J. Physiol. (1957) 135, 655–672

THE ACTION OF TETANUS TOXIN ON THE INHIBITION OF MOTONEURONES

By V. B. BROOKS,* D. R. CURTIS and J. C. ECCLES

From the Department of Physiology, Australian National University, Canberra, Australia

(*Received* 9 *November* 1956)

INTRODUCTION

The action of tetanus toxin on spinal reflexes was described by Sherrington (1905) as a conversion of inhibition into excitation. In general the clinical effects produced by the actions of tetanus toxin and of strychnine are very similar, and because of this Sherrington (1905, 1906) suggested that it was likely that these two substances had the same action in the central nervous system. Sherrington's observations were based upon experiments in which the inhibition of extensor reflexes was replaced by facilitation, the conditioning volleys having been set up in the internal saphenous or hamstring nerves.

When strychnine is injected intravenously in subconvulsive doses it greatly diminishes the amount of inhibition produced by a 'direct' inhibitory volley, but has no significant effect on the testing monosynaptic reflex (Bradley, Easton & Eccles, 1953). Intracellular records from motoneurones reveal that strychnine diminishes inhibitory action by depressing the inhibitory postsynaptic potential (IPSP) produced by impulses in inhibitory presynaptic fibres (Coombs, Eccles & Fatt, 1955).

In view of the similarity of the central effects of strychnine and of tetanus toxin it was thought of interest to investigate the effect of tetanus toxin upon spinal inhibitory mechanisms. Five main types of inhibition have been investigated: the 'direct' inhibition of motoneurones by impulses in the group Ia afferent fibres of antagonistic muscles; the inhibition by impulses in the group Ib afferent fibres from muscles of the same limb; the inhibition of extensor motoneurones by impulses in the groups II and III muscle afferent fibres and in cutaneous afferent fibres; and the inhibition of motoneurones

* Visiting Fellow, supported by a travel grant from the National Research Council of Canada.
Present address: The Rockefeller Institute of Medical Research, New York 21.

following activation of Renshaw cells by volleys in axon collaterals. A preliminary report of the results has already been published (Brooks, Curtis & Eccles, 1955).

METHODS

Cats lightly anaesthetized with pentobarbitone sodium have been used in the experiments. The spinal cord was severed in the lower thoracic region and the ventral roots of segments L5–S1 were cut and mounted on platinum electrodes for recording purposes. The spinal cord was covered by paraffin oil contained in the elevated skin flaps. The nerves to the posterior biceps and semitendinosus muscles (henceforth *BST*), sural nerve (*S*), gastrocnemius-soleus (*G*), plantaris (*P*), and flexor digitorum longus (*FDL*) were mounted on stimulating electrodes in another paraffin pool, while the quadriceps nerve (*Q*) was stimulated through a buried electrode. In several experiments the preparation was bilateral. As these experiments usually extended for 24 hr or longer, large doses of crystalline penicillin G were administered to the cat to reduce infection of the exposed tissues.

When recording the reflex responses, superimposition of about twenty to forty traces was used so that the mean response could be directly measured from the photographs. Reflexes were elicited at either 2 or 3·5 sec intervals and the testing monosynaptic responses were always maximal. The thresholds of the afferent fibres used for inhibitory volleys were checked from time to time.

Both non-crystalline and crystalline tetanus toxins were used and the doses administered are given in the text as mg or ml. of toxin. Owing to the instability of these toxins, regular assays were performed on mice. It is possible to relate cat to mouse minimum lethal doses (MLD) on a weight for weight basis by use of the factor 600 (Fildes, 1929), neglecting in so doing the variability reported by Llewellyn-Smith (1942). However, in view of the variations in the susceptibility to tetanus toxin of the domestic cats used in these experiments no figures are given relating the doses used to cat lethal doses. The non-crystalline toxin XW 1322 T 166 (henceforth XW) contained 10^6 mouse MLD/mg of powder and the crystalline toxin L61 which has been described by Pillemer, Wittler, Burrell & Grossberg (1948) was found to contain 10^4 mouse MLD/ml. of stock solution. There was no significant alteration in the toxicity of either of these toxins during the course of this investigation.

Local tetanus was produced in the animals by injection of the toxin either into the sciatic nerve or the spinal cord. The sciatic injections were performed aseptically under ether anaesthetic using a 27-gauge needle and taking care that there was no leakage to surrounding muscles (Abel, Hampil & Jonas, 1935). 5×10^{-2} ml. of a solution of toxin in saline was injected into the left nerve trunk at a level between the hamstring and sural branches. Following such an injection the typical symptoms of local tetanus developed in 18–20 hr (cf. Fildes, 1929; Acheson, Ratnoff & Schoenbach, 1942; Hutter, 1951; Davies, Morgan, Wright & Wright, 1954). Consequently, the preparation of both hind limbs and of both sides of the cord was commenced 8–12 hr after the injection so that a comparison of reflexes on the two sides could be made before the development of local tetanus and at various times thereafter. In several such experiments the L7 ventral root was left intact for a further 12 hr in order that the movement of toxin into the cord should not be prematurely interrupted.

Tetanus toxin was injected into both white and grey matter of the spinal cord through glass micropipettes of 5–20 μ external diameter at the tip. The injection device permitted the ejection of volumes as small as 10^{-5} ml. and depended on the displacement of a thin Perspex diaphragm by a micrometer (Brooks, V. B., Curtis, D. R. & Winsbury, G. J.—in the course of publication). By employing the micropipette also as a microelectrode it was possible to determine the relation of its tip to known areas in the ventral horn. As any one segmental ventral root samples the discharge of motoneurones extending over this segment, it was necessary to split the root into two or three portions in order to demonstrate the very localized effects, or alternatively to make a longitudinal series of injections 1 or 2 mm apart throughout the segment.

RESULTS

Effect of tetanus toxin on the excitation of motoneurones

When tetanus toxin was injected into the sciatic nerve or directly into the spinal cord, there was no significant change in maximal monosynaptic reflexes that were elicited by stimulation in group Ia fibres of a muscle nerve and recorded from the appropriate ventral root or peripheral nerve. For example, in Fig. 8 the record A was taken before the injection of L61 toxin into the dorso-lateral aspect of the L7 and S1 segments of the spinal cord. This maximal monosynaptic reflex of *FDL* motoneurones was virtually unchanged 2·45 and 7 hr later (D, G), whereas the maximal inhibition of the reflex by impulses propagated antidromically from the *BST*, *P* and *G* nerves was reduced considerably (see later).

When toxin was injected into the sciatic nerve, its effects developed much more slowly, hence considerable variations were likely to occur in the size of maximal monosynaptic reflexes, apart from any possible effect of the toxin. In the experiment illustrated in Fig. 1, *BST* monosynaptic reflexes were recorded from the left and right side of the spinal cord at various times after the intrasciatic injection of 7 mg XW toxin on the left side. The records A and G show the control reflexes on the left and right sides respectively 21 hr after the injection. The corresponding pairs C and J, E and L were recorded at 22 and 33 hr respectively at the same amplification. Over this period, especially on the left side, there was a large and progressive diminution in the amount of inhibition displayed in the records B, D and F, as will be described later, while there was no significant trend in the size of the maximum monosynaptic reflex.

In contrast to the lack of effect upon monosynaptic reflexes, polysynaptic reflexes are increased both by tetanus toxin and strychnine. With tetanus toxin there is considerable variation in the magnitude of the increment of polysynaptic reflexes. This is seen particularly after the toxin has been injected into the cord and probably depends both on the site of injection and the spread of the toxin from this area. The increase of polysynaptic reflexes raises the possibility of suppression of inhibition along polysynaptic pathways by tetanus toxin. These results, together with a consideration of tetanus dolorosus, will be discussed more fully in a later paper.

Fig. 2 shows polysynaptic reflexes elicited by maximal stimulation of sural fibres and recorded from the L7 ventral root before (A) and 38 min after (B) 4×10^{-4} ml. of a 1 in 10 dilution of L61 toxin was injected into the dorsolateral column of the same segment. At 38 min the area under the monophasic recording of the polysynaptic reflex was increased 6–8 times.

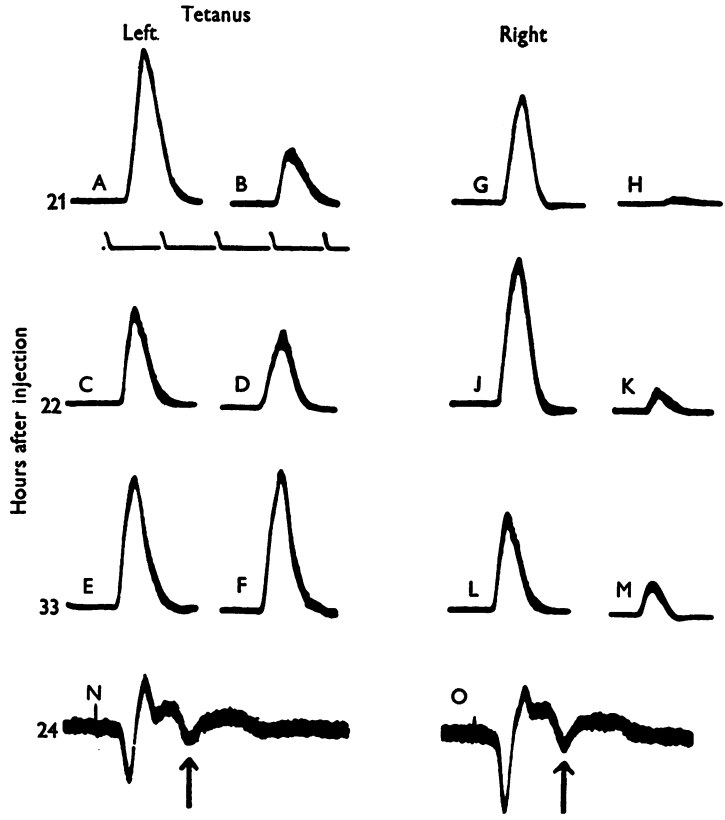

Fig. 1. A–M, maximal monosynaptic reflexes recorded at constant amplification from the left (A–F) and right (G–M) L7 ventral roots and evoked by stimulation of the left and right biceps-semitendinosus nerves respectively. The left-hand records of each pair (A, C, E, and G, J, L) are control reflexes while the right-hand records (B, D, F and H, K, M) show the same responses inhibited maximally by a preceding volley in the group Ia afferent fibres of the quadriceps nerve of the same side. The figures on the left side give the time in hours, after 7 mg of XW toxin had been injected into the left sciatic nerve, at which the responses at the same horizontal level in the figure were recorded. N, O, potentials recorded from the dorsal surface of the L7 segment of the spinal cord in the same experiment as A–M and evoked by a volley in the group Ia quadriceps afferent fibres; the arrows mark the positive notch; records taken 24 hr after the intrasciatic injection of toxin Time in msec for all records, and all consist of twenty superimposed traces. The word 'Tetanus' in this and other figures denotes the side either of the sciatic nerve or of the spinal cord into which the toxin was injected; 'left' and 'right' refer to the side of the spinal cord from which the reflexes were recorded.

Effects upon the various types of inhibitory action on motoneurones

Direct inhibition. Inhibitory curves can be drawn showing the percentage inhibition of a *BST* monosynaptic reflex caused by a single volley in the group Ia afferent fibres of quadriceps (Lloyd, 1946; Bradley *et al.* 1953; Bradley & Eccles, 1953). The maximum inhibition occurs when the volley in quadriceps fibres arrives at the spinal cord about 1·0 msec before the *BST* volley. In Fig. 1A–F are shown monosynaptic reflexes evoked by a *BST* volley and which in B, D and F are directly inhibited by a maximum volley in *Q* Ia fibres at the optimal interval for inhibition. When first examined, 21 hr after the intra-sciatic injection of tetanus toxin on the left side, the inhibition of the reflex amounted to 65% of its control value (A, B). However, 1 hr later the

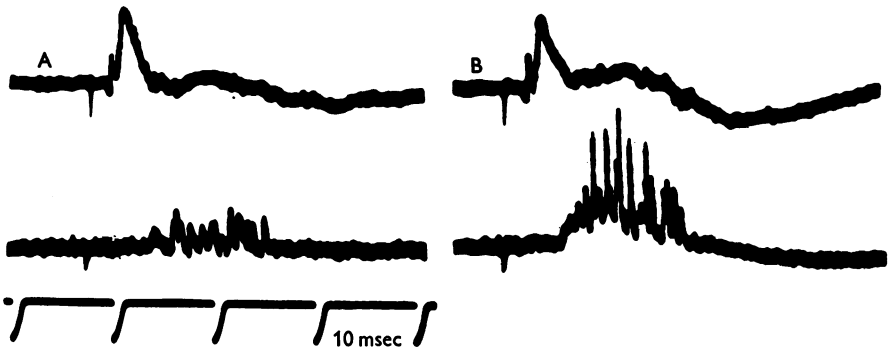

Fig. 2. The lower records show polysynaptic reflexes, recorded monophasically from the left L7 ventral root and evoked by stimulating the sural nerve at an intensity equalling ten times its threshold. The upper records show potentials recorded simultaneously from the dorsal surface of the L7 segment. A, before and B, 38 min after L61 toxin had been injected into the dorsolateral column in the same segment.

inhibition only amounted to 40% (C, D) and at 33 hr the inhibition had been completely abolished (E, F). At similar intervals on the right side there was much less diminution in the amount of inhibition. The complete inhibitory curves of this experiment are plotted in Fig. 3, where responses of the left and right sides of the animal are shown in the left and right sets of curves respectively. The earliest test revealed that inhibition amounted to 65% on the injected side in contrast to 95% on the contralateral side. Probably the tetanus action had already begun, for subsequent tests at 22 and 23·5 hr showed a rapid diminution of the inhibition on the left side to about 35 and 15% respectively. At this time inhibition was still 87% on the contralateral side. By 33 hr inhibition had disappeared on the left side, but still amounted to 75% on the right. The slow loss of inhibition on the right side was kept under observation until 43 hr, when inhibition was about 35%. During all this time the testing maximal monosynaptic reflexes showed little variation in size.

It has been shown by Eccles, Fatt & Landgren (1956) that the inhibition of motoneurones by impulses in the Ia afferent fibres from muscles of antagonistic function is mediated by short inhibitory interneurones. In the case of the inhibition of biceps-semitendinosus neurones by impulses in Q Ia fibres, impulses in the synaptic terminals of these interneurones generate a positive 'notch' which can be recorded from the surface of the L7 and upper S1 segments and which is marked by an arrow in Fig. 1N and O. Twenty-four hours after the toxin injection, these two notches are seen to be approximately equal and yet the direct inhibitory action amounted to only 15% on the left side, but was still about 80% on the right.

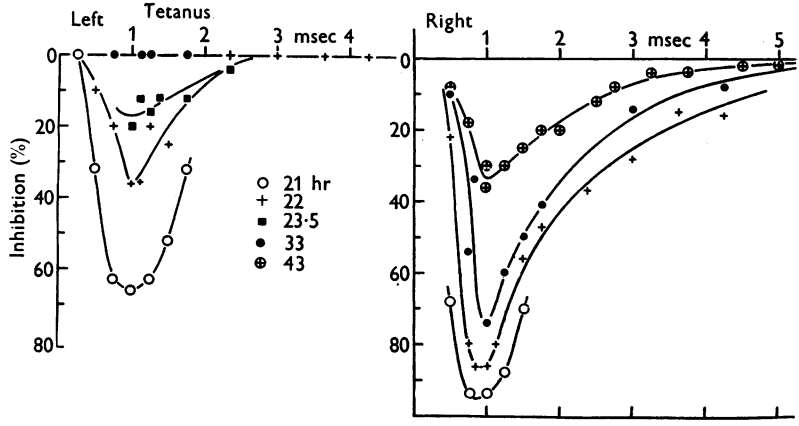

Fig. 3. Series of inhibitory curves showing the effects of a volley in the group Ia afferent fibres of the left and right quadriceps nerves upon maximal monosynaptic reflexes recorded from the L7 ventral roots of the corresponding sides of the spinal cord and evoked by stimulating the respective biceps-semitendinosus nerves. Ordinates: inhibition (%) = 100% minus the inhibited reflex size as a percentage of the control reflex; this convention has been used in all similar graphs of this paper. Abscissae: intervals between the inhibitory and the excitatory volleys as recorded by an electrode on the dorsal surface of the mid-L7 segment. Every plotted point is the mean of 10–20 observations. The symbols denote the inhibitory effects observed at the times indicated after the injection of 7 mg of XW toxin into the left sciatic nerve; part of this series is illustrated in Fig. 1.

A similar depression of direct inhibitory action was observed when toxin was injected directly into the spinal cord; the onset of the loss of inhibition, however, being much more rapid. The rapidity of action depended on the proximity of the injection to the motoneurones used for testing (see later). The reflexes B, E, H and K of Fig. 4 were maximally inhibited by volleys in Q Ia fibres before and at the stated times after an intraspinal injection of L61 toxin. The amount of inhibition, relative to the control reflexes A, D, G and J, was reduced progressively from 75% in B and E to 42% in H and zero in K. Shortly after each set of reflexes was recorded, potentials generated by the inhibiting quadriceps volley were recorded from the dorsolateral surface of the

L7 segment and it is apparent that the positive 'notch' marked with an arrow in C, F, I and L is relatively unaltered by the toxin although direct inhibition was abolished. Hence the depression of inhibitory action could not be attributed to blockage of impulse transmission through the intermediate neurones of the direct inhibitory pathway. The failure must occur in the inhibitory action which these impulses exert on the motoneurones.

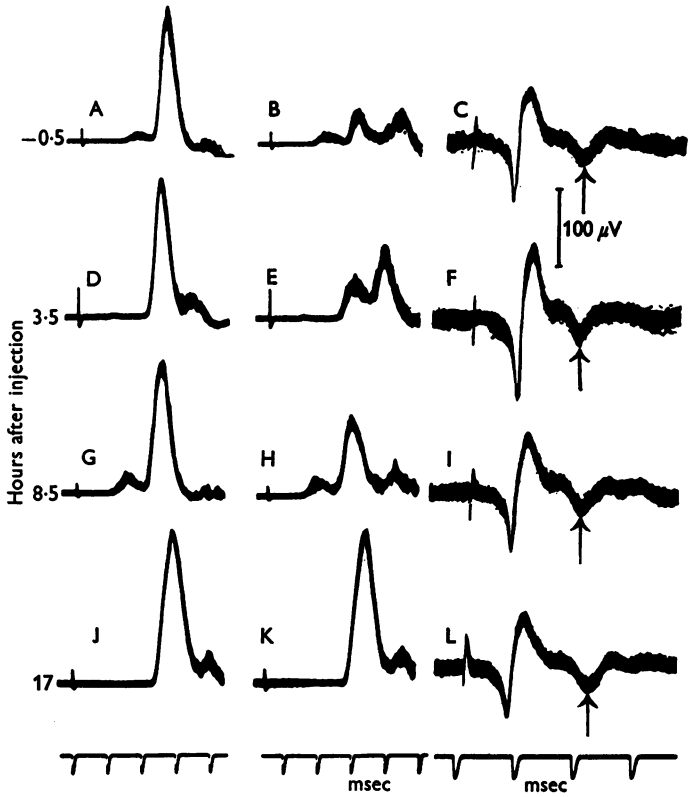

Fig. 4. Records taken 0·5 hr before (A, B, C); 3·5 hr (D, E, F); 8·5 hr (G, H, I); and 17 hr (J, K, L) after the injection of 10^{-3} ml. of L61 toxin into five sites in the dorsolateral column of the L7 and S1 segments of the cord: A, D, G, J, monophasic records of the maximal monosynaptic reflex, recorded from the *BST* nerve and evoked by stimulation of the L7 and S1 dorsal roots; two stimuli were used at an interval of 1 msec, the first being submaximal. B, E, H, K, as for A, D, G, and J respectively, but the reflexes are inhibited by a preceding volley in the group Ia afferent fibres of the quadriceps nerve. The volley interval was arranged for maximal inhibition. C, F, I and L, potentials recorded from the dorsolateral surface of the L7 segment when the quadriceps nerve was stimulated at the intensity that was used to obtain the corresponding records B, E, H and K; the positive notch is marked with an arrow. Time in msec for all records, which consist of 20 superimposed traces. A, B, D, E, G and H were recorded at the same amplification; J and K were recorded at an amplification 1·4 times higher.

Similar results were found in all seven experiments in which volleys in *Q* Ia fibres were used to inhibit maximal monosynaptic *BST* reflexes and also in the one test in which volleys in *BST* Ia fibres directly inhibited maximal monosynaptic *Q* reflexes, although in this latter experiment it is not possible to test for the activity of the intermediate neurones on this direct inhibitory pathway.

Inhibition due to impulses in group Ib muscle afferent fibres. Impulses in group I muscle afferent fibres that probably arise from tendon organs exert an

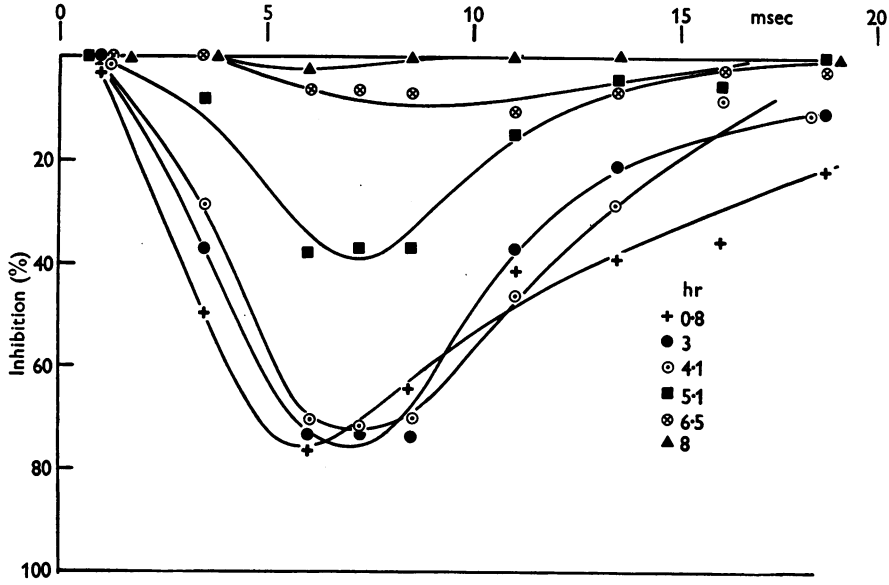

Fig. 5. The curves plot the inhibitory effect of volleys in the group Ib (and probably some group II) afferent fibres of quadriceps upon the motoneurones of gastrocnemius. The maximal monosynaptic *G* reflex was recorded peripherally and evoked by stimulating the L7 and S1 dorsal roots. The inhibition is plotted at the various times after L61 toxin was injected into the L7 and S1 segments (same experiment as Fig. 4).

inhibitory action that is widely distributed to motoneurones of that limb (Laporte & Lloyd, 1952). Usually when a volley from quadriceps nerve is recorded where the L5 or L6 dorsal root reaches the spinal cord, these fibres (designated group Ib) give a spike potential which can be distinguished from that of the group Ia fibres (Bradley & Eccles, 1953). In several experiments maximal monosynaptic reflexes of gastrocnemius and quadriceps motoneurones were inhibited by group Ib volleys of quadriceps and gastrocnemius nerves respectively. By using stimuli of graded intensity and by accurately measuring the latency, it is often possible to distinguish inhibition due to impulses in group Ib fibres from that due to impulses in group II fibres (cf. Laporte & Lloyd, 1952).

In the inhibitory curves of Fig. 5, reflexes elicited by stimulating the L7 and S1 dorsal roots and recorded from the gastrocnemius nerve were inhibited by volleys in quadriceps afferent fibres which included group Ib and possibly some group II. During the 8 hr of observation the test control reflexes were of constant size. The percentage inhibition was approximately constant for 4 hr after the intraspinal injection of toxin into the dorsolateral column of white matter of the L7 and S1 segments but thereafter diminished rapidly so that at 8 hr it was only 1%. Similar results were obtained after intrasciatic injection and in the inverse experimental arrangement where quadriceps monosynaptic reflexes were inhibited by volleys in gastrocnemius group Ib fibres.

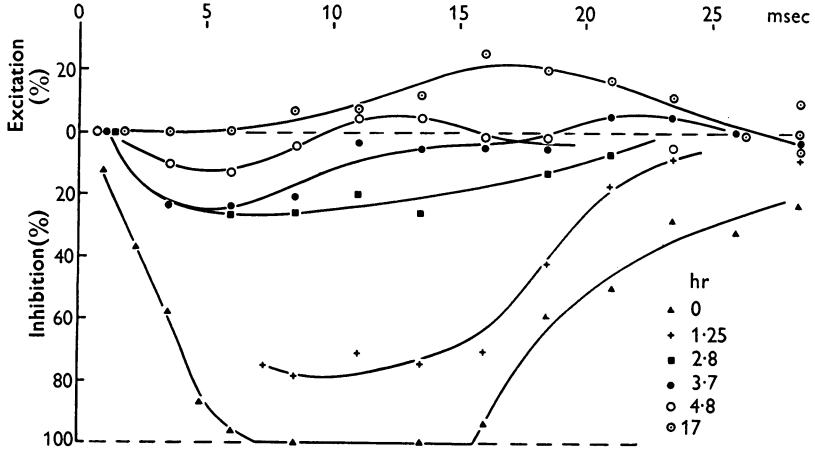

Fig. 6. Inhibitory curves from the same experiment as Fig. 5. The maximal *FDL* reflex, recorded from the *FDL* nerve and evoked by stimulating the L7 and S1 dorsal roots was conditioned by a prior volley in all the quadriceps myelinated afferent fibres. The curves plot the inhibition before and at various intervals after tetanus toxin was injected into the L7 and S1 segments of the cord.

Inhibition due to impulses in groups II and III muscle afferent fibres. Monosynaptic reflexes of extensor motoneurones are inhibited by impulses in the groups II and III afferent fibres from muscles of the same limb (Lloyd, 1946). Volleys in groups I, II and III quadriceps afferent fibres were used to inhibit the maximum monosynaptic reflexes of *FDL* motoneurones. The effect is shown in Fig. 6, from the same experiment as Fig. 5. Initially the maximum inhibition was 100% but this progressively diminished following the intraspinal injection of toxin and was abolished by 17 hr. At this time some *FDL* motoneurones were excited by impulses in the slower quadriceps fibres. Again, after the intrasciatic injection of toxin, similar results were found for the inhibition of maximal monosynaptic reflexes of *G*, *P* and *FDL* motoneurones by volleys in groups II and III fibres of the quadriceps nerve.

Inhibition due to impulses in cutaneous nerve fibres. Extensor reflexes are

inhibited by impulses in cutaneous fibres, although Hagbarth (1952) has shown that if the fibres arise from skin overlying the extensor muscle, facilitation may occur. The effect of volleys in the sural nerve has been tested upon the maximal monosynaptic reflexes of quadriceps, gastrocnemius, plantaris and flexor digitorum longus motoneurones. The inhibitory curves of Fig. 7 demonstrate the effect of tetanus toxin upon the inhibition of monosynaptic responses of quadriceps motoneurones of the L5 segment by maximal volleys in the sural nerve. Tetanus toxin had been injected into the left sciatic nerve (same experiment as Fig. 1) and first on the left and then on the right sides inhibition was depressed and finally abolished. Similar results have been found for the inhibition of gastrocnemius motoneurones by sural volleys.

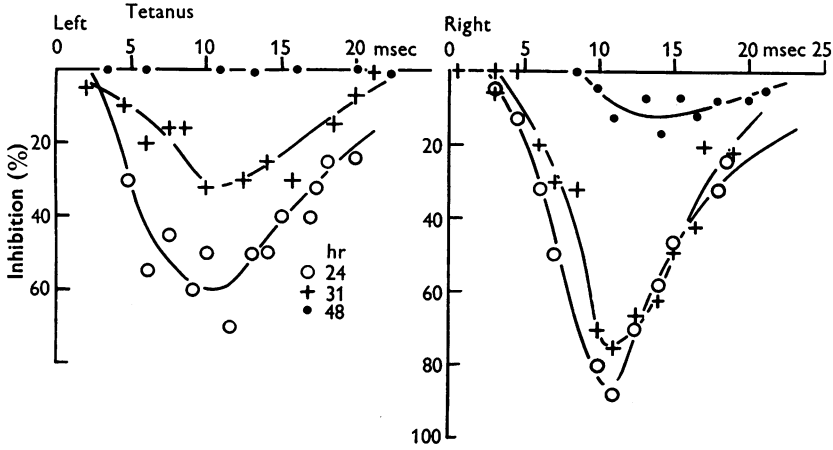

Fig. 7. Volleys elicited by stimulating the left and right sural nerves maximally were used to inhibit the monosynaptic responses of quadriceps motoneurones recorded from the L5 ventral roots of the left and right sides respectively. Tetanus toxin had been injected into the left sciatic nerve 24 hr before the first curve (same experiment as Figs. 1 and 3).

Inhibitory action by impulses in motor nerve fibres. The depression of excitability of spinal motoneurones after the antidromic activation of adjacent motoneurones (Renshaw, 1941) has been shown to be due to an inhibition produced by interneurones (Renshaw cells) that are activated cholinergically by impulses in motor-axon collaterals (Eccles, Fatt & Koketsu, 1954). The repetitive interneuronal discharge at about 1000/sec is revealed as a decrementing repetitive wave that can be recorded from the dorsolateral surface of the spinal cord in the appropriate segment. Eccles *et al.* (1954) showed that the inhibitory post-synaptic potential of the motoneurones after activation of Renshaw cells is depressed by subconvulsive doses of strychnine. It has also been demonstrated (Eccles, Eccles & Fatt, 1956) that these doses of strychnine have little effect on the discharge of Renshaw cells.

In the present series of experiments on tetanus toxin, dorsal roots were

severed. Monosynaptic reflexes were evoked by dorsal root volleys and were recorded peripherally from the muscle nerves. Conditioning volleys in the motor nerve fibres were set up in muscle nerves and propagated antidromically into the spinal cord. Where two or more nerves were used, volleys were timed for simultaneous arrival at the cord. Fig. 8 illustrates reflexes recorded from the *FDL* nerve and evoked by stimulating the L7 and S1 dorsal roots. The maximal control reflexes A, D and G were inhibited by an antidromic volley propagated from the biceps-semitendinosus, plantaris and gastrocnemius nerves and the maximally inhibited reflexes (B, E and H) are shown together with the potentials due to the repetitive discharge of Renshaw cells evoked by the same antidromic volley (C, F and I). The reflexes and surface potentials at the same horizontal levels in Fig. 8 were recorded shortly after each other

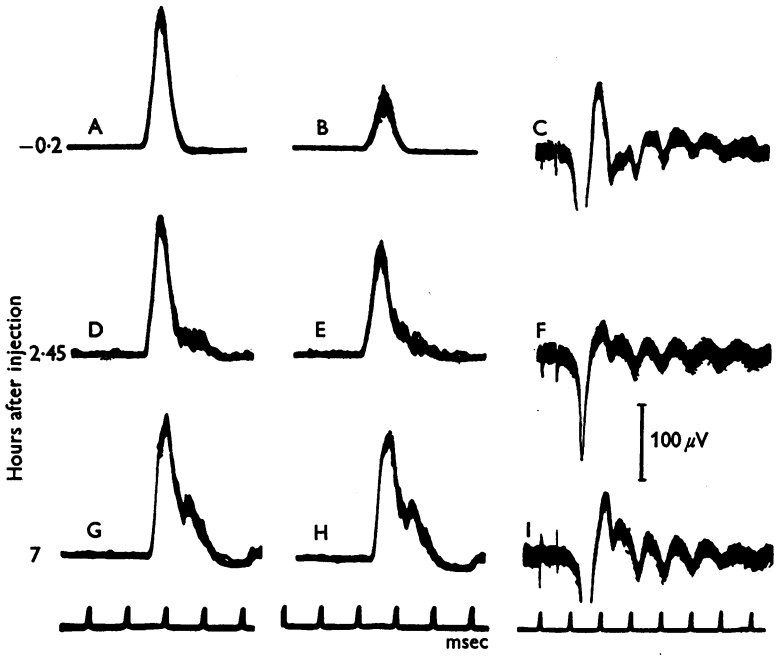

Fig. 8. Records from the same experiment as Fig. 4. A, B and C were taken 0·2 hr before; D, E, F, 2·45 hr after; and G, H, I, 7 hr after the intraspinal injection of L61 toxin into the L7 and S1 segments. A, D, G, maximal monosynaptic reflexes evoked by stimulating the L7 and S1 dorsal roots and recorded peripherally from the *FDL* nerve; B, E, H—as for A, D, G, respectively, but the reflex is inhibited by an antidromic volley propagated from the *BST*, *P* and *G* nerves. The volleys were arranged for simultaneous arrival at the cord and the stimulus intervals arranged for maximal inhibition. C, F, I, potentials recorded from the lateral aspect of the L7 segment when the antidromic volley, used for inhibiting the *FDL* reflex, was evoked at a rate of 5/sec. Time in msec for all records; A, B, D, E, G and H, recorded at the same amplification and consist of 20 superimposed records; C, F and I taken at the one amplification and consist of 25 superimposed traces.

before, and 2·45 and 7 hr after injections of L61 toxin were made into the dorsolateral aspect of the L7 and S1 segments of the cord.

The full time courses of the inhibitory action at various times are plotted in Fig. 9 where it is apparent that the inhibition was abolished at 17 hr. During this time, but illustrated in Fig. 8 only for the first 7 hr in which the maximal inhibition was reduced from 63% to about 5%, the Renshaw cell activity was normal and it is therefore apparent that, as with direct inhibition, tetanus toxin does not alter the responses of the inhibitory interneurones of this antidromic pathway but prevents their inhibitory action on the motoneurone. A special interest in this situation is the lack of effect of tetanus toxin

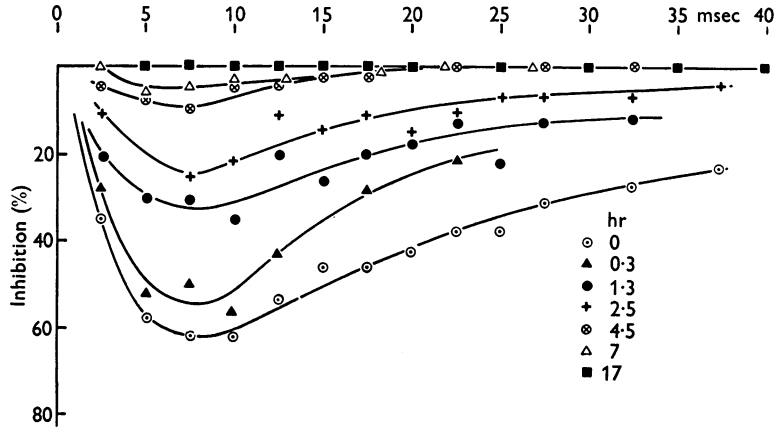

Fig. 9. Inhibitory curves constructed from the series partly illustrated in Fig. 8. The curves plot the percentage inhibition of the monosynaptic reflex from *FDL* motoneurones by antidromic volleys propagated from *BST*, *G* and *P* nerves at various times after an intraspinal injection of L61 toxin.

upon such cholinergically excited cells. These findings were also confirmed after intrasciatic injection of the toxin, and in all experiments where the effect of toxin was assessed using this antidromic inhibition the inhibition was depressed and finally abolished without being converted into excitation (cf. Fig. 9).

DISCUSSION

Until the demonstration (Bradley *et al.* 1953) that subconvulsive doses of strychnine depressed the direct inhibitory action of volleys in group Ia fibres of quadriceps nerve on biceps-semitendinosus motoneurones, there was considerable uncertainty as to the action of this drug. Sherrington (1905, 1906) recognized the similarity of action of strychnine and tetanus toxin, and had noted that with small doses it was possible to diminish reflex inhibition without replacing it by excitation. He suggested as alternative explanations either that these agents favoured central excitatory action and

depressed inhibitory action or that they converted central inhibition into central excitation. This dilemma arose because of the mixed excitatory and inhibitory effects of the conditioning volleys used in his tests.

It has been shown in the foregoing sections that, in spinal segments affected by tetanus toxin, monosynaptic reflexes are not significantly altered, polysynaptic reflexes are increased and the five investigated forms of inhibition upon motoneurones are depressed. Although it is often difficult to obtain a pure inhibitory conditioning volley when stimulating peripheral afferent nerves (cf. Fig. 6), antidromic volleys propagated up the motor axon have no demonstrable excitatory effect upon adjacent motoneurones (Eccles *et al.* 1954). That tetanus toxin abolishes the 'Renshaw' inhibition of motoneurones without converting it to excitation proves conclusively that the toxin specifically depresses central inhibition.

Several previous investigations have anticipated these findings. Acheson *et al.* (1942) demonstrated that intramuscular injection of toxin into cats produced local tetanus associated with increased polysynaptic reflexes, whereas the monosynaptic reflexes were almost normal. The loss of inhibition was demonstrated in the rabbit, although not recognized as such, by Davies *et al.* (1954). They showed that tetanus toxin, acting centrally, disorganized flexor reflexes so that pressure on the foot caused simultaneous reflex activation of antagonistic muscles instead of reciprocal inhibition. They also demonstrated increases in polysynaptic reflexes.

It is clear from the results presented here that in the spinal cat the effects of strychnine and tetanus toxin are similar. Although satisfactory intracellular records have not yet been obtained from motoneurones affected by tetanus toxin, it may be assumed that, like strychnine, tetanus toxin diminishes inhibitory action by depressing the process by which impulses in synaptic terminals of inhibitory interneurones generate an inhibitory post-synaptic potential of motoneurones (Coombs *et al.* 1955). Conceivably these substances could depress the inhibitory action by blocking the excitatory synaptic action on the inhibitory neurones which are interpolated in the inhibitory pathway. However, this possibility has been excluded by the observation that there is no diminution in the electrical responses which are produced on the surface of the spinal cord by the activity of the inhibitory interneurones both in the direct and antidromic inhibitory pathways. When impulses in quadriceps group I a afferent fibres have been used to inhibit biceps-semitendinosus motoneurones, the positive notch recorded from the dorsal surface of the cord in the L7–S1 segments has remained unchanged when inhibition was abolished by tetanus toxin. Similarly, the inhibitory cells on the antidromic pathway continued to be excited by antidromic impulses to give the characteristic rhythmic wave even when antidromic inhibition had been abolished. With other types of inhibition, interneuronal activity does not produce recognizable

surface potential waves, hence it has not been possible in this way to show that the depressant action of tetanus toxin is exerted on the inhibitory synaptic mechanism. However, with both strychnine and tetanus toxin a similarity of action upon these inhibitions must be extremely probable.

The highly specific and rapid action of strychnine after intravenous administration in relatively low doses suggests that it acts on the subsynaptic membrane in a similar fashion to the action that curare has at cholinergic synapses (Eccles, Katz & Kuffler, 1941). Thus it may act competitively with the inhibitory transmitter for the receptor patches of the subsynaptic membrane. It is difficult to assess accurately the time course of action of tetanus toxin because, even when injected into the ventral horn, some time is necessary for it to spread throughout the nuclei of the motoneurones used for testing purposes. Possibly tetanus toxin might act in the manner postulated for strychnine and combine sterically with the receptors of the subsynaptic inhibitory areas. However, in view of the general similarity of tetanus and botulinum toxin (van Heyningen, 1950; Wright, 1955) both in regard to molecular weight and clostridial origin, a possible alternative is that tetanus toxin acts in the same manner as botulinum toxin. The toxin of *Clostridium botulinum* prevents the release of acetylcholine at the neuromuscular junction by an action on the presynaptic terminals (Burgen, Dickens & Zatman, 1948; Brooks, 1954, 1956) and it is conceivable that tetanus toxin acts on the presynaptic terminals of inhibitory interneurones preventing either the production or the release of the inhibitory transmitter substance.

The report of Ambache, Morgan & Wright (1948) concerning the selective paralysis of cholinergic endings of the rabbit's iris by tetanus toxin is difficult to reconcile both with the present result that Renshaw cell activity is not altered by the toxin and with the findings of others upon the neuromuscular junction. Although Harvey (1939) stated that local tetanus has certain features resembling the phenomena seen with the denervation of skeletal muscle, further investigations of the effect of the toxin on neuromuscular transmission (Göpfert & Schaefer, 1940; Hutter, 1951; Mackereth & Scott, 1954) have yielded negative results.

There is very little information regarding the biochemical effects of tetanus toxin in the spinal cord, but presumably it acts specifically on a surface membrane or on an enzyme system. This is apparent from the small quantities necessary to obtain effects (cf. van Heyningen, 1950) and from the effects of variations in temperature in the progress of tetanus in poikilothermic animals. These creatures, including frogs and lizards, are comparatively resistant to high doses of tetanus toxin when kept at low temperatures but become susceptible when the temperature is raised (Cowles & Nelson, 1947; Wright, 1955). However the actual mode of action of both strychnine and tetanus toxin is open to question and will ultimately depend on the isolation and

application of the inhibitory transmitter substance. Tetanus toxin resembles strychnine in that it greatly increases polysynaptic reflexes. It has been argued that for strychnine this is due to the depressant action on inhibitory synapses along polysynaptic pathways (cf. Bradley *et al.* 1953). Possibly the same mechanism is responsible for the increased reflexes in tetanus, but further investigation is desirable.

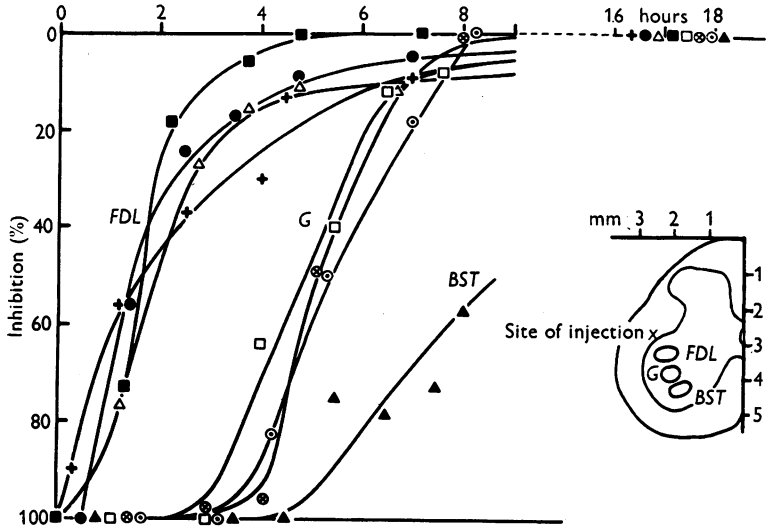

Fig. 10. Ordinates: maximum inhibitions plotted as percentages of the values obtained before L61 toxin was injected into the L7 and S1 segments, as shown in the inset diagram. Abscissa: time in hours, after the injections were made, at which the following inhibitions were determined: the inhibition of *FDL* motoneurones by antidromic volleys from the *BST*, *P* and *G* nerves +, by volleys in *Q* Ib (and II) afferent fibres ●, by volleys in all the myelinated *Q* fibres △ and by volleys elicited by stimulating the saphenous nerve maximally ■; the inhibition of *G* motoneurones by antidromic volleys in the *BST*, *P* and *FDL* nerves □, by volleys in the groups Ib (and II) afferent fibres of *Q* ⊗ and by volleys in all the myelinated *Q* afferent fibres ⊙; the inhibition of *BST* motoneurones by volleys in *Q* Ia fibres ▲. Serial observations were made for the first 8 hr only, but all inhibitions were abolished when further determinations were made 8–10 hr later. The approximate locations of the motor nuclei in the inset diagram have been derived from Romanes (1951) and are shown in the inset cross-section of the cord. Adjustment has been made for the slight variation found in the amount of inhibition upon *G* and *BST* motoneurones before the toxin had effect.

When assessed by the methods used in the present investigation there is no difference between the action of tetanus toxin injected directly into the cord or reaching it after injection into the sciatic trunk. This observation lends further support to the findings of Firor & Jonas (1938), Acheson *et al.* (1942), Hutter (1951), Wright, Morgan & Wright (1952) and Davies *et al.* (1954) that tetanus toxin has a central action; and to the investigations showing that the toxin enters the spinal cord from peripheral sites of administration by passing

along nerve trunks (Teale & Embleton, 1919; Friedemann, Zuger & Hollander, 1939*a*, *b*; Friedemann, Hollander & Tarlov, 1941; Baylis, Joseph, MacIntosh, Morgan & Wright, 1952; Baylis, MacIntosh, Morgan & Wright, 1952).

That tetanus toxin spreads within the spinal cord is indicated by the curves of Fig. 10, which have been derived from the experiment illustrated in Figs. 4–6 and 8. Inhibitions have been plotted as percentages of the values obtained before the toxin was administered and the graphs illustrate the effect upon the listed inhibitions of *FDL*, *G* and *BST* motoneurones of intraspinal injections of L61 toxin. Five injections were made at longitudinal intervals of 1–2 mm and at approximately the same locus in the transverse section as indicated in the inset diagram, which also shows the locations of the three motoneurone nuclei. It is apparent that inhibitions upon motoneurones located near the site of injection are affected earlier than those located at a distance. All the inhibitions of *FDL* motoneurones were reduced to 20% of their original values within 5 hr of the injection. At this time, however, inhibitions upon *G* motoneurones were approximately 60% of their control value but this figure was reduced to 20% after a further 2 hr. The reduction in the inhibition of *BST* motoneurones by impulses in the quadriceps Ia fibres was slow to develop and the full time course was not observed, though at 18 hr the inhibition was found to be completely abolished. These results indicate that tetanus toxin diffuses very slowly across the spinal cord, taking several hours to move 1 mm. The longitudinal movement in nerve trunks is much faster as is also the longitudinal movement within the spinal cord.

SUMMARY

1. Spinal reflexes and their inhibition have been investigated in anaesthetized spinal cats after the injection of tetanus toxin either into the sciatic nerve or into the spinal cord.

2. Tetanus toxin is virtually without effect upon monosynaptic reflexes, but like strychnine it increases polysynaptic reflexes.

3. The five forms of spinal inhibition investigated are all diminished and eventually abolished. The effect of the toxin, injected either peripherally into a mixed nerve trunk or directly into the spinal cord, is similar on all these types of inhibition.

4. Tetanus toxin exerts its effect near the synaptic junctions between the specific interneurones of the inhibitory pathway and the motoneurone. Both for 'direct' inhibition and for 'antidromic' inhibition this agent has no effect on the inhibitory interneurone involved.

The authors wish to thank Miss Molly Barr, of the Wellcome Research Laboratories, Beckenham, Kent, for the gift of toxin XW 1322 T 166; and Professor L. Pillemer, of Western Reserve University, Cleveland, Ohio, for the gift of toxin L61.

REFERENCES

ABEL, J. J., HAMPIL, B. & JONAS, A. F. (1935). Researches on Tetanus III. Further experiments to prove that tetanus toxin is not carried in peripheral nerves to the central nervous system. *Johns Hopk. Hosp. Bull.* **56**, 317–336.

ACHESON, G. H., RATNOFF, O. D. & SCHOENBACH, E. B. (1942). The localized action on the spinal cord of intramuscularly injected tetanus toxin. *J. exp. Med.* **75**, 465–480.

AMBACHE, N., MORGAN, R. S. & WRIGHT, G. PAYLING (1948). The action of tetanus toxin on the rabbit's iris. *J. Physiol.* **107**, 45–53.

BAYLIS, J. H., JOSEPH, J., MACKINTOSH, J., MORGAN, R. S. & WRIGHT, G. PAYLING (1952). The effect of transection on the ascent of tetanus toxin in the rabbit's spinal cord. *J. Path. Bact.* **64**, 47–52.

BAYLIS, J. H., MACKINTOSH, J., MORGAN, R. S. & WRIGHT, G. PAYLING (1952). The effect of sclerosis of the nerve trunk on the ascent of tetanus toxin in the sciatic nerve of rabbits and on the development of local tetanus. *J. Path. Bact.* **64**, 33–45.

BRADLEY, K., EASTON, D. M. & ECCLES, J. C. (1953). An investigation of primary or direct inhibition. *J. Physiol.* **122**, 474–488.

BRADLEY, K. & ECCLES, J. C. (1953). Analysis of the fast afferent impulses from thigh muscles. *J. Physiol.* **122**, 462–473.

BROOKS, V. B. (1954). The action of botulinum toxin on motor nerve filaments. *J. Physiol.* **123**, 501–515.

BROOKS, V. B. (1956). An intracellular study of the action of repetitive nerve volleys and of botulinum toxin on miniature end-plate potentials. *J. Physiol.* **134**, 264–277.

BROOKS, V. B., CURTIS, D. R. & ECCLES, J. C. (1955). Mode of action of tetanus toxin. *Nature, Lond.*, **175**, 120–121.

BURGEN, A. S. U., DICKENS, F. & ZATMAN, L. J. (1948). The action of botulinum toxin on the neuro-muscular junction. *J. Physiol.* **109**, 10–24.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The inhibitory suppression of reflex discharges from motoneurones. *J. Physiol.* **130**, 396–413.

COWLES, R. B. & NELSON, N. B. (1947). Studies on thermal sedation in suppression of symptoms of tetanus toxin. *Proc. Soc. exp. Biol., N.Y.*, **64**, 220–224.

DAVIES, J. R., MORGAN, R. S., WRIGHT, E. A. & WRIGHT, G. PAYLING (1954). The effect of local tetanus intoxication on the hind limb reflexes of the rabbit. *Arch. int. Physiol.* **62**, 248–263.

ECCLES, J. C., ECCLES, R. M. & FATT, P. (1956). Pharmacological investigations on a central synapse operated by acetylcholine. *J. Physiol.* **131**, 154–169.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

ECCLES, J. C., FATT, P. & LANDGREN, S. (1956). The central pathway for the direct inhibitory action of impulses in the largest afferent nerve fibres to muscle. *J. Neurophysiol.* **19**, 75–98.

ECCLES, J. C., KATZ, B. & KUFFLER, S. W. (1941). Nature of the 'endplate potential' in curarized muscle. *J. Neurophysiol.* **4**, 362–387.

FILDES, P. (1929). *A System of Bacteriology.* Medical Research Council, Vol. 3, 308–372. London: H.M. Stationery Office.

FIBOR, W. M. & JONAS, A. F., Jr. (1938). Researches on tetanus. VI. The production of reflex motor tetanus by intraspinal injections of tetanus toxin. *Johns Hopk. Hosp. Bull.* **62**, 90–109.

FRIEDEMANN, U., HOLLANDER, A. & TARLOV, I. M. (1941). Investigations on the pathogenesis of tetanus. *J. Immunol.* **40**, 325–364.

FRIEDEMANN, U., ZUGER, B. & HOLLANDER, A. (1939*a*). Investigations into the pathogenesis of tetanus. I. The permeability of the C.N.S. barrier to tetanal toxin. *J. Immunol.* **36**, 473–484.

FRIEDEMANN, U., ZUGER, B. & HOLLANDER, A. (1939*b*). Investigations into the pathogenesis of tetanus. II. The influence of section of the nerve on the neutralization of intramuscularly injected tetanal toxin by circulating antitoxin. *J. Immunol.* **36**, 485–488.

GÖPFERT, H. & SCHAEFER, H. (1940). Über die Mechanik des Wundstarrkrampfes. *Arch. exp. Path. Pharmak.* **197**, 93–122.

HAGBARTH, K. E. (1952). Excitatory and inhibitory skin areas for flexor and extensor motoneurones. *Acta physiol. scand.* **26**, Suppl. 94.

HARVEY, A. M. (1939). The peripheral action of tetanus toxin. *J. Physiol.* **96**, 348–365.

HUTTER, O. F. (1951). Effect of denervation on development of local tetanus. *J. Physiol.* **112**, 1*P*.

LAPORTE, Y. & LLOYD, D. P. C. (1952). Nature and significance of the reflex connections established by large afferent fibers of muscular origin. *Amer. J. Physiol.* **169**, 609–621.

LLEWELLYN-SMITH, M. (1942). Note on the complexity of tetanus toxin. *Quart. Bull. Hlth Org. L.o.N.* **10**, 104–112.

LLOYD, D. P. C. (1946). Facilitation and inhibition of spinal motoneurones. *J. Neurophysiol.* **9**, 421–438.

MACKERETH, M. B. & SCOTT, D. J. (1954). Local tetanus in the rat diaphragm. *Proc. Univ. Otago Med. Sch.* **32**, 13–14.

PILLEMER, L., WITTLER, R. G., BURRELL, J. I. & GROSSBERG, D. B. (1948). The immunochemistry of toxins and toxoids. VI. The crystallization and characterization of tetanal toxin. *J. exp. Med.* **88**, 205–221.

RENSHAW, B. (1941). Influence of discharge of motoneurones upon excitation of neighbouring motoneurones. *J. Neurophysiol.* **4**, 167–183.

ROMANES, G. J. (1951). The motor cell grouping of the lumbo-sacral spinal cord of the cat. *J. comp. Neurol.* **94**, 313–363.

SHERRINGTON, C. S. (1905). On reciprocal innervation of antagonistic muscles. VIIIth note. *Proc. Roy. Soc.* B, **76**, 269–297.

SHERRINGTON, C. S. (1906). *The Integrative Action of the Nervous System.* New Haven and London: Yale University Press.

TEALE, F. H. & EMBLETON, D. (1919). Studies in infection. II. The paths of spread of bacterial exotoxins with special reference to tetanus toxin. *J. Path. Bact.* **23**, 50–68.

VAN HEYNINGEN, W. E. (1950). *Bacterial Toxins.* Oxford: Blackwell Scientific Publications.

WRIGHT, E. A., MORGAN, R. S. & WRIGHT, G. PAYLING (1952). The site of action of the toxin in local tetanus. *Lancet*, **263**, 316–319.

WRIGHT, G. PAYLING (1955). The neurotoxins of clostridium botulinum and clostridium tetani. *Pharmacol. Rev.* **7**, 413–466.