PHOSPHORUS COMPOUNDS IN THE MUSCLES OF THE REPLANTED LIMB IN THE EARLY AND LATE STAGES AFTER THE OPERATION

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Replantation of the amputated limb has now reached such a high level of development by surgeons in experiments on animals that its clinical application is beginning to be considered [17]. The clinical and physiological state of the replanted limb has been studied in adequate detail, especially during the first months after the operation [2, 4, 5, 6, 8, 10, 15]. So far as biochemical investigations are concerned, however, information here is very scanty [11, 12].

In face of the important role of phosphorus compounds in the functional activity of muscles, the present investigation was carried out to study several phosphorus compounds in the limb muscles at various times after replantation.

EXPERIMENTAL METHOD

A limb was amputated through the middle third of the thigh in adult mongrel dogs of both sexes, weighing 10-15 kg. The limb was kept at 18-22° for 1.0-1.5 h and then replanted by the method described previously [9]. Mechanical sutures were used. Biochemical analysis of the muscles was carried out 1.5, 3, 9, and 12 months and 5-7 years after the operation. The muscles investigated were the thigh muscles proximally to the line of suture (the muscles of the former stump) and distally to line of suture (in both cases the muscle samples were taken 1.0-1.5 cm away from the scar) and the gastrocnemius muscle of the replanted limb. The thigh muscles and gastrocnemius of the intact limb acted as controls.

Estimations were made of the content of total, acid-soluble, and inorganic phosphorus, creatine phosphate, ATP [7], phospholipids, and nucleic acids [16]. Phosphorus was determined by the method of Fiske and Subbarow [14].

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results are given in Tables 1 and 2. The thigh muscles were severely traumatized as a result of their complete division. Whereas the proximal part of the thigh muscle retained its normal nerve and blood supply to some extent, the distal part was completely denervated, and in addition, its blood and lymph circulation was disturbed. This led to the development of atrophy. The complete muscle, restored after suture and healing, consisted of two heterogeneous morphological parts. This naturally complicated the restoration of the functional activity of the muscle. Analysis of samples taken from the proximal and distal part of such a muscle showed that trauma of this nature is accompanied by profound and prolonged changes in the content of all the phosphorus compounds studied: high-energy phosphates, nucleic acids, phospholipids, etc. In both the early and the late stages after the operation the content of phosphorus compounds are low. Comparison of the two parts of the repaired muscle showed that the biochemical changes in both the distal and the proximal part took place in the same direction, but they progressed further in the distal part.

After reinnervation, the disturbances of metabolism of the phosphorus compounds in the replanted limb were usually less severe in the gastrocnemius muscle which was not injured during the operation than in the thigh muscles which were injured. Experiments on rabbits also showed [3] that the return of the biochemical processes to normal in the denervated traumatized muscle took place less favorably after reinnervation than in the untraumatized, reinnervated muscle.

The external appearance of the muscles at this time also differed slightly. The atrophy was more marked in the distal part of the thigh muscles: they were flabby, they were palely stained, and they contained much fat and

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TABLE 1. Content of Phosphorus Compounds in the Muscles of the Replanted Limb at Different Times after Operation (in mg% of dry weight)

Number of experiments	Time after operation	Thigh muscles					Gastrocnemius muscle		
		control	proximally to scar	changes (in %)	distally to scar	changes (in %)	control	on the side of operation	changes (in %)
					Total phosphorus				
4	1 1/2 Months	835,0	549,2	—34,4	641,7	—23,2	741,4	522,4	—29,6
1	3 »	613,0	406,0	—33,8	422,0	—31,2	780,0	406,0	—48,0
1	9 Months	480,2	440,0	—43,6	530,0	—32,2	862,0	379,0	—56,0
4	12 »	723,0	710,0	—18,4	384,6	—46,9	750,0	638,3	—14,9
3	5—7 Years	892,0	535,8	—40,4	524,3	—41,3	886,0	630,0	—28,9
					Acid-soluble phosphorus				
4	1 1/2 Months	369,2	335,7	—9,8	271,9	—26,4	399,8	371,5	—7,1
1	3 »	556,0	417,0	—25,0	187,0	—66,3	500,0	151,5	—70,0
1	9 Months	532,0	314,0	—41,1	358,0	—32,8	497,0	219,3	—55,7
4	12 »	380,0	235,7	—34,2	250,8	—34,1	401,6	329,6	—18,0
3	5—7 Years	394,8	382,1	—3,2	409,6	+9,1	467,0	367,0	—21,3
					Inorganic phosphorus				
4	1 1/2 Months	75,6	65,3	—13,6	49,0	—35,0	—	—	—
1	3 »	102,5	140,8	+37,4	61,0	—40,5	102,5	34,2	—67,4
1	9 Months	134,7	127,1	— 5,6	137,1	+ 1,8	137,4	184,7	+34,4
4	12 »	85,5	73,9	—13,6	50,0	—41,6	85,5	58,1	—32,1
3	5—7 Years	81,1	82,6	+ 1,9	55,1	—32,1	77,9	64,4	—17,3
					Creatine phosphate				
4	1 1/2 Months	92,5	58,5	—36,8	48,2	—47,8	—	—	—
1	3 »	147,2	163,0	+9,7	66,1	—55,0	147,2	23,6	—83,8
1	9 Months	168,8	127,1	—24,6	111,9	—33,7	168,6	162,6	—3,6
4	12 »	89,9	62,1	—30,9	32,4	—63,0	89,9	49,1	—45,5
3	5—7 Years	86,9	68,6	—21,1	69,6	—19,9	127,9	90,2	—29,5
					ATP				
4	1 1/2 Months	75,5	36,0	—52,3	37,6	—50,2	—	—	—
1	3 »	120,5	140,3	+14,1	72,4	—39,9	120,5	111,5	—7,5
1	9 Months	125,5	53,2	—53,6	46,7	—62,8	125,5	99,0	—21,1
4	12 »	104,8	56,5	—46,2	48,2	—54,0	104,8	40,5	—61,3
3	5—7 Years	97,9	57,9	—41,0	50,5	—48,6	99,8	89,4	—10,4

many bands of connective tissue. The gastrocnemius muscle was also pale, but very little deposition of fat could be seen in it.

The maximal biochemical changes in the muscles of the replanted limb were observed 3-9 months after the operation. In contrast to the other phosphorus compounds, the content of nucleic acids in the muscles of the replanted limb was increased 1 year after the operation: RNA by 14% and DNA by 24%. This increase in the DNA content was possibly due to an increase in the number of nuclei in the muscle at this time[1].

It can be clearly understood from the presence of such marked disturbances in the phosphorus composition of the muscles why the function of the limb remains so impaired one year after the replantation [13].

The content of phosphorus compounds in the muscles of the replanted limb in the later period after operation (5-7 years) did not return to normal (Tables 1 and 2).

The normal content of phosphorus compounds likewise was not restored in the gastrocnemius muscle of the replanted limb. In the proximal parts of the thigh muscle (the former stump) the content of phosphorus compounds also did not become normal.

TABLE 2. Content of Nucleic Acids and Phospholipids in the Muscles of the Replanted Limb of Dogs at Different Times after Operation (in mg% of dry weight)

Number of experiments	Time after operation	Thigh muscle					Gastrocnemius muscle		
		control	proximally to scar	changes (in %)	distally to scar	changes (in %)	control	on the side of operation	changes (in %)
					P-RNA				
4	1½ Months	55,4	66,2	+19,5	82,9	+49,6	74,8	97,8	+30,7
1	3 »	101,0	94,9	—6,0	43,3	—47,3	88,9	83,6	—5,9
1	9 Months	57,0	45,5	—20,2	50,6	—11,2	71,5	27,2	—61,8
4	12 »	56,0	47,2	—15,7	64,1	+14,5	67,8	56,6	—16,5
3	5—7 Years	78,1	57,9	—25,9	90,5	+15,9	78,9	61,3	—22,3
					P-DNA				
4	1½ Months	19,8	22,5	+13,6	21,8	+10,1	14,6	17,7	+21,2
1	3 »	13,4	15,1	+12,7	16,3	+22,1	23,1	13,9	—39,8
1	9 Months	12,0	18,5	+5,3	24,8	+106,8	17,8	27,5	+54,5
4	12 »	13,0	14,5	+11,5	16,1	+23,9	18,8	22,0	+17,0
3	5—7 Years	17,1	23,3	+36,3	16,9	—11,7	15,3	18,1	+18,3
					Phospholipids				
4	1½ Months	96,0	71,4	—25,7	54,3	—43,6	85,6	70,3	—17,9
1	3 »	106,8	64,6	—39,6	68,8	—25,5	116,0	91,0	—21,6
1	9 Months	123,0	24,1	—80,5	77,4	—37,1	105,7	36,9	—65,2
4	12 »	108,0	72,3	—33,0	86,4	—20,0	103,0	81,5	—20,9
3	5—7 Years	132,5	101,3	—23,6	101,3	—23,6	85,5	89,9	+5,1

Hence, in the muscles of the replanted limb, for the period of a year after the operation, marked disturbances of the metabolism of various phosphorus compounds were present. In the late periods after the operation the severity of the disturbances was slightly reduced. However, complete recovery of the phosphorus metabolism was not observed. Nevertheless, the animal actively used its replanted limb; it ran, jumped, and outwardly it was hardly distinguishable from ordinary dogs. Evidently the compensatory and adaptive properties of the locomotor apparatus of animals are extremely great, and despite the marked changes in the phosphorus metabolism of the muscles of the replanted limb, its function as a whole outwardly appeared unimpaired.

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