ULTRASTRUCTURAL AND ENZYME-CHEMICAL CHANGES IN DOG TESTES DURING TEMPORARY CIRCULATORY ARREST AND CARDIAC MASSAGE

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Transplantation of cadaveric testes is widely used in clincial practice to make good the hormonal deficiency in patients with androgenic insufficiency [2]. The use of cadaveric testes to restore the recipient's reproductive function has been delayed because of rapid death of the spermatogenic epithelium as a result of heat-induced ischemia.

The technique of direct mechanical cardiac massage (DMCM) of the cadaver has been successfully used for short-term conservation of various organs to permit their removal and transplantation under both experimental and clinical conditions [4, 10]. However, no data could be found in the literature on the state of the testes and the possibility of using them for transplantation after the use of DMCM.

The aim of this investigation was to study the ultrastructure and metabolism of the dog testis after cardiac arrest and DMCM.

EXPERIMENTAL METHOD

Altogether 12 experiments were carried out on adult mongrel dogs weighing 15-26 kg. Under pentobarbital anesthesia thoracotomy was performed on the animals in the fifth left intercostal space, the pericardium was opened, and electrical ventricular fibrillation was induced. The systemic circulation was arrested for 10 min, after which a cardiac massager was applied to the heart and DMCM carried out for 1 h. Pieces of testis were taken before cardiac arrest, after 10 min of ischemia from the same testis, and after operation of the cardiac massager for 1 h from the other testis, i.e., the same animal served as both control and experimental. Histological and electron-microscopic investigations were undertaken. Activity of the key enzymes of the Krebs cycle (succinate dehydrogenase, SDH), of glycolysis (lactate dehydrogenase, LDH), of the pentose shunt (glucose-6-phosphate dehydrogenase, G6PDH), and of terminal oxidation (NAD- and NADP-diaphorases) was determined quantitatively in the tissue homogenate. The same reactions were carried out in parallel on frozen tissue sections.

EXPERIMENTAL RESULTS

Light-optical study of the testes 10 min after cardiac arrest revealed no visible changes in the spermatogenic epithelium of the convoluted tubules and Leydig's cells compared with the control. The walls of some arterioles were in a state of moderate spasm. Qualitative enzyme histochemical analysis revealed no differences from the control in the distribution of formazan granules and the intensity of the reactions in the testicular cells. Quantitative enzyme-histochemical investigation revealed a small (not statistically significant) decrease in activity of LDH (by 19.3%), G6PDH (by 24.3%), NAD-diaphorase (by 11.4%), and NADP-diaphorase (by 13.2%) activity, whereas SDH activity remained at the control level (Table 1). The electron-microscopic study also revealed minor changes (not significant) in the spermat-

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TABLE 1. Activity of Oxidation-Reduction Enzymes in Testicular Tissue after Cardiac Arrest and Cardiac Massage in Dogs

Enzyme	Normal	Cardiac arrest for 10 min	Cardiac massage for 1 h
SDH	68±22	68±15	71±19
LDH	306±60	247±25	265±34
G-6-PDH	58±12	44±5	40±8
NAD-diaphorase	799±50	708±91	707±99
NADP-diaphorase	250±46	217±31	189±36

Legend. Four analyses done at each time. Enzyme activity expressed in micrograms formazan formed by 1 gram net weight of tissue in 1 minute.

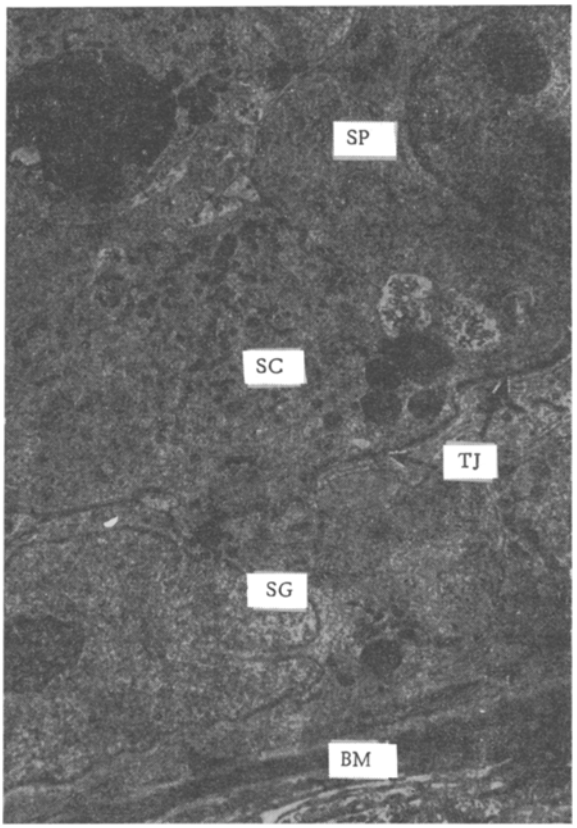

Fig. 1. Ultrastructure of testicular tubules 10 min after cardiac arrest: indented shape of nuclei of spermatogonia (SG), narrow intercellular spaces; tight junctions (TJ) between Sertoli cells (SC) have the usual structure. SP) Spermatocyte, BM) basement membrane. 6000×.

ogenic epithelium: Nuclei of the spermatogonia were indented in shape although the chromatin was finely granular, and the endoplasmic reticulum in the spermatids was moderately widened. Tight junctions between neighboring Sertoli cells were indistinguishable in structure from the control (Fig. 1). Myoid cells and noncellular components of the tunica propria of the seminiferous tubules did not undergo any visible changes. The electron density of the cytoplasm of the endothelium of the capillaries and venules was increased a little, many finger-shaped outgrowths appeared on the luminal surface, and moderately intensive pinocytosis was present.

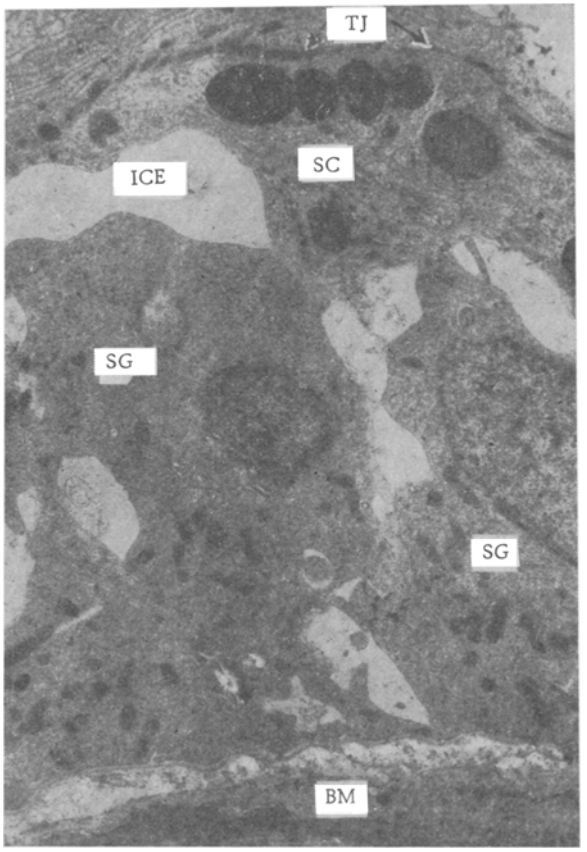

Fig. 2. Ultrastructure of testicular tubules 10 min after cardiac arrest and after cardiac massage for 1 h: appearance of intercellular edema (ICE); tight junctions (TJ) between Sertoli cells (SC) have the usual structure. SG) Spermatogonia, BM) basement membrane. 9000×.

Light-optical investigation of the testicular tubules 1 h after restoration of the systemic circulation by DMCM revealed no changes in the spermatogenic epithelium or Leydig's cells. The lumen of some blood vessels was contracted a little by spasm, whereas that of others was dilated. Qualitative enzyme histochemical analysis likewise revealed no differences from normal in cells of the tubules and stroma. Quantitative determination showed that LDH activity had a tendency to rise compared with the period of ischemia, but it was still 13.4% lower than the normal level. SDH activity was 4.4% above the control value. Activity of enzymes of the pentose shunt (G6PDH) and of terminal oxidation (NAD- and NADP-diaphorases) was a little lower than during ischemia (by 9.1, 0.1, and 12.9% respectively) and with normal (by 31.0, 11.5, and 24.4%). However, none of the variations observed was statistically significant. Electron-microscopic investigations showed that the nuclei of the epithelium of the seminiferous tubules had acquired the usual round shape and the structure of the cytoplasmic organelles likewise was the same as in the control. However, in some tubules moderate edema and widening of the intercellular spaces appeared. The edema fluid extended as far as tight junctions between the Sertoli cells and did not spread beyond, and the structure of the tight junctions themselves was the same as in the control (Fig. 2). Myoid cells and noncellular components of the tunica propria of the seminiferous tubules remained visibly unchanged. Capillaries and arterioles in a state of slight dilatation or spasm were found: The lumen was narrow and had a corrugated appearance, the endothelial nuclei were indented in shape, and the cytoplasm formed numerous finger-shaped outgrowths into the lumen.

Tolerance of the germinative epithelium of the testes toward heat-induced ischemia is known to be limited, although views of permissible exposures to ischemia differ. Kettle and Harrison [9] consider that after occlusion of the testicular artery for 30 min the structure of the testicular tubules is not restored. According to Hinmann and Smith [7], irreversible

destructive changes in the testicular parenchyma arise after 1-2 h of ischemia, whereas Attaran et al. [5] consider that transplantation of the testis even after ischemia for 2 h does not disturb normal spermatogenesis, and in Smith's opinion [11], the "critical time" for testicular structure is 4 h of ischemia.

It follows from the facts described above that the problem of allowable exposures of the testis to ischemia, after which recovery of spermatogenesis is possible, remains unsolved. Despite this, the technique of DMCM, on the one hand, enables the time spent by the organ in the cadaver, limited to a 10-min period while the cerebral cortex is dying after cardiac arrest, to be reduced to a minimum, and on the other hand, it enables short-term conservation of the testes to be undertaken *in situ*. The results of the present investigation showed that cardiac arrest for 10 min and cessation of systemic circulation give rise to mild ultrastructural changes in the tubular epithelium and the walls of the blood vessels and to a small but not significant decrease in activity of the enzymes of glycolysis and the pentose shunt in the testicular cells. These ischemic structural changes in the cells are considered to be completely reversible [6].

Restoration of the circulation by DMCM in the course of 1 h leads to normalization of the ultrastructure of the tubular epithelium and to an increase in activity of enzymes of glycolysis and the Krebs cycle. However, the ischemic changes are not completely abolished. Phenomena of spasm and dilatation of the blood vessels persist, edema of the intercellular spaces remains in the tubules, and values of parameters of the pentose shunt and terminal oxidation did not reach the control levels. This was probably connected with some special features of the general and local hemodynamics after application of the cardiac massager, and their incomplete normalization. The writers showed previously [1] that the cardiac massager can maintain a mean arterial pressure equal to 75% of its initial value. In some experiments this pressure could be obtained during the first minute of operation of the cardiac massager, in others it rose gradually and stabilized after 15-20 min in operation. The testicular blood flow was restored by the 3rd-5th minute of DMCM and amounted to 60-130% of the initial level during 1 h of DMCM. It is also known that after restoration of the circulation in an ischemic organ, tissue damage is drastically increased. This can be explained by the action of accumulated products of anaerobic metabolism on the vascular system, leading to paralysis of sphincters, a retrograde blood flow, congestion of the vessels, and a sharp rise of hydrostatic pressure, and even rupture of small vessels [3]. Intercellular edema in the testicular tubules after application of the cardiac massager can be linked with the above-mentioned processes. Despite this, however, the structure of the specialized tight junctions between neighboring Sertoli cells remained well preserved. This is very important because it is these junctions which constitute the anatomical barrier that under normal circumstances is impermeable for different substances from the blood, whereas the myoid and noncellular structures of the tunica propria of the tubules are a partially permeable barrier for tracers such as ferritin, peroxidase, and lanthanum [8]. Tight junctions between Sertoli cells separate the spermatogenic epithelium into two compartments: basal, containing spermatogonia, and luminal, containing spermatocytes and spermatids. The integrity of this barrier ensures normal spermatogenesis, whereas its disburbance provides access to the luminal compartment for various ions and immune factors, leading to disturbance of spermatogenesis.

It can be concluded from integrity of the tight junctions of the Sertoli cells both after cardiac arrest for 10 min and after operation of the cardiac massager for 1 h, that normal spermatogenesis is possible in the future graft. The mild hypoxic changes discovered in the structure and metabolism of the testis are no obstacle to its use in transplantation. Maintenance of the circulation in the potential donor by means of DMCM is evidence that this is an effective method for the purpose of conservation of the testis *in situ*.

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