AN INVESTIGATION OF SYNAPTIC TRANSMITTER SUBSTANCES IN THE SPINAL CORD

By

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Thesis submitted for the degree of Doctor of Philosophy in the Australian National University
1956

Owing to the nature of the experimental procedures used, most of the research reported in this thesis has been done in collaboration with other members of this department. However the attempted extraction of transmitter substances from central nervous tissue (Section I G (v)), the results of Section II C embodying the use of strychnine and NP 13 and portion of the design and construction of the equipment used in Section III are my own original work.

The following papers have appeared or are in the course of publication:-

Brooks, V.B., Curtis, D.R. & Eccles, J.C. (1955). Mode of action of tetanus toxin. Nature Lond. 175, 120-121.

Brooks, V.B., Curtis, D.R. & Eccles, J.C. (1956). The action of tetanus toxin on the inhibition of motoneurones. In the course of publication.

Coombs, J.S., Curtis, D.R. & Eccles, J.C. (1956). The duration of transmitter activity on spinal motoneurones. In the course of publication.

Coombs, J.S., Curtis, D.R. & Landgren, S. (1956). Spinal cord potentials generated by impulses in muscle and cutaneous fibres. J.Neurophysiol. accepted for publication.

Curtis, D.R. & Eccles, J.C. (1956). Post-activation potentiation of intracellularly recorded potentials of motoneurones. In the course of publication.

Curtis, D.R., Eccles, J.C. & Eccles, R.M. (1955). Pharmacological studies on reflexes. Am. J. Physiol. 183, 606.

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GENERAL INTRODUCTION

It is now generally accepted that synaptic transmission in the spinal cord takes place by the release of chemical substances from the presynaptic terminals. The nature and mode of action of these transmitter substances together with the factors controlling their release are therefore of great significance, and this thesis presents the results of three lines of investigation, each directed at an aspect of synaptic transmission between the neurones of the spinal cord.

The first is concerned with pharmacological studies on spinal reflexes. In particular, the effects of pharmacological agents operating on the chemical transmitter mechanisms of peripheral junctions have been studied in an attempt to determine whether they also have actions at central synapses. The second section reports investigations which establish that tetanus toxin and strychnine have a potent depressant action on the various inhibitory mechanisms of the spinal cord and which strongly suggest that this action is specifically exerted in relation to the inhibitory transmitter substance. The last section, based upon an electrophysiological determination of the properties of the motoneuronal membrane, considers the actual time course of the excitatory and inhibitory transmitter actions on spinal motoneurones. This latter section also includes a study of the enhanced release of the transmitter substances which is observed when test stimuli are applied after tetanic activation of the pathways concerned.

It is convenient at this stage to discuss briefly three principles which may be postulated in an attempt to give some order to the many problems relating to the operation of chemical transmitters

at central synapses.

The first may be called Dale's principle, for in 1935, Dale stated that any one class of nerve cell operates at all of its synapses by the same chemical transmitter substance. This principle stems from the metabolic unity of a single cell which extends to all of its processes. It is probable that the synaptic vesicles and the mitochondria of the terminals all derive ultimately from the nucleus and cell body (Young, 1956). An interesting example of this principle is provided by the motoneurone, which acts by the liberation of acetylcholine both at the motor nerve endings on muscle fibres and at the synaptic endings of axon collaterals on Renshaw cells (cf. Section ID). A further consequence is that the group Ia afferent fibres from muscle will be acting by the same excitatory substance at the synaptic endings that they make with three different classes of nerve cells, motoneurones, group Ia intermediate cells and the neurones of Clarke's column. Dale's principle has been utilized in an attempt to test centrally active substances isolated from dorsal root fibres (cf. Section I G (iii) and (v)). According to the principle, the chemical compound released from the peripheral collateral branches of certain primary afferent fibres during the axon reflex, and responsible for the vasodilation, should also be released from the synaptic terminals of these fibres within the spinal cord.

The second principle is that any one transmitter substance always has the same synaptic action, i.e. excitatory or inhibitory, at all synapses on nerve cells. According to this postulated principle,

any one nerve cell will function exclusively either in an excitatory or in an inhibitory capacity at all of its synaptic terminals. It has been found that in all the spinal inhibitory pathways that have been investigated (cf. Section II), the synaptic inhibitory action on motoneurones is exerted by a short-axon interneurone - the "inhibitory neurone". Before this recent discovery it was postulated that group Ia afferent impulses exerted a direct inhibitory action on motoneurones by mediation of the same transmitter that exerted a synaptic excitatory action on other motoneurones (cf. Eccles, 1953), specialization of the respective subsynaptic membranes accounting for the inhibitory or the excitatory action. The presence of the specialized interneurone in the direct inhibitory pathway renders such a postulate unnecessary. Moreover the specific action on the inhibitory transmitter mechanism of such agents as tetanus toxin and strychnine indicates that the excitatory and inhibitory synaptic transmissions are mediated by different substances.

The Renshaw cells (Sections I and II), the group Ia intermediate neurones and the other intermediate neurones on the inhibitory pathways for group Ib and cutaneous impulses (Section II) are neurones that are exclusively inhibitory in function whilst the dorsal root ganglion cells with their primary afferent fibres, neurones of ascending and descending long tracts, motoneurones and many interneurones can probably be classified as "excitatory neurones".

The third postulate, which is as yet purely speculative, is as yet purely speculative, is that any one nerve cell responds to one

excitatory substance only and is inhibited by one inhibitory substance. At the neuromuscular junction there is no evidence of any excitatory substance other than acetylcholine, and with smooth muscle, no evidence that both acetylcholine and adrenaline have the same excitatory effect on any one fibre. Although closely related substances may operate in the same fashion on cells, as has been shown to occur for the peripheral actions of adrenalin and nor-adrenalin (Euler, 1954) there are no instances of cells capable of giving the same response to two dissimilar transmitters. However Lundberg (1955), recording from the cells of the submaxillary gland, has shown that the same potential change may be recorded from the one cell following the impingement on it of impulses in both the parasympathetic and the sympathetic fibres. Different receptors are involved, for the parasympathetic response is effectively blocked by atropine whilst the sympathetic response continues unaltered. The complex nature of these cells and their electrical responses, suggest that impulses in these two groups of fibres, operating by different chemical transmitters, may activate different secretory processes.

There is good experimental evidence for the first of these two principles but the third as yet lacks any direct experimental support. It will be possible to test it adequately only when central excitatory and inhibitory transmitter substances have been isolated. Further discussion of the problems raised by these principles will be resumed in the conclusion and summary of this thesis.

SECTION I. PHARMACOLOGICAL STUDIES UPON SPINAL REFLEXES.

(A) INTRODUCTION

It is proposed in this section to review briefly some of the more recent investigations and to present some results concerned with the effects of drugs on spinal reflexes. There is now convincing evidence that the transmission at synapses in the central nervous system takes place by means of chemical transmitter substances (cf. Eccles, 1953; Fatt, 1954; Perry, 1956). Not only is this likely when the chemical nature of peripheral synaptic mechanisms is considered, but the more recont studies of the responses of cells using micro-electrodes for intracellular recording make untenable the postulate that synaptic action is due to the flow of electric currents generated by presynaptic impulses (Brock, Coombs & Eccles, 1952; Bullock & Hagiwara, 1956; Coombs, Eccles & Fatt, 1955b, 1955c, 1955d). As yet there is no evidence as to the nature of the chemical substances responsible for synaptic transmission in the spinal cord. The problems involved in their isolation and testing are considerable (cf. Gaddum, 1955). However it was a natural consequence of the discovery of the peripheral role of acetylcholine and adrenaline that the effects upon spinal transmission of these and related substances were studied at an early stage. A wide range of substances has been tested in an attempt to find chemical compounds with specific actions on spinal neurones. The investigations described here have been confined to spinal reflexes, for it is considered that these reflexes are sufficiently well understood for a precise examination to be made of the properties of the synaptic mechanisms involved.

(B) METHODS

In this investigation monosynaptic reflexes have been recorded from lumbar ventral roots following stimulation of the corresponding dorsal root. The experiments have been performed on the seventh lumbar (L7) and first sacral (S1) segments of the spinal cord of the cat under light pentobarbital anaesthesia. All other dorsal roots on both sides of the cord from the second lumbar to third sacral segments were crushed, without damaging the nearby blood vessels, in order to eliminate complications which the injections may evoke by discharges from receptor organs. The S1 and L7 ventral roots were sectioned, leaving the accompanying arteries intact. The spinal cord was transected at the thoracolumbar junction.

Since the effects of drugs were often fleeting, maximal monosynaptic reflexes were elicited as frequently as every second or even twice a second. Occasionally the testing reflex was facilitated by an earlier submaximal excitatory volley in order to obtain a stable record. In several experiments the stimulus intensity was increased so that polysynaptic reflexes could also be recorded. Alternatively these reflexes were recorded from an adjacent segment in the absence of a monosynaptic response (cf. Fig. 19).

Intra-arterial injections were made into the lower aorta with a polythene cannula inserted via the right femoral and right external iliac arteries. All the branches of the aorta below the renal arteries were ligated with the exception of the lumbar arteries as shown in Fig. 1. During the exposure of the spinal cord as many as possible of the branches of these vessels supplying the lumbar muscles were ligated, but it was

important to preserve the blood supply of the lumbo-sacral segments of the spinal cord by leaving intact the radicular arteries accompanying the ventral roots. Usually there was one vessel accompanying each root, but in several preparations one larger artery supplied two segments. The polythene cannula was flushed from time to time with saline containing heparin and the spinal cord circulation was occasionally checked by the injection of 1:1000 Evans blue solution in saline, which under the microscope could be seen to flow through the segmental arteries and then the dorsal surface veins. The carotid blood pressure was recorded with a mercury manometer and the animal received 100 units of Heparin per kilo every 3-4 hours.

The arterial injections were of constant volume (1cc) and made at a fairly constant rate (1cc in 2 seconds). Usually in any one series, five control reflexes were photographed prior to the injection. In order to secure a control level of the reflex spike height, the reflex was recorded on the lower beam of a two beam oscilloscope and the upper beam adjusted until, over a period of several minutes it was at the level of the average reflex spike (cf. Fig. 2). On account of random fluctuations the five "control" reflexes as illustrated (cf. Figs. 5 and 11) did not exactly conform to this average spike height which has been designated 100% in figures such as Fig. 3. No response was ever obtained with injections of physiological saline or buffered Ringer-Locke solution. The drugs used were dissolved in a buffered Ringer-Locke of pH 6.9 - 7.1 and the injected solutions were in this pH range. Both acetylcholine chloride and bromide (Hoffmann-La Roche) were used and gave the same results. Nicotine was used as the pure alkaloid.

(C) PREVIOUS INVESTIGATIONS OF CHOLINERGIC TRANSMISSION IN THE SPINAL CORD.

The numerous investigations upon spinal reflexes with substances having known effects at peripheral cholinergic junctions have been summarized by Feldberg (1945, 1950). The great variability and often contradictory nature of the results of some of these investigations are partly explicable by the various types of preparation used, for very few workers established that the effects produced were due to a direct action of the drug investigated on the spinal cord.

The findings of Brown and Gray (1948) did not directly implicate acetylcholine as the transmitter involved at peripheral sensory endings, but nevertheless showed that nicotine and acetylcholine, when injected into the arterial system of both skin and mesentery, caused a discharge in the afferent nerves of the injected area. This was thought to be due to an action upon the actual nerve terminations and was not blocked by atropine. Armstrong, Dry, Keele & Markham (1953) investigated pain-producing agents after application to the exposed base of a cantharidin blister, and found that pain could be produced peripherally by the action of acetylcholine, histamine and serotonin. Similar findings have been reported by Buchthal (1954). In view of the peripheral action of these drugs, the early reports of the effects of nicotine and acetylcholine on the spinal cord, especially when administered intravenously (Feldberg & Minz, 1932; Schweitzer & Wright, 1937 a & c, 1938), must be viewed with caution. Similarly, results are of doubtful significance when injections were made into some arterial system other than that of the spinal cord (McKail, Obrador & Wilson, 1941; Calma & Wright, 1944).

The special perfusion system of Bulbring & Burn (1941) excluded the muscles from which flexor and extensor reflexes were recorded, but did not exclude the possibility that the reflexes would be influenced by discharges from receptors in areas of skin and muscle which derived blood from the spinal perfusion system.

In an attempt to overcome some of these problems, Bulbring, Burn & Skoglund (1948) developed a method of applying drugs directly to the lumbar cord by injection into the arterial system. The effects of the stimulation of peripheral afferent fibres were prevented by deafferentation of the spinal cord. They were able to evoke flexion movements of the hind limbs with intra-arterial injections of acetylcholine and to modify reflexes elicited by medullary stimulation (Skoglund, 1947). The results are complex and often so conflicting that full assessment is impossible. One main finding was, however, that acetylcholine and adrenaline had opposite effects. Feldberg, Gray and Perry (1953) investigated the effects of intra-arterial injection of acetylcholine into the upper cervical segments of the spinal cord. The consequent spontaneous activity recorded from ventral roots and the modification of polysynaptic reflexes led to the suggestion that there were cholinergic synapses on polysynaptic pathways from dorsal to ventral roots. However no account was taken of the possibility that the injection would stimulate receptor organs and so reflexly act on the spinal cord.

Acetylcholine has been applied in solution to the dorsal surface of the spinal cord (Bernhard, Skoglund and Therman, 1947). These results are of doubtful significance, but gave the same conclusions as those experiments performed earlier (Bulbring et al., 1948) using intra-arterial

injections. Kennard (1953) investigated the effect of injecting acetylcholine directly into the cord, but his method of recording from whole muscle masses could account for his indefinite results. Investigations with nicotine (Libet & Gerard, 1938; Schweitzer & Wright, 1938; Bulbring & Burn, 1941; van Harreveld & Feigen, 1948 and Taugner & Culp, 1953) suggest that this drug has the same action on spinal reflexes as has acetylcholine: Bulbring and Burn (1941) found that carbamino-choline and acetyl-ß-methylcholine had actions resembling those of acetylcholine.

Of the anticholinesterases, eserine and prostigmin have been investigated to the greatest extent. Feldberg (1950) suggested that both these substances act as anticholinesterases when in small doses, but in larger doses may have "direct" effects. Evidence for a central action of these substances is confusing. Calma and Wright (1947) injected eserine into the spinal theca producing an increase in the knee jerk and crossed extensor reflex, but the effects on flexor reflexes were variable. They interpreted their results partly in the light of an earlier hypothesis (Schweitzer, Wright & Stedman, 1938) in which acetylcholine was postulated to be a universal transmitter, the observed effect of inhibition or excitation depending on the concentration. This hypothesis was evoked to explain the differences in action between tertiary and quaternary ammonium anticholinesterases. Calma (1949) applied prostigmin intrathecally and found that flexor reflexes were generally increased. The intra-arterial injection experiments of Feldberg et al. (1953) demonstrated that eserine behaved very similarly to acetylcholine in increasing the polysynaptic discharge. More recently Taverner (1954), using an intra-arterial method of applying eserine to the lumbar cord

of the cat, found that polysynaptic and monosynaptic extensor reflexes were increased, whereas flexor monosynaptic reflexes were depressed. These effects were abolished by atropine.

Other anticholinesterases have not been so extensively investigated. Chennels, Floyd and Wright (1949) found that HETP and TEPP had central excitatory actions similar to those of eserine and DFP (Chennels & Wright, 1947; Chennels, Floyd and Wright, 1951). These findings caused a revision of their earlier postulate that such lipoid insoluble compounds were central depressants. The same difficulty arises from the results of Holmstedt and Skoglund (1953), who found that intra-arterial injections of Tabun facilitated monosynaptic flexor reflexes, inhibited extensor reflexes and had a variable effect on polysynaptic reflexes, depending on the dose used. The possibility of central action due to the stimulation of receptor organs was excluded, and effects were obtained with doses far less than those necessary if intravenous administration had been used. The action was slow in onset, as would be expected from an anticholinesterase. It is however uncertain that these irreversible inhibitors of cholinesterase are acting by virtue of their anticholinesterase activity, for Holmstedt (1954) reports that under similar circumstances DFP increased both monosynaptic and polysynaptic reflexes.

The blockage of the central effects of acetylcholine, eserine and prostigmine by atropine are common findings (Schweitzer and Wright, 1937a and 1937c; Calma & Wright, 1944; Bulbring & Burn, 1941), although contrary results have been reported (Merlis & Lawson, 1939). Bulbring & Burn (1941) were unable however to abolish the central effects of nicotine with atropine. It is interesting in this regard that Marrazzi

(1953) found that atropine blocked the effect of acetylcholine on certain cortical neurones. Bernhard and Taverner (1951), Bernhard, Taverner & Widen (1951) and Bernhard, Gray and Widen (1953) have found that an intravenous injection of D-tubocurarine increased the monosynaptic extensor reflex, while the polysynaptic reflexes were unaltered, which is in contrast with earlier work by Naess (1950) who was unable to demonstrate any effect on these reflexes by the same drug.

The foregoing brief summary of the recent literature demonstrated the confusing results produced by different types of experiment. It is however fairly evident that, when administered by intra-arterial injection and even intravenously if the cholinesterase is inactivated, acetylcholine depresses monosynaptic extensor reflexes (Schweitzer & Wright, 1937a, c; 1938; Bulbring & Burn, 1941). Nicotine has a similar effect (van Harreveld & Feigen, 1948; Taugner & Culp, 1953). Both drugs have a variable effect on flexor reflexes but usually a potentiation occurs. It has been shown that both of these substances can stimulate peripheral receptors, especially those concerned with pain. In many of the experiments, no allowance has been made for this possibility and it is suggested that the effects produced by intra-arterial injections may arise on account of the central actions of impulses generated in this manner. By chemical stimulation of receptors, impulses could be set up in the group II or III muscle afferent fibres and in cutaneous afferent fibres which would be expected, in general, to have a central excitatory action upon flexor motoneurones and an inhibitory action upon extensor motoneurones (Lloyd, 1946).

Feldberg (1945 & 1950) has suggested that the presence of

acetylcholine and cholinesterase in central nervous tissue together with the ability of such tissue to synthesize and release acetylcholine provide strong evidence that acetylcholine is a major transmitter in the central nervous system. This idea has been extended and has led to the theory of alternate cholinergic and non-cholinergic transmission, which has been based mainly on the distribution of acetylcholine, cholinesterase and choline acetylase (Feldberg & Vogt, 1948; Feldberg, Harris and Lin, 1951; Feldberg, 1954). The relevant facts for the spinal cord can be summarized as follows. Acetylcholine is unevenly distributed (MacIntosh, 1941) and is mainly confined to the grey matter. Primary afferent fibres in dorsal roots, and the dorsal column and the medulla, and the pyramidal tract contain little or no acetylcholine whereas high concentrations occur in the anterior roots. This distribution is almost identical with that of choline acetylase (Feldberg & Vogt, 1948; Feldberg, et al., 1951; Wolfgram, 1954). Originally localization of cholinesterase was dependent on the macroscopic hydrolysis of acetylcholine (cf. Feldberg, 1945, Table 2), but has now gained precision with the histochemical methods of Koelle. Separation into true or acetyl-cholinesterase and pseudo-cholinesterase (cf. Koelle, 1955) has enabled acetyl-cholinesterase distribution to be determined accurately, and it appears that in general it resembles that of acetylcholine and choline acetylase (Burgen and Chipman, 1951; Koelle, 1954). A notable dissimilarity is that in cattle the dorsal and ventral roots have almost the same content of acetyl-cholinesterase (Wolfgram, 1954) but the cholineacetylase activity is twenty times greater in the ventral roots than in the dorsal roots.

It is important when considering these studies to realise that

apart from the histochemical localization of cholinesterase, the localizations and estimations are gross and open to considerable error. Although it has generally been agreed that dorsal root fibres are not cholinergic in their central synaptic action (Eccles, 1948; Bremer, 1953a; Feldberg, 1954) Burgen (1954) has pointed out that not all dorsal root ganglion cells have a low cholinesterase content (Koelle, 1951) and has suggested that some primary afferent fibres may have cholinergic terminals. The postulates of Feldberg and his co-workers depend on the correlation between the distribution of the three components of the acetylcholine system (Feldberg & Vogt, 1948; Feldberg, et al., 1951). It is suggested that those neurones which are rich in the components of this system exert synaptic activity by means of acetylcholine. The primary afferent neurones are non-cholinergic, but the cells of the cuneate and gracile nuclei and of the lateral geniculate body would exert a cholinergic synaptic action on the next neurone in the series. It is assumed that acetylcholine, choline acetylase of acetyl-cholinesterase are concentrated in the bodies and axones of cholinergic neurones rather than in the presynaptic endings upon these cells (cf. Brugen, 1954). Koelle (1954) has demonstrated that in spinal motoneurones acetylcholinesterase is intracellular, but it is important to realise that, although at the neuromuscular junction cholinesterase is predominantly on the post-synaptic cell surface (Koelle & Friedenwald, 1949) and in autonomic ganglia it is mainly in the presynaptic terminals, (Sawyer & Hollinshead, 1945), no such accurate localization has been possible for the synapses of the spinal cord.

The theory of Feldberg, based on estimations and localizations

that are made at a relatively crude level, lacks proper pharmacological and electrophysiological investigation. It fits moderately well with observations on the sensory pathways, but the evidence for such alternation of cholinergic and non-cholinergic junctions is less satisfactory for other parts of the central nervous system.

Before acetylcholine was fully accepted as the transmitter agent at the neuromuscular junction and in autonomic ganglia it was necessary to establish its release at these sites. Using eserinized Locke's solution for perfusion, Dale and his co-workers (cf. Dale, Feldberg & Vogt, 1936) were able to demonstrate this for neuromuscular transmission and Feldberg (Feldberg & Gaddum, 1934; Feldberg & Vartiainen, 1934) for autonomic ganglia. Many investigators have detected acetylcholine both in the cerebro-spinal fluid and venous blood from brain and spinal cord, but it is doubtful whether the small amounts involved originated from central nervous tissue. Bulbring and Burn (1941) detected acetylcholine when they perfused the lower spinal cord of a cat with eserinized saline and the amount was increased when the central end of the cut sciatic nerve was stimulated. Feldberg (1945) has brought together the evidence that during alterations in central activity there are changes in acetylcholine metabolism. The findings are conflicting and not particularly applicable to spinal transmission. It will be difficult to obtain a preparation of spinal cord in isolation from other tissue for the direct determination of acetylcholine output under different circumstances, as has been attempted for the superior cervical ganglion (MacIntosh, 1938) and for the cerebral cortex (MacIntosh & Oborin, 1953).

(D). PHARMACOLOGY OF A CENTRAL CHOLINERGIC SYNAPSE IN THE SPINAL CORD.

It has been established (Eccles, Fatt and Koketsu, 1954) that a group of interneurones in the ventromedial region of the ventral horn discharge repetitively in response to impulses in motor axones. The discharge of these cells had been described by Renshaw (1946), who had also demonstrated (Renshaw, 1941) the inhibitory effect of antidromic volleys in motor axons upon all types of motoneurones at that segmental level. Kölliker (1891), Lenhossek (1893) and Cajal (1909) had described the presence of collateral branches from motor axons as they emerged from the ventral horn, and in view of the negative results of experiments designed to test whether Renshaw cells were activated by the antidromic volley after it had traversed the motoneurone cell body and invaded the dendrites (Toennies & Jung, 1948; Toennies, 1949; Jung, 1953), it was postulated that impulses in these collateral branches were responsible for the activation of the interneurones. Since the terminals of motor axons release acetylcholine at the neuromuscular junction, in conformity with Dale's principle (Dale, 1935) the same chemical transmitter would be responsible for excitation of these neurones, provided the excitation was monosynaptic. It has now been shown (Eccles, Fatt and Koketsu, 1954; Eccles, Eccles & Fatt, 1956) that these interneurones (designated Renshaw cells) are activated by acetylcholine and act as a non-specific negative feed-back control of motoneuronal activity. Collaterals from many motor axons converge on any one Renshaw cell and excite the discharge of impulses. This discharge is often prolonged with an initial frequency of over 1000/sec.

This inhibitory system is the first example of a specifically

defined cholinergic synaptic mechanism in the spinal cord, and the pharmacology of these synapses has been determined by applying drugs by an intra-arterial route. The responses of Renshaw cells to synaptic stimulation are depressed by dihydro-ß-erythroidine which blocks cholinergic transmission (Unna, Kniazuk & Greslin, 1944) and prolonged by anticholinesterases such as eserine, TEPP, DFP and NU 2126. The cells are directly excited by nicotine and acetylcholine, the excitatory action of acetylcholine being prolonged by anticholinesterases. Dihydro-ß-erythroidine decreases the excitatory action of both acetylcholine and nicotine (Eccles, Eccles & Fatt, 1956). Pharmalogical studies have shown however that certain drugs, known to be effective at peripheral cholinergic junctions, exhibit anomolous behaviour at these central cholinergic synapses. Not all Renshaw cells can be activated by intra-arterial injections of acetylcholine although all are sensitive to nicotine. The cells do not respond to succinylcholine, decamethonium, mecholyl or arecholine. Of the anticholinesterases administered intravenously, eserine, TEPP and NU 2126 are most effective, DFP being less so, whilst prostigmin is much less effective. The gradation of effect of eserine, NU 2126, TEPP and DFP may be related to their known peripheral actions. ß-erythroidine was not as efficient in blocking the discharge of Renshaw cells as dihydro-ß-erythroidine, as might be expected (cf. Unna et al., 1944), but d-tubocurarine chloride, dimethyl-d-tubocurarine iodide, and gallamine triethiodide were ineffective. Atropine had a small effect in depressing the discharge of the cells, when used in large doses.

Because of the prolonged repetitive firing of Renshaw cells in response to a single antidromic volley and the relative resistance of the

first few discharges to the action of pharmacological agents such as dihydro-ß-erythroidine, it has been postulated that there is a diffusional barrier intimately related to the cholinergic synaptic terminals on Renshaw cells (cf. Eccles, Eccles & Fatt, 1956). The repetitive firing of Renshaw cells in response to an antidromic volley in motor axons might be due to the excitation of a chain of interneurones. However timing of the first Renshaw discharge and the onset of antidromic inhibition of motoneurones allows of no interneurone except the Renshaw cell between the axon collatera and the motoneurone. The Renshaw cell could be re-excited from an internuncial chain also set in action by impulses in the axon collaterals. It is possible that Renshaw cells are excited by volleys in group III afferent fibres from muscle (Eccles, Fatt & Koketsu, 1954) although definite evidence is lacking, and Frank and Fuortes (1956a) suggest that they may be also excited monosynaptically by volleys in larger fibres but present no definite evidence. This raises the possibility of some primary afferent fibres releasing acetylcholine at their synapses with Renshaw cells (cf. Burgen, 1954), but it is just as probable that impulses from these fibres do not directly excite Renshaw cells but are relayed by cholinergic interneurones. It is not known however whether acetylcholine is the only excitatory transmitter substance for Renshaw cells. When recording from a single Renshaw cell it is difficult to show that volleys in the group III muscle afferent fibres directly excite it as these volleys may activate motoneurones that in turn excite the Renshaw cells. The firing of Renshaw cells by impulses in the slow muscle afferent fibres is blocked by dihydro-ß-erythroidine (Curtis - personal observation) but these investigations are far from complete.

There is however a further barrier to penetration of nervous tissue by drugs administered intravenously or intra-arterially. The blood-brain barrier (Friedemann, 1942; Tschirgi, 1952) may be concerned with the pia-glial membrane surrounding the cerebral vessels (Patek, 1944; Woollam & Millen, 1954) the capillary endotheleum (Rodriguez, 1955), or with the actual inter-cellular substance of the nervous system (Hess, 1953; 1955). Whatever the nature of the barrier, the ineffectiveness of the intra-arterial and intravenous injections of some drugs can be explained by a selective diffusional barrier between the plasma and the synaptic areas on the Renshaw cells. It had been pointed out by Schweitzer, Stedman and Wright (1939), when investigating the central action of anti-cholinesterases, that the quaternary ammonium compounds occurred in ionic form, were insoluble in lipids and were central inhibitors of spinal reflexes. However salts of the tertiary ammonium bases undergo some hydrolytic dissociation in solution and therefore are partly soluble in lipid, and were excitors of spinal activity, perhaps because they penetrated cells. Later work by this group (Chennels et al., 1949) demonstrated that the lipoid-soluble condensed alkyl-phosphonates HETP and TEPP had a similar action to the tertiary bases eserine and DFP although the decomposition products of HETP when dissolved in water are lipoid-insoluble. Hence the property of lipoid solubility may not be important when dealing with the penetration of drugs either through this blood-brain barrier or through specialised diffusional barriers.

The salts of tertiary bases such as eserine and other substances with similar solubilities such as dihydro-β-erythroidine, erythroidine, Nu 2126 and nicotine are effective on Renshaw cells whereas quaternary

ammonium compounds such as prostigmine and tubocurarine are not. It has been observed (Eccles, Eccles & Fatt, 1956; Curtis, personal observation) that, when prostigmin is injected locally into the region of Renshaw cells, using the injection device of Fig. 20, it is as effective as intravenously administered eserine. This method, using Renshaw cells as a sensitive detecting mechanism has been extended to the study of other substances that are unable to penetrate the blood-brain barrier. It is probable that the ineffectiveness of intravenously injected prostigmine and tubocurarine is due predominantly to the blood-brain barrier which presumably has much the same permeability to these substances as has been found for the squid giant axon (Bullock, Nachmansohn & Rothenberg, 1946; Rothenberg, Sprinson & Nachmansohn, 1948). The effectiveness of this barrier may also explain the insensitiveness of some Renshaw cells to acetylcholine but this insensitiveness may also be associated with the diffusional barrier around the synaptic areas of Renshaw cells.

(E). EXPERIMENTAL RESULTS WITH CHOLINERGIC SUBSTANCES.

When all the lumbar dorsal roots were either cut or crushed, the testing monosynaptic reflex in L7 and S1 segments was almost invariably depressed transiently by intra-arterially injected acetylcholine or nicotine. Fig. 2 shows several series of maximum monosynaptic reflexes from an atropinized cat. These reflexes, recorded from the L7 ventral root at second intervals, were depressed by 100 μg of Acetylcholine (top records) and later by 25 μg of Nicotine (lower records). The drug was injected at the time of the arrow and the onset of the depression has a variation depending on the rate of injection, the spinal cord circulation and the blood pressure of the cat. The full time sequence of these

events are graphed in Fig. 30. The depressions due to both acetylcholine and nicotine are fleeting but in almost all experiments it was found that for doses of nicotine and acetylcholine giving approximately the same degree of depression, the nicotine depression was much longer in duration. In Fig. 3, after 25 μg of nicotine intra-arterially the reflex was not back to its original value until 60-65 seconds. Fig. 4 from the same experiment shows that, though smaller, the nicotine depression lasts slightly longer than that due to acetylcholine.

The shapes of the curves illustrated in Fig. 3 and 4 are typical of those obtained in all experiments. The depression is usually rapid in onset and then slowly subsides. Because of the considerable variations found for any one dose, it has been difficult to compare the resultant effects. The magnitude depended partly on the dose of either drug, as compared in Figs. 3 and 4, but the impression was gained that above a certain dose there was only a prolongation of the duration of the effect and no increase in its magnitude. In a few experiments little or no effect was found with doses of acetylcholine up to 200 μg whereas 10-50 μg of nicotine depressed the reflex as usual. This is illustrated in Fig. 5.

It was necessary to allow ten to fifteen minutes between injections in order to obtain consistent results. It has been found (Eccles, Eccles & Fatt, 1956) that an injection of nicotine evokes a long lasting discharge from Renshaw cells and in this present series of experiments the injection of 25-50 μg of nicotine immediately after obtaining a depression of the testing reflex by a similar dose usually resulted in very little or no additional depression. Fig. 6 shows the effect of 20 μg

of nicotine upon the Renshaw cell "rhythm" recorded on the dorso-lateral surface of the L7 segment when the ventral root of this segment was stimulated maximally at a rate of 5/sec. The intra-arterial injection of nicotine would be expected to activate the whole population of Renshaw cells for some time and in so doing render them insensitive both to volleys in the axon collaterals and to further intra-arterial injections. In Fig. 6 the effect on the total Renshaw cell population is slow to develop and lasts at least 10 minutes. This can be correlated with the extremely poor circulation of the experimental animal used, for usually after the same dose of nicotine, Renshaw cells can be activated by antidromic volleys after several minutes.

The reflex depression was usually well on the way to recovery before any change occurred in the systemic blood pressure. This was observed for acetylcholine in several animals before atropine had been given. There was however no method for the detection of local vascular changes within the segments involved. In the preliminary experiments all the dorsal roots were not severed and depressions seen after injections of acetylcholine and of nicotine were occasionally followed by a 10-20% increase in the size of the reflex which persisted for 10-30 seconds. Such an effect was presumably due to afferent impulses reaching the cord from peripheral structures stimulated by the injected drugs, for it was always diminished or abolished by a more complete severing of the dorsal roots.

Further pharmacological studies were necessary to establish the true nature of the effects of intra-arterial injections of acetylcholine and nicotine. Since the only known effects of such injections are the activation of Renshaw cells (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles

& Fatt, 1956), it is tempting to ascribe the depression of monosynaptic reflexes to the inhibition of motoneurones that is brought about by the activation of Renshaw cells. It is important to distinguish between a true inhibition of motoneurones due to the action of an inhibitory transmitter substance and any other process depressing their responsiveness to excitation, as for example the effect of anoxia or of a metabolic poison. The use of strychnine permits such a distinction, as this drug diminishes the effect of the inhibitory transmitter in setting up an inhibitory post synaptic potential (cf. Section II). Both acetylcholine and nicotine fail to depress the testing monosynaptic reflex after sub-convulsive doses of strychnine. For example in Fig. 7 the depression following the injection of 50μg of nicotine does not occur after 0.08 mgm/Kgm of strychnine had been administered intravenously, while ninety minutes later the same dose of nicotine was again effective in depressing the testing reflex, i.e. the effect is reversible.

It is therefore highly likely that the observed effects of intra-arterially injected acetylcholine and nicotine depend ultimately upon the release of an inhibitory transmitter at the motoneuronal membrane. This finding further emphasizes the link between the present findings and the possibility that activation of Renshaw cells is responsible for it. Extensive searching of the ventral horn region has failed to disclose any cells that can be activated by intra-arterially injected acetylcholine and nicotine, and not stimulated by an antidromic volley in the ventral root. The Renshaw cells are the only known cholinergically excited inter-neurones which can inhibit motoneurones. It was necessary however to establish more pharmacological similarities.

Most animals were atropinized in order to prevent gross blood pressure alterations due to acetylcholine. Comparison of a series of reflexes which are inhibited by acetylcholine before and after the intravenous injection of 0.5 mgm/Kgm of atropine sulphate shows usually a slight diminution of the acetylcholine inhibition. As illustrated in Fig. 8, this effect is small and hard to distinguish from the variation found with successive doses of the same amount of acetylcholine. It was, however, seen in several experiments and is probably of significance. Eserine sulphate given intravenously in doses of 0.2 - 1.0 mgm/Kgm had no significant effect on the inhibition of the reflex following an intra-arterial injection of nicotine (Fig. 9). However, as shown in Fig. 10, the same dose of eserine prolonged and increased the depression evoked by 200 µg of acetylcholine. An intra-arterial injection of 200 µg of acetylcholine produced approximately a 20% depression of the testing reflex, this was almost doubled after 0.2 mgm/Kgm of eserine was injected intravenously.

Dihydro-ß-erythroidine blocks the cholinergic activation of Renshaw cells (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956). Following the injection of 0.8 mgm/Kgm of dihydro-ß-erythroidine hydrochloride intravenously both nicotine and acetylcholine when injected intra-arterially have no depressant effect on the testing monosynaptic reflex (Fig. 11). This observation has been found to be reversible for both drugs.

The above pharmacological studies give good evidence that the observed inhibition of the monosynaptic reflex by intra-arterially injected acetylcholine and nicotine is due to activation of Renshaw cells. Both the depression of the reflex and firing of Renshaw cells

are prevented by dihydro-β-erythroidine. It is true that the initial firing of the cells when activated by a volley in the ventral root fibres is resistant to this agent (Eccles, Eccles & Fatt, 1956), but this period lasts for a few milliseconds only and would not be detected in the present comparatively gross study. Atropine and eserine have the expected effects when acetylcholine is used to inhibit the reflex but are ineffective when nicotine is used. Finally strychnine reversibly prevents the depression of the monosynaptic reflexes by intra-arterially injected acetylcholine and nicotine just as it prevents the inhibitory postsynaptic potential of motoneurones set up antidromically. As will be shown later, polysynaptic reflexes are depressed in a similar manner to the monosynaptic responses.

The properties of the Renshaw cell system can in part explain some of the earlier investigations on cholinergic mechanisms in the spinal cord. The depressant action of acetylcholine and nicotine on monosynaptic reflexes (Schweitzer & Wright, 1937a and c; 1938; Bulbring & Burn, 1941; van Harreveld & Feigen, 1948) could have been due to the excitation of Renshaw cells and the consequent non-selective inhibition of motoneurones. However, as explained earlier, this result together with the alteration in flexor monosynaptic and polysynaptic reflexes can also be explained by the peripheral actions of the drug used. In this respect it is of interest to compare the actions of acetylcholine and 5 hydroxy-tryptamine (5 HT) before and after deafferentation of the spinal cord. These substances were both able to stimulate receptor organs and produce pain when tested by Armstrong et al. (1953). In Fig. 12A all the dorsal roots except those of L7 and S1 segments on the left side were intact and 200 μg of either acetylcholine or 5 HT

when injected intra-arterially depressed the monosynaptic reflex of the L7 segment. Subsequent crushing of the left L5 and L6 dorsal roots altered the responses considerably (Fig. 12B). Probably crushing of all remaining dorsal roots on both left and right sides would have abolished the depression of the reflex due to 5 HT (cf. Section I G (i)).

Other drugs with known actions at peripheral cholinergic junctions were tested and were found to be ineffective in altering spinal reflexes. Succinyl choline, methylcholine and carbominocholine in doses of 50-200 µg are also without effect on Renshaw cells (Eccles, Eccles & Fatt, 1956). In view of the inability of prostigmin and of tubocurarine to penetrate the blood-brain barrier as detected by the failure of these drugs to influence Renshaw cell discharges, it is difficult to explain the findings of Schweitzer and Wright (1937a and c, 1938), Bulbring and Burn (1941) and Calma (1949) using prostigmin, and Bernhard and Taverner (1951), Bernhard et al. (1951) who administered d-tubocurarine intravenously. It may be concluded that under the circumstances used by these experimenters the drugs were either not acting centrally or else were acting on central spinal structures not separated from plasma by such a barrier. This latter postulate is unlikely. The variable effects of other anticholinesterases are equally inexplicable, as most are able to influence Renshaw cell discharge. They may however be acting directly and not predominantly as anticholinesterases.

(F). CENTRAL ADRENERGIC TRANSMISSION.

In contrast to the extensive literature on cholinergic transmission in the spinal cord, there has been very little investigation of the possibility of adrenergic transmission. Schweitzer and Wright (1937b)

demonstrated a depression of the knee jerk by intravenous adrenalin and Bulbring and Burn (1941) using a double perfusion system found that the knee jerk and flexor reflexes were increased both by adrenalin and ephedrine. Their main finding however was that in the presence of adrenalin, small doses of acetylcholine caused a spontaneous discharge of flexor motoneurones. Bernhard, Skoglund and Therman (1947) found adrenaline and acetylcholine had opposite effects when applied to the exposed lumbar region of the cord. Bulbring et al., (1948) again demonstrated an interaction between acetylcholine and adrenaline and found that in animals in which flexor or extensor movements were elicited by medullary stimulation and increased by intra-arterial acetylcholine, these effects were modified by a simultaneous intravenous perfusion of adrenalin or even reversed by intra-arterial adrenalin alone. These results were extended by Bernhard and Skoglund (1953) who demonstrated an augmentation of extensor monosynaptic reflexes but a diminution of flexor monosynaptic and polysynaptic reflexes.

There are two main results of these investigations. Firstly that adrenalin potentiates the action of acetylcholine in the cord and secondly that it has opposite effects to acetylcholine. It has been suggested above that many of the demonstrated effects of acetylcholine are not central in origin and it is probable that these "central" actions of adrenalin are spurious. Insufficient attention has been applied to general and local changes in the arterial supply of the cord. Burn (1945) has reviewed the evidence that adrenalin potentiates the effect of acetylcholine at peripheral junctions but there is little justification for extending this to the problem of central transmission.

Vogt (1954) has determined the distribution of adrenalin and nor-adrenalin in the central nervous system. These substances occur together, adrenalin being usually in lower concentration, and their distribution parallels that of 5-hydroxytryptamine. High concentrations occur in the hypothalamus, mid-brain and area postrema whereas the cerebrum, cerebellum and spinal cord contain very little. The dorsal and ventral roots have roughly equal amounts, the lowest concentration in the spinal cord occurring in the dorsal horn. Since there is little evidence for a central action of adrenalin and nor-adrenalin, Vogt (1954) suggests that the high concentrations found in both the area postrema and certain gliomas may indicate that these substances are not concerned with synaptic transmission but perhaps have some other function in relation to sympathetic centres.

In the present series of experiments adrenalin, nor-adrenalin and ephedrine in doses of 5-100 µg usually had no effect on a monosynaptic reflex when given by the intra-arterial route. Occasionally there was a slight increase in the reflex starting 5-10 seconds after the injection and lasting for thirty seconds. This is illustrated in Fig. 13 for both adrenalin and nor-adrenalin. The monosynaptic reflex of the L7 segment was evoked every two seconds and the late increase in the reflex, associated with a rise in the blood pressure, was presumably vascular in origin. In other experiments the increase was not as marked but was usually also associated with a rise in the blood pressure. Both these drugs and ephedrine, when injected intravenously have no effect on Renshaw cells (Eccles, Eccles & Fatt - personal communication).

(G). OTHER POSSIBLE TRANSMITTER SUBSTANCES.

(1) 5-Hydroxy-tryptamine.

There has been considerable speculation recently concerning the possible function of 5-hydroxy-tryptamine (5 HT) as a central transmitter substance. Investigated independently by Erspamer as the substance responsible for the properties of chromaffin cells of the gastro-intestinal mucosa (Enteramine) and by Rapport and his colleagues as the vasoconstrictor found in mammalian serum when blood clots (Serotonin), both substances were isolated and identified with 5-hydroxy-tryptamine (cf. Erspamer, 1954; Page, 1954). Pharmacologically 5 HT is a smooth muscle stimulant. The antagonists of this action were investigated by Gaddum and Hameed (1954) and can be divided into three main groups - the ergot alkaloids including lysergic acid diethylamide (LSD 25), the harmalma alkaloids and yohimbine. It was soon pointed out (Wooley & Shaw, 1954, a & b) that some of these alkaloids, when administered to man or animals, caused certain forms of mental aberration. This together with the investigation of the distribution of 5 HT in the nervous system (Amin, Crawford & Gaddum, 1954) led to the suggestion by Wooley & Shaw, in particular, that 5 HT was a central transmitter, the action of which was disturbed by such substances as LSD 25.

As far as the spinal cord is concerned these investigations are of little import. No action of the ergot alkaloids has been demonstrated on spinal reflexes. Amin et al. (1954) found no 5 HT in either dorsal or ventral roots or white matter. However grey matter contained a considerable amount, and there was a high concentration in the nuclei cuneatus and gracilis. The highest concentrations were found in the

hypothalamic area and mid-brain, especially the area postrema. It has been established by Erspamer (1955) that amine oxidase is responsible for one stage in the metabolic destruction of 5 HT, the eventual product 5-hydroxy-indole acetic acid (5 HIAA) being excreted in the urine. It may be that some drugs act by interfering with this metabolic destruction of 5 HT rather than by competing with 5 HT at receptor sites. There is some evidence that 5 HT will not penetrate the blood-brain barrier (Wooley & Shaw, 1954b), and Erspamer (1954) is reluctant to attribute any significance to its presence in the central nervous system. He considers that the comparatively low concentrations could be due to that in the blood contained in the brain tissue.

It has recently been demonstrated that gastro-intestinal argentaffin carcinomas are often associated with a raised blood level of 5 HT and an increased urinary excretion of 5 HIAA (cf. Pernow & Waldenstrom, 1954; Goble, Hay & Sandler, 1955; Snow, Lennard-Jones, Curzon & Stacey, 1955). The clinical manifestations are predominantly cardiac, respiratory and gastro-intestinal and the known antimetabolites of 5 HT are usually without effect. The absence of neurological disturbances is further evidence that 5 HT has no function in central nervous transmission.

Although LSD 25 evokes schizophrenic-like states in man, not all substances which antagonize the action of 5 HT on smooth muscle when tested in vitro lead to such conditions. Consequently the theory advanced by Wooley and Shaw (1954 a & b) needs revision. Cerletti and Rothlin (1955) have demonstrated that BOL 148, a simple bromids derivative of LSD 25, is an antagonist of 5 HT when tested in vitro but has no central effects. This evidence is further confused by the finding that the

central action of the rauwolfia alkaloids may be concerned with the release of 5 HT. Brodie, Pletscher & Shore (1955); Pletscher, Shore & Brodie (1955, 1956) consider that reserpine causes 5 HT to be released both from rabbit intestine and brain. The released 5 HT is excreted as 5 HIAA, and they postulate that reserpine and related alkaloids release 5 HT from a bound to a more active form. Whatever the outcome of all these investigations, it is unlikely that the central behaviour of certain known anti-metabolites of 5 HT can be satisfactorily explained by such an action in the central nervous system.

The findings of Slater, Davis, Leary & Boyd (1955) give no satisfactory evidence towards the role of 5 HT in the cord. Their experimental arrangement does not exclude the possibility of uncontrolled afferent impulses reaching the cord, and indeed their results on the alteration of the flexor reflex could be due to the peripheral stimulation of afferent fibres. It has been shown by Armstrong et al (1953) that 5 HT was able to cause pain when applied to the exposed base of a blister raised by cantharidin. Although slow in onset, this effect persisted for several minutes. Slater et al found that a preliminary dosage of LSD 25 modified the effects which they presumed to be due to 5 HT.

In the present series of experiments, 5 HT in doses up to 500 µg (expressed as 5 HT-creatine sulphate) intra-arterially had no effect on the testing monosynaptic reflex unless dorsal root fibres were intact. As shown in Fig. 12 200 µg of 5 HT depressed the reflex to a greater extent than did the same dose after several lumbar dorsal roots were crushed. In other experiments where de-afferentation was complete, 5 HT had no effect as illustrated in Fig. 14 for 500 µg of 5 HT. Occasionally there

was a 10-20% increase in the reflex 2-3 seconds after the injection. This persisted for 10-20 seconds and was not associated with the immediate rise in systemic blood pressure.

Direct extracellular recording from several Renshaw cells showed that 5 HT had no effect on either their normal discharge in response to an antidromic volley in the ventral root or the discharge due to 20 µg of nicotine injected intra-arterially. In Fig. 15 are shown extracellular records from a single Renshaw cell. This cell was firing spontaneously at a rate of one every two seconds and this rate was not altered by the intra-arterial injection of 500 µg of 5 HT (A). The response of this cell to stimulation by an antidromic volley in the ventral root (B) was not altered by a similar injection (C). Likewise the discharges from the cell (D) produced by the intra-arterial injection of 20 µg of nicotine were not significantly altered by a further injection of 5 HT (E). Until 5 HT has been injected locally into the region of Renshaw cells it is impossible to say whether they are influenced by it, for Wooley and Shaw (1954b) have suggested on the basis on analysis of the brain content of 5 HT of a mouse after 5 HT had been injected intra-peritoneally, that 5 HT does not penetrate the blood-brain barrier.

(ii) Substance P.

Euler and Gaddum (1931) isolated in alcoholic extracts of plain muscle and brain a substance which stimulated plain muscle. This effect was not prevented by atropine and the material was named substance P. The pharmacological properties and distribution have now been determined (Pernow, 1953, 1955; Kapera & Lazarini, 1953; Amin et al., 1954). Substance P can be purified by chemical or chromatographic techniques and can be separated pharmacologically from acetylcholine,

histamine, 5 HT and tryptamine. It is a polypeptide, and, although destroyed enzymatically by trypsin and chymotrypsin, no specific enzyme has yet been isolated.

The distribution in nervous tissue suggests a possible function as a transmitter agent (Lembeck, 1953). The concentration is high in dorsal roots, dorsal columns, spinal grey matter and gracile and cuneate nuclei whereas little is present in ventral roots. Hellauer (1953) suggests that substance P is the active vasodilator extracted from dorsal roots.

Up to the time of writing the effect of substance P on spinal transmission has not been recorded. Euler and Pernow (1954) have observed central effects with intraventricular injections but were unable to obtain significant results when substance P was injected into the para-ventricular tissue. If effects on spinal cord transmission are not obtained with intra-arterial injections of substance P, direct application will have to be made to specific areas in the grey matter.

(iii) Adenosine triphosphate (ATP).

Dale (1935) suggested that the substance causing antidromic vasodilation at the peripheral terminals of collaterals from sensory fibres would probably be the transmitter substance at their central terminals. Various extracts of dorsal roots have been tested, capillary vasodilation of the rabbit ear being the method of evaluation (cf. Hilton & Holton, 1954). Hellauer and Umrath (1947, 1948), Hellauer (1953) and Umrath (1953) extracted a substance from dorsal roots which dilated the vessels of the rabbits ear and which was destroyed by an enzyme also present in dorsal roots. They further reported that this enzyme was inhibited by strychnine and other convulsants. This substance could

not be extracted from ventral roots.

Recent investigations (Holton & Holton, 1953; Harris & Holton, 1953) have defined the actual capillary vasodilation with both antidromic stimulation and extracted substances, and have shown that the vasodilator substance was present both in dorsal and ventral roots in inverse proportion to the distribution of acetylcholine and choline acetylase. Holton (1953) demonstrated that the anticholinesterases eserine, prostigmin and DFP reduced the vasodilator response to both chemical and antidromic stimulation. Holton and Holton (1953, 1954) have shown that the vasodilator activity of extracts from dorsal and ventral roots is probably due to ATP and ADP with small amounts of further breakdown products. For example arterial injections of ATP and ADP gave a vasodilation which resembled that produced by antidromic nerve stimulation and by the injection of the extracts. The vasodilator substance was found to be concentrated predominantly in dorsal roots, dorsal columns and cuneate and caudate nucleus (Harris & Holton, 1953).

The results of experiments designed to evaluate the effect of ATP on spinal reflexes are indecisive. Buchthal, Engbach, Sten-Knudsen & Thomasen (1947) injected ATP into the vertebral artery of cats and obtained movements of the forelimbs with doses of 300-6000 µg. Similar results occurred with inorganic triphosphate and pyrophosphate but none with creatine phosphate or adenylic acid. Sodium orthophosphate and acetylcholine (1-100 µg) had no effect. It is possible that some of these results could be due to an increase in afferent inflow that arises on account of the stimulation of peripheral structures. Emmelin and Feldberg (1948) found that in spinal cats with the cervical cord intact, intravenous or intra-vertebral injections of ATP caused muscular

contractions particularly in the hind limbs resembling the scratch reflex. Occlusion of the vertebral artery caused the same movements. The peripheral effects of ATP are extremely complex (Emmelin & Feldberg, 1948) and it is difficult to ascertain whether the demonstrated "central effects" are due to a peripheral action. The effect of intra-arterially injected sodium-ATP upon monosynaptic reflexes in the lumbar cord of the spinal cat has been tried several times, but only in the one preparation. When dorsal root crushing was complete, 200 µg had no effect. This is shown in Fig. 16, the testing monosynaptic reflex being evoked in the S1 segment.

There is thus little precise evidence that ATP is a central transmitter substance. Its occurrence both in dorsal and ventral roots makes it unlikely that it is the transmitter released by primary afferent fibres either in the spinal cord or at the terminations of their peripheral collateral branches.

(iv) Histamine.

There is little evidence that histamine is a central transmitter substance. Kwiatkowski (1943) was unable to demonstrate appreciable quantities of histamine in either dorsal roots or the spinal cord. Hausler (1953) was able to excite motoneurones of the isolated frog's spinal cord by injecting relatively small amounts of histamine, but, in view of the low concentrations in mammalian nervous tissue, it is doubtful whether histamine has a significant function.

In the present series of experiment histamine dihydrochloride injected intra-arterially in doses of 10-500 µg did not alter the testing monosynaptic reflex in the lumbar region of the spinal cat. The effect of 500 µg on the reflex of the L7 segment is illustrated in Fig. 17,

the reflex not being altered significantly. This dose of histamine is also without effect on the discharge of Renshaw cells (Eccles, Eccles & Fatt - personal communication).

(v) Tissue extracts.

The relative ease with which acetylcholine was extracted from tissue by Dale and his collaborators led to a search for chemical transmitters in the spinal cord by many investigators without proper appreciation of the factors involved both in the preparation and in the testing of extracts. Gaddum (1955) has stated some of the criteria necessary when analysing extracts of tissue. The biochemical aspects of separation of the components of such extracts can be complicated and time consuming, hence it is important that the mothod of testing be relatively simple and give unambiguous results. As indicated above, intravenous and some methods of intra-arterial injection give results of doubtful validity, but, even when precautions are taken to exclude peripheral effects from simulating central actions of the substance under test, application via the spinal arterial system may fail on account of non-penetration of the blood-brain barrier. In these cases direct application to spinal structures by micro-pipettes may be indicated, but here again adequate methods must be used in order to detect the effects (cf. Kennard, 1953). It is surprising that some investigators (cf. Bernhard et al., 1947; Florey & McLennan, 1955b) have applied drugs or extracts directly on to the exposed spinal cord, for under such conditions it is probable that the described effects have largely been due to actions on dorsal root fibres.

The extraction of vasodilator substances from dorsal roots has

led to considerable confusion. Holton and Holton (1954) have produced strong evidence that their active substance is mainly ATP, but the active principle of Hellauer and Umrath (1948), Umrath (1953) and Hellauer (1953) is certainly not ATP and may be substance P. Florey (1954) has extracted both "inhibitory" (I) and "excitatory" (E) factors from mammalian central nervous system and assays these materials upon a single crustacean stretch receptor (Florey & Florey, 1955). Florey (1954) considers that the "excitatory" substance may be substance P. Recently Florey and McLennan (1955a) have reported that the "inhibitory" substance is released from mammalian cortex. Florey and McLennan (1955b) have also reported that their factor I inhibits spinal reflexes but the mode and site of application of this material makes the significance of their results extremely doubtful. The blockage of tendon reflexes of the sixth and seventh lumbar segments within 5 seconds of the application of a solution of factor I to the dorsal surface of the second lumbar segment is more likely to be due to a direct blocking action on dorsal root fibres than to an inhibitory action on motoneurones.

This factor I has been tested by intra-arterial injection into the spinal cord circulation (Florey - personal communication) without effect. Samples of factor I supplied by Dr. Florey and other samples prepared by his methods have been used in the present investigation and also gave a negative result. This may be attributable to the failure of factor I to penetrate the blood-brain barrier, for a central transmitter substance need not have this ability. For example, when applied intra-arterially, acetylcholine occasionally is unable to influence the discharge of Renshaw cells, though other tests indicate that these cells

are activated cholinergically (Eccles, Eccles & Fatt, 1956). However there is no definite evidence that this "inhibitory" substance inhibits anything but the crustacean stretch receptor, and it is doubtful whether either factors E or I are of significance.

A major problem in the study of the pharmacology of spinal reflexes is the extraction of transmitter substances from central nervous tissue. These substances would be expected to be extremely labile and to occur in low concentration together with the specific enzymes responsible for their destruction. Several attempts have been made to isolate an inhibitory substance from the brains of cattle but it is apparent that it is not readily obtainable with standard techniques. In the spinal cord, it seems likely that inhibitory interneurones lie entirely within the grey matter, hence it may be expected that the inhibitory transmitter would be extractable from grey matter.

Whole brains were removed within three minutes of the animal's death and the covering membranes, large blood vessels and blood clots dissected free. The brains were then sliced thinly and separated, as far as was possible, into grey and white matter. The pieces of tissue that were predominantly grey matter were wrapped in thin polythene sheets so that the individual packets were no thicker than one centimetre and packed in dry ice. The brain slices were usually solid within two minutes and the chemical extractions were commenced within two hours. In this way it was hoped to preserve as much as possible of the transmitter substances during the transport of the collected material to the laboratory.

Standard watery extracts were made, using either heat, trichloracetic acid or perchloric acid to prevent the enzymatic destruction

of any active principle during the process. After filtration and removal of these latter two agents, the solutions of 2-3 litres in volume were reduced at low temperatures to a volume of about 50 ml. This concentrate was then treated with different solvents in turn and the material either precipitated or soluble in ethanol, ether, acetone or chloroform separated. In this way from any one extraction process many samples were obtained, and after the sodium, potassium and chloride contents of each had been estimated they were dissolved in a suitable buffered solution so that the final pH and ionic content resembled that of Ringer-Locke solution.

These extracts were tested by determining their effect on the monosynaptic reflex when applied by intra-arterial injection. This method, although attractive, may be inadequate, for the transmitter substances need not necessarily penetrate the blood-brain barrier. The majority of extracts had no effect at all on the testing reflex. During one process, three similar extracts did depress the testing monosynaptic reflex (Fig. 18 - filled circles). As it is likely that strychnine acts in the spinal cord by blocking the action of the inhibitory transmitter on the post synaptic membrane of motoneurones and interneurones it would be expected that the depression of the testing reflex by the true inhibitory substance would be prevented by the intravenous administration of subconvulsive doses of strychnine. However, in this experiment, 0.1 mgm/Kgm of strychnine administered intravenously had no significant effect on the depression of the reflex following a further 2 mgm. of the extract and it may be concluded that the active constituent of this extract was not the inhibitory substance. Further extracts made in the same fashion were without effect.

(H). PHARMACOLOGY OF POLYSYNAPTIC PATHWAYS.

In the previous sections the effects of some pharmacological agents on both monosynaptic and polysynaptic reflexes have been described. There have been frequent attempts to discriminate between the actions of drugs on monosynaptic and polysynaptic reflexes.

The effects of intra-arterially injected acetylcholine and nicotine would be expected to be similar for both flexor and extensor motoneurones because the Renshaw cell inhibitory mechanism has not been found to have any selective organization with respect to flexor and extensor motoneurones (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954). It would be expected that the effect of Renshaw cell activation on polysynaptic reflexes would also be a depression, provided that there were no other cholinergically excited synapses along the polysynaptic pathway. Feldberg et al. (1953) and Bulbring and Burn (1941) have found that flexor reflexes increased with intra-arterial injections of acetylcholine but it is probable that this effect is due to its stimulating action on receptor organs. Most investigations on antidromic inhibition have dealt exclusively with the inhibition of monosynaptic reflexes (cf. Eccles, 1955). However Konig (1952) illustrates a concomitant reduction of polysynaptic and monosynaptic reflexes by a preceding antidromic volley in the ventral root which could be due in part to activation of Renshaw cells.

Fig. 19 based on the results from two experiments, illustrates the effect of both acetylcholine and nicotine on polysynaptic reflexes. The drugs were administered intra-arterially and the reflexes, evoked by the stimulation of the dorsal roots once every two seconds were recorded

from the appropriate ventral root. All other dorsal roots were crushed and the monosynaptic reflex in both the L7 and S1 segments (Fig. 19 - upper and lower records) was depressed by nicotine and acetylcholine respectively as previously reported (cf. Section I E). It is apparent from these figures that the polysynaptic reflexes were also depressed at the same time as the monosynaptic responses. Detailed pharmacological studies have not been carried out, but it is highly probable that this depression is due to activation of the ventral horn Renshaw cells, the inhibition taking place at the motoneurones, and not along the polysynaptic pathways.

There are certain substances having different pharmacological actions upon monosynaptic and polysynaptic pathways, which may suggest that the excitatory transmitters differ in these systems. Relatively large doses of myanesin (tolserol, mephenesin) selectively depress polysynaptic reflexes whilst monosynaptic reflexes are muc less affected (Berger & Bradley, 1946; Henneman, Kaplan & Unna, 1949; Kaada, 1950; Taverner, 1952; Wright, 1954). A similar action is seen with glyketal (Berger, 1949), miltown (Berger, 1954) and parpanit (Gruber, Kraatz, Gruber & Copeland, 1949). On the other hand strychnine, thebaine bruceine and tetanus toxin increase polysynaptic reflexes while the monosynaptic reflexes are slightly increased or even decreased (Hoffmann, 1922; Bremer, 1944; Kaada, 1950; Naess, 1950; Bernhard et al., 1951; Brooks & Fuortes, 1952; Bradley, Easton & Eccles, 1953; Fatt - personal communication; cf. Section II). This effect of strychnine is reduced by myanesin (Berger & Bradley, 1946; Kaada, 1950; Taverner, 1952) and parpanit (Gruber et al., 1949).

The only specific action known for strychnine is that it depresses the effectiveness with which activated inhibitory synapses generate the inhibitory postsynaptic potential of motoneurones. It is tempting therefore to ascribe the large increase in polysynaptic reflexes to the depression of inhibitory control at synaptic relays along the pathways and the minor changes in monosynaptic reflexes to the change in background barrage of impulses upon motoneurones (cf. Bradley et al., 1953).

Similarly, for myanesin and related substances, the relatively slight alteration in monosynaptic reflexes could be due to a depression of background interneuronal discharge. The specific action of these substances may therefore be one of depression of excitatory transmitter action on interneurones without a specific action on the motoneurone. This however is unlikely, and, as Wright (1954) has suggested, myanesin may be effective at all central excitatory synapses, the greater depression of polysynaptic pathways arising from the serial arrangement of depressed synapses. For example Kaada (1950) has shown that complex polysynaptic pathways are more vulnerable to the action of myanesin that the pathways with fewer synapses.

SECTION II. THE EFFECTS OF STRYCHNINE AND TETANUS TOXIN UPON SPINAL INHIBITORY MECHANISMS.

(A) INTRODUCTION

It has recently been demonstrated (Coombs, Eccles & Fatt, 1955b & d) that the inhibitory transmitter substance acts on motoneurones by increasing the permeability of "inhibitory patches" of the postsynaptic membrane to some ions, and not at all or very slightly to others. The manner in which the excitatory responses of motoneurones are depressed by inhibitory volleys, has been demonstrated only for the "direct" inhibitory action of impulses in the group Ia afferent fibres on motoneurones of antagonistic muscles. However for other types of inhibition the postsynaptic inhibitory membranes have been shown to have the same permeabilities to ions, and it can therefore be postulated that there is the same manner of inhibitory-excitatory interaction at the motoneurone irrespective of the actual pathway of the inhibitory action. It may further be postulated that the same inhibitory transmitter is released at the motoneurone regardless of the origin of the impulses in the presynaptic terminals releasing the transmitter.

The acceptance of chemical synaptic transmission implies that a primary afferent fibre cannot at one group of synapses excite motoneurones and at another inhibit (cf. General Introduction). On the basis of inaccurate timing and on analogy with the monosynaptic activation of motoneurones, it has been accepted that the "direct" inhibitory action that impulses in the fastest afferent fibres from the quadriceps (Q) muscle have on biceps-semitendinosus (BST) motoneurones is monosynaptic (Lloyd, 1941; 1946; Bradley, Easton & Eccles, 1953). However recent

investigation (Eccles, Fatt & Landgren, 1956) has demonstrated conclusively that "direct" inhibition is not in fact direct, but is relayed through an interneurone located in the intermediate nucleus of the spinal grey matter. With the direct inhibitory pathway from quadriceps Ia fibres to BST motoneurones the activity of this interneurone and the subsequent current flow into the presynaptic terminals is indicated by a brief positive wave on the dorsolateral surface of the spinal cord (cf. arrows in Figs. 22 & 26). Again with the inhibitory pathway from motor-axon collaterals to motoneurones an interneurone (the Renshaw cell) is interpolated, and there is good evidence that the inhibitory actions on motoneurones by impulses in groups Ib, II and III afferent fibres are also relayed by cells of the intermediate or dorsal horn region.

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 et al., 1953). 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 these two substances acted similarly in the central nervous system. This similarity was also noted by Simpson in 1854 (cf. Wright, 1955). Sherrington described the action of tetanus toxin on spinal reflexes as a conversion of inhibition into excitation. This was based upon observations 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.

There are five main types of local spinal inhibition that have

been investigated both with strychnine and tetanus toxin. It is convenient to include with these, the effects of these agents on polysynaptic reflexes because it is probable that the concomitant loss of spinal inhibition and the increase in polysynaptic reflexes, due to both these highly specific agents, have a similar basis (cf. Bradley et al., 1953).

(B) 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 the 5th, 6th, 7th lumbar (L) and 1st sacral (S) segments 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), flexor longus digitorum (FLD) and common peroneal (Per) 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.

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 second intervals and the monosynaptic responses were always maximal. The thresholds of the afferent fibres used for inhibitory volleys were checked from time to time.

Strychnine hydrochloride, dissolved in Ringer-Locke solution was administered intravenously. Both non-crystalline and crystalline tetanus toxins were used and the doses administered are given in the text

as mgm of toxin. Owing to the instability of these toxins, regular assays were performed using mice. The factor of 600, relating cat to mice MLD on a weight basis, has been used (Fildes, 1927) neglecting in so doing the variability found by Llewellyn Smith (1942). The non crystalline toxin XW 1322 T 166 (XW) contained 10^6 mouse MLD per milligram of powder and the crystalline (L61) which has been described by Pillemer, Wittler, Burrell & Grossberg (1948) was found to contain 10^4 mouse MLD/ml of solution.

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). The toxin was dissolved in 5×10^{-2}ml of saline, and the injection was made 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 hours (cf. Fildes, 1929; Acheson, Ratnoff & Schoenbach, 1942; Hutter, 1951; Davies, Morgan, Wright & Wright, 1954). Consequently both hind limbs and both sides of the cord were prepared 8 to 12 hours 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 hours in order that the movement of toxin into the cord would not be prematurely interrupted.

Tetanus toxin was injected into both white and grey matter of the spinal cord through glass micropipettes of 5-20μ 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 (Fig. 20). 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.

In some experiments conventional glass micro-electrodes of tip diameter less than 0.5μ were used for intracellular and extracellular recording. These were pulled from 3 mm pyrex tubing using a machine of new design (Winsbury, 1956) and filled with 0.6 M.K_2SO_4 solution by boiling under partial vacuum.

(C) RESULTS

(1) Effects on the excitation of motoneurones

(a) Monosynaptic reflexes

Subconvulsive doses of strychnine (0.1 mgm/Kgm) have no significant effect on the monosynaptic excitatory action in anaesthetized low spinal cats (Bradley et al., 1953; Coombs, et al., 1955c, Fig. 8). This was confirmed in the present series of experiments.

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 of group Ia fibres of a muscle nerve and recorded from the appropriate ventral root. This is illustrated in Fig. 21 for the maximal monosynaptic reflex evoked by a volley in the BST nerve and recorded from the caudal filaments of the

left L7 ventral root. The record A was taken prior to the injection of 0.2 mgm of XW toxin near the left ventral horn and at a segmental level between L7 and S1. Nine and a half minutes after this injection the reflex was virtually unchanged (C) whereas the maximal inhibition of the reflex by impulses in quadriceps Ia fibres had been 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. 22 BST monosynaptic reflexes were recorded from the left and right side of the spinal cord at various times after the intrasciatic injection of 7 mgm XW toxin on the left side. The records A and G show the control reflexes on the left and right sides respectively 21 hours after the injection. The corresponding pairs C & J, E & L were recorded at 22 and 33 hours 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 (see later) while there was no significant trend in the size of the maximum monosynaptic reflex.

(b) Polysynaptic reflexes

In contrast to the lack of effect upon monosynaptic reflexes, polysynaptic reflexes are increased both by tetanus toxin and strychnine. With strychnine the earlier findings of Bremer (1944), Kaada (1950), Naess (1950), Bernhard, Taverner & Widen (1951), Scherrer (1952) and Brooks and Fuortes (1952) were confirmed. Reflexes were usually elicited by stimulating the sural nerve at an intensity of twice threshold, five

times threshold and maximally for A-type fibres. Comparisons were made between integrated records of the areas beneath monophasic recordings of the polysynaptic reflexes, and increases of 2 - 4 times normal were found depending on the dose of strychnine used. After intravenous strychnine the increase of polysynaptic reflexes and consequent return to normal had the same time course as the loss of inhibition of monosynaptic reflexes. The increase in the polysynaptic reflexes elicited by stimulation of sural fibres is paralleled by the increase in the polysynaptic reflexes elicited by stimulating group II and III afferent fibres.

With tetanus toxin there is considerable variation in the magnitude of the increment of polysynaptic reflexes. This is seen particularly after the toxin had been injected into the cord and probably depends on the spread of the toxin. Fig. 23 shows polysynaptic reflexes elicited by maximal stimulation of sural fibres and recorded from the L7 ventral root 14 minutes (A) and 38 minutes (B) after 4×10^{-4}ml of a 1 in 10 dilution of L61 toxin was injected into the dorso-lateral column of white matter of the same segment. The reflex at 14 minutes (A) was unaltered from a control taken 1 hour before. At 38 minutes the area under the monophasic recording of the polysynaptic reflex was increased 6 - 8 times.

(2) Effects on the various types of inhibitory action on motoneurones

(a) 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. Very little of this time is concerned with intramedullary conduction of the quadriceps impulses, most being taken up by the excitation and firing of the intermediate neurone on the "direct" inhibitory pathway (cf. Eccles, Fatt & Landgren, 1956).

In any one preparation the actual percentage of maximum inhibition is partly dependent on the segmental level from which the reflex is recorded. In Fig. 24 are shown inhibitory curves plotted when maximal BST monosynaptic reflexes in the partial or whole ventral roots, as listed, were inhibited by a maximal volley in the group Ia afferent fibres of quadriceps. The maximal inhibition appears to be slightly later with more cuadal reflexes, but more evident is the diminished amount of inhibition of these reflexes compared with those more cranial. For example impulses in Q Ia fibres completely inhibited the BST reflex recorded from the lower L6 segment but were only 15% effective in inhibiting the reflex from the S1 segment.

Strychnine, in subconvulsive doses reversibly diminishes the "direct" inhibition by a Q Ia volley on a BST reflex (Bradley, et al., 1953). Fig. 25 plots the amount of inhibition as a percentage of the original inhibition at various time intervals after 0.05 mgm/Kgm of strychnine had been administered intravenously. The effect of strychnine is rapid in onset, the maximum effect usually being obtained within one minute. Thereafter the amount of inhibition slowly recovers to the original level in about 4 hours. Intracellular recording from BST motoneurones reveals that strychnine diminishes the inhibitory action by depressing the inhibitory postsynaptic potential (IPSP) (cf. Coombs,

et al., 1955d, Fig. 8). In Fig. 26 the records A and C were taken with the microelectrode tip inside a BST motoneurone before and after 0.2 mgm/Kgm of strychnine had been administered. The amplification of the lower record of C is about twice that of A and the upper record in each shows the arriving volleys at the surface of the L7 segment of the cord. The records B and D were taken under similar circumstances with the microelectrode tip just outside this cell. The amplifications are equal. Comparing A and C it is evident that the IPSP is completely abolished and replaced by the potential which is recorded outside the cell (cf. D). The arriving quadriceps volley is unchanged and the strychnine has no effect on the small positive notch recorded at the surface (marked with an arrow in the upper records of Fig. 26), this notch being due to impulses from the inhibitory interneurone reaching the presynaptic terminals in the motoneurone nucleus (Eccles, Fatt & Landgren, 1956). Consequently the prevention of the "direct" IPSP formation is not due to the failure of the inhibitory interneurone to discharge, but either to the failure of release of inhibitory transmitter from its terminals or to a post-synaptic block preventing this transmitter from affecting the motoneurone membrane.

When potentials are recorded in the region of the BST motor nucleus in the L7 segment, a volley in quadriceps group Ia fibres gives rise to a positive potential of several milliseconds duration (Brooks & Eccles, 1948; Eccles, Fatt & Landgren, 1956; Coombs, Curtis & Landgren, 1956). Fig. 27 shows potentials recorded in the BST nucleus consequent upon volleys in both the BST and the Q nerves, the stimulus intensity being given by the figures to the left. The proximity of the electrode tip to the BST motoneurones is indicated by the large negative focal synaptic potential due to excitation of motoneurones by the BST volley.

At the same site, a volley in low threshold Q fibres evoked the usual positive-negative-positive potential (indicated by arrows) due to the primary afferent volley, followed by a positive potential having several small spikes in its trough. These small spikes are seen also in the records of Fig. 26 A, B and D. When the intracellular and extracellular records of Fig. 26 A & B are compared, the IPSP of A is preceded by a small spike potential (SP). The latency of SP is equal to that of a similar spike in the trough of the positive potential of Fig. 26 B and these latencies exceed that of the positive notch recorded from the surface by only 0.2 msec. It is reasonable to conclude that these small spike record impulses in the presynaptic terminals of the inhibitory interneurones and their presence in Figs. 26 D & C after strychnine confirms that strychnine does not affect the discharge of impulses by these interneurones.

It has been suggested (Coombs, Curtis & Landgren, 1956) that the later positive potential may arise from two causes. When recording inhibitory hyperpolarizations extracellularly a positive potential would be expected because the areas of the neurone actively generating the IPSP would be sources of current to other areas of the motoneurone. Also impulses in Q group Ia afferent fibres activate interneurones in the intermediate nucleus which consequently draw current from their axons and their axonal terminals on the motoneurones. The evidence of Fig. 28 lends some weight to the second of these explanations. The upper and lower records A and E are from the surface of the L7 segment and show the spike potentials generated by the group Ia volleys from the quadriceps and biceps-semitendinosus nerves. The traces B, C and D are recorded

by the microelectrode at a fixed position in the BST motor nucleus, being respectively before, after 0.05 mgm/Kgm and after 0.15 mgm/Kgm of strychnine was injected intravenously. Records A and B were taken simultaneously as also were D and E. The injections are seen to produce very little alteration in the focal synaptic potential due to the excitation of BST motoneurones, in the positive notch after the Q volley recorded on the surface and in the initial portion of the positive focal potential due to this Q volley, but the time course of this positive potential is shortened. This is also evident when Fig. 26B and D are compared hence it is unlikely that the slow positive potential is predominantly the extracellular recording of an IPSP for as such it should be very effectively diminished by strychnine. It is probable that the early portion of the positive potential is related to the discharge of the interneurone and that a small fraction of the later portion is an extracellular recording of the consequent IPSP.

Tetanus toxin has the same effect on "direct" inhibition as strychnine. In Fig. 22A to F are shown monosynaptic reflexes evoked by a BST volley and in B, D and F, directly inhibited by a maximum volley in Q Ia fibres at the optimal interval for inhibition. When first examined 21 hours after the intra-sciatic injection of tetanus toxin on the left side the reflex was inhibited by 65% of its control value (A, B). However one hour later the reflex was inhibited only by 40% of this value (C, D) and at 33 hours 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. 29, 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. Subsequent tests at 22 and 23.5 hours showed a rapid diminution of the inhibition on the left side, to about 35 and 15 per cent respectively. At this time inhibition was still 87% on the contralateral side. By 33 hours inhibition had disappeared on the left side, being 75% on the right. The slow loss of inhibition on the right side was kept under observation until 43 hours, when inhibition was about 35%. During this time the testing maximal monosynaptic reflexes showed little variation in size.

When N and O of Fig. 22 were recorded, 24 hours after the toxin injection, the direct inhibitory action was only 15% on the left side but was still about 80% on the right. However in both records the positive notch, marked by an arrow, was well developed indicating that the depression of inhibitory action could not be attributed to failure of activation of the intermediate neurones on the direct inhibitory pathway. It thus appears that, although the inhibitory interneurone on this "direct" inhibitory system continues to function, there is a failure of inhibition of the motoneurone. This implies that an inhibitory postsynaptic potential is not generated, due to either pre- or postsynaptic causes.

Similar results were found in all six 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 experiment there is no counterpart of the positive notch recorded in the L7 segment following a Q volley. A similar depression of 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 reflexes on the right in Fig. 21 are maximally inhibited by volleys in Q Ia fibres and were recorded prior to (B) and nine and a half minutes after (D) an intraspinal injection of toxin. The amount of inhibition relative to the control reflexes A and C was reduced from 27% to 8% in this time.

(b) Inhibition due to impulses in group Ib muscle afferent fibres

Impulses in group I muscle afferent fibres that probably arise from tendon organs, inhibit motoneurones of synergistic muscles and also of other muscles 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 group Ib fibres 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 possible to distinguish inhibition due to impulses in group Ib fibres from that due to impulses in group II fibres (cf. Laporte & Lloyd, 1952). This is often difficult and was not possible in every experiment.

Fig. 30A (filled circles) plots an inhibitory curve when the maximal monosynaptic reflex recorded from the S1 ventral root and elicited by stimulating the gastrocnemius nerve was inhibited by volleys in the quadriceps nerve, which was stimulated at an intensity of 2.5 times threshold. This ensured that all the group I, as well as some group II afferent fibres, were stimulated. Before the administration of strychnine the reflex was

inhibited to about 35% of its control value. Five minutes after 0.075 mgm/Kgm of strychnine hydrochloride was injected intravenously this inhibition was almost abolished (open circles). This effect was reversible and the time course of the diminution of this type of inhibition from another experiment is plotted in Fig. 30 (B).

The effect of tetanus toxin was similar. In Fig. 31 are plotted inhibitory curves from the left and right sides of the spinal cord, where reflexes elicited by stimulation of gastrocnemius nerve and recorded from the L7 ventral roots were inhibited by volleys in quadriceps Ia and Ib afferent fibres. Test control reflexes on the two sides were approximately equal. Forty hours after the intrasciatic injection of 5.7 mgm of XW toxin on the left side, there was almost no inhibition on this side (upper line) where 85% inhibition remained on the right side. Similar results were given in the converse experiments, quadriceps reflexes being inhibited by volleys in gastrocnemius Ib fibres.

(c) Inhibition due to impulses in group II and III muscle afferent fibres

Monosynaptic reflexes of extensor motoneurones are inhibited by impulses in group II and III afferent fibres from muscles of the same limb (Lloyd, 1946). The volley evoked by stimulating the quadriceps nerve at an intensity of 8 times threshold inhibits the monosynaptic gastrocnemius reflex as shown by the curve through the open circles in Fig. 32. The inhibition was as large as 90% but was reduced to 20% seven minutes after 0.075 mgm/Kgm of strychnine had been given intravenously.

The same system of inhibitory volley and testing reflex was used for assessing the effect of tetanus toxin. In Fig. 33 maximal monosynaptic reflexes were elicited by stimulating the L7 dorsal root on

each side and were recorded peripherally from the appropriate gastrocnemius nerve. Tetanus toxin was injected into the right side of the spinal cord, near the ventral horn at the L7 level. Progressive alterations in the inhibition produced by a maximum volley in the quadriceps nerve are shown in the curves of Fig. 33. The inhibition on the right side, originally nearly 100%, was abolished within 16 hours. The effect was slower to develop on the left. Similar results have been found for the inhibition of maximal reflexes of both flexor longus digitorum and plantaris motoneurones by volleys in group II and III fibres of the quadriceps nerve.

(d) 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 have been tested upon the maximal monosynaptic reflexes of quadriceps, gastrocnemius, plantaris and flexor longus digitorum motoneurones. The inhibitory curves of Fig. 34 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. 22) and firstly on the left and then on the right sides inhibition was depressed and finally abolished.

The curves of Fig. 35 from the same experiment illustrate that the effect upon the inhibition of gastrocnemius motoneurones by sural volleys is similar. Here, however, at the time of the initial curve at 22 hours, most of the inhibition had been abolished on the left side, but on the right side inhibition was nearly 90% and then progressively

diminished. The asymmetry of the initial curves on the two sides arises because the first test of inhibition was too late after the administration of the toxin. It is probable that had this inhibition been determined before the injection of toxin it would have been equal on both sides. The early excitation due to impulses in sural fibres arising from skin areas overlying the gastrocnemius muscle (Hagbarth, 1952) is distorted and accentuated by the concomitant increase in polysynaptic reflexes due to these sural impulses. This effect is also seen when strychnine is used. The curves of Fig. 36 plot the effect of volleys in sural fibres, stimulated at 10 times the threshold intensity upon the monosynaptic responses of gastrocnemius motoneurones in the S1 segment. The lower control curve (open circles) shows that the early excitation of about 30% passes over at 10 to 15 msec into almost complete inhibition. Twelve minutes after the intravenous injection of 0.075 mgm/Kgm of strychnine this latter inhibition was approximately 50%, whereas the excitation had increased 2-3 times. At the same time the polysynaptic reflexes, recorded from the S1 ventral root when the same intensity of stimulus was used to excite sural fibres, were increased by about 160% as judged by the area under the monophasic recording of such reflexes.

(e) Inhibitory action by impulses in motor nerve fibres

The depression of excitability of spinal motoneurones following 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; cf. Section I). The repetitive interneuronal discharge at about 1000/sec is revealed by recording from the dorsolateral surface of the spinal cord in the appropriate segment.

Eccles, Fatt & Koketsu (1954) showed that the post-synaptic inhibitory potential of the motoneurone following 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. 37 plots the time course of the inhibitory action on gastrocnemius motoneurones that is produced by an antidromic volley propagating from biceps-semitendinosus, posterior tibial and plantaris nerves. Maximal inhibitions are shown from both sides of the spinal cord at the stated times after 5 mgm of XW toxin had been injected into the left sciatic trunk. The curves of the left side show that, at equivalent times, the loss of inhibition is greater on this side than that on the right. For the initial curves, the inhibition is less on the left side than on the right. This again is due to the long time elapsing between the administration of the toxin on the left side and the first test of inhibition. During this experiment the surface records from the L7 segment, of the repetitive discharge of Renshaw cells at 23.5 hours after the toxin was administered are reproduced in Fig. 38A. They were evoked by the same antidromic volley that was employed for the inhibition and at this stage the inhibition was 4-5 times greater on the right than on the left side.

However the surface records show no diminution of the Renshaw cell activity on the left side.

The records of Fig. 38 B are from another experiment. Renshaw cells were activated by a ventral root volley and recorded from the dorsal surface of the L7 segment. In this case, 38 hours after 5.7 mgm of XW toxin had been injected into the left sciatic nerve, the direct inhibitory action of quadriceps Ia volleys on biceps-semitendinosus motoneurones was still 90% on the right side whereas it was absent on the left, but the Renshaw cell activity appears normal and even larger on this side.

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 inhibition of the motoneurone. The special interest in this situation is the lack of effect of tetanus toxin upon such cholinergically excited cells.

(3) Action of other convulsants

Both strychnine and tetanus toxin are included under the general term of convulsant drugs. In this group are several other alkaloids with actions similar to that of strychnine. Brucein has a similar, but less potent action, as also has thebaine (Fatt, 1954). On the other hand such convulsant drugs as picrotoxin and metrazol have no appreciable depressant action on the inhibitory synaptic mechanisms in the spinal cord. The effect of ß ß-methyl-ethylglutarimide (NP 13) is illustrated in Figs. 39, 40 and 41. This drug, used in the treatment of barbiturate intoxication (Shulman, Shaw, Cass & Whyte, 1955) produces convulsions when administered in large doses.

In the experiment illustrated in Figs. 39, 40 and 41, the dosage

of NP 13 produced convulsions in the lightly anaesthetized spinal cat, yet the inhibitory curve of Fig. 39 shows that the inhibition of biceps-semitendinosus motoneurones by volleys in quadriceps Ia fibres was unaltered by 8 mgm/Kgm of NP 13 administered intravenously. This dose was also without effect on the inhibition of gastrocnemius motoneurones by volleys in all of the quadriceps afferent fibres (Fig. 40). Polysynaptic reflexes, evoked by stimulating the sural nerve at an intensity ten times the threshold and recorded from the S1 ventral root are not appreciably altered by NP 13. The records of Fig. 41A were taken before the drug was administered, those of Fig. 41 (B) being taken 5 minutes after. The very slight alteration in the polysynaptic reflexes is almost certainly the increase that would be expected when the depth of anaesthesia is reduced by the antagonism between NP 13 and the barbiturate used for the anaesthetic. There was no significant increase in the size of the monosynaptic reflexes used in the plotting of Figs. 39 and 40. It is therefore clear that the antagonism between NP 13 and the barbiturate is not due directly to an increase in synaptic excitatory action by the NP 13 because if this was the case a much greater increase in both the polysynaptic and monosynaptic reflexes would be expected.

(D) 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) had recognized the similarity of action of both strychnine and tetanus toxin and although noting that in small doses it was possible to diminish reflex excitation without replacing it by excitation, he was unable

to distinguish whether these agents favoured central excitatory action and depressed inhibitory action or whether central inhibition was converted to central excitation.

This difficulty arose because of the mixed excitatory and inhibitory effects of the conditioning volleys used in his tests. Later investigations (Owen & Sherrington, 1911; Liddell & Sherrington, 1925; Cooper & Creed, 1927) endeavoured to overcome this problem and showed that subconvulsive doses of strychnine did not reverse inhibitory actions which were virtually uncontaminated by excitatory action. This was confirmed by others (Magnus & Wolf, 1913; Bremer, 1925; Creed & Hertz, 1933; Denny Brown, 1932; Bremer & Bonnet, 1942). Thus, although there is good evidence that strychnine does not convert the process of central inhibition to one of excitation, very few observers noted any diminution in the inhibition by even convulsive doses of strychnine. The results of Liddell and Sherrington (1925), Cooper and Creed (1927) and Denny Brown (1932) are difficult to explain, but it is doubtful whether their experiments were designed to show any diminution in inhibitory processes. Liddell and Sherrington (1925) did report that the inhibitory effect on quadriceps motoneurones resulting from stretching the biceps muscle was diminished by strychnine. Creed and Hertz (1933) however, found that the relaxation of a diaphragm slip consequent upon inflating the lungs of a rabbit was not diminished by convulsive doses of strychnine. One experiment did however show some diminution in this inhibition.

Dusser de Barenne (1933) in reviewing the literature concerning strychnine, and considering his own experiments, concluded that the typical strychnine effects seen after intravenous injection occurred only when both dorsal and ventral horn cells were affected. There was no reason

to believe that in the doses used the drug had any effect on the transmission of impulses in peripheral nerve. This has been confirmed by more recent investigations (Peugnet & Coppee, 1936; Heinbecker & Bartley, 1939; Coppee & Coppee-Bolly, 1941), and it is unlikely that doses of 0.1 - 0.2 mgm/Kgm in the cat affect transmission in even the smaller diameter afferent fibres.

It has been shown that all five of the spinal inhibitory systems investigated are affected by strychnine in the same fashion. However there are numerous reports in the literature of spinal inhibitory mechanisms relatively refractory to the action of strychnine. In many early investigations (cf. Owen & Sherrington, 1911; Liddell & Sherrington, 1925; Cooper & Creed, 1927) the criterion of strychnine action was the reversal of inhibition to excitation, the actual diminution of inhibition not being fully investigated. Sherrington (1906) when reporting the inhibition of motoneurones by cortical stimulation, considered that this inhibition was less easily converted to excitation by either strychnine or tetanus toxin than purely spinal inhibition, operating on the same motoneurones. Magnus and Wolf (1913), and Bremer (1922) found that strychnine did not reverse the inhibition of extensor muscles in decerebrate rigidity which was elicited by stimulation of the anterior cerebellar cortex. The rhythmical discharge of motoneurones produced by the injection of strychnine into decerebrate cats, designated "strychnine tetanus" (Bonnet & Bremer, 1952; Bremer, 1953b) is still inhibited by stimulation of the reticular formation, the cerebellum, the vestibular apparatus and the neck proprioceptors (Bremer, 1941 a & b, 1953b; Terzuolo, 1952, 1954; Gernandt & Terzuolo, 1955). The results of Gernandt & Terzuolo (1955) show that when strychnine

tetanus is produced by a dose of strychnine of 0.1 mgm/Kgm body weight in the cat, the rhythmical activity of motoneurones can still be inhibited by vestibular stimulation or by stimulation of the proprioceptive receptors of the neck muscles. However when used in spinal animals this dose may not abolish "direct" inhibition but only diminish it by about 60-80%. The reported results show that inhibition from these descending pathways is not always complete and is not obtainable with larger doses of strychnine or even during the early stages of strychninization with doses of 0.1 mgm/Kgm (cf. Gernandt & Terzuolo, 1955).

It therefore may be concluded that in the investigations of the inhibition of spinal motoneurones by volleys in descending pathways the effects of strychnine have not been fully investigated. The reported failure of strychnine to influence these inhibitory processes is of importance because all types of spinal inhibition so far investigated have been diminished by both strychnine and tetanus toxin. More precise investigation reveals that there is suppression of production of an inhibitory post-synaptic potential, but that the responses of the specific inhibitory interneurone are unaffected. Hence all these inhibitions are presumably mediated at the motoneurone by the same inhibitory transmitter substance. The presence of inhibitory processes on motoneurones refractory to these agents raises the possibility of another inhibitory transmitter, but further investigation is necessary before such a postulate can be entertained.

The more recent investigations (Brooks & Fuortes, 1952; Frank & Fuortes, 1955a, 1955b; Fuortes & Frank, 1955) and discussions (Frank & Fuortes, 1955c) ascribe to strychnine the property of dendritic depolarization leading to accentuation of the "auto-rhythmicity"of motoneurones.

However there is no evidence whatsoever for this assumption. The results described could be due to depression of inhibition and to the consequent increase in the background discharge of interneurones. Similarly Wall, McCulloch, Lettvin and Pitts (1955) have not produced any evidence substantiating their suggestion that strychnine is effective by virtue of its ability to raise the threshold of the terminal arborizations of afferent fibres. The only elemental effect of strychnine that has been conclusively demonstrated is the depression of the effectiveness with which activated inhibitory presynaptic terminals can generate the inhibitory post synaptic potential. This occurs in low doses and the consequent disturbances of inhibition are adequate to explain all the observed phenomena.

It has been shown in the foregoing sections that in spinal segments affected by tetanus toxin, monosynaptic reflexes are unaltered, polysynaptic reflexes are increased and the five investigated forms of inhibition upon motoneurones are depressed. As Sherrington (1905) pointed out, this effect is indistinguishable from that of strychnine. In spite of the observations of Meyer and Ransom (1903) and Sherrington (1905, 1906) in which local tetanus was produced by intra-neural injection of the toxin into the motor nerve trunks, there has been considerable confusion in the arguments concerning the site of action of tetanus toxin. These workers demonstrated that the action was central but this was not readily accepted owing to the inability of others to accept the intra-neural spread of tetanus toxin.

Clinically, tetanus is almost invariably seen as a result of a peripheral wound contaminated with the Clostridium tetani. It has long

been known that if very few organisms are involved with the consequent low production of toxin, or if early treatment is instituted, tetanus may remain local (Courtois-Suffit & Giroux, 1918; Millard, 1954). This local tetanus, confined to one limb, or part of it, can be produced in animals by innoculating the limb with small doses of tetanus toxin (Ranson, 1928, Abel, Hampil & Jonas, 1935). There has been no confirmation of the reports by Abel and his colleagues (Abel, Evans, Hampil & Lee, 1935; Abel & Hampil, 1935; Abel, Hampil & Jonas, 1935; Abel, Evans & Hampil, 1936)that local tetanus did not occur when the toxin was injected into a motor nerve, but only when muscles were contaminated by the toxin. There is considerable confusion in the literature between the early spasms of local tetanus and the rigid contractures of the limbs late in the disease. It may be (cf. Ranson, 1928) that in the later stages there are alterations in the fibres of the muscles concerned, and Abel appears to have overlooked the early manifestations of tetanus in his experimental animals.

Because of these difficulties it has been concluded that tetanus toxin acted at the neuromuscular junction (Harvey, 1939) or at proprioceptive sensory nerve endings (Schaefer, 1944; Perdrup, 1946). However, the central action of tetanus toxin was proved conclusively by the researches of Firor and Jonas (1938), Acheson et al. (1942), Hutter (1951), Wright, Morgan & Wright (1950, 1952) and Davies et al. (1954). Further investigations of the effect of the toxin on neuromuscular transmission (Göpfert & Schaefer, 1941; Hutter, 1951; Mackereth and Scott, 1954) have yielded negative results and there is consequently little reason for postulating a peripheral action for tetanus toxin. 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 in the sciatic trunk. In this series of experiments the toxin travelled in the peripheral nerve at a rate of about 4 mm per hour. When investigating the spread of tetanus toxin after subcutaneous inoculation, Teale & Embleton (1919) proved that only motor nerve fibres were involved, because ventral root section prevented the development of local tetanus, if it was performed shortly after the inoculation. Dorsal root section had no effect on the onset of local tetanus. This has been confirmed by Friedemann and his colleagues (Friedemann, Zuger & Hollander, 1939a; 1939b; Friedemann, Hollander & Tarlov, 1941) who also showed that local tetanus does not result from the effect of toxin circulating in the blood stream. Wright (1953) has discussed the means by which particulate and dissolved matter can travel in the spaces of nerve trunks. Investigations in his department (Baylis, Joseph, MacIntosh, Morgan & Wright, 1952; Baylis, MacIntosh, Morgan & Wright, 1952) have demonstrated that chemical sclerosis of a nerve trunk, leaving axons relatively intact, prevents the development of local tetanus when the toxin is injected peripheral to the site of sclerosis. Further, it has been shown that transection of the spinal cord prevents the further development of general tetanus when large amounts of toxin enter the spinal cord below the level of section.

It can be safely concluded that tetanus toxin reaches the spinal cord from peripheral sites of formation or administration by travelling along motor nerve trunks, and that it also reaches "higher centres" of the nervous system by passage in the spinal cord. The large size of its molecule probably prevents its penetration of the blood brain barrier

(Friedemann, 1942). The actual mode of travel is uncertain. Other substances, when injected into a mixed peripheral nerve can be detected later in the central nervous system and it is possible that the perineural space in a nerve trunk communicates with the perineuronal material in the spinal cord (Brierley & Field, 1949; Brierley, 1950). Wright (1953) has suggested that substances injected into a nerve trunk are pumped by "the physiological mechanism of gross oscillations of tissue fluid pressure in certain peripheral structures" particularly muscle. This is unlikely as several preliminary experiments have shown that when tetanus toxin is injected into the sciatic nerve midway between the hamstring and the sural branches, it can later be detected not only central to this site but also peripherally in all the muscle branches derived from the sciatic nerve.

Several previous investigations have anticipated the present findings of the effect of tetanus toxin upon reflexes. Acheson et al., (1942 demonstrated in cats that local tetanus, produced by the intramuscular injection of toxin, was 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 again 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 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. Conceivably both 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 Ia 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 both by strychnine and tetanus toxin. Similarly the inhibitory cell on the antidromic pathway continued to be excited by antidromic impulses and to produce unchanged field potentials when antidromic inhibition had been abolished.

Such localizations have not been possible for other types of inhibition, because no surface potentials are recognized that signal activity of the inhibitory interneurones involved. With both strychnine and tetanus toxin the properties of the test reflexes and therefore probably of motoneurones remain unchanged in all cases investigated; hence it is possible that these substances have a uniform action upon all types of inhibition of monosynaptic reflexes. 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. A possible explanation of the convulsant action of strychnine and bruceine has been suggested by Hellauer and Umrath (1948) and by Umrath (1953) who find that these convulsants inhibit an enzyme which destroys an "excitatory substance" extracted from dorsal roots. However the results presented above demonstrate that strychnine has no direct effect on excitatory processes and this explanation can be rejected.

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 nucleus 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 sub-synaptic 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 Cl. botulinum prevents the release of acetylcholine at the neuromuscular junction by an action on the presynaptic terminals (Burgen, Dickens and Zatman, 1941; 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 findings of Ambache, Morgan and Wright (1948a) concerning the selective paralysis of cholinergic endings of the rabbit's iris by tetanus toxin are difficult to reconcile both with the present finding that Renshaw cell activity is not altered by the toxin and with the

findings of others upon the neuromuscular junction. The occasional occurrence of ophthalmoplegia and facial paralysis, together with certain clinical features of tetanus such as tachycardia, constipation and atonic bladder (cf. Courtois-Suffit & Giroux, 1918) suggest that tetanus toxin may have an action at certain cholinergic junctions. Harvey (1939) stated that local tetanus has certain features resembling the phenomena seen with the denervation of skeletal muscle but Ambache et al (1948b) were unable to detect any diminution in the choline esterase content of the iris muscle and Torda and Wolff (1947) present evidence that the toxin possibly increases the in vitro synthesis of acetylcholine. It is apparent that a full investigation of the problems involved is necessary.

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. It has been established that tetanus toxin does not pass into the spinal cord along the dorsal root if injected peripheral to the dorsal root ganglion (Fletcher, 1903; Teale & Embleton, 1919) or into a purely sensory nerve (Zupnik, 1905). However if tetanus toxin is injected either into the dorsal root itself (Fletcher, 1903) or into the dorsal horn of the spinal cord (Meyer & Ransom, 1903; Abel, Evans & Hampil, 1936) the clinical manifestations of "tetanus dolorosus" result. In this condition, the skin areas and deeper structures corresponding to the spinal segments involved are hypersensitive to all forms of stimulation. This state is not unlike that seen in animals in which strychnine is applied locally to the dorsum of the spinal cord (cf. Dusser de Barenne, 1933) and inview of the similarity in the action of these substances on ventral horn cells it is highly likely that they also depress inhibitory mechanisms in the dorsal horn and intermediate nucleus of the spinal grey matter. In this case both the increased polysynaptic reflexes and the hypersensitivity in tetanus dolorosus could be due to the inactivation of inhibitory control along polysynaptic pathways.

Another similarity between the actions of strychnine and tetanus toxin is that myanesin, which diminishes polysynaptic reflexes and the increase in them due to strychnine (cf. Section I H) has been found useful in controlling the convulsions of tetanus (Torrens, Edwards & Wood, 1948; Parkes, 1954).

The clinical manifestations of both strychnine poisoning and the various forms of tetanus are explainable by the known actions of

both of these convulsants. Strychnine reaches the central nervous system from the circulation and consequently the signs of poisoning are generalized. Tetanus is often observed to be local, in the first instance, and later becomes generalized as the toxin involves higher spinal centres and the brain stem, at a rate depending on the actual amount of toxin present. If however large amounts of toxin are produced or administered, with a consequent high level in the blood stream, the signs of involvement of bulbar nuclei may be early, due either to the toxin penetrating the blood brain barrier or reaching the motor nuclei along the comparatively short motor cranial nerves.

There is however one difference observed between intoxication by strychnine and tetanus toxin. In the intervals between the convulsions of strychnine poisoning the muscles relax completely whereas in cases of severe, generalized tetanus rigidity of the musclature of the limbs and trunk is a marked feature (Brain, 1951). Although there is evidence that the muscle fibres themselves may become altered (Ranson, 1928) this is unlikely in the early stages of generalised tetanus. It is probable, that, when considering animals having spinal segments under the influence of either strychnine or tetanus toxin, the same clinical signs would be observed for doses of each giving equivalent alterations in any one spinal inhibitory mechanism. Once "higher centres" are involved however, this dose relationship would be lost as strychnine would have an effect over the whole cerebrum and brain stem, tetanus toxin affecting predominantly the bulbar and midbrain motor nuclei. Consequently for equivalent doses as judged by the effect on a spinal inhibition the clinical manifestations of convulsions would be more apparent for strychnine, and if an extremely

high dose was used a rapidly fatal outcome would mask the muscular rigidity between spasms. It is likely therefore that when the signs of strychnine poisoning are compared with those of tetanus, in particular the absence or presence of rigidity between spasms, the percentage alteration in any one spinal inhibitory system might be far less in the case of strychnine than with tetanus - the manifestations of strychnine poisoning being predominantly a result of its effect on the cortex.

Not all "convulsants" tested have the ability to diminish spinal inhibition and presumably they have a direct effect on cortical neurones although there is no evidence that NP 13 directly excites spinal motoneurones. Strychnine has been used extensively in a investigation of cortical pathways (cf. Mendelow & Wright, 1955), and strychnine neuronography depending on the application of a saturated solution of strychnine to a minute area of the cerebral cortex. It is probable that the "strychnine" spikes evoked are a result of a depression of local inhibitory processes but it is also possible that the high dose of strychnine, applied locally, has a direct effect on the cortical neurones.

SECTION III. THE DURATION OF ACTION OF TRANSMITTER SUBSTANCES IN THE SPINAL CORD

(A) INTRODUCTION

It is now generally accepted that central excitatory and inhibitory actions essentially are effective by the potential changes that are set up across the motoneurone membrane (Coombs, Eccles & Fatt, 1956 b & c). Both the excitatory post-synaptic potential (EPSP) and the inhibitory post-synaptic potential (IPSP) have a short latency following activation of the respective presynaptic terminals and a comparatively short time course but are of opposite sign, the EPSP being a depolarization of the post-synaptic membrane and the IPSP a hyperpolarization. These potentials explain adequately excitatory action in motoneurones and also the inhibitory suppression of the reflex discharges of these cells (Coombs et al., 1955 b, c & d).

There is now good evidence that post-synaptic potentials are caused by the operation of chemical transmitter substances. There transmitters, released from the presynaptic terminals, cause brief increases in the ionic permeability of the underlying subsynaptic membranes; to all ions with the EPSP (Coombs et al., 1955c) and with the IPSP only to such small hydrated ions as K^+ and Cl^-, Na^+ in particular being excluded (Coombs et al., 1955b). These changes in permeability, providing the only adequate explanation for the effects observed when the membrane potential and the ionic composition of motoneurones are altered, are dependent on the sieve-like structure of the activated subsynaptic membrane and differ from the highly specific ionic carrier mechanisms occurring during and after an impulse evoked by an electrical excitation of the

membrane. Once the currents causing the EPSP depolarize the membrane to a critical level, there is a specific increase in permeability to Na^+ ions during the rising phase of the spike, and during the falling phase of the spike and the afterhyperpolarizations that follow there is an increased permeability to K^+ ions (Hodgkin, 1951; Hodgkin & Huxley, 1952 a & b; Coombs et al., 1955a).

However the ionic redistributions that occur during the EPSP and the IPSP are of the type that are known to result from the action of chemical transmitters at other junctional regions. For example there is good evidence that with amphibian muscle, the end plate potential is generated because the endplate membrane becomes permeable to all ions (Fatt & Katz, 1951; Castillo & Katz, 1954c), acetylcholine being the chemical transmitter responsible. On the other hand the inhibitory responses at a variety of junctions, crustacean stretch receptor cells (Kuffler & Ezyaguirre, 1955), crustacean neuromuscular junctions (Fatt & Katz, 1953) and vagal junctions in cardiac muscle (Castillo & Katz, 1955c; Hutter & Trautwein, 1955) are probably produced by a selective permeability to the small ions K^+ and Cl^-, Na^+ ions being largely excluded.

It is therefore reasonable to postulate that the specific chemical substances released at the excitatory and at the inhibitory synapses convert the subsynaptic membrane into a sieve-like structure, the pores being much larger with excitatory than with inhibitory junctions. In order to produce the EPSP, the activated synapses must cause a current to be generated which depolarizes the post-synaptic membrane. If this depolarization reaches a critical level the Na^+ carrier mechanism is activated and the cell discharges an impulse (Coombs et al., 1955c). The current resulting from ionic movement at activated inhibitory synapses,

hyperpolarizes the membrane and this hyperpolarization together with the increased ionic permeability of the inhibitory subsynaptic membrane accounts satisfactorily for the observed interaction between the EPSP and the IPSP (Coombs et al., 1955d).

Hitherto the time course of action of transmitters has been derived from analyses based upon the assumption that the exponential decay of the EPSP and of the IPSP is determined by the passive decay of the charge that has been placed on the membrane capacity during the brief initial phase of transmitter action. It is known that the end-plate potential at the amphibian neuromuscular junction decays with a time constant equal to that of the muscle membrane (Katz, 1948; Fatt & Katz, 1951) and as the time constants of the decay of the EPSP and of the IPSP were considered to be approximately equal, it was argued that the time constant of the motoneuronal membrane was approximately 4 msec (Coombs et al., 1955a). The time course of transmitter actions evaluated in this fashion agreed moderately well with the values derived by more indirect means. However more accurate investigation, depending on the use of pure excitatory and inhibitory volleys shows that while the time constant of decay of the EPSP is approximately 4 msec, that of the IPSP is usually about two-thirds of this and becomes even briefer when the IPSP is converted to a depolarizing response by diffusion of ions into the cell (Coombs et al., 1955b). Hence the evaluation of the time constant of the motoneuronal membrane is open to doubt and it becomes necessary to redetermine the properties of the membrane by direct methods in order to obtain a true determination of transmitter action.

Further a study has been made of the post-synaptic responses

of motoneurones following tetanic activation of the presynaptic terminals in order to obtain some evidence towards the factors governing the release of transmitter substances from these terminals.

(B) METHODS

All the experiments considered in this section were performed on the lower lumbar region of the cat's spinal cord under pentobarbital anaesthesia. The cord was transected at the lower thoracic level and the exposed lumbar region set up in a paraffin pool. The ventral roots of the 5th, 6th and 7th lumbar (L5, L6 and L7) and 1st sacral (S1) segments were severed and mounted on electrodes. The nerves to posterior biceps and semitendinosus (BST), the sural nerve (S), the nerve to gastrocnemius-soleus (G), the nerve to plantaris (P) and the nerve to flexor longus digitorum (FLD) were mounted on stimulating electrodes in a paraffin pool while the quadriceps muscle nerve (Q) was stimulated with a buried electrode.

The whole preparation was clamped in a frame of extremely rigid construction to which was also attached the micromanipulator used in inserting the glass micro-electrodes into the cord (cf. Eccles, Fatt, Landgren & Winsbury, 1954). At sites where electrodes were passed into the spinal cord, a small area of pia-arachnoid was removed without damaging the blood vessels on the surface of the cord.

Capillary micro-electrodes were made from pyrex glass tubing of 3 mm external diameter using a new machine designed for the purpose (Winsbury, 1956). Double-barrelled micro-electrodes (Coombs et al., 1955a) were pulled in the same fashion and both types of electrodes were filled with concentrated aqueous solutions of either KCl (3M) or K_2SO_4 (0.6M) by

boiling at 70°C under reduced pressure. After filling, the resistances of the electrodes were determined with their tips immersed in 0.9% saline and only those with resistances of 10-30 megohms were used in the experiments. The difficulties associated with the use of double-barrelled microelectrodes have been fully described (Coombs et al., 1955a). In particular the resistive coupling between the barrels raises problems when one barrel was employed to pass extrinsic current into the impaled cell and the other was used to record its membrane potential. Electrodes having values of 50-200 x 10^3 ohms for this coupling resistance were selected.

Connection of the microelectrode with the electrical equipment was made through an Ag-AgCl junction. The indifferent lead from the cat (earth plate) was made also through an Ag-AgCl junction and a saline soaked cotton gauze pad which made a low resistance contact with the lumbar muscles.

Fig. 42 is a block diagram of the electrical and display equipment used. The microelectrode was attached to a small probe unit containing a cathode follower input stage (CF 1) which was itself rigidly fixed to the micromanipulator. This input stage was connected to three amplifiers Two direct coupled units of fixed low gain (AMP C, AMP D) recorded resting potential; AMP C giving a meter reading and AMP D feeding the upper beam of the second oscilloscope (CRT 2). This beam also recorded action potentials. The third amplifier, having a differential input, controlled the upper beam of the first oscilloscope (CRT 1). This was a capacitatively coupled unit (AMP A) having a greater and more variable range of amplification and a time constant of 2 seconds. In addition a

platinum electrode was employed to lead potentials from the surface of the appropriate dorsal roots as they entered the spinal cord. These potentials were amplified by another capacitatively coupled amplifier B, and recorded on the lower beam of CRT 1.

In order to determine the electrical characteristics of the motoneurone membrane, it is necessary to pass current through it and record the consequent alterations in membrane potential. Rectangular pulses of up to 30×10^{-9}A were passed through both single and double electrodes. The current generating equipment on the left side of Fig. 42 was locked to the sweep through a square wave generator driving a Carpenter Relay (Type 3 GI). The polarizing unit (POL) provided voltages of zero to 45 volts, either positive or negative with respect to earth, and was connected via a short length of shielded cable to the micro-electrode by a 100 megohm resistance. This enabled current to be passed using either a single electrode or one side of a double electrode with minimal distortion in the recording of potential changes arising from responses of the cell. The current flowing in this circuit was measured by means of the direct coupled amplifier (AMP E) which measured the potential drop across a resistance of 1 megohm and displayed it on the lower beam of CRT 2.

Two synchronized Grass Kymograph cameras were used and AMP C was occasionally connected to an Esterline-Angus recording voltmeter.

When investigating the electrical properties of the membrane of an impaled cell by passing rectangular pulses of current through it, it is necessary to be able to record the potential changes that occur across the membrane as soon after the onset of the pulse as possible.

Both with single and double electrodes, voltages of up to 1-2 volts have to be applied in order to obtain the necessary currents. In the case of the single electrode this voltage is applied directly to the input grid of CF 1, while with the double electrode special problems arise because of the capacitative coupling between the two barrels. Consequently a) bridge network was developed for both types of electrodes. This was fed from the polarizing unit through a cathode follower (CF 2) so that the current measured by AMP E was predominantly that flowing in the micro-electrode circuit. The network was then connected through another cathode follower input stage (CF 3) to the other side of the differential amplifier.

The network was designed so that potentials at the output of CF 1 could be exactly balanced by the potentials at the output of CF 3. The equivalent circuit for single electrodes is represented in Fig. 43. The effective input capacity of CF 1, with an attached microelectrode penetrating 3 mm into the spinal cord was 5-10 x 10^{-12} F. It is obviously impractical to make a network using these values of capacity but as it was essential to reproduce potentials at CF3 with the same intensity and time course as those at CF 1 the network was made with capacities one hundred times those of the microelectrode system and resistances one hundredth of the corresponding values. In this way the stray capacities of the network were rendered negligible but the time constants were equal in the two sides of the reactance bridge.

All components of the network were made readily adjustable in order to match capacities and resistances that had considerable variation. With the microelectrode tip in an extracellular position in the motor

nucleus under investigation, R1 and C4 were adjusted so that when current pulses were applied, the output of the differential amplifier was zero. When the tip of the electrode entered a motoneurone, the potentials appearing on the upper beam of CRT 1, in response to current pulses of 10-15 msec duration were due entirely to current flow through the cell membrane, e.g. Fig. 45 C & D. The time constant of rise and fall of the potential is the time constant of the membrane, provided that the current pulse is rectangular. The membrane resistance is determined by dividing the final maximum value of the potential by the current used. In practice the current pulse at the microelectrode was not quite rectangular, its rising phase being distorted by the resistance and capacity (C2 of Fig. 42) of the line carrying current to the microelectrode. However the time constant of the rise of the pulse was rarely greater than 150 μ sec. Not only is this comparatively negligible when compared with the membrane time constant, but, as explained shortly, the final corrected membrane time constant was obtained by subtracting potentials recorded extracellularl and hence this factor can be disregarded. In Fig. 42 the variable capacitor (C1) was used to compensate for the capacity (C2) between the line carrying the current and earth. In this way the actual record of the current pulse, measured as a potential across the resistance of 1 MΩ, was made to be rectangular. When the electrode tip was in the intracellular position, it was possible by adjusting C3 and R4 (Fig. 43) to compensate completely for the cell membrane properties until again the differential amplifier output was zero. The values of C3 and R4 could be read directly and gave in this case the approximate corresponding values for the motoneurone. However more accurate values were obtained by an

analysis of potentials such as those of Fig. 45C & D.

Because the electrode resistance fluctuates in value during its passage through the cord, it was necessary to withdraw the electrode from the cell and redetermine the potentials with the tip in an extracellular position and with the same adjustments of R1 and C4. Full compensation was rarely found. Usually potentials such as those of Fig. 45 E & F were obtained. For any one current these potentials have then to be subtracted from the corresponding values recorded intracellularly in order to obtain the time course of the potential change actually occurring across the cell membrane.

For double microelectrodes a comparable circuit was used (Fig. 44). In the absence of a compensating device, the capacity between the two barrels (about 20 x 10^{-12}F) caused a large artefact to appear in the recording system when current was passed through the other barrel. Consequently it was impossible to follow potential changes over the first 2-3 msec of the pulse (cf. Coombs et al., 1955a). However the circuit illustrated in Fig. 44 gave adequate compensation and with any one cell potentials were recorded both intra- and extracellularly with the same values of R1, R2, R3, C4, C5 and C6 (cf. Fig. 46). When the electrode was moved in the cord, the resistance coupling between the two barrels represented as 50K and R3 fluctuated considerably and it was necessary not only to make frequent checks of its value, but also to reset R3 from time to time when the tip was extracellular. The ancillary equipment for the double electrode was considerably larger than that for a single one and a small switch was made so that either barrel could be used for recording or for passing current. Consequently the capacity C6 was used

to compensate for the extra capacity to ground on the current-passing side of the electrode. This value was rarely greater than 5×10^{-12}F and C6 was often omitted.

These circuits were tested by means of an artificial network that effectively duplicated the electrical properties of the microelectrode and the cell. Provided that compensation was reasonable with the circuits equivalent to an extracellular position of the microelectrode tip, the potentials recorded when a "cell" was added had time courses and magnitudes that would be expected from the electrical properties of the circuit representing the cell.

(C) RESULTS

(1) Preliminary investigation of motoneuronal properties.

(a) Membrane potential and resistance

The resting membrane potential of motoneurones impaled with microelectrodes filled with KCl or K_2SO_4 were in the range of -50 to -80 mV. The uncertainty in the evaluation of this potential has been discussed by Coombs et al. (1955a), and it is probable that -70 mV is nearer the true membrane potential. This is the average value found for cells in which the microelectrode was securely impaled with minimal cell damage, as judged by the length of time that one can record intracellular potentials and responses from the cell.

When measured as above with both single and double microelectrodes and with the aid of a compensating network, the membrane resistance was in the range of 0.5 - 1.5 megohm. Voltage current curves such as those of Fig. 47 were obtained using currents of up to 30×10^{-9}A, the potentials being measured under steady state conditions. This was

done both with the electrode tip inside and outside the cell and the corrected membrane resistance was obtained by subtraction. Microelectrodes often changed their electrical properties during the passage of currents as has been observed by Castillo and Katz (1955b), Weidmann (1955) and Frank and Fuortes (1956b). This was particularly obvious with currents greater than 30 x 10^{-9}A and would be expected to arise on account of the ionic migration caused by the currents and of the interaction with the glass surface at the most constricted part of the microelectrode i.e. at the orifice. Usually there was an increase in electrode resistance, particularly when negative pulses were used. Sometimes the same effect was observed when positive potentials were applied to the microelectrode.

When phenomena of this nature were observed, the best procedure was to record the potential-time courses produced by currents applied in both directions intracellularly, and then to repeat the observations immediately after withdrawal from the motoneurone (Figs. 45 & 46). However in a few instances this procedure was unsatisfactory, for the properties of the electrode altered between the two positions of its tip. Usually, when cells had a low resting potential due to damage the membrane resistance was also low, being in the range 0.2 - 0.4 x 10^{6} ohms.

(b) Membrane time constant.

The surface membranes of giant axons and of muscle fibres have been shown to approximate to a simple electrical system (cf. Katz, 1948; Hodgkin, 1951), with time constants ranging from 1 to 30 msec. It is likely that the surface membrane of the soma, dendrites and initial segment of a motoneurone would exhibit similar properties. If a ractangular

current pulse (I) is applied to a simple network consisting of a resistance R in parallel with a condenser C, the potential across the condenser will increase exponentially with a time constant given by the RC product to a final value equal to the IR product. On cessation of the current the potential across the condenser decays with the same time constant. The most direct method of determining the time constant for the motoneurone would be to record with an intracellular microelectrode the time course of the potential generated by the passage of a rectangular current across the membrane. This is a relatively simple proposition when it is possible to use two independent microelectrodes, as has been used by Fatt and Katz (1951) for the end plate and by Tauc (1955) for the giant ganglion cells of Aplysia. However for technical reasons it is impossible to use two independent electrodes for motoneurones, and either a single or double microelectrode must be used for passing current and recording the consequent potential change.

When using a single microelectrode, the neuronal membrane usually has a resistance of less than 10 per cent of that of the electrode. If the assumption is made that the electrode properties are not altered during the passage of a current, the potential due to current flowing through the electrodes and other resistances in series with the neuronal membrane can be balanced out by a bridge circuit. However microelectrodes do not behave as simple resistances. When a rectangular current pulse is passed through a microelectrode in an extracellular position, a small potential is observed which has an exponential rise and fall resembling that observed with the tip in an intracellular position (cf. records E & F of Fig. 45). Furthermore, reversal of the current sometimes does not give a mirror image change in the recorded potential. Thus, for the determinatio

of the membrane time constant, using the circuit of Fig. 43, the best approximation that could be obtained was to record the potential time courses produced by rectangular currents applied in both directions intracellularly (Fig. 45 C & D) and then repeat the observations immediately after withdrawal from the motoneurone (Fig. 45 E & F). It may be assumed that the differences between the two series of curves are due to the motoneurone membrane, which in this manner was observed to have a time constant of about 2.5 msec (range 1.4 to 4 msec) for motoneurones in good condition.

Similarly a reactance bridge (Fig. 44) was used to eliminate the artefacts due to the capacitative coupling between the two barrels of double microelectrodes when current was passed through one barrel and potential changes were recorded from the other. Potentials were recorded intracellularly (Fig. 46 C & D) and again immediately afterwards extracellularly (Fig. 46 E & F) and the differences are assumed to give the actual time course of the membrane potential change that is produced by the onset and cessation of a rectangular current pulse. As determined in this manner the time constant of the motoneuronal membrane has also been about 2.5 msec.

For cells in poor condition due to damage by the electrode, not only was the membrane resistance low, but also the time constant. Figures of 0.8 to 1.4 msec were obtained and correspondingly the time constants of decay of both the EPSP and of the IPSP were shorter than normal. Some cells were impaled for several hours by an intracellular electrode, and towards the end of this period a falling membrane potential was associated with a similar shortening of the membrane time constant.

(c) Membrane capacity

A figure for the capacity of the surface membrane of a motoneurone may be derived from the average time constant of 2.5×10^{-3} sec for a neuronal membrane that has a resistance of about 10^{6} ohms. The value of 2.5×10^{-9}F so obtained gives a specific membrane capacity of 5×10^{-6}F cm^{-2} for a standard neurone having a surface area of $5 \times 10^{-4} cm^{2}$. The surface area is calculated by considering that the standard neurone is a sphere 70 microns in diameter, having six cylindrical dendrites of 5 microns in diameter radiating for approximately 300 microns and an axon arising from a conical axon hillock. For the purpose of this calculation the dendrites can be neglected over distances greater than 300×10^{-6}cm, as this is approximately their length constant (cf. Coombs et al., 1955a), and the number six, derived from Balthasar (1952) represents the average number of primary dendrites per motoneurone.

The value of 5×10^{-6}F cm^{-2} is higher than the specific membrane capacity of giant axons (cf. Hodgkin, 1951) but is lower than the value found for muscle fibres by Fatt & Katz (1951, 1953). There may be considerable error in assessing the surface area of a motoneurone and therefore this standard value of specific membrane capacity may be in error by a factor of about 2, i.e. the value may be approximately 2.5×10^{-6}F cm^{-2}.

(2) Time course of central transmitter action

It has been shown (Coombs et al., 1955b & c) that both the excitatory and inhibitory transmitters alter the permeability of the subsynaptic membrane of the motoneurone towards certain ions for extremely short periods of time. The duration of the effective transmitter action exerted by a volley in group Ia afferent fibres of a muscle upon the

motoneurones of that muscle is so brief that a single volley never sets up more than one discharge. This same afferent volley also stimulates interneurones of the intermediate nucleus, whose discharge consequently inhibits motoneurones of antagonist muscles. Usually a single primary afferent volley evokes a single discharge from the interneurone. By using the threshold discrimination between the group Ia and Ib afferent fibres of a muscle (Bradley & Eccles, 1953), it is possible to evoke direct inhibitory responses due to impulses relayed from the Ia fibres, uncomplicated by the effects produced by impulses in the group Ib fibres. Both with excitatory and inhibitory action virtually synchronous pre-synaptic volleys are obtained and the time course of the post-synaptic potentials produced by subsynaptic currents set in action by the excitatory and inhibitory transmitters approximate to the time course of the change produced by a single impulse at a single synapse. The more prolonged IPSP's produced by other types of inhibitory action may be assumed to be produced both by a temporal dispersion of many milliseconds in the activation of the inhibitory synapses and by the repetitive activation of these synapses.

In order to produce the observed excitatory and inhibitory post-synaptic potentials, current must flow respectively outwards and inwards across the motoneuronal membrane and there must be a corresponding opposite current flow across the subsynaptic membrane of the activated synapses. It has been shown (Coombs et al., 1955a, b & c) that the transmitter substances generate this latter current by altering the ionic permeability of the subsynaptic membrane, and it can be assumed that the duration of the action of the chemical transmitter corresponds approximately to that

of the current flow. Since a potential placed on the motoneurone membrane decays approximately exponentially with a time constant that can be measured, the EPSP and the IPSP can be analysed on the basis of Hill's local potential theory (Hill, 1936; Eccles, Katz & Kuffler, 1941). It is implicit in this calculation and in the determination of the time constant of the membrane that the intracellular electrode gives a record of the mean alteration in the membrane potential due either to activation of synaptic knobs, that are uniformly distributed over the surface of the soma and dendrites of the cell, or to currents passed across the membrane. A more accurate analysis of the rising and decaying phases of the potentials resulting from rectangular pulses of current passed across the membrane, shows that these are not truly exponential. This would be expected from the shape of the cell and the distribution of its synapses. Usually the onset of the rising phase is faster and the termination of the rising phase slower than an exponential time course. Consequently the time constant has been measured, from figures such as Fig. 45 and 46, one millisecond after the commencement of the pulse. The value obtained is still only approximate.

At any time t, two factors are responsible for the rate $(\frac{dP}{dt})$ at which the EPSP or the IPSP (P_E, P_I) is changing. The potentials are proportional to the intensity of the subsynaptic currents (A) but simultaneously are decaying exponentially with a time constant (T) equal to that of the membrane.

Hence

$$\frac{dP}{dt} = KA - \frac{P}{T} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$KA = \frac{dP}{dt} + \frac{P}{T} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

(where K is a constant)

give the relation between the subsynaptic current and the potential it develops. The equation (2) allows of the calculation of the current at different time intervals after its onset. The broken lines of Figs. 48 and 49 plot this current in arbitrary units with reference to time, for the EPSP and the "direct" IPSP respectively of a cell having a measured membrane time constant of 2 msec. The EPSP decayed roughly exponentially with a time constant of 4.2 msec and the corresponding value for the IPSP was 2.8 msec.

These graphs are typical of those found for other cells. The subsynaptic current rises steeply to a maximum in about 0.5 - 0.7 msec of its onset and rapidly declines. The current corresponding to the EPSP usually rapidly declines for about 1 msec, but then the decline becomes slower so that at 2 msec the current is still about 10 per cent of its maximum and thereafter it slowly decays for many milliseconds. This residuum causes the EPSP to decay with a time constant that is about double that of the membrane. The time course of the current that produces the IPSP shows that the high intensity phase has a longer duration than that of the excitatory subsynaptic current. Also in contrast to the EPSP, there is less residual current after 2 msec. The longer duration of the high intensity phase of the current producing the IPSP could be due to double firing of a few of the interneurones on the inhibitory pathway with the consequent duplicate release of transmitter. In the cell illustrated in Figs. 48 and 49 the differences between the high intensity phases of the transmitters are more marked than usual, and in most cells there was very little difference at all. In some cells there was a slight reversal of the inhibitory current between 2 and 3 msec

after its onset, that is presumably attributable to the unequal distribution of inhibitory synaptic knobs between the soma, axon hillock and dendrites. The negligible size of the residual component of the inhibitory subsynaptic current may be correlated with the finding that the time constant of decay of the IPSP is very little longer than the electric time constant of the motoneuronal membrane, the respective mean values being 2.8 msec and 2.5 msec.

The curves of Fig. 50 show the extent of the variation in the duration of transmitter time when different time constants are used in its calculation. The continuous black line plots the average of several EPSP's of a gastrocnemius motoneurone with a resting potential of 55 mV. This potential decays with a time constant of 4.2 msec. The electrical time constant of the membrane, measured by passing current pulses with a single KCl filled microelectrode was 2 msec. The dotted line plots the time course of the subsynaptic current generating the EPSP when $T = 2$ (Equation 2) whereas for the broken line the value of $T = 4.2$ was used, corresponding to the time constant of the decay of the EPSP. With both these values the initial current flow is not dissimilar but there is a large difference in the residual current flow. When the time constant of decay of the EPSP is used, the current ceases to flow at 4 msec, but using the true value, corresponding to the electrical time constant of the membrane the current is still about 10% of its maximum value at 4 msec.

In the determination of the time course of the subsynaptic current from equation (2) it is possible to give actual current measurements, and so calibrate the ordinate scales of Figs. 48, 49 and 50. If the membrane capacity is assumed to be constant at roughly 2.5×10^{-9}F, the

derived value of KA, being in Volts sec^{-1} can be converted to Amperes. This has not been done, because at the present time the figures are given primarily as an indication of the time course of the transmitter action. A full analysis of the currents involved will be attempted at a later date.

(3) Post-activation potentiation.

At most junctional regions, following a single or repetitive presynaptic impulse there is often potentiation of the post-synaptic response that is elicited by a test impulse in the same presynaptic fibres. The potentiation is assessed by an increase in either the post-synaptic potential or in the discharges of impulses, it is elicited only by impulses in those presynaptic fibres that had been initially stimulated, and it is due to an increased presynaptic action and not to an increased excitability of the post-junctional membrane. Considerable evidence concerning the phenomena of post-tetanic potentiation has been collected (Schaefer & Haas, 1939; Feng, 1941; Eccles, Katz & Kuffler, 1941; Larrabee & Bronk, 1947; Lloyd, 1949, 1952b; Eccles & Rall, 1951b; Strom, 1951; Jefferson & Benson, 1953; Job & Lundberg, 1953; Liley & North, 1953; Eccles, 1953; Castillo & Katz, 1954a; Beswick & Evanson, 1955; Wilson, 1955). It is more aptly called post-activation potentiation since it may be seen after a single conditioning volley (Larrabee & Bronk, 1947; Job & Lundberg, 1953).

When recording monosynaptic reflexes from ventral roots, the potentiation of the response following tetanic stimulation of the pathway concerned implies post-tetanic activation of motoneurones in the subliminal fringe. This type of system has been investigated by Lloyd (1949) who showed that monosynaptic reflexes could be potentiated, but that there was

very little potentiation of polysynaptic reflexes although there was a subliminal fringe for the motoneurones concerned. Lloyd considered that his results using monosynaptic reflexes led to the conclusion that the phenomena was due to an increase in the size of the presynaptic nerve impulses brought about by the hyperpolarization following intense repetitive activity (cf. Lloyd, 1949; Eccles & Rall, 1951b; Lloyd, 1952b). Eccles and Rall (1951b) recorded synaptic potentials of motoneurones either focally or from the ventral root and found that these were also potentiated, by a previous tetanus, in approximate proportion to the reflex potentiation. By using conditioning tetani of different duration they were able to demonstrate that the increase of the presynaptic volley size failed to explain the post-synaptic increase in either the synaptic potential or the reflex size and suggested that the presynaptic impulse could become more effective on account of a temporary alteration in the spatial relationship between the synaptic knob and the underlying subsynaptic membrane (cf. Eccles, 1953).

Beswick and Evanson (1955) again recording reflex responses of motoneurones from ventral roots, were able to show that this method of assessing an increase in response following a tetanus could be unreliable, as motoneurones of synergistic muscles may be stimulated in the post-tetanic period and give a false impression that the increase was due only to the motoneurones belonging to the muscle whose group Ia afferent fibres were used for the conditioning and testing volley. This difficulty is overcome by recording intracellular responses from single motoneurones, the great advantage of this method being that it gives a direct measure of the increased synaptic efficacy.

The graphs of Fig. 51 are derived from records of the post-

synaptic potentials of a biceps-semitendinosus motoneurone. This cell had a resting potential of -70 mV and an electrode filled with 0.6M K_2SO_4 solution was used. The EPSP of 7.5 mV was evoked by stimulating maximally the group Ia fibres of the biceps-semitendinosus nerve. The IPSP, due to stimulating the Ia afferent fibres of the quadriceps nerve was 3 mV. Each potential was elicited at two second intervals and 4 controls were recorded before the tetanus. The afferent nerves were tetanized at a rate of 660/sec for 15 seconds, the stimulus intensity being increased by a factor of two. The EPSP's and the IPSP's were then recorded at 2 second intervals for a duration of several minutes and the potential sizes plotted at the times that the records were taken, without regard to the different sign of the two responses. Comparison of the graphs showsthat whereas with the EPSP there was considerable potentiation with a maximum at about 10 seconds after the end of the tetanus, there was very little increase in the size of the IPSP. The EPSP (A) and the IPSP (C) before the tetanus are contrasted in Fig. 52 with those afterwards (B & D). This result has been observed in four motoneurones, two of which showed no post-tetanic increase in the inhibitory post-synaptic potential even when a range of tetanus frequencies and durations was used. With both the EPSP and IPSP no alteration in the resting membrane potential occurred after the tetanus and in several instances the potentiation of the EPSP was sufficient for it to generate spike discharges in the motoneurone.

Lloyd (1949) was able to obtain potentiation of inhibition when impulses in the group I afferent fibres of gastrocnemius were used to inhibit the motoneurones of the pre-tibial muscles. This observation has

not been confirmed in the present series of experiments where volleys in quadriceps Ia fibres have been used to inhibit biceps-semitendinosus motoneurones. The inhibitory curve of Fig. 53 (A) shows the percentage inhibition of the maximal monosynaptic reflex, recorded from the S1 ventral root and evoked by stimulating the biceps semitendinosus nerve, by a preceding volley in the quadriceps group Ia afferent fibres. The maximal inhibition is approximately 75%. This maximally inhibited reflex, elicited every 2 seconds was then recorded as the "control" and the quadriceps nerve tetanized at a rate of 300/sec for 20 seconds. Thereafter every "control reflex" was recorded and the heights plotted in Fig. 53 (B) using the same ordinate scale as in A. There was no alteration in the actual size of the biceps-semitendinosus reflex over this time. Similarly Fig. 53 (C) plots the maximal inhibition following a tetanus of 300/sec for 30 seconds. It is apparent that there is no significant alteration in the amount of inhibition and this would be expected from the results of intracellular recording of the IPSP evoked in biceps semitendinosus motoneurones by impulses in the quadriceps Ia fibres (Fig. 51).

Usually only a single stimulus has been employed in testing for post-activation potentiation. However when repetitive presynaptic volleys are used for the testing stimulus, there has been a rapid decline in the response to successive volleys; so that there has been little or no potentiation after the first few volleys (Strom, 1951; Eccles & Rall - unpublished observations; Liley & North, 1953). Fig. 54 illustrates this finding for the EPSP recorded from the same biceps-semitendinosus cell as used for Figs. 48 and 49. The two control records (A & B) show a repetitive series of six EPSP's, evoked at a rate of 45/sec by stimulating the biceps semitendinosus nerve every 3.5 seconds. This nerve

was then tetanized with a stronger stimulus at a rate of 660 stimuli per second for 15 seconds. The records C & D are the fourth and fifth series after the end of the tetanus and show that the first EPSP is potentiated by about 60% and thereafter there is a rapid decline of the potentiation so that there is less than 10% with the sixth EPSP in the repetitive series.

The graphs of Fig. 55 are derived from another biceps semitendinosus cell wherein a repetitive series of three EPSP's at a rate of 66 per second was evoked every 2 seconds prior to and after a tetanus of 660 per second for 15 seconds. The lines plot the actual size of the post tetanic EPSP's and show the great increase in the first EPSP of the series compared with the much smaller increase in the second and third.

(D) DISCUSSION

Apart from a direct determination of the electrical time constant of the motoneuronal membrane and the consequent different value derived for the capacity of the membrane, the electrical properties of the motoneuronal membrane as here described are not radically different from those previously described (Coombs et al., 1955a). It would be expected that depolarized motoneurones would have lower values of membrane resistance than normal, and this is reflected in the lower time constants both of the decay of post-synaptic potentials and of the membrane itself. It is probable that under these altered conditions the capacity of the membrane would remain fairly constant (cf. Cole & Curtis, 1939; Hodgkin, Huxley & Katz, 1952). By a bridge technique Araki and Otani (1955) have determined the membrane resistance and time constant of toad's motoneurones that were impaled by a single microelectrode. The values of resistance

obtained were considerably higher (3.0 - 5.8 x 10^6 ohm) than the present findings and they have calculated a mean membrane resistance of 270 ohm cm^2 on the basis of a very low estimate (6 x 10^{-5} cm^2) for the membrane area. Furthermore their estimate of the time constant of the membrane may be in error owing to a failure to appreciate the possibility of alterations in the electrode characteristics. However they obtain a specific membrane capacity of 17.5 x 10^{-6} F cm^{-2} which is considerably higher than values obtained for other membranes.

The short time course of the subsynaptic currents causing the post-synaptic potentials, derived from the interaction both of the excitatory and inhibitory post-synaptic potentials (Coombs et al., 1955d) and also from the interaction of the EPSP and a spike action potential (Coombs et al., 1955c) appeared to justify the argument that the EPSP decayed passively with the electrical time constant of the membrane. However calculation of the time course of the subsynaptic currents in the light of the directly determined time constant for the membrane explains both of these phenomena and the shorter time course of the IPSP compared with that of the EPSP in a more satisfactory manner. In particular, accurate determination of the interaction between an EPSP and the antidromic spike potential confirms the presence of the later residual subsynaptic current responsible for the slow decay of the EPSP.

The records of Fig. 56A show the intracellularly recorded responses of a motoneurone in which an antidromic volley was propagated over the surface of the cell at various times relative to an EPSP. As shown in Fig. 56A, even after many milliseconds, during the after-hyperpolarization that follows the antidromic spike potential, the EPSP is smaller

than the control response (Fig. 56C) and in addition, as shown in the subtracted records of Fig. 56B, it reaches an earlier summit and decays more rapidly. As the EPSP is moved earlier relative to the spike, all of these changes are accentuated, but the EPSP is still of considerable size (about 30% of the control) when it is generated on the later part of the falling phase of the spike at the second arrow of Fig. 56B. However when the presynaptic volley is timed so that it would set up an EPSP at the first arrow (about 1.5 msec earlier), there is only the very small response shown by the broken-line curves of Fig. 56 A & B.

On the basis of similar observations Coombs et al. (1955c) concluded that the synaptic mechanism is not able to generate any appreciable EPSP if it is prevented from doing so for 1.2 msec and hence that the EPSP is generated by a transmitter whose depolarizing activity persists for no longer than 1.2 msec. By virtue of the enormously increased Na^+ and K^+ fluxes during and after the spike, any charge on the membrane due to the subsynaptic current generated by the excitatory synaptic transmitter would be very effectively removed. Thus the subsynaptic current is not effective in building up an EPSP until it is late on the declining phase of the spike potential. At the second shortest test interval in Fig. 56 A the maximum subsynaptic current operated at this earliest effective stage after the spike, the alterations in the EPSP presumably being due to the effect that the spike and its after-hyperpolarization have on the movement of the excitatory transmitter in the synaptic cleft. On the other hand at the shortest test interval, the initial subsynaptic current coincides with the spike potential and therefore is ineffective in setting up an EPSP whereas the slowly declining residuum of this current (cf. Fig. 48) would

be responsible for the small and relatively delayed EPSP (broken lines of Figs. 56 A & B) at this interval.

It might be expected from the nature of the direct inhibitory pathway that the transmitter giving rise to the IPSP would have a longer duration than that causing the EPSP. It has been shown (Eccles, Fatt & Landgren, 1956) that the relay interneurone on the direct inhibitory pathway between quadriceps Ia afferent fibres and biceps-semitendinosus motoneurones is capable of following frequencies of stimulation up to 600 per second and may discharge repetitively in response to a single afferent volley. However this repetitive discharge must be unusual, for the time course of the inhibitory transmitter released at the motoneurone is relatively short and probably in most cases the cell is only fired once or twice. Differences in the duration of the inhibitory transmitter would be expected from different levels of anaesthesia and only a few intermediate cells relaying group Ia impulses have the property of repetitive firing (cf. Eccles, Fatt & Landgren, 1956).

Recent electron microscope studies have provided evidence of the structural features of central synapses. The surface of the motoneurone is studded with synaptic knobs of approximately 2μ in diameter (Wyckoff & Young, 1956) and these are distributed less intensely as the dendrites are followed more peripherally (Lorente de No, 1938; Barr, 1939; Bodian, 1952). The knobs themselves are covered with a continuous membrane about 50 A thick and contain small vesicles of 300A diameter together with numerous mitochondria (Palay & Palade, 1955, 1956; de Robertis & Bennett, 1955). The mitochondria of the knob suggest a high level of metabolic activity and probably, as with the motor nerve endings on muscle (Palade & Palay, 1954; Castillo & Katz, 1955b; Robertson, 1956) the vesicles

may be regarded as containing the chemical substances that are responsible for transmission across the synaptic junctions. Vesicles are often clustered on the synaptic surface of the knob and occasionally appear to be bursting on the surface. The knob is separated from the subsynaptic membrane by the synaptic cleft of approximately 200A in width. The subsynaptic membrane of width 50A presumably has very different properties from the remainder of the soma-dendritic membrane as it is specifically affected by transmitter substances, and on analogy with the end plate membrane of the neuromuscular junction (Kuffler, 1943; Fatt & Katz, 1951; Castillo & Katz, 1954c, 1955a), it may be unable to respond to an impulse.

The area of the synaptic contact and the width of the cleft are important because the function of a synapse is to apply small amounts of a transmitter substance to the specialized receptor area of the subsynaptic membrane. This then becomes highly permeable to ions and the resulting electric current flows through the synaptic cleft and so to the remainder of the post-synaptic membrane. Fig. 57 shows the direction of this current flow when the excitatory transmitter substance makes the subsynaptic membrane permeable to ions. The synaptic cleft should be narrow enough so that the transmitter substance is applied rapidly and efficiently to the subsynaptic membrane; it should also be wide enough so that the subsequent post-synaptic currents may flow as freely as possible. By assuming a high specific conductance for the activated subsynaptic membrane and a specific resistance of about 100 ohm-cm for the fluid in the synaptic cleft it can be calculated that with a cleft of about 200A, the resistance offered to current flow through the cleft is only about 5% of that offered in its passage through the subsynaptic membrane. There is

thus an adequate safety margin for the efficient operation of the cleft in carrying current to the whole subsynaptic membrane.

With a transmitter substance released in a cleft of 200A, the diffusion time across it would be of the order of a microsecond and it can be calculated that, if the substance is free to diffuse and has approximately the diffusion coefficient of acetylcholine, it will diffuse away from a focus of the dimensions of a synapse so rapidly that its concentration will have fallen to a negligible level within 1 msec of its liberation (Fatt, 1954; Ogston, 1955). The slightly longer time course of the excitatory and inhibitory transmitter action on motoneurones (Figs. 48 & 49)suggests a presynaptic volley might cause the release of transmitter substances for a period as long as 1 msec, while the small residual action indicates that the removal of the transmitter substance might be delayed by a diffusional barrier around the synapse. This barrier has been postulated to exist around the synapses on Renshaw cells (Eccles, Eccles & Fatt, 1956) and would also explain the prolonged duration of transmitter action of cutaneous impulses on interneurones of the intermediate nucleus and on the neurones discharging up the dorsal spino-cerebellar tract. A diffusional barrier around synapses would not only increase the effectiveness of synaptic transmitter action but also prolong the duration of temporal facilitation. There is no structural correlate for these diffusional barriers but possibly they may be due either to the close glial investment of synapses (Wyckoff & Young, 1956) or to the ground substance described by Hess (1953, 1955).

At the neuromuscular junction the crowding of the synaptic vesicles towards the synaptic surface of the knob, together with the

occurrence of vesicles appearing to burst on the surface of the knob makes attractive the postulate that the bursting of these vesicles, containing the transmitter agent acetylcholine, accounts for the miniature end-plate potentials that occur spontaneously in a random manner at the end-plate (Fatt & Katz, 1952; Castillo & Katz, 1955b). Nerve impulses reaching the knob would cause the simultaneous release of a large quantity of acetylcholine, and it has been found that depolarization of the nerve terminals either by extrinsically applied current or by raising the extracellular potassium concentration increases the frequency of the miniature end-plate potentials (Castillo & Katz, 1954b; Liley, 1956b). In the light of these concepts one can argue by analogy that in the central nervous system, transmitter agents exist in the synaptic knobs as vesicles and are released by depolarization of the knobs. Random release may occur and would account for certain forms of synaptic noise. This noise is however difficult to analyse owing to the possibility of the simultaneous random impingement of excitatory and inhibitory impulses on the motoneurone under examination.

At the neuromuscular junction it seems likely that the vesicles are positively charged because depolarization of the presynaptic membrane increases the frequency of miniature end-plate potentials i.e. the frequency with which vesicles are emitted. There is some evidence to suggest that the excitatory and inhibitory transmitter substances of the spinal cord are also charged. In Fig. 58 A & B EPSP's have been recorded in a gastrocnemius motoneurone at the resting potential of -70 mV and also when the membrane potential was hyperpolarized to -100 mV. It is evident that hyperpolarization caused the summit of the EPSP to be earlier and

also considerably shortened the time constant of its decay. Such changes have been previously reported (Coombs et al., 1955c). Simultaneous measurements showed that there was no appreciable diminution of the membrane resistance at a potential of -100 mV, and it is unlikely that any change would occur in the membrane capacity. Hence it appears justifiable to assume that the time constant of the membrane was the same at both potentials. In this cell the time constant of the membrane was not directly determined, but it can be assumed to be approximately 2.8 msec, which on the basis of other experiments, is a reasonable value for a cell having an EPSP which decays with a time constant of 4.6 msec at the resting membrane potential. The EPSP's of Fig. 58 A & B have been plotted as the continuous lines of Fig. 58 C & D respectively, and using the assumed value of the membrane time constant as in Fig. 48, the time courses of the subsynaptic currents have been calculated (broken lines). When the cell was hyperpolarized (B & D) the current decayed much more rapidly than normal and its summit was lower than would be expected for a linear relationship to membrane potential (cf. Coombs et al., 1955c). Hence it may be assumed that the hyperpolarizing current was interfering with the time course of the excitatory transmitter action either by removing the transmitter from the proximity of the subsynaptic membrane or by preventing its attachment to the subsynaptic receptors. It is attractive to postulate that the transmitter is negatively charged, and hence is carried away from the subsynaptic membrane and the synaptic cleft by the hyperpolarizing current that is passing inwards through the cleft and penetrating the subsynaptic membrane. This postulate accounts not only for the deficiency in the initial summit but also for the greatly increased rate of decay of

the subsynaptic current in Fig. 58 D. It also contributes to the explanation of the diminished size, the earlier summit and the faster decay of an EPSP that is set up during the hyperpolarization following a spike potential (cf. Fig. 57), for, if this hyperpolarization is set up by the regions of the post-synaptic membrane that are invaded by the spike, it will cause an inward current flow through the subsynaptic areas of the motoneurone, which effectively will be the same as an extrinsically applied hyperpolarizing current.

As soon as the excitatory transmitter alters the permeability of the subsynaptic membrane, the current set up (cf. Fig. 56) will flow across the subsynaptic membrane in the same direction as an extrinsic hyperpolarizing current. Initially this current will be of great intensity and so may contribute to the initial very rapid decline of the transmitter concentration if the transmitter molecules carry a negative charge. Similarly when the IPSP of a motoneurone is converted to a depolarizing response its time course of decay is usually shortened (Coombs et al., 1955b). This might be attributable to the rapid removal of the transmitter substance from the synaptic cleft under the influence of subsynaptic currents but a full analysis of the problem is not yet complete.

The findings of post-activation potentiation are of interest when considering the storage and release of transmitter substances. As far as has been investigated (Brooks et al., 1950; Brock et al., 1951; Eccles & Rall, 1951a & b; Jefferson & Schlapp, 1953), during repetitive monosynaptic excitation of motoneurones there is a depression of the successive responses which parallels that of sympathetic ganglia (Eccles, 1943; Larrabee & Bronk, 1947; R.M. Eccles, 1955), and which likewise may

be attributed to depletion of transmitter substance. However, subsequent to a prolonged repetitive stimulation, there is a large and prolonged increase in the response evoked by a monosynaptic testing volley, the post-activation potentiation. This potentiation suggests that there is an increased amount of transmitter agent available in the terminals, but the results of experiments using repetitive presynaptic volleys as the testing stimulus suggest that there is not a large increase in the total amount of available transmitter, for the potentiation falls off rapidly with all responses subsequent to the first (Fig. 55).

It appears that there is an increased output of transmitter substance per impulse because potentiation is associated with a single testing presynaptic volley, but the time course of its action is not unlike that for single stimulus prior to the tetanus. It might be considered that following a tetanus, the decay of the potentiated EPSP might be slower than normal owing to the greater amount of transmitter substance released. The time constant of decay of an EPSP is usually evaluated by the measurement of 10-20 superimposed records so that random variations of potential due to synaptic noise are cancelled. It was therefore hard to measure the value for the single testing EPSP's used in this study and in some cases the decay was faster than normal (cf. Fig. 52 A & B). The actual measurement is difficult and numerous errors can arise because the testing volley is supramaximal for the group Ia afferent fibres and in most cases the decline of the EPSP is not exponential but distorted by the post-synaptic potentials evoked by volleys in higher threshold fibres. The same difficulty arises in the accurate measurement of the time constant of the decay of the IPSP and no significant figures have been obtained.

In part an explanation of the potentiation is provided by the increased size of the presynaptic spike that occurs during the after hyperpolarization that follows activity (Lloyd, 1941; 1952a; Eccles & Rall, 1951b) for Castillo and Katz (1954b) have suggested recently that an impulse causes a greater quantal release of transmitter from hyperpolarized nerve terminals. The phenomena is not associated with postsynaptic alterations in membrane potential. However this explanation does not cover the whole duration of the potentiation (Eccles & Rall, 1951b; Liley & North, 1953) and under some conditions a large potentiation is associated with a diminished presynaptic spike. Alterations in either the effectiveness of presynaptic impulses or in the relation of the synaptic knob to the subsynaptic membrane do not account for the observation that only the first of a repetitive test series of responses is greatly potentiated after a tetanus.

At the neuromuscular junction it has been demonstrated that activation of the presynaptic terminals is followed by an increase in the frequency of the miniature end-plate potentials (Liley, 1956a). This potentiation, unlike that seen for the end-plate potential in curarized muscles is largest immediately after the end of the conditioning tetanus and thereafter declines with the same time course as does the potentiation of the end-plate potential. It can therefore be postulated that a tetanus of presynaptic impulses "crowds" the synaptic vesicles in the knob towards the cleft so that not only will a further impulse release more transmitter, but also there will be an increase in the spontaneous emission. The delayed development of post-activation potentiation indicates that the presynaptic impulse is not immediately effective in operating the release mechanism for the transmitter. The fact that there is a rapid decline in the potentiation

during a repetitive series of testing impulses suggests that following the depletion of the reserve supply of chemical transmitter by the tetanus, there is not an excessive replenishment of available transmitter. Thus post-activation potentiation seems to be attributable to two events localized to the immediate region of the presynaptic membrane, a mobilization there of a relatively small number of synaptic vesicles, and an increased size of the presynaptic spike.

The small amount or even absence of post-activation potentiation of "direct" inhibition would be expected from an understanding of the anatomical pathway concerned. If "direct" inhibition was monosynaptic it might be expected that the IPSP should be potentiated in the same fashion as the EPSP. However, the impulses in the group Ia muscle afferent fibres of a muscle do not impinge directly upon the motoneurones of an antagonist muscle but are relayed by an interneurone which normally fires once or twice only to each volley. These cells are capable of following high frequencies for short periods, but are probably not efficient in transmitting a high and prolonged frequency to the presynaptic terminals that release the inhibitory transmitter substance. In an investigation of this pathway, Eccles, Fatt & Landgren (1956) found no significant post-tetanic potentiation of the responses of the group Ia intermediate neurones. The technique used did not allow of refined measurements, and it is evident from graphs such as Fig. 51 that there is a small potentiation which conforms with the observations of Lloyd (1949). This potentiation could occur either at the relay in the intermediate nucleus or there could be a potentiation of the synaptic mechanism for generating the IPSP. Both Lloyd (1949) and Wilson (1955) have found that there is minimal

post-tetanic potentiation of polysynaptic pathways, and this can be accounted for in the same fashion. After a conditioning tetanus there is possibly a potentiation of the firing of the first one or two cells in the pathway but in the absence of a high level of frequency transmission, potentiation goes no further towards the final motoneurone.

CONCLUSIONS AND SUMMARY

The researches reported in this thesis have been presented in three sections. The investigations of Section I demonstrate that among the pharmacological agents with known specific actions at peripheral junctional regions, acetylcholine is the only one that has been shown to have an action upon cells within the spinal cord. The effects of intra-arterially injected acetylcholine (and of nicotine) upon both monosynaptic and polysynaptic reflexes (Section I E) are those that would be expected from the activation of the cholinergically excited Renshaw cells (cf. Section I D). There is no evidence that acetylcholine acts either as an excitatory or an inhibitory transmitter at synapses upon motoneurones. The relatively slight action of intravenous tubocurarine and prostigmine on the cholinergic junctions of axon-collaterals upon Renshaw cells can be explained by the presence both of the blood-brain barrier and of diffusional barriers more intimately related to the synaptic junctions. Although Renshaw cells can be activated by volleys in the slow muscle afferent fibres and this activation appears pharmacologically similar to that resulting from volleys in the collaterals of the motor axon, there is insufficient evidence as yet to give support to the third postulate of the general introduction to this thesis in which it was suggested that cells react to one type of excitatory synaptic transmitter only.

Other possible central transmitter agents are discussed and the available evidence, together with the findings of Section I F and G, suggests that adrenaline, nor-adrenaline, 5-hydroxy-tryptamine, ATP and histamine have no such function in the spinal cord of the cat. However the reported findings concerning the distribution of substance P

(Section I G (ii)) suggest that this polypeptide has a central function but difficulties in the production of a pure sample have so far prevented adequate testing.

The preliminary results of attempts to extract transmitter substances from central nervous tissue have not been encouraging. The chemical methods used may not have distinguished between the excitatory and the inhibitory substances and also between these and the other known constituents of brain tissue such as acetylcholine, adrenaline, noradrenaline, 5 HT and substance P. Because of this difficulty, methods of extraction and consequent separation using paper chromatography are being developed. The problem of the non-penetration of the blood-brain barrier when testing extracts might be overcome by using the isolated, oxygenated spinal cord of the frog (Eccles, 1947). This preparation, divided by a medial longitudinal incision, could be soaked in solutionsof extracts. Since strychnine has the same effect on its reflex activity as it has on that of the spinal cat, it can be presumed that the inhibitory transmitter of the two preparations is similar. Consequently any extract found to inhibit the reflexes of the frog's spinal cord would be worth further testing, either by intra-arterial injection or by direct micro-injection into the cat's spinal cord.

Both strychnine and tetanus toxin have been shown in Section II to diminish central inhibition although the mode of action may be different for these two agents. Strychnine is virtually without effect on the excitation of motoneurones (Section II C (1)), but diminishes all five types of inhibition investigated (Section II C (2)): 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 of synergistic muscles; the inhibition of extensor motoneurones by impulses in the group II and III muscle afferent fibres and in cutaneous afferent fibres; the inhibition of motoneurones following activation of Renshaw cells by volleys in axon collaterals.

The effect of tetanus toxin, either injected peripherally into a motor nerve or directly into the spinal cord, is similar on all these types of inhibition, but the reversibility is much slower. In several instances some recovery has been observed following the injection of very small doses of tetanus toxin directly into the spinal cord, but as yet the results cannot be regarded as being conclusive and further investigation is desirable. However, recovery in man, following purely local tetanus, often takes weeks (Courtois-Suffit & Giroux, 1918) and would not be readily observable in the experimental preparations used in this investigation.

As it has been shown that the ionic movements repsonsible for the inhibitory post-synaptic potentials for these different types of spinal inhibition are similar (Coombs et al., 1955b), it can be postulated that a common inhibitory transmitter substance is involved. This postulate is strengthened by the finding that the effects of both strychnine and tetanus toxin are similar on all these inhibitions. The evidence is presented (Section II D) that strychnine has little effect upon the inhibition of motoneurones mediated from higher centres by descending tracts. However the experimental studies are incomplete and are considered to present no clear evidence that more than one inhibitory transmitter is involved in the spinal cord.

It is shown that both strychnine and tetanus toxin exert their

effects near the synaptic junctions between the specific inhibitory interneurone and the motoneurone. Both for "direct" inhibition (Section II C 2(a)) and for "antidromic" inhibition (Section II C 2 (e)) it has been proved that these agents have no effect on the inhibitory interneurone. In the discussion it has been suggested that these two agents may have different modes of action,

It has been suggested (Section II C (3)) that the convulsant action of some drugs, in particular NP 13, cannot be explained by their effects on either the excitatory or the inhibitory processes of the spinal cord. It is possible that they are convulsants by virtue of their action upon cells in higher centres, but as the investigations are far from complete a possible alternative suggestion is that these drugs cause a mild intensification of excitatory synaptic activity, just as some anaesthetics have a mild depressant activity upon the same processes. This mild intensification would produce a very small effect when assessed by the increase in a monosynaptic reflex, but would become more cumulatively amplified when it occurred at every synaptic relay along a polysynaptic pathway.

It is argued in Sections I H and II D that the effects of drugs such as strychnine, tetanus toxin and myanesin on polysynaptic and monosynaptic reflexes can be accounted for by the different anatomical structure of these two types of reflex, in particular the number of synaptic relays involved. There is therefore little evidence of more than one inhibitory and one excitatory substance (apart from acetylcholine) in the spinal cord.

The direct measurement of the properties of the motoneuronal membrane in Section III C (1) shows that it has a shorter time constant than had been considered previously. The calculation of the time courses of action of the inhibitory and of the excitatory transmitter substances

gives a more satisfactory explanation of the interaction both between the EPSP and the IPSP and between the EPSP and an antidromic spike potential of the motoneurone.

Observations upon the post-activation potentiation of post-synaptic potentials recorded intracellularly from motoneurones (Section III C (3)) show differences between the potentiation of the EPSP and IPSP which are explainable by the nature of the anatomical pathways concerned and by the known properties of the relay interneurone on the direct inhibitory pathway. The evidence has been collected (Section III D) for the presence in the synaptic terminals of vesicles which can release quanta of charged transmitter material into the synaptic cleft. The discharge of these vesicles possibly accounts for some forms of synaptic noise and forms the basis of a reasonable explanation of post-activation potentiation.

Apart from the suggestion that the monosynaptic excitatory transmitter substance is negatively charged, there is no evidence as to the nature of the excitatory and inhibitory transmitter substances of the spinal cord. However there are findings to suggest the occurrence of common transmitter agents at several sites in the cord. It has been suggested (Section II D) that the same inhibitory transmitter substance is released by impulses reaching motoneurones over at least five different pathways.

According to Dale's principle (see General Introduction) the same excitatory transmitter is involved at the three sites of termination of the group Ia afferent fibres from muscle. These fibres pass directly to the motoneurones of the muscle and branch extensively so that any one presynaptic fibre can secure a large area of synaptic contact on the same

neurone (Cajal, 1909, 1934; Lorente de No, 1938; Bodian, 1942; Chang, 1952). The synaptic knobs are about 2μ in diameter and the release of the synaptic transmitters has been shown to be brief and in the case of excitation, the post-synaptic potential can generate the discharge of only one post-synaptic spike. For monosynaptic excitatory action to fire a motoneurone considerable summation of excitatory impulses is necessary and the motoneurone will not follow effectively frequencies of stimulation greater than about 40/sec.

Primary afferent impulses in the group Ia fibres synaptically excite interneurones of the intermediate nucleus of Cajal (Eccles, Fatt & Landgren, 1956). These cells respond to high frequencies of synaptic stimulation, often respond repetitively to a single afferent volley and may also be excited by impulses in group III muscle afferent fibres and by cutaneous impulses. Intracellular records from these cells show, that as a consequence of the impingement of impulses upon them, graded EPSP's are set up and one or more impulses are generated if the EPSP is sufficiently large. Similarly the group Ia afferent fibres of muscle synaptically excite the cells of Clarke's column (Grundfest & Campbell, 1942; Lloyd & McIntyre, 1950; Laporte, Lundberg & Oscarsson, 1956). There is general agreement that these cells are excited to discharge impulses by a very few afferent fibres, but again the discharge may be repetitive.

Although the nature of the ionic movements responsible for the EPSP of the motoneurone is known (Coombs et al., 1955c), nothing is known about the ionic mechanisms responsible for the EPSP's both of group Ia intermediate cells and of Clarke's cells. However the differences between the excitatory processes presumably are explainable by an

understanding of the different types of synaptic endings, the properties of the subsynaptic membrane and the different properties of the whole neurone including its after-hyperpolarization.

The structure of the synaptic knob and of the synaptic cleft of the motoneurone has now been described in detail, and the transmitter time of action, derived from a knowledge of the post-synaptic membrane, explains the observable phenomena at the junction. Little is known concerning the synaptic terminals of the group Ia afferent fibres upon the intermediate relay cells and until the actual characteristics of these comparatively small cells can be measured, the action time of the transmitter remains unknown. However, either the post-synaptic membrane or the actual amount and time course of the action of the transmitter must differ from the values at the synapses on the motoneurones because these intermediate cells may fire repetitively to single presynaptic volleys. This property of repetitive response is presumably due to the small or absent after-hyperpolarization following a spike discharge.

The histological correlate of the powerful synaptic excitatory action of single impulses in group Ia afferent fibres upon Clarke's cells is provided by the "giant synapses" in which synaptic contact apparently is made for several hundred microns along the dendrites by the giant terminal end bulbs of the presynaptic fibres, so giving areas of contact of many hundreds of square microns (Szentagothai & Albert, 1955). Such synaptic contacts are very many times larger than any made by the branched endings of a single presynaptic fibre on a motoneurone. The actual size of the synaptic cleft and also the characteristics of the subsynaptic membrane are unknown, but it is apparent from the repetitive responses of

the relay cell that the time course of the transmitter and the characteristics of the neuronal membrane differ from those occurring at the motoneurone.

It is apparent therefore that the same transmitter substance, released from the terminals of the group Ia fibres, can, depending on the nature of the synapse and the cell involved, activate different cells in a slightly different fashion although the basic ionic redistributions involved could be similar. It is probable that the same transmitter substance and ionic movements are responsible for the EPSP's of motoneurones set up by impulses relayed by intermediate and dorsal horn cells but definite proof is lacking.

It therefore can be postulated (cf. General Introduction) that, besides acetylcholine as an exciter of Renshaw cells, there is only one excitatory substance existing in the spinal cord. However a difficulty in the acceptance of this arises from a consideration of the specificity of synaptic action on neurones that have overlapping dendritic fields. The reflex pattern of the hind limb is now reasonably clear, but the specificity of operation is not readily understandable on anatomical grounds. For example, exploration with a microelectrode reveals that the soma of a gastrocnemius motoneurone may be as close as 50 - 100 microns to a biceps-semitendinosus motoneurone and yet these two neurones exhibit their quite distinctive responses to afferent volleys. Each is monosynaptically activated by the corresponding group Ia volleys and inhibited by the group Ia volleys from muscles having antagonistic function.

Again, the small motoneurones of a muscle are interspersed within the motor nucleus of that muscle (Eccles, 1953; Fig. 3E; Brooks & Curtis,

personal observation), yet these small motoneurones show no trace of the powerful monosynaptic excitatory action which is exerted on large motoneurones by impulses in the group Ia afferent fibres of that muscle or its synergists. However extensive investigations have shown a fairly close parallelism between the responses of the small and large motoneurones of a muscle to impulses in the other groups of afferent fibres (Hunt, 1951; Granit & Kaada, 1952; Eldred, Granit & Merton, 1953; Eldred & Hagbarth, 1954).

In order to explain the specific action of impulses in the afferent fibres of a muscle it might be postulated that a number of different transmitter substances exist and that some property of the post-synaptic membrane is responsible for the determination of specific pathways. For example the small motoneurones of a muscle would not be activated by the release of the excitatory transmitter from the terminals of the group Ia afferent fibres of a muscle. However in view of the complexity of some spinal reflex mechanisms, the number of different transmitter substances necessary for this postulate becomes prohibitive. Hence, for the present, the view must be entertained that the specific action of afferent impulses is determined purely on anatomical grounds. It is clear from recent observations in this laboratory (Eccles, Eccles & Lundberg - personal communication) that the reflex pattern of the hind limb is not as clear cut as was originally thought and many aberrant monosynaptic connections exist. It is probable that early in development, relations of the different motor nuclei to the ingrowing fibres are more distinctive, and both the number of synapses and their relation to the initial segment of the motoneurone may be important in determining the responses of the cell to presynaptic impulses.

It is obvious that a full investigation of the chemical substances responsible for synaptic transmission in the spinal cord must include a knowledge of the actual structure of the different synapses. In particular the size of the synaptic connection, the width of the cleft and the electrical and electrochemical characteristics of the subsynaptic membrane are capable of determination. However the nature of the transmitters, the conditions governing their release, the properties of diffusional barriers and of the blood-brain barrier are problems about which the lack of evidence precludes further speculation.

ACKNOWLEDGEMENTS

I wish to thank Professor J.C. Eccles for his constant advice and encouragement during his supervision of this research. Mr. J.S. Coombs has given invaluable aid and advice upon the electrical and mechanical equipment used. Most of the apparatus used in the experiments has been constructed by Mr. G.J. Winsbury and Mr. H.C. Daynes and during the preparation of this thesis the technical assistance rendered by Miss R. Burkitt, Mr. A. Chapman and Mr. V. Paral has been most helpful.

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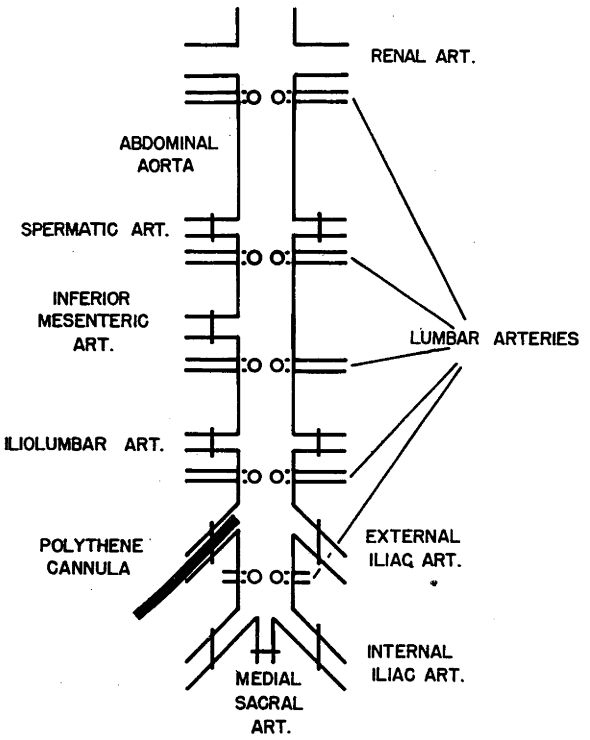

Fig. 1. Diagrammatic representation of the abdominal aorta viewed from the ventral side showing the branches ligated and the position of the tip of the polythene cannula. The lumbar arteries are shown arising from the dorsal surface of the aorta and then passing laterally.

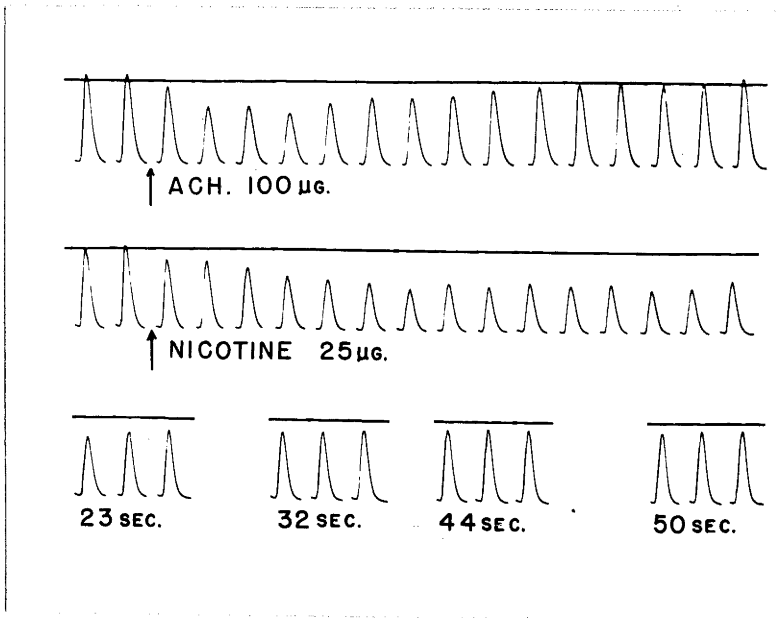

Fig. 2. Monosynaptic reflex spikes were evoked by a L7 dorsal root volley at a frequency of 1/sec and recorded monophasically in the L7 ventral root. The individual spikes had a duration of about 1 msec but the brief gaps between them represent 1 second. In the upper series the arrow signals the intra-arterial injection of 100 µg of acetylcholine which results in a depression of the reflex for about 10 seconds. In the lower series 25 µg of nicotine produced a larger depression of greater duration. The first fifteen reflexes after this injection were recorded and then groups of three at the times shown. The horizontal line formed by the other beam of the oscilloscope was adjusted at the mean summit heights for a long series of reflex spikes immediately before the injection.

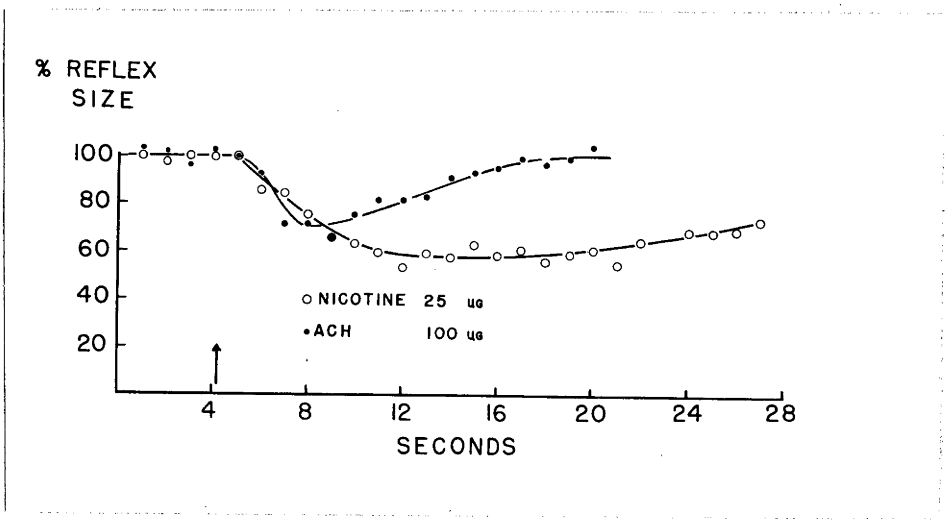

Fig. 3. Graphs plotting the size of the reflexes of Fig. 2. The injections were made at the time of the arrow and the average reflex size beforehand is shown as 100% on the ordinate scale. Each point represents a single reflex response.

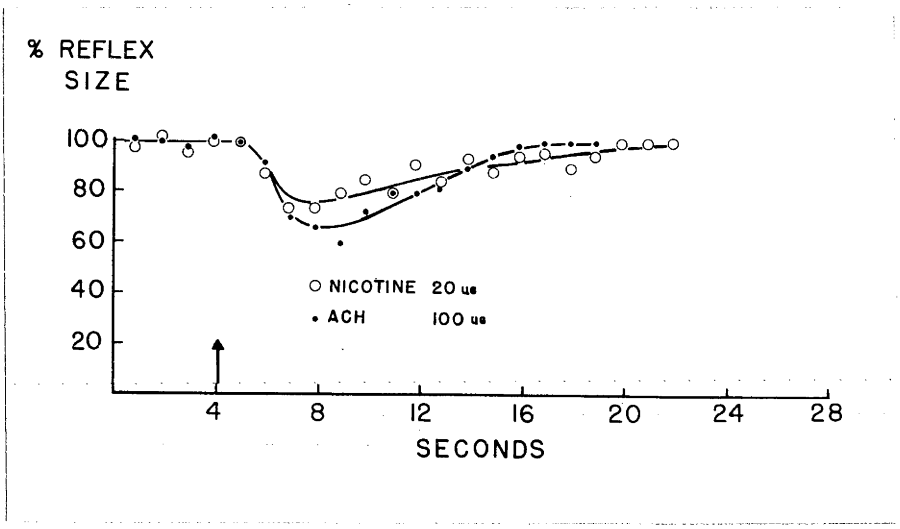

Fig. 4. From the same experiment as Fig. 2 and 3 but different doses of nicotine and acetylcholine were injected intra-arterially.

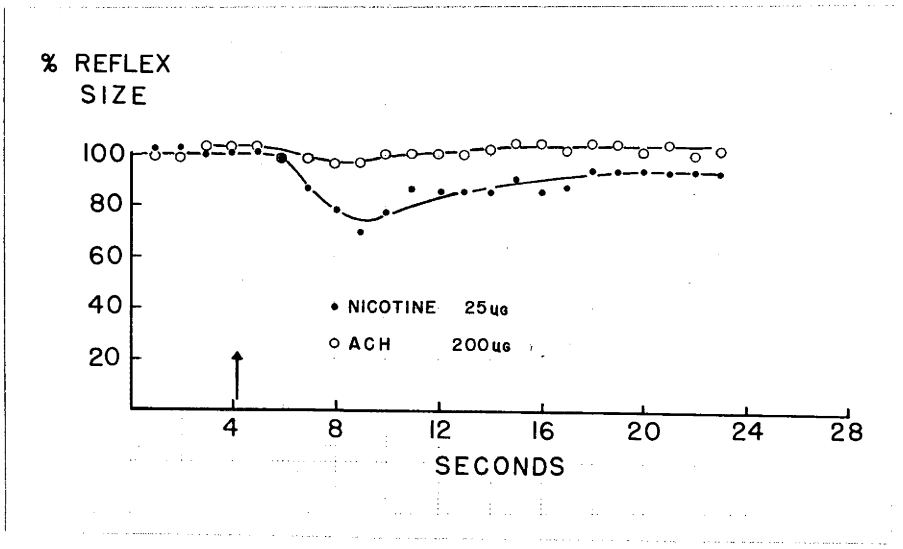

Fig. 5. As for Fig. 3 but from another experiment showing the small effect of 200 µg of acetylcholine compared with that of 25 µg of nicotine following intra-arterial injection.

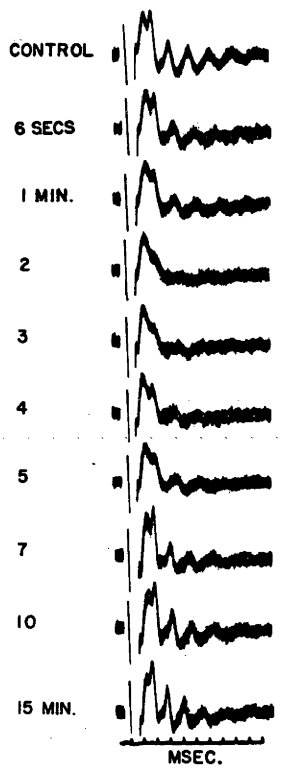

Fig. 6. Records from the dorsolateral surface of the L7 segment of the spinal cord when the L7 ventral root was stimulated maximally at a rate of 5/sec. Each trace represents forty superimposed records. The upper "control" record was taken prior to, and the other records at the stated times after, 20 µg of nicotine had been injected intra-arterially.

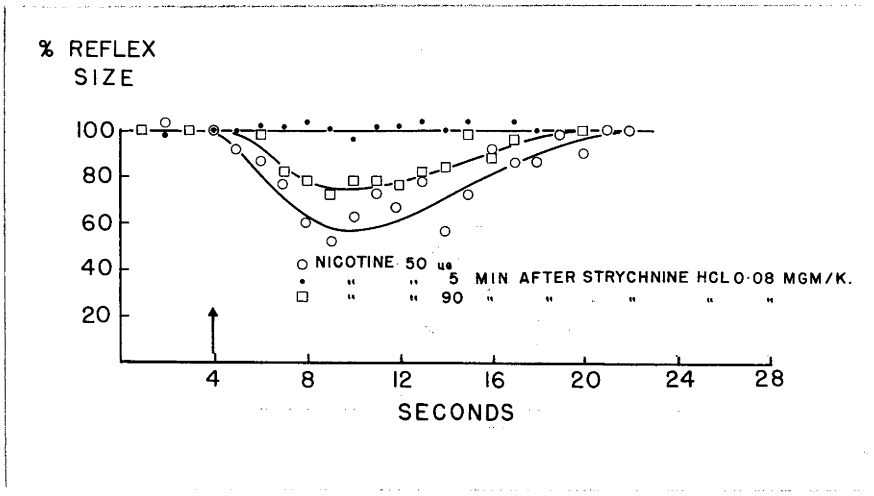

Fig. 7. From an experiment where the maximal monosynaptic reflexes of the L7 segment were depressed by the intra-arterial injection of 50 µg of nicotine. This depression was abolished by an intravenous injection of strychnine but ninety minutes later the reflex was again depressed by a further intra-arterial injection of nicotine.

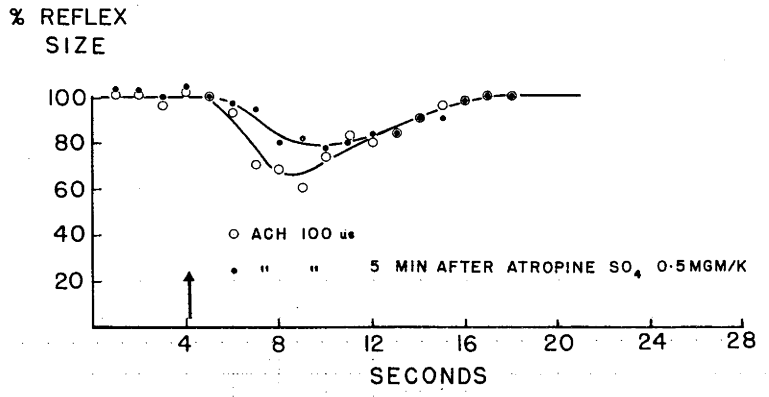

Fig. 8. The effect of an intra-arterial injection of 100 µg of acetylcholine, before and after an intravenous injection of atropine, upon the maximal monosynaptic reflex of the S1 segment.

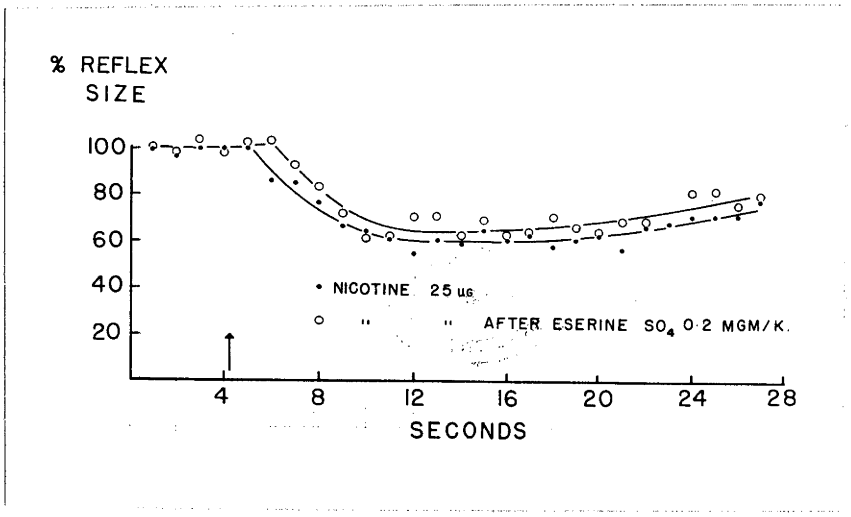

Fig. 9. From the same experiment as Figs. 2 and 3 showing that the depression of the monosynaptic reflex following an intra-arterial injection of nicotine is not significantly altered by a previous intravenous injection of eserine.

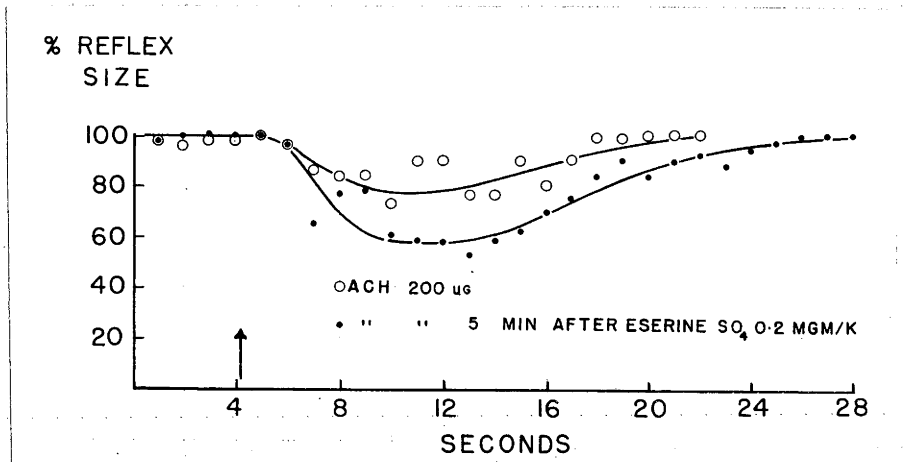

Fig. 10. Same experiment as Fig. 9 showing the alteration produced by an intravenous injection of eserine in the time course of the depression of the monosynaptic reflex of the L7 segment of the spinal cord due to an intra-arterial injection of acetylcholine.

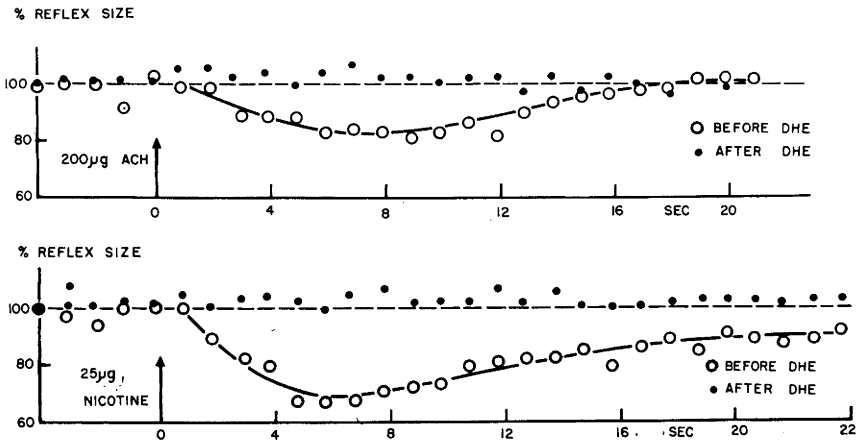

Fig. 11. Two series of monosynaptic reflexes from the same experiment. The open circles plot the depression of the reflex due to intra-arterial injections of 200 µg of acetylcholine (upper series) and 25 µg of nicotine (lower series). The filled circles show the complete abolition of both depressions following the intravenous injection of 0.8 mgm/Kgm of dihydro-ß-erythroidine (DHE).

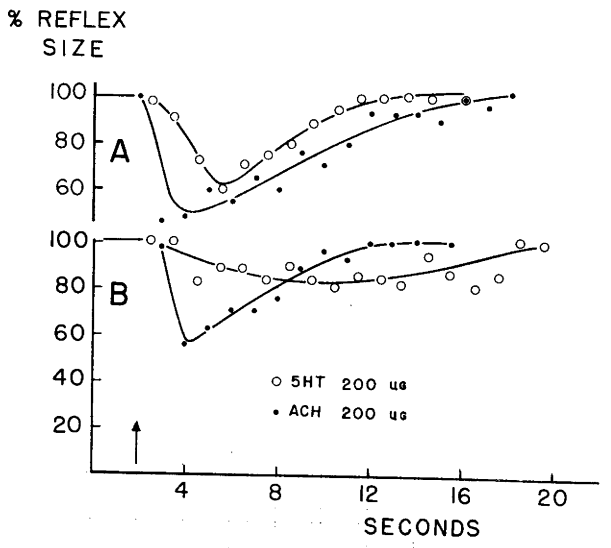

Fig. 12. A. The effect on the monosynaptic reflex of the L7 segment of intra-arterial injections of acetylcholine (filled circles) and 5-hydroxy-tryptamine (5HT) (open circles). All dorsal roots except those of the left L7 and S1 segments were intact.

B. Same experiment but after the left L5 and L6 dorsal roots were crushed. There is considerable alteration in the depressions particularly in that following an intra-arterial injection of 5HT.

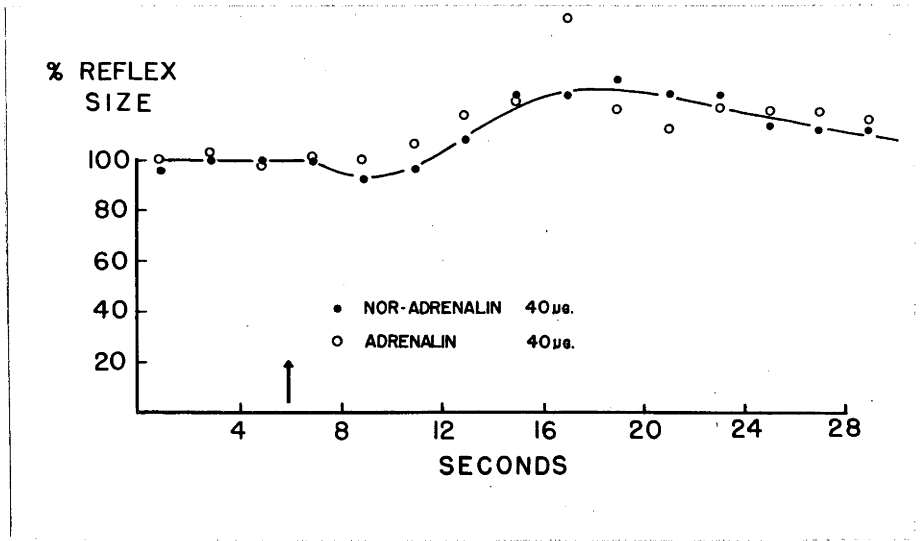

Fig. 13. The effect of 40 µg of nor-adrenalin (closed circles) and of adrenalin (open circles) administered intra-arterially, on the maximum monosynaptic reflex evoked by stimulating the L7 dorsal root and recorded from the corresponding ventral root.

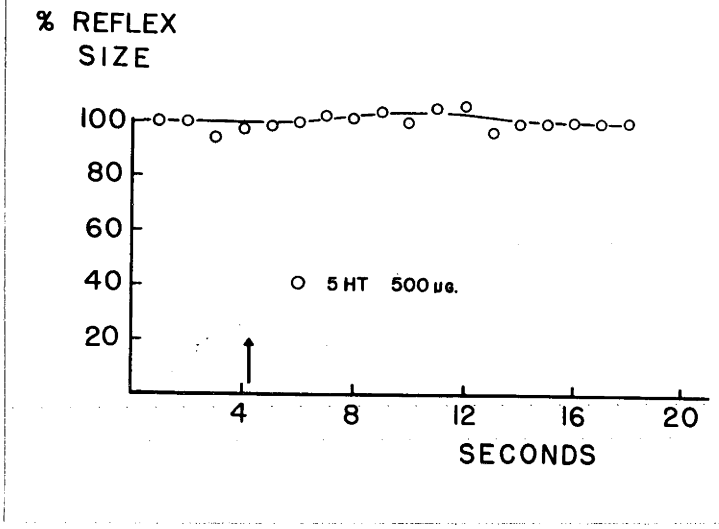

Fig. 14. At the time of the arrow. 500 µg of 5HT was injected intra-arterially, the open circles plotting the height of the maximal monosynaptic reflex of the L7 segment.

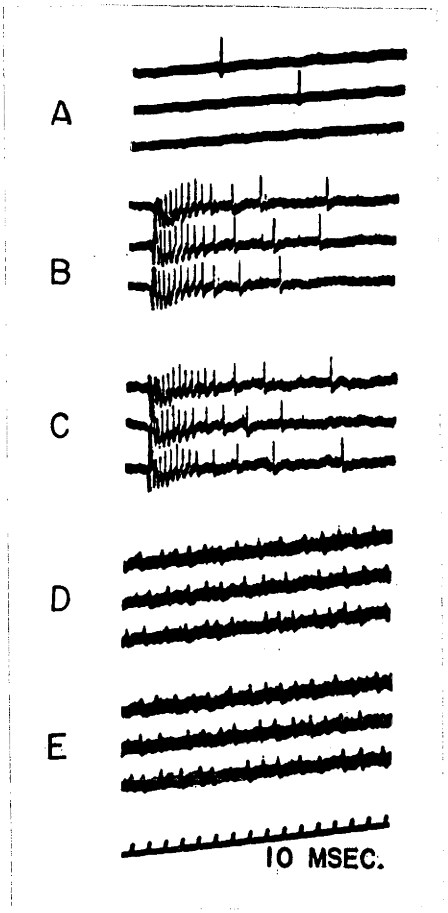

Fig. 15. Groups of three consecutive sweeps illustrating the responses of a single Renshaw cell recorded extracellularly.

A. - following the intra-arterial injection of 500 μg of 5HT. There was no increase of the firing rate above that occurring spontaneously.

B. - response to a single antidromic volley in the L7 ventral root.

C. - similar to B but five seconds after 500 μg of 5HT had been injected intra-arterially.

D. - discharge evoked by 20 μg of nicotine injected intra-arterially. In this and the subsequent record there was some movement of the electrode tip in relation to the cell and the potentials are smaller than in A, B & C.

E. - the effect of a further 500 μg of 5HT intra-arterially injected 10 seconds after the records of D.

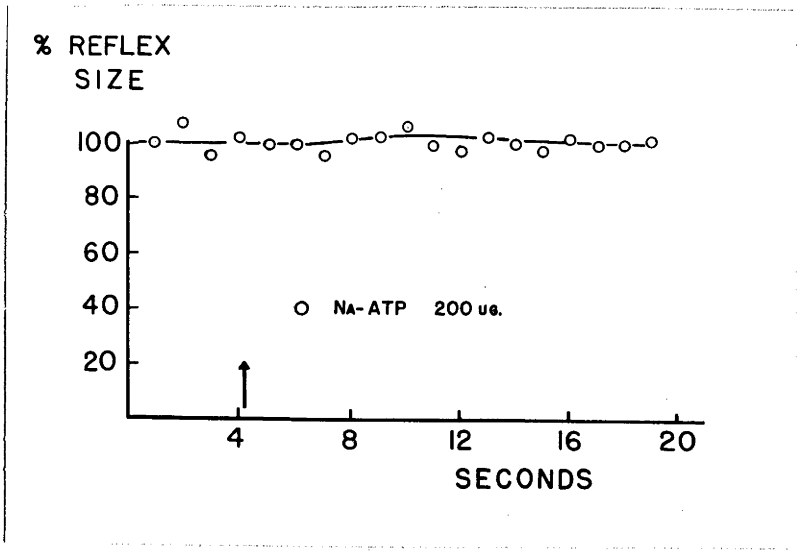

Fig. 16. The effect of 200 μg of sodium - ATP injected intra-arterially on the maximal monosynaptic reflex of the S_1 segment. Injection started at the arrow.

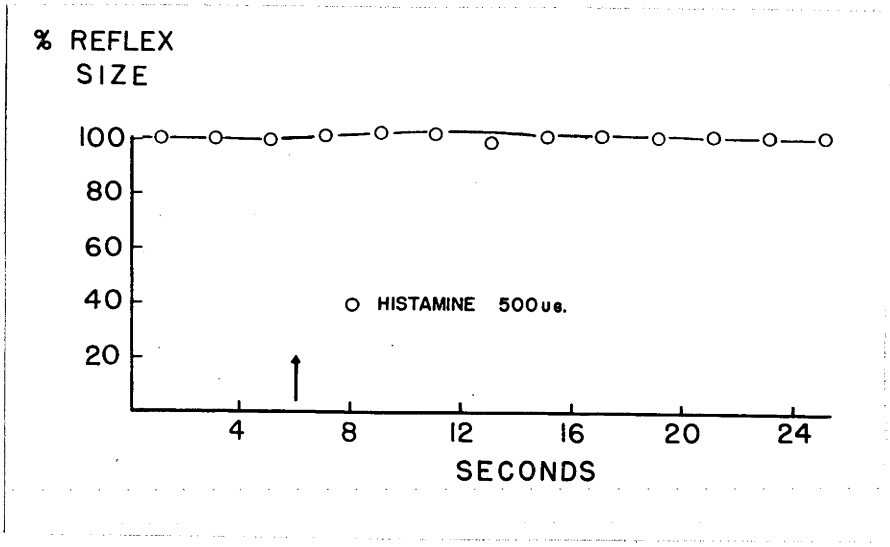

Fig. 17. The open circles plot the size of the maximal monosynaptic reflex of the L7 segment and at the time of the arrow 500 μg of histamine dihydrochloride was injected intra-arterially.

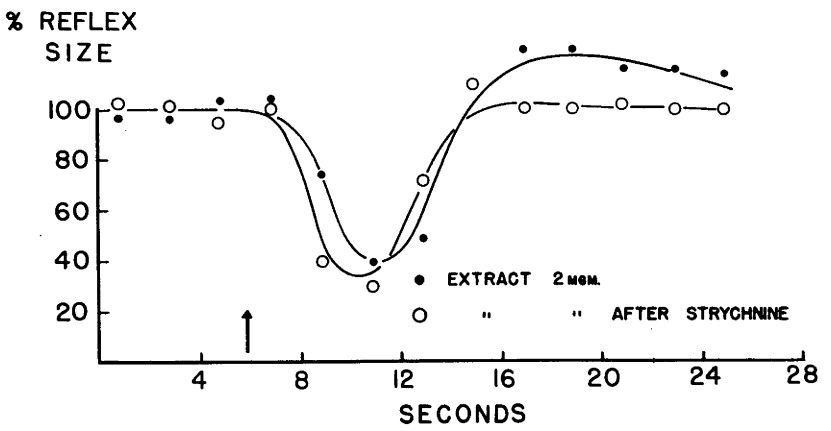

Fig. 18. The maximal monosynaptic reflex of the S_1 segment, evoked every 2 seconds, was depressed by an intra-arterial injection of 2 mgm of an extract (filled circles), and a similar result (open circles) obtained with a further intra-arterial injection after 0.1 mgm/Kgm of strychnine had been administered intravenously.

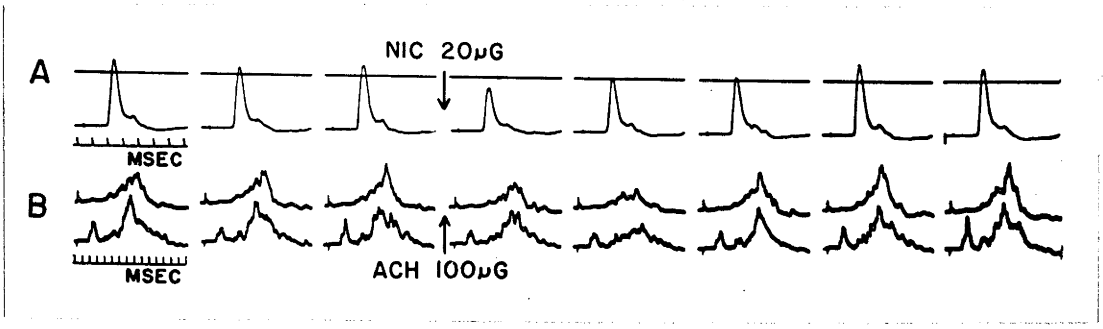

Fig. 19. A. Monophasic records of the reflexes recorded from the L7 ventral root and evoked by stimulation of the L7 dorsal root at 2 second intervals. Upper beam set as an indication of the size of the monosynaptic spike. 20 μg of nicotine was injected intra-arterially at the time of the arrow and both the monosynaptic and following polysynaptic reflexes are depressed transiently.

B. Reflexes recorded monophasically from the L7 (upper) and S1 (lower) ventral roots in response to stimulation of the S1 dorsal root at 2 second intervals. Not the same experiment as A. 100 μg pf acetylcholine was injected intra-arterially at the arrow and there is a depression of both the monosynaptic and the polysynaptic reflexes in these segments. Time in msec for each individual record only. Two second intervals between records.

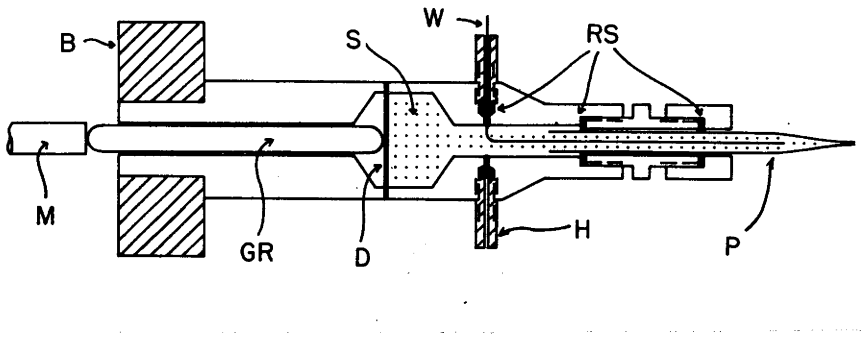

Fig. 20. Micro-injection device. This was made of perspex and clamped in a brass block (B) which was held in the manipulator. The micropipette (P) was sealed in the device by rubber seals (RS) held under pressure. The perspex diaphragm (D), 4 x 10^{-3} inches in thickness, was deformed by movement of a micrometer spindle (M) transmitted by a glass rod (GR). The complete capsule was filled with saline (S) and two hollow studs (H) were screwed into the side. One contained a platinum wire (W) connecting the micropipette to an external cathode follower for recording purposes and the other acted as a bleeder valve when the pipette was being replaced. These studs were also sealed with rubber under pressure.

The glass rod and the length of perspex between B and D were interposed in order to reduce the input capacity of the electrode system - the brass block and attached micromanipulator being at earth potential.

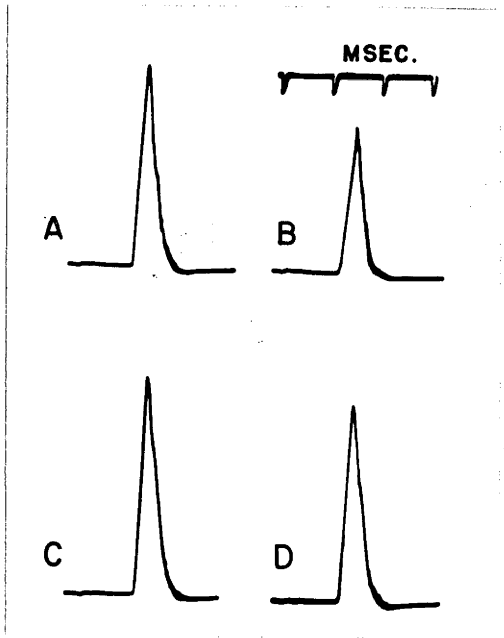

Fig. 21. Monophasic records of the maximal monosynaptic reflex, recorded from the caudal portion of the left L7 ventral root and evoked by stimulation of the biceps-semitendinosus nerve. A is the maximal control reflex and B the same reflex inhibited by a preceding volley in the group Ia afferent fibres of the quadriceps nerve. C & D - as for A & B respectively but nine and a half minutes after the injection of 0.2 mgm of XW toxin into the spinal cord at the junction between the L7 and S1 segments (see text). Each record 10 superimposed traces.

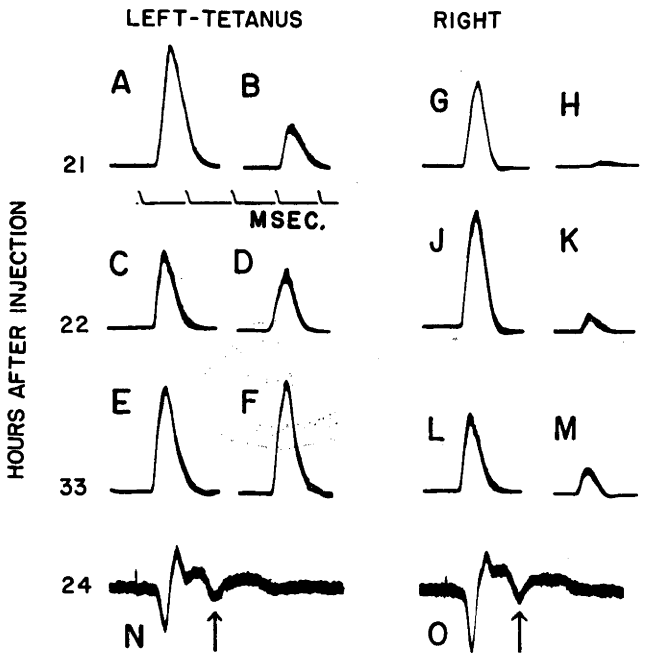

Fig. 22. A - M. Maximal monosynaptic reflexes recorded from the left (A - F) and right (G - M) L7 ventral roots and evoked by stimulation of the left and right biceps-semitendinosus nerves respectively. The left hand records of each pair (A,C,E and G,J,L) are control reflexes while the right hand records (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 mgm 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 hours after the intra-sciatic injection of toxin. Time in msec for all records and all consist of 20 superimposed traces.

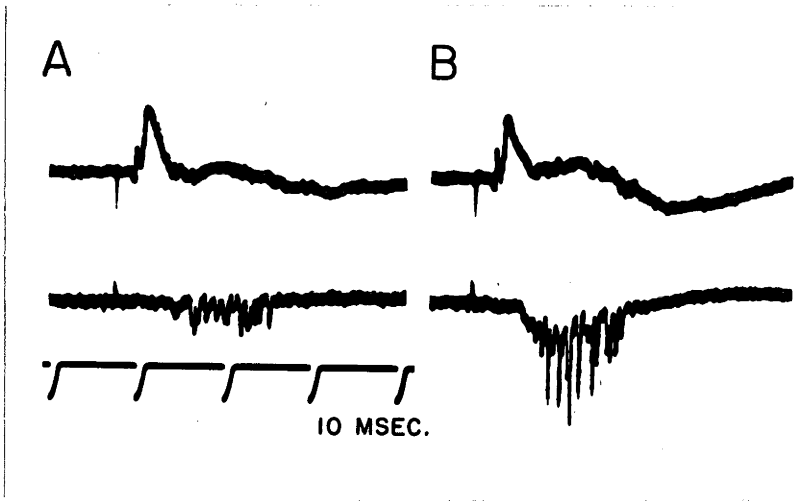

Fig. 23. The lower records show polysynaptic reflexes, recorded monophasically from the left L7 ventral root and evoked by stimulating the sural nerve at an intensity equalling ten times its threshold. The upper records show potentials recorded simultaneously from the dorsal surface of the L7 segment. A - before and B, 38 minutes after L61 toxin had been injected into the dorsolateral column in the same segment.

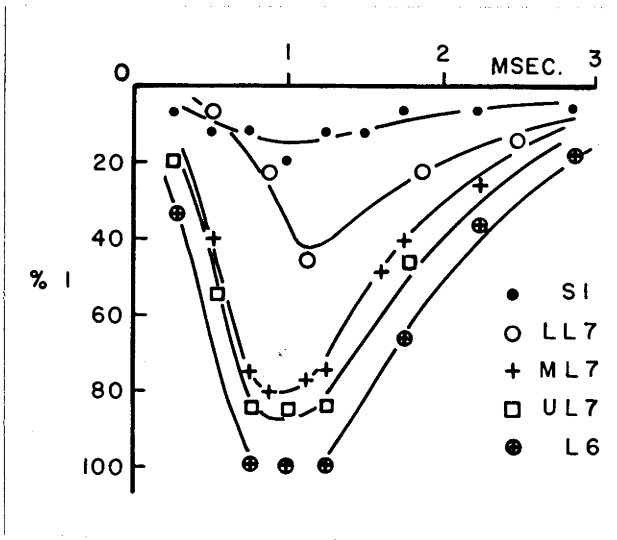

Fig. 24. Series of inhibitory curves showing the effects of a volley in the group Ia afferent fibres in the left quadriceps nerve upon the maximal monosynaptic reflexes recorded from the S1, lower L7 (LL7), mid L7 (ML7), upper L7 (UL7) and L6 ventral roots and evoked by stimulating the biceps semitendinosus nerve. Ordinates - percentage inhibition (%I) equalling 100% minus the inhibited reflex size as a percentage of the control reflex. This convention has been used in all similar graphs of this series. Abscissae - intervals between the inhibitory and excitatory volleys as recorded by an electrode on the dorsal surface of the mid L7 segment. Every plotted percentage is the mean of ten to twenty observations and curves have been drawn for each segmental reflex considered.

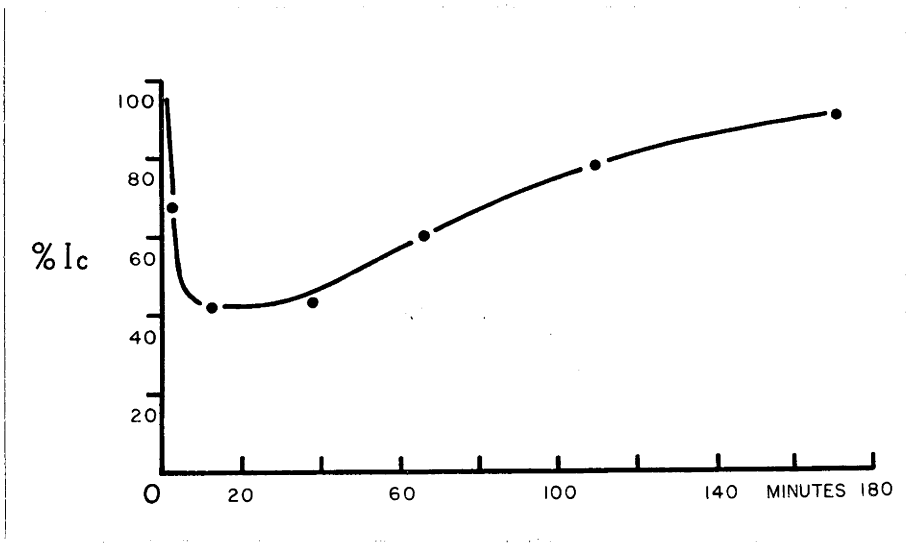

Fig. 25. Time course of the change produced by an intravenous dose of strychnine (see text) in the direct inhibition of biceps-semitendinosus motoneurones due to a volley in quadriceps group Ia fibres. Ordinates - inhibition as a percentage of the maximal control inhibition (Ic) occurring before the injection. The reflex was recorded from the S1 ventral root. Abscissae - time in minutes after the injection. Each point plots the maximum inhibition determined from an inhibitory curve constructed as in Fig. 24.

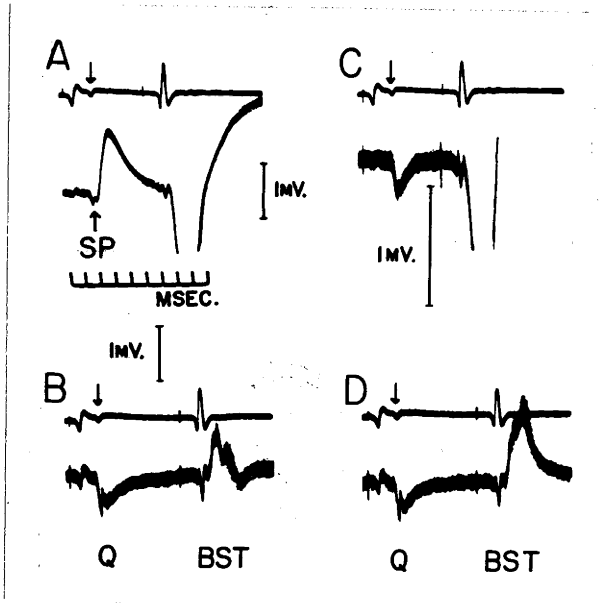

Fig. 26. Potentials recorded from the dorsal surface of the L7 segment of spinal cord (upper records) and from within the ventral horn of this segment (lower records). Fixed amplification for the surface records (below msec timer) but amplification of records B,C & D is more than double that of A. The potentials were evoked by volleys in the group Ia fibres of quadriceps and biceps semitendinosus nerves and the positive notch in the surface record following the quadriceps volley is marked with an arrow. The lower beam of A records the intracellular responses of a biceps semitendinosus motoneurone to the arriving volleys. Increased negativity being recorded in an upward direction. The full spike potential evoked by monosynaptic excitation is not shown in either A or C. The electrode was then withdrawn from the cell and record B obtained - with increased amplification. Following the intravenous injection of strychnine (0.2 mgm/Kgm) record D was taken again extracellularly and then C when the microelectrode had been re-inserted in the cell. The resting potential for both A and C was -65 mV.

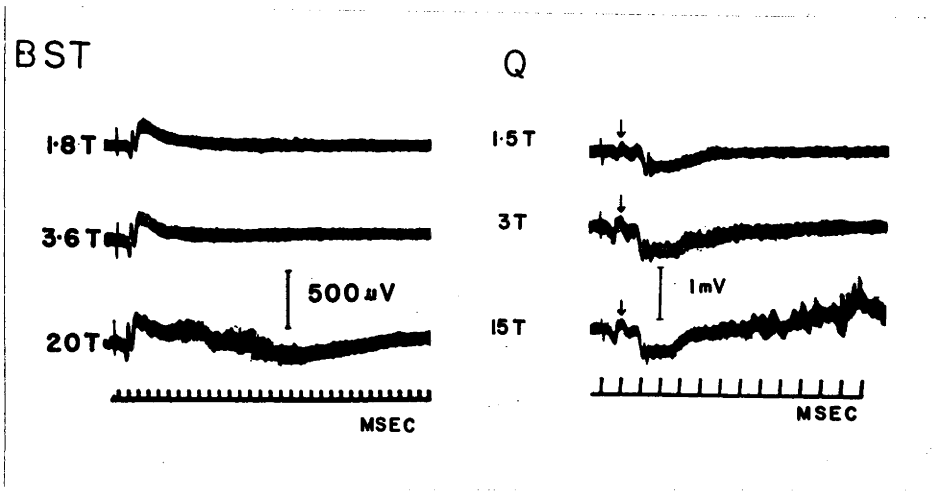

Fig. 27. Focal synaptic potentials recorded in the region of the biceps semitendinosus motor nucleus of the L7 segment and evoked by volleys in both the biceps semitendinosus (BST) and quadriceps (Q) nerves. Increased negativity upwards. The intensity of stimulation for each record is given on the left side as the number of times this stimulus was increased above the threshold intensity (T) of the nerve concerned. Note the different time scale for the two series.

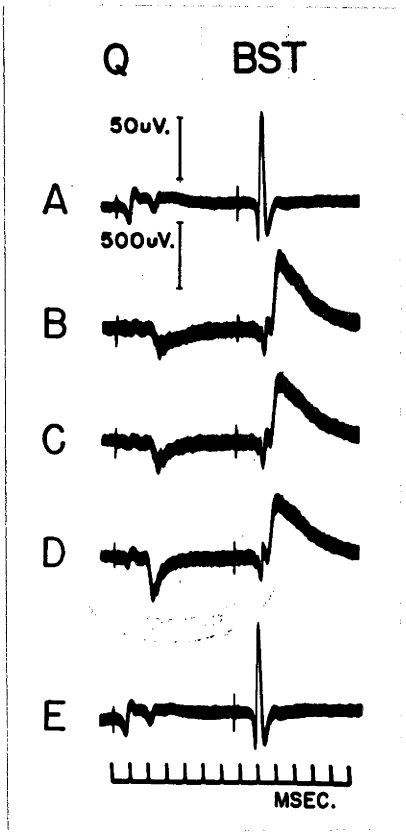

Fig. 28. Potentials recorded in a similar fashion to those of Fig. 26 B and D and evoked by volleys in quadriceps (Q) and biceps-semitendinosus (BST) group Ia afferent fibres. A and E are from the surface of the spinal cord (upper voltage calibration) and B, C & D from the biceps semitendinosus motor nucleus at lower amplification. A & B before; C after 0.05 mgm/K of strychnine and D and E after 0.15 mgm/Kgm of strychnine intravenously.

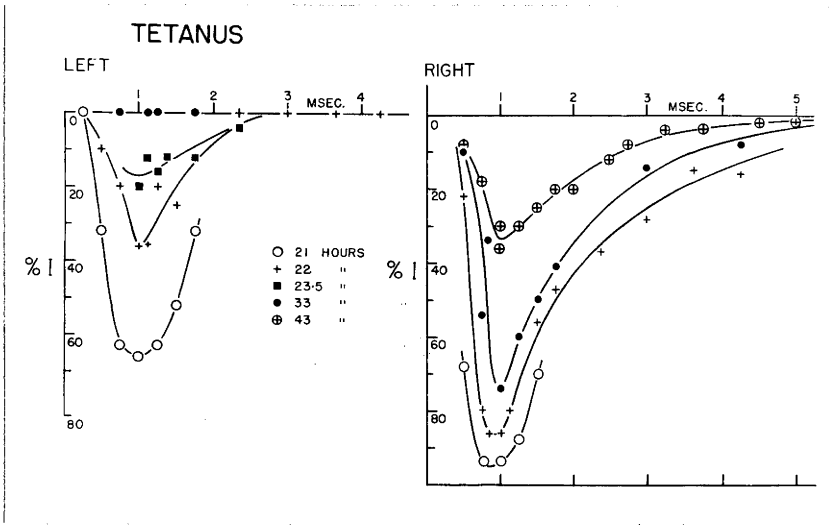

Fig. 29. Curves as in Fig. 24 showing the time course of the direct inhibition of the monosynaptic reflex of biceps semitendinosus motoneurones of the L7 segment on the left and right sides of the spinal cord. The symbols denote the inhibitory effects observed at the times indicated after the injection of 7 mgm of tetanus toxin into the left sciatic nerve. Part of this series illustrated in Fig. 22. The word "TETANUS" in this and similar figures denotes the side of either the sciatic nerve or 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.

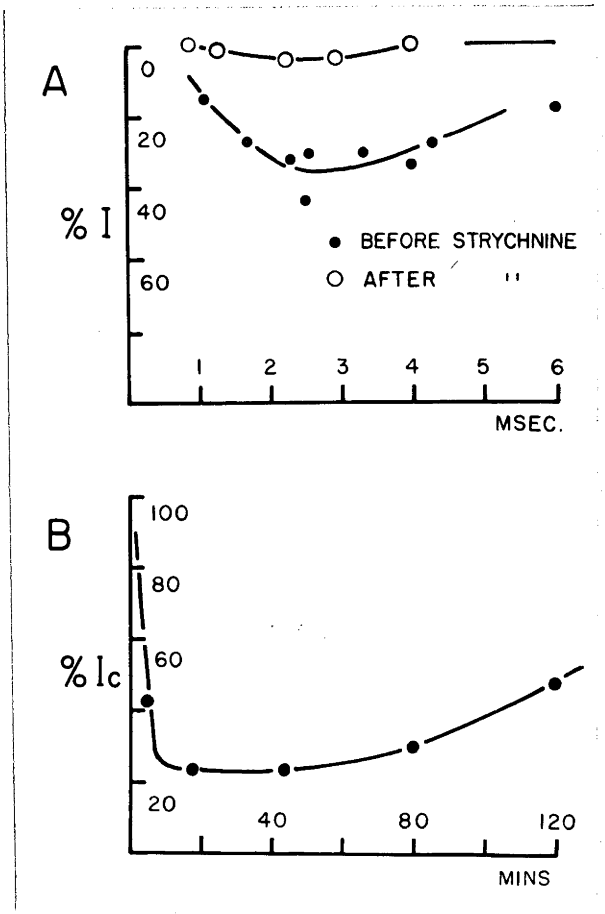

Fig. 30. A. Inhibitory curve for the effect of volleys in the group Ib afferent fibres of quadriceps on the monosynaptic reflex of gastrocnemius motoneurones of the S1 segment before and after 0.075 mgm/Kgm of strychnine.

B. Time course of the effect as in (A). Ordinates plot the inhibition as a percentage of the maximal value found before strychnine was administered (cf. Fig. 25).

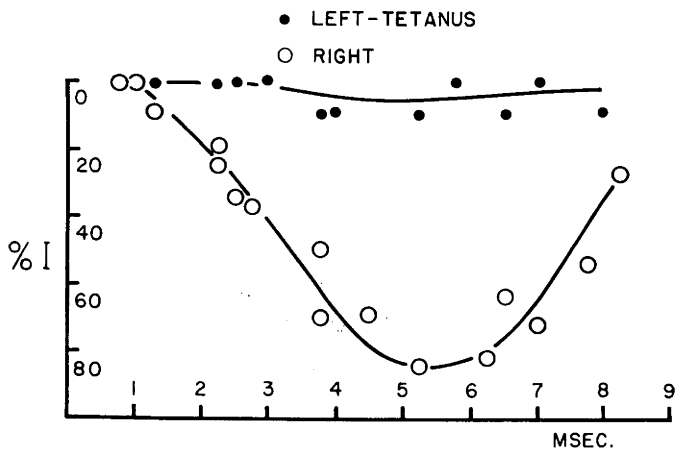

Fig. 31. Inhibitory curves as in Fig. 30A for the effect of volleys in quadriceps group Ib fibres upon the monosynaptic responses of gastrocnemius motoneurones. Forty hours after 5.7 mgm of XW toxin had been injected into the left sciatic nerve the upper and lower curves were obtained from the left and right sides of the spinal cord respectively. The maximal control reflex of the gastrocnemius motoneurones was approximately equal on the two sides at this time. The longer duration of inhibition shown by the lower curve when compared with that of Fig. 30A is almost certainly due to the quadriceps volley containing impulses in group II fibres as well as in group Ib.

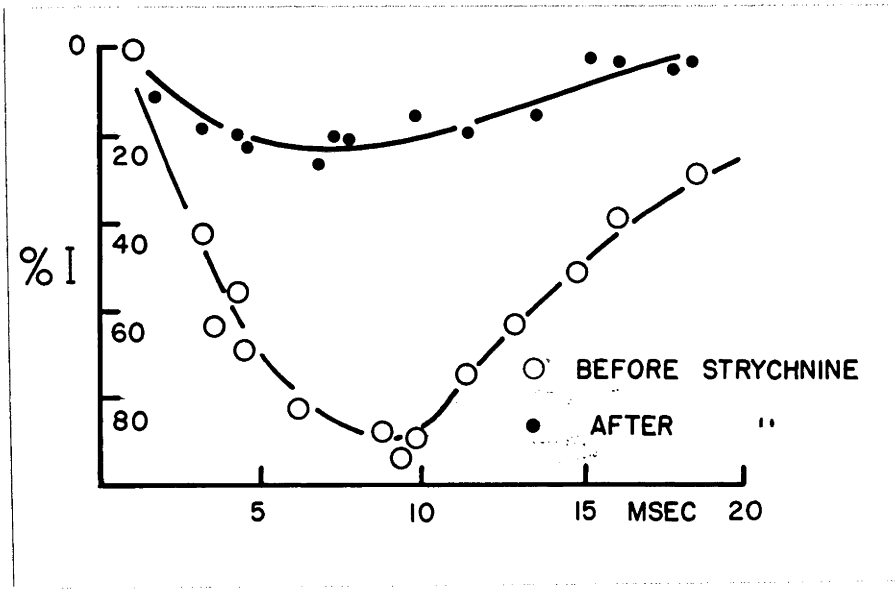

Fig. 32. Inhibitory curves before and after 0.075 mgm/Kgm of strychnine intravenously. Volleys in groups I, II and III afferent fibres of quadriceps were used to inhibit the monosynaptic responses of gastrocnemius motoneurones of the L7 segment.

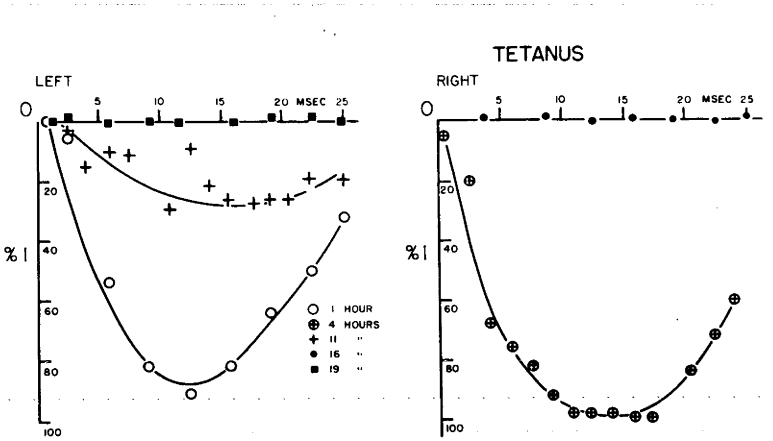

Fig. 33. Inhibitory curves, as for Fig. 32, from the left and right sides of the spinal cord at the times indicated after tetanus toxin was injected into the right side of the cord in the L7 segment. Volleys in all quadriceps afferent fibres were used to inhibit the monosynaptic reflexes of gastrocnemius motoneurones.

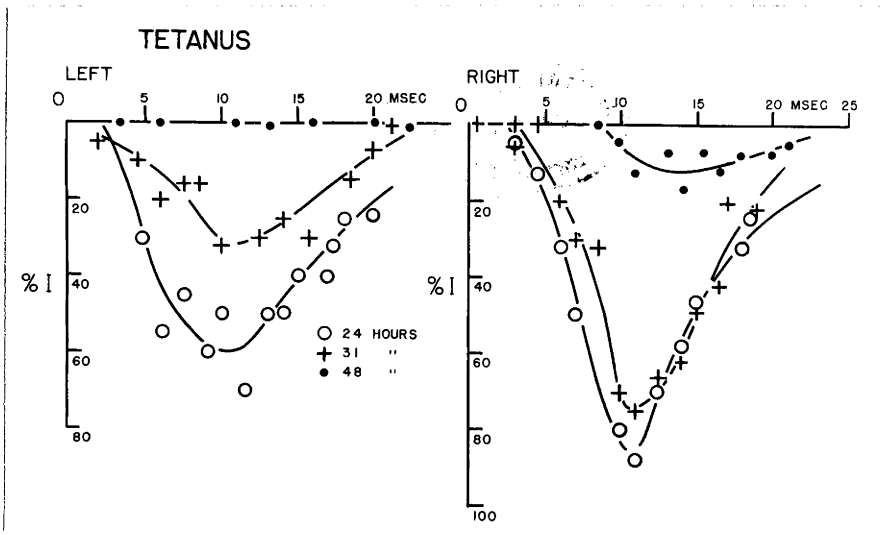

Fig. 34. Volleys elicited by stimulating the left and right sural nerves maximally were used to inhibit the monosynaptic responses of quadriceps motoneurones recorded from the L5 ventral roots of the left and right sides respectively. Tetanus toxin had been injected into the left sciatic nerve 24 hours prior to the first curve (same experiment as Fig. 22).

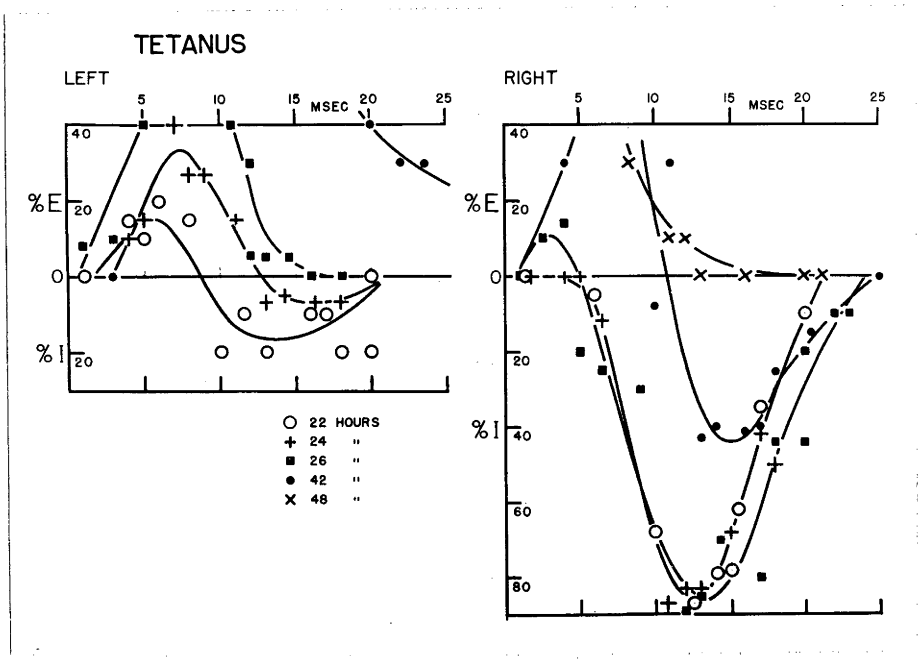

Fig. 35. Inhibitory curves from the same experiment as Fig. 34, but sural volleys were used to inhibit the monosynaptic responses of the gastrocnemius motoneurones of the L7 segment. Tetanus toxin had been injected into the left sciatic nerve 22 hours prior to the first curve and even at this time there was excitation of gastrocnemius motoneurones which increased progressively on the two sides. Ordinates below zero plot percentage inhibition (%I) as in other graphs whereas above zero the points plot the percentage increase in the size of the reflex (%E).

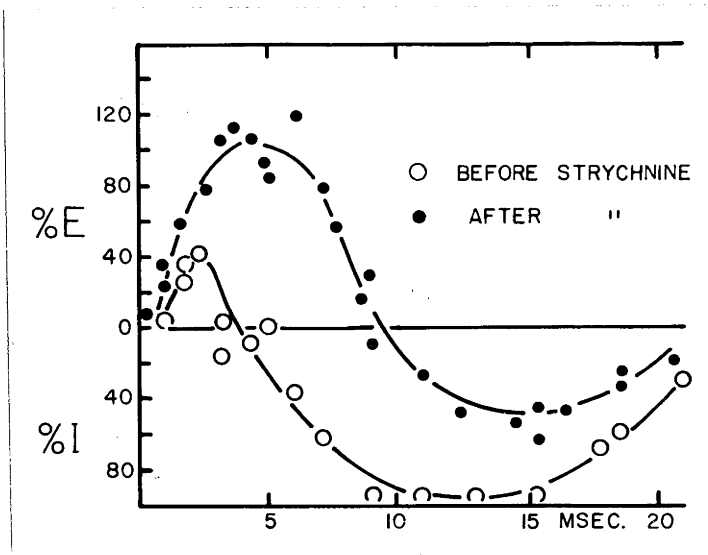

Fig. 36. Curves as for Fig. 35 before and after strychnine had been injected intravenously (0.075 mgm/Kgm). Volleys in sural fibres were used to condition the monosynaptic reflex of gastrocnemius motoneurones.

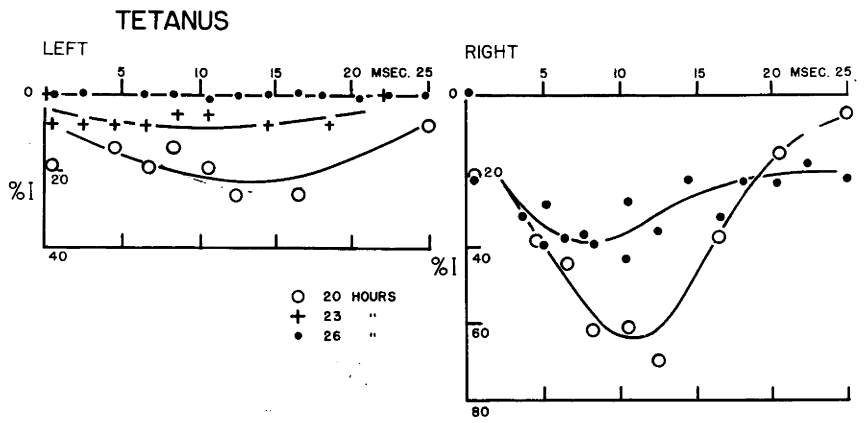

Fig. 37. Antidromic volleys propagating from the biceps-semitendinosus, posterior tibial and plantaris nerves were used to inhibit the monosynaptic reflex response of gastrocnemius motoneurones of the L7 segment on the left and right sides of the spinal cord. Twenty hours prior to the first determination, 5 mgm of XW tetanus toxin had been injected into the left sciatic trunk.

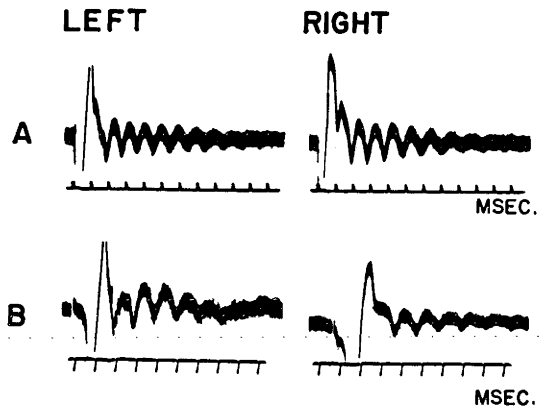

Fig. 38. A. From the experiment used in Fig. 37. The same antidromic volley was used on the left and right sides and potentials were recorded from the dorsolateral surface of the L7 segment 23.5 hours after the toxin administration. B. Potentials recorded from the dorsal surface of the L7 segment and evoked by stimulation of the L7 ventral root on the left and right sides respectively. 38 hours prior to this, XW toxin had been injected into the left sciatic nerve.

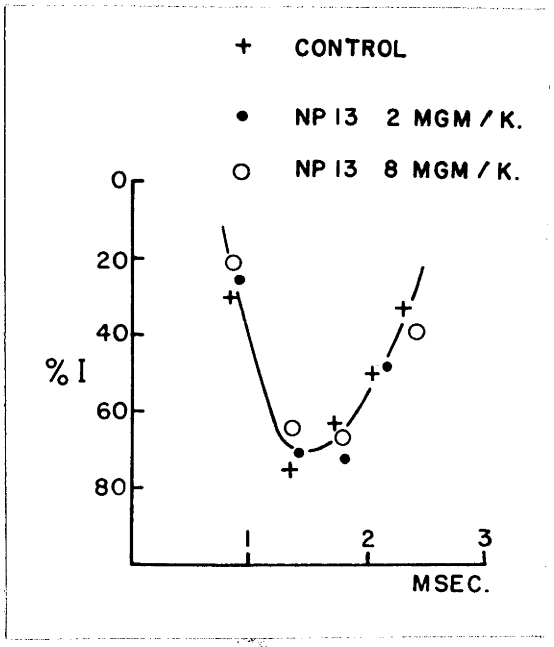

Fig. 39. Inhibitory curves constructed as for Fig. 24 plotting the inhibitory effect of a volley in quadriceps Ia afferent fibres upon the monosynaptic reflex of the biceps semitendinosus motoneurones of the L7 segment. The points + were determined before, and those marked • and O 3 minutes after 2 and 8 mgm/Kgm of NP 13 respectively. All points lie reasonably on the one inhibitory curve.

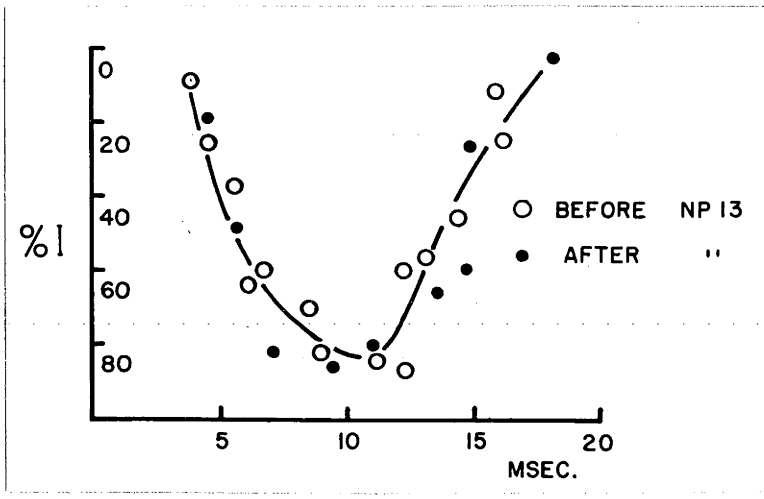

Fig. 40. Inhibitory curve of the effect of volleys in all the quadriceps afferent fibres upon the monosynaptic reflex recorded from the S1 ventral root and evoked by stimulating the gastrocnemius nerve. The one curve fits points taken before and after 8 mgm/Kgm of NP 13 had been administered intravenously.

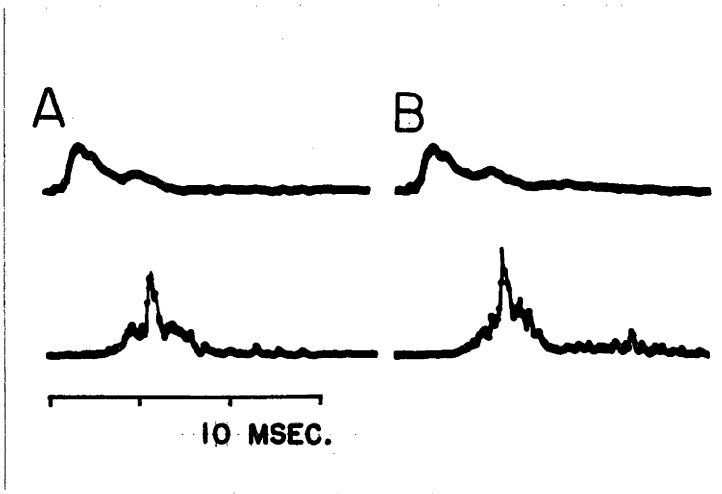

Fig. 41. Potentials recorded from the surface of the S1 segment (upper beam) and polysynaptic reflexes (lower beam) evoked simultaneously by stimulating sural nerve at an intensity ten times its threshold and recorded monophasically from the S1 ventral root. The records of A were taken before and those of B 5 minutes after 8 mgm/Kgm of NP 13 had been injected intravenously.

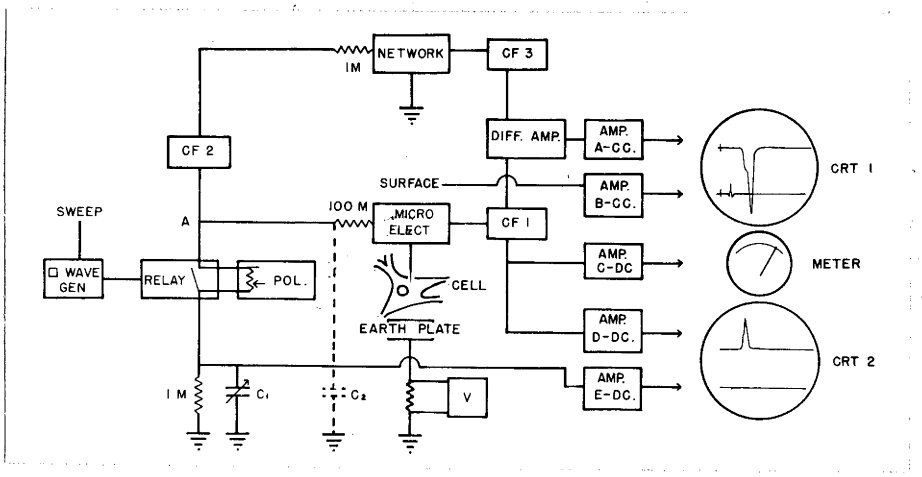

Fig. 42. Block diagram of the circuit used for the determination of the electrical properties of motoneurone membranes. The microelectrode and the input cathode follower valwe (CF1) were combined in a perspex probe unit held rigidly by the micromanipulator. Five amplifiers were used, three being direct coupled (DC) and two capacitatively coupled (CC). Both the microelectrode and the compensating network derived current pulses from the polarizing unit (POL). The two cathode ray tubes (CRT 1 and 2) were photographed by separate cameras and the meter, indicating resting potential, was placed so that both it and CRT 1 could be viewed simultaneously. V is the voltage calibration unit. Timing pulses could be fed to either beam of CRT 1 (see text).

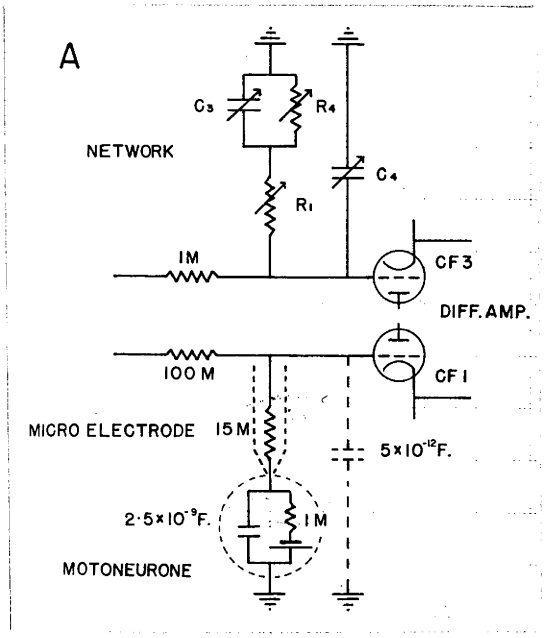

Fig. 43. The equivalent electrical circuit of a motoneurone impaled by a single microelectrode (enclosed by dotted line) and the associated compensating network. The cell is represented by a condenser of 2.5 x 10^{-9}F, resistance of 1 megohm and a battery of -70 mV. The total input capacity of the cathode follower (CF 1) was 5 - 10 x 10^{-12}F and the electrode resistance is represented as 15 megohms. Current pulses were fed to both sides of the bridge system and the variable components of the network adjusted, as described in the text, until the potentials at the output of CF 1 and CF 3 were equal in amplitude and time course. Stray capacities have been drawn as a broken line.

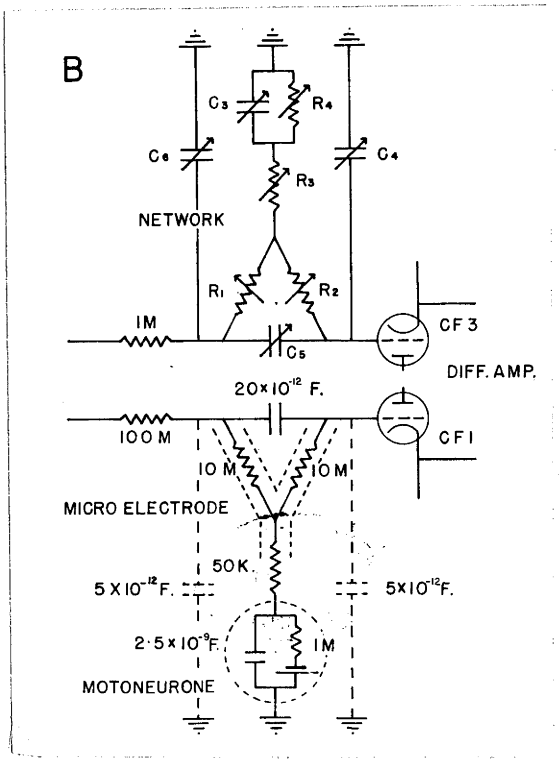

Fig. 44. Similar electrical diagram to Fig. 43 but for a double microelectrode having a coupling resistance of 50 x 10^3 ohms and a capacitative coupling between the two barrels of 20 x 10^{-12}F. Each barrel has a resistance of 10 megohms and both sides of the electrode assembly have stray capacities to earth of approximately 5 x 10^{-12}F. The network components were adjusted as described in the text.

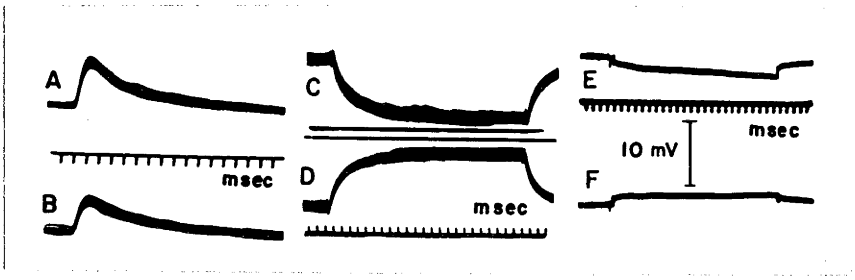

Fig. 45. A and B show intracellularly recorded excitatory post synaptic potentials which were generated monosynaptically by a single volley and recorded from a Plantaris motoneurone by means of a single microelectrode filled with 0.6M K_2SO_4 solution. Intracellular potential change in a positive direction is shown as an upward deflection. In C and D hyperpolarizing and depolarizing rectangular current pulses of 8.5×10^{-9}A were applied through the same electrode and the compensating network of Fig. 43 was used to give approximate compensation for all potential changes occurring in the system apart from those on the cell membrane. In E and F the same current pulses were applied immediately after the electrode was withdrawn from the cell, the amplification being the same as in A, B, C & D but with slower time course. The relative potential changes in E & F have to be subtracted from those in C & D respectively to determine the potential time course for the cell membrane. Records formed by superposition of about forty faint traces. Note changes in time scales for the three sets of records.

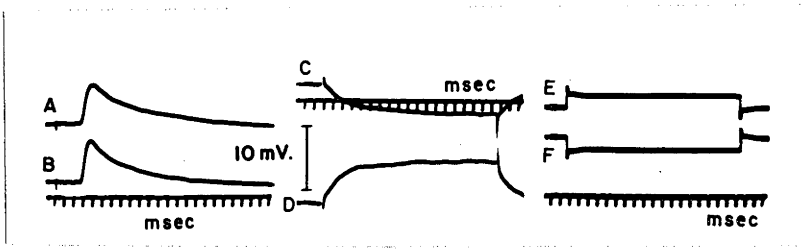

Fig. 46. Potentials recorded from a gastrocnemius motoneurone with a double microelectrode, both barrels being filled with 0.6 M K_2SO_4 solution. A and B are excitatory post synaptic potentials generated monosynaptically, the presynaptic volley being a little below maximum strength in B. C and D show potentials generated by rectangular current pulses of 12.5×10^{-9} A. which are in the hyperpolarizing and depolarizing directions respectively. As shown in the extracellular records E and F, the compensating network virtually eliminated all potential changes except those within 1 msec after the onset and cessation of the pulses. The currents in E and F were 16×10^{-9} A in the hyperpolarizing and depolarizing directions respectively.

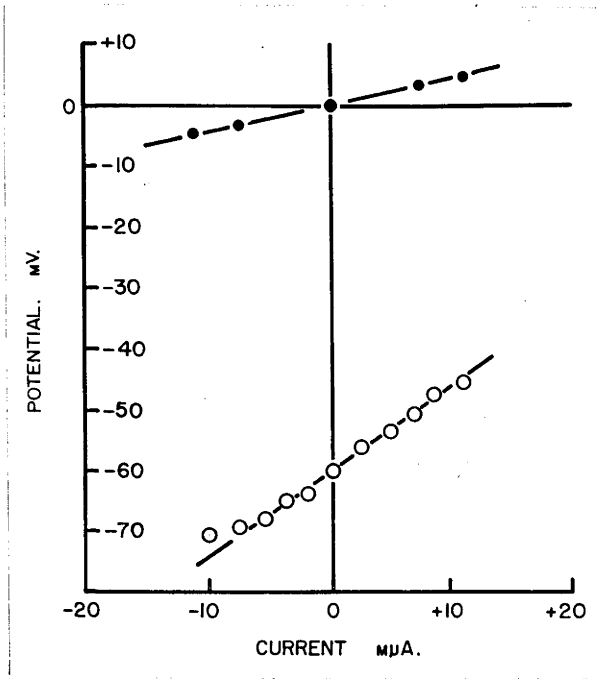

Fig. 47. Directly recorded potentials, plotted against current, from a Plantaris motoneurone with a single K_2SO_4-filled electrode. Current is indicated to be positive when directed out of the microelectrode. The potential is that at the microelectrode tip with respect to a distant indifferent electrode. The points designated O were obtained with the microelectrode in the motoneurone. The resting potential was -60 mV and the compensating circuit of Fig. 43 was used. Following withdrawal of the electrode from the cell and without alteration of the network the points ● were obtained. It is seen that straight lines fit both sets of points quite reasonably and the difference in the gradients of the two sets of measurements gives a membrane resistance of 1 megohm for the cell.

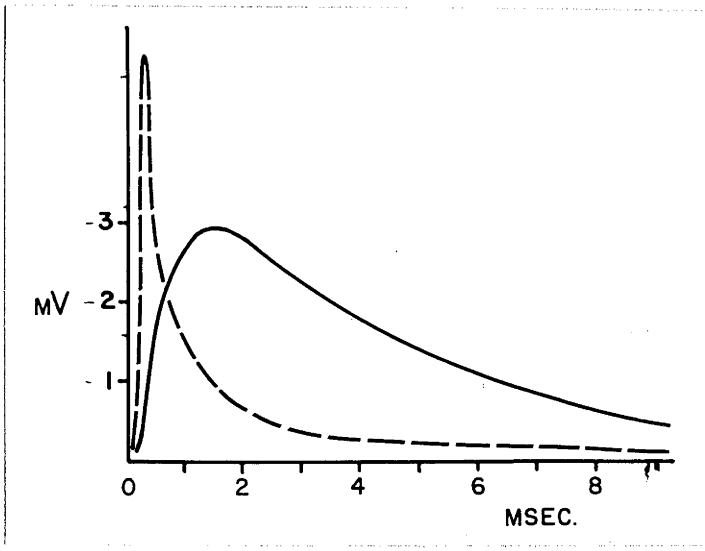

Fig. 48. The continuous line is the mean of five monosynaptic EPSP's of a biceps-semitendinosus motoneurone recorded with a single K_2SO_4 -filled electrode. Ordinate in mV. The broken line shows the time course of the subsynaptic current required to generate this potential change. Ordinate in arbitrary units (see text). The time constant of the membrane is 2 msec.

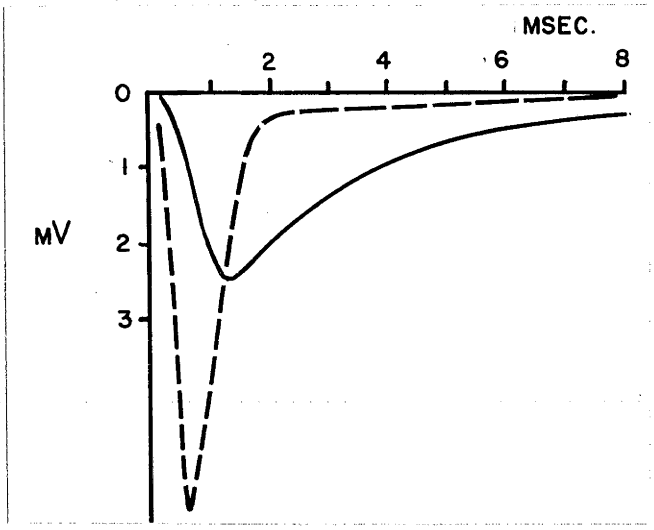

Fig. 49. The continuous line is the mean of five "direct" IPSP's of the same cell, as in Fig. 48. The broken line plots the time course of the subsynaptic current required to generate this potential change.

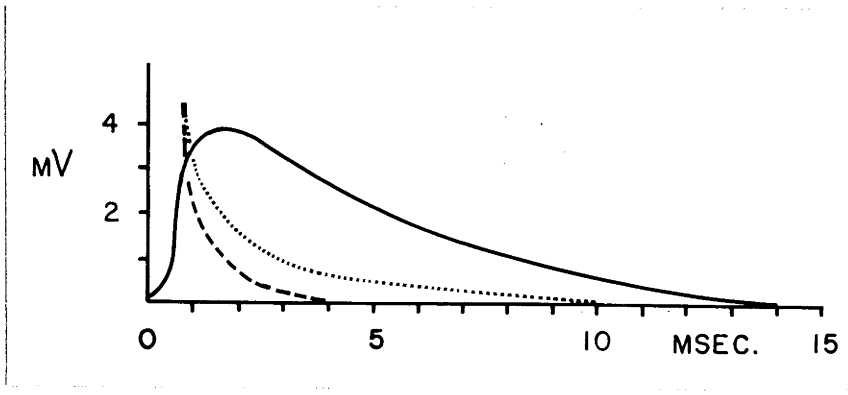

Fig. 50. The continuous line is the mean of several monosynaptic EPSP's of a gastrocnemius motoneurone having a membrane time constant of 2 msec. The time constant of decay of the EPSP is 4.2 msec and this value has been used to determine the time course of the transmitter shown by the broken line. The dotted line is the true transmitter time course assessed using the membrane time constant in the calculation. The initial current flow is approximately equal in both cases and the transmitter course is not drawn. Ordinates in mV for the EPSP (see text).

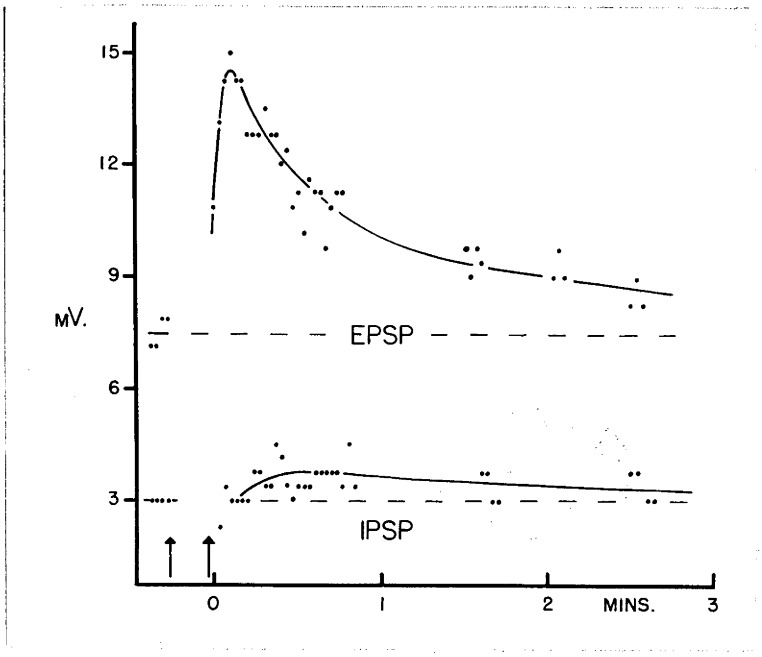

Fig. 51. Curves plotting the size in mV of the EPSP and IPSP of a BST motoneurone. For convenience EPSP and IPSP plotted in same direction. Between arrows the respective afferent nerves stimulated for 15 sec at a rate of 660/sec.

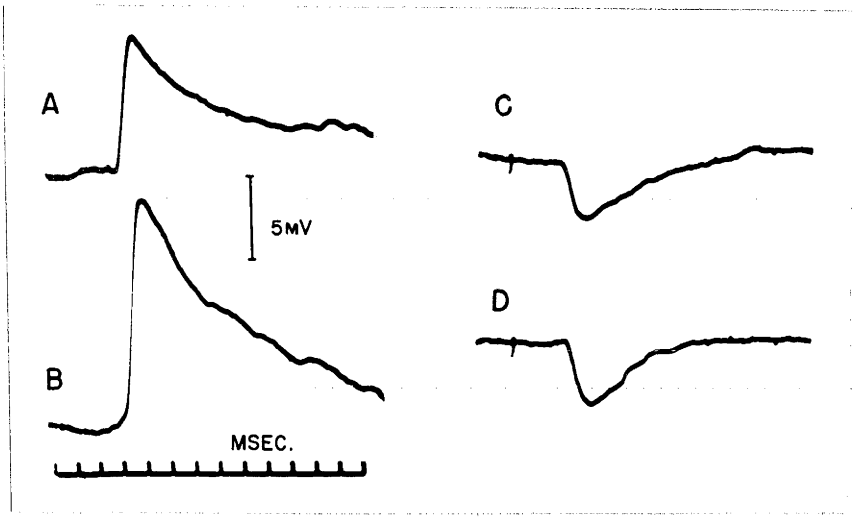

Fig. 52. A & B are single records of the EPSP's used to derive the upper graph of Fig. 51 before (A) and after (B) tetanic stimulation of the monosynaptic pathway. Similarly C & D are records of the IPSP before and after the tetanus (lower graph of Fig. 51). Increased intracellular positivity plotted upwards.

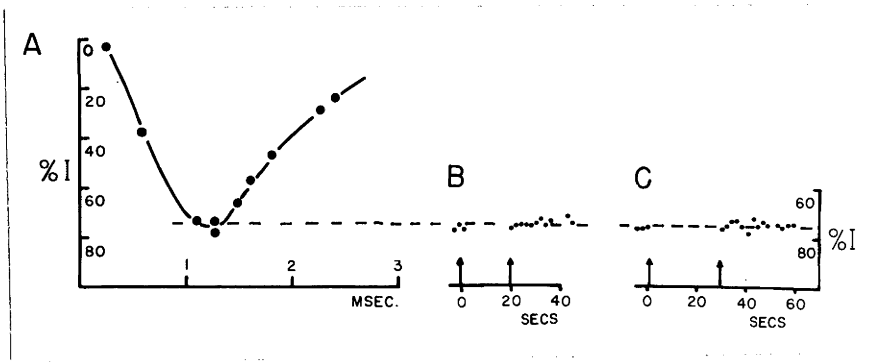

Fig. 53. (A) Curve showing time course of the direct inhibition of the monosynaptic reflex of biceps-semitendinosus motoneurones, recorded from the L7 ventral root, by a volley in the quadriceps group Ia afferent fibres. Ordinates plot percentage of inhibition, each plotted point being the mean of ten observations at two second intervals. Abscissae-interval between excitatory and inhibitory volleys recorded at the L7 dorsal root entry.

(B) Points plot single maximally inhibited biceps semitendinosus reflexes before and after the quadriceps nerve was stimulated at a rate of 300/sec for 20 seconds (between arrows).

(C) As for (B) but the tetanus lasted 30 seconds.

The broken line is at the level of the maximal inhibition before the tetanus. Abscissae of B and C in seconds.

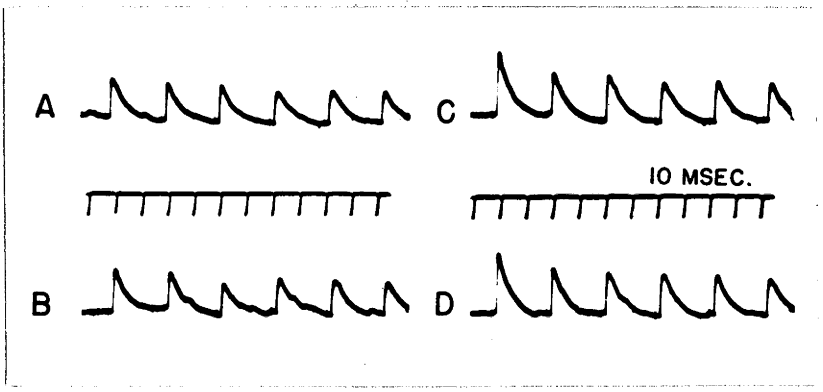

Fig. 54. A and B. EPSP's recorded from a biceps-semitendinosus motoneurone in response to a repetitive monosynaptic excitation, six volleys at 45/sec. The records C and D were taken 14 and 17.5 seconds respectively after a prolonged high frequency stimulation of the testing afferent nerve, 660/sec for 15 seconds.

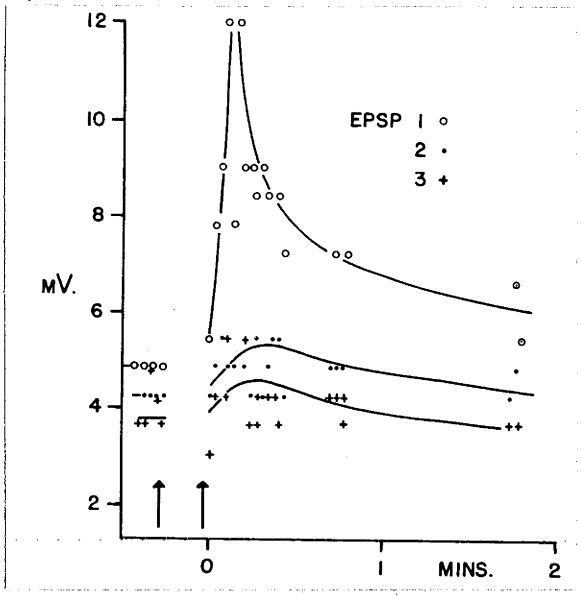

Fig. 55. The three sets of curves plot the size in mV of three successive repetitive EPSP's of a biceps semitendinosus cell evoked at a rate of 66 per second. Between the arrows the afferent nerve fibres were stimulated maximally at a rate of 660/second for 15 seconds. Note that before the tetanus the EPSP's were not of equal size, the first being the largest. The testing volleys were evoked every two seconds.

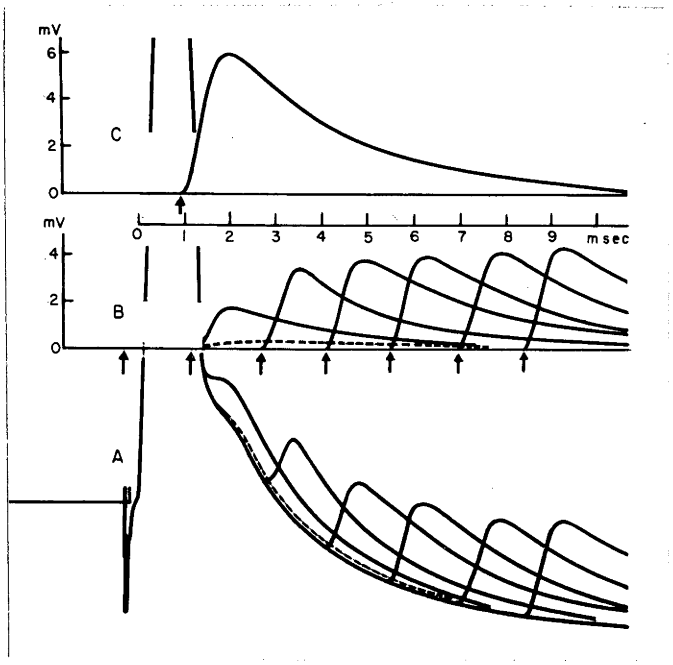

Fig. 56. A. Combined tracing of the motoneurone action potential alone (spike and after potential) and the action potential with monosynaptic EPSP's superimposed on it at various times from its onset. Broken line is response to a presynaptic volley that would set up an EPSP beginning at the first arrow of Fig. 56B.

B. EPSP's set up at various times during the action potential, obtained by subtracting the control action potential from the superimposed action potential and the EPSP's shown in A.

C. Isolated EPSP obtained in the absence of the action potential. Note that the spike potential of A is shown by interrupted lines extending up through B and C.

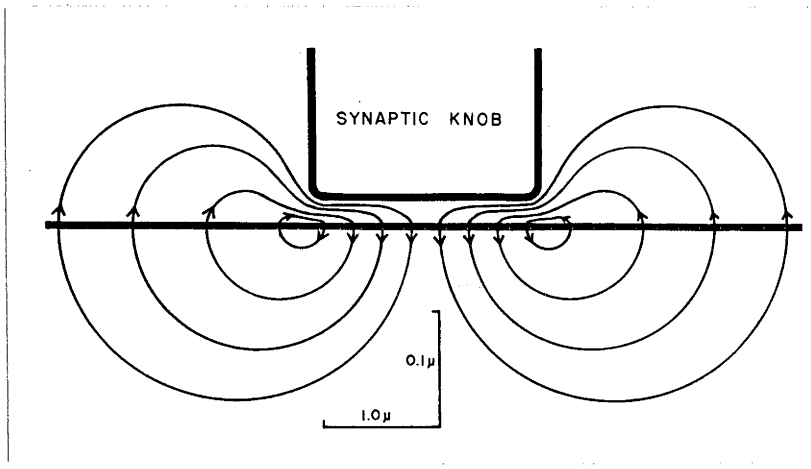

Fig. 57. Diagram showing an activated excitatory synaptic knob and the post-synaptic membrane. As indicated by the scales, the synaptic cleft is shown at ten times the scale for width as against length. The current generating the EPSP passes in through the cleft and inwards across the activated subsynaptic membrane but outwards across the remainder of the post-synaptic membrane.

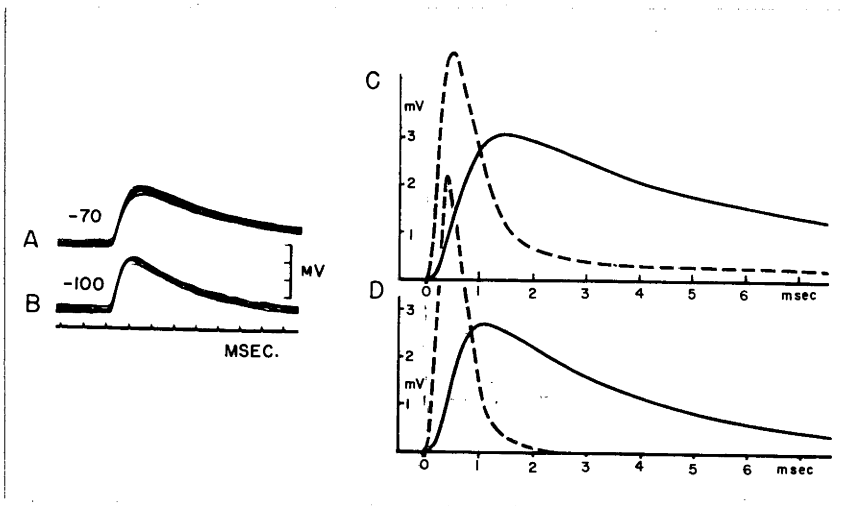

Fig. 58. A & B are monosynaptic EPSP's of a gastrocnemius motoneurone, A being at the resting potential of -70 mV while with B the membrane was hyperpolarized by about 30 mV (Fig. 4; Coombs et al, 1955c) i.e. the membrane potential was -100 mV. C & D. The continuous lines plot the EPSP's of A & B respectively whereas the broken lines plot the time courses of the subsynaptic currents causing these EPSP's (see text). For economy of plotting the curve of D overlaps that of C.