CHAPTER 3

THE STRUCTURE OF THE PECTORALIS MAJOR MUSCLE OF THE PIGEON IN DISUSE ATROPHY

The two types of fibres in the pectoralis major muscle of the pigeon have been shown to be fundamentally different and the reactions of these fibres in muscular atrophy are is, therefore, of special interest. The study of developmental abnormalities and pathological malformations resulting from natural and experimentally induced causes, has been an important tool in the study of the normal and has contributed much to the great advances in embryology and morphology. In the case of the pigeon breast muscle, therefore, a study, elucidating how far the major differences in the two types of fibres are maintained and what changes they undergo under experimentally induced pathological conditions, is attempted in this chapter.

According to Adams, Denny-Brown and Pearson (1954), the differences between "the pigmented and pale muscle fibres" in a mixed type of muscle are no longer? more evident at the end of two months after denervation. All muscle fibres contain some fat granules and pigment as in the foetus. Knoll and Hauer (1892) showed that the great differences between the " pale large" and the "dark small" fibres vanish by the thirty-fifth day after denervation. The present work deals with the structural changes in the two types of fibres in disuse atrophy.

Material and Methods

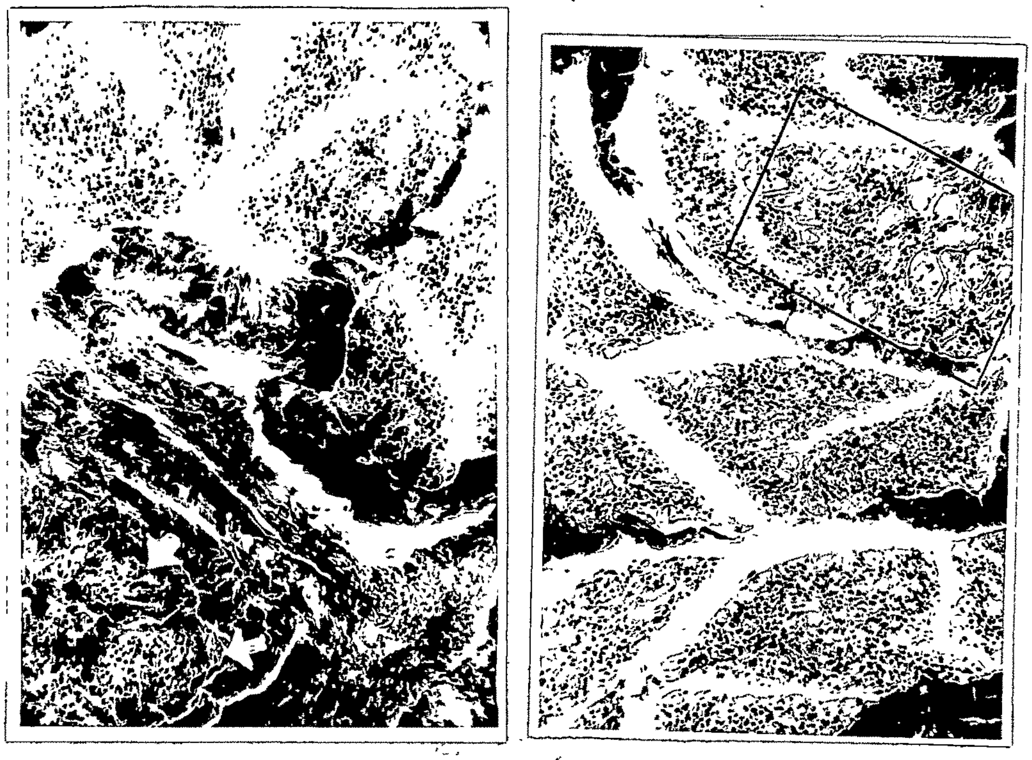
Of the two pigeons utilised in the present work one (pigeon no.1) was kept along with a female in a rectangular cage (18" X 24" X 18") for about two years. Due to the small size of the cage, the birds could only move about on the floor and flight was impossible. The only possible movement of the wings was (that for) the adjustment of feathers and a few flaps in the morning and occasionally at other times of the day, apparently for 'warming up'. During the entire period of captivity, they were fed regularly with their normal diet, were normal in every respect, and ~~they~~ even reared ~~up~~ several broods of young ~~ones~~. At the end of two years of captivity, the male was sacrificed and the structure of its pectoralis studied from sections (t.s. as well as l.s.) of fresh frozen muscle ~~pieces~~ and of those fixed in Zenker-Formol and embedded in paraffin. Fresh frozen sections were studied unstained, whereas the paraffin sections were stained with Ehrlich's Haematoxylin and Van Gieson's Picro-Fuschin.

Another male pigeon (pigeon no.2) had its right shoulder joint fixed in a plaster cast, which restricted to a very great extent the movement of the humerus at that joint, though a few extremely slight movements were still possible. Moreover, since the plaster cast was put all around the joint in the form of a broad circular ring, the wing could not be flexed completely, with the result that, the upper arm remained in a slightly extended position. The pigeon was kept in this manner in a large cage along with some ~~other~~ normal pigeons, on a normal diet. ~~In the~~ begining, it had some difficulties in balancing itself for a few days, but soon got accustomed to it. It was observed to flap the left wing, with ease, quite often for balancing while running about in the cage and in movements of courtship, offence and defence. At the end of three months the animal was decapitated and the pectoralis major of both sides were cut out. The left pectoralis was used as control and the muscle pieces cut out from this muscle (left pectoralis) were studied in the same manner as those from the right pectoralis. For immediate inspection fresh frozen sections were used, whereas frozen sections of muscle pieces, from different regions of the muscle, fixed in Baker's Calcium-Formol and embedded in gelatin (Clark, 1947), were stained, with Ehrlich's Haematoxylin and Eosin, Sudan Black B and Eosin and Oil Red O and Haematoxylin.

Observations

In the normal pigeon pectoralis, the red and white fibres, due to their sharp and clear cut differences and definite pattern of arrangement, could be easily distinguished in stained or unstained histological preparations of fixed or unfixed muscle. Throughout the present work the sections of the normal pectoralis were used as an useful guide.

In ~~the~~ pigeon no. 1 the pectoralis muscle of each side was extremely reduced in size and paler in colour and likewise, in fresh frozen sections, the narrow fibres appeared paler and less granulated compared to those in normal pigeons, but the granulation in the white fibres, just as in normal ones was negligible or nil.



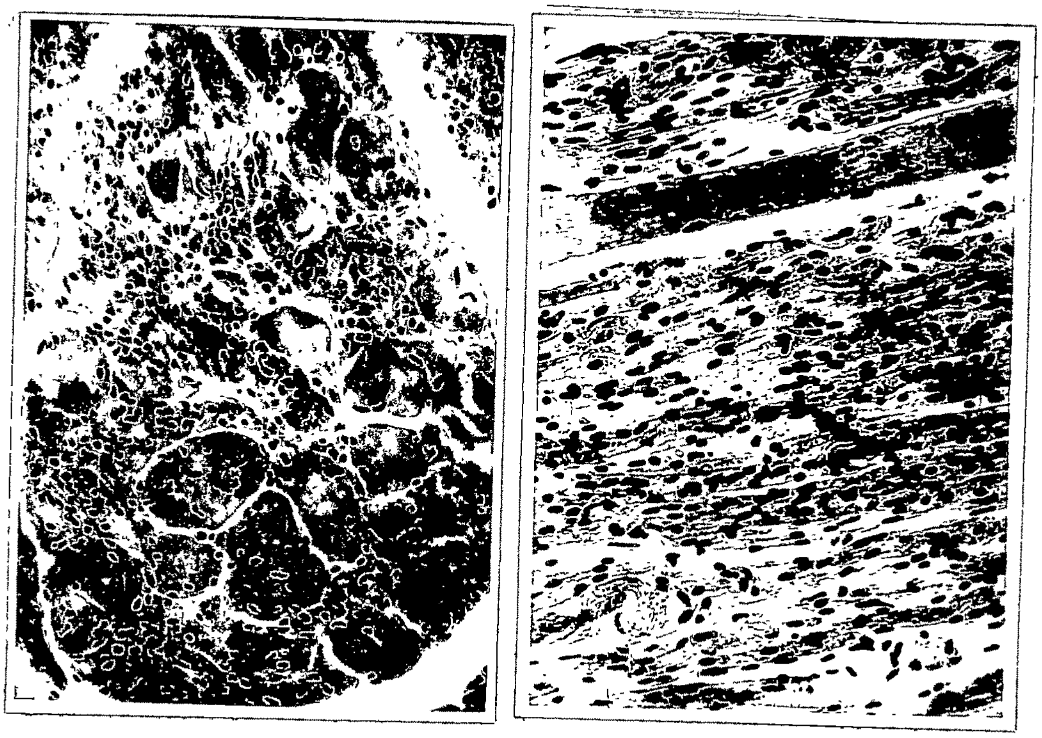
Figs. 3.1 & 3.2. T.S. of the pectoralis of the pigeon no.1 (Two arrows in fig. 3.1. indicate clumps of nuclei of degenerated muscle fibres, surrounded by dense aggregate of connctive tissue) x 180

Figures 3.1 to 3.5 present the structure of the pectoralis of pigeon no.1, showing the signs of atrophy. Both types of fibres were affected. All the red fibres were extremely reduced in diameter. Among the white fibres a few ~~fibres~~ here and there were reduced but little, ~~while~~ but the majority ~~of them~~ showed (varying some extent of) reduction in size, with the

Fig. 3.3 L.S. of the pectoralis of ~~the~~ pigeon no. 1 stained with Haematoxylin - Van Gieson x 180

result that in any preparation, white fibres of all sizes from broadest to narrowest could be found. The striations were clear in all the fibres however reduced and none of the fibres showed any sign of segmentation.

The nuclei stood out prominently in all the preparations and were oval or rounded in shape (fig.3.5). On observing under oil-immersion the nuclei appeared granulated and with prominent nucleolus. Some elongated nuclei, beaded at two ends with an intervening narrow portion, gave the indication of an amitotic nuclear division. But from the present work it is difficult to say whether there was any definite increase in the number of nuclei or not. The red fibres appeared as tubes filled with nuclei. In the white fibres some nuclei were seen away from the periphery, while others were at their original position (figs. 3.4 and 3.5).



Figs. 3.4 and 3.5 Enlarged microphotographs of the portion marked in figures 3.2 and 3.3 respectively. Haematoxylin-VanGieson x430

A definite increase in the amount of connective tissue in the perimysium was seen. In certain regions thick bands of connective tissue extended in between the fasciculi (figs. 3.1 and 3.2). In figure 3.1, towards the left at the bottom, two large clumps of nuclei (indicated by arrows) resembling those of muscle fibres, are seen in the midst of a thick aggregate of connective tissue, suggesting the possible replacement of muscular tissue by connective tissue. Large aggregates of fat cells, as are usually associated with the degenerative changes in fibres of denervated muscle were however not observed in the muscle.

In ~~the~~ pigeon no.2, the right pectoralis was somewhat more reduced in size than the left one; which was used as control. Histological observations showed that throughout the latter muscle the fibres were normal in every respect. On the other hand, white fibres in the right pectoralis showed signs of atrophy which at different depths of the muscle varied in magnitude. Figure 3.6 shows portions of two fasciculi from the region proximal to the ventral face of the muscle, where the effect of atrophy was very slight. Both types of fibres appeared similar to those in the control, except that in many of the broad fibres some of the nuclei had migrated away from the periphery.

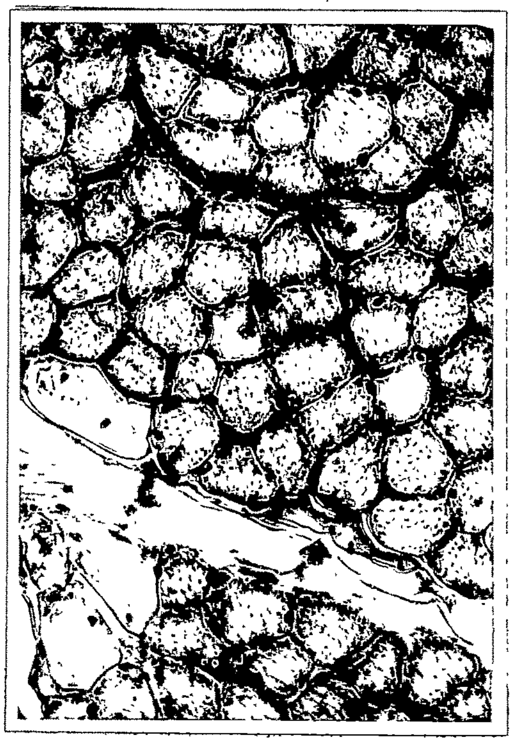


Fig.3.6 T.S.passing through the portion of the muscle proximal to the ventral face. Ehrlich Haematoxylin - Eosin. x430.
Fig.3.7 T.S.passing through the deeper region — at about mid-depth of the muscle. Ehrlich's Haematoxylin - Eosin. (of the four white fibres seen in the fig.3.7, the one in advanced state of atrophy is shown by arrow). x430

Figure 3.7 represents a typical portion in the deeper layer — at about mid-depth of the muscle. All broad fibres show varying reduction in size. Three broad fibres at the bottom of the figure are smaller in diameter (compared than to) those in figure 3.6, while in the upper part of the figure a white fibre can be seen very much reduced in size and flattened out against the border of the fasciculus. Migration of nuclei away from the periphery of white fibres was a prominent feature in this region. Size of the red fibres was unaffected and, except for an occasional fibre, most of them had nuclei in the normal position.

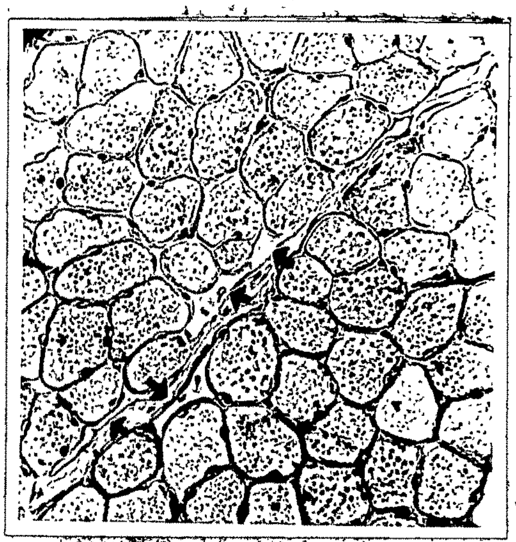


Fig. 3.8 T.S.passing through the portion of the muscle proximal to the dorsal face of the muscle. Ehrlich's Haematoxylin - Oil Red O. (white fibres are indicated by arrows) x 430

Figure 3.8 presents the picture of a still deeper layer of muscle. Here all the white fibres show great reduction in size. Those on the periphery of the fasciculi (indicated by arrows) can be seen (in the form of as ?) flattened out ribbons, while those in the middle of the fasciculi (not shown in the figure) were not flattened but instead deeply indented, angular and much reduced. Still the red fibres showed, almost no sign of atrophy — they were still normal in size and in the position of their nuclei. In this region as well as in another still proximal to the dorsal face of the muscle, at several places white fibres had disappeared altogether, leaving little or no remnant of the muscle fibre with the result that the borders of the fasciculi were broken up and gaps formed in the regions where the white fibres degenerated. Whether the fibres were replaced by connective tissue or not, however, could not be ascertained.

Figure 3.6 clearly shows the differences in the structural details of the two types of fibres as in the normal muscle. In the red narrow fibres, the Cohnheim's areas were very prominent and irregular, whereas in the white fibres they were less conspicuous and formed a regular pattern. Almost the same differences in the structure of the two types of fibres remained at any depth of the muscle even where the white fibres were in an advanced state of atrophy.

As observed in the previous chapter, the red fibres of the pigeon breast muscle, have greater affinity for Sudan dyes, unlike the white fibres. In the present investigation, the red fibres towards the deeper layers of the right pectoralis of pigeon no.2 showed more fatty inclusions, the lipoid globules being more numerous, than those in the superficial prominent?

layers or in the normal muscle. Figure 3.8 shows lipoid inclusions in the red fibres, prominently, whereas in the white fibres, even in the advanced state of degeneration, no such inclusions are seen.

Fig. 3.9 T.S. passing through the deeper region of the muscle. Sudan Black B - Eosin (white fibres indicated by arrows) x430
3.10 L.S. passing through the deeper region of the muscle showing fat deposition around branches of a blood vessel. Haematoxylin - Eosin. x180

Figure 3.9, which also is of the deeper layer of the muscle, shows the relative affinity of the two types of fibres distinctly. All along the bottom and left margin of the figure aggregates of fat cells are seen. Between intensely stained fat cells and the darkly stained red fibres are three flattened white fibres (indicated by arrows) with almost no deposition of Sudan Black B. It should however, be noted, that the higher deposition of fat is mainly in the 'intercolumnar' cytoplasm of the red fibres and that this deposition should not be confused with the replacement of the muscular tissue by fat cells, as it usually happens in certain degenerative processes of the muscular tissue. Nowhere in the present work the replacement of muscle fibres by fat cells was observed. No doubt in the deeper layers of the muscle, large aggregates of fat cells were present in the perimysium (fig. 3.9) and isolated fat cells were present in the endomysium (visible near the upper border of fig. 3.9). Wherever the large aggregates of fat cells were present they were mainly in the vicinity of large blood vessels. Figure 3.10 shows such deposition around several branches of a blood vessel.

Discussion

Knoll and Hauer (1892) reported that the great differences between the 'pale large' and 'dark small' fibres of the pigeon after denervation, no longer existed. Present observations show that in disuse atrophy the main differences, mainly those ~~that~~ of colour and lipoid inclusions, are well maintained.

In ~~the~~ pigeon no. 1, where the animal was caged, in a limited space for a long period, inactivity produced atrophy in both ~~the~~ types of fibres in the same manner. Since the white fibres were originally broader than the narrow ones at all ~~the~~ stages in the process of atrophy, they should be greater ~~bigger~~ in diameter than the red fibres (which ? too should reduce in size in a like manner.

On the other hand in ~~the~~ pigeon no.2, where the activity of the pectoralis was limited due to control of movements at the shoulder joint, changes due to atrophy were much more profound in the white fibres than in the red ones, which seem to be less suceptible to atrophy. The white fibres proximal to the ventral face of the muscle were comparatively less affected than those of the deeper portion of the muscle. This suggests that under the present experimental conditions, the activity of the muscle in the deeper region has been more restricted than in the superficial part (proximal to the ventral face) of the muscle. This experiment, doubtless, reveals that the white fibres are more suceptible to atrophy than the red ones, but ~~however~~ no definite reason for it can be given because of the lack of data from similar experiments under altered conditions. The mode of action of some factors involved in the conditions of the present experiment also need to be checked. For instance, the movement at the shoulder joint was totally eliminated and since the plaster cast caused ~~rendered~~ the upper arm to be in a slightly abducted position, the muscle was obliged to remain slightly stretched and not in its usual resting position. The present findings nevertheless clearly indicate the possibility of existence of some basic difference, still unknown, possibly in the physico-chemical properties of the two types of fibres in the muscle.

Summary

1. Observations on the effect of disuse atrophy on

the *pectoralis major* muscle of the pigeon is reported.

2. The *pectoralis major* of a pigeon kept captive captivated in a small cage for two years, showed the effect of atrophy on both the red, narrow and white, broad types of fibres. Another pigeon in which muscular atrophy was produced by fixing a plaster cast on the shoulder joint, showed at the end of three months high degree of atrophy in the white broad fibres whereas the red narrow ones remained unaffected.