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HOMOTRANSPLANTATION OF TENDONS PRESERVED BY LYOPHILIZATION

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For lengthening tendons plastic surgical techniques are generally used. However, in case of major tissue defects caused by trauma, extensive pyogenic infection or neoplasma the tendon defect should be repaired by some other method. In such instances autoplasty (transplantation of free tendon) is most often the method of choice, which, however, has the disadvantage of exposing the patient to additional stress. Homo- and heterotransplantations have been carried out infrequently, mainly because of the difficulties involved in the preservation of the graft. The use of alloplastic materials in the repair of tendon defects failed to gain wide-spread acceptance.

In earlier transplantation experiments [1, 2, 3] we have found lyophilization to be a procedure suitable for the preservation of tendon. The method, which has been applied to other tissues as well, has been developed by our associate, chemist I. Szilágyi [4]. Being a braditrophic tissue (one with low metabolism), the tendon is exceptionally amenable to preservation by lyophilization and as we have shown it experimentally, it can be used with success as a homograft, particularly if we provide for it the proper function.

The first experimental homotransplantation of tendon was carried out in 1881 by Glück [5]. Seggel [6] performed autotransplantation experiments in 1903. According to M. Lange [7] Lexer in 1908 was the first to carry out such operations in man, but after being successful with the first cases later he experienced failure. Bunnel [8] obtained better results, operating on the flexor and extensor muscles of the hand. For this purpose one can make use of the tendon of the palmaris longus, peroneus tertius, or other muscles. Kirschner [9] repaired tendon defects with fascia, which being a braditrophic tissue, could be used with success. Tube-shaped fascial autografts have proved to be useful in the repair of short and thick tendons, but yielded less favourable results in operations on the hand. As mentioned by M. Lange [7], Rehn tried to repair tendon defects by the use of skin strips, whereas Ritter transplanted a piece of the saphena magna vein into on artificially created tendon defect. Most of these experiments ended in failure, but disclosed interesting morphological observations. It has been found that under the influence of function the cutaneous and venous grafts, which are

remote from tendon as far as structure is concerned, became similar to tendon in histological pattern. These observations support the view put forward recently by KROMPECHER *et al.* [10] that the fate of grafts depends in a high degree on function. We, too, have observed this in our experiments.

The first successful attempt to repair tendon defects by the use of materials foreign to the body was made by F. LANGE [7]. After extensive trials he found that silk impregnated with mercury oxycianate is not ejected from the organism but causes chemical stimulation resulting in the formation of a connective tissue sheath around the silk. With time this sheath becomes similar to tendon in structure under the influence of function. The "tendon" thus formed takes over the role of the silk, which loses its original tensile strength. LANGE's results were good (ejection of silk having not occurred in more than 2,4 per cent of cases), but others were unable to reproduce them.

In the course of the tendon repair experiments we have endeavoured to elucidate a number of problems. First of all, we examined the fate of tendon homografts preserved by lyophilization, particularly from the point of view of function and regeneration, because in the literature available to us we have found no experiments dealing with these problems. At the same time, we have made attempts at finding new materials suitable for suturing tendon. The formation of adhesions around grafts has been also investigated.

One of the greatest problems in the surgery of tendons is that of suture material and technique. The essential precondition of success is that the tendon ends be united firmly, but with as little traumatization as possible. The commonly used sutures, such as silk, catgut, etc. are poorly tolerated by tendinous tissue and BUNNEL [8] observed that the reactive hyperaemia accompanying the healing of the wound in the tendon (i. e. the swelling of stumps) is the more marked, the more, and less suitable, suturing material has been used. Recently, KÓS [11] has published a report on this problem. Tendons lying superficially can be united by suture, which can be removed percutaneously after the stumps had healed, i. e. about 3 weeks after operation. However, this technique is unfeasible with tendons located deeper. In the latter cases the suture material of choice should cause no disturbance even if left in place. In cases of incisional hernia, strips of fascia lata have been used for suturing by GALLIE and Le MESURIER [12], and more recently by USHER [13]. Since to our best knowledge nobody has given a trial to this kind of suturing material in tendon surgery, we have carried out experiments with it. Recently, alloplastic plastic materials, first of all nylon and perlon threads, have been used for suturing tendons. These materials caused problems in other relations [14] and this is why we have subjected also them to study.

The formation of adhesions is another problem in transplantations of tendon. Earlier observations [15] and our own experiments offered proof that the regeneration of the transplanted tendon takes place not from the recipient tendon

ends, but from the environment, from the paratenon. Thus the adhesions which are formed between the tendinous graft and its environment cannot be considered an useless complication, but must be looked upon a past of the regeneration process. Such adhesions, however, interfere with the motions of the tendon and for this reason several (mostly unsuccessful) attempts have been made to eliminate them. Sheathing with various kinds of auto- or homoplastic tissue (e. g. fat, pleura, etc.) increases rather than decreases adhesions, because these, too, are regenerating grafts around which adhesions develop. The use of alloplastic sheathing (cellophane, polyethylene, etc.) is theoretically well-founded, but did not prove feasible in practice, because, isolating the graft from its environment, such sheaths caused destruction of the graft and failure of sutures usually in 2 weeks [16]. Attempts have been made to reduce adhesions by active and passive exercise, with certain success in some cases. This treatment must namely overcome opposite difficulties : if exercise is begun too soon, the freshly united tendon may break as a result of suture insufficiency. If exercise is begun too late, the adhesions may be too firm and the tendon may be immobilisable.

In order to elucidate the above problems, experiments were carried out in 30 dogs. Under intraperitoneal Evipan anaesthesia the previously shaved left hind leg was isolated and operated on under the strictest asepsis. The grafts were somewhat smaller in size than was the defect created, because, according to data in the literature, the grafts may strech later. After operation the talocrural joint was immobilized in a plaster of Paris cast for 2 weeks, partly to spare the graft from weight-bearing and partly to prevent infection from outside. When the operation was successful, the animals began to use their operated limb as early as 2 to 3 days after operation and were moving about unrestrictedly in about 3 weeks.

Our experiments and the results obtained are described as follows.

1. In group 1 (10 dogs) tendon grafts preserved by lyophilization were transplanted and sutured in place with nylon thread (thin fishing-line). Prior to implantation the lyophilized graft was maintained in a penicillinized physiologic saline solution at 37° C for about 1 hour, because otherwise the graft was too rigid and hard to be transplanted. The two ends of the nylon suture were threaded on a needle and both ends of the graft were sutured by the common technique shown in Fig. 1. As it is rather difficult to knot the rigid nylon thread, surgical knots were made, which held well in every case.

In this series two problems were subjected to study *a)* the behaviour and healing of the graft, and *b)* the histological patterns around the nylon sutures.

a) Prior to grafting, histological sections were made from the lyophilized graft wetted as specified above. These sections showed patterns absolutely identical with that of normal, fresh tendon, with perfect nuclear staining. Following up the fate of the graft, it was found that one day after transplantation nuclear staining was absent and the collagen fibres were straight and parallel, like in

normal tendon (Fig. 2). After 4 days nuclear staining was still absent, but the collagen fibres ran a more undulating course than that seen in the former specimens. The graft was enclosed in a capsula composed of clotted blood and leucocytes, but no cellular invasion was seen among the collagen fibres (Fig. 3). After 20 days the graft was surrounded by young connective tissue, with slightly elongate nuclei, which did not show a regular arrangement ; a few blood vessels were also visible. The collagen fibres, which appeared around the graft, were not arranged either. At sites lymphocytes and leucocytes could be seen. Within the above loose capsule the graft could be clearly distinguished, with its deeper, cell-free layers, and wavy, intact collagen fibres (Fig. 4). The cellular invasion in the marginal areas was composed mainly of fibroblasts with spindle shaped

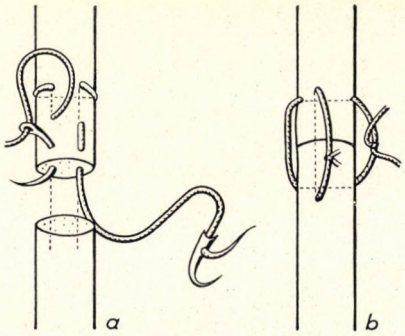

Fig. 1. Suturing of tendon graft *a)* with nylon thread, and *b)* with strip of fascia lata

nuclei and a smaller number of lymphocyten (Fig. 5). 2 months following transplantation the collagen fibres showed a regular arrangement, the graft was revascularized, the nuclei of the immigrated fibroblasts were arranged longitudinally and were similar to the nuclei of tendon cells. At the ends the young connective tissue penetrated also in between the recipient fibres of tendon, establishing firm connection with them. The recipient tendon was histologically distinguishable from the graft and connective tissue because it took the eosin stain better and its cells had longer and thinner nuclei. The transition was absolutely gradual. After 4 months the graft almost completely regained its original structure and became similar to the recipient ends of tendon. After 6 to 8 months it was only the site of sutures by which it could be determined which is the graft and which the original tendon. The revascularization of 3 months old or older grafts has been examined by injecting dilute China ink into the femoral artery of the anaesthetized dog with normal circulation, through a long cannula reaching into the popliteal artery. The total volume of China ink injected varied from 100 to 150 ml. After injection the thigh of the animal was compressed by applying a strong bandage, then another dose of 100 to 150 ml dilute China ink was injected through the cannula which had been left in place and then the animal

was sacrificed. The preparations were treated with Ol. gaultheriae and examined by stereomicroscopy. It has been found that in the recipient tendon ends the

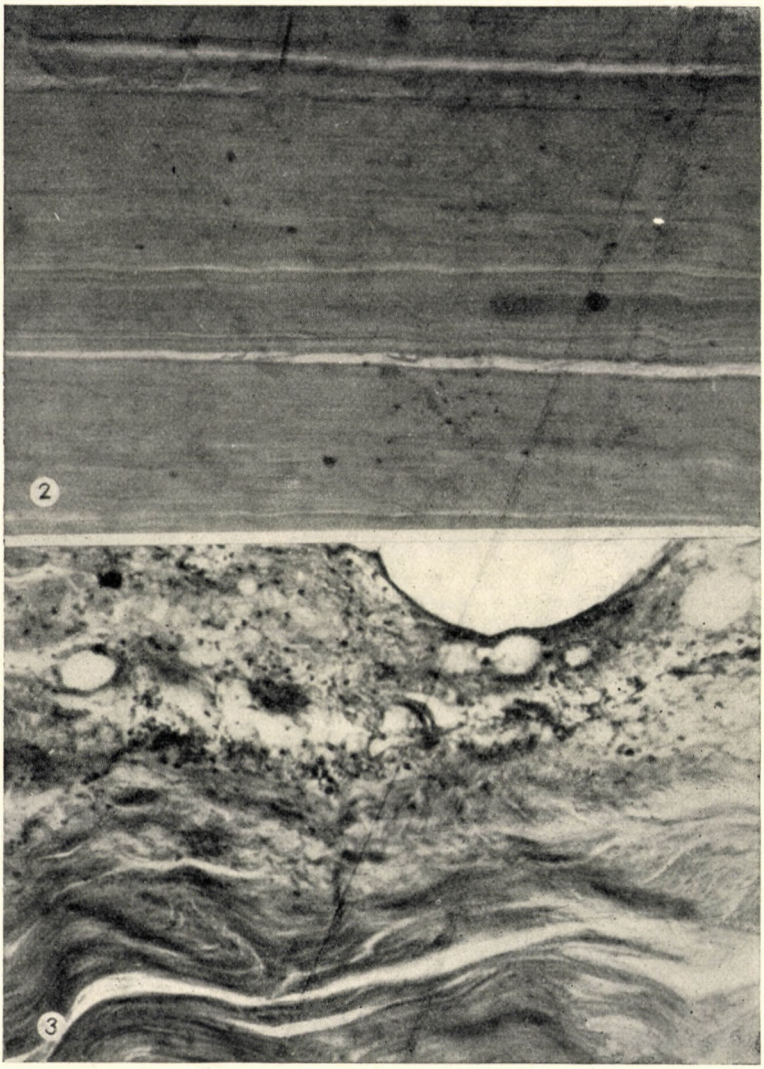

Fig. 2. Histological appearance of lyophilized homograft of tendon, one day after transplantation
Fig. 3. Environment of four-day old tendon graft, composed mainly of coagulated blood and leucocytes

blood vessels run parallel with the longitudinal axis of the tendon, whereas in the graft two zones can be distinguished. Vessels invade the external layers of the graft from the paratenon to form there a dense plexus (Fig. 6). In the centre the blood vessels run grossly parallel with the longitudinal axis of the tendon,

originate partly from the vessels in the recipient tendon ends and partly from those of the external cone, forming bulges at sites (Fig. 7).

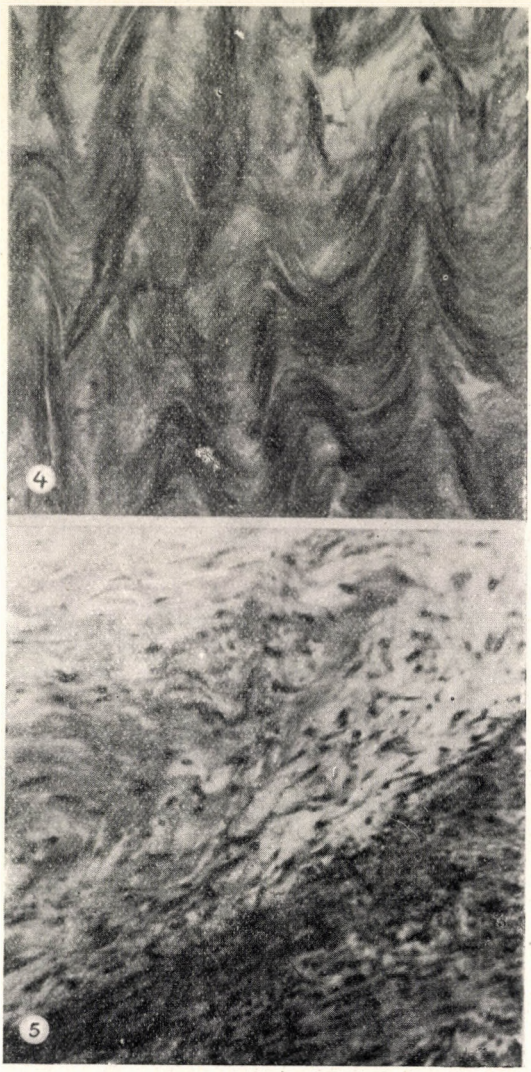

Fig. 4. After 20 days no immigration of cells is visible in the graft
Fig. 5. Fibroblasts with spindle-shaped nuclei, invading the margins of the 20-day old tendon graft

The nylon sutures, which were clearly distinguishable in the sections, were enclosed in circular, thin connective tissue capsules. Foreign body giant cells were not visible anywhere and the sutures held well (Fig. 8).

Nine of these 10 experiments were successful. In one case suppuration developed and the graft was ejected.

2. In group 2 of the experiments the graft was sutured in position with strips of fascia lata preserved by lyophilization. The procedure is illustrated in

Fig. 6. Marginal vascularization of tendon graft 3 months after transplantation
Fig. 7. Central vascularization of tendon graft 3 months following transplantation

Fig. 1*b*. Ten dogs were used. The fascia lata strip, which was about 1 mm wide and 6 to 8 cm long, was threaded on needle, knotted once and the knot was stitched with a very fine nylon thread taken from a nylon stocking. The sutures did not cut through and held well. The process of regeneration in the fascia lata sutures was identical with that described for the tendon graft. The nuclear

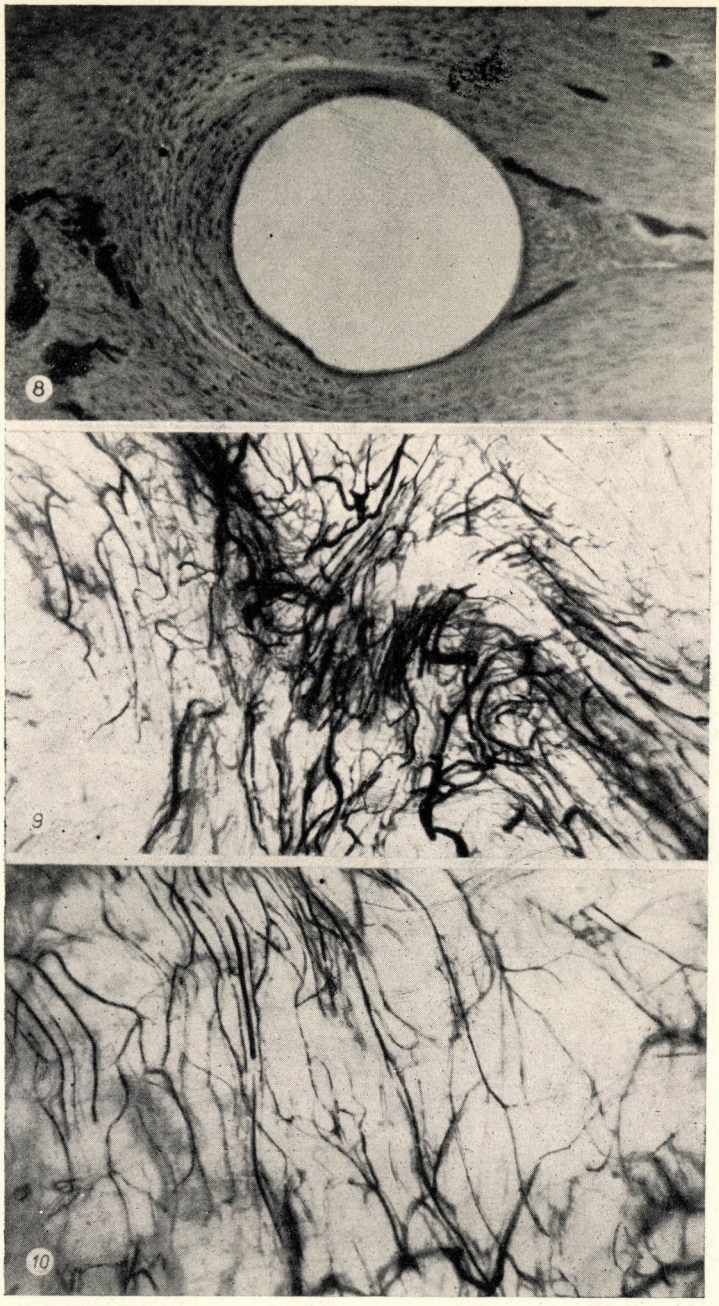

Fig. 8. Environment of nylon suture 2 months after transplantation
Fig. 9. Vascularization of built-in fascial suture 3 months after operation
Fig. 10. Vascularization of tendon formed from lyophilized homoplastic fascia lata 3 months after transplantation

staining disappeared in the fascial suture on the first day, just like in the graft and later it was gradually transformed structurally, as a result of the invasion by fibroblasts of the collagen fibres. Three months after operation the strip of fascia lata was not any more discernible, it having been built in completely. On injecting China ink intraarterially the vascular network at the site of the suture was found to be more abundant than that in its environment (Fig. 9). In this group 8 experiments were successful, 1 dog was bitten to death by its kennel mates and in 1 dog the strip of fascia broke, because it was too thin.

3. In group 3 the lyophilized fascia lata was used not only as a suture, but also as a plate fashioned like a cuff, for the repair of tendon defects. In 5 of these 10 experiments the graft was sutured with nylon thread and in the other 5 experiments strips of fascia were used. The margins of the fascial cuff were sewn with fine nylon taken from a stocking. The process taking place in the inside of the cuff-shaped graft was similar to that described for tendon grafts. In the course of this process the lumen of the cuffs filled out by a mass of erythrocytes, leucocytes and networks of fibrin as early as 4 days after operation. This mass developed gradually into young connective tissue and 3 months later no histological distinction could be made between the fascial graft and a tendon graft of the same age. Intraarterial China ink injections have shown also these kinds of graft to exhibit abundant vascularization (Fig. 10). In this group all the 10 experiments were successful and, like in the former series, the grafts did not stretch significantly.

4. In group 4 of the experiments the aim was to eliminate the adhesions which developed around the grafts described in the former groups. Although the above experiments yielded excellent results from the point of view of function, even the early use of the grafted tendon could not prevent the development of adhesion between graft and adjacent tissue. This, however, did not interfere with the motion of animals. As it has been pointed out, adhesions constitute part of the regeneration process, their development cannot be prevented, but it would be unreasonable to do so, anyway. We are namely of the opinion that exercise begun at the proper time will result in the formation of more or less elastic adhesions which, unlike the rigid ones, do not interfere with free motion. As a matter of fact, adhesions may develop with autografts, or even with simple tendon sutures. We have abolished them by tenolysis. We have started out from the observation that in three months' time the transplant is supplied with sufficient blood vessels not only from its environment (i. e. from adjacent tissue), but also from the recipient tendon ends and thus will not be devitalized even if the lateral vascular supply is eliminated, unlike it happens when the graft is sheathed at the time it is transplanted. To obtain corroborative evidence, the grafts in 6 animals have been exposed 3 months after operation. After freeing the loose adhesions, the graft was covered by cellophane. One to six weeks after this operation the animals were killed. It was found that the grafts sheathed

with cellophane were absolutely intact, showing no evidence of necrosis anywhere and, of course, no new adhesions had formed. From this we have drawn the practical conclusion that in cases when tendon grafting is followed by the development of adhesions interfering with function, the latter can be eliminated by the above procedure without the risk of causing necrosis.

As it has been shown in earlier fascial graft experiments [17] the fate of cellular elements can be elucidated by the methods described above, but it is more difficult to form an opinion as to the fate of the collagen fibres in the tendon graft. As far as we know, there is no method available to supply reliable information concerning the survival time of transplanted collagen fibres. The impregnation methods did not clarify this problem, although the differences in impregnation suggested that the fibres were not uniform in age. A careful analysis of our experimental material created the impression that just like in bone grafts [2], here, too, break-down and build-up processes are involved; the collagen fibres are broken down gradually and are replaced by new collagen produced by the fibroblasts, which immigrate. Function will establish a dynamic equilibrium in this process, as a result of which the graft will be fully capable of carrying out the function it is intended to perform, and this alone is important from the point of view of success. When judging problems of this kind, not only the fibres themselves, but also their environment should be taken into consideration, as it has been emphasized in the electronmicroscopic studies of PAHLKE [18]. PEER [19] suggested that the collagen fibres in tendon grafts killed by preservation would survive for 6 months and would undergo umbau after that time. PEER tried to preserve tendon by treatment with 70 per cent alcohol and found some foreign body reaction to develop after transplantation. This complication has never occurred in our lyophilization experiments.

Tendon preserved by lyophilization can be used not only for the repair of tendon defects, but also as suture. Broken up into fine bundles of fibre, it will yield very thin, yet highly resistant suture, which can be used successfully under highly variable conditions. Experiments are in progress to obtain more information concerning this point.

Although the results of our animal experiments cannot be held fully valid in human therapy, and although we have experimented on healthy dogs, whereas sick men are operated on, we nevertheless believe that lyophilized tendon grafts will prove useful in human surgery.

Summary

After describing in brief the possibilities of tendon grafting experiments are presented in which the fate of tendon homografts preserved by adsorptive lyophilization has been studied from the point of view of regeneration and function. The preserved tendon and cuff-shaped fascial grafts were sutured in place partly with nylon thread, and partly with strips of preserved fascia lata. Although the nylon suture did not produce any significant foreign

body reaction, the strips of fascia lata proved to be a superior suturing material. In view of the fact that the tendon grafts regenerate from the environment, from the paratenon, and not from the recipient tendon ends, we disapprove of the techniques in which the tendon grafts are sheathed either with other tissue or with alloplastic substances, because in the former case even more abundant adhesions may be formed and in the latter case the graft may undergo necrosis. Experimental evidence is presented showing that 3 months following transplantation the graft is sufficiently supplied with blood vessels not only from the paratenon, but also from the recipient tendon ends. If in spite of exercise begun at a proper time than develop adhesions interfering with function to such a degree that tenolysis becomes necessary, after three months the operation can be performed without the risk of causing necrosis of the graft.

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ГОМОТРАНСПЛАНТАЦИЯ КОНСЕРВИРОВАННЫХ ЛИОФИЛИЗАЦИЕЙ СУХОЖИЛИЙ

ДЬ. БОРНЕМИСА, Г. БАКО и Л. ФАРКАШ

После краткого описания возможностей замещения недостающих сухожилий, излагаются собственные эксперименты авторов. В течение этих экспериментов исследовалась гомотрансплантация, проведенная консервированными адсорбционной лиофилизацией сухожилиями, с точки зрения регенерации и функции. Консервированные сухожилия и трубчато оформленные апоневрозы для пересадки зашивались, отчасти нейлоновыми нитками, а отчасти также консервированными гомопластическими полосками широкой фасции (fascia lata). Хотя нейлоновая нитка не вызывала достойной упоминания реакции на инородное тело, то все же полоски апоневроза оказались лучшим швейным материалом. Ввиду того, что регенерация пересадочных сухожилий происходит со стороны окружающей среды, с сухожильного влагалища, а не с воспринимающих концов сухожилий, авторы считают, что те методы, при которых пересаживаемые сухожилия уже при пересадке окружаются другими тканями или аллопластическими веществами, следует считать неправильными, так как в первом случае в повышенной мере могут появляться сращения, а в последнем случае трансплантат может отмирать. Эксперимен-

тально было доказано, что три месяца после пересадки, трансплантированная ткань не только со стороны сухожильного влагалища, но и со стороны воспринимающих концов сухожилий получает соответствующее кровообращение, и если даже вопреки своевременно начатого движения появляются сращения, требующие позднейшее проведение тенолиза, то в это время можно провести операцию и без опасности некроза трансплантированной ткани.

HOMOTRANSPLANTATION VON MIT LYOPHYLISATION KONSERVIERTEN SEHNEN

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Nach kurzer Darstellung der Ersetzungsmöglichkeiten von fehlenden Sehnen, werden Experimente beschrieben, im Laufe welcher das Schicksal der mit adsorptiver Lyophilisation konservierten Sehnen durchgeführten Homotransplantate, im Hinblick auf Regeneration und Funktion untersucht wurde. Die konservierten Sehnen- und röhrenförmig ausgebildeten Fascientransplantate wurden zum Teil mit Nylonfaden, zum Teil mit ebenfalls konservierten homoplastischen Streifen der Fascia lata genäht. Obzwar der Nylonfaden keine nennenswerte Fremdkörperreaktion hervorrief, erwies sich Fascia lata doch als das bessere Nähmaterial. Da die Regeneration der Sehnentransplantate von der Umgebung, vom Paratenon aus erfolgt, und nicht von den aufnehmenden Sehnenenden aus, sind die Verfasser der Meinung, daß diejenigen Verfahren, bei welchen die Sehnentransplantate bei der Verpflanzung mit anderen Geweben oder alloplastischen Stoffen umgeben werden, nicht zu empfehlen sind. Im ersteren Fall können in erhöhtem Maße Verwachsungen auftreten und im letzteren kann das Transplantat absterben. Es wurde bewiesen, daß drei Monate nach der Verpflanzung das Transplantat nicht nur vom Paratenon, sondern auch von den aufnehmenden Sehnenenden aus einen entsprechenden Blutkreislauf erhält, und wenn trotz der rechtzeitig angefangenen Bewegung solche Verwachsungen auftreten, welche eine spätere Stenolyse erfordern, so kann zu dieser Zeit die Operation auch ohne Absterbegefahr des Transplantats durchgeführt werden.

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