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Comparative study of three different kinds of heart: vascular, avascular and mixed

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RESUMEN

Usando técnicas estereológicas se midieron el volumen del lumen y el área de superficie endotelial de tres diferentes tipos de miocardio: vascular (ratón), avascular (rana) y mixto (pez). La principal pregunta es saber si en vertebrados inferiores la ausencia de capilares es compensada por una mayor extensión de área de superficie endotelial y saber cómo están relacionados con su capacidad metabólica y volumen del lumen. Los resultados mostraron que el área de superficie de la pared de las "venas" es mayor en ratones cuando se compara con pez y rana, mientras que las densidades de superficie y volumen del endotelio "capilary" y volumen "capilar" son mayores en el corazón avascular que en el mixto, y mas aún cuando se compara con el corazón vascular. Las mediciones estereológica y bioquímica mostraron que el corazón vascular tiene mayor capacidad metabólica que los tipos mixto y avascular.

Palabras clave: estereología, vascular, avascular, mixto, corazón.

ABSTRACT

The lumen volume and endothelial surface area of three different kinds of myocardium: vascular (mouse), avascular (frog) and mixed (fish), were measured by using stereological techniques. The main question is, whether in low vertebrates the lack of capillarization is compensated by more endothelial surface area extension and to know, how their luminal volume and metabolic capacity are related. The results show that, the endothelium "veins" wall surface area was greater in mouse when compared with fish and frog, while the "capillary" endothelium, volume "capillary" and surface densities in "capillaries", were greater in the avascular heart than in the mixed one, and bigger when compared with the vascular heart. The stereological and biochemical measurements showed that the vascular heart has higher metabolic capacity than the mixed and avascular type.

Key words: stereology, vascular, avascular, mixed, heart.

The frog myocardium has a spongy type and it lacks of an arterial blood supply.[1] On the other hand, the teleost can be divided into two groups[2-5] those with only spongy type, like the frog, and those with a compact outer layer with a coronary supply in addition to the inner spongy myocardium.[6]

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It's well known that the density of capillarity in mammal's myocardium is relatively higher when compared to other tissues and is proportional to the heart oxidative activity.[7] Nevertheless, nobody has compared the capillarity density in the hearts with another kind of oxidative capacity. In addition it has been shown that a higher aerobic capacity is matched with a proportional larger complement of mitochondrial units.[8] Furthermore, the mitochondrial density correlates well with the capillary supply.[9] The degree of the myocardium capillary density, is closely related to: a) the absolute supply of oxygen and other metabolites, and b) the rate of blood flow through the microvasculature in the heart.[10]

In order to find a correlation between the microvasculature morphology and functionality heart, and taking into account the different metabolic capacity and the evolution grade of the selected group, we need a comparative study of three different types of heart: vascular, avascular and mixed, which is made on the basis of their heart morphological differences and by making quantifications of the next parameters: "veins" volume density (*Vvv*),

endothelium "veins" surface density (*Svv*), muscular mass volume density (Vvvm), "capillary" volume density (Vvc), and endothelium "capillary" surface density (Svc), mitochondrial volume density (Vvmi), mitochondrial outer membrane surface (Svmo) and mitochondrial cristae density (Vvmc). The stereological mitochondrial results were correlated with the biochemical determinations of the cytochrome "C" oxidise activity. The results were related to 100g body weight, and that is why the specific heart volume (S.H.V, specific heart volume) was used.

MATERIAL AND METHODS

Experiments were performed on the three different kinds of heart: vascular (mouse, *Mus musculus*), avascular (frog, *Rana temporaria*) and mixed (tench, *Tinca tinca*). To avoid the heart rate changes along the year seasons, the animals were collected and processed during the same season (October-November). Frogs and mice were anesthetized with ether, while fishes were sacrificed by severing the neck. The animals were weighed and after immediate thoracotomy the abdominal aorta was cannulated.

The animals heart was arrested in diastole by perfusion of buffer phosphate 0.1M, pH 7.4, with KCl 3x.[11] The weight and volume of the hearts before and after fixation were determined by weighting hearts and volume was measured by volume displacement.[12]

Five hearts were used for each technique. For light microscopy (level I of magnification), Bouin as fixative solution was perfused through the each heart for 5 minutes, and kept for two days immersed in the same fixative at 20ºC, followed by dehydration with ethanol and finally embedded in paraffin. The hearts were tangentially sectioned (45º from the central axis). The slices (3micras), were stained with Hematoxylin and Eosin. For electron microscopy (level II and III of magnification), the hearts were perfused for 15 minutes with 3% glutaraldehyde in buffer phosphate 0.1M, 4. The tissue was postfixed with 1% osmium tetroxide in the same buffer, one hour in cold and then two hours in glutaraldehyde 3%. After dehydratation in graded ethanol and embedding in araldite, thin sections were obtained with an LKB ultra microtome. Sections were placed on 200 mesh cooper grids and stained with uranyl acetate and lead citrate. Observations and electron micrographs were made in a transmission electron microscope TESLE.

SAMPLING AND STEREOLOGY

The stereology was performed by using the methods of Weibel E.R.[13, and see 14] Point counting techniques were used to estimate the volume density (*Vv)*. The surface density (*Sv*) was calculated by using linear intersection and in the case of mitochondria the membrane intersections were counted as a single point.

The cascade sampling designed for analyzing cardiac tissue[15] was necessary. At level I, "veins" relative volume (*Vvv*), mass muscular relative volume (*Vvmm*) and relative surface area of the "veins" endothelium wall (*Svv)* were evaluated. A multipurpose test system containing 96 test points was used. At level II "capillary endothelium volume density (*Vvc*) and "capillary surface density (*Svc*), the multipurpose system containing 64 points was used at 4,000x magnification. At level III, The mitochondrial volume density (*Vvmi*), the mitochondrial outer surface density (*Svmo*) and the mitochondrial cristae surface density (*Svcr)*, were counting at 24000x magnification.

BIOCHEMICAL ASSAY

The animals were perfused with buffer phosphate 0.1M, pH7.4. The Cytochrome "C" oxidase was assayed by the modified method of Glick D.[16] The relaxed heart[1], was dissected, weighted and chilled on ice. Tissue was homogenized in buffer phosphate 0.05M, pH 7.0. Centrifugation was carried out for 10 minutes at 2800 rpm. The supernatant absorbance was recorded at 5500 nm.

RESULTS

The mean relative heart volume (R.H.V.) of these animals was: *Tinca tinca* 439µl, *Rana temporaria* 92µl and *Mus musculus* 103µl (Table 1). The relative weight, in the same ascendant evolutive order, was: 267.3g, 17.34g and 14.51g, respectively. The differences among groups were evident when the specific heart volume (S.H.V.-ml/100g body weight) was calculated: Fish (0.165ml) < Frog (0.555ml) < Mouse (9.706ml) ($p<0.1$). At Level II (Table 2), the *Vvv*, did not show any difference among groups, but when the body weight was considered, the statistical differences among groups made clear that the vascular type > avascular type > mixed type ($p > 0.01$). Similar situation was found for the muscular mass quantifications (Table 2), where only

Table 1. Animals basic data

Animal	*Body weigth (Bw) (g)*		*Heart (mg)*	*(µl)*	*SHV (ml/100g BW)*
Fish	X	267.300	416	439	0.165
	S.D.	18.526	19	31	0.017
Frog	X	17.34	79	92	0.555
	S.D.	5.615	16	23	0.162
Mouse	X	14.510	101	103	9.709
	S.D.	2.029	18	16	0.029

SHV: specific heart volume defined as heart volume In 100 g. body weight. N=5 for each group of animals.

Table 2. "Veins" and muscular mass

	Densities			*Specific values per 100g/BW*		
Heart Types	Vvv (%)	Svv (end, cm^{-1})	Vvmm (%)	*Vv (µl)*	*Sv (cm^{-2})*	*Vmm (µl)*
Mixed	x28.6	210.36	71.4	52	39	137
	S.D. 8.9	39.81	8.9	12	6	42
Avascular	x44.8	174.44	55.2	110	42	132
	S.D14.0	32.96	14.0	13	12	52
Vascular	x28.2	103.22	69.8	180	66	448
	S.D 2.6	25.42	5.1	21	18	67

Vvv: luminal "veins" volume density; Svv: "veins" wall surface density; Vvmm: muscular mass density volume; Vv: "veins" specific volume; Sv: "veins" wall specific surface and Vmm muscular mass specific volume.

the specific values showed the differences among groups turning out that the vascular type > mixed type = avascular type ($p > 0.01$). The same as in the vascular heart, the mixed heart in which the capillarity is present in a half of it total percentage, the volume occupied by the muscular mass of myocardium was proportionally larger than the volume occupied by the lumen (Table 2). On the contrary, in the hearts of spongy type, in which the capillarity is absolute absent, an equilibrium of 55% to 45% (Vvmm versus Vvv respectively) is kept (see Table 2). Regarding, the Svv (Table 2), of the avascular and mixed heart types were similar and larger than the vascular type ($p > 0.01$). However, when the BW (body weight) was considered, the specific surface density (Sv) was: vascular > mixed = avascular ($p > 0.054$) (Table 2).

Quantifications of the Vvc at Level II (Figure 1), showed that the avascular type > mixed type > vascular type ($p > 0.05$), and even more when the specific value, was considered. The differences in Svc, were evident ($p > 0.01$) the avascular type was found to be 35% larger than the Svc of the mixed type, and 71.44% larger than the vascular type (Figure 1). Furthermore, when considered the Sv, the avascular type result to be 82% and 69% larger than the mixed and vascular types respectively ($p > 0.05$).

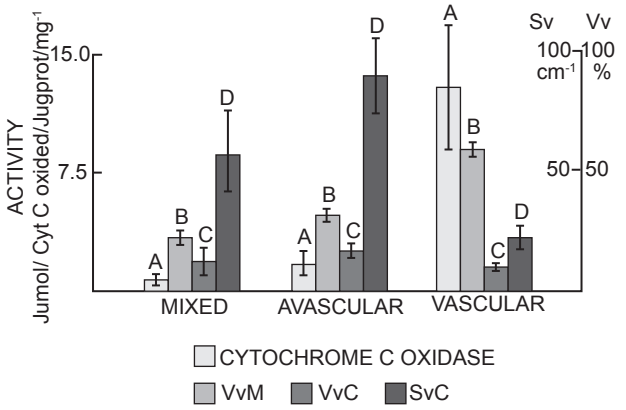

Figure 1. Metabolic capacity and "capillary" densities.
A: Cytochrome C oxidase activity. B: mitochondrial volume density (Vvmi%). C: "capillary" volume density (Vvc%). D: "capillary" surface density (Svc cm).

The percentage of Vvmi with respect to the myocyte total volume (Table 3), was vascular type (48%) > avascular type (29.30%) > mixed type (17.60%), ($p < 0.01$), and the specific values (Smo) show that mouse = frog > fish ($p < 0.01$). with respect to Svmc and it specific value (Smc) the differences were statistically the same among groups, finding that mouse > frog > fish ($p < 0.01$).

The biochemical analysis of the ventricular Cytochrome "C" oxidase activity (reported in nml/Cyt C/g prot/ mg),

Table 3. Steriological values of mitochondria

	Densities			*Specific values per 100g/BW*		
Heart Types	Vvmi (%)	Svmo (cm^{-1})	Svmc (cm^{-1})	Vmi (µl)	Smo (cm^2)	Smc (cm^2)
Mixed	x 17.60	26.73	194.82	29	4.3	32
	S.D 1.70	6.15	22.87	2	1.0	3
Avascular	x 29.30	36.64	227.33	161	20.36	154
	S.D4.40	4.42	29.03	45	7	51
Vascular	x 48	31.95	453.60	346	22.5	320
	S.D 3.40	3.32	20.50	22	2.0	21

Vvmi: mitochondrial volume density; Svmo: mitochondrial outer membrane surface density; Svmc: mitochondrial cristal surface density; Vmi: mitochondrial specific volume; Smo: mitochondrial outer membrane specific surface and Smc: mitochondrial cristal specific surface.

showed that mouse (12.818, S.D = 3.94) > frog (1.64, S.D = 0.77) > or = than fish (0.74, S.D 0.22). Statistics calculations were performed by the Jerrold bioestatistical analysis (17).

CONCLUSION

Three types of myocardium, vascular, avascular and mixed, are compared. The hearts belong to different kinds of vertebrates: active (mouse) and less active (frog and fish). Because of the differences in BW (body weight) and HW (heart weight), the S.H.V. (specific heart volume) was used. The S.H.V. effect is implicated in each one of the present results and is evident especially at Level I (Table 2), that there are no differences among the hearts, but when the specific values were applied the differences were clarified, showing that the vascular type has higher Vv and Vmm per 100g of body weight than the avascular and mixed types. On the other hand, it is well known that the S.H.V. is inversed to the heart rate, and that is why it is higher in mammals than in lower vertebrates and similarly in fish and frog.

When comparing the ratio Vvmm%/Vvv% (Table 2) we found that in both vascular and mixed the Vvmm is almost three times higher than the Vvv, while in the spongy type this ratio keeps equilibrium of 50% to 50%. These results could support the hypothesis that the presence of capillarity is explained to appear in the history of the vertebrates, as the response of a higher demand of blood supply and, like a consequence of a more demanding style of living which is also together with an increase of the muscular mass as a necessity of a higher fibre systolic force. The body mass of animals and the heart rate are proportional to the metabolism; the heart rate depends mainly on the oxygen supply necessities, tending to be low in larger animals and high in warm blooded animals, and that is why the heart rates of reptiles, amphibians and fishes are less than those of equivalent mammals size.

The microvascular arrangement in the vascular type has been always related to the absolute supply of oxygen and other metabolites, to cardiac myocites. The absence of capillarity in the frog spongy heart according with the results seems to be accompanied with a morphological response where, greater endothelial surface area and luminal volume are invested. The frog life style, in which the metabolic activity and demand for oxygen are poor, and where the pulmonary breathing is helped by the characteristic skin breathing, it takes an important role especially during the hibernation, and is together with a low heart output. In this way the oxygen distribution can be regulated by varying the diameter of the capillary system especially on the heart rate. The large luminal volume in frog could be partly compensated by the reduced oxygen period of hibernation of this organism. And then, the lack of vascularization also is compensated by the high solubility of oxygen at low temperature, cutaneous respiration and low basal metabolic rate.

High volume density of the mitochondria ventricle has been correlated with the energy demand of a high cardiac output. Both the biochemical and the stereological results showed that *Tinca tinca* and *Rana temporaria* have less than one half of the *Mus musculus* metabolic capacity. The present results accord with the previous literature studies, showing that the heart of small mammals has a greater mitochondrial content than in the heart of lower vertebrates. Is also showed that if the additional effect of organ size is considered, the metabolic capacity of the mammalian heart is further increased when compared to the amphibian and fish heart.

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