THE JOURNAL OF NEUROLOGY, NEUROSURGERY AND PSYCHIATRY

FOUNDED BY S. A. K. WILSON

VOL. 8. [New Series]

JANUARY-APRIL, 1945

Nos. 1 and 2

METABOLIC FACTORS AFFECTING FIBRILLATION IN DENERVATED MUSCLE

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(RECEIVED 11TH JANUARY, 1945)

Introduction

FIBRILLATION, the spontaneous rhythmical twitching in voluntary muscles following denervation, has been the subject of many observations since Schiff (1851) first described the phenomenon. Some workers have dealt with the time of onset and distribution of the activity, and others have concerned themselves with the unit in action and the frequency of the contractions. The electrical activity accompanying the twitches has also been investigated and a number of observers have been concerned with the chemical factors which influence fibrillation. Finally the relation of fibrillation activity to muscle atrophy following denervation has received considerable attention.

In this paper we present the results of a study, by means of action potential records, of the relation between muscle fibrillation and metabolic activity. Different species of experimental animal have been used and observations have also been made from experimentally denervated muscles in man.

A Review of the Literature

(a) *The Direct Observations of Denervated Muscle.*—Schiff (1851) was the first to give an adequate description of the phenomenon of fibrillation. His observations were made on the tongues of dogs after section of both hypoglossal nerves. He notes that for three days after nerve section the tongue is completely paralysed. Between three and five days after section, a few spontaneous surface twitches are seen. These rapidly increase in number until the whole tongue is in a state of continuous agitation. He says that the tongue as a whole appears to be undulating but that this is due to summation of the spontaneous asynchronous rhythmic twitches of individual muscle bundles. After three or four months the activity is somewhat weaker and at the end of six months has ceased; the tongue is then pale and grossly atrophied. In the rabbit, fibrillation commences between the third and fourth day,

earlier than in the dog, and Schiff relates this to the loss of "electrical conductivity" of the severed nerve fibres which also, he says, disappears earlier in the rabbit. He also makes the observation that fibrillation occurs in limb muscles after interruption of their nerve supply, but that it can only be seen when the skin and fascia are removed. He says of the limbs, that air or other external stimulus increases fibrillation activity, but that this is not a constant phenomenon. Finally, he notes that after section of the facial nerve in the rabbit the vibrissæ of the affected side start to quiver on about the fourth day. When the nerve regenerates, this spontaneous quivering ceases.

Following Schiff's observations, the phenomenon was recorded again by both Rogowicz (1885) and Ricker (1901), but they added nothing to the account given by Schiff. Langley and Kato (1914-15*b*) re-investigated the phenomenon in some detail. They confirmed Schiff's general observations, and added the following details. The onset of fibrillation is established by the fifth day in the limb musculature of the rabbit and is best seen by reflected light from the muscle surface. As a rule, fibrillation at any one spot has a constant rhythm. The rate, rhythm, and intensity of contraction vary considerably in different parts of the surface of the same muscles, but the phenomenon is generally similar in both red and white muscles. Fibrillation ceases a few minutes after death, before the phenomenon of indirect excitability disappears. The rapidity of fibrillation is lessened by feeble circulation, and the surface application of adrenalin for this reason may stop it. Indeed, if the blood-flow through a muscle be stopped for ten to fifteen minutes, on restoration of circulation fibrillation remains greatly diminished or absent.

In a later paper Langley (1917) mentions that fibrillation is increased by warmth and decreased by cold, but gives no details of experimental findings in this connection. He also states that the activity

varies from muscle to muscle; for instance, in the cat it is always less in the soleus than in the gastrocnemius, although he did not find this particular difference in the rabbit. He observes that a few fibrillary twitches can be seen in the cat for a considerable time after death and he believes that he and Kato "did not observe for long enough on the rabbit." He also finds that fibrillation is slight in muscles in the later stages of recovery following denervation.

Langley and Kato (1914-15a) and Langley and Hashimoto (1918) observe that fibrillation varies in different parts of the same muscle, but that it is relatively constant at any one spot. The contracting unit they believe to be very localized, 0.5 to 1 mm. in length, and from this they infer that a portion only of a muscle fibre is involved in the contraction. Eccles and his collaborators (1941) have shown conclusively, however, that the whole of the muscle fibre is involved in the contraction.

Hines and Knowlton (1933) state that, following division of the sciatic nerve in the rat, typical fibrillary contractions are seen as early as the third day after denervation in the gastrocnemius and continue for at least forty-two days, which was the longest period during which observations were made. Denny-Brown and Pennybacker (1938) confirm previous observations that fibrillation commences in the cat's gastrocnemius on the fifth day at the earliest.

Tower (1939) has reviewed the literature on the subject and states that she has observed fibrillation in a muscle kept experimentally denervated for a year. She has also shown (1941) that, after isolation of the lumbar spinal cord and dorsal root section in the monkey, fibrillation does not occur within two weeks of the operation in the paralysed muscles unless, in addition, the sciatic nerve or other lower motor neuron is sectioned. Solandt and Magladery (1942) made similar observations after high thoracic cord section with dorsal root section in rats, but it is interesting to note that on the side opposite to that, on which the sciatic nerve was cut, transient muscle atrophy and increased acetylcholine sensitivity were recorded. Solandt, Partridge and Hunter (1943) noticed that following the fixation of a skeletal muscle (nerve supply intact), atrophy took place, and although there was an increased sensitivity to acetylcholine, fibrillation did not occur. The fixed muscles contracted only occasionally, and after the second day were usually flaccid. They suggest that, if fibrillation is due to an increased sensitivity to acetylcholine, as claimed by Denny Brown and Pennybacker, these fixed muscles should have exhibited the phenomenon. Reid (1941) has shown that denervated muscles in the frog do, on occasions, fibrillate, and has made the interesting observation that the phenomenon never occurs before the forty-sixth day and, moreover, is only seen in "summer" frogs, i.e. those operated on between November and January (Australia). He also notes that fibrillation in one frog was increased from 100 to 140 twitches per minute on the application of warm Ringer solution.

(b) *The Electrical Activity accompanying Fibrillation of Denervation.*—Schäffer and Licht (1926) noted fine oscillations of the string galvanometer when recording from four-days' denervated tongue musculature in the dog, but their apparatus was relatively insensitive and thus the form of the action potentials was uncertain.

Proebster (1928) described spontaneous action currents recurring at intervals of about 1/9 second, which he obtained from the relaxed weak left biceps muscle of a thirteen-year-old boy who had probably suffered a traumatic birth injury to the brachial plexus. Recordings from normal muscles of the opposite side of the body showed no such action potentials. He also reports that action potentials of a similar type can be obtained from cases of long-standing post-poliomyelitic paralysis. Proebster considered that these action potentials were due to periodic discharge from the central nervous system. It is, however, more probable that they were fibrillation action potentials.

Brown (1937) has described the electrical activity of denervated (cat gastrocnemius) muscle recorded with concentric needle electrodes. It consists of a continuous series of diphasic action potentials repeating at from two to seven times per second. The insertion of the needle excites a number of fibres, and for a while rapid discharges of some 30 per second may be encountered. The highest voltage recorded was 30 microvolts, and Brown remarks how close this figure is to that recorded from single muscle fibres excited by acetylcholine (25 microvolts). This, he says, leads to the conclusion that these spontaneous action potentials are to be attributed to the activity of single muscle fibres.

Rosenblueth and Luco (1937), also using cats, recorded from tongue, face, and leg muscles five to forty days after denervation. They also used concentric needle electrodes. They found that from all the denervated muscles they studied a continuous asynchronous series of spike potentials could be led off with concentric needle electrodes. The spontaneous activity was greatest 10 to 30 days after nerve section. They also describe the effect of drugs on the spontaneous activity (*vide infra*). Denny-Brown and Pennybacker (1938) have summarized the literature relating to the action potentials from denervated muscles. They have shown that action potentials in no way differing from those obtained from completely denervated muscles in animals can be obtained from the affected muscles of patients suffering from amyotrophic lateral sclerosis, or from facial palsy with partial recovery. Tower (1939) also summarizes the literature on the action potentials from fibrillating muscle, and finds that the rate of contraction of the unit responsible for the activity is around 9 per second in the cat. This rate remained unchanged throughout a year of observation; only the amplitude of the action potential decreased as the muscle atrophied.

(c) *The Effect of Drugs on the Activity of Fibrillation of Denervation.*—Langley and Kato (1914-15b)

state that curare and nicotine are without effect on fibrillation in the gastrocnemius of a rabbit. Physostigmine does not cause any marked increase in spontaneous fibrillation, and curare after physostigmine does not cause any marked decrease. They were not certain that these drugs had no effect. Langley (1915-16) states that calcium chloride causes the cessation of fibrillation when applied locally or parenterally, and Langley (1917) found that the injection of pituitary extract, although rendering the muscle pale, was without effect on fibrillation, whereas the surface application of adrenalin decreased the activity markedly.

Frank, Nothmann and Hirsch-Kauffmann (1922) have shown that the intravenous injection of acetylcholine increases greatly the spontaneous fibrillation of the denervated dog's and cat's tongue. Brown (1937) has shown the increase of fibrillation activity in response to the intra-arterial injection of small doses of acetylcholine; large doses give rise to "electrical silence."

Rosenblueth and Luco (1937), using cats, confirm Brown's findings, and, in addition, show that atropine has no effect on spontaneous fibrillation activity; both eserine and prostigmine cause a transitory increase in activity. In addition, both eserine and prostigmine, especially the latter, sensitize muscle to acetylcholine. They also state that in the early stages following denervation (5-6 days), acetylcholine even in large doses only increases spontaneous fibrillation activity and no contracture takes place. Fifteen to 30 days after denervation large doses of acetylcholine cause contracture and "electrical silence." It thus appears that the contracture response to acetylcholine develops more slowly than the contraction response. Finally they found that relatively small doses of curare were required to reduce markedly the effects of the intravenous injection of acetylcholine.

Denny-Brown and Pennybacker (1938) showed that a drop of saline containing acetylcholine, diluted 1 : 500,000, allowed to fall on the surface of a muscle just commencing to fibrillate on the 7th day after nerve section, brought about an immediate local contraction lasting some twelve to twenty seconds. As the contraction subsided, all the muscle fibres in the area remained fibrillating for many minutes, and gradually the disturbance passed by *imperceptible transition* of moderate fibrillation into the original state of a few isolated fibres fibrillating.

Solandt and Magladery (1940) tested the influence of a large number of drugs, anaesthetics, and procedures such as electrical exercise, adrenalectomy, etc., on the spontaneous fibrillation activity of the rat's gastrocnemius-soleus group. They recorded the fibrillation action potentials with needle electrodes through the skin, in addition to checking their results by visual examination of the muscle on a number of occasions. They found electrical recording to be more sensitive than visual inspection. Anaesthetics such as ether and the barbiturates were without effect on fibrillation activity, as were

atropine, curare, and eserine. Potassium chloride, in doses which are frequently lethal, temporarily abolishes fibrillation; calcium chloride temporarily arrests the activity but there is rapid adaptation. Quinine hydrobromide abolishes the activity for one-half to four hours whereas quinidine sulphate is effective for nine hours. Acetylcholine given intravenously in comparatively large doses can abolish the activity for 10 to 30 minutes.

Levine *et al* (1942), also using the denervated gastrocnemius-soleus complex of rats, obtained results somewhat different from those of Solandt and Magladery. They ordinarily used the method of visual inspection of the muscles although in some experiments action potentials were recorded. In particular they found that atropine, in addition to quinine and quinidine, inhibited fibrillation. They also found that ether and nembutal anaesthesia inhibited the activity 30 minutes and longer following induction. They found that prostigmine noticeably increased the activity.

Magladery and Solandt (1942) have shown that spontaneous fibrillation is increased by the intra-arterial injection of small concentrations of potassium chloride. The excitation, like that produced by acetylcholine, is abolished by quinidine in doses which arrest spontaneous fibrillation. They suggest that acetylcholine, potassium, or both may be the casual agents of fibrillation of denervation.

(d) *The Relation of Denervation Atrophy to Fibrillation Activity.*—Langley (1915-16) was of the opinion that fibrillation contributes markedly to denervation atrophy, even if it is not the only factor involved. In support of this is the higher oxygen consumption of denervated rabbit's muscle compared with normal muscle (Langley and Itagaki, 1917). Knowlton and Hines (1934), however, failed to find a raised oxygen consumption in denervated rat's muscle up to 28 days. Langley and Hashimoto (1918) say of rabbits that the degree of fibrillation seen at the time of death corresponds in general to the degree of atrophy of the muscle, but there are exceptions; for instance, the fibrillation in the soleus is usually less than might be expected in relation to its atrophy. This confirms previous observations in the cat (Langley, 1917). Solandt and Magladery (1940) prevented fibrillation in the rat's denervated gastrocnemius-soleus complex with quinidine, but found that the loss of weight due to atrophy was still marked. They concluded that fibrillation was not responsible for the denervation atrophy. Levine *et al.* (1942) on the other hand, state that the administration of prostigmine, which increased spontaneous fibrillation in the denervated gastrocnemius-soleus complex of the rat, was accompanied by an increase in the rate of atrophy by 47 per cent.; by contrast, atropine, which decreased spontaneous fibrillation, was accompanied by a decrease in the rate of atrophy by 39 per cent. Fischer (1939), however, has shown that electrically induced exercise decreases muscle atrophy following denervation and also increases the oxygen consumption of the affected muscle.

Summarizing this review of the literature, there is general agreement as to the main features of fibrillation activity, but there are some aspects which are not yet satisfactorily explained. For instance, the time of onset of fibrillation varies in different species of animal. In general, the onset is more rapidly established in small animals, but no analysis of the factors responsible for the variation has been made. In addition, the fact that fibrillation activity differs from muscle to muscle in the same animal and even in different parts of the same muscle has not been accounted for, but it is clear that workers using the method of visual inspection have been more concerned with this variation than those using the method of electrical recording through the intact skin.

The literature contains contradictory statements on the effect of drugs, particularly with regard to atropine. However, in no case have observers reported that the factor of local or body temperature has been taken into account during the course of their experiments. Discrepant findings have also been reported with regard to the relation of denervation atrophy to fibrillation, although the weight of evidence is against any direct relationship between the two phenomena.

Material and Methods

Material.—The laboratory animals used were mice, rats, guinea-pigs, rabbits, and an adult rhesus monkey. In addition, one of us (R. E. P.) submitted to a series of observations carried out on the experimentally denervated brachio-radialis and extensor carpi-radialis muscles in his left forearm.

Operative Procedures.—1. In the mice, rats, guinea-pigs and rabbits, the sciatic nerve was exposed in the thigh under intravenous nembutal anaesthesia, the sural nerve was separated from the sciatic complex, and the tibial and peroneal nerves were crushed 10 times with smooth-bladed forceps at the mid-femur level. In two rabbits the peroneal nerve only was crushed at its point of entry into the anterior tibial muscle group, and in one rabbit 3 cm. of the peroneal nerve were resected and the muscle group which it supplies was kept denervated for two years.

2. In the monkey, the right radial nerve was exposed in its course in the radial groove of the humerus under ether anaesthesia. The nerve was crushed 10 times with smooth-bladed forceps.

3. In one of us (R. E. P.) under local anaesthesia, the nerves of the left brachio-radialis and extensor carpi-radialis longus muscles were crushed 10 times with smooth-bladed forceps 5 cm. from the points of entry into their respective muscles.

Recording Apparatus.—The action potentials are led off with concentric needle electrodes (Adrian and Bronk, 1929) in all cases. The insulated core of the needle is connected to the amplifier through a lead shielded by earthed flexible steel gas piping, itself in electrical contact with the hypodermic needle. The amplifier and recording apparatus follow conventional lines. An all-mains high and low tension supply is used. Hum is eliminated by feeding high tension to the pre-amplifier valves through a four-stage resistance capacity smoothing circuit; the filaments being supplied with smoothed rectified alternating current. Resistance capacity networks are also necessary to eliminate disturbances due to 1–5 cycle modulation of the mains voltage. The pre-amplifier feeds voltage and power amplifiers driving respectively a cathode ray oscilloscope and loud speaker. The frequency response of the amplifier is linear from 30 to 2,000 cycles. There is a small falling off between

2,000 and 4,000 cycles. The low-frequency cut-off makes it possible to record action potentials within less than half a second of the insertion of the needle electrode into the muscle.

Recording Technique.—With the amplifier switched on, the needle electrode was pushed rapidly through the skin into the denervated muscles. Recordings were made from the day following denervation up to functional recovery. In animals submitted to peroneal and tibial nerve crushing, recordings were made from the anterior tibial group of muscles; in the monkey, they were made from the extensor communis digitorum muscle, and in man, from both the muscles which had been denervated. A number of recordings were made from different parts of the selected muscles on each occasion. Anaesthetics were not used for making recordings, for the procedure is less painful than giving a hypodermic injection. Functional recovery was estimated in the usual way in man and monkey. In the remaining animals the toe-spreading reflex was found to be the most reliable guide, although it cannot be directly related in time to the onset of functional recovery in the anterior tibial group (Altschul and Turner, 1942). Care was taken that the room temperature in which the recordings were made was between 18° C. and 22° C. and the animals were brought into the room three hours before observations were made.

EXPERIMENTAL OBSERVATIONS

(1) The Electrical Activity of Normal Muscle

It is necessary to give a brief description of the electrical activity recorded by concentric needle electrodes from normal muscle in order to contrast it with that obtained from denervated muscle.

The insertion of the electrode into a normal muscle in any of the animals used—including man—causes a brief outburst of electrical activity which in no way differs from that due to motor unit activity related to reflex or voluntary movement. The number and frequency of the action potentials and duration of the outburst depend both on the speed and depth of insertion of the electrode, and we have assumed that it is caused by the mechanical stimulation of terminal nerve fibres. It is heard as a rumble from the loud speaker. When the electrode is at rest in the muscle and the animal quiet, there is “electrical silence.” When muscles contract, the motor unit activity gives rise to action potential spikes up to 1 millivolt in amplitude and from 5 to 10 milliseconds in duration (Fig. 1); these are also heard as a series of low-pitched sounds. The description given of motor unit activity in normal muscle in man by Weddell *et al.* (1944) is in general applicable to animals. Mono-di-, and triphasic spikes and occasional polyphasic spikes, which are of high amplitude, are seen. The amplitude of motor unit action potentials in the same muscle varies, but lies between 100 microvolts and 1 millivolt; the duration is from 5 to 10 milliseconds. The only notable difference is the smaller average amplitude and shorter average duration of the action potentials obtained from corresponding muscles in the smaller animals (Fig. 2). This leads to a series of rather higher pitched sounds being heard from the loud-speaker in small as compared with large animals. In small animals, fewer muscle fibres must compose motor units in corresponding muscles, for we have found, on histological examina-

FIG. 1.—Motor unit action potentials from a normal human brachio-radialis muscle during a minimal voluntary contraction.

FIG. 2.—Motor unit action potentials from the normal tibialis anterior muscle of a guinea-pig. The muscle was only contracting feebly.

FIG. 3.—A motor unit and fibrillation action potentials from an 8 days denervated tibialis anterior muscle (rabbit).

FIG. 4.—Fibrillation action potentials from the tibialis anterior muscle of a mouse denervated 6 days previously.

FIG. 5.—Fibrillation action potentials from a human brachio-radialis muscle denervated 30 days previously.

FIG. 6.—Fibrillation action potentials from the tibialis anterior muscle of a rabbit weighing 1.5 kilogrammes 20 days following denervation.

tion, that individual muscle fibres vary but little in diameter in the species of mammal referred to in this paper. We have related this fact to our findings in man, that in muscles which have to perform delicate movements (i.e. face and larynx) motor units are composed of fewer fibres than in the limb muscles, and give rise to correspondingly smaller and sharper action potentials (Weddell *et al.*, 1944).

(2) The Time-course of the Electrical Activity in Denervated Muscle

1. *The Activity aroused by insertion of the Needle Electrode.*—Following denervation, no spontaneous motor unit activity is recorded from muscles, but the insertion of the electrode still gives rise to an outburst of motor unit action potentials. This mechanically aroused activity can be provoked for

varying times in the different species of animal used; for instance, in the mouse it can be obtained for 48 hours and in man for 18 days (see Table I). The larger the animal the longer this activity can be aroused. As already stated, it has been assumed that the activity in the intact muscle is due to stimulation of terminal nerve fibres by the needle point, but this can hardly be true in man two weeks after denervation. It may be that the motor unit is a morphological entity which retains its identity for a considerable time after denervation, and responds as a whole to mechanical stimulation. This mechanically aroused motor unit activity rapidly decreases in amount with the onset of fibrillation. Fibrillation action potentials appear at varying times after denervation, depending upon the size of the animal under observation; in small animals (mouse) they appear within 48 hours, in man they do not appear for 16 days (see Table I).

At first fibrillation action potentials only appear on insertion of the needle electrode and overlap by varying periods the mechanically aroused motor unit action potentials. They take the form of mono- or diphasic spikes 3 to 100 microvolts in amplitude and 1 to 2 milliseconds in duration and are heard as sharp clicks from the loud-speaker. It is difficult to distinguish both visually and audibly the chief type of action potential early in the overlap period, particularly in the mouse. Within 12 hours of the commencement of change of type of action potential, however, the two varieties can be clearly distinguished (Fig. 3). When motor unit

action potentials disappear, spontaneous fibrillation action potentials make their appearance. A few days after the appearance of spontaneous fibrillation action potentials, insertion of the electrode evokes a large number of high frequency fibrillation action potentials (frequencies up to 40/second have been recorded) which gradually die away in the course of 3-4 seconds, leaving a residue of 4-5 spontaneous repetitive fibrillation action potentials.

(2) *The Spontaneous Activity.*—This increases in amount (number and frequency of the action potentials) for two weeks following its commencement, but the recording methods used do not allow of a quantitative estimate of the increase. It is also significant that the amount of activity is related to the size of the animal under investigation. For instance, it is far more vigorous in the mouse than in man (see Figs. 4 and 5). Moreover, in rabbits of the same breed, the amount of fibrillation obtained is smaller in large animals of the same age (see Figs. 6 and 7). Spontaneous fibrillation activity has been obtained in the anterior tibial group of muscles of a rabbit which had been kept denervated for two years. The average number of action potentials recorded is, however, somewhat less than in recently denervated muscle (Fig. 8). The frequency of spontaneous fibrillation action potentials when fully developed is from 2 to 10/second. In man, atrophy was easily recognizable clinically within two weeks of operation, and the circumference of the forearm had diminished by 2 cm. but spontaneous fibrillation had not yet commenced.

TABLE 1.—THE TIME-COURSE OF THE ELECTRICAL ACTIVITY OF DENERVATED MUSCLES

Species and number of animals	Weight in kg.	Motor unit action potentials: mechanical	Fibrillation action potentials: mechanical	Fibrillation action potentials: spontaneous	Motor unit action potentials: voluntary or reflex	Functional recovery	
						Days after operation	Days after appearance of first motor unit action potential
Mice (5)	40-45 gm.	Up to 3 days. Precise time difficult to estimate.	20 hours	40 hours	8 days	11	3
Mice (5)	45-50 gm.	Up to 3 days. ditto	24 hours	48 hours	9 days	12	3
Guinea-pigs (5)	300-450 gm.	Up to 3 days. ditto.	40 hours	2 days	26	30	4
Guinea-pigs (5)	450-600 gm.	Up to 3 days. ditto.	48 hours	3 days	29	34	4
Rabbits (10)	1.5-2.0 kilos.	Up to 8 days. ditto.	4 days	5 days	38 days	45	7
Rabbits * (10)	2-2.8 kilos.	Up to 8 days. ditto.	5 days	7 days	41 days	53	12
Rabbits (2)	2.0 and 2.1 kilos.	Up to 8 days. ditto.	4 days	5 days	13 days	22	9
Monkey (1)	6.8 kilos.	Up to 8 days. ditto.	8 days	9 days	42 days	49	7
Man (1)	76.3 kilos.	Up to 18 days. ditto.	16 days	18 days	55 days	57 and 70	2 and 15

* Nerve crushed close to muscle.

FIG. 7.—Fibrillation action potentials from the tibialis anterior muscle of a rabbit weighing 2.8 kilogrammes 20 days following denervation

FIG. 8.—Fibrillation action potentials from the tibialis anterior muscle of a rabbit 2 years following denervation.

FIG. 9.—Fibrillation action potentials from the tibialis anterior muscle of a thyroidectomized rabbit 20 days following denervation.

FIG. 10.—Fibrillation action potentials from the tibialis anterior muscle of a rabbit fed on desiccated thyroid 20 days following denervation.

FIG. 11.—Fibrillation action potentials from the tibialis anterior muscle of an anaesthetized (ether) eserinated rabbit 14 days following denervation. (When this record was made the needle electrode had been in position for one half-hour.)

FIG. 12.—As for Fig. 11, but three seconds after the injection of 20 mg. of acetylcholine intravenously. The position of the electrode was unaltered.

In cases where the nerves are crushed, spontaneous fibrillation continues unabated until about two weeks before motor unit action potentials first appear, when a marked decrease in the number of

the action potentials recorded occurs. It has not been possible to measure this decrease quantitatively for it would require an excessive number of needlings and recording material, but it is very definite. The

first motor unit action potentials appear close to the point of entry of the nerves into the muscles, and subsequently spread from these points through the muscles. Between 5 and 6 days after the first motor unit action potentials are recorded in the rabbit, the activity has spread throughout the muscle group. An appreciably longer time (12 days) was recorded in man, and shorter time (3 days) in the mouse due to the size of the muscles under examination. During the spread of motor unit activity, fibrillation virtually disappears, although in some cases a few fibrillation action potentials can be recorded from isolated areas for as long as a year following re-innervation.

When motor unit action potentials first appear following re-innervation, they are not sustained for longer than a few seconds at a time; moreover, they are of low amplitude and short duration and can only be distinguished from fibrillation action potentials in their relation to voluntary or reflex movement. Some weeks following re-innervation, motor unit action potentials assume their characteristic form, although their range of amplitude is greater than in normal muscles and a considerable number of polyphasic action potentials of medium amplitude are seen (Weddell *et al.*, 1944). Functional recovery in the animals submitted to sciatic crush cannot be detected until some time after the appearance of motor unit activity. We have found that the toe-spreading reflex appears about the same time as the anterior tibial muscle group can be felt to contract.

In the monkey the early stages of functional recovery were difficult to assess, so that the figures given in Table I are only approximate. In man, in the brachio-radialis muscle, the first onset of functional recovery was stated by the subject to have occurred on the day the first motor unit action potentials were elicited. Two days later minimal movements were observed. The assessment of recovery was, however, easier in the case of this particular muscle owing to its having become adherent to the overlying scar. In the extensor carpi radialis muscle, which was not attached to skin, recovery was not obvious for some days. A

more detailed analysis of our findings in man has already been given (Weddell *et al.*, 1944).

In the rabbits in which the peroneal nerves were crushed close to their entry into the muscle groups, there was no essential difference in the time-course of electrical activity.

(3) Factors Influencing the Time-course of Fibrillation of Denervation

(1) *The Effect of Thyroidectomy.*—Our finding that the time of onset and quantity of spontaneous fibrillation in denervated muscles depends on the size of the animal and not the species, suggests that a metabolic factor is concerned in the activity. We therefore submitted 5 rabbits to thyroidectomy 4½ weeks before the tibial and peroneal nerves were crushed. This period was chosen as the result of the observations of Marine (1922) that the metabolic rate following thyroidectomy in rabbits is by this time lowered 35 to 40 per cent. Our observations are given in Table II, and show clearly that thyroidectomy delays the onset of fibrillation activity.

A curious finding which we are at a loss to explain is that the amplitude and frequency of fibrillation action potentials after thyroidectomy, both spontaneous and mechanically aroused, are greater than in normal control animals. The frequency of the action potentials is sometimes high enough to give rise to musical notes from the loud-speaker (Fig. 9).

(2) *The Effect of Feeding Desiccated Thyroid.*—Four grams of desiccated thyroid were fed daily to two rabbits of known weight for a week, by which time they had started to lose weight daily. The peroneal and tibial nerves were then crushed in the usual way. On the day following denervation, the insertion of the electrode gave rise to a more vigorous outburst of motor unit activity than in the control animal, but the same was true of muscles with intact nerve supplies. Fibrillation commenced earlier in the animals fed with thyroid and was more vigorous when fully established than in control animals, although not so vigorous as that in thyroidectomized animals (Fig. 10). Table III summarizes our findings.

(3) *The Effect of Temperature.*—In our pre-

TABLE II.—THE TIME-COURSE OF THE ELECTRICAL ACTIVITY OF DENERVATED MUSCLES.
(Thyroidectomized rabbits)

Species and number of animals	Weight in kg.	Motor unit action potentials: mechanical	Fibrillation action potentials: mechanical	Fibrillation action potentials: spontaneous	Motor unit action potentials: voluntary or reflex	Functional recovery	
						Days after operation	Days after appearance of first motor unit action potential
Rabbits control (4) ..	1.6-2	up to 7 days	4 days	5 days	38 days	45	7
Rabbits thyroidectomized (5)	1.9-2	up to 8 days	8-10 days	12-14 days	30 days	34	4

MUSCLE FIBRILLATION AND METABOLIC ACTIVITY

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TABLE III.—THE TIME-COURSE OF THE ELECTRICAL ACTIVITY OF DENERVATED MUSCLES
(Rabbits fed on desiccated thyroid)

Species and number of animals	Weight in kg.	Motor unit action potentials: mechanical	Fibrillation action potentials: mechanical	Fibrillation action potentials: spontaneous	Motor unit action potentials: voluntary or reflex	Functional recovery	
						Days after operation	Days after appearance of first motor unit action potential
Rabbits control (4) ..	1.6–2.0	up to 7 days	4 days	5 days	38 days	45	7
Rabbits fed on desiccated thyroid (2) ..	1.62–1.7	up to 3 days	2 days	3 days	36 days	43	6

liminary experiments with rabbits we compared the electrical activity with the activity seen visually on each occasion. In this way we confirmed the observations of previous workers and were able to establish conclusively that the visual picture was reflected in the action potential picture. Following these preliminary observations we conducted a second series of experiments with the skin intact. Our observations under these conditions were far more consistent and, although we found that fibrillation did vary from place to place in a muscle, the variation was far less than when the muscle was exposed; moreover, the difference in the average activity in various muscles was not so marked as that reported by Langley (1917) using the method of observation of the exposed muscle.

In the next series of experiments we tried the effect of warming and cooling exposed fibrillating muscles, both locally and as a whole. We found that the activity was extremely sensitive to temperature change. Bathing with cool Ringer's solution (18° C.) stopped the activity, which was restored by bathing with a solution at 38° C. Exposure to the air in a cool room (15° C.) for five minutes stopped the activity, but it was restored on shining a lamp (100 watt bulb in an "Anglepoise" lamp 2 ft. distant) on the muscle for one minute. It rapidly died away again on removal of the lamp. Further experiments showed that cold air currents would stop a local region of muscle fibrillating. A reduction of 1.5° to 2° C. in local muscle temperature (measured by buried thermo-couples) was effective in the rabbit. The effect of warmth and cold was next tried without removal of the skin, by applying warm (39° C.) and cold pads (18° C.) around the denervated limb of a rabbit. The electrode was first inserted in the muscle and a steady stream of impulses recorded; on application of the cold pad, the activity gradually died away and within five minutes had ceased. It gradually returned as the limb grew warm again, but was rapidly restored by the application of the warm pad. The assessment of the quantity of fibrillation activity by inspection is clearly liable to grave errors unless precautions are taken to keep the muscle moist and at a *uniform* temperature.

The Effect of Drugs on Fibrillation of Denervation

We have re-investigated the effect of a few drugs under controlled temperature conditions in the rat. In particular we were interested in the effect of atropine, for opinion in the literature is divided as to its effects. Table IV summarizes our results. We find that our observations coincide with those of Solandt and Magladery (1940) but are at variance with those of Levine *et al.* (1942). In addition we tried the effect of prostigmine intravenously in an unanæsthetized rabbit. 2.5 mg. were injected into an ear vein at the same time as recordings were being made from the left anterior tibial group of muscles denervated 2 weeks previously. There was a rapid increase in fibrillation activity which reached a maximum in 120 seconds, and lasted for some 15 minutes. The rabbit became weak following the injection and could not maintain its upright position, and the limbs felt quite flaccid. All normal muscles were fasciculating vigorously at this time. Recovery was complete within 35 minutes of the injection.

TABLE IV

Drugs	Effect of fibrillation determined visually and by action potential records	Dose (rats, 150–210 gm.)
Ether anæsthesia.	Nil.	In full anæsthetic doses.
Nembutal anæsthesia.	Nil.	In full anæsthetic doses.
Atropine sulphate.	Nil.	Up to 100 mg.
Quinine.	Abolishes the activity up to 5 hours.	20 mg.
Quinidine sulphate.	Abolishes the activity up to 10 hours.	25 mg.

Finally in 3 rabbits (weighing approximately 2 kilogrammes each) with sciatic nerves crushed two weeks previously, recordings were made from the denervated muscles with the animal under nembutal anæsthesia and at constant temperature. 20µg of acetylcholine were injected intravenously.

There was no measurable increase in fibrillation. 0.2 mg. of eserine were next injected, again without obvious effect. The acetylcholine injection was then repeated. There was a great increase of fibrillation activity which reached a maximum within 3 seconds (Figs. 11 and 12). In 30 seconds the activity had returned to normal. These experiments confirm the observations of Rosenblueth and Luco (1937).

Discussion

Summarizing our observations we have shown that the time of onset of fibrillation following denervation varies with the size of the mammal; the smaller the mammal the earlier the onset. We have shown that the quantity of fibrillation decreases as the size of the animal increases. Thyroidectomy delays the time of onset of fibrillation, while the administration of desiccated thyroid advances it. Warmth increases fibrillation; cold decreases it. In short, we have shown that fibrillation is dependent on a metabolic factor. Finally, we have demonstrated that from the clinical point of view denervation atrophy is detectable before fibrillation action potentials are recorded. These observations probably account for some of the inexplicable and discrepant findings in the literature reviewed above. They certainly explain the varying time of onset of fibrillation in different species of mammal.

The effect of temperature on fibrillation activity is presumably directly related to metabolism, and we have shown that very small changes have a profound effect on the activity. This probably explains the puzzling variations in the activity reported by Langley and his collaborators. It may also account for the decreased activity resulting from the surface application of adrenalin, an effect not shared by pituitrin given intravenously, although both drugs cause visible vaso-constriction (Langley and Kato, 1914-15b). It probably also explains why fibrillation activity was found to last so much longer after death in the dog than in the rabbit (Langley, 1917).

Reid's observations are of particular interest in relation to our findings. It had long been thought that fibrillation does not occur in poikilothermic animals, but it is now clear that in "summer" frogs fibrillation does occur 46 days following denervation. The long interval between denervation and the onset of fibrillation has previously been related to species differences, and not to a metabolic factor.

The discrepancies in the literature relating to the effect of drugs, particularly atropine, may have been due to a neglect of the temperature factor during the observations, for we have found using both electrical and visual methods of recording that atropine has no effect on fibrillation in the rat. It will be remembered that Solandt and Magladery used chiefly the visual method. In addition, the observations of Levine *et al.* on the effect of anaesthetics are probably related to a lowered temperature or metabolic rate, for decreased fibrillation activity

was not detectable until approximately 30 minutes after the induction of anaesthesia.

Finally, we have confirmed the findings of Solandt and Magladery that there is no close relationship between atrophy and fibrillation, for muscle atrophy in man can be observed before the onset of fibrillation.

It is clear that the method of action potential recording with concentric needle electrodes is a valuable means of detecting the presence of denervated muscle fibres. It can thus be used to determine minimal lesions of motor nerves. It can also be used to demonstrate minimal degrees of re-innervation following motor nerve interruption and subsequent regeneration. In each case the determination can be made without exposure of the muscle concerned. The clinical application of action potential recording with concentric needle electrodes has already been given (Weddell *et al.*, 1944).

Summary

(1) The time-course of fibrillation of denervation is dependent on the size of the mammal under investigation. The onset occurs in the mouse in 24 hours and in man (limb musculature) 16 days.

(2) Thyroidectomy in rabbits delays the onset of fibrillation following denervation.

(3) Feeding desiccated thyroid to rabbits decreases the time before the onset of fibrillation following denervation.

(4) The amount of fibrillation of denervation (number of frequency of action potential spikes) is greater in small than in large mammals, regardless of species.

(5) The amount of fibrillation depends on the temperature of the denervated muscle under investigation. A small fall in temperature causes a marked decrease of activity.

(6) Our observations on the effect of drugs on fibrillation confirm the work of Solandt and Magladery (1940). Muscle temperature must remain constant during the assessment of the action of a drug on fibrillating muscle.

(7) Muscle atrophy in man can be detected before the onset of fibrillation. Thus atrophy is not solely dependent on "overwork" due to fibrillation.

(8) The discrepant findings in the literature have been discussed in the light of the metabolic factor affecting fibrillation of denervation.

Acknowledgments

Our thanks are due to Professor W. E. Le Gros Clark, F.R.S., and H. J. Seddon, F.R.C.S., for their help and encouragement. Mr. P. Peade, of the Department of Anatomy, University of Oxford, rendered much valuable technical assistance; to him we also offer our sincere thanks. The expenses of this investigation were defrayed by a grant to one of us (G. W.) by the Medical Research Council, which is gratefully acknowledged. (B. F.) was in receipt of a personal grant from the Carnegie Research Fund and (R. E. P.) from the Nuffield Fluid Research Fund; these are also gratefully acknowledged.

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