Virchows Arch (1998) 433:167–172

ORIGINAL ARTICLE

T.M. Mayhew · C. Gregson · A. Pharaoh · D.G. Fagan

Numbers of nuclei in different tissue compartments of fetal ventricular myocardium from 16 to 35 weeks of gestation

Received: 9 February 1998 / Accepted: 10 March 1998

Abstract The aim of this study was to examine mechanisms of growth in different tissue compartments of the ventricular myocardium of prenatal human hearts. To this end, stereological methods were applied in order to estimate tissue volumes and total numbers of myocyte, connective tissue and endothelial nuclei in hearts collected after death at between 16 and 35 weeks of gestation. Volumes of tissue compartments were obtained after multiplying volume densities (estimated by test-point counting) by ventricular volumes (estimated from ventricular mass and tissue density). Absolute numbers of nuclei were calculated in similar fashion from corresponding nuclear packing densities (estimated using physical disectors). The volumes of all three tissue compartments increased linearly over the period of gestation examined, and in each case, the increase in tissue volume appeared to be due entirely to proliferation. Numbers of all three types of nuclei increased linearly whilst tissue volumes per nucleus remained constant. The net rate of production of myocyte nuclei was 35×10^7 per week (2.1 million nuclei per hour). The net rate of production of connective tissue nuclei was 12×10^7 per week (0.7 million nuclei per hour) and that for endothelial cell nuclei was 5.1×10^7 per week (0.3 million nuclei per hour). Predictions are made about the postnatal ages at which adult ratios of different nuclear types might be attained.

Key words Ventricles · Myocardium · Compartments · Nuclei · Number

T.M. Mayhew (✉) · C. Gregson · A. Pharaoh
School of Biomedical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK
e-mail: terry.mayhew@nottingham.ac.uk
Tel.: (+44)115-970-9415, Fax: (+44)115-970-9732

D.G. Fagan
Division of Pathology, School of Clinical Laboratory Sciences, Queen's Medical Centre, Nottingham, UK

Introduction

Previous studies on growth of the mammalian ventricular myocardium during pre- and post-natal periods have concentrated on either the total ventricle or merely the myocytes within it. There is relatively little information on cells in other tissue compartments [1–6, 10–13, 17, 20, 26–29, 31–34, 43, 44]. For myocytes at least, there is a consensus that fetal and perinatal growth occurs principally by hyperplasia. Soon after birth, a transitional period in which proliferation ceases gives way to a phase in which myocardium grows mainly by myocyte hypertrophy [5, 12, 38]. A similar picture, of prenatal growth principally by an increase in nuclear number (expressed as DNA content), is seen in the case of the entire heart [43]. Protein-to-DNA ratios suggest that, from 13 weeks to term, the amount of tissue protein per nucleus also increases. More recent evidence suggests that ratios of connective tissue nuclei to myocyte nuclei vary according to developmental age before stabilising at between 2:1 and 3:1 in adults [1, 2].

Estimates of DNA and protein contents do not distinguish nuclei in different tissue compartments or with different ploidy levels [43]. In recent studies [1, 2], attempts have been made to cater for variable ploidy status by conducting differential counts of nuclei in muscle and connective tissue. Unfortunately, they relied on counts of nuclear profiles appearing on single tissue sections. This approach is not satisfactory, because single sections provide a biased sample of nuclei in which apparent number also depends on nuclear size, shape and spatial orientation [14, 15]. Single sections sample nuclei with probabilities determined by their longest dimensions normal to the plane of sectioning, a problem that can be overcome by applying a design-based stereological device, the disector. Using *pairs* of sections, this provides unbiased estimates of numbers of nuclei in 3-dimensional space by sampling them with identical probabilities [22, 37, 41]. Previously, we and others have applied the disector successfully to count myocyte nuclei in the ventricles of normal and abnormal human hearts [5, 33, 38].

The present investigation uses physical disectors to estimate the numbers of myocyte, connective tissue and endothelial nuclei in human ventricles separately at 16–35 weeks of gestation in utero. Results provide evidence that myocardial growth in all these tissue compartments is essentially hyperplastic over this period.

Materials and methods

We obtained 6 post-mortem hearts from an archive maintained by the Department of Pathology. They were from apparently normal-for-age subjects varying in gestational age from 16 to 35 weeks and in fetal weight from 46 g of 2.5 kg [38]. Weights were within the 50th–90th centiles as established by ultrasound examination. In all subjects, the heart and great vessels were in the anatomically correct positions and showed no evidence of abnormal morphology.

Heart morphometry was performed using stereological methods [16, 24, 25, 35, 36]. The mammalian heart is not homogeneous in composition and, during development, does not grow uniformly in different segments or tissue compartments [3, 19, 27, 31, 42]. Therefore, it was necessary to ensure that all parts of a given ventricle were given an equal change of being selected. To this end, random sampling was employed. Each heart was opened by lateral incisions (to examine morphology and evacuate blood coagula) and then fixed in 10% buffered calcium-formalin solution. Thereafter, ventricles were dissected free of atria, pericardium, vessels and fat before weighing [18]. Left and right ventricles were weighed separately after division of the interventricular septum.

Sampling was uniformly random in location. Each ventricle was cut into 2- to 3-mm^3 blocks and stored in coded pots prior to being analysed blind. Later, blocks were cut into smaller pieces, from which 2–5 per ventricle were selected in a systematically random manner [23]. These were embedded in JB4 acrylic resin and cut on a Reichert-Jung Autocut microtome using Latta-Hartmann glass knives to provide ribbons of 4–6 serial sections. The nominal section thickness (3 μm) was chosen to satisfy the technical requirement that the distance between the section planes of a physical disector [22, 37, 41] should be less than the smallest linear dimension of nuclei orthogonal to the plane of sectioning. Ribbons were mounted on glass microslides and stained with haematoxylin and eosin [7].

Light microscopical fields of view were selected in a systematic random manner from reference sections and matching sites on consecutive parallel look-up sections. The section planes of each reference and look-up pair were separated by one section thickness. Colour slide transparencies of the fields were viewed by dual flat projection at a final linear magnification of ×5730. Magnifications were calibrated using stage micrometer scale standards. Pairs of transparencies were viewed side by side. Each reference section was projected onto pieces of stiff white card on which unbiased 'forbidden line' counting frames were drawn [21]. Each frame sampled a tissue subfield and 21–110 (mean 54) subfields were sampled from a given ventricle.

The numerical density , N_V, of nuclei of a given type (whether from a myocyte, connective tissue, or endothelium) within a myocardium was estimated using the ventricle as the total containing volume. Myocyte nuclei were identified as large, relatively euchromatic, spheroidal or ellipsoidal nuclei occupying a central position within the cell. Connective tissue nuclei were similar in size to those of myocytes, but more irregular in shape and heterochromatic. Endothelial cell nuclei were small, highly heterochromatic and even more variable in shape. In myocardium, they belong predominantly to the rich blood capillary plexus, but they also include endothelial cells of lymphatic vessels. The nuclei of the tunica media of vessels were excluded from counts, but this had no great impact on the estimates of numbers because most of the larger arteries and veins are found in the epicardium and not in the myocardium.

On the disector principle, nuclei appearing in frames on reference sections were eligible for counting if they did not touch either the forbidden lines of the frames or their extensions or appear on the look-up sections. This number is contained in a volume of ventricle given by the product of the tissue area bounded by the unbiased counting frame and the mean distance between section planes [41]. A convenient way of estimating ventricular sectional area is to count test points, each of which is associated with a known constant area given on the scale of the specimen, i.e. after taking into account the areal magnification. Nuclear and point counts were summed over all counting frames sampled from a given ventricle.

The mean distance between section planes in this study was equal to mean section thickness on the microslide, estimated as the difference in height of pre- and post-sectioned resin blocks measured with a micrometer screw gauge [39]. The mean thickness of a set of 100 microtome sections was estimated to be between 2.7 and 2.9 μm.

Numbers of nuclei per ventricle were estimated by multiplying N_V by the volume of the corresponding ventricle, which was determined from ventricular mass assuming a tissue density of 1.05 g/cm^3. Nuclear number obtained in this way is subject to bias, because ventricular mass was measured on fixed but not processed tissue, whilst N_V was determined for fixed and processed tissue. However, relative biases are common, so that the final numbers are valid for drawing comparisons. Finally, nuclear number is equal to cell number only if each cell contains, on average, one nucleus. Whilst this applies to connective tissue and endothelial cells, cardiomyocytes vary in nuclear content [3, 9, 12, 26, 31]. Therefore, estimates are confined to numbers of nuclei, and no attempt is made to convert these to numbers of myocytes [38].

To calculate volumes of individual tissue compartments per nucleus, the fractions of ventricle volume occupied by myocytes, connective tissue and blood vessels were estimated separately by test-point counting [35, 36] using sections from the set cut for the nuclear counts. As with numerical density estimation, the validity of volume density depends on randomness of section location (but not orientation).

Numbers and volumes were estimated per heart by combining values obtained in both ventricles. Coefficients of error (CE=standard error expressed as a fraction, or percentage, of its mean) were calculated for estimated numerical densities and total numbers of nuclei [5]. For nuclear numerical densities, individual values of CE(N_V) were calculated according to Braendgaard et al. [8], and from these an overall mean was determined. Repeat estimates of total number were made on ventricles from two subjects so as to monitor the reproducibility of number estimation, CE(N).

Relationships between age and nuclear number or volume per nucleus were assessed by Pearson's correlation coefficient and linear regression analysis [40]. The latter was also used to assess relationships between fetal weight (the independent or predictor variable) and various structural quantities (the dependent or outcome variables) using log-transformed data. The regression equation for a straight line can be expressed in the form

$$\log D = \log A + (B \times \log I)$$

where D is the dependent and I the independent variable. B denotes the slope of the regression line and represents the exponent (or scaling factor) of the allometric equation $D=A\times I^B$. Log$A$ is the intercept of the regression line on the log$D$ axis, and A is the integration constant, i.e. the value of D for a unit amount of I. A standard error of the mean (SEM) was calculated for B.

For all statistical tests, the null hypothesis was rejected if the probability level, P, was less than 0.05.

Table 1 Coefficients for regression lines of gestational age against various morphometric variables (SEM)

Variables	Intercept	Slope	Correlation coefficient
Fetal weight (g)	–2253 (145)	135 (6.09)	0.996
Tissue volumes (cm^3)			
Ventricles	–7.52 (0.405)	0.460 (0.017)	0.997
Myocytes	–4.36 (0.201)	0.268 (0.008)	0.998
Connective tissue	–2.67 (0.199)	0.159 (0.008)	0.994
Blood vessels	–0.356 (0.086)	0.021 (0.004)	0.945
Nuclear numbers ($\times 10^9$)			
All nuclei	–8.70 (0.509)	0.536 (0.021)	0.997
Myocytes	–5.64 (0.304)	0.350 (0.013)	0.997
Connective tissue	–2.19 (0.202)	0.133 (0.009)	0.992
Endothelium	–0.87 (0.065)	0.053 (0.003)	0.995
Tissue volume per nucleus (μm^3)			
Overall	701 (181)	3.53 (7.60)	0.226
Myocytes	647 (151)	3.63 (6.37)	0.274
Connective tissue	971 (266)	4.92 (11.2)	0.215
Blood vessels	551 (305)	–5.87 (12.8)	–0.223

Results

Changes with gestational age

Findings indicate that volumes of tissue compartments and numbers of their nuclei increased linearly from 16 to 35 weeks of gestation, as summarised in Table 1 and Figs. 1 and 2. From 16 to 35 weeks, there were highly significant positive correlations between gestational age and fetal weight, tissue volumes and numbers of nuclei (correlation coefficient, *r*, equal to or greater than 0.94 in each case). The intercepts and slopes for the regression lines are also provided in Table 1. With the exception of the total volume of blood vessels in myocardium (intercept significant at $P<0.05$ and slope at $P<0.01$), all intercepts and slopes were significantly different from zero at a probability level of $P<0.001$.

In contrast, there was no evidence that tissue volume per nucleus altered significantly between 16 and 35 weeks. The volume of myocardium per nucleus, connective tissue per nucleus and myocyte per nucleus (not equal to mean myocyte volume, because some cells have more than one nucleus) appeared to increase from 16 to 35 weeks, whilst the volume of blood vessel per nucleus seemed to decline (Fig. 2). However, the slopes of the regression lines were not significantly different from zero.

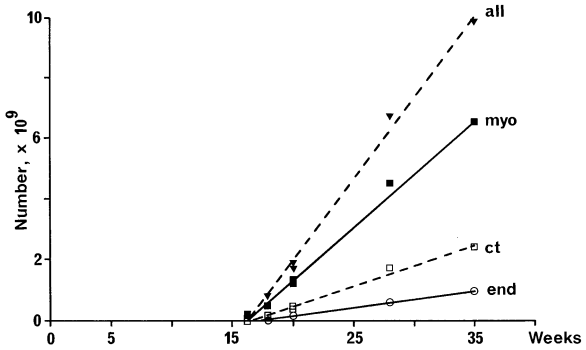

Fig. 1 Relationships between nuclear number and gestational age for different types of nuclei in fetal ventricles (*myo* myocyte nuclei, *ct* connective tissue nuclei, *end* endothelial cell nuclei, *all myo+ct+end*)

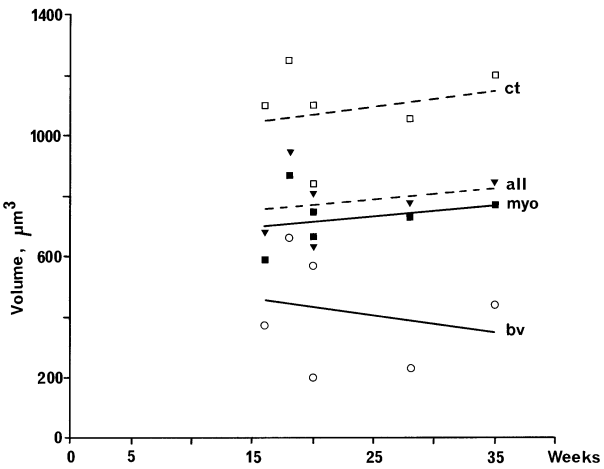

Fig. 2 Relationships between compartment volume per nucleus and gestational age for different tissue types in fetal ventricles (*myo* myocytes, *ct* connective tissue, *bv* blood vessels, *all myo+ct+bv*)

Changes with fetal weight

Allometric relationships were examined using log-transformed variables (Table 2). The volumes of all tissue compartments increased more or less isometrically with fetal weight, as evidenced by the facts that exponents for different tissues differed significantly from zero ($P<0.001$) but not from unity or each other (Fig. 3). Similar relationship were established between fetal weight

Table 2 Coefficients for regression lines of log W (fetal weight) against various morphometric variables. Mean (SEM)

Variables	Constant	Exponent	Correlation coefficient
Tissue volumes (cm^3)			
Ventricles	0.00334	1.014 (0.050)	0.995
Myocytes	0.00215	1.003 (0.040)	0.997
Connective tissue	0.00079	1.051 (0.025)	0.999
Blood vessels	0.00006	1.138 (0.137)	0.972
Nuclear numbers ($\times 10^9$)			
All nuclei	0.00451	0.996 (0.043)	0.996
Myocytes	0.00387	0.959 (0.037)	0.997
Connective tissue	0.00076	1.045 (0.053)	0.995
Endothelium	0.00009	1.216 (0.132)	0.977
Tissue volume per nucleus (μm^3)			
Overall	655	0.028 (0.048)	0.277
Myocytes	557	0.044 (0.041)	0.472
Connective tissue	1040	0.005 (0.058)	0.047
Blood vessels	605	–0.078 (0.165)	–0.229

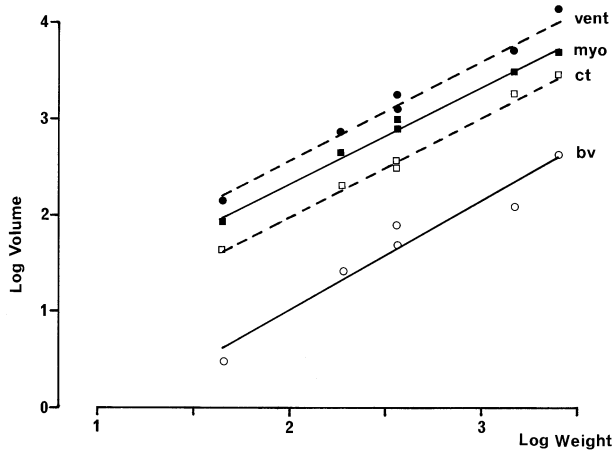

Fig. 3 Log–log plot of relationships between tissue compartment volumes and fetal weight (*vent* ventricles, *myo* myocytes, *ct* connective tissue, *bv* blood vessels)

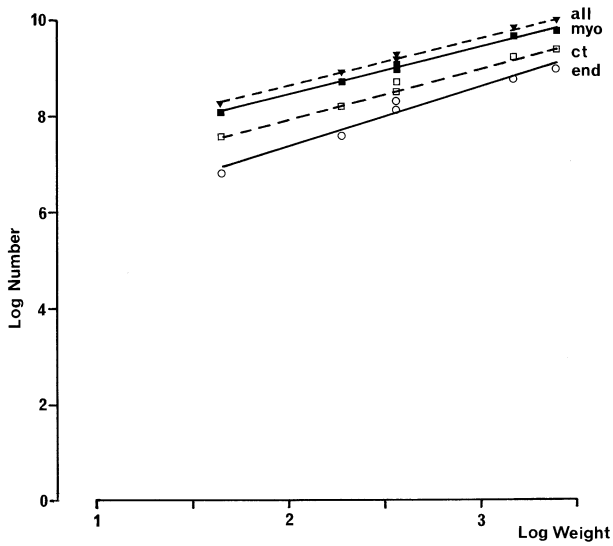

Fig. 4 Log–log plot of relationships between nuclear number and fetal weight (*myo* myocyte nuclei, *ct* connective tissue nuclei, *end* endothelial cell nuclei, *all myo+ct+end*)

and nuclear numbers (Fig. 4). When all nuclear types were considered together, total number increased with fetal weight raised to the power 0.996 (0.043) with a correlation coefficient of r=0.996. When considered separately, the number of myocyte nuclei increased with fetal body weight raised to the power 0.959 (0.037), r=0.997. For connective tissue and endothelial nuclei, corresponding figures were 1.045 (0.053), r=0.995 and 1.216 (0.132), r=0.977. Again, all constants and exponents were significantly different from zero (P<0.001) but slopes were not significantly different from unity or from each other.

In contrast, and as for gestational age, tissue volumes per nucleus did not increase significantly with fetal weight. Exponents of the regression lines of weight against tissue compartment volumes per nucleus were not significantly different from zero.

Precision of number estimation

Between 65 and 402 (mean 182) nuclei (all three types together) were counted per ventricle, and 280–592 (mean 363) nuclei were counted per subject. Assuming, for the sake of calculation, that the myocardium is homogeneous and sampling was independent, the maximum predicted coefficient of error is about 7% for a total count of 182 nuclei per ventricle and 5% for a count of 363 nuclei per heart. However, the nuclei of myocytes and connective tissue cells occurred more frequently than those of endothelial cells. Consequently, the numerical densities and absolute numbers of myocyte and connective tissue nuclei were estimated with greater precision. Predicted group mean coefficients of error for the numerical densities, $CE(N_V)$, of different nuclear types were 8% (con-

nective tissue), 9% (myocytes) and 27% (endothelium). Predicted group mean CE(N) values amounted to 10% (myocytes), 17% (connective tissue) and 28% (endothelium).

Discussion

The present study has provided direct evidence, using unbiased stereological counting procedures, that growth of the human ventricular myocardium from 16 to 35 weeks of gestation involves continuous proliferation of myocyte, connective tissue and endothelial nuclei. The results suggest that the three main tissue compartments (myocytes, connective tissue, blood vessels) expand linearly during this period of gestation and the volume changes are commensurate with the increases in fetal and ventricular mass. Similarly, numbers of different types of nuclei increase linearly with gestational age and are commensurate with the increase in fetal mass. When the changes in compartment volumes are related to nuclear type and number, there were no significant differences in compartment volume per nucleus with time or fetal size. This implies that the mechanism underlying the increases in compartment volumes is proliferation, and not cell hypertrophy or interstitial growth. Clearly, these findings hold for the period from 16 to 35 weeks and should not be extrapolated to earlier or later stages of gestation without experimental verification. However, there is some evidence that changes in myocyte number continue to term and into the early postnatal period [5].

Our findings concerning total compartment volumes (connective tissue, myocytes, blood vessels) and numbers of myocyte nuclei are entirely in accord with recent estimates obtained in an investigation of human fetuses from 12 to 36 weeks of gestation [33]. These authors demonstrated significant increases in numbers of myocyte nuclei and the relative volume of interstitium (defined as connective tissue, non-myocyte cells, vessels and nerves). Our regression equation for myocytes predicts a complement of about 7×10^9 nuclei at 36 weeks of gestation and 9×10^9 nuclei by full term. In earlier studies, the actual numbers observed at 42 gestational weeks and 40 postnatal weeks were $9–10\times10^9$ [5, 38]. There is also excellent agreement with the numbers of nuclei found at 36 weeks (almost 6×10^9) in an independent stereological study based on the use of optical directors (see Fig. 3 in [33]). These predictions and findings are consistent also with the results of studies on hearts from various mammals, mainly rodents, which have suggested that the proliferation of myocyte nuclei stops at 2–3 postnatal weeks and that thereafter, growth occurs by hypertrophy of individual myocytes. There is evidence that cardiac interstitium is responsible for producing factors that stimulate the growth of cardiomyocytes [30].

It is predicted further that connective tissue nuclei would be numerically equal to myocyte nuclei by about 50 postnatal weeks, whilst endothelial nuclei would not attain parity until the 3rd postnatal year. In a recent study of nuclearity estimated from DNA contents [1], it was estimated that the ratio of connective tissue nuclei to myocyte nuclei in adults hearts is roughly 2.5:1. The present study suggests that this ratio would be achieved by about 3 years of age.

The net rate of production of myocyte nuclei from 16 to 35 weeks was constant at about $3–4\times10^8$ per week. Corresponding figures for connective tissue and endothelium nuclei were $1–2\times10^8$ and $5–6\times10^7$ per week. These are equivalent to production rates of roughly 2 million (myocytes), 0.7 million (connective tissue) and 0.3 million (endothelial cells) nuclei per hour. If we take a simplistic model in which nuclei arise from a single ancestor that divides into two daughters, each of which leads to subsequent generations by successive karyokineses, it is possible to make predictions about the number of generations (g) needed in order to produce the numbers of nuclei observed. On this scheme, the nuclear populations present at 35 weeks of gestation would be attained by 32.6 (myocytes), 31.2 (connective tissue) and 29.9 (endothelium) generations. These values imply that nuclear complements are produced by an average of 0.85–0.93 generations per week. Alternatively, the nuclear complements double every 7–9 days. The generation and doubling rates vary at different stages of gestation [38]. Fewer generations per week (longer doubling times) are required as gestation advances, reflecting the expanding pools of different nuclear types available for each successive generation. It seems likely that a progressively smaller proportion of nuclei (the growth fraction) is needed at each successive stage in order to generate the subsequent complement of nuclei of any given type.

Other findings confirm some, but not all, of the conclusions drawn by Mandarim-de-Lacerda et al. [33] in a study conducted on subjects between 12 and 36 weeks of gestation. They noted a decrease in the volume density of myocytes and an increase in the volume density of interstitium during this period, and our observations on compartment volumes are consistent with that interpretation. However, they also found a small but significant increase in the volume of myocyte per nucleus between 12 and 36 weeks of gestation. Whilst our regression equations for myocyte volumes between 16 and 35 weeks appear, at first sight, to be consistent with this observation, the slopes of the regression lines were not significant. Nor did myocyte volume per nucleus increase significantly with fetal weight over the range encompassed in the present investigation. Clearly, further studies with larger sample sizes are required to resolve this inconsistency.

In future studies, these baseline values will be compared with stereological estimates obtained from hearts associated with cases of intrauterine growth retardation.

Acknowledgements Parts of this work were supported by The Foundation for the Study of Sudden Infant Death.

References

1. Adler C-P, Friedburg H, Herget GW, Neuberger M, Schwalb H (1996) Variability of cardiomyocyte DNA content, ploidy level and nuclear number in mammalian hearts. Virchows Arch 429:159–164
2. Adler C-P, Neuberger M, Herget GW, Muhlbach D (1997) Regeneration processes in human myocardium after acute ischaemia – quantitative determination of DNA, cell number and collagen content. Virchows Arch 430:149–153
3. Anversa P, Olivetti G, Loud AV (1980) 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
4. Anversa P, FitzPatrick D, Argani S, Capasso JM (1991) Myocyte mitotic division in the aging mammalian rat heart. Circ Res 69:1159–1164
5. Austin A, Fagan DG, Mayhew TM (1995) A stereological method for estimating the total number of ventricular myocyte nuclei in fetal and postnatal hearts. J Anat 187:641–647
6. Bai S, Campbell SE, Moore JA, Morales MC, Gerdes AM (1990) Influence of age, growth, and sex on cardiac myocyte size and number in rats. Anat Rec 226:207–212
7. Bancroft JD, Stevens A (1990) Theory and practice of histological technique, 3rd edn. Churchill Livingstone, Edinburgh, pp 503–504
8. Braendgaard H, Evans SM, Howard CV, Gundersen HJG (1990) The total number of neurons in the human neocortex unbiasedly estimated using optical disectors. J Microsc 157: 285–304
9. Chacko S (1973) DNA synthesis, mitosis, and differentiation in cardiac myogenesis. Dev Biol 35:1–18
10. Claycomb WC (1975) Biochemical aspects of cardiac mucle differentiation. Deoxyribonucleic acid synthesis and nuclear and cytoplasmic deoxyribonucleic acid polymerase activity. J Biol Chem 250:3229–3235
11. Claycomb WC (1977) Cardiac muscle hypertrophy. Differentiation and growth of the heart cell during development. Biochem J 168:599–601
12. Clubb FJ, Bishop SP (1984) Formation of binucleated myocardial cells in the neonatal rat. An index for growth hypertrophy. Lab Invest 50:571–577
13. Cluzeaut F, Maurer-Schultze B (1986) Proliferation of cardiomyocytes and interstitial cells in the cardiac muscle of the mouse during pre- and postnatal development. Cell Tissue Kinet 19:267–274
14. Cruz-Orive LM (1980) On the estimation of particle number. J Microsc 120:15–27
15. Cruz-Orive LM (1987) Particle number can be estimated using a disector of unknown thickness: the selector. J Microsc 145:121–142
16. Cruz-Orive LM, Weibel ER (1990) Recent stereological methods for cell biology: a brief survey. Am J Physiol 258:L148–L156
17. Dowell RT, McManus RE (1978) Pressure-induced cardiac enlargement in neonatal and adult rats. Left ventricular functional characteristics and evidence of cardiac muscle cell proliferation in the neonate. Circ Res 42:303–310
18. Fulton RM, Hutchinson EC, Morgan-Jones A (1952) Ventricular weight in in cardiac hypertrophy. Br Heart J 14:413–420
19. Gerdes AM, Moore JA, Hines JM, Kirkland PA, Bishop SP (1986) Regional differences in myocyte size in normal rat heart. Anat Rec 215:420–426
20. Gillette PC, Claycomb WC (1974) Thymidine kinase activity in cardiac muscle during embryonic and postnatal development. Biochem J 142:685–690
21. Gundersen HJG (1977) Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. J Microsc 111:219–223
22. Gundersen HJG (1986) Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc 143:3–45
23. Gundersen HJG, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147:229–263
24. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394
25. Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ (1988) The new stereological tools: disector, fractionator and point-sampled intercepts and their use in pathological research. APMIS 96:857–881
26. Katzberg AA, Farmer BB, Harris RA (1977) The predominance of bi-nucleation in isolated rat heart myocytes. Am J Anat 149:489–500
27. Knaapen MWM, Vrolijk BCM, Wenink ACG (1996) Nuclear and cellular size of myocytes in different segments of the developing rat heart. Anat Rec 244:118–125
28. Korecky B, Rakusan K (1978) Normal and hypertrophic growth of the rat heart: changes in cell dimensions and number. Am J Physiol 234:H123–H128
29. Limas CJ, Limas C (1978) DNA polymerases during postnatal myocardial development. Nature 271:781–783
30. Long CA, Henrich CJ, Simpson PC (1991) A growth factor for cardiac myocytes is produced by cardiac interstitium. Cell Regul 2:1081–1095
31. 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
32. Mandarin-De-Lacerda CA, Costa WS (1993) An update of the stereology of the myocyte of the baboon's heart: analysis of the crista terminalis, interatrial and interventricular septa, and atrio-ventricular bundle. Ann Anat 175:65–70
33. Mandarim-De-Lacerda CA, Dos Santos MB, Le Floch-Prigent P, Marcy F (1997) Stereology of the myocardium in human foetuses. Early Hum Dev 48:249–259
34. Marino TA, Cao W, Lee J, Courtney R (1996) Localization of proliferating cell nuclear antigen in the developing and mature rat heart cell. Anat Rec 245:677–684
35. Mayhew TM (1991) The new stereological methods for interpreting functional morphology from slices of cells and organs. Exp Physiol 76:639–665
36. Mayhew TM (1992) A review of recent advances in stereology for quantifying neural structure. J Neurocytol 21:313–328
37. Mayhew TM, Gundersen HJG (1996) "If you assume, you can make an ass out of u and me": a decade of the disector for stereological counting of particles in 3D space. J Anat 188:1–15
38. Mayhew TM, Pharaoh A, Austin A, Fagan DG (1997) Stereological estimates of nuclear number in human ventricular cardiomyocytes before and after birth obtained using physical disectors. J Anat 191:107–115
39. Simpson RA, Mayhew TM, Barnes PR (1992) From 13 weeks to term, the trophoblast of human placenta grows by the continuous recruitment of new proliferative units: a study of nuclear number using the disector. Placenta 13:501–512
40. Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research. Freeman, San Francisco
41. Sterio DC (1984) The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 134:127–136
42. Stoker ME, Gerdes AM, May JF (1982) Regional differences in capillary density and myocyte size in the normal human heart. Anat Rec 202:187–191
43. Widdowson EM, Crabb DE, Milner RDG (1972) Cellular development of some human organs before birth. Arch Dis Child 47:652–655
44. Zak R (1973) Cell proliferation during cardiac growth. Am J Cardiol 31:211–219