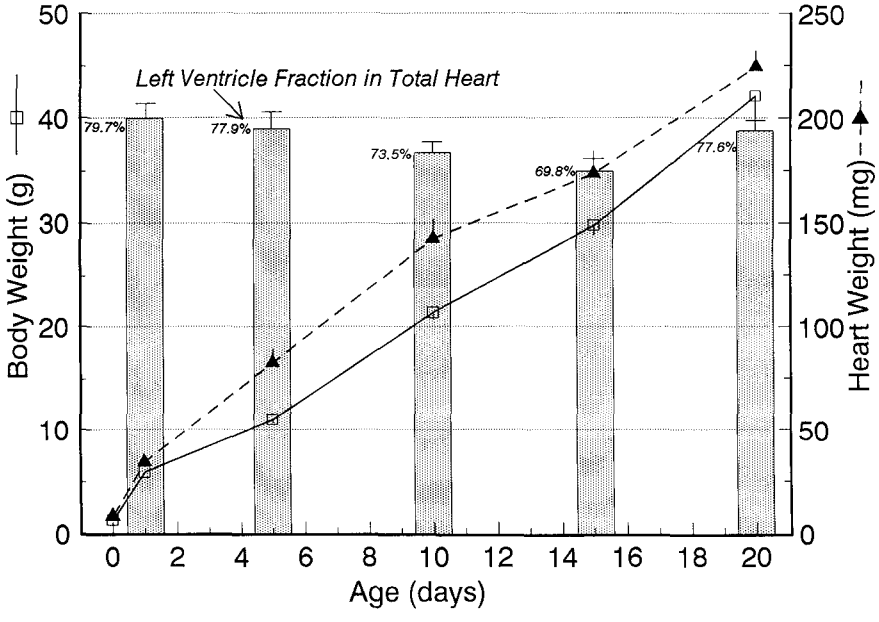

Fig. 1. Evaluation of body weight and heart weight in groups of neonatal rats ($n=5$ in each group) during normal growth; mean ± standard error

mass, a continuous increase in left ventricular mass as a proportion of total heart mass is observed (Table 2). Left ventricular wall thickness also increases with ageing (Table 3). These effects can be attributed to the well-known phenomena of changes in circulation and peripheral resistance, which begin abruptly shortly after birth and continue throughout life (Anversa et al. 1980a; Olivetti et al. 1980).

During total caloric deprivation there was a steady decrease in body weight to 65.5 ± 1.1% of initial body weight in starved rats, compared to a further rise in body weight to 112.4 ± 2.4% of initial weight in normal fed controls. Rats dying of malnutrition had a mean body weight at death of 52.3 ± 3.3% of initial weight with a range of 44–63% ($n=7$). The linear fall in weight during starvation was not followed by a parallel change in heart weight (Fig. 2). This evolution was reflected in

Table 3. Thickness of left (LV) and right (RV) free ventricular wall (mm) (mean ± SEM) in young adult rats (16 weeks) before and after 10 days starvation and in normal older rats (24 months)

	Groups		
	Young-fed	Young-starved	Old
LV (mm)	1.83 ± 0.21	1.18 ± 0.08***	2.06 ± 0.05*
RV (mm)	0.77 ± 0.11	0.49 ± 0.04***	0.94 ± 0.04**

* $P<0.05$: compared to young-fed group
** $P<0.01$: compared to young-fed group
*** $P<0.001$: compared to young-fed group

Table 4. Heart weight parameters in separate groups of starved rats ($n=5$) expressed as mean ± standard error of the mean

	Day of starvation			
	0	6	10	D
BW (g)	320 ±10	229 ±5****	181 ±6****	175 ±7****
HW (g)	1.09±0.04	1.02±0.02	0.78±0.03****	0.64±0.06****
LV (g)	0.87±0.04	0.82±0.01	0.64±0.03****	0.51±0.05****
RV (g)	0.20±0.02	0.18±0.01	0.14±0.01***	0.11±0.01****
H/B (10^{-3})	3.43±0.18	4.45±0.11***	4.31±0.15***	3.62±0.21
LV/B (10^{-3})	2.73±0.17	3.58±0.08***	3.57±0.17**	2.90±0.20
RV/B (10^{-3})	0.62±0.04	0.81±0.07*	0.77±0.04*	0.60±0.04
LV/H (%)	81.3 ±1.5	81.7 ±1.1	82.1 ±1.1	82.6 ±1.2
RV/H (%)	18.7 ±1.5	18.3 ±1.1	17.9 ±1.1	17.4 ±1.2

D, Group of rats that died spontaneously of malnutrition; BW, body weight; HW, heart weight; LV, mass of left ventricular and interventricular septal heart; RV, mass of free right ventricular wall; H,B: heart and body weight symbols

Statistical significance between value and control group (day 0):
* 2 $P<0.05$, ** 2 $P<0.01$, *** 2 $P<0.005$, *** 2 $P<0.001$

Table 5. Regional differences of volume fraction (V_v) of myocytes in rat heart in the subendocardial (endo) and the subepicardial (epi) zone in the left (LV) and right (RV) ventricle in normal fed rats (control), after 10 days fast, and in aged rats.

	Endo	Epi	P
Control			
LV	83.4±4.2	84.6±3.1	NS
RV	85.0±2.1	82.4±3.8	NS
Fasting			
LV	88.2±3.5	87.9±4.4	NS
RV	86.7±2.0	86.5±1.0	NS
Aged			
LV	85.9±2.1	86.9±1.6	NS
RV	89.3±2.8	88.1±0.9	NS

Data expressed as percentage V_v (mean ± SD)

NS, Not significant

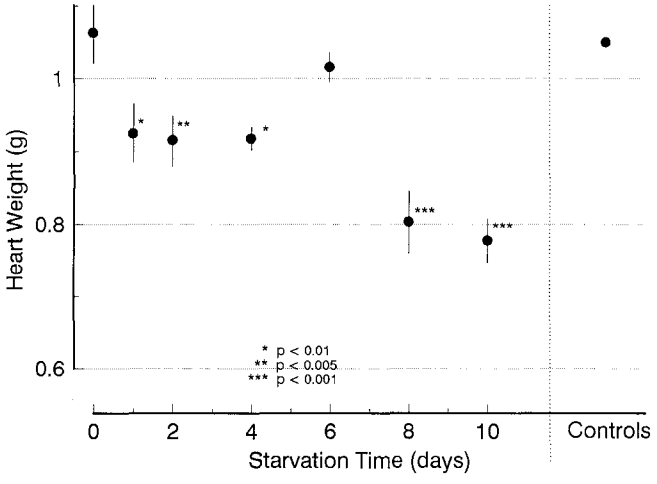

Fig. 2. Heart weight in rats during total caloric deprivation ($n=5$–7 in each subgroup). *C*, Control group. Mean ± standard error; *P* compared to control group at day 0

Table 6. Myocyte volume fraction of left (LV) and right (RV) heart ventricle in the rat: effect of age and starvation. Data expressed as percentage volume fractions (mean ± SD)

	LV	RV
Control	84.0±3.6	83.7±3.2
Fasting	88.0±3.7*	86.6±1.5**
Aged	86.4±1.8*	88.7±2.1***,****

* $P<0.05$, ** $P<0.01$, *** $P<0.005$: compared to control group
**** $P<0.01$: compared to aged LV

a continuous rise of the ratio heart/body weight in the initial phase of starvation (until day 6). Later in starvation, this ratio stabilized at a high level. In the animals that spontaneously died of malnutrition, the ratio at the time of death was equal to that in the control group. This evolution is shown in Table 4. The changes in wall thickness and mass of right and left ventricles were comparable (Tables 3, 4).

Morphometric data related to regional differences in myocyte volume fraction show no statistical differences between the subendocardial and subepicardial zones. This holds for the left and the right ventricle in the control group, the starvation group, and the aged group (Table 5). When analysed for the effect of ageing, an increase was seen in the volume fraction of myocytes, which was more pronounced in the right ventricle and eventually reached a statistically significant difference between right and left ventricle ($P<0.01$) in the older group (Table 6). Also during fasting a significant rise in myocyte volume fraction is seen both in right ($P<0.01$) and left ($P<0.05$) ventricle (Table 6).

The effect of starvation on non-vascular collagenous tissue was very limited and did not give rise to any significant change (Table 7). In every subgroup, however, the right ventricle contained more collagenous tissue than the left; a finding which was not changed by fasting. With ageing there was a striking increase in the volume

Table 7. Effect of fasting and ageing on the volume fraction of non-vascular collagenous tissue in rat heart in the left (LV) and right (RV) ventricle, analysed for regional differences in the subendocardial (endo) and subepicardial (epi) zones

		Control	Fasting	Aged
A. Data				
LV	Endo	1.40 ± 0.16	2.00 ± 0.33	7.83 ± 0.72
	Epi	2.80 ± 0.39	3.20 ± 0.57	6.08 ± 0.66
RV	Endo	4.60 ± 0.58	6.30 ± 0.83	8.83 ± 0.86
	Epi	3.90 ± 0.48	5.40 ± 0.56	7.58 ± 0.76
B. Significances $P <$				
Intra-group:				
LV	Endo – Epi	NS	NS	0.05
RV	Endo – Epi	NS	NS	NS
Endo	LV – RV	0.005	0.005	NS
Epi	LV – RV	0.05	0.01	NS
To control group:				
LV	Endo	–	NS	0.01
	Epi	–	NS	0.001
RV	Endo	–	NS	0.001
	Epi	–	NS	0.001

Data expressed as percentage volume fraction (mean ± standard error)

Table 8. Volume fraction of the myocyte components in the left and right ventricle in young control rats compared with that in young rats starved for 10 days and in old rats

	Control	Starved	Old
Left ventricle			
Nucleus	1.37 ± 0.32	1.47 ± 0.47	1.14 ± 0.39
Sarcoplasm	98.6 ± 0.3	98.5 ± 0.5	98.9 ± 0.4
Mitochondria	33.4 ± 0.8	26.3 ± 0.6****	37.0 ± 1.0**
Myofibrils	63.0 ± 0.8	69.2 ± 0.6****	60.5 ± 1.0**
T-system	2.04 ± 0.16	2.80 ± 0.23*	1.19 ± 0.21**
SR	1.60 ± 0.18	1.74 ± 0.27	1.35 ± 0.25
Right ventricle			
Nucleus	1.66 ± 0.45	1.91 ± 0.47	0.71 ± 0.24***
Sarcoplasm	98.3 ± 0.5	98.1 ± 0.5	99.3 ± 0.3***
Mitochondria	33.9 ± 0.8	29.4 ± 0.8****	37.9 ± 1.4*
Myofibrils	63.1 ± 0.8	65.3 ± 0.8****	59.1 ± 1.4*
T-system	1.53 ± 0.12	3.27 ± 0.28****	1.57 ± 0.27
SR	1.47 ± 0.17	2.06 ± 0.25	1.42 ± 0.27

SR, Sarcoplasmic reticulum. Values are expressed as percentage volume fraction per reference space (men ± SEM)

* $P < 0.05$: compared to control group
** $P < 0.01$: compared to control group
*** $P < 0.005$: compared to control group
**** $P < 0.001$: compared to control group

fraction of collagen with a much more uniform distribution in the different regions of the aged heart so that the collagen content was practically the same in both ventricles.

At the cellular level, expressed per myocyte, the volume fraction of the nucleus did not change with fasting.

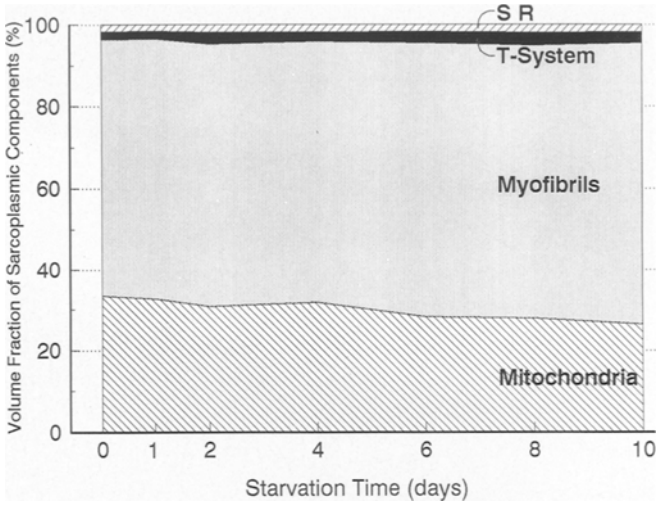

Fig. 3. Relative volume fractions of sarcoplasmic components in rat ventricle myocytes during total starvation. *SR*, Sarcoplasmic reticulum

With ageing, however, a decrease was observed both in the right and left ventricle, which was statistically significant in the right ventricle (Table 8). The changes of the major components of the sarcoplasm during fasting are presented in Fig. 3. The most striking change was the important and abrupt increase in volume fraction of the T-system ($P < 0.001$) with no change in the sarcoplasmic reticulum (Fig. 4). Further, a more progressive decline in mitochondrial volume fraction ($P < 0.001$) during fasting with a reciprocal rise of the myofibril intracellular compartment was observed. The changes in the T-system were comparable both in the left and right ventricle (Fig. 5). After the initial peak rise in volume fraction there was a stabilization of value during the later stages of starvation at lower, but still statistically significant, levels compared to control values.

The changes in volume fraction of the myocyte components in older rats compared to that of younger rats, both fed and starved for 10 days, are shown in Table 8. The mitochondria represent a statistically higher fraction of the myocyte at the expense of the myofibrillar compartment in older rats. There are no major changes in the sarcoplasmic reticulum, but there is a lower volume fraction of the T-system in the left ventricle.

Although myocyte surface density during fasting was only significantly high in the later stage of starvation, a gradual increase was seen after an initial rise and fall (Fig. 6). This was observed in both right and left ventricle. No significant differences were observed between values of the two ventricles.

The changes in surface to volume ratio of major cell organelles are given in Table 9. With fasting, an increase in surface density was seen in the nucleus ($P < 0.05$) and the mitochondrion with respect to outer membrane ($P < 0.05$), while no significant change is seen in the mitochondrion with respect to inner membrane. There was an immediate reduction in surface density of the T-system in both ventricles during early starvation, but the

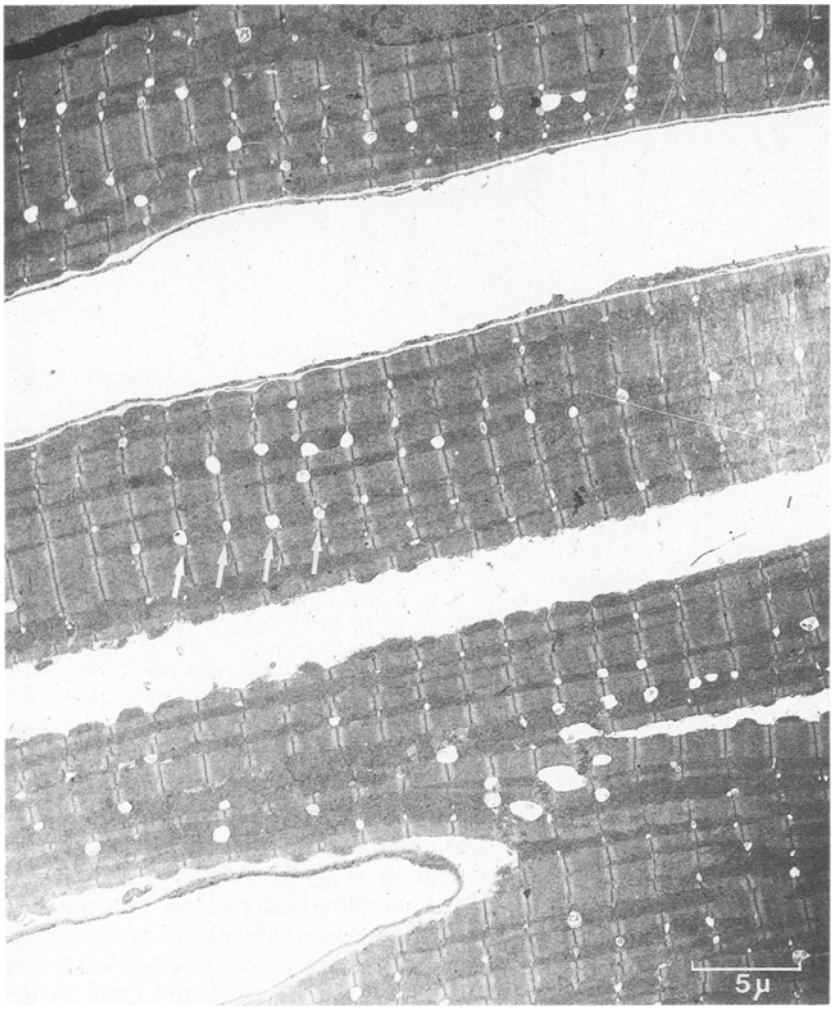

Fig. 4. Longitudinal sections of myocytes from rat left ventricle after one day caloric deprivation. Note the regular array of the distended T-tubules at the Z-discs (*arrows*), ×4,450

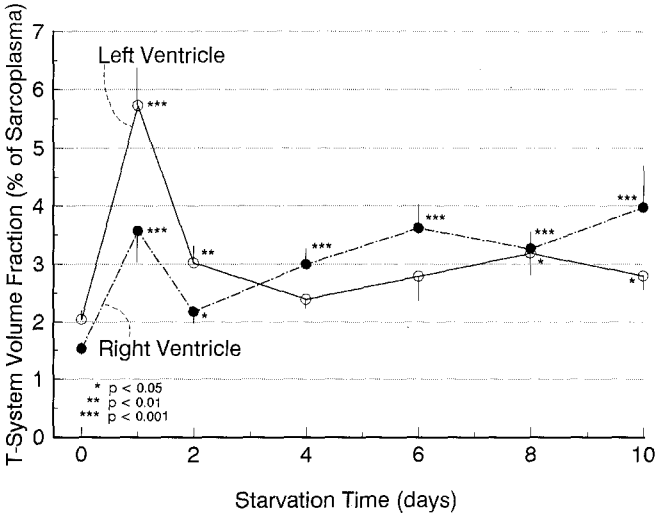

Fig. 5. Volume fraction of the T-system expressed as percentage of the sarcoplasma in rat myocytes from the left and right ventricle during total starvation

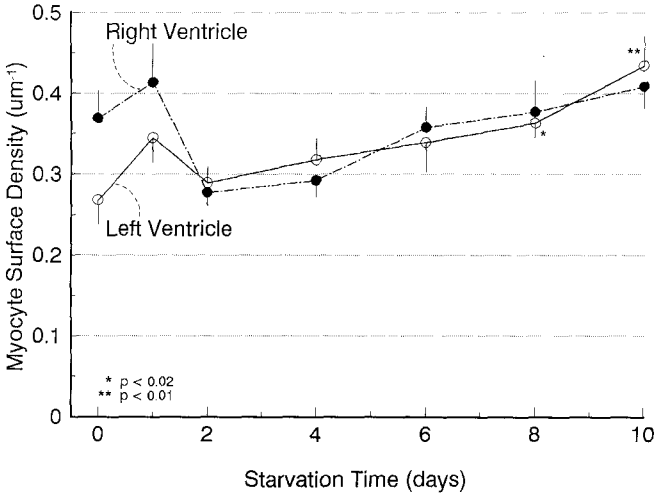

Fig. 6. Surface density of myocytes of the rat left and right heart ventricle during total starvation

Table 9. Surface density (μ^{-1}) of cellular organelles in the left and right ventricle in control, starved (1 and 10 days), and old rats

	Control	1 day fast	10 day fast	Old
Left ventricle				
Nucleus	0.93±0.17	1.25±0.13	1.33±0.10*	1.19±0.21
Mitochondrion				
OM	4.99±0.34	6.30±0.42*	6.76±0.43*	5.41±0.23
IM	38.1 ±2.5	31.1 ±2.6	40.4 ±3.2	22.3 ± 2.0***
T-system	22.2 ±3.1	11.5 ± 0.6***	5.9 ±3.7	28.2 ±4.1
SR	21.5 ±3.2	12.5 ±2.1	20.4 ±2.6	21.9 ±1.9
Right ventricle				
Nucleus	1.34±0.17	1.75±0.37	1.94±0.09*	1.29±0.14
Mitochondrion				
OM	6.13±0.45	5.98±0.61	6.51±0.40	6.52±0.68
IM	40.0 ±4.5	31.5 ±3.9	35.8 ±3.3	40.1 ±3.3
T-system	22.7 ±2.9	13.4 ±1.8**	18.9 ±2.9	31.3 ±1.9*
SR	21.6 ±2.0	30.2 ±3.5*	21.2 ±2.3	26.4 ±3.0

Data expressed as mean±standard error. OM, Outer mitochondrial membrane; IM, inner mitochondrial membrane; SR, sarcoplasmic reticulum

* $P<0.05$, ** $P<0.01$, *** $P<0.005$

normal ratio is restored later on. T-system surface density was higher in older rats than in younger rats, and the sarcoplasmic reticulum showed no consistent changes in surface density with ageing or fasting.

Discussion

Although malnutrition is a very frequently encountered problem in medicine, very few studies deal with the morphometric changes induced by malnutrition. Ultrastructural analysis of myocardium is mostly performed to evaluate the hypertrophy induced by hypertension (Bishop et al. 1979a, b; Breisch et al. 1980; Dalen et al. 1987) or exercise (Anversa et al. 1983; Loud et al. 1984; Frenzel et al. 1988). Other conditions, however, have also been studied, such as toxicity (Buja et al. 1973; Mall et al. 1980a, b; Hertsens et al. 1984), specific metabolic changes (Davies and Jennings 1970; McCandless et al. 1970; Cluzeaud et al. 1981; Factor et al. 1983; Gerdes et al. 1987; Hsiao et al. 1987; Mall et al. 1987), and central nervous system changes (Jacob et al. 1972; Smith and Page 1976) but only a limited number of studies have been done on the changes during starvation (Abel et al. 1979; De Waal et al. 1986; Vandewoude et al. 1988).

From the data on heart weight during starvation, it is clear that there is a direct effect of fasting on the heart. There seems to be a proportional weight loss in both ventricles as the ratio between right and left ventricle weights does not change. So, the percentage loss of wall thickness is the same in the left and right ventricle. This proportional change is not seen when ventricular weights are compared to the fall in body weight, which is more rapid and documented by a progressive rise in heart/body weight ratio. The ratio, in the later stage of starvation, however, remained stable, at a higher level. In rats that died of malnutrition spontaneously the heart/body weight ratio was again the same as in the normally fed rat. This evolution can be explained by an initial sparing of the heart relative to the rest of the body, an advantage that is lost in later phases when the heart loses weight at the same rate as the body. In the ultimate stages cardiac weight loss is even greater, probably due to structural breakdown of tissue.

Volume fractions of different components at the tissue level were not significantly different in the region of the subendocardium and subepicardium. Neither starvation nor ageing induced differences in morphometric parameters between the subendocardial and subepicardial zones. However, there was a higher content of nonvascular collagenous tissue in all regions of the right ventricle in normal and starved rats. With ageing this content increased in both ventricles, but particularly in the left ventricle so that in the aged rat the difference between left and right ventricular content of collagenous tissue disappeared.

Apart from the above reported changes in starvation, a striking increase was noted in the volume fraction of the T-system. During early starvation a global dilatation of the T-tubes was observed, extending into the interior of the cell. Qualitative analysis showed the protrusion and shedding of sarcoplasm and parts of cell organelles into the T-system (Fig. 7). The possibility of an "excretory" function of the T-system for expulsion of degraded cell elements should be considered, apart from its well-known role in the excitation-contraction coupling (Forbes and Sperelakis 1982). The process of expelling cytoplasmic constituents could be synergistic with the previously reported phenomenon of autophagic vacuoles where cytoplasmic constituents are degraded after sequestration within lysosome-like structures. These vacuoles are regularly found in heart muscle and are subject to a circadian variation (Pfeiffer and Strauss 1981; Pfeiffer et al. 1987). The dilatation of these T-tubules actually reflects the change in the relation between the

Fig. 7. Protrusion of mitochondrial (*M*) and sarcoplasmic components in a dilated T-tubule (*T*) in a rat myocyte from the left ventricle after 1 day total starvation, ×123,750

myocyte and its immediate environment, so essential for its vital function.

At the cellular level, expressed per myocyte, the volume fraction of the nucleus did not change with fasting. The volume fraction of the nucleus observed was comparable to earlier reported volume fractions (Anversa et al. 1979). Inside the cell, changes are more progressive, with an increasing myofibril/mitochondria ratio. This progressive reduction in mitochondrial fraction could lead to a deficient energy supply that may compromise contractility in the malnourished heart. However, changes in mitochondria and nuclei during starvation lead to an increase in surface density of the outer membranes, thereby creating more surface area per volume for exchange. The same mechanism of increasing exchange surface is seen here at subcellular and the cellular levels during fasting.

The opposite is true in the aged rat where larger and more voluminous myocytes have less exchange capability. These morphological alterations are quite comparable to the changes observed in experimental hypertrophy models in the rat heart (Julian et al. 1981; Tomanek and Hovanec 1981; Engelmann et al. 1987; Mattfeldt and Mall 1987).

In summary, we can say that the modulating effects of malnutrition and ageing on the rat myocyte are important. The major changes, regarding cell morphology, occur in the longitudinal dimensions of the cell. This directly influences the capacity for metabolic exchange which is lower in the aged cell and relatively improved in the starved cell. This may also be reflected in the altered mitochondrial/myofibril ratio in these two different situations. In old rats more mitochondria are available per contractile element than in control rats. The opposite is true in starved rats. Changes in the aged rat resemble the changes reported in literature during hypertrophy. From the morphometric view point these changes seem to be less favourable. Relative hypotrophy may be a mechanism to preserve the functional state of the cell and may be one of the factors which favours survival.

Appendix

The surface area per unit volume (S_v) of a component with a curved surface in space can be estimated by placing a straight line probe at random in space, and counting the number of intersections be-

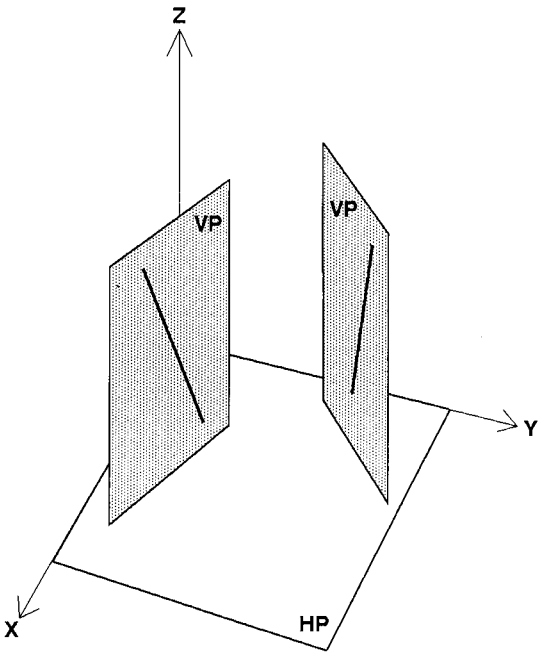

Fig. 8. The vertical direction z is chosen as perpendicular to an arbitrary horizontal plane (*HP*). Any plane parallel to this z-axis is nominated as a vertical plane (*VP*)

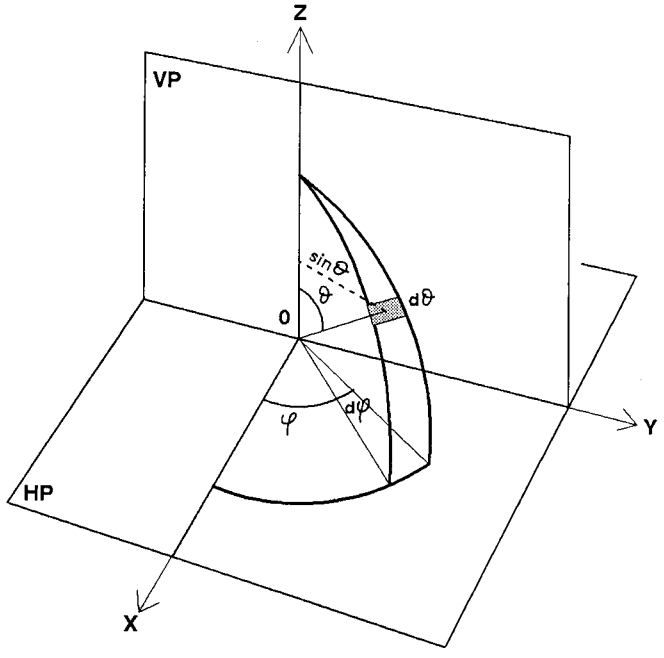

Fig. 9. The horizontal plane (*HP*) is normal to the vertical axis z. Any spatial direction is determined by the longitude Φ and the co-latitude θ. The probability of a random direction determined by the pair (Φ, θ) is described by the hatched area. Values of Φ are equally likely, larger values of θ are more likely than small values. The probability of θ is proportional to sin θ

tween the surface and the line probe. However, unlike the estimation of volume fraction, the determination of surface area requires a kind of isotropy. Muscle tissue is an example of a tissue with a very high degree of anisotropy and therefore an error will be induced systematically in the estimation of S_v using this basic relation. This can be overcome by an approach that deals with the orientation distribution of the surface under study (Cruz-Orive et al. 1985). To obtain an isotropic, uniformly random line in the three dimensions in an anisotropic structure the concept of vertical planes is necessary (Baddeley et al. 1986). A vertical section is any plane section perpendicular to a given horizontal x, y plane (Fig. 8). The only meaning attached to the horizontal plane is that of an arbitrary chosen reference plane, which defines the orientation of the section. Except from lines parallel to the vertical axis, every straight line in three dimension is contained in a unique vertical plane. So, we can say that S_v can be statistically estimated by taking only vertical sections of a three-dimensional specimen and drawing test lines within these sections. When we draw only straight lines, however, they can not be considered to be uniformly spread over all spatial directions.

To express a set of uniformly spread, isotropic lines, we can imagine that every point of a sphere's surface corresponds to a direction in space of a particular line originating in the sphere's centre, O. A position on the surface of the sphere can be described in spheric coordinates with a combination of two angles: the longitude Φ (angle around an arbitrary vertical axis) and the co-latitude θ (angle away from the vertical axis) (Fig. 9). The values of Φ are all equally likely, the values of θ, however, are not. Larger values are more likely than smaller values. The probability of finding a line inclined at an angle θ away from the vertical axis is proportional to sin θ. When in vertical sections, test lines through the central point O are unevenly drawn, so that the density of lines with an angle θ to the vertical is proportional to sin θ, these test lines are isotropic in three dimension. When finally the vertical sections are made with uniformly random positions within the reference space, the test lines are isotropic, uniformly random lines, whose intersections can be used adequately in the basic stereological relation $S_v = 2I_L$ to estimate surface area density, S_v.

References

Abel RM, Grimes JB, Alonso D, Alonso M, Gay WA Jr (1979) Adverse hemodynamic and ultrastructural changes in dog hearts subjected to protein-calorie malnutrition. Am Heart J 97:733–744

Anversa P, Olivetti G, Melissari M, Loud AV (1979) Morphometric study of myocardial hypertrophy induced by abdominal aortic stenosis. Lab Invest 40:341–349

Anversa P, Olivetti G, Loud AV (1980a) Morphometric study of early postnatal development in the left and right ventricular myocardium of the rat: I. Hypertrophy, hyperplasia and binucleation of myocytes. Circ Res 46:495–502

Anversa P, Olivetti G, Melissari M, Loud AV (1980b) Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 12:781–795

Anversa P, Levicky V, Beghi C, Mc Donald SL, Kikkawa Y (1983) Morphometry of exercise-induced right ventricular hypertrophy in the rat. Circ Res 52:57–64

Anversa P, Beghi C, Kikkawa Y, Olivetti G (1986) Myocardial infarction in rats: infarct size, myocyte hypertrophy and capillary growth. Circ Res 58:26–37

Baddeley AJ, Gundersen HJG, Cruz-Orive LM (1986) Estimation of surface area from vertical sections. J Microsc 142:259–276

Bishop SP, Drummond J, Reynolds R (1979a) Regional cardiac myocyte growth in normotensive and spontaneously hypertensive rats. J Mol Cell Cardiol 11 [Suppl]:8

Bishop SP, Oparil S, Reynolds RH, Drummond JL (1979b) Regional myocyte size in normotensive and spontaneously hypertensive rats. Hypertension 1:378–383

Breisch EA, Bove AA, Phillips SJ (1980) Myocardial morphometrics in pressure overload left ventricular hypertrophy and regression. Cardiovasc Res 14:161–168

Buja LM, Ferrans VJ, Mayer RJ, Roberts WC, Henderson ES (1973) Cardiac ultrastructural changes induced by daunorubicin therapy. Cancer 32:771–788

Cluzeaud F, Laplace M, Moravec J, Rakusan K, Hatt PY (1981) Hypertrophie cardiaque secondaire à une anemie par carence en fer chez le rat. Pathol Biol 29:11–18

Cruz-Orive LM, Hoppeler H, Mathieu O, Weibel ER (1985) Stereological analysis of anisotropic structures using directional statistics. J R Stat Soc Ser C 34:14–32

Dalen H, Saetersdal T, Odegarden S (1987) Some ultrastructural features of the myocardial cells in the hypertrophied human papillary muscle. Virchows Arch [A] 410:281–294

Davies MJ, Jennings RB (1970) The ultrastructure of the myocardium in the thiamine-deficient rat. J Pathol 102:87–95

De Waal EJ, Vreeling-Sindelarova H, Schellens JPM, James J (1986) Starvation-induced micro-autophagic vacuoles in rat myocardial cells. Cell Biol Int Rep 10:527–533

Engelmann GL, Vitullo JC, Gerrity RG (1987) Morphometric analysis of cardiac hypertrophy during development, maturation and senescence in spotaneously hypertensive rats. Circ Res 60:487–494

Factor SM, Minase T, Bhan R, Wolinsky H, Sonnenblick EH (1983) Hypertensive diabetic cardiomyopathy in the rat: ultrastructural features, Virchows Arch [A] 398:305–317

Forbes MS, Sperelakis N (1982) Association between mitochondria and gap junctions in mammalian myocardial cells. Tissue Cell 14:25–37

Frenzel H, Schwarzkopff B, Hotermann W, Schnurch HG, Novi A, Hort W (1988) Regression of cardiac hypertrophy: morphometric and biochemical studies in rat heart after swimming training. J Mol Cell Cardiol 20:737–751

Gerdes AM, Moore JA, Hines JM (1987) Regional changes in myocyte size and number in propranolol-treated hyperthyroid rats. Lab Invest 57:708–713

Hertsens R, Jacob W, Van Bogaert A (1984) Effect of hypnorm, chloralosane and pentobarbital on the ultrastructure of the inner membrane of rat heart mitochondria. Biochem Biophys Acta 769:411–418

Hsiao Y, Suzuki K, Abe H, Toyota T (1987) Ultrastructural alterations in cardiac muscle of diabetic BB Wistar rats. Virchows Arch [A] 411:45–52

Jacob WA, Van Bogaert A, De Groodt-Lasseel MHA (1972) Myocardial ultrastructure and haemodynamic reactions during experimental subarachnoid haemorrhage. J Mol Cell Cardiol 4:287–298

Julian FJ, Morgan DL, Moss RL, Gonzalez M, Dwivedi P (1981) Myocyte growth without physiological impairment in gradually induced rat cardiac hypertrophy. Circ Res 49:1300–1310

Lais LT, Rios LL, Boutelle S, Dibona GF, Brody MJ (1977) Arterial pressure development in neonatal and young spontaneously hypertensive rats. Blood Vessels 14:277–84

Loud AV, Anversa P, Giacomelli F, Wiener J (1978) Absolute morphometric study of myocardial hypertrophy in experimental hypertension. I. Determination of myocyte size. Lab Invest 38:586–596

Loud AV, Beghi C, Olivetti G, Anversa P (1984) Morphometry of right and left ventricular myocardium after strenuous exercise in preconditioned rats. Lab Invest 51:104–111

Mall G, Mattfeldt T, Volk B (1980a) Ultrastructural morphometric study on the rat heart after chronic ethanol feeding. Virchows Arch [A] 389:59–77

Mall G, Reinhard H, Stopp D, Rossner JA (1980b) Morphometric observations on the rat heart after high-dose treatment with cortisol. Virchows Arch [A] 385:168–180

Mall G, Klingel K, Baust H, Hasslacher C, Mann J, Mattfeldt T, Waldherr R (1987) Synergistic effects of diabetes mellitus and renovascular hypertension on the rat heart: stereological investigations on papillary muscles. Virchows Arch [A] 411:531–542

Mattfeldt T, Mall G (1987) Growth of capillaries and myocardial cells in the normal rat heart. J Mol Cell Cardiol 19:1237–1246

McCandless DW, Hanson C, Speeg KV, Schenker S (1970) Cardiac metabolism in thiamine deficient rats. J Nutr 100:991–1002

Merry BJ (1986) Dietary manipulation of ageing: an animal model. In: Bittles AH, Collins KJ (eds) The biology of human ageing. Cambridge University Press, pp 233–242

Olivetti G, Anversa P, Loud AV (1980) Morphometric study of early postnatal development in the left and right ventricular myocardium of the rat. II. Tissue composition, capillary growth and sarcoplasmic alterations. Circ Res 46:503–512

Paulus G (1984) The relationship between proliferation and maturation in transplantable tumours in the mouse and in man, Thesis, UIA, Antwerpen

Pfeifer U, Strauss P (1981) Autophagic vacuoles in heart muscle and liver. A comparative morphometric study including circadian variations in meal-fed rats. J Mol Cell Cardiol 13:37–49

Pfeifer U, Fohr J, Wilhelm W, Dammrich J (1987) Short-term inhibition of cardiac cellular autophagy by isoproterenol. J Mol Cell Cardiol 19:1179–1184

Reynolds E (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Smith HE, Page E (1976) Morphometry of rat heart mitochondrial subcompartments and membranes: application to myocardial cell hypotrophy after hypophysectomy. J Ultrastruct Res 55:31–41

Romanek RJ, Hovanec JM (1981) The effects of long-term pressure overload and ageing on the myocardium. J Mol Cell Cardiol 13:471–488

Vandewoude MFJ (1990) Modulating effects of ageing and malnutrition. A biochemical, morphometrical and clinical study. PhD Thesis, UIA, Antwerpen

Vandewoude MFJ, Cortvrindt RG, Goovaerts MF, Van Paesschen MA, Buyssens N (1988) Malnutrition in the heart: a microscopic analysis, Infusionstherapie 15:217–220