TRANSPLANTATION OF WHOLE MUSCLES IN TOLERANT RATS

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Whole gastrocnemius muscles were transplanted in tolerant and intolerant rats belonging to the same or different litters at the age of 15 days. Muscle tissue of newborn rats was used to induce tolerance. In every case during the first 2-3 weeks structural changes occurred in the grafts with the formation of a muscle organ. Some of the grafts, especially in intolerant and tolerant animals of different litters, subsequently died because of an incompatibility reaction. The highest percentage (up to 50) of survival of the grafts was observed in experiments in which muscles were grafted in tolerant rats of the same litter.

Experiments have shown that after injection of a foreign antigen in the embryonic period the organism loses its ability to give an immune response to that particular antigen and acquires a state of immunologic tolerance [6, 9]. It was later found that the period during which tolerance can be induced in some animal species extends into early postnatal ontogeny [2, 7]. This has led to a search for new way of overcoming immunologic tissue incompatibility [3-5, 8,10-12].

In this investigation an attempt was made to graft whole gastrocnemius muscles in animals using muscle tissue as the inducer of tolerance.

EXPERIMENTAL METHOD

Altogether 3 series of experiments were carried out on noninbred albino rats. In series I whole muscles were grafted in unprepared animals taken from the same litter (22 rats). In series II whole gastrocnemius muscles were homografted in tolerant rats taken from the same litter (24 rats). In series III whole gastrocnemius muscles were grafted in tolerant rats taken from different litters (34 rats). To create tolerance in the series II and III, pieces of the thigh muscle of one young rat were cross-grafted beneath the skin of the abdominal wall of another rat at the age of 1 day. Each rat was used simultaneously as donor and recipient. In all 3 series the grafting of the whole muscle was performed on rats aged 15 days by the method described previously [1]. At various times (from 15 days to 4 months) after the operation the contractile activity of the graft was verified as a physiological control. The mean weight of the grafts relative to the body weight was determined. The muscles were fixed in Zenker's fluid and 12% neutral formalin. Sections were stained with azocarmine by Heidenhain's method, with iron-hematoxylin after Regaud, and with azure-eosin after Romanovsky.

EXPERIMENTAL RESULTS

In series I (control) by the 15th day much of the newly formed muscle tissue had undergone secondary disintegration with replacement of the graft by connective tissue. Only about 25% of the grafted muscles still remained 4 months after grafting.

In series II 15 days after transplantation in every case the transplanted muscles had united with the stumps of the removed muscles. The relative weight of the graft was $0.47 \pm 0.08\%$. The weight of the control muscle also was $0.47 \pm 0.03\%$. Six of the 8 grafted muscles responded by contraction to stimulation of the tibial nerve with an induction current.

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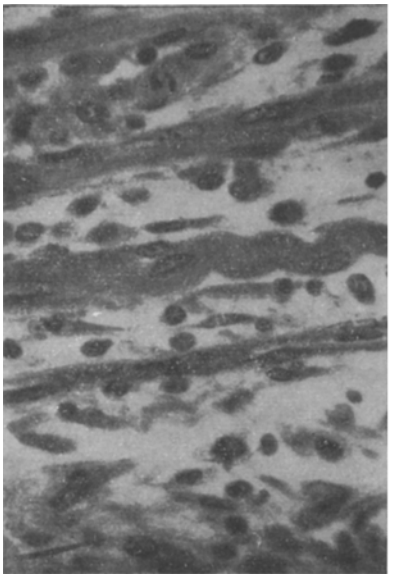

Fig. 1

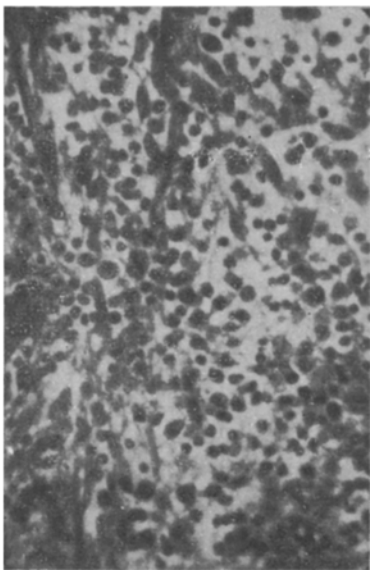

Fig. 2

Fig. 1. Myoblasts and young muscle fibers in graft 15 days after transplantation (360×).

Fig. 2. Lymphoid infiltration and secondary disintegration of newly formed muscle 30 days after transplantation (192×).

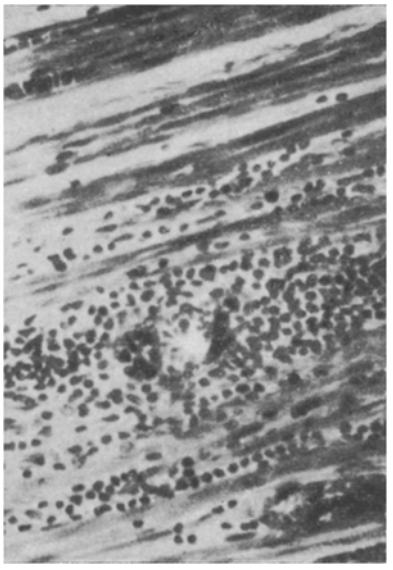

Fig. 3. Young muscle fibers and clusters of lymphocytes in graft 21 days after transplantation (192×).

Microscopic examination of the preparation showed that the muscles had undergone morphological changes with liberation of myoblasts from the viable areas of the muscle fibers and the subsequent differentiation of myogenic cells. Six of the grafted muscles consisted mainly of young muscle fibers separated by bands of dense or loose connective tissue. The process of development continued in the grafts: besides differentiated muscle fibers there were also myoblasts and young muscle fibers of the muscle tubule type (Fig. 1). Many of the cells were in a state of mitotic division. Hypervascularization of the newly formed muscles was observed.

Two of the grafted muscles developed a well-marked tissue incompatibility reaction – the muscles were abundantly infiltrated with lymphocytes. Connective tissue was activated in these grafts and many dividing fibroblasts could be seen. Later these newly formed muscles underwent destruction, degeneration, and replacement by scar connective tissue.

The relative weight of the graft 1 month after transplantation was $0.25 \pm 0.02\%$. Most of the newly formed muscles contracted distinctly in response to stimulation of the nerve by an induction current. These grafts were of muscle type. They contained closely packed bundles of young differentiated striated muscle fibers. Some variation in the diameter of the muscle fibers was noted. Fewer of the newly formed muscles were profusely infiltrated with lymphocytes, and among the white blood cells and connective tissue only small fragments of muscle fibers remained (Fig. 2).

After 4 months more than 50% of the grafted muscles retained their structural integrity and functional activity. The relative weight of the graft was $0.27 \pm 0.03\%$. The newly formed muscles were regular in shape, the belly was narrower than normally, and the Achilles' tendon was lengthened.

A study of the histological section showed that the muscles were feather-shaped and contained fully differentiated muscle fibers. Their diameter was increased although it remained variable, and the muscle fibers were chaotically arranged in some parts of the muscle. Small clusters of lymphocytes could be seen in some of the grafts around large blood vessels.

The results of the experiments of series III showed that 15-21 days after transplantation, just as at the previous period, structural changes were present in all the grafted muscles. The relative weight of the graft was $0.39 \pm 0.05\%$. Nearly all the grafts responded by contraction to stimulation of the tibial nerve with an induction current.

The grafts consisted chiefly of muscle tissue. However, side by side with areas containing differentiated and closely packed muscle fibers, other areas could be seen with thin, palely stained muscle fibers and large numbers of connective-tissue cells and white blood cells (Fig. 3). The number of blood vessels was increased.

One month after transplantation half of the newly formed muscles had undergone destruction as the result of an incompatibility reaction and had been replaced by connective tissue. The rest of the muscles retained their structure and functional activity. The relative weight of the grafts was $0.25 \pm 0.01\%$.

Later the number of muscles undergoing destruction and connective-tissue replacement increased. Compared with the control series, the grafts of this series remained viable for longer.

About 25% of the newly formed muscles remained structurally intact 4 months after transplantation.

It can accordingly be concluded from these results that after transplantation of whole muscles in tolerant young rats of the same litter the grafts take in 50% of cases and undergo structural changes, resulting in the structural and functional restoration of a muscle organ, whereas in unprepared young rats of the same litter, the grafts took in only 25% of cases. This shows that muscle tissue can be used to induce tolerance as a prelude to free muscle grafting.

LITERATURE CITED

1. N. N. Bosova, Byull. Éksperim. Biol. i Med., No. 6, 85 (1971).
2. O. E. Vyazov, The Immunology of Embryogenesis [in Russian], Moscow (1962).
3. O. I. Vyazova and V. I. Govallo, in: Problems in Tolerance and Homografting [in Russian], Moscow (1966), p. 52.
4. I. N. Kryukova, Byull. Éksperim. Biol. i Med., No. 4, 78 (1957).
5. A. G. Lapchinskii, in: Proceedings of the 3rd All-Union Conference on the Transplantation of Tissues and Organs [in Russian], Erevan (1963), p. 49.
6. W. Boyd, Fundamentals of Immunology, Wiley (1967).
7. O. Grosjean and D. Blumenstock, C. R. Soc. Biol., 161, 733 (1967).
8. M. Gashek, Zh. Obshch. Biol., No. 5, 405 (1955).
9. T. V. Puza, Byull. Éksperim. Biol. i Med., No. 10, 100 (1961).
10. D. Subbarayudu and J. V. Craig, Transplantation, 161, 733 (1968).
11. M. F. Woodruff and L. O. Simpson, Brit. J. Exp. Path., 36, 494 (1955).