OBSERVATIONS ON DENERVATED AND ON REGENERATING MUSCLE. BY J. N. LANGLEY, F.R.S.

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ALL the observations were made on cats. Some of the sources of experimental error in estimating the degree of muscle atrophy I have mentioned in a previous paper (1) and I need not recapitulate them. In all cases the muscles were taken as nearly as possible alike on the two sides, but as the experiments were made at different periods in the last year and a half, the amount of tendon taken has varied somewhat.

A certain amount of tendon is necessarily taken. Since in some cases the muscle fibres only gradually cease in the tendinous mass, and the point of disappearance varies, I have cut the lower tendons at the ankle joint. The foot was placed at right angles to the leg, the tendo Achillis (after separation of the ligaments and isolation of the soleus tendon) was cut where it begins to enlarge; the tendons of the extensor dig. com. and the tibialis anticus were cut at the annular ligament of the former. The peronei were cut at the joint so that two of the tendons were long. About ·5 cm. of the upper tendon of the exten. dig. com. was taken.

It may be noted that the estimated percentage difference in weight on the two sides is more liable to error the smaller the muscles and the more extensive their attachment to bone or to a common tendon. Thus the probable error in the estimated percentage difference is greater in the popliteus and in the peronei than in the gastrocnemius or extensor dig. com. The tibialis anticus has some deep fibres which are cut away unless care is taken.

The muscles were weighed to a milligram, and the estimated percentage difference calculated from this. In the tables, the weights are only given to the nearest decigram, so that in Tables V and VI the estimated p.c. difference does not exactly correspond with the weights. The 'rest of flexor group' in the tables includes such of the four muscles —popliteus, flexor longus dig., tibialis posticus and flexor longus hallucis —as are not separately mentioned. I have used the usual nomenclature, but the term flexor l. hallucis is singularly little descriptive of the muscle so called.

1. *Normal difference of weight of muscles on the two sides.* In Table I, observations are given of the difference in weight of corresponding muscles on the two sides of the body in two unoperated animals.

In the nine muscles weighed, the difference in weight on the two sides is from 1 to 3 p.c. in five cases, about 5 p.c. in three cases, and nearly 9 p.c. in one case. I have not made more observations on unoperated animals, since thirty-two undenervated muscles on each side of the body were weighed in the experiments in which nerves to other muscles were cut, and these give results which are sufficient for the conclusions drawn in this paper. The results are given in Table II. The percentage difference in these is no doubt somewhat greater than in unoperated animals since in unilateral partial denervation, the limb for a time is less used and in this limb there may be slight disuse wasting in the undenervated muscles; and moreover in all these cases the tension of the muscles was unequal on the two sides in consequence of more or less paralysis of the antagonists on one side. Generally it may be said that in considering the degree of atrophy, a difference of 5 p.c. on the two sides must be disregarded, and a difference of 5 to 10 p.c. treated with caution to an extent varying in the several muscles.

TABLE I. Difference of weight of muscles on the two sides in normal cats.

	Exp. 1		Exp. 2	
Body weight in kilos ...	2·76		2·79	
	Weight on right in grams	Percentage difference on left	Right	p.c. diff. on left
Gastrocnemius and plantaris	19·52	−0·23	20·76	+3·0
Soleus	2·15	−1·77	2·51	−6·8
Rest of flexor group ...	6·86	−1·02	7·03	+1·4
Tibialis anticus	—	—	4·60	+5·3
Extensor dig. com.... ...	—	—	2·24	+4·5
Peronei	—	—	2·30	+8·7

TABLE II. Percentage difference in weight in undenervated muscles on the two sides in cases in which there was more or less paralysis of the antagonistic muscles on one side. (See Tables III, IV, V, VII.)

Gastrocnemius and plantaris	Soleus	Popliteus	Rest of flexors	Tibialis anticus	Extensor dig. com.	Peronei
0·8	1·1	3·0	3·0	2·2	8·0	3·3
0·9	1·2	4·8	3·0	7·0	9·2	4·0
2·1	1·8	8·8	4·3	8·4	10·1	5·6
2·2	4·1					
3·0	5·0	0·9				
4·0	7·1	1·3				
5·1	9·3	5·4				

2. *Weights of muscles on denervated and undenervated sides in absence of regeneration.* Observations on four animals are given in Table III. Two others on the effect of section of the internal popliteal nerve for

twenty-five and twenty-seven days have been given in an earlier paper ((1) p. 343). In all six cases the estimated loss of weight of the soleus was considerably less than that of any other muscle. Whilst the soleus is a red muscle it would not be safe to conclude that all red muscles behave like the soleus. The variations in the weight of the other muscles are not sufficiently constant to allow any definite conclusion to be drawn from them. It may be noticed however that in the extensor group of muscles the estimated loss of weight is roughly proportional to the time of denervation and that there is no regularity in this respect in the flexor group.

TABLE III. Loss of weight in denervated muscles in absence of regeneration. Section on the left side; in Exp. 4 of internal popliteal nerve; in Exps. 3, 5, 6 of both popliteal nerves. The percentage difference of the denervated muscles are in black type.

	Exp. 3		Exp. 4		Exp. 5		Exp. 6	
Duration of denervation	17 days		18 days		21 days		29 days	
Weight at operation	—		2·70 kilos		3·47		3·52	
Weight at death ...	3·8 kilos		2·51 ,,		3·35		3·50	
	Right	Percentage difference on left	Right	p.c. diff. left	Right	p.c. diff. left	Right	p.c. diff. left
Gastrocnemius and plantaris	31·21	**-41·5**	18·94	**-45·6**	27·04	**-41·3**	23·50	**-43·0**
Soleus ...	3·45	**-14·8**	2·05	**- 6·1**	2·80	**-12·6**	3·84	**-25·5**
Flexor long. hall. ...	8·86	**-38·1**	5·69	**-32·1**	8·93	**-39·3**	4·92	**-47·7**
Flexor longus dig. and tibialis post. ...							2·08	**-36·4**
Popliteus ...	1·60	**-43·3**	0·97	**-25·8**			1·58	**-46·9**
Tibialis anticus	7·16	**-42·8**	3·92	- 2·2	6·14	**-45·6**	5·73	**-54·4**
Extensor dig. com. ...	3·22	**-30·7**	2·18	- 9·2	3·09	**-36·1**	3·14	**-44·4**
Peronei ...	3·20	**-28·2**	2·25	- 3·3	3·36	**-32·1**	2·90	**-41·8**

In all cases there was more or less active fibrillation in the gastrocnemius and very little in the soleus. The extensors were only observed in Exps. 3 and 6 and only three to four hours after the beginning of anæsthesia; the fibrillation was considerably less than in the gastrocnemius and was slight in the peronei. In Exp. 6 the cat was long-haired and there was skin disease on the outer surface of the right thigh; the skin was much roughened and the hair had fallen out.

Table IV gives two experiments which are nearly of the same kind as the preceding. In Exp. 7 the peroneal nerve was compressed but not quite completely, and stimulation above the neurome caused trifling contraction in all the muscles. In Exp. 8 stimulation of the nerve caused weak contraction (more than in Exp. 7), so that nervous connexion was beginning to be established. It will be seen that in Exp. 8 as in the other experiments the estimated atrophy of the soleus is much less than that of the other muscles of the flexor group.

TABLE IV. Loss of weight in nearly complete absence of nervous connexion. Exp. 7, nearly complete compression left peroneal nerve. Exp. 8, section of internal popliteal on left side; beginning nervous connexion.

	Exp. 7		Exp. 8	
Duration of denervation ...	9 days		56 days	
Weight at operation in kilos	2·09		3·60	
„ death	2·08		3·30	
	Right	p.c. diff. on left	Right	p.c. diff. on left
Gastrocnemius and plantaris	13·31	− 0·8	23·35	−66
Soleus	1·83	+ 9·3	3·03	−42
Rest of flexor group ...	5·38	+ 5·4	8·37	−60
Tibialis anticus	3·74	−14·7	5·21	− 7
Extensor dig. com.	2·06	−17·8	3·17	− 8
Peronei	2·12	−13·7	3·00	− 4

In Exp. 7, slight and infrequent fibrillation was seen but only after careful observation. In Exp. 8, the fibrillation was slight but fairly obvious. In Exp. 8 the heel could be raised slightly in walking, but it collapsed with very little weight (cp. Exp. 12).

3. *Weights of muscles on denervated and on undenervated sides during regeneration after interruption of the continuity of the nerve by compression.* I have not found it practicable to determine with any accuracy the progress of regeneration by the behaviour of the animal, though marked recovery is recognisable, and I have relied mainly on the effect of nerve stimulation in the anæsthetised animal shortly before killing it.

A cat a few days after section of the peroneal nerve walks on a level surface in a manner not at once distinguishable from normal. On close observation it will be seen that there is a tendency for the ankle joint to bend a little laterally or medially and for the paw to rest more on the phalanges. On uneven ground the toes are now and then bent dorsal surface downwards. If the animal is held up with the hind toes just touching the ground and gently pushed forward, the toes double over; this lasts longer than the other symptoms, but it also decreases apart from regeneration. Sometimes the feet are lifted abnormally high in walking but this is not constant.

Two normal actions are recovered very slowly; the recovery was not complete in any of my cases.

i. The animal can soon make scratching movement with the leg, but the foot at first does not touch the body, and later though it touches it, the toes remain more or less flexed.

ii. If a normal animal is held up by the forepart of the body, the toes are usually more or less separated from one another, and in all cases the toes separate, though to a varying extent in different trials, on suddenly lowering it in the air. The separation increases when the toes just touch the ground and the cat endeavours to obtain foothold. After section of the peroneal nerve the separation of the toes (except that of the 2nd digit which can be abducted by the posterior tibial nerve) does not occur until there is some recovery of nervous connexion. In the early stage of recovery, abduction of the 5th digit occurs as the foot touches the ground; distinct separation of the 3rd and 4th digits is only obtained later. But when the movement is slight, a number of trials is required to make certain that the separation of the toes is not due simply to the weight of the body. In a later stage, separation of the toes occurs on lowering the animal in the air, its extent depends on the suddenness of the downward movement.

After section of the tibial nerve, the recovery symptoms are much more distinct. For some time, the heel touches the ground in walking; as regeneration takes place it is kept off the ground more and more, but the change in the position of the limb is so gradual that the date of beginning regeneration cannot be definitely fixed. The abduction of the 5th digit is usually great when the animal is simply held up in the air.

In order to determine the effect of re-establishing nerve connexion on the progress of muscular atrophy the continuity of the nerve was broken by compression, since this gives the best conditions for uniformity of regeneration. The peroneal nerve was compressed in three experiments about ·75 cm. proximally of its dipping down under the peroneus longus. The method adopted was to place a sterilised metal rod longitudinally on the nerve, tie the nerve tightly round the rod and then to remove the ligature. The rod was used because the peroneal nerve in this region is a flattened band, and compression around a rod would be less likely to break the neurilemmal sheaths. I estimate that the latter were emptied for about ·5 mm. The internal popliteal nerve was tied firmly with a slip knot and the thread released. The completeness of severance of continuity was tested by microscopical examination after death. The musculo-cutaneous nerves at the ankle and one or two muscular nerves were teased out, and all the fibres in them were found to have undergone Wallerian degeneration.

The results of the experiments in Table V are in general features such as might be expected.

Table V. Muscle weight in partial regeneration after compression of peroneal nerve in Exps. 9, 10, 11, of internal popliteal nerve in Exp. 12.

	Exp. 9			Exp. 10			Exp. 11			Exp. 12	
Duration of denervation ...	21 days			36 days			38 days			48 days	
Weight at operation in kilos	2·52			2·53			2·6			3·48	
„ death	2·67			3·45			3·1			3·37	
	Right	Lf.	p.c. diff. left	Right	Lf.	p.c. diff. left	Right	Lf.	p.c. diff. left	Right	p.c. diff. left
Gastrocnemius and plantaris	18·43	—	+ 2·2	23·93	—	– 4	21·05	—	– 5·1	27·37	–23·8
Soleus	2·20	—	+ 1·8	2·42	—	+ 5	1·98	—	+ 7·1	2·95	– 1·1
Flexor long. hall.	6·20	—	– 1·3	4·28	—	– 3	—	—	—	7·98	–22·4
Rest of flexor group ...				3·47	—	– 3	—	—	—	6·19	–19·4
Tibialis anticus	4·61	3·01	–34·6	4·95	3·81	–23	4·55	3·45	–24	7·06	– 8·4
Extensor dig. com.	2·50	1·69	–32·4	2·88	2·34	–18·8	2·50	2·04	–18·3	3·56	–10·1
Peronei	2·24	1·97	–12·0	2·78	2·44	–12·2	2·50	2·06	–17·6	3·36	– 5·6

In Exps. 9, 10, 11, slight abduction of the 5th digit was obtained on the nineteenth day on suddenly lowering the animal when the feet touched the ground. Stimulation of the peripheral end of the peroneal nerve above the point of compression caused contraction in all the extensor muscles of the leg; this was much stronger in Exps. 10 and 11 than in Exp. 9.

In Exp. 9, there was slight but distinct fibrillation, its degree in the several muscles did not correspond with the estimated percentage loss. In Exp. 10, the fibrillation was very slight and infrequent. In Exps. 11 and 12 there was apparently none in the leg muscles, but in Exp. 12 there was active fibrillation in the plantar muscles; stimulation of the posterior tibial nerve caused contraction in these though a strong current was required. The cat in Exp. 12 walked with the heel raised from the ground in an approximately normal manner.

After interruption of nerve continuity by compression the recovery of nervous connexion with the muscle begins very soon. It is indeed surprisingly rapid, for in three weeks slight power of abduction of the 5th digit was recovered, and nerve stimulation caused considerable contraction of the muscles.

It is known that with recovery of nervous connexion, recovery of muscle weight occurs. A comparison of Table V with Table III shows that in all the cases of nerve compression there was more or less recovery of muscle weight. But the results also emphasise the gradual nature of the recovery in nearly all the muscles. Nervous connexion was considerable three weeks after compression, but the tibialis anticus a fortnight later was 23 to 24 p.c. less in weight than that of the other side. The gastrocnemius and the flexors of the toes had about the same deficiency seven weeks after compression. It will be noted that in Exp. 8 (Table IV) in which nervous connexion was not established till there was great muscular atrophy, and then only to a slight extent, the estimated loss of the gastrocnemius and of the flexor group of muscles was about 60 p.c.

An exception to the general gradual rate of recovery is apparently afforded by the soleus muscle. In Exp. 12 the soleus on the side of nerve compression was practically of the same weight as that of the undenervated side, which itself was of normal weight. A similar equality between the weights of the soleus on the two sides occurred in an experiment which I gave in a former paper ((1) p. 343) in which a certain degree of nervous connexion had been established, although the cut nerve had not been sutured. Whilst further experiments are required on this point the results tend to show that in the cat the soleus recovers more quickly from atrophy than the other muscles. And we have seen that in the experiments it atrophied more slowly.

It is known that the regeneration of nerve is centrifugal, and that in general the longer the nerve the longer the time taken in recovery. After suture, as Howell and Huber(2) have shown, the shorter nerve course is not always the first to regenerate, since the time of regeneration depends upon the co-aptation of the ends of the nerve. But after uncomplicated nerve compression, it can hardly be doubted that the shorter the nerve course to a muscle, the sooner its atrophy will cease. On this account I should be inclined to attribute the slower recovery of the flexor muscles (except the soleus) in Exp. 12 than of the extensor muscles in Exps. 10 and 11 to the longer stretch of nerve between the point of compression and the muscle. The same factor may possibly also account for the (apparent) less atrophy of the peronei amongst the extensor muscles since the nerve course to the peroneus long. is the shortest. The difference in nerve response after three weeks and after five weeks shows that the nervous control of the muscles increased in the interval. This is probably due partly to increased nervous supply and partly to increased muscular response. The rate of regeneration of nerve fibres depends, amongst other factors, on the vitality of the nerve cells from which they arise, and this varies, as is shown by the unequal chromatolysis in the cells when the nerves are cut. It is possible too that the block caused by simple compression is unequal in small and in large fibres. So far as there is a difference of time in the establishment of nervous connexion with the several muscle fibres of any one muscle, it must tend to prolong the time required for the whole muscle to recover its normal weight, so that the rate of regeneration of muscle fibres is no doubt somewhat more rapid than is indicated by the total increase of weight.

In Exp. 10 the cat was given 340 mgms. of calcium lactate daily in milk on the lines of earlier experiments (1) in the hope that it would reduce the fibrillation and thus possibly reduce the atrophy. It was compared with the cat of Exp. 11. No certain difference was found in the recovery symptoms. At the time of death there was slight fibrillation, and none, so far as was seen, in the control. As will be seen from the table, the atrophy was practically the same. In Exp. 10, however, the nerves appeared to respond to a somewhat weaker current than in Exp. 11.

4. *Estimation of the original weight of atrophied muscles, when the nerves have been cut on both sides.* In my previous experiments (1) on the effect of electrical stimulation and of passive movements on muscle atrophy, the nerves were cut on both sides and treatment applied to one side, since it might fairly be expected that the rate of atrophy of corresponding muscles on the two sides of the body, would, apart from the treatment, be nearly alike. I have followed the same method in comparing the effects of different forms of suture. In such experiments estimations of the *degree* of change from normal on either side lose the guide afforded by the knowledge of the approximate original weight which is given in unilateral denervation by the weights of the muscle on the normal side.

Probably in any one breed of animals, there is a general relation between the body weight and the weights of the several muscles, but there are no sufficient data with regard to this. The body weights in my experiments take no account of the contents of the alimentary canal, or of the amount of fat, or of the breed.

In cases in which some of the muscles only on the two sides are denervated, a rough estimate of their original weight can be made by noting the relation of their weight to that of the undenervated muscles. In Table VI, I give the weights of the extensor muscles of the leg in relation to the weight of the gastrocnemius and plantaris gathered from the experiments given in this paper and from three previously published. As the second decimal is here of no value, the numbers are given to the nearest first decimal. The not inconsiderable variations shown in some of the cases are in part due to the different conditions of the experiments and to the somewhat varying amount of tendon taken, but to what extent can only be settled by direct experiment. Notwithstanding the variations it will be seen that there is a general relation between the weights of the several extensors and that of the gastrocnemius and plantaris, sufficient at any rate to allow some conclusions to be drawn as to the original weights of the extensors in the cases of suture given in the following section.

It will be noticed that with increasing weight of the gastrocnemius, the weight of the tibialis anticus increases more than does that of the extensor dig. com. or the peronei, *i.e.* with increasing body weight the extensor of the toes increases in weight relatively less than the main flexor and extensor of the foot.

TABLE VI. Relative weights of muscles.

Gastrocnemius and plantaris	Tibialis anticus	Extensor dig. com.	Peronei
13·3	3·7	2·1	2·1
15·5	3·4	2·1	1·8
18·4	4·8	2·5	2·2
18·9	3·9	2·2	2·3
19·6	4·4	2·5	2·3
20·7	4·6	2·3	2·3
21·1	4·6	2·5	2·5
22·9	5·4	2·9	2·7
23·4	5·2	3·2	3·0
23·5	5·7	3·1	2·9
23·9	5·0	2·9	2·8
27·0	6·1	3·1	3·4
27·4	7·1	3·6	3·4
31·2	7·2	3·2	3·2

5. *Weights of muscles during regeneration after different forms of suture on the two sides.* Three experiments were made in which the peroneal nerve was sutured on both sides in varying conditions. In all cases the method of suture was the same. A catgut ligature was passed transversely through one end, then similarly through the other end, and tied so that the ends of the nerve were just in contact. The peroneal

nerve in each case was exposed in the latter part of its course over the gastrocnemius. The objects of the experiments and the method of making them were as follows:

Exp. 13. Since the hindrance to recovery in primary nerve suture is largely due to connective tissue formed at the central end, it seemed possible that by killing a small portion of the central end the formation of connective tissue might be retarded more than the down growth of the axons. An equal length of nerve was isolated on each side and cut. On the left side a piece of indiarubber was placed under about 5 mm. of the central end and 95 p.c. alcohol brushed over it for two to three minutes; the nerve was then well washed with sterilised Ringer's fluid. On the right side the nerve was cut and sutured.

Exp. 14. The peripheral part of the peroneal nerve consists for the most part of separate nerves for the muscles of the legs (cp. Langley and Hashimoto(3)). In suturing it, corresponding ends cannot be kept in contact with any certainty. It seemed possible that the wandering of nerve fibres into wrong channels might be lessened by separating the nerves and cutting away as much as possible of the epineurium, though this is but small in amount. The peroneal nerves were exposed, and 1 cm. isolated. On the left side the nerve bundles, seven in number, were separated, cut, and spread out fanwise, the ligature being passed just above and below the isolated portion. On the right side the nerve was simply cut, and ligatured in the same manner.

Exp. 15. When a gap has to be filled up in a nerve, another piece of nerve is usually used (interposition suture). The nerves taken to fill the gap are in fact always bundles of nerves bound together by connective tissue. A nerve in a perineurial sheath, but without epineurium would offer much less resistance to regeneration. Such nerves of considerable size are present in the dog and cat, and if the nerve, after removal of the epineurium, is heated to 70°–80° C. the diameter is increased, the nerve shrinks to about one-third of its length without becoming bent and it can be kept in alcohol. Whilst it is known that dead nerve is less favourable than live nerve for interposition suture, it seemed possible that a dead nerve consisting of a single bundle might serve as well as a live nerve consisting of many bundles. In order to test this 1 cm. of the peroneal nerve was cut on the left side of a cat, and 1 cm. of dead nerve treated as above (the alcohol being removed by Ringer's fluid) was sewn in its place. On the right side 1 cm. of the peroneal nerve was cut out, and sewn in again.

Different times were allowed for regeneration in order to form some idea of the degree of recovery after suture. The weights of the muscles at the time of death are given in Table VII.

TABLE VII. Muscle weight in regeneration after various forms of nerve suture. Suture on both sides.

	Exp. 13			Exp. 14			Exp. 15[1]		
Operation ...	Alcohol on ·5 cm. of central end, left side			Nerves isolated on left side			Graft—dead nerve on left side Graft—live nerve on right side		
Duration of denervation	58 days			137 days			124 days		
Weight at operation	2·98 kilos			2·73 kilos			3·23 kilos		
" death	3·15 "			2·67 "			3·82 "		
	Right	Lf.	p.c. diff. left	Right	Lf.	p.c. diff. left	Right	Lf.	p.c. diff. left
Gastrocnemius and plantaris ...	25·69	—	+ 2·1	19·43	—	− 3·0	25·61	—	− 0·90
Soleus	3·12	—	− 1·1	3·01	—	+ 1·2	3·01	—	+ 4·05
Popliteus ...	8·87	—	− 0·9	1·09	—	− 8·8	1·42	—	+ 4·80
Rest of flexor group				4·81	—	− 3·0	5·88	—	− 4·29
Tibialis anticus ...	2·18	2·31	+ 6·1	3·56	3·90	+ 9·7	3·63	1·89	−47·82
Extensor dig. com.	1·73	1·41	−19·0	1·72	2·11	+22·2	0·97	0·73	−24·76
Peronei	1·44	1·64	+14·0	1·71	1·64	− 4·1	0·93	0·86	− 7·24

In Exp. 13 (58 days) there was moderate to slight fibrillation in the muscles; in corresponding muscles it was greater on the side on which the muscle was less in weight.

In Exp. 14, the fibrillation was not looked for. It was noticed in this experiment that the irritability of the tibial nerve lasted much longer after death than that of the peroneal nerve above the neurome.

In Exp. 15, there was slight fibrillation on the left side (very slight in the peronei); it was infrequent and barely visible on the right side.

It is known that recovery is much slower after nerve suture than after nerve compression. The slower recovery was very distinct in Exps. 13 and 14 as judged by the symptoms, and in Exp. 13 as judged by nerve stimulation. In Exp. 13 the nerve on the right side was cut and at once sutured, yet nerve stimulation after fifty-eight days had less effect than that obtained after twenty-one days of nerve compression (Exp. 9). The slower recovery is also brought out by a comparison of the weights of the muscles on the right side in Exps. 13 and 14 in which there was direct suture, with those given in Table V after nerve compression, bearing in mind what has been said in § 6 as to the relation between the normal weight of the extensor muscles and the gastrocnemius. The recovery from atrophy after 137 days suture was on the

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[1] The muscles in this experiment were kindly weighed for me by Dr **Hashimoto**.

whole of about the same order as that after thirty-six days compression.

The comparison also suggests that the rate of recovery of muscle weight in the several experiments was more unequal after nerve suture than after nerve compression, but the method of estimating the original weight of the muscles in the former case is not sufficiently accurate to make this certain, and in any case the rate of recovery must depend largely on the degree of apposition of the central and peripheral ends of the several nerve bundles, in suture.

Evidence of the straying out of nerve fibres into wrong channels was investigated in Exp. 13 on the left side, and in Exp. 15 on the right side. In both, stimulation of the central end of the cutaneous branch of the musculo-cutaneous nerve after section of the peroneal nerve above the neurome caused an axon reflex. This was very marked in Exp. 15 in which 124 days were allowed for regeneration; the stimulation caused lively contraction both in, the tibialis anticus and peroneus longus muscles. In Exp. 15 the three bundles which are present in the peroneal nerve centrally of the neurome (cp. Langley and Hashimoto(3)) were stimulated separately on the right side; the cutaneous branch had no motor effect (it gave as mentioned above axon reflexes from the peripheral end). The anterior tibial bundle and the bundle for the peronei, instead of causing contraction confined to their own group of muscles caused contraction in all the muscles of the leg. It may be recalled that Osborne and Kilvington(4) found that on simple suture of the sciatic in the dog, nerve fibres of the flexor division may spread over into the extensor division. The results bring out the drawback attached to suturing as a whole the peripheral bundles in a nerve trunk.

A comparison of the experiments in Table VII tend to show that when even a very short piece of nerve is sutured between the cut ends (Exp. 15 right side) the recovery is distinctly slower than in simple suture. The recovery of the tibialis anticus was far greater than that of the other muscles, indicating that the farther the ends are apart the less regular the regeneration of the several muscles will probably be.

With regard to the special objects of the experiments:

A comparison of the weights of the muscles on the two sides in Exp. 13 shows that the application of alcohol to the central end of the nerve before suture had no certain effect in either hastening or retarding recovery. The differences might be due to the unequal growth of the central axons down the several peripheral nerves.

In Exp. 14 the symptoms indicated a better recovery on the side on which the nerve bundles had been separated. The left leg was used more normally in scratching and at the time of death sudden lowering of the animal in the air caused much wider separation of the toes on the left side than on the right. A comparison of the weights of the muscles with that of the gastrocnemius, and with the relative weights given in Table V, shows that the recovery on both sides was considerable. The better recovery on the left side was the more remarkable since the swelling at the point of suture was slight on the right side and rather large on the left. Thus the isolation of the nerve bundles had caused increased connective tissue growth, but since the nervous connexion was on the whole greater it may be presumed that the axon growth largely preceded the connective tissue formation.

In Exp. 15 the recovery of muscle weight was much less on the side on which the ends of the nerve had been joined by a piece of dead nerve taken from another cat than on that on which they had been joined by the cat's own live nerve. Nerve stimulation just before death caused on the right side strong contraction of the tibialis anticus, moderate contraction of the extensor dig. com. and less contraction of the peronei. The small extent of recovery in the weight of the extensor dig. com. suggests that nervous connexion with it had taken place at a late stage. On the left side nerve stimulation caused rather weak contraction of the peroneus longus, weaker of the extensor dig. com. and a trace only in the tibialis anticus. Both inserted nerves were completely absorbed so far as ocular inspection went. The doubtful point in this experiment was whether the dead inserted nerve was completely sterilised; the skin wound was well joined and dry five days after the operation, but on the tenth day it was found open and slightly septic; after treatment it healed in three days. The sepsis may have seriously interfered with the result.

6. *Irritability and conductivity of regenerating nerve.* All observers have found that a stronger induction shock is required to cause contraction when applied below the point of union of a regenerating nerve than when applied above it. In the regenerating portion, the connective tissue is increased, there are more or less numerous phagocytes, and lumps of myelin are present. Whilst it did not seem probable that this change could offer sufficiently greater resistance to the passage of the current to influence its effect, it seemed worth while to determine whether if a few normal fibres were left in a degenerating nerve, their irritability would be altered. In Exp. 7 as I have said the nerve was not completely compressed, and a few, but only a few, fibres remained

uninjured. The nerve was stimulated below and above the point of compression and the threshold was found to be practically the same.

I suggested in an earlier note on this subject that the lack of irritability of regenerating nerve to induction currents was probably due to their short duration. But there is a similar low irritability of regenerating nerve to galvanic currents. These have been used by some early observers. I confirmed the observation in Exp. 9. The current was made and broken by a contact key put down by hand. A very much stronger current was required to cause contraction when the nerve was stimulated below the point of union than when it was stimulated above.

With the induction current, contraction was obtained on stimulating above the neurome with the sec. coil at 34–35 cm. from the primary; a little below the neurome with the sec. coil at 11. An accumulator cell was then joined up to a rheochord-wire 1 metre. Above the neurome, contraction was obtained with the rheochord contact at 14 cm. Below the neurome, no contraction was obtained with two cells and the rheochord contact at 100 cm. More slowly rising currents have still to be tried.

7. *Fibrillation.* In the experiments of Kato and myself (5) fibrillation in denervated muscle did not begin for a day or more after the nerve section. I have made one other experiment to test this. The left internal popliteal nerve was cut in a cat, and the muscles were examined seventy-one hours later. No fibrillation could be seen. Stimulation of the nerve caused no contraction. Thus the changes produced by denervation must reach a certain intensity before they lead to fibrillation.

Variations in the degree of fibrillation are not easy to be certain about, since its distinctness depends upon the degree of illumination, the angle of incidence of the light, and the amount of surface fluid. When fibrillation is feeble it requires very careful observation to see it at all. It was mentioned by Kato and myself that in some cases the fibrillation varied considerably in different parts of the gastrocnemius. There is in fact, usually some difference in intensity in different parts in all muscles. In the tibialis anticus, the fibrillation is usually most active in the upper portion. Further, adjoining muscle fibres appear to be differently affected, for when the fibrillation is not active it may be distinct in some small patches, slight and very rare (if not absent) in others. When fibrillation is active, it causes a short indentation (about 1 mm.) on the surface; when it is slow, the indentation may either be of the same length or be longer (3 to 4 mm.); in the former case the contraction is apparently weaker; in the latter case apparently stronger.

The immediate cause of fibrillation is probably an increased permeability to salts. I have mentioned in an earlier paper(1) that intravenous injection of 150–300 mgms. of calcium chloride lessens and may stop it. But the effect varies in different animals. The cessation so far as I have seen is always brief, commonly the heart is stopped before the fibrillation. Potassium iodide 1 p.c. locally applied in one case caused a decrease, in another it had no effect. A similar result was obtained with 5 p.c. guanidine chloride. The injection of pituitary extract (1 c.c. vaporole of Burroughs and Wellcome said to contain ·2 grm. of fresh pituitary) caused pallor of the muscles lasting half-an-hour but did not affect the fibrillation. I had expected that it would reduce the fibrillation since Kato and myself had found this effect with adrenaline which causes less protracted pallor. The variations which occur on injection suggest that the fibrillation is influenced by something set free in the body.

Fibrillation is increased by warmth and decreased by cold; it decreases during prolonged anæsthesia and this may in part be due to a direct action of the anæsthetic. Notwithstanding the decrease on fall of temperature, it is remarkable how long the fibrillation may continue after death. Kato and myself described it in rabbits as lasting for a few minutes but we did not watch it to its cessation. I have paid attention to a few cases in cats, and find that it may last for half-an-hour after bleeding to death an anæsthetised animal, gradually becoming more feeble and infrequent and ceasing in the different muscles at different times.

If fibrillation is the cause of atrophy, the atrophy, other things being equal, would be greater when fibrillation is most active. One difficulty of determining any relation of this kind is that the fibrillation observed at the time of death is not necessarily that which has occurred during the previous period. My observations have been made at various times during the course of anæsthesia so that they are not strictly comparable. The results, so far as they go, are as follows:

There was a striking difference between the degree of fibrillation of the soleus and gastrocnemius; in each of the eight cases which I have observed, the fibrillation was much less in the soleus than in the gastrocnemius and as said above the atrophy was less. It may be mentioned that in the rabbit Kato and myself(5) did not find any constant difference in fibrillation in the two muscles.

In the experiments on denervation without regeneration (Table III), a comparison of the fibrillation in the gastrocnemius and the extensor muscles was only made in two experiments and this three to four hours after the beginning of anæsthesia; it was much less in the extensor muscles, and there was no direct relation between the degree of fibrillation and the estimated atrophy of the muscles. It may be noticed however that in one of these experiments (Exp. 6) the pathological condition of the skin may have affected the estimated percentage difference of weight.

In the experiments on nerve compression with regeneration (Table V) the fibrillation was slight after twenty-one days, there was very infrequent slight fibrillation after thirty-six days, and it was doubtful whether there was any after thirty-eight and forty-eight days. The results point to a fairly rapid cessation of fibrillation—as of atrophy—in this form of regeneration. But a puzzling point is that there was very little fibrillation in Exp. 7 (Table IV) nine days after a nerve compression which was nearly complete.

In suture the conditions are more complex, since some fibres may receive nervous connexion and others may not. Further on the theory that fibrillation is the cause of the atrophy, the fibrillation should decrease in prolonged denervation since the rate of atrophy decreases. In the experiments given above some evidence was obtained that both these factors are operative, but there were considerable differences in the duration and extent of the fibrillation in the different cases. In Exp. 8 (Table IV, 56 days) there was great atrophy with just beginning regeneration, and the fibrillation was slight; but in Exp. 13 (Table VII, 58 days) with considerably greater recovery there was better fibrillation. In the latter case the degree of fibrillation on the two sides corresponded with their degree of atrophy. In Exp. 15 (124 days) there was slight fibrillation on the left side on which there was very little regeneration, but there was much less on the right side on which there was much more but still incomplete regeneration, and on this side the fibrillation did not correspond with the degree of atrophy.

Whilst then the theory that fibrillation is the cause of atrophy is in harmony with some of the facts, it is not, so far as the experiments have gone, in harmony with them all.

8. *Regeneration in muscles stretched by their antagonists.* In none of the experiments given in this paper was a splint put on to prevent extension of the paralysed muscles. The cats with paralysed extensor muscles were kept in cages for about a week, they were then put in a run opening into an out-of-doors enclosure and the animals walked freely. Thus whilst there were frequent passive movements of the

paralysed muscles, they were for the greater part of the time stretched by their antagonists. The cats with paralysed flexors were kept in cages until in walking they could raise the heel slightly from the ground, they were then let out for intervals depending on the degree of recovery.

The rapid progress of recovery in the cases given in Table V shows that the stretching of the paralysed muscles by their antagonists had little if any injurious effect. If there was any it must have been approximately compensated by the beneficial effect of the movements which were allowed to take place.

It seems to me incorrect to speak of paralysed muscles stretched by their antagonists as being in a state of over-extension. They are not more extended than they sometimes are by perfectly harmless movements in life, and their tension is less than in such movements since their own tonic contraction is abolished. What is injurious is not the extension itself, but its long unbroken continuance, just as the tonic shortening of the antagonists is not injurious unless it is protracted. As the muscle atrophies it is less and less able to withstand abnormal conditions and protracted extension becomes more and more harmful.

In the early days after suture, movement of the joints tends to draw the nerve ends apart; whether it does so or not depends upon the firmness of the suture and its position in the length of the nerve. But the ends of the nerve are soon fixed by connective tissue growth, this fixation indeed is an important function of the connective tissue, and moderate movement then has no harmful effect.

So far as these statements can be applied to man, I conclude that in cases of primary suture, whilst the paralysed muscles should on the whole be kept relaxed, moderate passive movements might be carried out with advantage two or three times a day as soon as the movement does not strain the skin ligatures. Probably in many cases a splint allowing slight movement would be better than a rigid one.

SUMMARY.

The effect of nerve degeneration and regeneration was studied chiefly from the point of view of changes in the muscle weight. All the observations were made on cats.

The corresponding muscles of the leg of the two sides in normal animals usually differed in weight from 1 to 5 p.c., but occasionally there was a larger difference. Consequently in considering the atrophy of muscles all differences up to 5 p.c. must be neglected, and differences from 5 to 10 p.c. considered only in proportion to the constancy of their occurrence.

The denervated soleus muscle lost weight less rapidly than the other muscles of the leg (8 Exps.). In the two experiments in which there was regeneration it recovered weight more rapidly than the other muscles.

On interruption of the continuity of the peroneal nerve by compression, nervous connexion with all the extensor muscles of the leg was rapidly established, even in three weeks marked contraction was obtained on nerve stimulation. The atrophy at this time had begun to disappear; its further decrease was gradual.

After nerve suture, the recovery from atrophy, as would be expected, was very much slower; the experiments suggest a greater inequality in the recovery of the several muscles than in nerve compression and a still greater inequality when a piece of nerve is cut out and sutured in again.

Three experiments were made on the comparative recovery on the two sides after suture of the peroneal nerve in various conditions.

(*a*) There was no certain difference in recovery when a small portion of the central end of the nerve on one side was killed by alcohol.

(*b*) Isolation of the bundles of the nerve appeared to favour recovery.

(*c*) There was much better recovery on insertion of a piece of the animal's live nerve than on insertion of a piece of dead nerve which had a perineurial sheath only, but in the latter case there was some sepsis.

When a few sound fibres are present in a nerve the remaining fibres of which have degenerated, the threshold of irritability is practically the same throughout. The observation that the irritability of regenerating nerve is low for galvanic as well as for induction currents was confirmed.

The activity of the fibrillation found at the time of death was much less in the soleus than in the gastrocnemius muscle; it was very slight or absent in all muscles in the later stages of recovery after nerve compression; it lasted much longer after suture, and was slight when the rate of atrophy was presumably slow on account of its long standing. But in some cases no correspondence was found between the activity of fibrillation in the several muscles and their degree of atrophy.

Although the paralysed muscles were not prevented from being stretched by their antagonists, the recovery after nerve compression was rapid, so that either the extension of the paralysed muscles had little or no injurious effect, or this effect was compensated by the more or less frequent passive movements which occurred in locomotion.

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