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# CAPILLARITY AND MITOCHONDRIAL DISTRIBUTION IN RAT MYOCARDIUM FOLLOWING EXERCISE TRAINING

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## SUMMARY

Rats were subjected to a laddermill running programme either once per week for 6 weeks, or daily for 6 weeks. Heart mass and maximal oxygen consumption rate increased relative to controls and with the frequency of the running programme. Mitochondrial distribution, measured in seven regions within fibres, was similar in all hearts, with a peak volume density of $0{\cdot}42 \pm 0{\cdot}01$ occurring 4–5 $\mu$m from the centre of a capillary. Capillary density decreased with increasing heart mass, but total capillary length and capillary-to-fibre ratio were constant. Thus the higher metabolic demands of the running programmes did not alter the volume density or distribution of mitochondria, despite significant increases in heart mass and decreases in capillarity.

## INTRODUCTION

The problem of energy transfer in muscle tissue has long occupied respiratory physiologists (Krogh, 1919). One element of this problem is to determine the effect of the distribution of mitochondria, first on the supply of oxygen from capillaries to mitochondria, and then on the delivery of high-energy phosphate compounds from mitochondria to the sites of energy consumption in cells (Eisenberg, Kuda & Peter, 1974; James & Meek, 1979; Hoppeler *et al.* 1981; Mainwood & Rakusan, 1982; Jones, 1984). This problem has not been solved yet due to difficulties in finding suitable morphometric methods for measuring mitochondrial distributions, difficulties in mathematically interpreting such data, and difficulties in experimentally testing mathematical predictions. Some advances have been made in the morphometry of mitochondrial distributions in mammalian myocardium. Rakusan (1984*a*) and Kayar & Banchero (1982, 1984) have reported that the volume density of myocardial mitochondria is several volumes percent higher near capillaries than in the centre of myocytes. Kayar & Banchero (1984) assigned a specific pattern to the distribution of mitochondria in guinea pig myocardium. A peak in the volume density was found at a distance of approximately 15 % of the total diffusion distance, as determined from capillary density estimates. Volume density then decreased fairly smoothly to a uniform, lower value in the spaces farthest from all capillaries. One intuitively suspects that such a pattern would favour the diffusion of oxygen and

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other metabolic substrates from capillaries to mitochondria. But is this pattern fixed anatomically, or can mitochondrial location be altered by altering the metabolic demands placed on the heart?

In another report, K. E. Conley, H. Hoppeler, E. R. Weibel & C. R. Taylor (in preparation) describe the results of an exercise training programme with rats using two training regimes. Heart mass of the rats increased proportionally to the frequency of the training, and whole-animal oxygen uptake rate increased proportionally to heart mass. However, the volume density of myocardial mitochondria was similar in both trained groups to values from the untrained rats. Thus we had an experimental situation in which we knew that metabolic demands on the hearts had increased sufficiently to induce increases in the total volume of the myocytes in a heart, but had caused no changes in the relative proportions of ATP-consuming myofibrils and $O_2$-consuming mitochondria. It is the purpose of the present study to report on concomitant changes in the capillarity and on the distribution of mitochondria in these hearts.

## MATERIALS AND METHODS

Fifteen 7-week-old male rats (Sprague-Dawley) were subjected to a test of their maximal oxygen consumption rate ($\dot{V}_{O_2}$max) by running on a laddermill inclined at either 55° or 70°. Respiratory gases were collected from the running rats by drawing air at a high flow rate (41 $min^{-1}$ STP) through a loosely-fitting mask that covered the head to the ears. The collected gases were drawn into an open-flow gas-analysis system through a calibrated Brooks Rotameter (Model R-2-25-D, Emerson Electric Co., Hatfield, Pennsylvania, U.S.A.) and passed through $H_2O$ (Drierite) and $CO_2$ absorbers (Ascarite) into an oxygen analyser (Servomex Paramagnetic $O_2$ Analyzer). The procedures of Fedak, Rome & Seeherman (1981) were used to check the calibration of the system and calculate $\dot{V}_{O_2}$, within an estimated accuracy of 3 % (K. E. Conley, H. Hoppeler, E. R. Weibel & C. R. Taylor, in preparation). After an initial minute of running at low speed, the speed of the laddermill was gradually increased over the next 2–3 min until there was no further increase in $\dot{V}_{O_2}$ with speed. Rats ran at this speed for an additional 1–2 min and then were removed from the laddermill.

The rats were divided into three groups. A control group performed duplicate $\dot{V}_{O_2}$max tests only at the end of a 6-week period. The second group of rats performed duplicate $\dot{V}_{O_2}$max tests once per week for 6 weeks. The rats in the third group performed duplicate $\dot{V}_{O_2}$max tests once per week for 6 weeks and also were made to run 25 min per day, 5 days a week at a speed that elicited 85 % of the $\dot{V}_{O_2}$max measured for that week.

All animals were anaesthetized; their hearts were removed, blotted dry and weighed. Samples of tissue 1–8 $mm^3$ were cut from the tip of the left ventricle. These samples were immersion-fixed in a solution of 6·25 % glutaraldehyde in 0·1 mol $l^{-1}$ sodium cacodylate, pH 7·4, post-fixed in 1 % $OsO_4$, dehydrated and embedded in Epon 812. Of the original 15 animals, the tissue samples of 3 rats from each of the

three groups were selected for morphometric analysis. These 9 rats were specifically selected to maximize the total range of $\dot{V}_{O_2}$max values represented. No other selection criteria were applied.

Ultrathin sections of the preserved tissues were cut from two blocks per animal with an LKB ultramicrotome and photographed on 35 mm film with a Philips 300 electron microscope. The sections were cut at an angle that appeared grossly to be a transverse section of the fibres. Since tissue samples were taken from the tip of the left ventricle where many fibre bundles converge at various angles, no tissue block was cut perfectly transversely for the entire block face. The actual angle of sectioning of a block was analysed by measuring the average distance between Z-lines seen in 10 micrographs of fibres at an approximate magnification of 10 000×. This length was compared to the estimated true length of sarcomeres (2·0 $\mu$m) measured in micrographs of sections cut longitudinally to the fibres. Angle of section ($\theta$) was taken to be the arcsine of the ratio of the latter value to the former (Eisenberg *et al.* 1974; Mathieu, Cruz-Orive, Hoppeler & Weibel, 1983). In this study $\theta$ ranged from 15° to 46°, when $\theta = 0°$ is a perfect transverse section.

For the analysis of capillarity, 12 to 16 micrographs (depending on total tissue section area and quality) from two tissue blocks were scored at a magnification of approximately 2300×. This gave an approximate total sample of 300–500 capillaries and 300–400 fibres per animal. The number of capillaries and the number of fibres were counted directly. The area occupied by fibres was estimated by point-counting. Mean fibre area ($A_F$) was corrected to that pertaining to a transverse section by multiplying the measured areas by the cosine of the angle of section. The reference space used for capillary density was the space occupied by fibres alone. Capillary density was corrected to that pertaining to a transverse section by dividing the measured capillary density by the cosine of the angle of section. Total capillary length ($J_C$) in metres in a heart was estimated from capillary density (CD) (in $mm^{-2}$) and heart mass ($M_H$) (in grams) as:

$$J_C = CD \times 1{\cdot}12 \times M_H / 1{\cdot}06,$$

where 1·12 is a dimensionless factor for the anisotropy of the capillaries and 1·06 g $ml^{-1}$ is the density of muscle (Hoppeler, 1984 and unpublished data; Pietschmann & Bartels, 1985).

For the analysis of the mitochondria, 18 to 20 micrographs from two tissue blocks were scored at a magnification of approximately 10 000×. The distribution of mitochondria relative to a capillary was determined by a method of point-counting in concentric rings (Kayar & Banchero, 1982, 1984; Fig. 1). A template of seven concentric circles forming six rings of equal area, each containing approximately 50 uniformly-spaced points, was centred over a capillary. The volume density of mitochondria in each ring was calculated by counting the number of points per ring that fell on mitochondria. When rings from neighbouring capillaries overlapped, these areas were discarded from the analysis. Volume density of mitochondria was also calculated in the spaces farthest from all capillaries in a micrograph, using the centremost circle of the template, which contained 21 uniformly-spaced points.

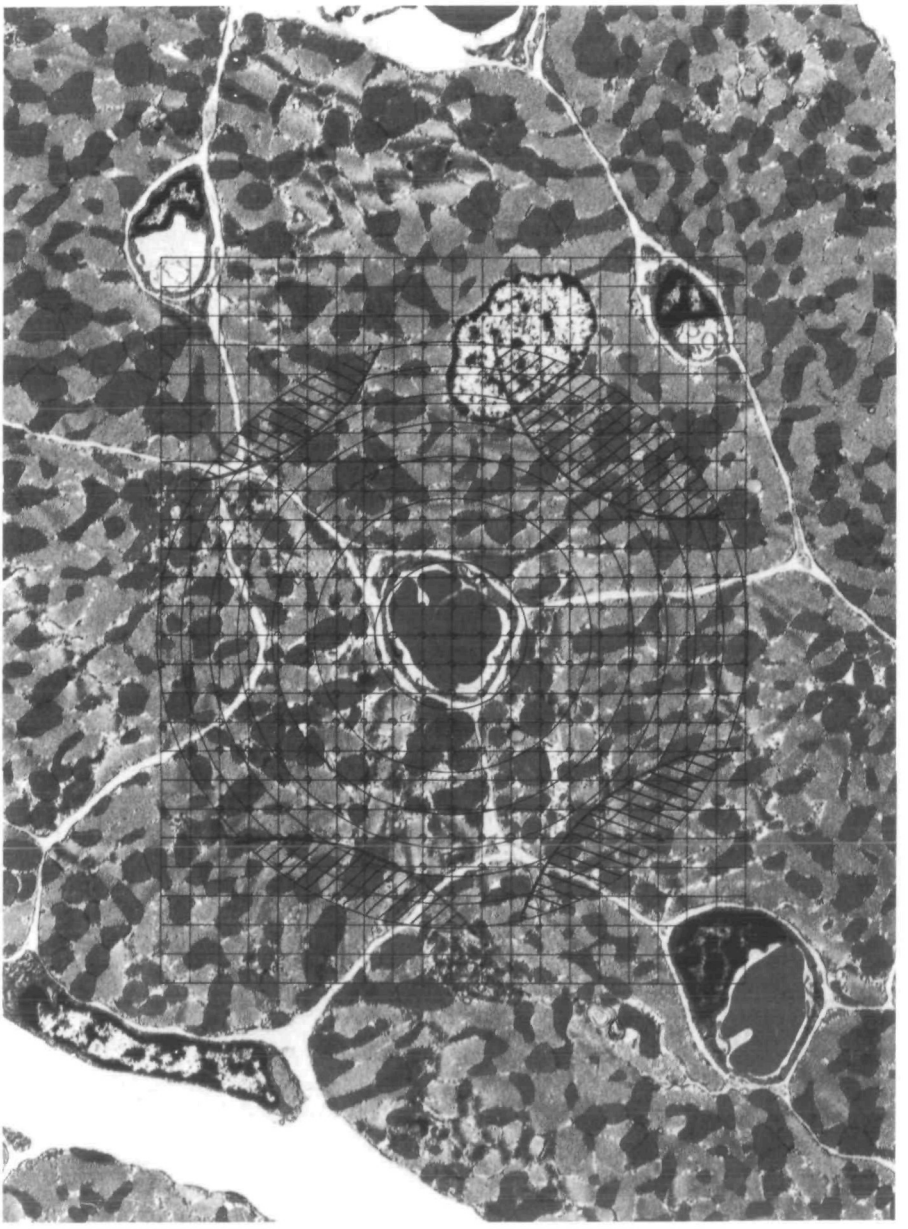

Fig. 1. Method of point-counting in concentric rings for determining mitochondrial distributions relative to capillaries (Kayar & Banchero, 1982, 1984). Template is centred over a capillary, and volume density of mitochondria is calculated in each ring. When rings from adjacent capillaries overlapped, these areas were excluded from the analysis (shaded areas).

Total mitochondrial volume ($V_m$) (in ml) was calculated from mitochondrial volume density ($V_{vm}$) and heart mass as:

$$V_m = d(V_{vm} \times M_H / 1{\cdot}06),$$

where d is a factor for the tissue volume represented by a template ring relative to total tissue volume. This factor was calculated from the fraction of the total diffusion distance represented by each ring, assuming a hexagonal array of capillaries (Kayar, Archer, Lechner & Banchero, 1982).

At the magnification of the micrographs, the original template radii were 2·5, 4·7, 6·2, 7·4, 8·4, 9·3 and 10·1 $\mu$m. However each of the two tissue blocks per animal had its own correction factor for the angle of section. To adjust all measurements to the distances that would pertain to a true transverse section, ring radii for each block were multiplied by a correction factor that was generated from the mean radius vector for the ellipse formed by the oblique angle of section (Abramowitz & Stegun, 1965). At the angles of section used in this study, these correction factors ranged from 0·98 to 0·84. Data from the two blocks per animal, and then the three animals per group were pooled, matching ring radii as closely as possible. However, the precision of the measurement was thereby reduced to approximately $\pm 1$ $\mu$m, making ring radii effectively 1 $\mu$m wide and 4, 5, 6, 7, 8 and 9 $\mu$m from the centre of a capillary. Data from the spaces farthest from all capillaries in a given myocardium were pooled directly. The farthest point from all capillaries in a given myocardium was estimated from its capillary density and an assumption of a hexagonal array of capillaries. This is $0{\cdot}8774 \times CD^{-1/2}$ (Kayar *et al.* 1982). Thus data from these farthest spaces were plotted as lying from 9 $\mu$m to this farthest distance.

### *Statistical analysis*

Data from the three groups of rats for capillary density, fibre area, capillary-to-fibre ratio, total capillary length and volume density of mitochondria were compared with a one-way analysis of variance. A least-squares regression was used to compare capillary density *versus* heart mass for all animals. Mean values are reported $\pm 1$ standard error. Mean volume densities of mitochondria between different rings were compared by use of Student's *t*-test.

## RESULTS

There were significant differences in heart mass among the three groups of rats, with heart mass increasing with training (Table 1). As heart mass increased, capillary density decreased significantly (Fig. 2). However the capillary-to-fibre ratio and total capillary length were not significantly different among the three groups. Mean fibre areas were also not significantly different among the three groups.

Mitochondrial volume density was highest (mean value $0{\cdot}42 \pm 0{\cdot}01$) at a distance of approximately 4–5 $\mu$m from the centre of a capillary in all hearts. Volume density then decreased significantly ($P < 0{\cdot}001$) over the next several micrometres to a mean value of $0{\cdot}33 \pm 0{\cdot}01$ in the spaces farthest from all capillaries (Fig. 3). There were no significant differences in mean volume densities among the three groups in any of the six rings or in the farthest spaces ($P$ ranging from 0·18 to 0·96). Thus the running programmes had no significant effect on either mitochondrial volume density or distribution.

## DISCUSSION

The primary effect of the exercise training on the myocardium that was observed in this study was the increase in heart mass following a 6-week running programme of either two brief runs once per week, or one extended run daily. Despite the evidently greater work load placed on the heart with the exercise, capillary density decreased and the volume density of mitochondria was constant as the hearts increased in mass.

The decreasing capillary density but constant capillary-to-fibre ratio and total capillary length are consistent with a situation in which the total capillary network in these hearts did not change with the cardiomegaly. Capillary density decreased as the growing myocytes pushed capillaries farther apart. Given heart mass increases of factors of 1·12 and 1·24 for the weekly-run and trained rats, respectively, we would have expected to see mean fibre areas increase approximately with the 2/3 power of mass, or by factors of 1·08 and 1·15 (assuming the cardiomegaly to be by hypertrophy of fibres, with length and area increases proportional to each other;

Table 1. *Values for heart mass ($M_H$), capillary density (CD), mean fibre area ($A_F$), total capillary length ($\mathcal{J}_C$) and capillary-to-fibre ratio (C/F) for control rats, rats made to run once per week for 6 weeks and rats made to run daily for 6 weeks*

| | Heart mass (g) | Capillary density ($mm^{-2}$) | Mean fibre area ($\mu m^{-2}$) | Total capillary length (m) | Capillary-to-fibre ratio |
|---|---|---|---|---|---|
| Controls | 1·157 | 3955 | 347 | 4835 | 1·376 |
| | 1·052 | 4305 | 264 | 4785 | 1·135 |
| | 1·129 | 4197 | 318 | 5007 | 1·333 |
| $\bar{x}$ | 1·113 | 4152 | 310 | 4876 | 1·281 |
| S.E. | 0·031 | 103 | 24 | 67 | 0·074 |
| Weekly-run | 1·311 | 2848 | 433 | 3945 | 1·233 |
| | 1·182 | 3524 | 400 | 4401 | 1·411 |
| | 1·239 | 3147 | 413 | 4120 | 1·300 |
| $\bar{x}$ | 1·244 | 3173 | 415 | 4155 | 1·315 |
| S.E. | 0·037 | 196 | 10 | 133 | 0·052 |
| Trained | 1·421 | 3271 | 369 | 4911 | 1·202 |
| | 1·358 | 3309 | 314 | 4748 | 1·039 |
| | 1·356 | 2444 | 452 | 3502 | 1·106 |
| $\bar{x}$ | 1·378 | 3008 | 378 | 4387 | 1·116 |
| S.E. | 0·021 | 282 | 40 | 445 | 0·047 |
| *P* | 0·003 | 0·008 | 0·09 | 0·24 | 0·11 |

Means and standard errors for each group are included. Level of statistical significance (*P*) from one-way analyses of variance among all three groups are also included.

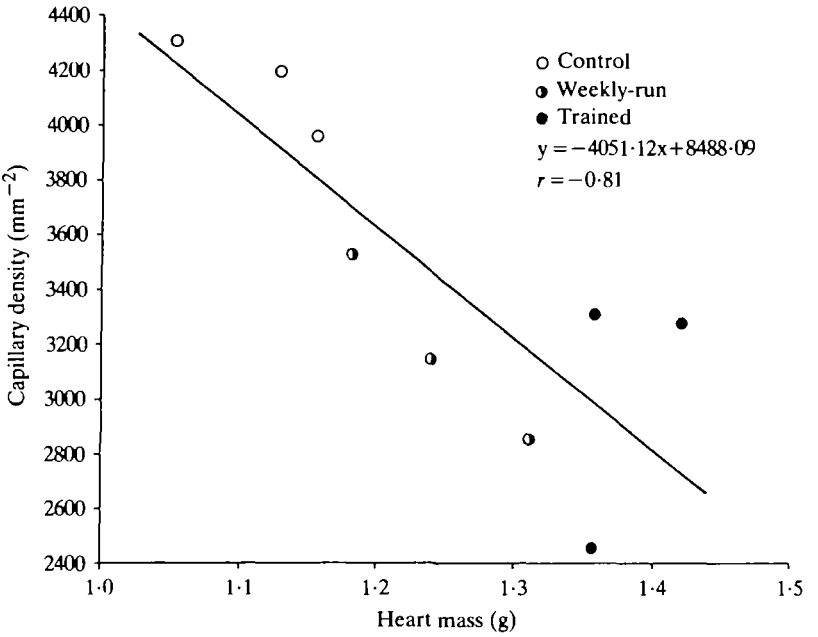


Fig. 2. Capillary density *versus* heart mass in the myocardium of three control rats, three rats made to run once per week for 6 weeks, and three rats made to run daily for 6 weeks (trained).

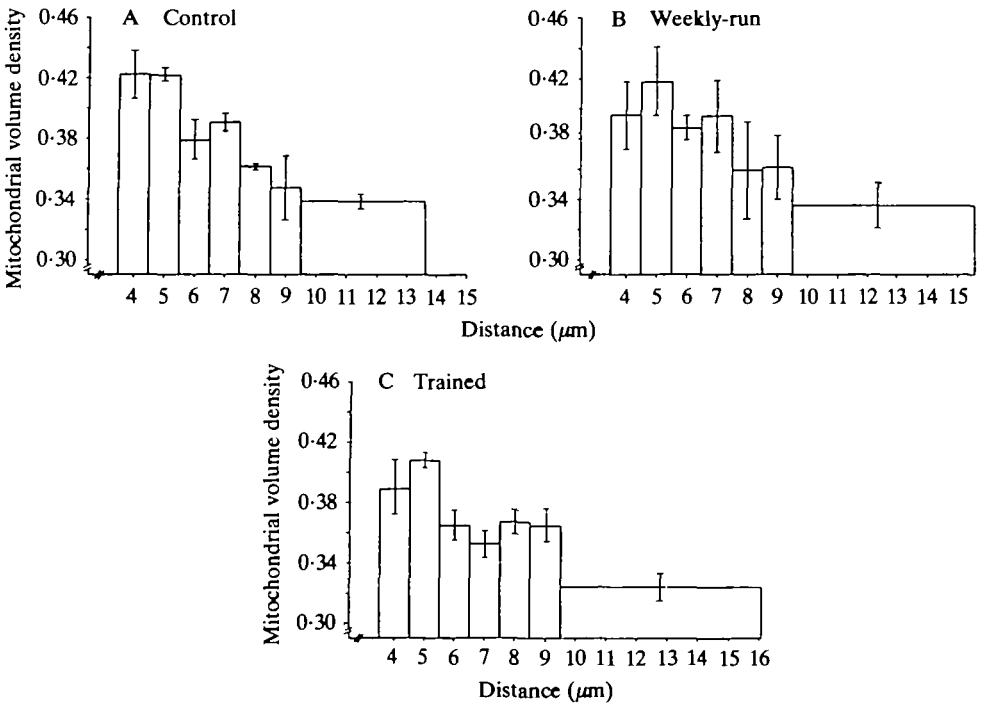


Fig. 3. Volume density of mitochondria *versus* distance from the centre of a capillary in the myocardium of (a) three control rats, (b) three rats made to run once per week for 6 weeks, and (c) three rats made to run daily for 6 weeks (trained).

Rakusan, 1984*b*). Given the inherent difficulties in accurately counting numbers of myocyte profiles in myocardium, we were not able to demonstrate statistically significant increases in mean fibre areas. However, our fibre areas are reasonable and consistent with our other results.

A decrease in capillarity with increasing heart mass has been reported in a number of studies (Rakusan, Moravek & Hatt, 1980; Wright & Hudlicka, 1981; Anversa *et al.* 1982; Tharp & Wagner, 1982; Tomanek, Searls & Lachenbruch, 1982). Increasing heart mass in this study was also associated with increasing $\dot{V}_{O_2}$max (K. E. Conley, H. Hoppeler, E. R. Weibel & C. R. Taylor, in preparation). We do not know if there were any other changes in the capillaries of these hearts, such as an increase in tortuosity or diameter, that might have affected total capillary surface area. However, the present data suggest that it is more likely that any increase in oxygen supply that might be needed by the myocardium of exercising animals with elevated $\dot{V}_{O_2}$max values would have to be provided by altering some aspect of capillary blood flow, rather than by increasing capillarity.

James & Meek (1979) and Mathieu *et al.* (1983) have pointed out that morphometric analyses of structures such as capillaries and mitochondria should ideally be performed either on sections cut at random angles or on both longitudinal and transverse sections. In the present case, this was not practical. We do not know if significant errors remained in our data after adjusting distances and areas for section angle. However our adjusted capillary densities are consistent with values reported by others for rat myocardium (Henquell & Honig, 1976; Rakusan & Korecky, 1982). Total mitochondrial volumes and total capillary lengths in this study fit the regression of these two parameters recently calculated by K. E. Conley, K. Roesler, H. Hoppeler, S. R. Kayar, E. R. Weibel & C. R. Taylor (in preparation) for a number of muscles and animal species. Weibel (1972) has shown that the volume density of anisotropic structures such as mitochondria should not be affected by the angle of section. Thus, within the limited scope of this study and for the parameters described, the variations in section angle did not appear adversely to affect our data.

As has been found in the past in the myocardium from several mammalian species (Rakusan, 1984*a*; Kayar & Banchero, 1982, 1984) mitochondrial volume density was higher near capillaries than in regions far from capillaries. Assuming a hexagonal array of capillaries, at an average capillary density for these rat hearts of approximately 3400 $mm^{-2}$, the peak in the mitochondrial distribution at a distance of 4–5 $\mu$m from the centre of a capillary corresponds to a relative distance of approximately 20% of the total diffusion distance in these hearts ($\pi \times 0{\cdot}0045^2 \times 3400$; Kayar *et al.* 1982). This is in keeping with values of 15% (Kayar & Banchero, 1984) and 25% (Kayar & Banchero, 1982) previously reported in guinea pig and dog heart, respectively. Thus our data indicate that there was no major redistribution of mitochondria following the exercise training programme. However, capillary density in these rat hearts actually ranged from 2444 $mm^{-2}$ to 4305 $mm^{-2}$. If the peak in the distribution of mitochondria had shifted in absolute distance away from a capillary in order to remain at the same relative distance, as reported by Kayar & Banchero (1984), such a shift would be too small for us to demonstrate as

statistically significant with the technique used in this study. At a capillary density of 4305 $mm^{-2}$, a relative distance of 20% of the total diffusion distance would be 3·9 $\mu$m from a capillary. The same 20% point at a capillary density of 2444 $mm^{-2}$ would be 5·1 $\mu$m from a capillary. Our measurement precision is only approximately $\pm 1$ $\mu$m. Thus, although clearly indicating that the exercise programmes induced no large shifts in mitochondrial distribution, our data cannot exclude the minor shifts that have been reported to occur with decreasing capillarity (Kayar & Banchero, 1984).

Total heart mass in the two groups of exercised rats increased during the running programmes and was significantly correlated with $\dot{V}_{O_2}$max (K. E. Conley, H. Hoppeler, E. R. Weibel & C. R. Taylor, in preparation). Thus, although the volume density of mitochondria in each template ring did not change, the total volume of mitochondria in these hearts increased with the cardiomegaly. If one examines the distribution of absolute mitochondrial volume (Fig. 4) rather than volume density, one obtains a somewhat different perspective. We calculated the total diffusion distance in these hearts (Kayar *et al.* 1982) and divided this into three equal portions, which then represent three equal volumes of tissue. The volume of mitochondria in each of these three spaces increases as the $\dot{V}_{O_2}$max of the rat increases. The volume of mitochondria is also greater near the capillary than it is in regions farther from the capillary in each animal. Thus, whether examining absolute volume or volume

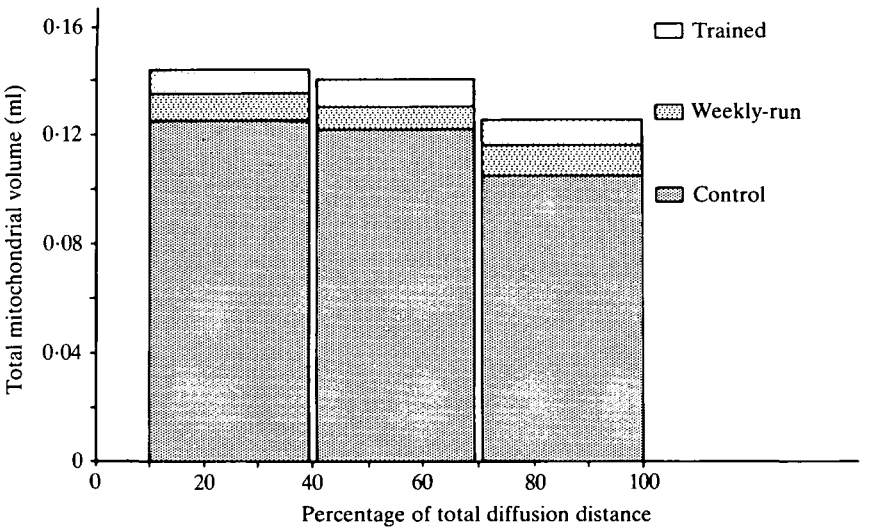


Fig. 4. Volume of mitochondria *versus* relative diffusion distance from the centre of a capillary in one control rat ($M_H$ = 1·129 g; $\dot{V}_{O_2}$max = 23·9 ml $min^{-1}$), one rat made to run once per week for 6 weeks ($M_H$ = 1·182 g; $\dot{V}_{O_2}$max = 30·5 ml $min^{-1}$), and one rat made to run daily for 6 weeks ($M_H$ = 1·358 g; $\dot{V}_{O_2}$max = 35·5 ml $min^{-1}$). Diffusion distance was calculated based on an average capillary density of 3400 $mm^{-2}$ and an assumption of a hexagonal array of capillaries (Kayar, Archer, Lechner & Banchero, 1982). Heart mass, $M_H$; maximal oxygen uptake rate, $\dot{V}_{O_2}$max (K. E. Conley, H. Hoppeler, E. R. Weibel & C. R. Taylor, in preparation).

density of mitochondria, it is evident that the distribution of mitochondria follows a consistent pattern in all these rat hearts. The increase in tissue volume with training is associated with an increase in mitochondrial volume, which is being deposited according to this pattern. This suggests that there is a cellular mechanism that regulates mitochondrial distribution. However, under the conditions of this experiment, the regulation appears more likely to be based on anatomy than on the metabolic demands placed on the hearts.

Mainwood & Rakusan (1982) have discussed the theoretical implications of a Krogh-type model for cellular respiration, but with a mitochondrial distribution entirely packed close to capillaries. They report that the effect of such a distribution would be to increase substantially the effectiveness of oxygen transfer from capillaries to mitochondria. Recent work by Honig & Gayeski (1984), however, suggests that oxygen concentration gradients in cells may be much smaller than one would assume from a Krogh model due to a buffering effect by myoglobin. This suggests that a distribution of mitochondria relatively closer to capillaries would be quite unimportant in oxygen delivery. The distribution of mitochondria may also be important for the transfer of ATP from mitochondria to myofibrils. Mainwood & Rakusan (1982) have calculated that distributing mitochondria close to capillaries need not be a disadvantage to high-energy phosphate transfer if creatine phosphate acts as an effective shuttle over relatively long intracellular distances. However, Meyer, Sweeney & Kushmerick (1984) have concluded that the image of creatine phosphate as a shuttle mechanism is not the most appropriate; at the distances that are actually found between mitochondria in the myocardium, the diffusion rate of ATP by itself is adequate.

Thus, it is not at present clear to us how to interpret our data on mitochondrial distribution relative to cellular respiration. The consistent finding of a single distribution in all our rat hearts, including those with cardiomegaly from animals with elevated $\dot{V}_{O_2}$max, suggests that this distribution is significant in the problem of energy transfer from capillaries to myocardial myofibrils.

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