OBSERVATIONS ON DENERVATED MUSCLE[1].

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In a Paper published not long since, some observations were recorded by Professor Kato[2] and myself on the effect of electrical stimulation on denervated muscle in rabbits. The experiments were not numerous enough to give decisive results, but so far as they went they were in general accord with the result deduced from observations clinically obtained in man. They tended to show that stimulation with condenser shocks delayed the atrophy, that rhythmic extension and flexion had a similar but less effect. The stimulation did not prevent fibrillation nor the sluggish contraction characteristic of denervated muscle. In these experiments the maximum degree of stimulation corresponded approximately to that commonly given in cases of paralysed muscles in man, viz. 200 condenser shocks each day, in the other three cases the degree of stimulation was less. The extent of the daily contraction thus produced is obviously very much less than that which occurs in normal muscles in life. A walk of 200 yards would cause more than 200 contractions in the leg muscles, and each of these would be a short tetanus, whilst the contractions with condenser shocks are single contractions and moreover in all probability do not involve the deeper muscle fibres. It is well known in man that electrical stimulation of muscle, in the extent to which it is carried on, does not prevent the wasting of denervated muscle though it is generally held to delay it. If the atrophy is simply a "disuse atrophy" caused by absence of contraction it cannot be expected to be prevented unless the degree of contraction is something like that which takes place in the normal muscle. In view of this I have tried the effect of more prolonged stimulation by electrical currents and more prolonged passive movements.

The plan of the experiment was to cut the nerves on both sides, to subject the denervated muscle on one side to treatment, and at the

[1] Some of the points dealt with in this account I have touched on in a short paper in the *Lancet*, 191, p. 6. July 1, 1916.

[2] Langley and Kato. This *Journal*, XLIX. p. 432. 1915.

end of a certain time to note the difference in the weight and other conditions of the muscles of the two sides. In such experiments there are several sources of error in determining accurately the weight of the muscles at the end of the period of stimulation. (*a*) The muscle must be carefully dissected out, and to do this takes time, and in consequence there is evaporation from the surface of the muscles and some loss of weight. I have equalised this as far as possible by dissecting in stages so that each pair of muscles was exposed to the air for an approximately equal time. Each muscle as soon as it was removed was weighed by an assistant. (*b*) It is not easy to clear the muscles from other tissue to exactly the same extent, nor to cut the tendons in exactly corresponding positions, without unduly exposing them, nor to separate muscles in exactly the same way when they are closely attached to bone. (*c*) Before the animal was killed, observations were made on the irritability of the muscles, the denervated muscle was stimulated for a somewhat longer period and was somewhat more exposed than the normal muscle and this may have caused a slight difference in weight.

These sources of error are however probably small, especially in the case of the larger muscles. A more important cause of difference in the weight of the muscles at death is I think a normal and variable difference in weight at the beginning of the experiment. It is true that Debedat (*op. cit. infra*) found in a month old rabbit that the biceps femoris, the semi-membranosus, and the semi-tendinosus on the two sides were of exactly the same weight. But supposing this result is confirmed by further observations it does not preclude an unequal development of the muscles in half-grown and adult animals. In order to form an estimate of the extent of the various sources of error I have weighed the muscles of normal animals, dissecting them out in the same way as in the experimental animals. The results are given in Table I. In this table the weight of each muscle on the left side is given. Taking this as standard the excess or deficit in weight of the corresponding muscle on the right side is given. The difference is reckoned as the percentage difference of the muscles on the right side from those on the left.

These observations show that by the method adopted there may be a difference of nearly 7 p.c. in the weight of the muscles on the two sides; but a difference to this extent only occurred in two out of 16 cases. It is of course possible that in individual cases there may be a wider difference. In the experiments of Prof. Kato and myself the combined tibialis anticus and extensor dig. ped. comm. on the

TABLE I. *Weight in grams of muscles on the two sides in normal rabbits.*

	No. 1			No. 2. Weight 2·3 kilos.			No. 3. Weight 2·1 kilos.		
	Left	Difference on right	p.c. diff.	Left	Difference on right	p.c. diff.	Left	Difference on right	p.c. diff.
Gastrocnemius and soleus	11·02	−·08	−0·7	9·16	+·33	+3·6	9·665	−·200	−2·1
Plantaris ...	4·20	+·24	+5·7	3·75	−·02	−0·5	4·020	−·065	−1·4
Flexor dig. ped. comm.	—	—	—	2·82	+·02	+0·7	2·705	−·090	−3·3
Tibialis ant. ...	2·6	+·15	+5·8	2·37	−·08	−3·3	1·940	+·120	+6·2
Extensor dig. ped. comm.	2·6	+·06	+2·3	2·02	−·04	−1·9	1·905	+·050	+2·6
Peroneii ...	—	—	—	1·91	+·13	+6·8	1·565	−·040	−2·6

It may be noticed that the relative development of the several muscles varies in different individuals; thus in No. 3 the plantaris was heavier, but the tibialis anticus lighter than in No. 2.

two sides were weighed in 12 cases; in eight of these the difference in weight was less than 2·7 p.c., in two only greater than 6·3 p.c., but in one of these it was 12 p.c.

We may now consider the effect of treatment. The first question to decide was its daily duration. It seemed to me that 1 to 2 hrs. would be adequate to produce a decided effect on the theory of disuse atrophy, and that this duration would not be sufficient to cause fatigue, since an animal can, without fatigue, take daily active exercise for much longer than 2 hours. The electrical stimulation was, then, carried on for 2½ hours daily. Since the electrodes were shifted about on the skin surface, no muscle was stimulated for the whole of the time. Judging from the position of the electrodes, the movements obtained, and the hardening of the muscles, the order of degree of stimulation was: the gastrocnemius (about 2 hours), then the tibialis anticus and extensor dig. ped. comm., much less frequently the more superficial peroneal muscles, and only occasionally the flexors.

In order to compare the effect of passive movement with that of the active movement produced by electrical stimulation, the leg of another operated rabbit was alternately flexed and extended for 2½ hours a day. In doing this, the foot was held, the leg extended and then pressed back against the body to the fully flexed position.

Nov. 29. The internal and external popliteal nerves cut on both sides in two adult rabbits under ether. The animals were in good health throughout.

Exp. 1. Stimulation with condenser shocks on the *right* side was begun on the following day for 2½ hrs., and continued each day till Dec. 20. On Dec. 21 stimulation for 1 hour. The stimulation was generally for three periods during the day, sometimes only for two at 9 a.m. and 3 p.m. The electrodes were round and covered with chamois leather, one about ½ cm. in diameter, the other about ¼ cm.; they were moved about for part of the

time on the moistened skin surface; for part of the time the smaller electrode was pushed down on the medial surface of gastrocnemius, slightly moved at intervals, whilst the larger electrode was placed on the outer part of the gastrocnemius or on the outer part of the shin. The direction of the current was reversed at short intervals. A Lewis Jones condenser apparatus was used, 100 volts; the currents in the first week were generally ·025–·062 microfarads, later ·05–·08 mf. Slight contraction could be obtained up to the last day with ·025 mf. The shocks were at the rate of 36 a min.

Dec. 21. Animal anæsthetised with chloroform, then with A.C.E. and urethane. On the left side the fibrillation was very active; on the right (stimulated) side it was slower, but the separate contractions apparently stronger. Condenser shocks, ·016 mf. with both electrodes on the exposed muscle caused weak contraction only in the left gastrocnemius or tibialis anticus, but obvious contraction on the right; shocks of ·025 mf. caused contraction in both muscles but stronger on the right. Single induction shocks just felt to the tongue caused local contraction on both sides (the threshold stimulus was not determined), but much less than in the biceps femoris and semi-membranosus. The injection of 3 c.c. of 5 p.c. $CaCl_2$ into the jugular vein decreased the fibrillation especially on the right side; an additional 3 c.c. caused a further decrease. The animal was bled and the muscles cut out. They were kindly weighed for me by Mr Winfield as soon as they were removed from the body.

Exp. 2. Extension and flexion began on the day following the operation and continued for 2½ hours a day till Dec. 22 inclusive. Rate of complete flexion and extension about 30 a minute. On Dec. 23 flexion and extension for 1 hour.

Dec. 23. Animal anæsthetised as in Exp. 1. There was little difference in the fibrillation on the two sides, but it was possibly a little more active on the right side. Condenser currents ·016 mf. caused contraction on both sides when the rounded electrodes just touched the muscle, but not when pressed on the muscle, *i.e.* when the current density was decreased. With platinum electrodes, single induction shocks not felt on the tongue caused local contraction, no difference in the threshold was found on the two sides. With the large electrodes, the threshold for single induction shocks appeared to be a trifle lower on the right side. Warm 5 p.c. calcium chloride in Ringer's fluid, applied to the surface of the right gastrocnemius, nearly stopped its fibrillation. Injection of 3 c.c. 5 p.c. calcium chloride stopped the fibrillation on the right side and nearly stopped it on the left side. Injection of another 3 c.c. did not completely stop the fibrillation on the left side. Animal bled and muscles cut out and weighed.

It is clear from the results given in Table II, that neither electrical stimulation nor passive movement had any definite effect in preventing loss of weight. The difference in the weight of the muscles on the two sides, though greater in a few cases than in the control animals (Table I), is not so much greater as to give any certainty that it was due to the treatment. Moreover in Exp. 1 the increase or decrease in weight bears no relation to the degree of stimulation, and in Exp. 2 it bears no relation to the degree of extension and flexion. In Exp. 1, there was contraction in some part or other of the gastrocnemius muscle about 4000 times a day; the extensor dig. I and popliteal were probably not made to contract at all, and the other muscles a very unequal number of times. If contraction were sufficient in itself to prevent muscular wasting, one or other of the muscles should have lost weight

much less than the corresponding muscle of the opposite side. As this did not occur, it may fairly be concluded that the atrophy of denervated muscle is not due to the absence of contraction. It does

TABLE II. *Effect of electrical stimulation and of passive movement on denervated muscles of rabbit.*

	Exp. 1	Exp. 2
Weight at beginning	3·05 kilos.	2·55 kilos.
Weight at end	3·25 „	2·88 „
	Nerves cut both sides 21 days. Electrical stimulation on right side.	Nerves cut both sides 23 days. Flexion and extension on right side.

The weights of the muscles are given in grams.

	Left side	Diff. on right side	p.c. diff. on right	Left side	Diff. on right	p.c. diff. on right
Gastrocnemius and soleus ...	9·77	+·48	+4·9	6·78	+·42	+6·2
Plantaris	3·55	+·05	+1·5	2·57	+·04	+1·5
Flexor dig. ped. comm. ...	3·48	−·31	−8·9	2·76	+·09	+3·3
Extensor dig. I and popliteal	1·46	−·025	−2·0	1·30	0	0
Tibialis ant.	2·43	−·15	−6·2	1·72	+·22	+12·2
Ext. dig. ped. comm. ...	3·03	−·23	−7·6	2·44	−·23	−9·4
Peroneii	2·17	−·145	−6·2	1·43	−·17	−12·0

not however follow that stimulating denervated muscle may not have a slight beneficial effect. In Exp. 1 the stimulated gastrocnemius seemed to be distinctly (though not greatly) more irritable than the unstimulated one.

Observations on the results following section of spinal cord in animals and clinical observations in man show that unused muscles decrease in weight; this is no doubt a true disuse atrophy but the process I consider is too slow to be of appreciable effect on the rapid loss of weight which follows denervation.

Experiments on the effect of electrical stimulation on the weight of normal muscles have been made by Debedat and Bordier. Debedat[1] experimented on five rabbits one month old and of the same litter. On one side the posterior thigh muscles were stimulated through the skin for four mins. daily, 30 times a minute. The kind of stimulation varied in the different cases. At the end of 20 days the muscles on the two sides were weighed, Debedat found an increase in weight on the stimulated side and a greater increase with faradic than with galvanic stimulation. The increases were:

	Faradic stim.	Galvanic stim.
Biceps ...	30·4 p.c.	18 p.c.
Semi-tendinosus	50·0 „	23 „
Semi-membranosus	15·4 „	15 „

Bordier's[2] experiments were made on man. He stimulated on one side the muscles of the upper arm with galvano-faradic currents. The stimulation was for alternate 30 secs. for 10 mins. three times a week for a month. He found that the circumference of the arm was appreciably increased.

[1] Debedat. *Arch. d'Élec. Méd.* II. p. 100. 1894.
[2] Bordier. *Ibid.* X. p. 331. 1892.

Whilst both sets of experiments tend to show that stimulation of normal muscles causes an increase in their weight, the results cannot I think be transferred to denervated muscles; further the additional contraction caused by stimulation must have been slight compared to the normal contraction so that it is difficult to refer the effect to the contraction itself.

Since the atrophy is not due to absence of contraction, *i.e.* is not a disuse atrophy in the ordinary meaning of the term, we have to consider to what other cause it may be due. There are three possibilities:

i. The theory that nutrition (assimilation and dissimilation) is directly affected by nervous impulses has long been a subject for discussion. In its later development it has two forms, one that nutrition is influenced by a special class of nerve fibres and the other that it is influenced by a special kind of nerve impulse in the common nerve fibres. As regards muscle, there is no independent evidence of a special nutritive class of nerve fibres, and the work on the characters of the nervous impulse does not suggest the existence of impulses of more than one kind. In consequence the theory of nutritive nerve impulses is hardly likely to be seriously considered until all other ways of explaining the atrophy have been disproved.

ii. The question of the continuity or discontinuity of nerve and muscle fibres has also long been discussed. If there were continuity, the nerve might have a direct nutritive action on the muscle by chemical interchange, of a somewhat similar nature to that which occurs between the nucleus (or body) of a nerve cell and its axon. I may incidentally point out that on the theory of continuity we might—on the lines of Nernst's theory of excitation—refer inhibition to discontinuity since the ions accumulating at the membrane would be of opposite sign in the two cases. But the histological evidence is now almost universally considered as against the theory of continuity, and until further evidence is forthcoming we must look for some other cause of the atrophy than a chemical interchange between nerve and muscle.

iii. A third possibility is that the atrophy is due to the continuous fibrillation which occurs in denervated muscle. The fibrillation was noted recently by Prof. Kato and myself (*op. cit. supra*) and we referred to Schiff's oft-quoted discovery of this in the muscles of the tongue after section of the hypoglossal nerve. I have since then looked up Schiff's original paper in 1851[1], and find that he gave a fairly complete account of the phenomenon. He described fibrillation in the muscles of the foot after section of the sciatic nerve, and oscillation of the large supra-ciliary hairs of the rabbit after section of the facial nerve. He

[1] Schiff. *Arch. f. physiol. Heilk.* x. p. 587. 1851; and *Ibid.* p. 665.

concluded that fibrillation was a general phenomenon in muscle after nerve section, and he stated that after regeneration of the nerve the fibrillation ceased.

At this time the old controversy of the independent irritability of muscle was still active. Schiff attributed the fibrillation—as all quick contraction—to nerve action. He considered that muscle itself was irritable, but that the irritability was shown by a slow and prolonged contraction.

Rogovicz in 1885 confirmed Schiff's result of the occurrence of fibrillation on section of the facial nerve, and described it in the sterno-mastoid after section of the spinal accessory. Further results were given by Schiff in 1892. These papers deal chiefly with "pseudomotor" action and I shall discuss them in a later paper on this question.

Since the separate muscle fibres are in rapid continuous rhythmic contraction, the total daily expenditure of energy must be considerable and it seems probable that the brief intervals between the contractions are insufficient to allow the wasting to be made good.

The change in the reaction of muscle which occurs after denervation is in most respects at any rate like that which occurs in progressive fatigue. The irritability to currents of very short duration decreases, the period of shortening becomes greater, and the period of relaxation is very greatly prolonged. I may remark that in physiological experiments it has frequently been noticed that denervated muscle still responds to faradic currents. In my own experiments the decrease in irritability to single induction shocks and to tetanising currents has been slow and gradual, and I think that muscle would be very near the stage of ceasing to contract with any stimulus by the time it ceased to respond to faradic currents provided they are sufficiently strong. If small pointed platinum electrodes are employed and placed direct on the muscle, induction currents not or barely felt on the tongue will cause contraction in a denervated muscle which has lost 50 p.c. of its weight. The contraction is of course confined to a few fibres. The minimal contraction is best seen by viewing the muscle by light reflected from its surface. The current causing the minimal contraction varies somewhat according to the position of the electrodes on the muscle fibres; probably because the neural region—as in the frog—is more irritable than the non-neural region.

Barcroft and Kato[1] have shown that the specific gravity of fatigued muscle is less than that of unfatigued. In the experiments mentioned in Table III, I determined the sp. gr. of the gastrocnemius (with plantaris) on the two sides; in each case the sp. gr. was less on the denervated side.

[1] Barcroft and Kato. *Phil. Trans.* (London), B. 207, p. 164. 1915.

In Exp. 3 the sp. gr. was determined immediately after the removal of the muscles from the body. On the normal side it was 1074, on the denervated 1064. In the other cases the sp. gr. was not determined at once, and the muscles, though covered up in small glass vessels, had lost weight by evaporation; the results however were practically the same.

Two other facts may be quoted as *prima facie* in favour of the fatigue theory, though further investigation of them is necessary. (*a*) The only other peripheral tissue which loses weight rapidly after section of its nerves is that of the salivary glands and in these the atrophy is accompanied by a continuous secretion. (*b*) In the frog, denervated muscle does not as a rule obviously diminish in size[1], and fibrillation, in the considerable number of cases in which I have looked for it, did not occur. Hofmann[2] noticed fibrillation in the denervated muscle of the toad, but not in that of the frog. It would be interesting to see if there is a difference in the rate of atrophy in the two animals.

It must however be mentioned that on the fatigue theory, the atrophy of the muscle would be hastened by additional contraction, and that in Exp. 1 in which there was additional contraction there was no clear evidence that the atrophy was hastened. It is however possible that stimulation has a counterbalancing effect, either by a direct nutritive action on the muscle, or by the consequent contraction driving on metabolites.

If the wasting is due to over-activity, the method of treatment is obviously to reduce the activity. In the protocols of the experiments given above, I have mentioned that calcium chloride solution applied locally, or injected into the circulation, decreased or stopped the fibrillation. It was therefore desirable to determine whether a soluble calcium salt given with the food would lessen the fibrillation and reduce the atrophy. Cats were taken for the experiments since the calcium could readily be given in the milk. The internal popliteal nerve was cut on the left side in three cats. Two of these were given milk containing calcium lactate. The control cat, and one of those to which calcium lactate had been given, were killed after about three weeks, and the muscles on the two sides compared. The third cat was left for two months. The animals thrived and at no time was there any indication of disturbance of nutrition.

Each cat was given 75 to 100 c.c. of milk morning and evening. An hour or two after the morning milk, fish or meat was given. The whole of the milk was not always

[1] Cp. Langley. This *Journal*, XXXVII. p. 289. I have since found, in one out of a number of cases, a considerable decrease in denervated frog's muscle. This was in the gastrocnemius 95 days after nerve section. The denervated muscle weighed 190 mgms., the muscle of the opposite side 280 mgms.

[2] Hofmann. *Arch. f. exp. Path. u. Pharm.* LXV. p. 12. 1911.

taken, the amount left was measured. At the end of the period, the animal was anæsthetised, the irritability of the gastrocnemius on the two sides compared and some other observations which it is unnecessary to mention here.

Exp. 1. Control. Left internal pop. n. cut 27 days. Animal anæsthetised. Left muscle in active fibrillation, injection of 3 c.c. $CaCl_2$ stopped the fibrillation in about 45 secs., this effect lasted for 10 mins. when other drugs were injected.

Exp. 2. Left int. pop. n. cut 25 days. Administration of calcium lactate in milk after first two days; average daily amount taken 570 mgms., at end of period, anæsthetised; left gastrocnemius in active fibrillation. Injected into jugular vein 3 c.c. 5 p.c. $CaCl_2$, fibrillation nearly stopped in 45 secs. (the duration of this effect was not observed, other drugs were injected and the fibrillation began again).

Exp. 3. Left int. pop. n. cut 60 days. Administration of calcium lactate after first two days; daily amount taken irregular, on average 355 mgms. daily. On 9th day cat had kitten, which flourished. At the end of the period there were signs of some return of voluntary power. The animal was anæsthetised. The left gastrocnemius much redder than right; fibrillation considerably less than in the other animals but individual contractions rather stronger. The central end of the internal popliteal nerve was found to be joined to the peripheral end by a swelling (neurome) about ·5 cm. long, tie and cut the nerve above the neurome and isolate it; stimulation above the neurome with secondary coil at 20 cm. (current not felt on tongue) caused backward movement of foot, flexion of toes and an obvious contraction of the gastrocnemius. Stimulation of the nerve below the neurome with the same currents caused a trace of movement only in the outer part of the gastrocnemius. Stimulation below the neurome with sec. coil at 10 caused good contraction of the gastrocnemius but no flexion of the toes, this current above the neurome caused stronger contraction, flexion of foot and protrusion of claws[1]. The nerve branches to the soleus muscle were placed in osmic acid and teased, they contained pale band fibres, but no myelinated fibres. The nerve a little below the neurome contained numerous myelinated fibres, most of them being 3–4 μ in diameter, a few 6–8 μ.

TABLE III. *Cats. Left internal popliteal nerve cut. Nos. 2 and 3 given calcium lactate twice a day in milk.*

	Exp. 1. Control 27 days			Exp. 2. Calcium lactate 25 days			Exp. 3. Calcium lactate 60 days		
Body weight at beginning of Exp.	2·00 kilos.			2·72 kilos.			2·42 kilos.		
Body weight at end of Exp.	2·48 „			3·28 „			2·65 „		
	Weight in grm right side	Diff. of weight on left side	p.c. diff.	Weight in grm right side	Diff. of weight on left side	p.c. diff.	Weight in grm right side	Diff. of weight on left side	p.c. diff.
Gastrocnemius and plantaris	15·49	−6·92	−47·7	22·9	−12·44	−45·7	19·950	−7·06	−35·4
Soleus ...	1·95	−0·34	−17·4	2·8	−0·73	−26·0	1·975	−0·025	− 1·3
Flexor muscles } Popliteal }	5·43	−2·47	−45·5	7·44	−3·51	−47·2	4·890 1·020	−2·095 −0·400	−42·8 −39·2
Tibialis anticus ...	3·35	−0·21	−6·3	5·35	−0·28	−5·2	4·375	−0·085	−1·94
Extensor dig. comm. ped.	2·05	−0·14	−6·8	2·91	−0·16	−5·5	2·540	−0·375	−14·76
Peroneii ...	1·81	−0·17	−9·4	2·68	−0·11	−4·1	2·335	−0·030	−1·3

[1] Physostigmine had recovered its power of causing fascicular contractions in the gastrocnemius; this I shall refer to in a later paper on the action of drugs on nerve endings.

A comparison of Exps. 1 and 2 shows that the administration of calcium lactate in the food did not delay the atrophy of the denervated muscles. But since at the time of death the muscles were in active fibrillation, the experiments are inconclusive as to the relation between fibrillation and atrophy. The absence of effect may have been due to imperfect absorption from the intestine, or to the excretion by the kidneys preventing sufficient accumulation of calcium salts in the blood. Further experiments on the effect of giving calcium salts in the food would probably be best made on the tongue, in which the fibrillation could be observed from day to day; but it is possible that the introduction of $Ca^{\cdot\cdot}$ by ionisation may be more effective in stopping the fibrillation than giving it in the food.

The regeneration in Exp. 3 was marked in 60 days although the central end of the nerve had to grow about ·5 cm. before joining the peripheral end; in the absence of comparative experiments it cannot however be said that the calcium feeding had anything to do with this rapid rate of regeneration. As is usual in regenerating nerve, the irritability to faradic currents was much greater centrally of the original point of section than peripherally of it; moreover the effects produced were somewhat different.

Summary and Conclusions.

Neither passive movement of denervated muscle, nor active movement caused by electrical stimulation, has any certain effect in delaying its loss of weight.

The atrophy of denervated muscle is not a disuse atrophy in the ordinary sense of the term, *i.e.* it is not due to absence of contraction.

Since denervated muscle is in constant fibrillation, it is possible that the atrophy is a fatigue atrophy.

The fibrillation is stopped, or nearly stopped, by local application and by venous injection of soluble calcium salts.

Two experiments were made on the effect of adding calcium lactate to the food. In one, fed for 23 days, there was no delay in the muscle atrophy, but the fibrillation was still active. In the other, fed for 60 days, considerable nerve regeneration occurred and some recovery of muscle weight. At the time of death the fibrillation was less active than in the other animals.