J. SUBMICROSC. CYTOL. PATHOL., 33 (1-2), 151-156, 2001

Ultrastructural alterations in the myocardium of patients with sickle cell anemia

Ö. TAP, M. ŞAN*, U.Ö. METE and M. KAYA

Department of Histology and Embryology and *Department of Cardiology, Faculty of Medicine, Çukurova University Balcalı, Adana, Turkey

SUMMARY - Endomyocardial biopsies of patients with sickle cell anemia (SCA) were examined ultrastructurally. Myocardial fibers of patients revealed prominent degenerative changes such as nuclear chromatin condensation, myofibrillar degeneration, sarcosomal changes, dilatation of sarcoplasmic reticulum, formation of autophagic vacuoles, increase of lipofuscin granules and occurrence of vacuoles derived from endoplasmic reticulum and/or transverse tubules in some micrographs. Furthermore, edematous changes were developed at the same area where the degenerative changes were prominent. All of these cellular alterations could be related with ischemia resulting from local microvascular occlusion.

KEY WORDS *heart - sickle cell anemia - ultrastructure - hypoxia - ischemia*

INTRODUCTION

Sickle cell anemia (SCA), which is a hereditary disorder, causes chronic organ damage generated by capillary occlusion. Moreover, heart failure in patients with SCA is characterized by cardiac hypertrophy, left ventricular dilatation, cardiac conduction system defects and degenerative myocardial changes (Simmons *et al.*, 1988; James *et al.*, 1994; Berezowski *et al.*, 1992). Also, it has been reported that sudden death in patients with SCA was associated with myocardial infarction (Martin *et al.*, 1983). Some investigators pointed out to the presence of a specific cardiomyopathy in patients with SCA (Falk and Hood, 1982) whereas others suggested that there was no specific cardiomyopathy associated with SCA (Lester *et al.*, 1990; Covitz *et al.*, 1995). Thus, the cardiac histopathology of patients with SCA is still unclear due to presence of different interpretations about this subject. Nevertheless, there are few studies related to cardiac morphology of patients with SCA. Therefore, the present study is designed to disclose the ultrastructural changes in the myocardium of patients with SCA.

Mailing address: Dr. Özgül Tap, Çukurova Üniversitesi Tıp Fakültesi, Histoloji and Embriyoloji AD, 01330 Balcalı-Adana, Turkey; e-mail: umete@mail.cu.edu.tr.

MATERIALS AND METHODS

In this study, endomyocardial biopsies of ten patients with SCA who had been diagnosed with sickling test and hemoglobin electrophoresis were examined. Patients included 8 males and 2 females and they ranged in age from 16 to 29 years. Detailed clinical evaluation and routine laboratory tests of patients were performed (Table 1) and to these results, student-*t* (paired) test was applied. The consent for all procedures was obtained from the patients who had cardiomegaly and echocardiographic diastolic dysfunction. Endomyocardial biopsies were taken from the right ventricle via femoral venous approach. The biopsies were fixed in 5% glutaraldehyde in Millonig phosphate buffer at pH 7.4 for four hours and postfixed in 1% osmium tetroxide (OsO_4) in phosphate buffer at pH 7.4 for 2 h at 4 °C. Tissues were dehydrated in graded ethanol and embedded in araldite. Sections were cut using Reichert OMU3 ultramicrotome, stained with both uranyl acetate and lead citrate and then examined with Zeiss EM 10 B electronmicroscope.

RESULTS

Some of myocardial fibers had a normal structure, while others exhibited various structural alterations (Figs. 1 and 2). These alterations were characterized with sarcosomal degeneration, dilatation of sarcoplasmic reticulum cisternae, increase of lipid droplets and glycogen particles and nuclear

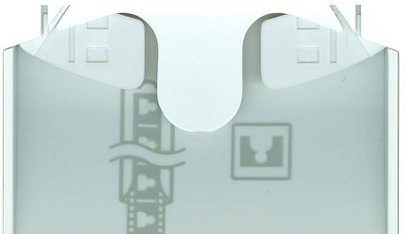


TABLE 1

Features of the patients who underwent endomyocardial biopsy. Hb. E: hemoglobin electrophoresis, HTC: hematocrit, MCV: mean corpuscular volume, ECG: electrocardiogram, Tele: telecardiogram, LVH: left ventricular hypertrophy, CMG: cardiomegaly

	Age	Hb. E.	HTC	MCV	ECG	Tele
Male (n = 8)	22 ± 7	SS	26 ± 3	97 ± 7	LVH	CMG
Female(n = 2)	27 ± 0	SS	28 ± 0	26 ± 0	LVH	CMG

pyknosis. Also, blood capillaries were generally obliterated with sickled and immature erythrocytes and blood platelets at myocardium.

The nuclei of myocardial fibers were generally indented and some of them exhibited increased heterochromatin granules that localized as aggregations (Figs. 1 and 2). Transverse striations were generally protected (Fig. 1), but they disappeared at degenerated myocardial fibers (Figs. 2 and 3). Sarcosomes localized in the vicinity of nucleus, peripheral sarcoplasm and between the myofilaments. The sarcosomes were clustered in peripheral sarcoplasm and perinuclear area, while they were aligned in rows between the myofilaments (Figs. 1 and 4). Sarcosomal cristae were generally closely packed, but they were disorganized in some myocardial fibers (Figs. 4 and 5). Furthermore, sarcosomal matrix showed small vacuoles or electron lucent areas (Figs. 2 and 4), but its density increased at some other micrographs as well (Fig. 5). Spheroidal shaped lipid droplets scattered through the cytoplasm and were generally in close association with sarcosomes (Fig. 6). Sarcoplasmic reticulum dilated in some myocardial fibers (Figs. 4 and 7). Glycogen granules were in small amounts in some myocardial fibers, while they were seen as large aggregates in some others (Figs. 1, 3 and 4). They were also observed as free or small aggregate between the myofilaments in which they were associated with sarcosomes (Figs. 4, 5 and 7), while they localized as large aggregates at the peripheral sarcoplasm, particularly around the discus intercalaries region (Fig. 4). Increased lipofuscin pigment granules were observed in perinuclear area and between myofibrils (Fig. 7). Furthermore, differently sized and shaped vacuoles were observed in the areas where abundant sarcosomes existed and

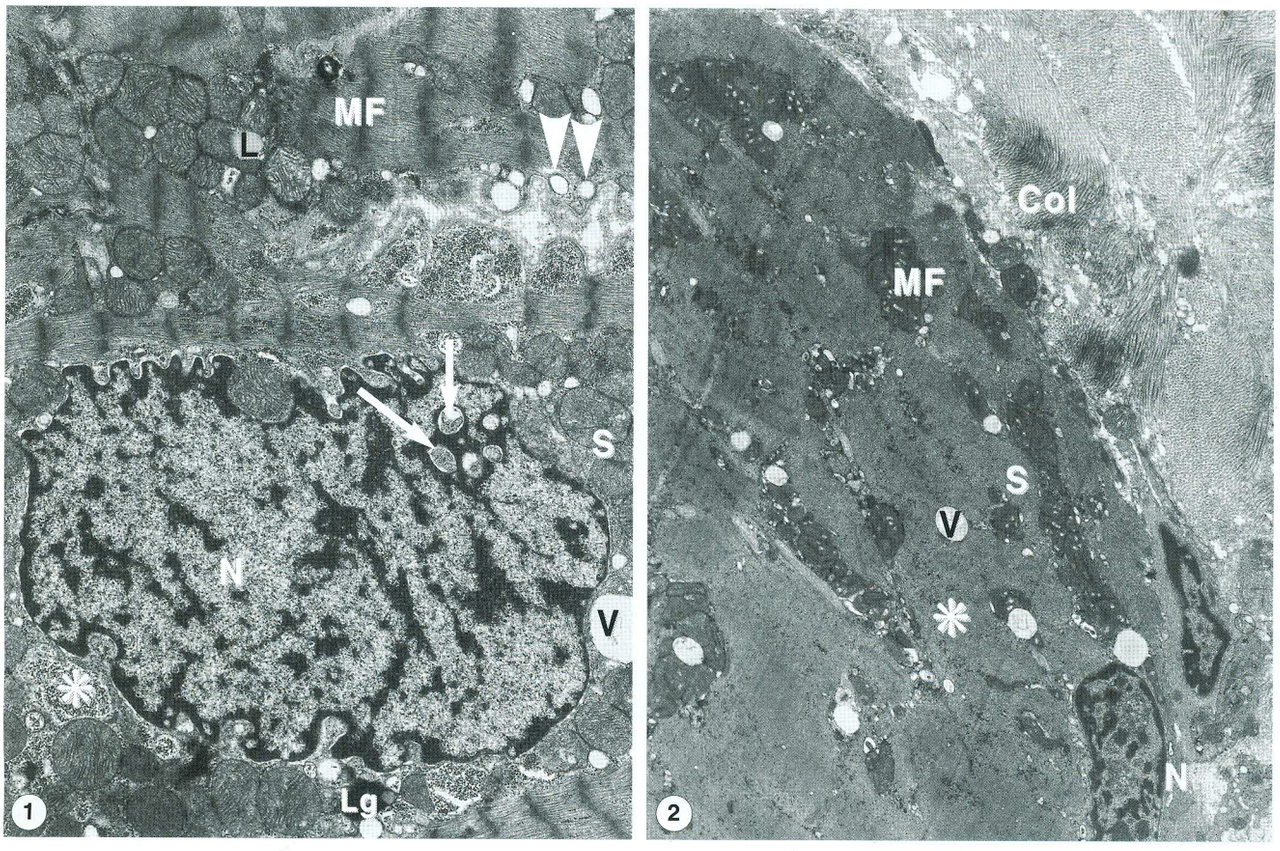


FIGURE 1 Intranuclear vacuoles (arrows) at the indented nucleus (N) of the myocardial fiber (MF) in the patients with SCA are seen. Sarcosome (s), sarcoplasmic reticulum (arrowheads), lipofuscin granule (Lg), glycogen particles (asterisk), lipid droplet (L), cytoplasmic vacuoles (V). × 9,300.

FIGURE 2 Degenerated myocardial fiber (MF) with hyperchromatic nucleus (N), increased sarcosomal matrix density of sarcosomes (s), disappeared striation (asterisk) and vacuolization (V) are observed. Also, increased collagen fibers (Col) are seen. × 7,575.

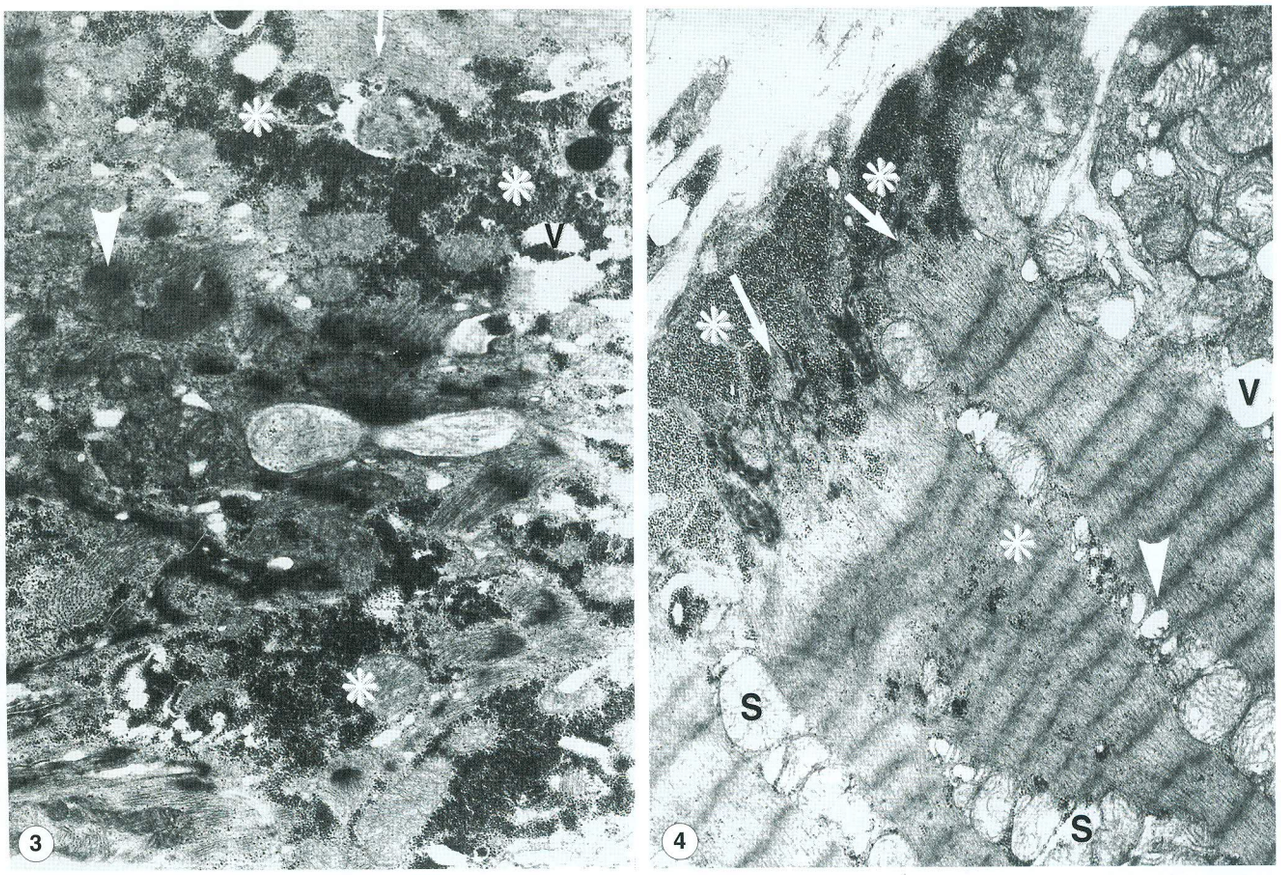


FIGURE 3 Myocardial fiber includes nonfibrillar areas (arrow), disorganized myofibrils (arrowhead), large aggregate of glycogen particles (asterisk) and variously sized vacuoles (V) in its sarcoplasm. × 15,000.

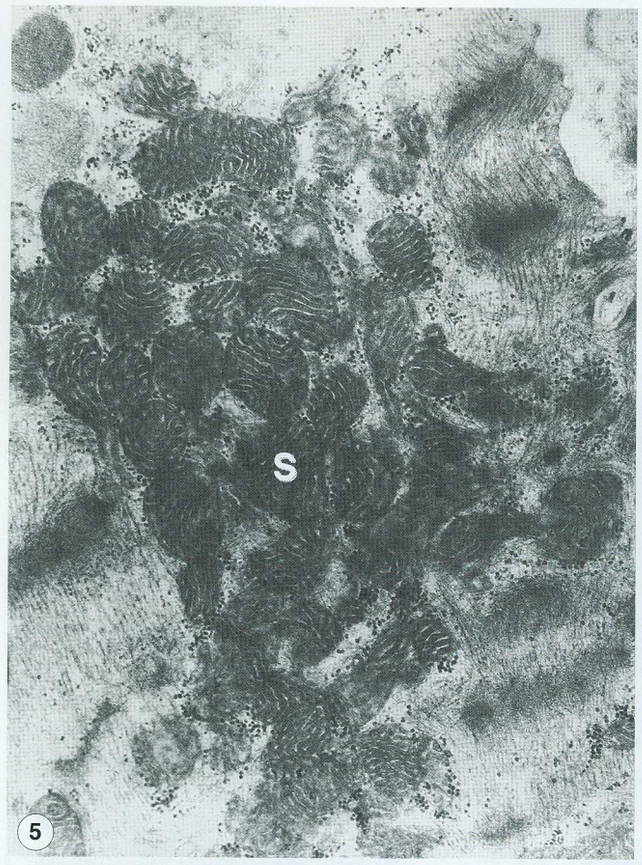
FIGURE 4 Dilated sarcosomes (s) and sarcoplasmic reticulum (arrowhead) among myofibrils, large aggregation of glycogen particles (asterisks) in the discus intercalaries region (arrows) and different shaped and sized vacuoles (V) appear in the myocardial fibers. × 15,000.

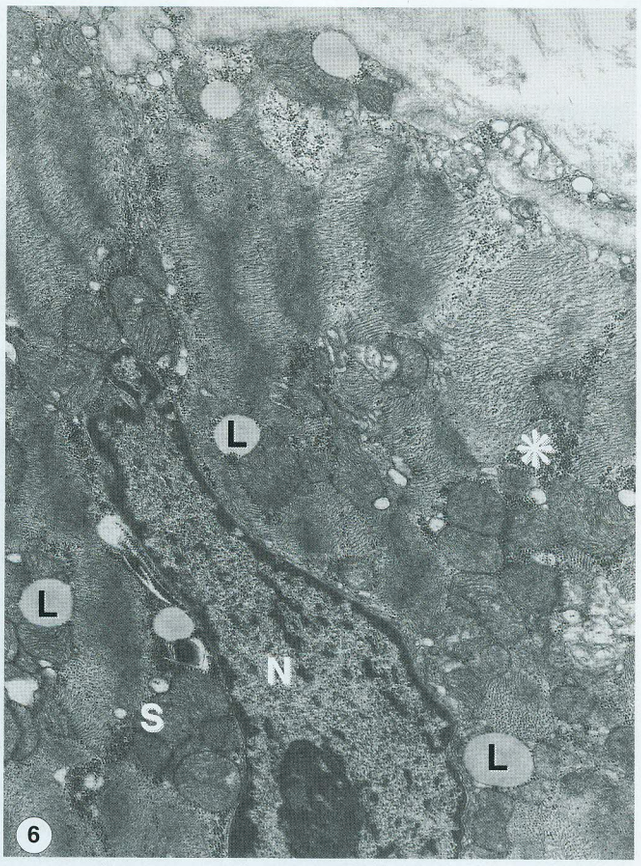
also in the nonfibrillar areas of sarcoplasm (Figs. 2, 4 and 8). In addition, several autophagic vacuoles appeared at some degenerated myocardial fibers (Fig. 9).

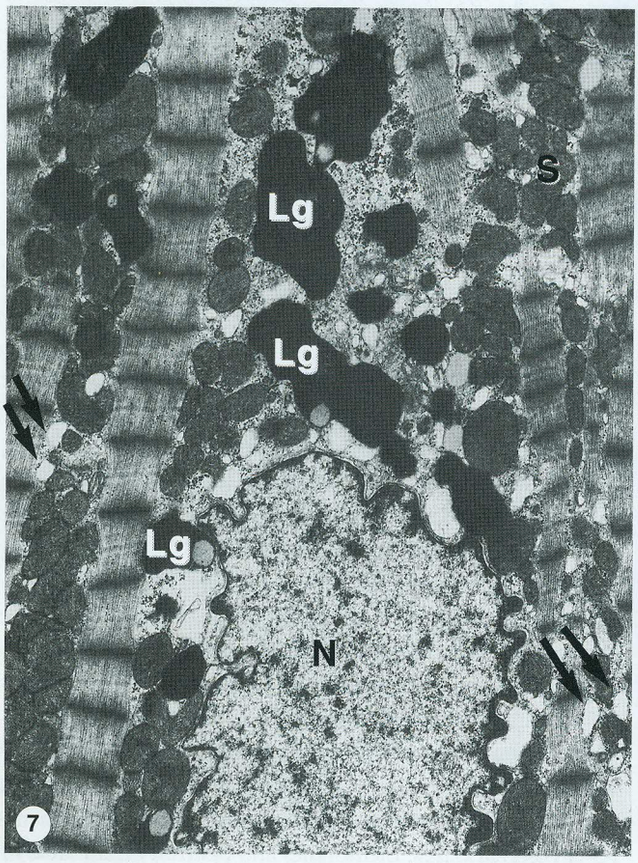
In some regions where the orientation of discus intercalaries was disturbed, there were several vacuoles containing membranous whorls and myelin figures (Fig. 10), and aggregated glycogen particles (Fig. 4). Collagen and reticular fibers increased in connective tissue, especially between the degenerated myocardial fibers (Figs. 2 and 11). Endothelial cells of myocardial capillaries were seen to include numerous micropinocytotic vesicles along both luminal and basal surfaces and filopodia (Figs. 11 and 12). The filopodia of endothelial cells extended into the capillary lumen. Moreover, capillary lumen generally contained sickled and immature erythrocytes and blood platelets. Sickled erythrocytes were generally spindle or irregular in shape, while immature erythrocytes contained filamentous web and both of them contained microvesicles in their cytoplasm. The capillaries generally obliterated because of sickled immature erythrocytes aggregation, and edematous changes developed at the connective tissue around the capillaries (Fig. 12).

DISCUSSION

The patients with SCA suffer from a deterioration at multiple organ systems resulting from vascular occlusion. Vascular occlusion causes local ischemia at some organs such as kidney, liver, skeletal muscle, heart, and lungs (Falk and Hood, 1982; Hasleton *et al.*, 1989; James *et al.*, 1994). In this study our findings proved that myocardial fibers had a normal appearance in some micrographs, but they seemed to have degenerative changes focally in many micrographs. These degenerative changes observed in myocardial fibers were characterized by nuclear chromatin condensation, sarcosomal changes, dilatation of sarcoplasmic reticulum, occurrence of autophagic vacuoles, increase in lipofuscin granules, structural alterations of discus intercalaries and presence of vacuoles. Sarcosomes appeared in different ultrastructural features such as: fragmented sarcosomal cristae membranes and electron lucent areas or amorphous matrix densities in their matrix. In their research, Decker and Wildenthal (1980) reported that there was dilatation and fragmentation in the cristae membranes of sarcosomes, and formation of amorphous matrix densities at differ-







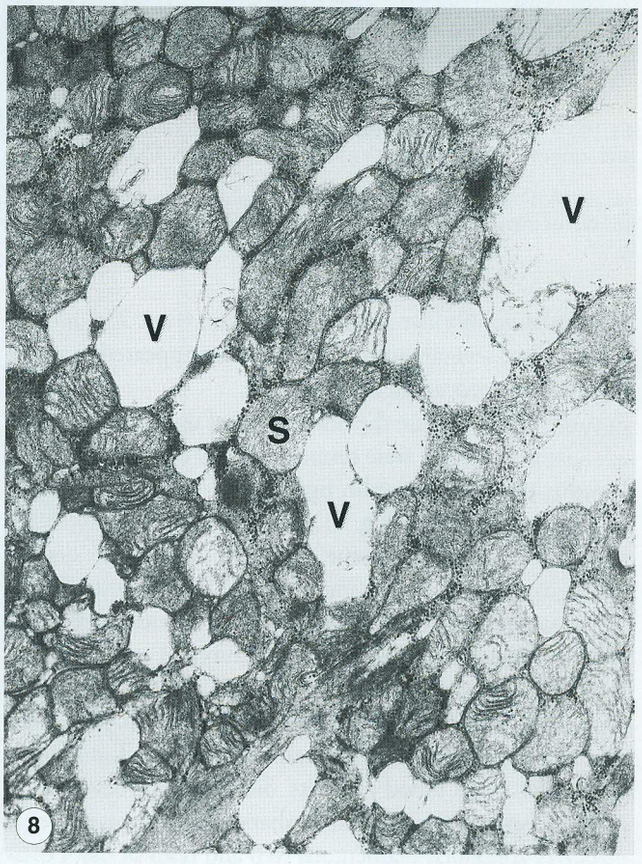


FIGURE 5 Increased sarcosomal matrix density of clustered sarcosomes (s) is seen in the myocardial fibers. × 18,600.

FIGURE 6 Few lipid droplets (L) are observed throughout the sarcoplasm of myocardial fiber. Nucleus (N), glycogen particles (asterisk), sarcosomes (s). × 12,075.

FIGURE 7 Increased lipofuscin granules (Lg) in the perinuclear area of myocardial fiber are seen. Nucleus (N), sarcosome (s), dilated sarcoplasmic reticulum (arrows). × 10,100.

FIGURE 8 Vacuoles (V), in different size and shape, appear among the clustered sarcosomes (s). × 16,100.

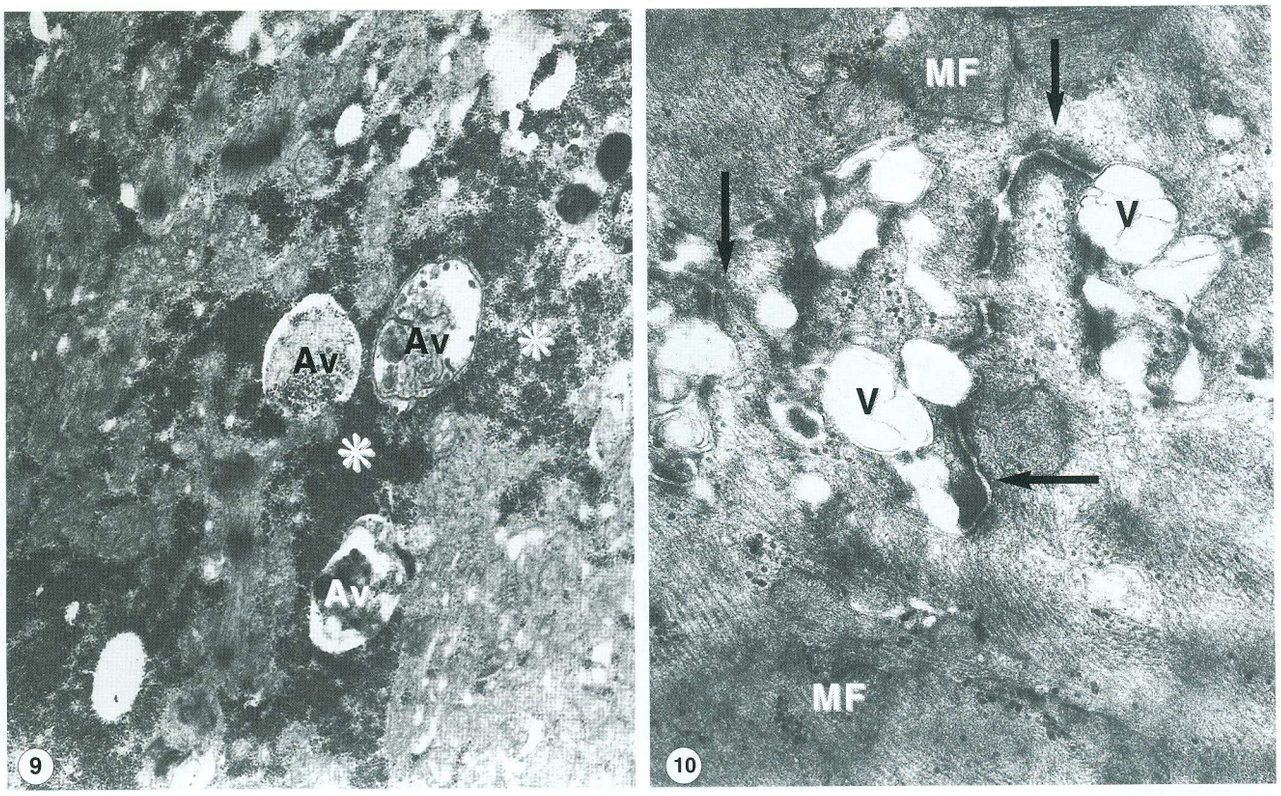


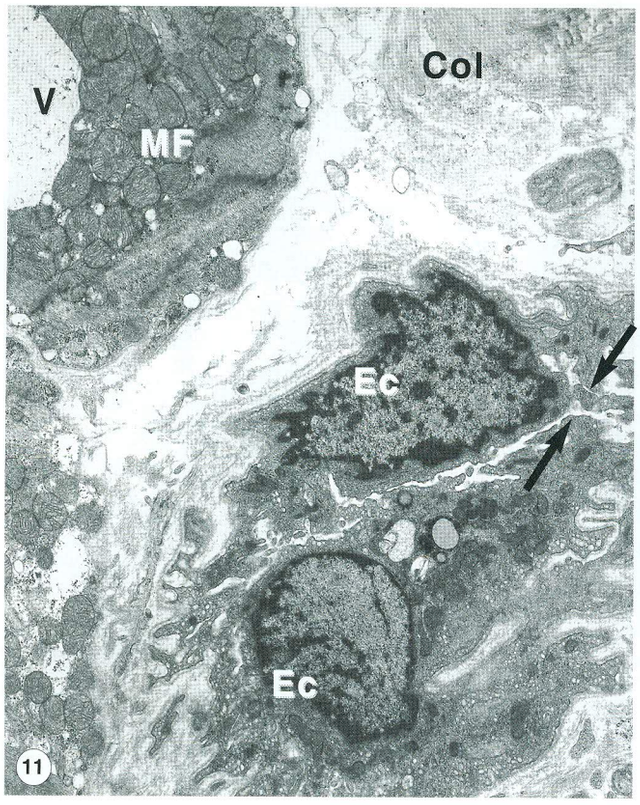
FIGURE 9 Autophagic vacuoles (Av) are seen in close association with glycogen particles (asterisks) in degenerated myocardial fiber. × 16,100.

FIGURE 10 Discus intercalaries (arrows) between the myocardial fibers (MF) are observed as related with several vacuoles (V). × 30,000.

ent durations of hypoxia. Thus, the sarcosomal alterations in this study presumably resulted from the exposition to hypoxia caused by vascular occlusion. In addition, it is obvious that the enzymes of the Krebs and fatty acid cycles are situated within the sarcosomal matrix, while respiratory enzymes are felt to be attached to the sarcosomal membranes (Mirsky *et al.*, 1974; Leeson *et al.*, 1988). Regarding these facts, this study also revealed that structural variations of sarcosomal matrix and sarcosomal membranes were ultimately accompanied by alterations in sarcosomal functions. Moreover, sarcosomal changes can cause inactivation of sarcosomal enzymes and depression of ATP synthesis from glycogen particles. Accordingly, large glycogen aggregates were not observed ultrastructurally in normal myocardial fibers, but they were generally observed at the periphery of sarcoplasm without sarcosomes and at degenerated myocardial fibers. In addition, sarcoplasmic reticulum showed dilatations which were attributed to hypoxic effect. In fact, some of sarcoplasmic vacuoles were likely to be generated from this dilated sarcoplasmic reticulum and transverse tubules of t-systems. Myocardial contraction requires an energy (ATP) supplied by sarcosomes and calcium ions stored in the sarcoplasmic reticulum (Leeson *et al.*, 1988). Thus, degeneration of these organelles might have played an important role in the depression of the contraction mechanism of the myocardial fibers. Additionally, Denenberg and coworkers (1983) suggested that left ventricular muscle contractile performance was depressed in SCA.

It was also observed that there was an increase in the amount of lipofuscin granules derived from secondary lysosomes and represented the deposits of indigestible substances. Lipofuscin granules are generally observed in association with regressive changes at atrophy and chronic degeneration (Robbins and Kumar, 1987). Moreover, the autophagic vacuoles may be formed to eliminate the damaged cellular organelles of injured myocardial fibers due to ischemia. In addition, it was reported the formation of lysosomal autophagic vacuoles responsible for the elimination of damaged, presumably nonfunctional organelles in minimal, injured myocardial cells (Decker and Wildenthal, 1980). Besides, alterations of intercalated discs are thought to cause the failure of impulse conduction between the myocardial fibers. Additionally, it was claimed that the development of intercellular widening in the region of discus intercalaries could affect electrical excitation, and thereby it might break down the efficiency of ventricular contraction (Mirsky *et al.*, 1974). Thus, in view of the primer effect of SCA on the heart left ventricular hypertrophy (Simmons *et al.*, 1988), structural alterations of the discus intercalaries were attributed to, that is left ventricular hypertrophy.

The blood capillaries were generally obliterated by the sickled and immature erythrocytes and platelets. James and colleagues



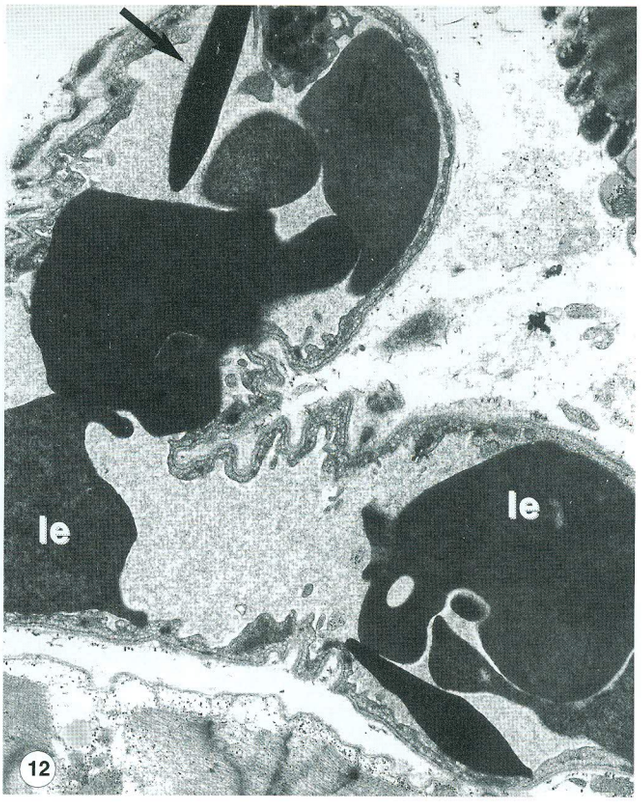


FIGURE 11 Endothelial cells (Ec) of capillary are revealed filopodia (arrows). Myocardial fiber (MF), increased collagen fibers (Col). × 6,300.

FIGURE 12 Immature (Ie) and sickled erythrocytes (arrow) in the capillary lumen are seen. × 6,600.

(1994) reported that sickled erythrocytes and aggregated platelets cause occlusion in the capillaries and small arteries. Additionally, vascular occlusion is responsible for local tissue hypoxia, which sometimes causes degeneration, tissue necrosis and fibrosis (Haynes *et al.*, 1994; James *et al.*, 1993). Thus, occurrence of both focal myocardial cell degeneration and increase of fibroconnective tissue among the degenerated myocardial fibers suggest that local fibrosis might have developed at the myocardium. Consequently, myocardial fibers were mildly degenerated in some areas, however heavily degenerated myocardial fibers and fibrotic areas were increased in some micrographs where vascular occlusion was widespread. Thus, in view of the results discussed above it is claimed that cardiac failure might be more distinctive at further ages, when vascular occlusion is more likely to develop.

REFERENCES

BEREZOWSKI K., MAUTNER G.C. and ROBERTS W.C., 1992. Scarring of the left ventricular papillary muscles in sickle-cell disease. *Am. J. Cardiol.*, **90** (15), 1368-1370.

COVITZ W., ESPELAND M., GALLAGHER D., HELLENBRAND W., LEFF S. and TALNER N., 1995. The heart in sickle cell anemia. The cooperative study of sickle cell disease. *Chest*, **108** (5), 1214-1219.

DECKER R.S. and WILDENTHAL K., 1980. Lysosomal alterations in hypoxic and reoxygenated hearts. 1. Ultrastructural and cytochemical changes. *Am. J. Pathol.*, **98**, 425-444.

DENENBERG B.S., CRINER G., JONES R. and SPANN J.F., 1983. Cardiac function in sickle cell anemia. *Am. J. Cardiol.*, **51**, 1674-1678.

FALK R.H. and HOOD W.B., 1982. The heart in sickle cell anemia. *Arch. Intern. Med.*, **142**, 1680-1684.

HASLETON P.S., ORR K., WEBSTER A. and LAWSON R.A.M., 1989. Evolution of acute chest syndrome in sickle cell trait: an ultrastructural and light microscopic study. *Thorax*, **44**, 1057-1058.

HAYNES J. JR., TAYLOR A.E., DIXON D. and VOELKEL N., 1993. Microvascular hemodynamics in the sickle red blood cell perfused isolated rat lung. *Am. J. Physiol.*, **264** (*Heart Circ. Physiol.*, **33**), H484-H489.

JAMES T.N., RIDDICK L.R. and MASSING G.K., 1994. Sickle cells and sudden death: Morphologic abnormalities of the cardiac conduction system. *J. Lab. Clin. Med.*, **124**, 507-520.

LEESON T.S., LEESON C.R. and PAPARO A.A., 1988. 'Text and Atlas of Histology'. W.B. Saunders Co., Philadelphia and London.

LESTER L.A., SODT P.C., HUTCHEON N. and ARCILLA R.A., 1990. Cardiac abnormalities in children with sickle cell anemia. *Chest*, **98** (5), 1169-1174.

MARTIN C.R., COBB C., TATTER D., JOHNSON C. and HAYWOOD L.J., 1983. Acute myocardial infarction in sickle cell anemia. *Arch. Intern Med.*, **143**, 830-831.

MIRSKY I., GHISTA D.N. and SANDLER H., 1974. Cardiac mechanics: Physiological, clinical and mathematical consideration. Chapt. II. Cardiac ultrastructure and function in the normal and failing heart. John Wiley & Sons Inc., New York, London, Sydney, Toronto.

ROBBINS S.L. and KUMAR V., 1987. 'Basic Pathology. Vol. 1. Cell Injury'. W.B. Saunders Co., Philadelphia and London.

SIMMONS B.E., SANTHANAM V., CASTANER A., RAO K.R.P., SACHDEV N. and COOPER R., 1988. Sickle cell heart disease: Two dimensional echo and doppler ultrasonographic findings in the hearts of adult patients with sickle cell anemia. *Arch. Intern. Med.*, **148**, 1526-1528.