Retrograde coronary perfusion of the isolated rat heart with calcium-deficient Hanks's solution leads to cardiac arrest. Microscopic examination revealed destruction of the intercalated disks, followed by segmentation of the myocardium. The dissociated segments contracted sharply but the myofibrils in them remained in a definite order. After further perfusion with complete Hanks's solution the contractile activity of the isolated heart was restored, but the segmentation of the myocardium did not disappear.

The inability of the myocardium to contract in the absence of calcium ions while preserving its bioelectrical activity is well-known [6, 8]. More recent investigations have demonstrated the complex role of calcium ions in the initiation of excitation, the coupling of excitation with contraction, and the regulation of permeability of the cell membrane. Calcium ions are essential for the functioning of several enzyme systems, including the ATPase system, and for the maintenance of contacts between the muscle cells of the myocardium.

Attempts have been made to demonstrate the importance of disturbances of the calcium balance in the development of myocardial injury. However, changes observed in the myocardium during perfusion of the isolated heart with calcium-free solutions have been interpreted quite differently. Gradually developing deaggregation of the myofibrils of the muscle cells with the formation of an optically empty sarcolemmal sheath has been described [9]. This phenomenon, known as "myofibrolysis," was attributed to the need for calcium ions for morphological integration of the myofibrils. After other observations on the myocardium when perfused under similar conditions severe injuries (contractures) to the muscle cells were described [7]. These changes were regarded as the result of a disturbance of cell adhesion.

Since contractural changes and myocytolysis have been shown to be clearly distinguishable types of injury to heart muscle cells [2-5], it is interesting to discover what type of changes are produced in the myocardium by calcium ion deficiency.

**EXPERIMENTAL METHOD**

The isolated hearts from 50 male albino rats weighing 120-150 g were treated by retrograde coronary perfusion with oxygenated Hanks's solution at 37°C. Besides complete Hanks's solution containing 1.2 mmole Ca++ per liter, solutions containing 0.6, 0.12, 0.06, 0.04, and 0.03 mmole Ca++ per liter and calcium-free solutions were used. The hearts were perfused for 5 min with complete Hanks's solution and then with one of the Ca++-deficient solutions for 5 min to 2 h. Some of the isolated hearts perfused with the Ca++-deficient solution were again perfused with the complete Hanks's solution for 1 h.

The methods of preparation of the material for microscopic investigation and the changes arising in the myocardium during perfusion with complete Hanks's solution for 5 min to 2 h were described previously [1].
Fig. 1. Area of myocardium from a heart perfused with calcium-free Hanks’s solution: a, b) commencing destruction of intercalated disks; c, d) dissociated segments in sarcolemmal sheath; e, f) contracturally changed muscle segments. PAS reaction combined with staining with colloidal iron and hematoxylin, 630×; a, c, e) photographed in ordinary light; b, d, f) photographed in polarized light.

EXPERIMENTAL RESULTS

The course of development of the distinctive changes arising in the contractile apparatus of the myocardium during perfusion of the isolated heart with Ca++-deficient solutions can be studied in detail by histological and, in particular, by polarization microscopic methods. These changes consisted of swelling of the intercalated disks with deaggregation of the myofibrils in areas lying next to the disks (Fig. 1a, b). Muscle cells previously joined together into a fiber became separated from one another, their myofibrils contracted sharply, their cross striation disappeared, and it was replaced by continuous anisotropy. These contracturally changed segments, preserving the cylindrical shape of the myocardial cell, each with one or two slightly pycnotic nuclei, were located in a well-preserved sarcolemmal sheath (Fig. 1c, d). An isotropic globular substance, varying in amount, could be observed between the segments inside the sarcolemmal sheath. If the perfusion continued for long enough, the orderly pattern just described was disturbed. The segments contracted still more, lost their connection with one another through the sarcolemma, and became rounded in shape (Fig. 1e, f). Despite such profound changes in the muscle cells, the myofibrils in them underwent neither complete lysis nor complete deaggregation but preserved a definite orderliness, as demonstrated by their anisotropy.

To give rise to the above-mentioned changes in the contractile apparatus of the muscle cells it was sufficient to perfuse for 15 min with Hanks’s solution containing 0.04 mmole Ca++ or less per liter. Raising the Ca++ concentration in the solution to 0.12 mmole/liter led to the development of changes such as these only after perfusion for 2 h. In this calcium concentration the contractile activity of the myocardium ceased however, after perfusion for 3-5 min. The segmentation of the myocardium described above spread to all parts of the heart if the isolated heart was perfused with solutions containing 0.03-0.06 mmole Ca++ per