ENDOCARDIAL SURFACE CHANGES OBSERVED WITH A SCANNING ELECTRON MICROSCOPE & PHOSPHOLIPID CHANGE DURING EXTRACORPOREAL CIRCULATION IN MAN*

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RECENT developments in cardiovascular surgery make it possible to open the heart by the aortic occlusion method with cardiopulmonary bypass.

The root of the aorta is occluded intermittently for 15-minute periods to produce cardiac arrest. It is an important problem to learn the influence of anoxic arrest upon myocardial morphology and simultaneously upon myocardial metabolism.¹

On the radical operation of tetralogy of Fallot, it is easy to obtain the fresh heart muscle samples from the stenotic infundibulum in the right ventriculum. For this purpose, 8 cases of tetralogy of Fallot were selected.

A muscle block was obtained immediately after ventriculotomy and at the end of intracardiac operative procedure. The total aortic occlusion time ranges from 40 to 100 minutes. The resected muscle blocks were fixed with 1% osmium tetroxide in phosphate buffer with an added 5% glucose solution. They were dehydrated with serial alcohols and anhydrous acetone. Then they were covered with gold in a vacuum evaporator and then the surface of the endocardium and myocardium were examined using a scanning electron microscope.²

1. Morphology

With the conventional electron microscope,

we cannot view the surface of the myocardium. But with the introduction of the scanning electron microscope we can view the three-dimensional fine details of the surface structure of the endocardium. The sectioned myocardium was also examined and the surface of myocardial fibers were viewed with the scanning electron microscope.

There are some reports about myocardial ultra-microscopic changes before and after cardiac arrest, using conventional electron microscopes, that is, diffuse swelling of mitochondria, clearing of the central matrix, with clumping of cristae, and condensation of the inner membrane.³

On the other hand, the normal endocardial surface, observed by a scanning electron microscope, were shown in Fig. 1 and 2. The endocardial surface changes due to prolonged anoxic condition were the destruction of the regular cellular arrangement and change from the fine granular appearance of the cellular surface to a coarse granular appearance (Fig. 3). With a prolonged anoxic condition, the loss of regularity of the endocardial cells and the irregular swelling of endocardial cells were viewed (Fig. 4).

The bundles of myocardial fibers are covered with plasma membrane and basement membrane. Each myofibril is divided into a series of repeating units, the sarcomeres. All the sarcomeres within one cell are in transverse register, giving the cardiac cells a striated appearance.

Using the scanning electron microscope, from the sectioned muscle bundle, we found the following surface changes of muscle fibers due to a prolonged anoxic condition. There was a loss of

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Fig.1. The normal endocardial surface observed by a SEM (scanning electron microscope) x2000

Fig.2. The normal endocardial surface. SEM x6000

Fig. 4. With a prolonged anoxic condition, the loss of regularity of the endocardial cells and the irregular swelling of endocardial cells were viewed. SEM X3000

Fig. 3. The endocardial surface changes due to a prolonged anoxic condition. SEM X1000

Fig.6. The surface of muscle fibers are shown. The regularity is damaged at the portion indicated by the arrows. SEM x5000

Fig.5. The surface of muscle bundle, a loss of regularity in the appearance of muscle fibers. The swelling of the muscle (arrow A) was also viewed. The regularity of striation is well preserved in the fiber indicated by the arrow B. SEM x1000

Fig.7. The relationship between the cell membrane and lipids. The unit membrane theory was changed to the theory of the repeating units.

Fig.8. Change of the percentage composition of phospholipid from heart muscle.

regularity in the appearance of muscle fibers, specially at the position which corresponded to Z-lines from external appearance. The swelling of the muscle fibers was also viewed (Fig. 5,6).

2. Metabolism

The surface membrane of the heart muscle cells measures approximately 100 Å in thickness and since 1961 it was considered the typical tri-layered "unit membrane" structure, consisting of a bimolecular layer of lipid molecules with associated protein layers on both surfaces, however, it is now postulated from recent studies that the lipids of membrane subunits are bound within the interior of the protein and the membrane is considered as a series of repeating units which contains the base piece and the head piece^{4,5} (Fig. 7).

Lipid in the membrane is predominantly phospholipid. The amount varies from 25 to 50% by weight. The fatty acid composition of phospholipid from membrane are undoubtedly polyunsaturated, such as linoleate and arachidonate.

Thus we have, first of all, intended to study the relationship between myocardial phospholipid changes and endocardial surface changes following prolonged anoxic condition during cardiopulmonary bypass.

The method employed for the phospholipid study was the extraction of lipid with Folch's solution from the resected heart muscle at the

radical operation of tetralogy of Fallot, using a homogenizer. Seven cases of tetralogy of Fallot were selected for this purpose. Separation of phospholipid from total lipids were performed with thin-layer chromatography.

Fatty acid were methylated and were analysed by a gas chromatograph with flame ionization detector. The details of the methods employed are found in the previous report of the author¹. The percentage compositions of phospholipids were compared according to the time when the heart muscles were resected in the operation, namely at the time of cardiectomy and about 60 to 70 minutes after the start of cardiopulmonary bypass. We found that the percentage of palmitate increased at 60 to 70 minutes after the start of cardiopulmonary bypass. On the other hand, the percentage of the unsaturated fatty acids, such as linoleate and arachidonate decreased as perfusion time prolonged (Fig.8). This might be the hypoxic response of cardiac muscle cells during extracorporeal circulation in man. This fact resembled the data obtained in the alteration of free fatty acids in arterial blood during extracorporeal circulation (Fig. 5 in the previous report¹).

Another useful method utilized in the study of myocardial metabolism is the coronary arterio-venous differences of free fatty acids⁶. For this purpose 9 cases of heart diseases mainly tetralogy of Fallot, were selected. A few minutes after the start of perfusion, the heart of almost every case extracted free fatty acids. Palmitate and oleate was mainly extracted. Forty seconds after the last declamp of the ascending aorta, the heart was resuscitated and began beating. From the study of arterio-venous difference at this time, the metabolism of the heart in anoxic or hypoxic state was estimated. Immediately after the release of anoxic occlusion, myocardium never extracted palmitate, however, mainly oleate and linoleate were extracted. Ten minutes thereafter, free fatty acids were easily extracted and that was mainly palmitate and oleate. This showed a metabolic recovery of the heart from anoxia.

But linoleate, because of the lack of quantity at the end of the perfusion time, showed no remarkable arteriovenous difference (Fig.6 in the previous report¹).

CONCLUSION

Endocardial and myocardial surface change due to prolonged anoxic arrest was observed, using a scanning electron microscope.

The destruction of the regular cellular arrangement and the change from the fine granular appearance of the cellular surface to a coarse granular appearance, was observed. There was a loss of regularity in the appearance of muscle fibers and the swelling of the fibers was also observed.

Phospholipid in the myocardium was studied as a membrane constituent and the percent composition of fatty acids showed significant change which was thought to be an influence of anoxic arrest. So the relationship between morphological change and metabolic change were simultaneously reported.

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