2. G. D. Zasukhina, G. N. L'vova, I. M. Vasil'eva, et al., Dokl. Akad. Nauk SSSR, 265, No. 5, 1261 (1982).
3. I. V. Kolonina, R. G. Desyatskova, I. M. Vasil'eva, and G. D. Zasukhina, Vopr. Virusol., No. 5, 580 (1983).
4. K. Kohn and R. Ewig, Cancer Res., 33, 1849 (1973).
5. E. Schröder and E. Magdon, Stud. Biophys., 64, 193 (1977).

SURVIVAL OF XENOGRAFTS IN ANIMALS

A.N. Studitskii and N. N. Bosova

UDC 612.6:02:612.74+616.74
-089.843-089.168-92.9

KEY WORDS: muscle tissue; xenoplasty.

Techniques of free autografting of muscles, developed in the writers' laboratory, have now become widely known and used both for research in experimental morphology and in surgical practice [8-12]. Various methods have been developed for preparing muscle tissue for transplantation of whole muscles and the conditions of its regeneration have been discovered [4, 6].

One favorable condition for free muscle grafting, as we know, is preliminary denervation, which induces a combination of changes in the muscle tissue that promote survival of autografted and homografted muscles [1-3, 5].

However, the most interesting subject from the theoretical point of view and the most promising for future use in surgical practice is interspecific grafting of whole muscles, to which this paper is devoted. We used the method of preparation of donor's tissue, suggested and developed by A. N. Studitskii, by wrapping it in cellophane film [7].

EXPERIMENTAL METHOD

There were three series of experiments. Wistar rats weighing 130-150 g were used as the donors. The recipients were C57BL mice, hamsters (*Mesocricetus auratus*), and guinea pigs.

Preparation of the donor's muscle: the right gastrocnemius muscle of the rats was wrapped in cellophane film, leaving the proximal and distal tendons free. Blood vessels and nerves supplying the muscle were left undisturbed. The muscle was kept under film for 9 to 12 months. Before grafting, the prepared muscle was removed from its bed, freed from the cellophane film, and cut into fragments corresponding in size to the excised muscles of the recipients. A fragment of donor's muscle was sutured by its end to the remnants of the proximal and distal tendons of the recipient's removed gastrocnemius muscle. The medial popliteal nerve was sutured to the graft.

In the experiments of series I rats were used as the donors and mice as the recipients. The material was fixed after 7, 14, 21, 30, 60, 270, and 330 days. In the experiments of series II the donors were rats and the recipients were hamsters. The material was fixed after 60 and 330 days. In the experiments of series III the donors were rats and the recipients were guinea pigs. The material was fixed after 3, 7, 14, and 60 days. Before fixation, contractile activity of the transplanted muscle was tested by stimulating the sutured nerves with an induction current. Histological preparations were stained with Heidenhain's azocarmine, with Romanovsky's azure-eosin, and Régaud's iron hematoxylin. Nerve endings were demonstrated by Bielschowsky's impregnation method.

EXPERIMENTAL RESULTS

A study of the state of the rat muscle after removal of the cellophane film at the end of 270-360 days showed that the muscles lost up to 40-60% of their weight. They were paler

Laboratory of Evolutionary Histology, A. N. Severtsov Institute of Evolutionary Morphology and Ecology of Animals, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. P. Avtsyn.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 197-199, February, 1986. Original article submitted January 24, 1985.

0007-4888/86/0002-0229$12.50

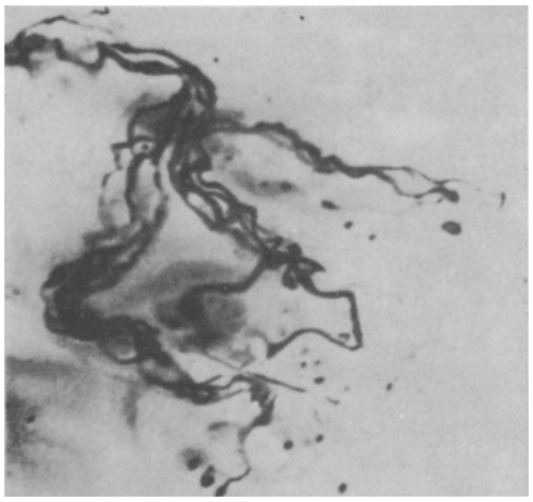

Fig. 1. Myoneural synapse in xenograft 60 days after transplantation in a hamster. Impregnation by Bielschowsky's method. 450×.

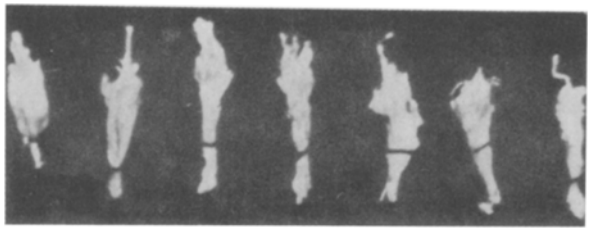

Fig. 2. Muscle xenograft 330 days after transplantation.

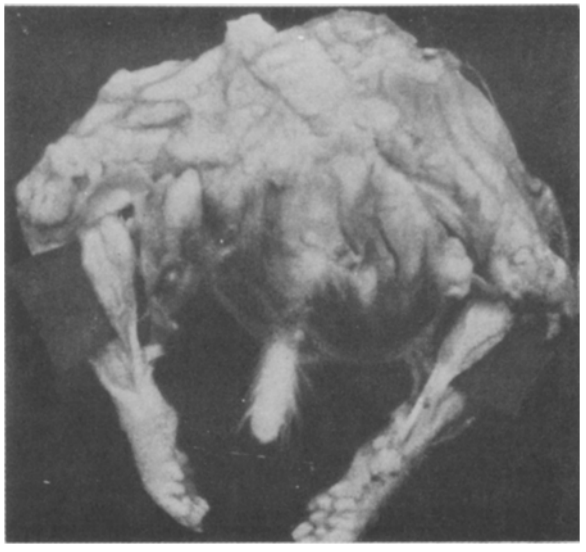

Fig. 3. Xenograft in a hamster 330 days after transplantation.

in color than intact muscle. Examination of histological preparations showed some abnormalities in the structure of the muscle. The muscle surface in contact with the film was abundantly vascularized. In some cases it could be separated from the cellophane by a dense connective-tissue capsule. Thinning of the muscle fibers was observed. In the peripheral zone disinte-

grating muscle fibers and foci of hemorrhage could be seen. The quantity of fibrous connective tissues increased. Fatty degeneration of isolated areas of muscle was observed. The whole muscle was innervated, although some differences in innervation were found compared with the intact muscle.

The intact gastrocnemius muscle has a pinnate structure and is abundantly vascularized and innervated. During the development of the transplanted muscles, in the first stages of fixation (up to 60 days) processes similar to those described by the writers previously after autografting of whole muscles were observed. Characteristically in the first week edema was found, leading to an increase in volume of the graft. Histologic investigation revealed necrotic changes in the central part and evidence of reconstruction of the muscle tissue at the periphery of the grafts, of the usual type studied in our laboratory: the development of myoblasts and the beginning of formation of muscle tubes. In the peripheral zone, ingrowth of blood capillaries could be seen by the end of the first week. During the next 2 weeks progressive development of the grafts was observed, accompanied by restoration of the structure of the transplanted muscle. A contraction response to stimulation of the sutured nerve by an induction current appeared 21-30 days after transplantation. Invasion of lymphocytes, leading to their destruction, was noted in some of the grafts.

In series in which the operations were performed on mice and hamsters as recipients, further differentiation of the muscle tissue was observed 60 days after the operation in the grafts, accompanied by absorption of the necrotic parts of the grafts. In both series abundant vascularization was found, together with neurotization, with the formation of abundantly branching motor endings (Figs. 1-3). After 330 days the external appearance of the grafts resembled the removed gastrocnemius muscle, and differed only in their smaller size. The characteristic thickened form of the proximal part and narrowed form of the distal part of the muscle were preserved (Figs. 2 and 3). Significant differences were observed in the ratio between muscular and connective-tissue components in both series: from predominance of connective tissue to its complete replacement by differentiated muscle tissue. Differentiation of nerve endings evidently continued in the grafts, for besides clearly differentiated motor end plates, developing synapses also were seen. A study of contractile activity of the xenografts in both series revealed the presence of a distinct motor response which differed in intensity, to stimulation by an induction current through the nerve.

The results of analysis of the immune response of the recipient to transplantation of muscles prepared by the writers' own method are evidence of the broad spectrum of relations between lymphoid cells and foreign tissue in the two series. During the first 2 months, in some cases the number of lymphocytes observed in the grafts was typical for alloplasty, whereas in other cases increased infiltration with lymphoid tissue was observed, corresponding to the mainly weak development of muscle tissue in the graft. In most cases, however, a muscle prepared by long-term keeping wrapped inside a cellophane cover acquires the ability to change its antigenicity, and so to block the recipient's immune response.

Data on the dynamics of development of xenografts in a guinea pig recipient are insufficient to allow final conclusions, although the phenomenon of survival of the grafts without rejection for up to 2 months after the operation shows that in this case also a positive result is possible.

Muscle tissue prepared with long-term (up to 330 days) disturbance of morphogenetic interactions with surrounding tissues, while preserving the normal vascularization and innervation, thus acquires the ability to survive under conditions of xenoplasty. The developing xenograft undergoes complete reorganization, as a result of which an organ of chimeric nature is formed, for its differentiation is accompanied by invasion of the recipient's blood vessels and nerve fibers. At the site of transplantation a functioning organ capable of replacing the removed muscle is formed *de novo*.

LITERATURE CITED

1. N. N. Bosova, Dokl. Akad. Nauk SSSR, 152, No. 1, 235 (1963).
2. N. N. Bosova, Byull. Éksp. Biol. Med., No. 6, 85 (1971).
3. N. N. Bosova, Byull. Éksp. Biol. Med., No. 11, 1377 (1976).
4. R. P. Zhenevskaya, Neurotrophic Regulation of Plastic Activity of Muscle Tissue [in Russian], Moscow (1974).
5. A. N. Studitskii and N. N. Bosova, Arkh. Anat., No. 12, 18 (1960).
6. A. N. Studitskii, Transplantation of Muscles in Animals [in Russian], Moscow (1977).

7. A. N. Studitskii (ed.), Experimental Histology of Tumor Growth [in Russian] (1966).
8. B. M. Carlson and E. Gutmann, Experientia, 30, 1272 (1974).
9. L. Hakelius, J. Gierup, G. Grotte, and H. Jorulf, Z. Kinderchir., 31, 141 (1980).
10. A. J. J. Lischka, J. Holle, and G. Freilinger, Anat. Anz., 138, 755 (1975).
11. S. Refsum and O. Knutrud, Z. Kinderchir., 31, 30 (1980).
12. N. Thomson, Plast. Reconst. Surg., 48, 11 (1971).

INTRALOBULAR DISTRIBUTION OF ALBUMIN SYNTHESIS IN HEPATOCYTES OF THE NORMAL AND REGENERATING MOUSE LIVER

V. N. Baranov, V. S. Poltoranina, and A. I. Gusev

UDC 612.352.3-015.36+616.36-003.
93-07:616.36-008.939.623-074

KEY WORDS: immune electron microscopy; liver; albumin; normal state; regeneration.

Mammalian serum albumin is known to be synthesized in the liver. Immune electron-microscopic investigations carried out in recent years [8, 11, 14] on rat liver have shown that all hepatocytes synthesize albumin, and that immunohistochemical data on the existence of specialized albumin-producing cells in the liver, obtained previously, are artefacts. In the investigations cited, no heterogeneity of distribution of albumin among the liver lobules could be found, which is not in agreement with the presence of marked gradients in the lobule affecting many features such as glycogen content, enzyme activity, and ability to detoxicate organic and inorganic substances [5, 7, 12].

This paper describes an immune electron microscopic investigation of intralobular differences in albumin synthesis in the normal and regenerating adult mouse liver.

EXPERIMENTAL METHOD

Noninbred mice and BALB/c/J mice weighing 20-30 g, obtained from the Nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used. The liver of normal mice and 70 h after poisoning with CCl_4 vapor [1] was studied.

An albumin preparation was obtained from adult mouse serum by double polyacrylamide gel electrophoresis [2]. No contamination with other serum proteins was found in the concentrated albumin preparation by precipitation in agar and electrophoresis. Antiserum against albumin was obtained by immunization of rabbits in the popliteal lymph nodes with a preparation of pure albumin [3]. Monospecific antibodies were obtained by means of an immunosorbent prepared from purified albumin, covalently bound with Sepharose CNBr-4B (from Pharmacia, Sweden) in accordance with the firm's instructions. Fab'-fragments of antibodies were obtained from the isolated antibodies [9]. The preparation of Fab'-fragments was conjugated with horseradish peroxidase type VI, RZ-2,9 (from Sigma, USA) [12].

The liver was fixed by our modified method of perfusion with saponin [10]. Under ether anesthesia, after application of a ligature a needle was introduced into the posterior pole of the spleen, and the organ perfused with 25 ml of a solution of 0.05% glutaraldehyde + 6% paraformaldehyde + 0.05% saponin in 0.15 M cacodylate buffer, pH 7.4. Perfusion was carried out with a peristaltic pump at the rate of 5 ml/min. After perfusion pieces of liver 1 cm^3 in volume were postfixed with a solution of 6% paraformaldehyde + 0.05% saponin for 3 h at 4°C. The pieces were then washed for 12-15 h with buffer with the addition of 3.5% sucrose and 0.05% saponin. The pieces were frozen with liquid nitrogen and cryostat sections cut to a thickness of 15 μ and treated with 0.1 M lysine solution for 30 min. Next, 20-30 freely floating sections were incubated in 0.3 ml of a solution of Fab + horseradish peroxidase con-

Laboratory of Tumor Morphology and Laboratory of Immunochemistry, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 199-201, February, 1986. Original article submitted March 14, 1985.

 0007-4888/86/0002-0232$12.50