REGENERATION IN CARDIAC MUSCLE

BY

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Received December 3, 1939

Proliferation of striated muscle, either in the form of regeneration of damaged tissue or hyperplasia of relatively normal cells, is generally thought not to occur. Such an opinion is an example of the general idea that more "specialized" tissues differ from less "differentiated" tissues in that they are unable to multiply in adult life.

This proposition, however, has gradually become more and more untenable during the past fifty years. At an early stage in the development of microscopy the capability of various forms of epithelium—skin, alimentary canal, etc.—for proliferation was appreciated. For some time such capacity was denied the more "specialized" epithelia such as the liver and renal cells. When hepatic and renal proliferation was at last recognized, the original view was still applied to other tissues, notably muscular and nervous cells.

Whether one regards a mucous cell of the intestine as being more differentiated than a fibrous tissue cell and less differentiated than a liver cell is purely a question of the point of view from which it is considered. The intestinal cell cannot, or in ordinary circumstances does not, produce collagen fibres, nor the liver cell mucin. Apart from this question and the presumed inverse capacity for proliferation, there is no doubt that certain cells may be observed to multiply more often than others. This has been recognized from the earliest days of histological study, but though many cells were thought not to multiply at all, it is now known that, to some extent, they do.

Regarding muscle cells, hyperplasia of smooth muscle was the first observed. More recently, regeneration of voluntary muscle, both naturally and experimentally, has been described, but knowledge of such changes in heart muscle has lagged behind.

HISTORICAL

The question of regeneration of cardiac muscle fibres has often been discussed in the last eighty years. The earliest writers—Kölliker (1852), Friedreich (1855), Paget (1865), von Rokitansky (1856), and Rindfleisch (1867)—accepted without question the occurrence of hyperplasia. Lebert (1857), however, emphasized the absence of any direct histological demonstration. Goldenberg
(1886) reviewed the previous reports and concluded that, though increase of cardiac tissue was largely by hypertrophy of muscle fibres, longitudinal splitting could occur. Zielonko (1875), from observations on the hearts of frogs and rabbits, thought that hyperplasia of cells took place.

Tangl (1889) and Wideröe (1911), and, more recently, Kaufmann (1928), Aschoff (1936), and Mönckeberg (1924) attributed all enlargement of the heart to hypertrophy of fibres without hyperplasia. They based their views mainly on the absence of mitotic figures. On the other hand, Ziegler (1889), Adami and Nicholls (1909), Saltykov (1905), Heller (1913), and Rössle (1923) maintained the probability of hyperplasia, but did not produce incontrovertible evidence of this. Counts and measurements of muscle fibres, however, made by Collier (1922) and Karsner et al. (1925) suggested that not only hypertrophy but also hyperplasia occurred.

Thus opinion was divided, and MacMahon (1937), reviewing the position recently, indicated that, since increase in fibres—probably by splitting—had been accepted by many writers, the principal difficulty was failure to demonstrate mitotic figures in muscle nuclei. He described cases in which such had been observed, and the illustrations leave little room for doubt regarding their occurrence in heart muscle of children.

Most of the material that has been studied was heart muscle obtained from various forms of toxic or inflammatory diseases of the myocardium. The muscle changes after injury have been reviewed by Hesse and Hesse (1924) and by Klose (1923). There is the same uncertainty in such cases, though the evidence appears somewhat to favour the occurrence of hyperplasia of muscle cells.

**Pathological Material**

The observations that form the basis of this paper were made on the heart muscle in the neighbourhood of a recent wound.

A man, æt. 19 years, received a penetrating knife wound of the chest, which caused a superficial injury to the anterior wall of the left ventricle. The heart was exposed and the wound sutured with silk. The patient recovered from the operation, but died from a streptococcal septicæmia on the fourth day.

A necropsy was performed six hours after death. There were small amounts of blood clot and of sero-purulent material in the anterior pericardial region; this was walled off by soft adhesions from the remainder of the pericardium which was unaffected. The lungs showed some patchy congestion and there was straw-coloured fluid in the left pleural cavity. There was congestion and toxic damage in the liver, kidneys, etc.

Pieces of heart wall were removed from the seat of the injury; it was healing well but could be recognized by the suture material used at operation.

Microscopically, the muscle cells appeared normal, though striations were indefinite in some areas—attributed to post-mortem change and the general toxæmia. Near the wound edge the muscle cells were separated from each other by fluid (œdema) (Figs. 1 and 2), and this made the examination of
Fig. 1.—Photomicrograph of muscle fibres near area of injury, cut in longitudinal section. The fibres are about half the thickness of those in neighbouring parts (cf. Figs. 3 and 4). Splitting of fibres may be seen, and they run, more or less, in pairs. Magnification ×350.

Fig. 2.—Photomicrograph of muscle fibres close to the injured area, cut in transverse section. Some are very small, only about 7μ in diameter (arrows). There is separation of some fibres by oedema and an absence of vessels in some of the groups of fibres. ×350.
individual fibres easier than usual. Immediately adjacent to the injured area the changes in the tissue were maximal.

At the site of injury there were numerous wandering cells of various kinds and many fixed cells with larger nuclei and more voluminous protoplasm than similar cells elsewhere. Their number steadily diminished in the surrounding tissue until at a distance of about 0.5 cm. the number was minimal and corresponded to that observed elsewhere. The affected area was arbitrarily divided into three zones: a central one with the greatest damage and greatest accumulation of connective tissue cells, an intermediate one, and a peripheral one where such connective tissue changes were definite and in excess of any seen in the normal myocardium, but less developed than the other zones of activity.

The muscle fibres in the intermediate—and to some extent in the peripheral—zone were much thinner than those elsewhere. They were arranged in pairs (Fig. 1) or in small groups (Fig. 2), sometimes separated from other similar groups, but at times in larger numbers due to the aggregation of smaller groups. Although blood capillaries were dilated and easily observed, these seemed to be fewer in proportion to muscle fibres than in other parts. These groups of small fibres were regarded as arising from the splitting of fibres.

In or close to the central zone muscle fibres appeared to end, and in many there was degenerative change of a hyaline type in this area (Fig. 3). From the ends of such fibres masses of protoplasm containing a number of large vesicular nuclei were observed. This protoplasm in some parts showed striations.

The nuclei in the fibres varied considerably in size, shape, and staining characters. In some of the cells there were double nuclei (Fig. 4). Many resembled the atypical nuclei seen in chronic myocarditis. A number of very irregular nuclei were found. Careful search of the fibres showed that in a number of them there were, replacing the nuclei, irregular masses of chromatin, deeply staining and consisting of an aggregation of small chromatin masses and rods (Fig. 5; at the top of p. 160). These appeared to be mitotic figures in various stages of evolution.

DISCUSSION

The case described was chosen partly because the local conditions seemed most suitable (as shown by the rapid proliferation of connective tissue and healing) for proliferative changes in the muscle, and also because such proliferation might be expected more readily in a young than an old subject.

The muscle changes found were of two sorts:

1. Those involving the whole muscle fibre—(a) splitting of the fibres, and (b) outgrowths in the neighbourhood of damaged tissue.

2. Those affecting the nuclei—(a) the presence of double nuclei, and (b) the presence of mitotic figures.
Fig. 3.—Photomicrograph of a section through the end of damaged fibres (showing some degenerative change), in which outgrowth of new fibres is occurring. × 350.

Fig. 4.—Photomicrograph through an area adjacent to the wound showing double nuclei (arrows). × 350.
1. (a) In cardiac muscle where fibres are of unequal size and where anastomosis of fibres is common, proof of splitting of these fibres is not easy to obtain. However, the fibres in the neighbourhood of the affected area may be compared with those in other parts. Two observations seem to be of importance in this regard.

Firstly, many of the fibres in the healing area are smaller than those in other parts. When cut in longitudinal sections they can be seen to run for some distance in pairs (Fig. 1), and in transverse sections the division of the fibre into a number of components can be seen (Fig. 2). Such appearances, of course, may be found in other parts of the heart, but in the affected zone the small fibres can be seen to be much smaller than those in other areas; in some cases they are about 7 μ in diameter. The way in which the fibres split is shown in the diagrams in Figs. 6 and 7. This is, of course, hypothetical, but was drawn from different sections and shows the way in which a fibre may separate into component parts, and because of the small size of the fibre was assumed to be occurring at the time of death. It will be seen that the new fibres are formed by separation of groups of Cohnheim’s areas.
Secondly, in the normal heart it is easy to demonstrate that there is one capillary for each muscle fibre, but in the affected tissue, although this was not proved by injection of the vessels, such vessels did not appear to be so numerous, and indeed there seemed to be only about half the number of vessels to the same number of muscle fibres. This arrangement and its mode of development is shown diagrammatically in Fig. 6.

(b) In the neighbourhood of damaged muscle tissue there are a large number of cells of different kinds, some wandering and some fixed, most of which are

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**Fig. 6.**—Diagram to show the probable method of formation of the new fibres (cf. Fig. 1). Note the relationship of capillaries to fibres.

**Fig. 7.**—Diagram to show the stages of division of muscle fibres, as seen in cross section (cf. Fig. 2). Serial sections through normal tissue will show a similar, though less developed, arrangement at sites of division of fibres.
of connective tissue origin. Some of these cells, recognized largely by the amount of protoplasm and also by its eosinophilic staining characters, have been regarded as arising from muscle cells. The protoplasmic outgrowths, however, from the ends of damaged muscle fibres, particularly since some of them show striations, seem to be attempts at formation of new muscle fibres (Fig. 3).

2. (a) In a number of the fibres the nuclei are arranged in pairs; these lie close together and overlapping, or side by side with their long axes parallel. These have been seen by many investigators and suggest multiplication of nuclei. However, double nuclei occur normally in Purkinje fibres, and one must exclude this possibility before it is assumed that they are in ordinary muscle fibres. This may be suggested by the absence of the other features of Purkinje fibres, but the more certain method is to compare the affected area with relatively normal tissue in the neighbourhood. When this is done it is found that the number of fibres containing double nuclei in the affected zone is greatly in excess of that in the neighbouring tissues.

(b) Finally there is the question of mitotic figures. In a number of the cells the nucleus is found to consist of an aggregation of small irregular deeper staining chromatin masses, usually in the form of rods. This area is surrounded by a zone of paler staining protoplasm. In the material examined these mitotic figures were rather irregular and did not show the characteristic features—the aster, diaster, etc.—of normal mitosis. In addition, there were a large number of very irregular nuclei—irregular in contour and much more deeply staining than their neighbours—and though it is tempting to regard these as related to the mitotic figures, this could not be proved. They closely resemble the irregular nuclei observed in cases of cardiac hypertrophy and chronic myocarditis.

Thus we have, in this damaged tissue, changes in the muscle cells which suggest that proliferation is taking place to some extent. There is indubitable evidence of nuclear activity, and the cells are undergoing hyperplasia in two ways—partly by protoplasmic outgrowths in the region of injury, and partly by splitting of fibres and the formation of new ones.

It is not intended to suggest that such proliferation of muscle plays a predominant part in the healing of an injured area; there can be no doubt that healing occurs by connective tissue activity and the formation of a fibrous scar. It is merely intended to indicate that muscular hyperplasia, which is often considered to be absent, does occur to some variable extent.

Summary

1. Examination of a recent heart wound in a young adult was undertaken to determine the possible presence of myocardial hyperplasia.
2. This was demonstrated by:
   i. The splitting of the fibres—as indicated by the size and arrangement of the fibres and their relationship to the capillary vessels.
ii. Protoplasmic outgrowths from the ends of damaged fibres,  
iii. The presence of double nuclei in some fibres.  
iv. The presence of mitotic fibres.

3. This does not, however, presuppose that such hyperplasia is responsible for the healing of wounds, which occurs by the usual connective tissue proliferation and by the formation of scar tissue.

REFERENCES


EDITORIAL NOTE

The general consensus of opinion is that hyperplasia of cardiac muscle does not take place. The subject was well reviewed by Karsner, Saphi, and Todd (1925) in adults and by McMahon (1937) in children. The editors have, however, decided to publish this paper as the subject's death so soon after the injury to his heart provided an unusual opportunity of re-examining the question, and the author has presented the evidence for his point of view fairly and scientifically. None of the points on which he bases his opinion seems to the editors to be final or conclusive, and the question is complicated by the actual cause of death from septicemia, with the possibility of resultant changes in the nuclei. Thus the splitting of fibres and their apparent arrangement in pairs might possibly be an artefact; the protoplasmic outgrowths and many of the changes seen in the nuclei might be the result of the infection and of degeneration; the double nuclei might be Purkinje fibres (though the author considers this improbable) or might be due to the superimposition of two nuclei. It is
only fair to add that the author has considered these explanations and rejected them; but the evidence seems inconclusive to the editors.

The two conclusive pieces of evidence would be a cell count, which is admittedly very difficult in cardiac muscle, or the presence of mitotic figures. The author thinks these last were observed, but admits that they were atypical, and the picture of mitosis with its spindles and regular formation is very unmistakable. Should a similar case occur with death resulting directly from the effects of the accident elsewhere without the complication of infection, an ideal opportunity of re-testing the question would be provided.