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# Fibre number and fibre size in a surgically overloaded muscle

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## INTRODUCTION

It is well known that striated muscle is capable of gaining weight in response to an increased work load in the form of exercise, and it is generally accepted that this hypertrophy results from enlargement of the constituent fibres rather than increase in their number (Morpurgo, 1897; Siebert, 1928; Eliot, Wigginton & Corbin, 1943; Goldspink, 1964; Walker, 1966; Gordon, 1967). Overloading a muscle by surgical incapacitation of its synergists has also been shown to increase muscle weight (Crawford, 1961; Hamosh, Lesch, Baron & Kaufman, 1967; Lesch *et al.* 1968; Mackova & Hnik, 1971, 1972, 1973) and to result in hypertrophy of the muscle fibres (Denny-Brown, 1960; Goldberg, 1967; Rowe & Goldspink, 1968; Gutmann, Schiaffino & Hanzlikova, 1971; James, 1973, 1976). This apparent similarity between the hypertrophy resulting from increased muscular work through exercise and the hypertrophy resulting from 'surgical overloading' of a muscle has led to the latter procedure being used experimentally to simulate the effects of exercise on muscle (Hamosh *et al.* 1967; Goldberg, 1967, 1968, 1969; Lesch *et al.* 1968; Binkhorst, 1969; Jablecki & Kaufman, 1973).

However, it has been reported that the hypertrophy of surgically overloaded muscles is sometimes accompanied by an increase in fibre number (Rowe & Goldspink, 1968; Sola, Christensen & Martin, 1973; Yellin, 1974). James (1976), on the other hand, states that the number of fibres decreases. There are several reports that severe overloading of this kind induces longitudinal fibre 'splitting' (Linge, 1962; Rowe & Goldspink, 1968; Reitsma, 1970; Hall-Craggs, 1970; Hall-Craggs & Lawrence, 1970; Sola *et al.* 1973; Yellin, 1974). Split (i.e. bifurcating or trifurcating) fibres have rarely been observed in normal or physiologically exercised muscles; Edgerton (1970), however, has seen them. Certainly there seems to be general agreement that some rather small fibres are present in cross sections of surgically overloaded muscles which are not represented in control muscles. A small fibre is often found within the same endomysial sheath as a larger fibre, as one would expect if the fibre had bifurcated unequally at a higher level. However, James (1973, 1976) has suggested that these small fibres arise independently from satellite cells rather than from splitting of existing fibres.

These conflicting opinions about fibre splitting, and its influence on fibre numbers counted in muscles overloaded by surgical means, require clarification. In the present work, the soleus of the mouse was overloaded by tenotomy of its synergists (plantaris and gastrocnemius) and examined histologically after 7, 55 and 208 days. The first post-operative interval of 7 days was chosen because the maximal weight gain in an overloaded muscle has been reported to occur during the first week after operation

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(Goldberg, 1967; Lesch *et al.* 1968; Mackova & Hnik, 1971, 1973). James (1976) states that there are no characteristic histological changes at this time, but Williams & Goldspink (1973) found an increase in muscle fibre length (resulting from an increase in the number of serial sarcomeres) in overloaded soleus muscles after one week. The second interval of 55 days was chosen because a preliminary experiment indicated increases in fibre diameters and the occurrence of split fibres at this time. The third interval of 208 days was chosen to determine whether or not the changes were permanent. James (1976) found that overloaded muscle fibres regained their normal histological appearance after 80 post-operative days, and Hall-Craggs (1970) observed a reduction in the number of split fibres after 80–120 days, but as neither author counted the total number of fibres directly, it was not possible for them to tell whether any new fibres had established themselves permanently in the muscle.

The soleus of the mouse is particularly suitable for a quantitative histological study of this nature. It is fusiform, with a long belly through which all the fibres pass; the fibres run from tendon to tendon and do not terminate or interdigitate in the muscle belly (Rowe, 1967). As the soleus normally has fewer than 1000 fibres, total fibre counts may be made quite readily from transverse sections. Counts were made at different levels in the muscle belly to ascertain whether or not total fibre numbers were affected by the operation. It also seemed that the nature of the small additional fibres might be cleared up by examining whole fibres teased from overloaded muscles, since under these conditions splitting and possible recombination should be directly observable. Some material was also prepared for electron microscopy.

## MATERIALS AND METHODS

### *Animals*

Male and female mice of the stock HUL TO were used. Operations were performed on 21 days old weanlings as the effects of overloading are said to be greatest in young animals (Mackova & Hnik, 1972). Six experimental and six control animals of each sex were killed after 7, 55 and 208 post-operative days and their soleus muscles weighed and processed for determination of total fibre number and fibre diameters. Some additional animals provided material for electron microscopy and for making teased preparations.

### *Surgical techniques*

The animals were anaesthetised with intraperitoneal sodium pentobarbitone (Pilgrim & DeOme, 1955) and operations were performed on both hind limbs of the experimental animals. The distal portion of the plantaris and its tendon of insertion were removed, and the tendon of insertion of the gastrocnemius was severed just proximal to its junction with the tendon of the soleus. The distal portion of the gastrocnemius was then removed. A sham operation was performed on both hind limbs of the control animals by making a skin incision and starting to separate the muscles, but without cutting them or their tendons.

### *Fixation of muscles and histology*

Glutaraldehyde fixation (2·5% glutaraldehyde in 0·1 M phosphate buffer at pH 7·3) was chosen as it tends to separate muscle fibres seen in transverse section and therefore facilitates identification of small fibres closely associated with larger fibres. The soleus muscles of the right hind limbs were dissected and weighed,

leaving the contralateral muscles for histological examination. The left hind limbs were cleared of skin and excess connective tissue and the soleus muscles exposed by removing the whole gastrocnemius in the experimental animals. The limbs were severed just above the knee and pinned on to pieces of cork with the foot fully dorsiflexed to extend the soleus. In this position the tendons of origin and insertion are separated as far as possible and a long belly is provided for sectioning. The limbs were immersed in fixative for 2 hours after which the soleus muscles were dissected free and returned to the fixative for a further 2 hours.

After fixation the muscles were washed in 0·1 M phosphate buffer at pH 7·3, dehydrated in a series of graded ethanols, cleared in cedarwood oil and embedded in Paraplast (Sherwood Medical Industries). Transverse sections 7 $\mu$m thick were cut serially along the whole length of the belly of the experimental muscles, but only a few sections from the mid-belly were cut from the control muscles. The sections were stained with Mallory's trichrome stain.

*Counting of fibres*

All the fibres in selected transverse sections of the muscles were counted. The sections were projected at a final magnification of about × 220 onto a screen, using a microprojector. As fibre numbers had been shown in a preliminary experiment to differ at the proximal and distal ends of the belly of the experimental muscles, one section from each of these regions was selected for study. The section from the proximal belly region, with the smaller number of fibres, was chosen just distal to the last traces of the origin tendon. The distal belly section was taken where the muscle belly had the largest number of fibres, not at the extreme distal end of the belly because, in overloaded muscle, it is difficult to distinguish between the new connective tissue and the tendon of insertion. In the control muscles it was sufficient to count the number of fibres in one section from the mid-belly region because the number of fibres in the belly region of the normal mouse soleus hardly varies (Rowe, 1967).

*Measurement of fibre diameters*

Fibre diameter measurements were obtained from the same sections as those used for counting fibres. Sections were examined under a microscope whose eyepiece contained graduated cross-hairs. The muscle was scanned from the deeper surface to the outer surface a number of times, and two estimates of the diameter of each fibre touched by the centre of the cross-hairs were made, the average value being recorded. The sample size was 100 fibres in each case.

*Preparation of single fibres (method after Williams & Goldspink, 1971)*

Some muscles were fixed as previously described, transferred to 20% (w/w) nitric acid for 3 days to hydrolyse connective tissue, then stored for a few days in 50% glycerol to soften the fibres. With the help of a dissecting microscope and some fine needles the muscle was teased and its elements mounted in glycerine jelly. These elements were examined microscopically for signs of splitting, recombination and degeneration of fibres.

*Electron microscopy*

Some experimental muscles were fixed with glutaraldehyde as previously described, but with the addition of 0·5% glucose to improve the preservation of the fibre

ultrastructure. After fixation the distal belly regions of the muscles were cut into small pieces and washed overnight at 4 °C in 0·1 M phosphate buffer at pH 7·3. The muscle pieces were post-fixed in Palade's osmium tetroxide fixative for 2 hours before being dehydrated in a graded series of alcohols. After further dehydration in 100% acetone the tissue pieces were embedded in Araldite (Ciba) and sectioned. Sections with silver interference colours were mounted on celloidin-coated copper grids and double stained with uranyl acetate and lead citrate. Sections were examined and photographed with a JEOL JEM 7A electron microscope.

*Statistical analysis*

Each post-operative time period was treated as a separate experiment for statistical purposes. Two-factor analysis of variance (anovar) was used except where otherwise stated. The anovar was sometimes followed by the test of least significant difference. The source for statistical procedures was Sokal & Rohlf (1969). Differences between sets were accepted as significant when the probability of obtaining such a result by chance was 5% or less.

## RESULTS

*Muscle weights* (*Table* 1)

Considerable differences were recorded between control and experimental muscle weights. At each post-operative time period the overloaded muscles were significantly heavier than controls; in some groups the mean muscle weight was increased by about 80%. Differences between the sexes were not significant.

*Muscle fibre number* (*Table* 1)

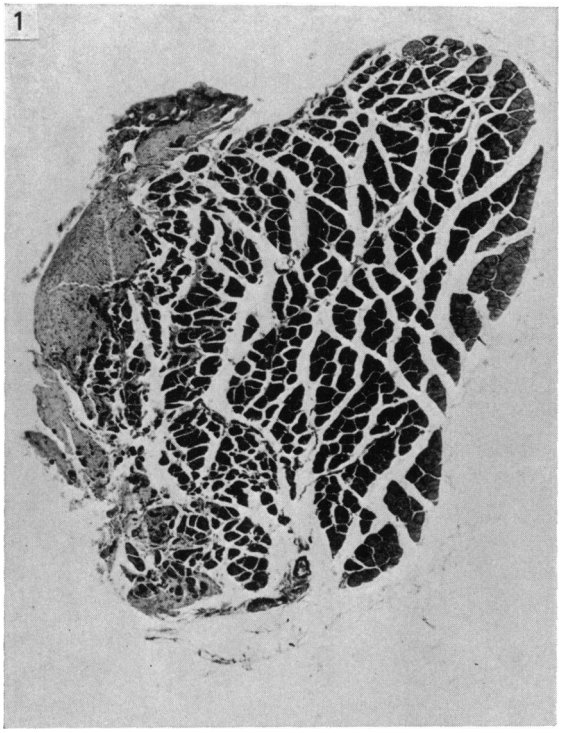
Total fibre number counts are shown in the Table; in experimental muscles, counts for the distal and proximal regions of the muscle belly are given. At the 7 days stage the differences in numbers between control and experimental groups were insignificant. However, the small increase in fibre number at the distal end of the experimental muscle bellies as compared with the proximal end was significant (paired *t*-test). At 55 and 208 days relative to normal control muscle, there was a marked increase in the number of fibres in the distal region of the overloaded muscles, but no significant difference in the number of fibres in the proximal region. At 55 days the females had significantly more fibres than the males, but such a sex difference was not found at the other post-operative times. Transverse sections from the three muscle regions after 55 days are shown in Figures 1–3.

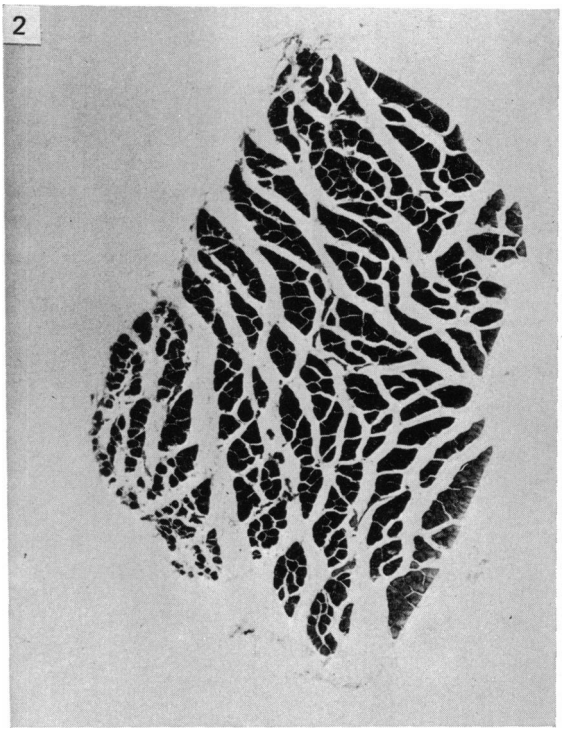
*Muscle fibre diameters* (*Table* 1)

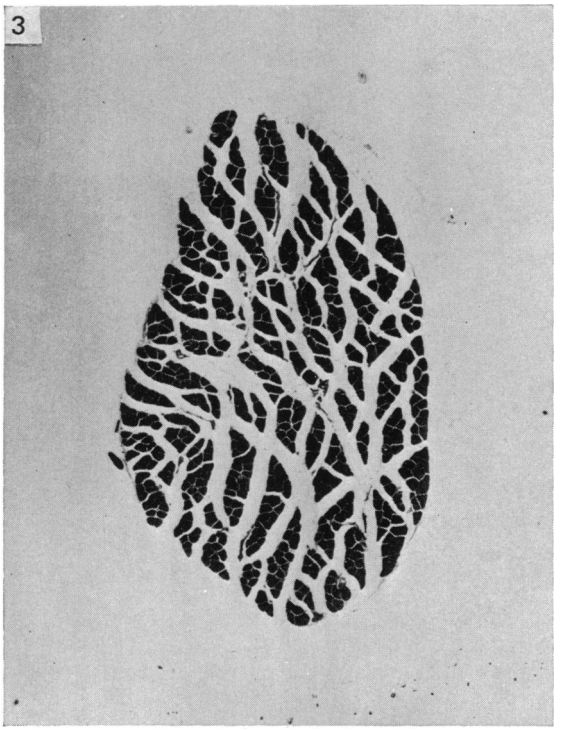
The data sets of 100 fibre diameter readings from each muscle had widely differing variances; distal experimental samples had the highest variances, and control samples the lowest. All the data groups could not, therefore, be analysed together using anovar, as the conditions for parametric statistics would have been violated. The following procedures were therefore adopted, since a suitable 2-factor non-parametric test is not available. Data sets from the same muscle regions of males and females had similar variances, so sex effects were examined using 1-factor nested anovar. These effects were found to be insignificant in all cases. Mean fibre diameters were calculated, and the effects of the treatment on the muscle regions were compared, using the Kruskal–Wallis test. After 7 days, overloading had not affected mean fibre diameters. After 55 days, fibre diameters had increased significantly in the

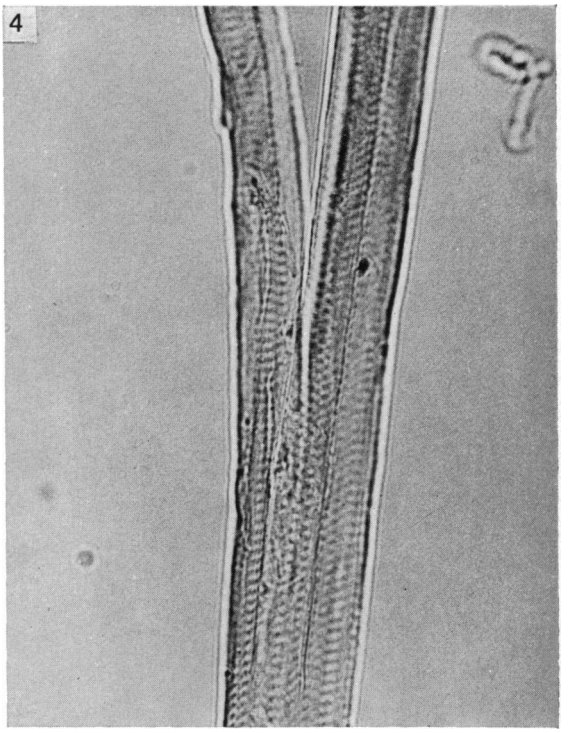
Table 1. *Effects of surgical overloading on total fibre number and fibre diameter in the m. soleus*

| | Control animals | | | | Experimental animals | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Distal muscle region | | Proximal muscle region | |
| Group | Body wt. (g) | Muscle wt. (mg) | Fibre number | Mean fibre diam. (μm) | Body wt. (g) | Muscle wt. (mg) | Fibre number | Mean fibre diam. (μm) | Fibre number | Mean fibre diam. (μm) |
| | | | | | 7 post-operative days | | | | | |
| Males | 19·0 | 5·3 | 758 | 14·9 | 22·0 | 5·1 | 655 | 14·8 | 686 | 15·4 |
| | 21·0 | 6·2 | 610 | 17·2 | 20·0 | 8·9 | 860 | 15·6 | 793 | 15·6 |
| | 21·0 | 5·0 | 792 | 15·0 | 21·0 | 8·5 | 713 | 14·4 | 660 | 14·3 |
| | 23·0 | 6·0 | 848 | 17·4 | 22·0 | 6·3 | 808 | 15·6 | 1743 | 16·7 |
| | 24·0 | 5·5 | 779 | 16·5 | 25·5 | 8·0 | 658 | 15·7 | 635 | 17·5 |
| | 25·0 | 6·5 | 959 | 16·2 | 15·5 | 5·3 | 842 | 14·8 | 779 | 14·8 |
| Group mean | 22·0 | 5·8 | 791 | 16·2 | 21·0 | 7·0 | 756 | 15·1 | 716 | 15·7 |
| Females | 19·0 | 5·3 | 783 | 15·4 | 19·0 | 8·5 | 781 | 15·0 | 690 | 16·5 |
| | 19·0 | 4·6 | 767 | 15·1 | 20·0 | 10·9 | 848 | 16·9 | 774 | 18·3 |
| | 19·5 | 5·7 | 833 | 15·7 | 20·0 | 10·7 | 685 | 14·8 | 618 | 15·5 |
| | 20·0 | 5·2 | 876 | 15·5 | 20·5 | 8·2 | 874 | 15·1 | 701 | 15·8 |
| | 21·0 | 4·3 | 773 | 15·8 | 21·0 | 7·7 | 715 | 14·3 | 655 | 16·5 |
| | 22·0 | 4·7 | 804 | 15·8 | 22·5 | 8·3 | 903 | 15·7 | 758 | 15·1 |
| Group mean | 20·0 | 5·0 | 806 | 15·6 | 20·5 | 9·1 | 801 | 15·3 | 699 | 16·3 |
| | | | | | 55 post-operative days | | | | | |
| Males | 29·5 | 6·4 | 662 | 20·8 | 38·0 | 12·7 | 928 | 22·2 | 775 | 24·5 |
| | 35·5 | 7·1 | 789 | 19·3 | 36·5 | 12·1 | 1105 | 23·9 | 616 | 24·9 |
| | 33·0 | 9·0 | 688 | 23·0 | 37·5 | 16·3 | 1264 | 24·7 | 880 | 28·0 |
| | 35·0 | 8·4 | 667 | 20·7 | 35·0 | 13·5 | 1037 | 23·0 | 764 | 25·8 |
| | 36·0 | 7·0 | 719 | 22·7 | 34·5 | 11·9 | 949 | 20·7 | 709 | 25·6 |
| | 33·5 | 7·2 | 695 | 20·7 | 36·0 | 12·4 | 961 | 25·9 | 771 | 26·0 |
| Group mean | 34·0 | 7·5 | 703 | 21·2 | 36·5 | 13·2 | 1041 | 23·4 | 753 | 25·8 |
| Females | 35·5 | 8·5 | 735 | 22·5 | 27·5 | 13·0 | 1186 | 23·2 | 873 | 28·0 |
| | 33·0 | 9·8 | 764 | 22·1 | 30·0 | 14·6 | 1089 | 22·9 | 889 | 25·1 |
| | 33·0 | 8·4 | 908 | 20·2 | 32·0 | 14·7 | 1333 | 20·0 | 864 | 24·1 |
| | 30·0 | 9·1 | 862 | 21·6 | 30·0 | 11·4 | 1153 | 23·0 | 697 | 25·7 |
| | 29·0 | 8·8 | 892 | 21·2 | 30·5 | 13·9 | 1060 | 23·8 | 789 | 26·8 |
| | 30·0 | 7·5 | 648 | 21·9 | 31·5 | 10·8 | 926 | 23·6 | 767 | 25·7 |
| Group mean | 32·0 | 8·7 | 802 | 21·6 | 30·5 | 13·1 | 1125 | 22·7 | 813 | 25·9 |
| | | | | | 208 post-operative days | | | | | |
| Males | 57·0 | 13·4 | 826 | 21·4 | 44·0 | 16·1 | 956 | 19·2 | 826 | 22·1 |
| | 49·0 | 11·8 | 988 | 21·1 | 47·0 | 17·8 | 1274 | 19·6 | 948 | 23·4 |
| | 46·0 | 11·5 | 780 | 19·9 | 50·0 | 17·0 | 837 | 21·2 | 782 | 23·6 |
| | 46·0 | 9·9 | 846 | 20·3 | 51·0 | 10·8 | 995 | 19·6 | 835 | 22·7 |
| | 45·0 | 8·9 | 693 | 20·6 | 44·0 | 15·1 | 1373 | 18·1 | 1077 | 20·6 |
| | 44·0 | 10·6 | 793 | 20·7 | 51·0 | 19·5 | 1112 | 20·7 | 925 | 23·6 |
| Group mean | 48·0 | 11·0 | 821 | 20·7 | 48·0 | 16·1 | 1091 | 19·7 | 899 | 22·7 |
| Females | 36·0 | 9·9 | 821 | 19·8 | 37·0 | 17·0 | 1051 | 20·4 | 862 | 23·3 |
| | 37·0 | 9·4 | 832 | 19·6 | 40·0 | 17·8 | 1052 | 19·2 | 907 | 21·6 |
| | 35·0 | 9·0 | 683 | 20·5 | 40·0 | 16·5 | 1071 | 20·6 | 861 | 23·0 |
| | 38·0 | 9·1 | 723 | 18·9 | 40·0 | 15·0 | 1168 | 18·7 | 904 | 22·0 |
| | 35·0 | 8·5 | 812 | 19·3 | 41·0 | 14·8 | 1025 | 20·6 | 935 | 21·9 |
| | 41·0 | 11·1 | 190 | 22·5 | 43·0 | 19·8 | 1048 | 21·8 | 942 | 23·7 |
| Group mean | 37·0 | 9·5 | 777 | 20·1 | 40·0 | 16·8 | 1069 | 20·2 | 902 | 22·6 |




Figs. 1–3. Transverse sections through the soleus of male mice after 55 post-operative days. × 55.

Fig. 1. Distal region of overloaded muscle belly: 1037 fibres.

Fig. 2. Proximal region of overloaded muscle belly: 764 fibres.

Fig. 3. Control muscle belly: 694 fibres.

Fig. 4. Single teased fibre from an overloaded soleus muscle after 55 post-operative days. The fibre is seen to split into two smaller fibres. × 600.

proximal experimental region relative to controls. Mean fibre diameters from the distal experimental region were intermediate between those from the two other regions (i.e. the proximal experimental and the control), but did not differ significantly from either (this was no doubt due to the increase in the number of small fibres in the distal region, which of necessity lowers the mean diameter). After 208 days the largest mean fibre diameters were again found in the proximal experimental region: these were significantly larger than in the distal experimental region (both sexes) and in controls (females only). Mean fibre diameter measurements from the control and distal experimental regions did not differ significantly.

*Observations on teased fibres*

Microscopic examination of fibres from muscles overloaded for 7 days did not reveal any longitudinal splitting. There was some evidence of damage, for fibres tended to taper towards their distal ends, and striations were indistinct. Fibres from muscles overloaded for 55 and 208 days, however, were frequently split distally into two or more smaller fibres (Fig. 4); and recombination was sometimes seen. There was little evidence of degenerative changes in these fibres. Splits were never observed in control fibres.

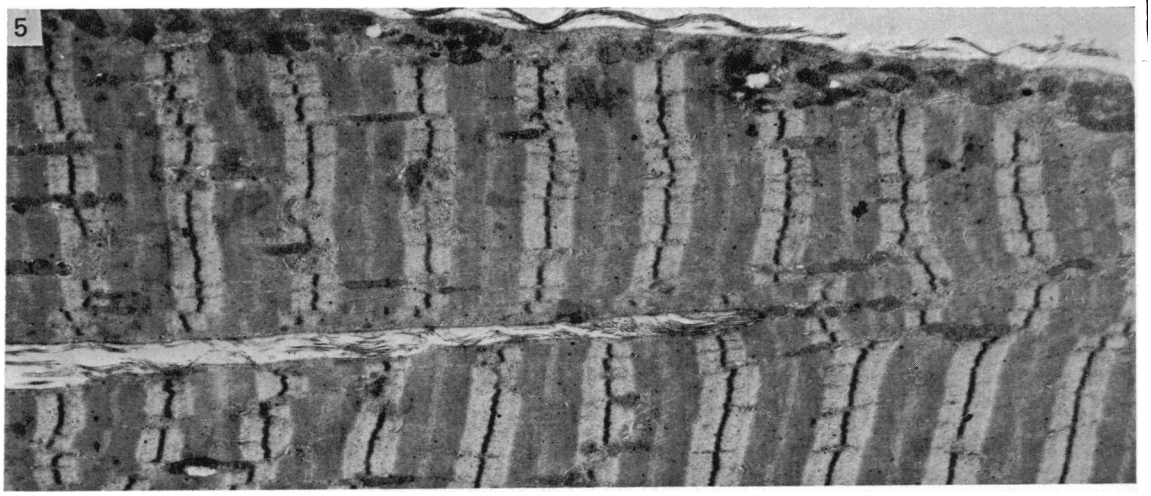
*Electron micrographs*

The electron micrographs (Figs. 5–8) were obtained from material taken from the distal belly region of overloaded soleus muscles of male mice after 55 post-operative days. Figure 5 shows part of a bifurcated muscle fibre with young connective tissue in the fork of the split. Figure 6 shows what appears to be a very short split within a fibre, the Z-discs locally being out of register. Figure 7 shows a split with intervening connective tissue. The discontinuity of the pattern of Z-discs (arrowed) indicates sarcomeres out of register. Figure 8 shows a damaged Z-disc (arrowed) and some 'lines of stress', which perhaps indicate incipient splitting. Once again the sarcomeres are out of register.

## DISCUSSION

The findings presented here indicate the existence of two stages in the response of a muscle to overloading. At an early stage (7 post-operative days) the overloaded muscles become considerably heavier, but the mean diameters of the fibres do not change and there is only a small increase in the number of fibres in each muscle. At a later stage (55 and 208 post-operative days) the increase in muscle weight is slightly less pronounced, but fibre diameters are greater at the proximal end of the muscle and there is an increase in the total number of fibres counted in transverse sections at the distal end of the muscle belly; here, however, some of the fibres are of smaller diameter than normal. The response of male and female muscles is similar.

It is clear that the increase in muscle weight at the early (7 days) stage cannot be attributed to transverse hypertrophy of fibres, as in exercised muscles. It *could* be a consequence of a lengthening of the muscle; Williams & Goldspink (1973) found that the number of serial sarcomeres along fibres from overloaded soleus muscle increased considerably during the first 10 post-operative days. However there is also oedema and accumulation of fibroblasts to be considered. In any event it seems that some workers who have studied surgical overloading of muscles have been misled by the initial weight change into thinking that there must have been rapid fibre width hypertrophy.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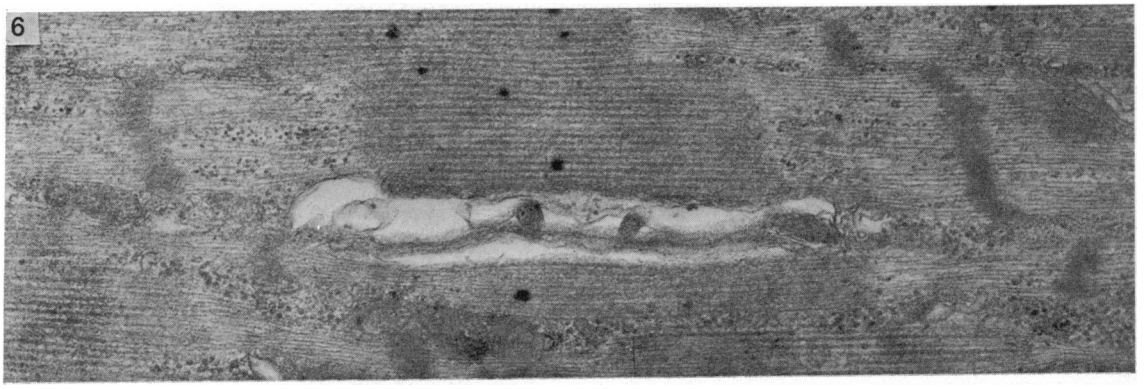
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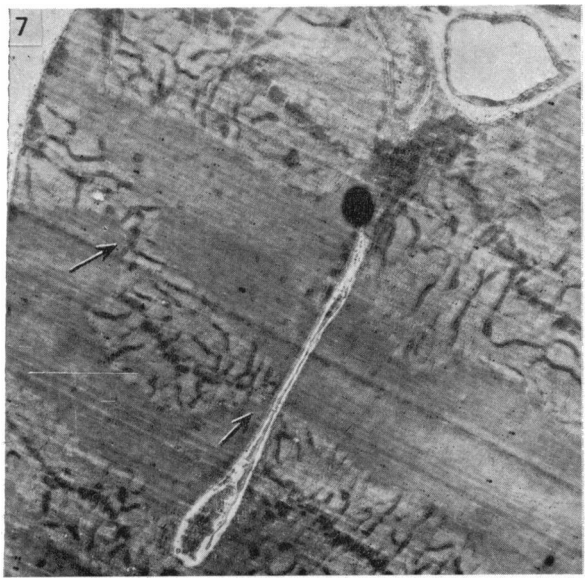
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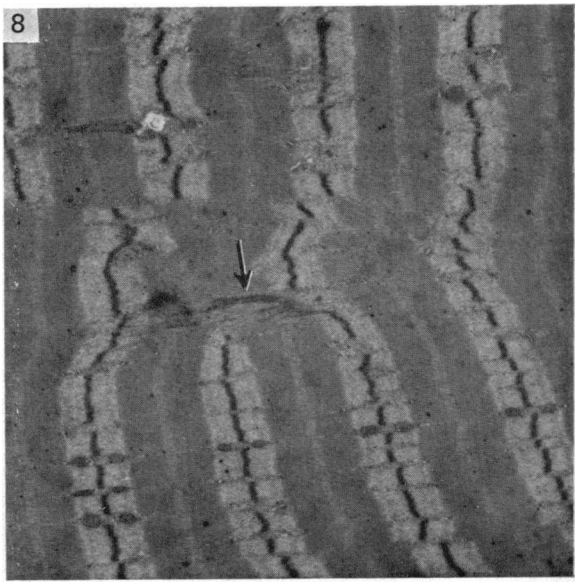
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There is no doubt that the soleus is initially stretched by its undamaged antagonists when its synergists are incapacitated; careful observation of the experimental animals as they moved around their cages revealed that their feet were more dorsiflexed than usual for the first few days following the operation.

Much later on fibre width hypertrophy does occur, presumably as a response to extra work load (cf. Mackova & Hnik, 1971, 1973). Surgical overloading might now appear to be a useful experimental method for studying fibre hypertrophy. However, the hypertrophy obtained in this way is complicated by the bifurcation of fibres distally, with the appearance of many thin fibres, and this reduces the mean fibre diameter there, confusing the issue as to the degree of fibre hypertrophy in the muscle as a whole.

The reasons for the different responses to overloading in the distal and proximal regions of the soleus are not immediately obvious. However, when the experimental muscles were dissected from the animals it was found that the residual proximal section of the gastrocnemius had 'healed' on to the soleus about half way up the belly by means of a proliferation of strong connective tissue. Possibly, therefore, this portion of the gastrocnemius was protecting the proximal section of the soleus from excessive stretch and (to an unknown extent) mitigating its work load.

The increase in fibre numbers at the distal end of the belly is consistent with the view that fibres have split. Splitting was clearly observed in teased whole fibres (Fig. 4), and the electron microscopic evidence was supportive. Moreover, the disrupted sarcomere patterns and damaged Z-discs (Figs. 5–8) suggested that there had been abnormal loading of the muscle fibres. Since muscle fibre splitting is rarely seen in normal muscles it would seem obvious that surgical overloading had induced the splitting (cf. Hall-Craggs, 1972). Moreover, the splits seem to be permanent. James (1973, 1976) is not convinced of the reality of muscle fibre splitting or the permanence of the new fibres, arguing that the appearances in transverse sections can be explained on the hypothesis that satellite structures have developed into small fibres which later either fuse with existing fibres or completely regress. Teased fibre preparations, however, indicate the reality of splitting beyond reasonable doubt.

It may be asked how and why the fibres split longitudinally. The answer may lie in the construction of the fibres. Peachey & Eisenberg (1978) have shown that the transverse tubular system in a fibre is arranged in several helices, and it is probably along these lines of relative weakness that a fibre would tend to split when overloaded. This view is supported in one of the electron micrographs (Fig. 8). Another possible explanation of the splitting is that some overloaded fibres tear across inside their epimysial sheaths at an early stage, and that the later appearances are a

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Figs. 5–8. Electron micrographs from overloaded soleus muscles after 55 post-operative days. The Z-discs, which usually lie in register in adjacent myofibrils, frequently appear to have been pulled out of line. This may be an indication of opposing forces within the muscle. The presence of connective tissue within the splits seen in the fibres indicates that endomysium is being formed between the daughter fibres.

Fig. 5. Part of a bifurcation of a fibre. × 5700.

Fig. 6. A very short longitudinal split. × 35000.

Fig. 7. A split seen in transverse section. Discontinuity of the Z-disc pattern is arrowed. × 5200.

Fig. 8. A muscle fibre with a damaged Z-disc (arrowed), and some lines of stress, suggesting regions of weakness where splitting might occur. × 7100.

consequence of end-regeneration of torn fibres, with multiple budding, as happens after many kinds of muscle injury.

In any case it cannot be inferred on present evidence that distal splitting of muscle fibres when a muscle is surgically overloaded is an adaptive phenomenon: it seems much more likely to be a consequence of physical damage. Finally, this work as a whole makes the surgically overloaded muscle a less attractive model on which to study muscle fibre hypertrophy than has been thought.

## SUMMARY

Soleus muscles of male and female mice were overloaded by surgical resection of parts of gastrocnemius and plantaris. The effects of overloading were examined histologically after 7, 55 and 208 post-operative days, and also in teased preparations.

Animals studied after 7 post-operative days showed a marked increase in muscle weight, but no significant change in mean fibre diameter or fibre number.

Animals studied after 55 and 208 post-operative days showed an increase in soleus muscle weight, with fibre hypertrophy (but no increase in fibre number) proximally, while distally there was an increase in the number of fibre profiles in cross sections, some being wider, some thinner than normal. The small diametered fibres seem to persist indefinitely. From the evidence, both direct and indirect, it was concluded that surgically overloaded fibres split longitudinally into unequal parts, and that this explains the increase in fibre profiles in distal cross sections as well as their variation in size. It is clear that, because of the splitting, a surgically overloaded muscle is a difficult model on which to study fibre hypertrophy.

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