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# EFFECT OF TRANSPLANTATION ON ENTIRE ANTERIOR TIBIALIS MUSCLE OF RABBIT, *ORYCTOLAGUS CUNICULUS**

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**Abstract:** Effect of transplantation on entire anterior tibialis muscle (ATM) has been studied in rabbits. It was observed that there was an initial phase of degeneration of almost all the original muscle fibres followed by a regeneration of new myotubes within 10 days of transplantation. The process of regeneration was slow in this muscle. All the morphometric parameters studied were decreased till the end of experiment. The DNA and RNA contents showed increase while protein contents decreased during whole period.

**Key words:** Regeneration, morphometric studies, nucleic acids, muscle DNA, muscle RNA, total proteins.

## INTRODUCTION

The transplantation of entire mammalian skeletal muscles has been accomplished in both the research laboratory (Carlson *et al.*, 1978; Sloper and Partridge, 1980; Albani and Vrbova, 1985; Martin *et al.*, 1990; Gill and Shakoori, 1995, 1996) and clinical practice (Thompson, 1971, 1974; Hakelius, 1974; Henriksson *et al.*, 1985). The degeneration and regeneration process proceeds after transplantation so that the graft regains its structural and functional characteristics (Faulkner *et al.*, 1980; Thomas *et al.*, 1984). The size of the muscle in relation to total body size of the animal is of considerable importance in terms of its ability to undergo regeneration in a transplanted condition. Many authors reported good regenerative ability of the anterior tibialis muscle (ATM) (Salafsky *et al.*, 1974; Sadeh *et al.*, 1985). The present paper describes the regenerative ability of ATM in rabbit, *Oryctolagus cuniculus*.

## MATERIALS AND METHODS

The male rabbits, *Oryctolagus cuniculus* of 1.00-1.30 kg wieght were used in this

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experiment. The animals were acclimatized for two weeks in a separate room of the Animal House of the Department of Zoology, University of the Punjab, under semi-controlled temperature conditions. The animals were provided with fresh green fodder (clover) and tap-water.

### *Transplantation*

The animals were operated upon under thiopentone sodium (50 mg/kg body weight) anaesthesia. The procedure for transplantation of ATM was same as described by Gill and Shakoori (1996). For control, both right and left legs were sham-operated and muscles were left intact. After various time intervals *viz.*, 1, 3, 5, 7, 10, 15, 30 and 60 days, the muscles were removed and processed for histological, morphometric and biochemical studies.

### *Morphological and histological studies*

For morphological studies both right and left legs were dissected, muscles were exposed, their proximal and distal connections were snipped, weighed, breadth, length and colour was noted. For histological studies, muscles were fixed, processed and sectioned at 6 $\mu$m.

### *Morphometric studies*

Following parameters were considered for morphometric studies: (*i*) total muscle area, determined by planimetry using Liesegang A 30S microprojector; (*ii*) number of muscle fibres / microscopic field and number of nuclei / muscle fibre was counted at a magnification of 500x and 1250x respectively; (*iii*) orthogonal diameter (major axis x minor axis) of muscle fibres, measured with the help of an ocular micrometer at a magnification of 500x and that of nuclei at 1250x.

### *Biochemical analysis*

For extraction of nucleic acids, the method described by Shakoori and Ahmad (1973) was adopted. The estimation of DNA and RNA was done according to Schmidt and Thannhauser procedure as described by Schneider (1957). The procedure of Lowry *et al.* (1951) was adopted for estimation of protein contents.

## RESULTS AND DISCUSSION

### *Body weight*

The weight of animal showed maximum decrease of 16% during first 15 days of transplantation.

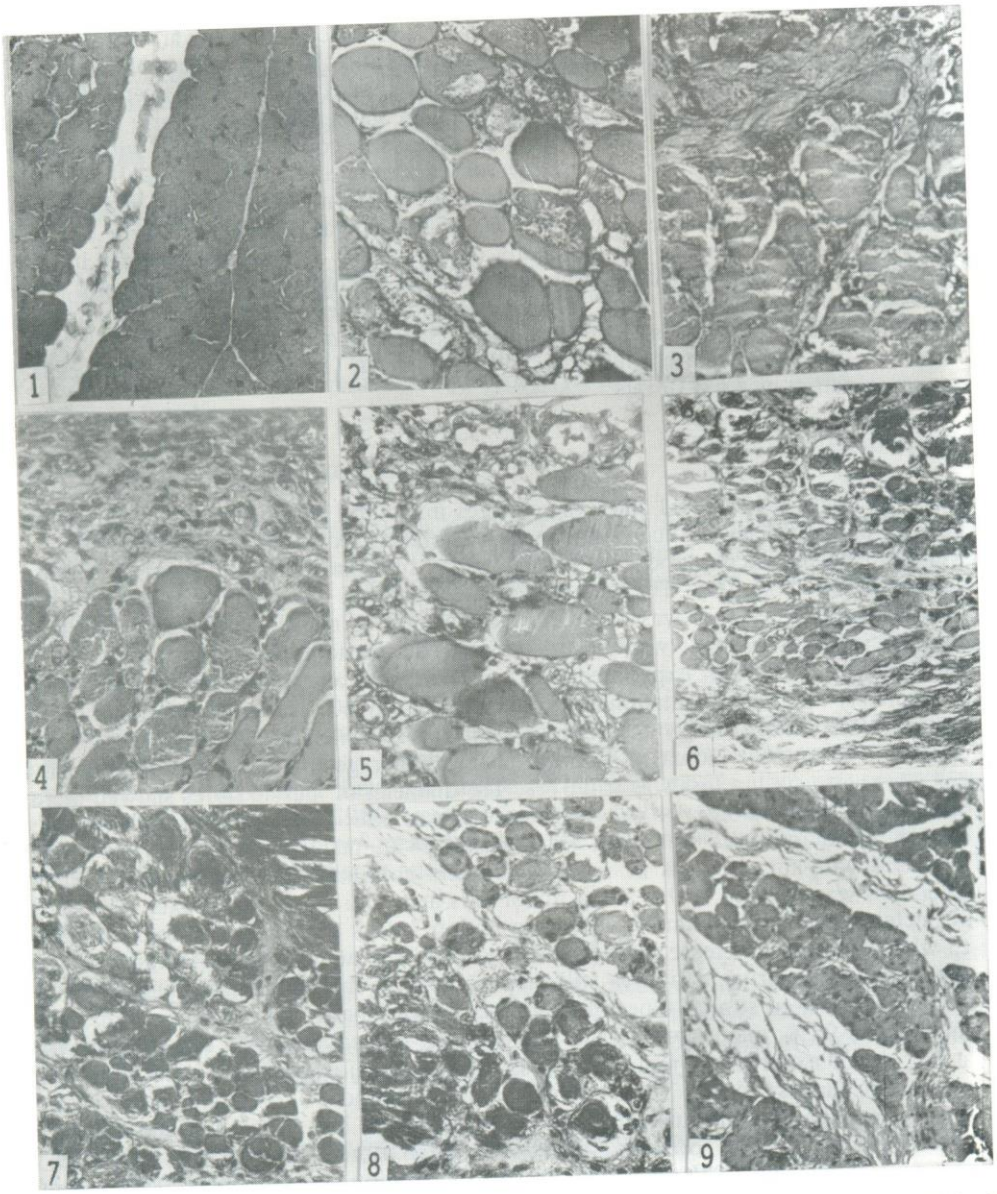
### *Morphological and histological studies*

Figures 1-9 show the sequence of various histological changes in control and

transplanted ATM of rabbits. In control muscle, muscle fibres were enclosed in muscle bundles which were compactly arranged. The nuclei were present toward the periphery. Morphologically, the wound was completely healed after 10 days of transplantation. The colour of the regenerate was pink with yellow patches. Histologically, twenty-four hour after transplantation, majority fibres hypertrophied and a few showed early degenerative changes. Many fibres were sarcolysed with increase in connective tissue within 3 days. The degenerative changes proceeded towards the centre after one week. Following this, regeneration initiated towards the periphery in the form of myoblasts and myotubes alongwith lot of connective tissue. By day 15, regenerated myotubes were basophilic with central nuclei. During the remaining days, amount of regenerated fibres increased with much reduced diameter and central nuclei. Lot of connective tissue was still present in between the regenerated fibres. The regeneration process was slow in this muscle and seems to be due to bigger size of the muscle. Sadeh *et al.* (1985) reported that muscle structure were restored after one week. Robertson *et al.* (1993) reported limited capacity for regeneration in ATM of rats.

### *Morphometric studies*

The Figures 10 and 11 show the changes in weight, dimensions, total muscle area, number and diameter of nuclei and muscle fibres in ATM after transplantation. The weight of muscle increased during first 3 days of transplantation and seems to be due to edema caused by surgery. The weight decreased within 5 days and continued till the end of experiment when loss was 48%. Just like weight of muscle, breadth of muscle also showed increase within twenty-four hours. The breadth decreased afterwards and continued till the end of experiment (28%). The length and total muscle area decreased immediately after transplantation and continued till day 60. The number of muscle fibres also decreased after one day and continued till day 10, when it was 56% less than that of control. The number increased with the progress of regeneration and continued till the end of experiment but loss was still 37%. The diameter of muscle fibres showed increase during first 3 days along two axes. It was decreased after degeneration and continued till day 15 when loss was maximum (minor axis 34%, major axis 38%). The diameter increased with the progress of regeneration and continued till the end of experiment when loss was reduced to 9% along minor axis and 20% along major axis. The number of nuclei / muscle fibre started increasing within 3 days and continued to do so till day 15 when maximum increase of 70% was recorded. The number decreased afterwards but at the end of experiment, it was still 32% more than that of control. The diameter of nuclei also showed similar types of results. It showed maximum increase within 15 days (minor axis 95%, major axis 61%). The diameter decreased during remaining days but at the end of experiment, minor axis was 51% and major axis 38% more than that of control. Changes in all these morphometric parameters correlate well with the histological studies. Gill and Shakoori (1996) observed loss in number and diameter of muscle fibres in ATM of rat. The reduced mean fibre diameter and fibre size was observed in minced ATM regenerate system (Neerunjun and Dubowitz, 1977; Salafsky *et al.*, 1974). The human muscle adaptation to physical demand occur by means of variation in fibre types, number of muscle fibres and fibre size over the muscle cross-section (Henriksson *et al.*, 1985).




**Figs.1-9:** Histological section through transplanted anterior tibialis muscle of rabbit; 1, control, 2, day 1, showing early degenerative changes; 3, day 3, note degenerated fibres with macrophage activity; 4, day 5, note replacement of muscle fibres by connective tissue; 5, day 7, showing degenerative changes without any traces of myoblasts; 6, day 10, showing early regeneration towards the periphery; 7, day 15, note regenerated fibres; 8, day 30, note regenerated fibres with mostly central nuclei; 9, day 60, note reduced diameter of regenerated fibres. Stain: Haematoxylin and Eosin; Magnification: all 100x.

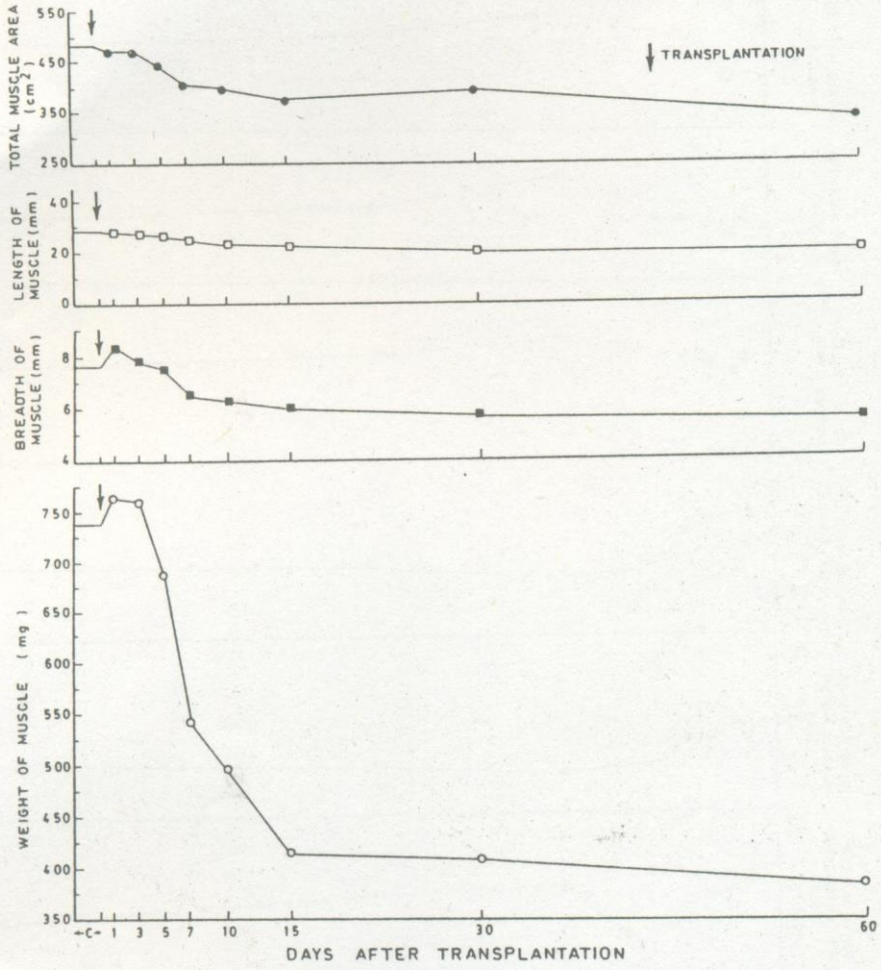



**Fig. 10:** **The weight of muscle, dimensions and total muscle area after transplantation of anterior tibialis muscle in rabbit.**

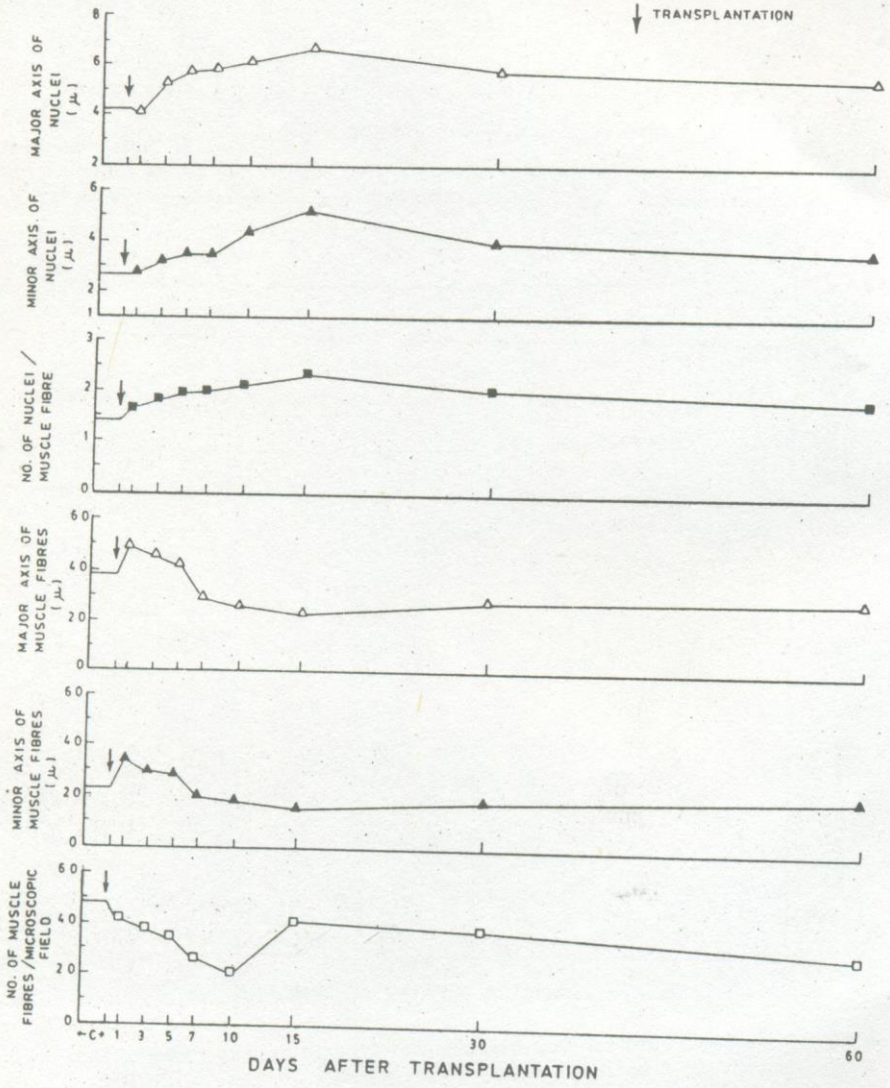



Fig. 11: **Changes in various morphometric parameters of anterior tibialis muscle of rabbit after transplantation.**

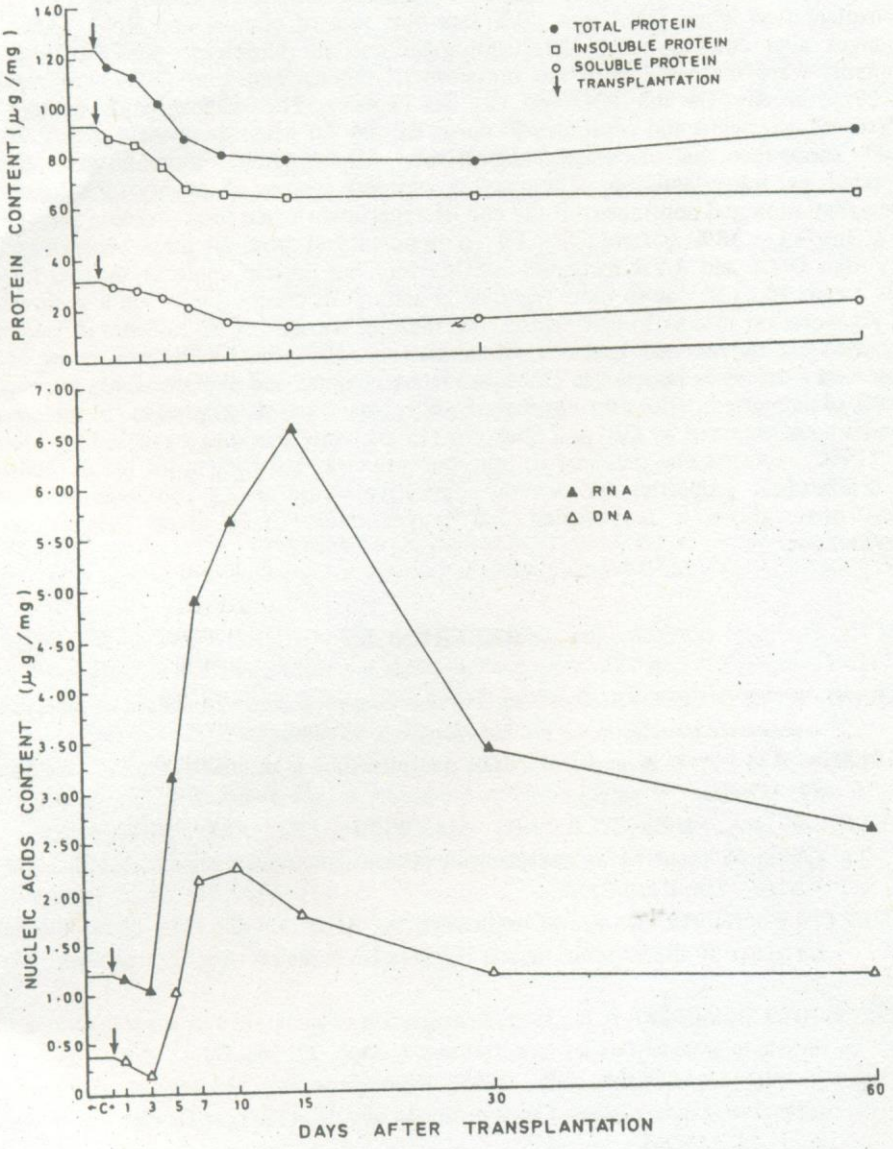



**Fig. 12:** **Effect of transplantation on the nucleic acids and protein content in rabbit anterior tibialis muscle.**

*Nucleic acids and protein contents*

Figure 12 shows changes in DNA, RNA and protein contents of control and transplanted ATM. The DNA and RNA contents decreased during first 3 days of transplantation when DNA was 50% less than that of control and RNA 14%. These changes also coincide with the histological and morphometric studies. Both these contents were increased with the initiation of regeneration and DNA was maximum (5.29x) on day 10 and RNA on day 15 (5.44x). The nucleic acids content were decreased afterward and continued to do so till day 60 when these were only 2.76x and 2.07x more than that of control, respectively. All the protein contents were adversely effected by transplantation. The protein contents decreased twenty-four hours after transplantation and continued till the end of experiment when total proteins showed 42% loss, insoluble 38% and soluble 53%. It is postulated from all these observations that although DNA and RNA increased considerably but protein contents showed loss. All this happened to be due to more increase in amount of connective tissue and slower rate of regeneration due to bigger size of the muscle. Variability in number of nuclei was obtained in regenerated muscles (Robertson *et al.*, 1993). Gallucci *et al.* (1966) observed 4-10 times increase in DNA and RNA contents and explained this increase as a result of increased cellularity particularly fibroblasts and macrophages. Similar type of results were obtained by Gill and Shakoori (1996) while working on rat ATM. Martin *et al.* (1990) reported that recovery of upto one year was insufficient for the normalization of biochemical properties and several connective tissue matrix components. From all these observations it is revealed that bigger muscle take much longer time for regeneration.

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