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'PHYSIOLOGICAL AND PHARMACOLOGICAL INVESTIGATIONS
ON SYAPTIC TRANSMISSION IN SYMPATHETIC GANGLIA'

By

Rosamond M. Eccles

Thesis submitted for the degree of Doctor of Philosophy in the Australian National University
I hereby declare that, with the exception of a few experiments on the action of pentamethonium and hexamethonium iodides (C₅ and C₆) in collaboration with Dr. J. W. Saunders, all of this thesis is my own original work.

Rosamond M. Eccles.

(Rosamond M. Eccles)
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I INTRODUCTION

In vertebrates, outside the spinal column, lies the long sympathetic chain of nerve fibres studded with ganglia whose neurones innervate the glands, heart and smooth muscle. The preganglionic fibres arise from neurones in the spinal cord and leave it in the ventral roots. Thence by way of the white rami they reach the sympathetic trunk and sometimes run up or down several segments of the chain before ending synaptically on ganglion cells. The sympathetic ganglion cells were shown by the histological studies of Cajal (1911), Castro (1932) and others to possess a branching dendrite system which is practically enclosed in a thick capsule, quite unlike the wide and free distribution of the motoneuronal dendrites. The intracapsular dendrites form an open network uniformly distributed around the cells and end in curious shaped swellings either on the capsule itself or amongst the small cells in the capsular sheath called satellite cells by Cajal (1911) and Castro (1932).

There is good experimental evidence that there are no interneurones in the ganglia between the preganglionic fibres and the ganglion cells. Thus Ranson and Billingsley (1918) found that section of the cervical sympathetic, i.e. the preganglionic fibres, produced degeneration of all the fine branching fibres in the ganglion, only the ganglion cells and their axons surviving. Secondly the uniformity of the preganglionic endings and the ganglion cells made it seem very unlikely that interneurones were present (Cajal, 1911 and Castro, 1932; also Ranson and Billingsley, 1918). Since the number of postganglionic fibres far exceeds the number of preganglionic fibres, each preganglionic fibre must innervate several ganglion cells (Langley, 1900). Billingsley and Ranson (1918a) estimated a 1:32 ratio on histological counts though later work indicated a 1:15 ratio (Wolf, 1941).
The following additional evidence also relates to the simple synaptic structure of the ganglion. When nicotine was painted on the superior cervical ganglion (Langley, 1900), transmission through the ganglion was blocked. Similarly, section of the preganglionic nerve trunk produced no degeneration beyond the ganglion, i.e., degeneration could affect only the preganglionic trunk and not the internal carotid nerve (Ranson and Billingsley, 1918). Electrical stimulation of the postganglionic nerve produced no activity in the preganglionic nerve—a very good indication, as Brown (1934) points out, for unidirectional conduction through ganglia. The sympathetic ganglion thus provides the simplest example in vertebrates of a synaptic system between two groups of nerve cells, the axons of the preganglionic neurones ending on the ganglion cells without the complication of interneurones.

In the cat there are two types of postganglionic fibres in the internal carotid nerve, a small group of myelinated fibres and unmyelinated fibres (Billingsley and Ranson, 1918b). On the other hand, the postganglionic nerve from the superior cervical ganglia of rabbits contains only unmyelinated nerve fibres (Bishop and Heinbecker, 1932). Occasionally a few sensory fibres traverse the ganglion without synapsing—these usually join the vagus and are not included in the postganglionic trunk. The electrical studies of Bishop and Heinbecker (1932) showed a correlation between threshold of the preganglionic fibres and the function of the ganglion cells they activate. In the cervical sympathetic trunk the low threshold fibres supply the smooth muscle in the iris and the nictitating membrane, the higher threshold fibres being responsible for piloerector and vasoconstrictor actions. The refractory period for the superior cervical sympathetic ganglia of the rabbit was given as 20 msec by Bishop and Hein-
becker, however Brown (1934) found a figure of only 2 msec for synaptic delay, and a value for refractory period in the order of two msec in the cat superior cervical ganglia.

It is fairly evident that there is normally an asynchronous barrage of activity on to the sympathetic cells because there is experimental evidence that section of the preganglionic or postganglionic cervical sympathetic produces dilatation of the ear blood vessels, reduction in the tone of the nictitating membrane and dilatation of the pupil. Adrian, Bronk and Phillips (1932) found an asynchronous firing of impulses in the cervical sympathetic of anaesthetized rabbits. Since the rate varied with the respirations it was suggested to be the effect of the respiratory centres on the vasomotor centres which was producing a slight alteration in the tone of the blood vessels.

As Bronk (1939) indicated, the most direct method of measuring the manner in which a ganglion cell behaves is to record the activity in different preganglionic fibres converging on a particular ganglion cell and the relationship between this activity and the impulse discharge down the axon. At present the method of recording the ganglionic response, either by the effector organ, or better by the impulses in the postganglionic fibres, remains the only possible way of examining the alteration in activity of ganglion cells. However it is not forgotten that investigation of the ganglionic response to a series of synchronized electrically initiated volleys in the preganglionic fibres may not provide a complete explanation of the effects of drugs on the normal asynchronous discharge of impulses.

The type of transmission to be expected in sympathetic ganglia was formulated in the first instance by Langley and his co-workers who
suggested that the post-synaptic membrane must have receptors for the transmitter substance. Interest in Ach as the probable transmitter was first aroused in 1932 when Kibjakow produced evidence that stimulation of the preganglionic fibres caused the appearance of a ganglionic stimulating substance in the perfusate. Feldberg and Vartiainen (1934b) observed in eserinized perfusates an Ach output on stimulation of the fibres but not the postganglionic fibres. Brown and Feldberg (1936b) found little difference between the responses of perfused ganglia and ganglia with their blood supply intact. Addition of potassium chloride in amounts greater than normal could liberate Ach from these ganglia in situ. Their preliminary studies on Ach content of denervated ganglia (Brown and Feldberg, 1936b), were confirmed by MacIntosh's studies on the effect of section of the preganglionic nerve which revealed a 25% decrease in Ach content of ganglion in 24 hours while 72 hours after section only 15% of the Ach was left (MacIntosh, 1938a). Kahlson and MacIntosh (1939) showed the dependence of Ach output on metabolism and the glucose requirements of the ganglion.

Running parallel with the development of the chemical transmitter story were the studies by Eccles (1935, 1936, 1937) and Bronk et al. (1936, 1937, 1938, 1939) on the electrical responses of ganglion cells. Leading from the postganglionic trunk of the superior cervical ganglion, rabbit or Belgian hare, Eccles showed that the generation of an action potential by the ganglion cells whether stimulated ortho- or antidromically, was followed by a negative and positive afterpotential. The long positive afterpotential with a peak at 100 msec coincided with a period of decreased excitability to antidromic volleys and preganglionic stimuli. However facilitation of the testing volley occurred if the second or testing stimulus was applied to the same preganglionic fibres as the condition-
The depression of the ganglion cell during the positive afterpotential was illustrated by the complete disappearance during the positive afterpotential of the asynchronous firing of the ganglion cells that was evoked by perfused Ach. The discharge of impulses to the perfused Ach increased as the positive afterpotential decayed (Bronk, 1939).

Preganglionic fibres are known to be capable of following frequencies of up to 300-400/sec. The postganglionic unmyelinated fibres have been shown to conduct impulses satisfactorily up to 150/sec. Ganglion cells are stated (Bishop and Heinbecker, 1932; Bronk and Pumphrey, 1935; Bronk, 1939) to be capable of following only very low frequencies. The failure of response seemed to be a property of the synaptic mechanism. This failure to respond to high frequencies may be due to several factors, anaesthetic, anoxia and temperature. Bishop and Heinbecker’s ganglia preparations are unlikely to be at a temperature greater than 30°C and at such a low temperature ganglia are known to fail rapidly (Eccles, 1935). Anaesthetics produce a reduction in the response of ganglion cells as Bishop and Heinbecker commented on the "sluggish behaviour" when urethane was used as the anaesthetic. However Brown (1936) found that there was very little alteration in ganglionic potentials when nembutal was employed as an anaesthetic. The effect of anaesthetics on ganglionic potentials was very carefully investigated by Larrabee and Holaday (1952), and Larrabee and Posternak (1952). No experimental evidence was found to support the theory that anaesthetics primarily affected the oxygen uptake of sympathetic ganglia (Larrabee, Ramos and Bulbring, 1952).

Like all nerve cells especially of warm blooded animals there is a high rate of metabolism and therefore a large requirement for oxygen.
Bronk, Larrabee, Gaylor and Brink (1938) indicated that oxygen lack led to a reduction of the ganglion response whether this response was evoked by the perfusion of Ach or potassium or by the preganglionic impulses. It seems then to be a failure in the excitability of ganglion cells rather than a reduction in the effectiveness of the preganglionic terminals though this could be simultaneously developing. Bronk (1939) reported that conduction of impulses in the fibres traversing the ganglion without synapsing failed at the same time as the transmission across synapses. Later work (Bronk, Larrabee, and Gaylor, 1948) verified this finding of simultaneous failure in transmission across synapses and conduction through ganglia. When high rates of stimulation are employed during anoxia, the development of blockage is greatly accelerated (Bronk, Larrabee, and Gaylor, 1948). The blockage of transmission is almost completely reversible if the asphyxia duration is not greater than two hours (Bronk, 1938; Bronk, Larrabee, Gaylor and Brink, 1938). Even after six hours (Bronk, 1939) 20% of the cells recovered. This remarkable ability to recover from a long period of asphyxia is in marked contrast to the neurones of the central nervous system (Gerard, 1932; Bronk, Larrabee and Davis, 1946) and the p-sympathetic ganglia, which were shown to be unable to recover their excitability after a short exposure to low oxygen pressure (Perry and Talesnik, 1953).

The energy requirements of ganglion cells are not as great as the neurones of the central nervous system but are very much greater than the requirements of peripheral nerves. Larrabee and Bronk (1952) showed little difference in the metabolic requirements of preganglionic and postganglionic fibres, or the ganglion cells themselves. Metabolic inhibitors reduce the oxygen uptake of ganglion cells as of any other tissue, however anaesthetics
block conduction across the synapses without having any effect on metabolism as measured by oxygen uptake and glucose utilization.

Certain changes in the characteristic discharge of ganglion cells after prolonged preganglionic tetanus were observed by Bronk (1939) and Larrabee and Bronk (1947). During the post-tetanic period a test volley could elicit a response up to four times normal. It was shown that the potentiation was due to alteration in the preganglionic terminals rather than to some change in post-synaptic characteristics i.e., properties of the ganglion cells. A full description of post-tetanic effects is reserved for a later section.

Further investigations on the effect of drugs on ganglionic transmission have substantiated the theory of Ach as transmitter. Curare in a concentration of 1.0 mg/Kg was found to block transmission across the synapses of the cat's stellate and yet presynaptic impulses still set up a post-synaptic negativity — the synaptic potential (Eccles, 1943). The synaptic potential was 10-15% of the spike discharge, with a time half-decay of 40-60 msec. On repetitive stimulation at frequencies greater than 10/sec the synaptic potentials summed to reach a negative plateau which was maintained throughout the period of stimulation. This plateau level was reached earlier and its height was higher, the faster the stimulus frequency until a maximum was obtained with rates of 120/sec. The negative plateau decreased in two phases, a fast decaying fraction and a slow one. An injection of eserine (1 mg/Kg) greatly prolonged the slow second fraction without a great increase of the duration of the fast decaying phase. This long period of depolarization was readily explained to be due to the presence of the Ach liberated from the preganglionic terminals by stimulation and still unhydrolysed as the enzyme has been inhibited by eserine.
Certain substances like potassium and adrenaline are known to alter the ganglionic response to preganglionic activity. Vogt found an increase in the potassium content of perfusate after stimulation (Vogt, 1936). Brown and Feldberg (1936) showed an increase in the amount of Ach in the perfusate when potassium chloride was injected. However, the sensitivity to potassium was not altered by curare which makes it seem probable that the higher sensitivity of ganglion cells is due to the depolarizing action of the potassium on the post-synaptic membrane. Adrenaline has been postulated to aid transmission in low concentrations (Bulbring and Burn, 1942b; Bulbring, 1944) and block when in high concentrations (Marrazzi, 1939; Marrazzi and Marrazzi, 1947; Bulbring, 1944).

Recently the discovery of new drugs has shown that the blocking action of different substances can be divided into three classes: 1) those that depolarize the post-synaptic membrane thereby producing block; 2) those that have no depolarizing action and yet actively compete with Ach for receptors on the post-synaptic membrane; and 3) those that block the preganglionic terminals. In 1953 Paton and Perry using a direct-coupled amplifier illustrated the difference between the depolarizing action of C₁₀ and nicotine and the blocking action without accompanying depolarization by C₅, C₆ and the toxiferins.

Laporte and Lorente de Nó (1950) reported the appearance of large positive potentials when stimulation was applied to the preganglionic fibres of deeply curarized turtle ganglia. These positivities were interpreted as an anelectrotonus imposed on the ganglion cells by blockage of the impulses invading the preganglionic terminals and it was suggested that this anelectrotonus was actually due in uncurarized ganglia to specific inhibitory fibres. It was in an effort to find whether similar potential changes occurred in mammalian sympathetic ganglia that
initiated the work on these isolated curarized ganglia (R. Eccles, 1952b). The discovery of identical potentials was followed by an attempt to analyse these by pharmacological means.

The pharmacology of mammalian sympathetic ganglia up to 1950, had only been explored in blood circulated or perfused ganglia. The work of Bronk (1939) indicated that ganglion cells were curiously resistant to oxygen lack and could recover their excitability after several hours of anoxia. However until recently isolated ganglia had been used for only one hour after excision (Larrabee, Bronk & Gaylor, 1948). Malcolm (1949), Saunders and Sinclair (1949) and Laporte and Lorente de Nó (1950) had kept excised amphibian and turtle ganglia in good condition for many hours. While this present work was in progress, Brown and Pascoe (1951) reported that isolated mammalian ganglia under favourable conditions could discharge impulses twenty-four hours after excision.

Complete isolation of the ganglion gives two advantages. Firstly, with the isolated ganglion it is possible to use any combination of leads for electrical analysis without the complications of electrical recording which arise from attachment of the ganglion to the animal. Secondly, the ganglion can be subjected to the action of substances in known concentration. Possible disadvantages are that the isolated ganglion would be a deteriorating preparation, and that a long diffusion time is required before there is equilibration with any fluid in which it is immersed. Deterioration has not been serious, because even 36 hours after excision the action potentials have not noticeably changed, and diffusion time for equilibration has been shortened to about 20 minutes by incising the sheath and partly removing it (cf. Rashbass and Rushton, 1949; Feng and Liu, 1949, 1950).
The rabbit's superior cervical ganglion has the advantage that a relatively long length of post-ganglionic trunk is available for experimental purposes. This advantage is somewhat offset by the occasional presence of double ganglia, already described by Bishop (1936). However it would seem (Bishop, 1936) that the post-ganglionic fibres from the first ganglia join the vagus and were not usually included in the post-ganglionic trunk used. Bishop and Heinbecker (1932) reported the absence of afferent fibres passing uninterruptedly through the ganglion, but only a few low threshold fibres of this type have been observed in some of the present experiments.
METHOD

The superior cervical ganglia were removed from rabbits that were anaesthetized by administration of paraldehyde by a stomach tube. The preganglionic trunk was freed in the neck and cut several centimeters proximal to the ganglion. The postganglionic trunk was separated from the internal carotid artery, freed from the surrounding connective tissue and cut at its point of entry into the base of the skull. During this operative procedure the blood supply of the ganglion was preserved. Just before the ganglion was exercised a loop was threaded under the common carotid. When the ganglion was freed from most of its attachments the carotid was tied and the ganglion cut free. This ligation of the carotid prevented time being wasted due to haemorrhage from the artery as the final dissection is done. The whole preparation was then removed into a Petrie dish of Ringer-Locke solution at room temperature, oxygenated by 95% O₂ 5% CO₂. The sheath was then carefully removed in order to allow free diffusion of substances into, and out of, the ganglion.

The perspex recording chamber (Fig. 1) has been constructed so that it incorporates the following features: air-tight seal with tubes for gas entry and outflow so that a constant predetermined atmosphere can be maintained for long periods; fixation of the preparation on the electrodes, but also ability to adjust the position of one electrode without opening the chamber; the preparation may be immersed in a solution or lifted up therefrom for recording merely by rotating the chamber to one or other side; the soaking solution may be changed by addition of substances in small volumes of solution also without opening the chamber. All the electrodes as well as the perspex posts are fixed in the roof of the chamber which is removed and inverted when mounting the preparation for recording.
The amplifiers used throughout this work were either a direct coupled amplifier or a capacity-coupled amplifier with a time constant of 8 sec. The former amplifier was employed for all the later work.

The ends of the pre- and postganglionic trunks were tied to the two perspex posts (Fig. 1), that on the preganglionic side being moveable in order to take up slack in the preparation and leave it at the minimal tension for fixation on the electrodes. The platinum electrodes were flamed immediately before use and adjusted so that they made contact with the preparation at the required positions. The roof of the chamber was then sealed in position, the preparation soaked in the solution through which the gas mixture (95% O₂ and 5% CO₂) was bubbled. Unfortunately, owing to the inadequacy of the heating system, the temperature was not accurately stabilized, the extremes varying from 33°-37°. Later the chambers were suspended in a perspex bath containing water at a constant temperature throughout the experiment which was usually about 39°C though in occasional experiments the stabilized temperature was as low as 37°C or as high as 41°C. The actual temperature of the chambers themselves was about two degrees lower than that of the surrounding bath. The modified Krebs solution (Krebs and Henseleit, 1932) used throughout these experiments was equilibrated with 95% O₂ and 5% CO₂ and had the composition shown in Table 1.

With the original concentration of bicarbonate (27.2 mM) blockage of ganglionic transmission often developed. The pH of the solution was 8.3 to 8.4 before the addition of 5% CO₂, and on vigorous bubbling with 5% CO₂ and 95% O₂ the pH only fell as far as 7.7. At such low hydrogen ion concentration Saunders and Sinclair (1949) showed that transmission through isolated amphibian ganglia failed rapidly. The concentration of
sodium bicarbonate was therefore reduced to 120 mM. Bubbling with 5% CO₂ then gave a pH of 7.2 to 7.3. The addition of glucose at approximately plasma level also proved necessary in order to maintain ganglionic function.

| TABLE I. |
| Composition of modified Krebs solution (mM/l) |

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Drugs and their sources

The following drugs were employed in the study of ganglionic transmission.

**Ganglion-blocking drugs**

- d-tubocurarine (Squibb; Burroughs Wellcome)
- dihydro-β-erythroidine (Merck)
- β-erythroidine (Merck)
- Pentamethonium iodide, C₅, (Eli Lilly and Co., U.S.A.)
- Hexamethonium iodide, C₅, (Eli Lilly and Co., U.S.A.)
- Curarine from Strychnos toxifera, (King, 1949)
- c-toxiferin II chloride (prepared by Wieland, cf. McIntyre, 1947)
- c-dihydro-toxiferin I chloride (prepared by Wieland, cf. McIntyre, 1947)
- Ro 2-4658 (Hoffmann La Roche).

**Anticholinesterases**

- Prostigmine (Hoffmann La Roche)
- Eserine (Merck)
- Nu 2126 (Hoffmann La Roche)
Nu 1250  (Hoffmann La Roche)
TEPP  (Eli Lilly and Co., U.S.A.)
DFP  (Defence Research Laboratories, Melbourne).

Sympathomimetic amines
Nor-adrenaline  (Steames and Co., Australia)
Pholedrine  (Imperial Chemical (Pharmaceuticals) Ltd.)
Methyl amphetamine  (Boots)
Phenylephrine hydrochloride  (Boots)
Veratrine  (Faulding and Co., Australia)
Nicotine  (British Drug House)
III. CLASSIFICATION AND NOMENCLATURE OF POST-GANGLIONIC POTENTIALS

Preganglionic impulses produce a very complex sequence of potentials in the sympathetic ganglion. It is necessary, therefore, to adopt a uniform nomenclature before attempting the present description of investigations. Seven distinct types of electrical response can be listed as follows. The first four are observed only if the ganglion cells discharge impulses.

1) The spike. Normally in the isolated ganglion, or the ganglion in situ, a preganglionic volley leads to the discharge of impulses from ganglion cells giving the characteristic negative spike potentials (Bishop and Heinbecker, 1932; Eccles, 1939; Brown and Feldberg, 1936; Bronk, 1939).

2) Following the negative spike there is often a brief positive wave which may be called the positive component of the spike. This component is observed in cephalopod axons (Hodgkin and Katz, 1949), in depolarized motoneurones (Coombs, Eccles and Fatt, 1954) and in depolarized ganglion cells (Paton and Perry, 1953 and this thesis).

3) Another accompaniment of the spike is the negative afterpotential or the N wave of Eccles (1936c). It is possible to distinguish between the synaptic potential and the negative afterpotential by veratrine which selectively increases and prolongs the negative afterpotential (Lloyd, 1939; Eccles, 1935c, 1944), without any action on the post-synaptic potential. The superposition of the negative afterpotential and the synaptic potential may explain why Eccles (1936a) found the N waves smaller on antidromic activation i.e. when no synaptic potentials were present compared to N waves following orthodromic stimulation (cf. Eccles, 1944).

4) The fourth potential related to the spike potential is a true positive afterpotential which always follows activation of ganglion cells
by antidromic or orthodromic volleys (Eccles, 1935a, 1936a, 1944; Brown and Feldberg, 1936; Bronk, 1939; Lloyd, 1939).

The following potentials are recorded in the absence of any spike discharge from the ganglion cells.

5) When the impulses in the preganglionic terminals are unable to set up a post-synaptic spike, e.g. in the presence of a ganglion blocking drug, a negative potential wave called a synaptic potential or post-synaptic potential can be recorded (Eccles, 1943; Laporte and Lorente de Nó, 1950; R. Eccles, 1952a and b).

6) There is even a positive potential generated in cells that have been partially depolarized by a synaptic potential. This positive potential is observed in the absence of any spike discharge (Eccles, 1943; Laporte and Lorente de Nó, 1950; R. Eccles, 1952b).

7) In certain isolated ganglion preparations a late negative wave has been described following the positive potential (Laporte and Lorente de Nó, 1950). This late negativity has been called an LN wave (R. Eccles, 1952b).

In order to conform to the previous descriptions of ganglionic potentials the N wave will remain as a negative potential apparent at the end of the stimulation. However it is understood that this N wave in uncurarized ganglia is derived from the sum of two negative potentials, the true negative afterpotentials of those cells that generated impulses and the synaptic potentials of all cells. In curarized ganglia the N wave is entirely due to the summation of the synaptic potentials of all cells. Similarly the slow positive potentials following a spike discharge or a synaptic potential will be designated as a P wave. It seems likely that they both arise from the same mechanism as the time courses are similar (cf. Eccles, 1955c and 1943). However the P wave does not include the positive
component of the spike, which has been described above to appear before the negative afterpotential. In the rabbit ganglia, the large spike potential is probably equivalent to the $S_1$ and $S_2$ spikes that are more readily separable in the cat (Bishop and Heinbecker, 1932; Eccles, 1935a). Possibly the fusion in the rabbit is attributable to the absence of postganglionic $S_1$ fibres in the cat's superior cervical ganglion (Ranson and Billingsley, 1918; Bishop and Heinbecker, 1932; Eccles, 1935a). Occasionally a later spike discharge appears with the rabbit ganglia. However this spike discharge requires such a strong preganglionic stimulus and has a longer preganglionic conduction time that it presumably is attributable to the non-medullated preganglionic fibres and so is equivalent to the $S_4$ spike of the cat (Eccles, 1935a). This higher threshold $- S_4$ discharge - is not considered in the responses to be described though it may sometimes appear as a small spike discharge superimposed on the N wave.

**IV A** RESPONSES OF NORMAL GANGLION TO PREGANGLIONIC VOLLEYS

Ganglionic and postganglionic leads ($R_4 R_5$ in Fig. 2)

Just as in the blood-circulated ganglion (Eccles, 1935 a,b, 1943; Bronk et al., 1938), a weak preganglionic stimulus set up a relatively sharp and synchronous spike attributable to the discharge of impulses by the ganglion cells, and later slow negative (N) and positive (P) waves (Fig. 3 a & b). Progressive strengthening of the stimulus caused the spike to increase and a later small and diffuse spike appeared superimposed on the N wave (Fig. 3a iii & 3b iii).

When recording with electrodes $R_3 R_5$, there is no noticeable contribution to the potentials from any preganglionic source, as is revealed by the complete absence of any potential changes before the spike discharge.
In Fig. 4 it is seen that the ganglion in isolation resembles the blood-circulated ganglion in its response to repetitive preganglionic stimulation (cf. Fig. 3, Bronk et al., 1938; Fig. 7, Eccles, 1944; and later in this section). As shown by the maintained ganglionic spike size, the majority of ganglion cells in all of the isolated preparations responded to all frequencies up to 50 per second, and a large number of cells responded even to 125/second. On cessation of stimulation the N wave and later P wave were much larger and more prolonged than after the response to a single preganglionic volley (cf. Bronk et al., 1938; Eccles, 1944). Even with such a critical test as response to high frequency of stimulation, the isolated ganglia have remained virtually unchanged for as long as 24 hours after excision. Deterioration during an experiment of several hours must therefore be negligible.

RECORDING OF THE ACTIVITY OF THE PREGANGLIONIC SPIKES

When the stimulating leads were placed on the preganglionic nerve and the recording leads also on the preganglionic trunk, one just as it enters the ganglion and the other more proximal (R₁R₂ position Fig. 2) only the activity in the preganglionic fibres will be recorded. Fig. 5a shows that the response of the preganglionic fibres to a single volley applied to the stimulating leads consists of a large spike followed by a smaller group of dispersed spikes. The fast group are low threshold fibres, the maximum travelling at a velocity of 20-25 m/sec. The strength of stimulus required to excite them maximally is of the same order of magnitude as that required for a maximum response of the low threshold ganglionic cells. The fibres forming the second group are of high threshold. Their maximum velocity is about 2m/sec.
If the potential recorded in this way is considered to be made up of several components, it would be found that the initial spike (Fig. 5i) is due to activity in the faster group of preganglionic fibres recorded as the impulses pass the first electrode, R₁. The following dispersed waves can be attributed to upward spikes produced by the impulses in the smaller preganglionic nerve fibres and recorded as a negative potential of R₁ and to the downward spikes caused by the invasion into the preganglionic terminals by the earlier preganglionic nerve impulses recorded as a negativity of R₂. These two potentials are recorded in opposite directions, the former upwards, and the latter downwards in the records of Figs. 5i and ii. Since both these spike potentials are usually irregularly dispersed in time, the net effect gives no clear picture of the invasion of the preganglionic terminals. Thus any attempt to gauge a depressant action on the invasion into the preganglionic terminals by drugs will not be very convincing, since the effects, if any, will be slight with the present recording system. It may be possible to record preganglionic activity more precisely with a microelectrode placed outside the ganglion cells close to the preganglionic terminals.

Preganglionic fibres were found capable of following frequencies up to 400/sec. The absence of a large positive afterpotential was confirmed by testing at intervals after a long conditioning tetanus. A potentiation of the preganglionic spike would have been expected during any positive afterpotential that would have followed the tetanus (Lloyd, 1929). Possibly an increased temporal dispersion could account for the slight depression observed in 90% of experiments (Fig. 5ii). Depressed preganglionic excitability can be excluded as an explanation, since the testing stimuli were several times stronger than maximum for the fast preganglionic fibres.
From the previous section it was seen that electrodes in the \( R_1 R_2 \) position (one on the entrance of the preganglionic trunk into the ganglion and the other a centimetre away on the preganglionic trunk) lead off the diphasic potentials which are generated by volley propagation along the preganglionic fibres. It was hoped that shifting of the second electrode \( R_2 \) to position \( R_3 R_4 \) or \( R_5 \), i.e. on the ganglion or postganglionic trunk, would lead off in part the potentials produced by invasion of the preganglionic terminals. Position of the recording electrodes, \( R_1 R_5 \), records identical potentials as when in position \( R_1 R_4 \), since the postganglionic trunk acts merely as an extension to the ganglion from the \( R_5 \) recording electrode.

It would at first appear that this extension of the effective lead from preganglionic fibres would extend right to the preganglionic terminals which would in this way be selectively recorded from with leads \( R_1 R_5 \) or \( R_1 R_4 \). However the ganglion itself has a much larger cross-section (about ten times) than either the pre- or postganglionic trunks, (Bishop, 1936). On account of its lower resistance per unit length, the potentials generated in the ganglion by preganglionic impulsaes will give a much lower density of longitudinal current. Hence, as pointed out by Bishop, the \( R_5 \) lead will in effect record preganglionic potentials as if it were applied at \( R_2 \), rather than those of the actual preganglionic terminals in the ganglion, whose potentials would be attenuated perhaps by a factor of 10 relative to preganglionic potentials at \( R_2 \). Furthermore the ganglionic spike discharge begins so soon after the arrival of the fast preganglionic spike that it obscures most of the diphasic preganglionic spike, which of course would be the component contributed in part by
the preganglionic terminals (Fig. 6). Hence this attempt has failed to give any reliable information on the conduction of impulses in the preganglionic terminals. Attempts were made without success to obtain more discrimination by employing weaker stimuli submaximal for the fast preganglionic spike.

The ganglion responses to preganglionic stimulation in a ganglion in situ (R-R<sub>5</sub> leads)

Figure 7 shows that there were no significant differences in the ganglionic responses to preganglionic stimulation in blood-perfused superior cervical ganglia. The response to a single preganglionic volley (i and ii) consisted of a large spike discharge followed by an N wave. The P wave was about the same size as in isolated ganglia, contrary to the more usual statement that afterpositivities are greater in isolated preparations (Paton, 1954). Increase in the frequency of stimulation from 32/sec produced a larger P wave. Even at 100/sec the majority of ganglion cells were still capable of responding to each preganglionic volley. We therefore can assume that the isolated ganglia investigated in this thesis are functioning under normal physiological conditions. The similarity of response to stimulation has already been shown for ganglia perfused by blood or Ringer-Locke solution (Brown and Feldberg, 1936b). In order to ensure the normal functioning of the isolated ganglia they must be bathed in a solution having ionic concentrations similar to those of plasma and be supplied with glucose (MacIntosh, 1938d; Bronk, 1939) and oxygen (Bronk and Larrabee, 1952).
It was important to investigate the effect of drugs on the preganglionic fibres to ascertain whether there is any action on the preganglionic nerve fibres or terminals. However, as already stated on page 19, recording the activity between the preganglionic nerve and the ganglion can only give information about the number of preganglionic fibres active, but no indication of the number of preganglionic terminals invaded.

The effect of drugs on the preganglionic nerve fibres is illustrated in Fig. 8. The preganglionic response to a single volley applied to the peripheral end of the preganglionic trunk, consisted of a small component, then a large spike which was the main preganglionic fibres setting up the fast ganglionic spike response. Following the spike wave a series of waves - probably the impulses in the very small medullated and non-medullated preganglionic fibres. The first small spike was ignored as it was not the group of fibres responsible for the fast ganglionic, Sa, discharge and possibly is due to the preganglionic fibres for cells whose axons were not included in the postganglionic trunk or fibres not relaying in the ganglion.

Tubocurarine in a concentration of $8.7 \times 10^{-5} \text{M}$ might have altered the later waves following the main spike though this could easily be due to a change in the position of the electrode on the preganglionic trunk, (i). Eserine in a concentration of $4.8 \times 10^{-5} \text{M}$ greatly affects ganglionic transmission and causes a considerable depolarization, yet it produced no noticeable alteration in the preganglionic spike potential (iii) evoked by a maximal stimulus. Strychnine in high concentrations acts like a blocking agent on transmission across ganglionic synapses yet in a concentration of $5 \times 10^{-5} \text{M}$ there was little sign of any effect on
the preganglionic fibres (iv). As will be illustrated later nicotine in concentrations up to $6.2 \times 10^{-5} \text{M}$ acted like acetylcholine, depolarizing the ganglion cells, while at a concentration of $1.4 \times 10^{-5} \text{M}$ there was complete absence of transmission across the synapses both of cat stellate and rabbit superior cervical ganglia. However in a concentration of $2.5 \times 10^{-4} \text{M}$ there was hardly any change in the maximum preganglionic spike potential (cf. v and vi).

The conclusions to be drawn from the above data are that the use of drugs in the concentrations that have been employed have not affected the threshold of the preganglionic fibres, the conduction velocity, or the size of a maximum preganglionic spike potential. However conduction into preganglionic terminals cannot be measured with external electrodes in this manner, so the possibility of drug action on these terminals has not been eliminated by these experiments.
ANALYSIS OF POTENTIALS FROM CURARIZED GANGLIA

There is much experimental evidence that curare blocks ganglionic transmission by depressing the depolarizing action on ganglion cells of preganglionically released acetylcholine (Feldberg and Vartiainen, 1935; Feldberg, 1950; Paton and Perry, 1951). However synaptic transmission in the isolated amphibian ganglion persists in the presence of relatively high concentrations of d-tubocurarine chloride $1.6 \times 10^{-4} \text{M}$, (personal observations) or acetylcholine $5.1 \times 10^{-4} \text{M}$ (Malcolm, 1949), and Laporte and Lorente de Nó (1950) postulated that tubocurarine in a concentration of $1.3 \times 10^{-4} \text{M}$ blocked transmission in the isolated turtle ganglion solely by blocking the propagation of impulses in the preganglionic fibres; however they gave no direct evidence for such an effect. There is now no doubt that curare blocks neuro-muscular transmission both in amphibia and mammals by depressing the depolarizing action of acetylcholine released from nerve terminals (Brown and Feldberg, 1936b; Kuffler, 1943, 1948, 1949; Eccles and Macfarlane, 1949; Feldberg, 1950; Pat and Katz, 1951), but some findings of Nastuk (1950) indicated blockage of motor nerve fibres also. In an earlier section it was shown that a high concentration of tubocurarine $8.7 \times 10^{-5} \text{M}$ had no effect on the spike size of the preganglionic volley, indicating that there was no blockage of the transmission of preganglionic impulses. However there seemed a slight reduction in the diphasic potential, as if there was a reduction in the number of preganglionic terminals invaded by a preganglionic volley; but this evidence is of doubtful significance.

In the following sections the blockage brought about by tubocurarine and dihydro-β-erythroidine as well as other curare-like substances will be described, though a full discussion of the potentials will be left
until later sections when the effects of potassium, anticholinesterases, adrenaline and its analogues and strychnine on the potentials found in ganglia blocked by a curare-like substance will be illustrated.

b. d-Tubocurarine-Chloride

A concentration of $3 \times 10^{-5}$ M tubocurarine blocked the transmission of impulses through the ganglion, but a preganglionic volley still caused the ganglion cells to become negative with respect to the postganglionic trunk, i.e. a synaptic potential was generated (Fig. 9a,ii). This potential, uncomplicated by spikes, was as large as 25% of the original spike height. It has a slower time course than an impulse and decrements steeply along the postganglionic trunk with a space constant of about 1.7 mm, given by Eccles (1943). The time for rise to half was 8-16 msec., the summit occurred at 25-35 msec, and the decay to a half at 80-110 msec from the onset. Following the synaptic potential there was a small after-positivity (Fig. 9b i), which is clearly the P wave previously described (Eccles, 1943). Finally the P wave terminated in a late negativity which attained a value of about 0.2 mV and persisted for about three seconds. Increase in the tubocurarine concentration led to a reduction in the size of the synaptic potential to as low as 1-2% of the control height, whereas the late negativity (LN wave) was not appreciably altered in size or in time course (Fig. 9b ii) until a depression occurred when doses of about ten times blocking concentration were used.

Responses to repetitive preganglionic volleys

Fig. 10. The preganglionic trunk was repetitively stimulated at varying rates of stimulation, the duration of the tetanus being kept constant. Small spikes were superimposed on the second and the third responses but the rest of the responses were relatively pure synaptic
potentials on a plateau negative relative to the baseline. These results are similar to those of Eccles (1943) on the blood-circulated ganglion. At cessation of stimulation there was a rapid decay of the negative plateau and reversal to a P wave. The full growth of this P wave appeared to be checked by the development of the LN wave. This LN wave (usually about 0.2 mV, extreme values from 0.05 to 0.3 mV) has not previously been recorded from mammalian ganglia and there is no sign of it in the records of Eccles (1943) which were not taken at a sufficiently slow speed. The possibility that it was a special feature of the excised rabbit was proved by the inability to record it in the majority of the experiments on the blood-circulated superior cervical ganglion of the rabbit (Fig. 17). Heavy dosage of d-Tubocurarine, however, could not then be employed on account of the circulatory failure that ensued. Iaporte and Lorente de Nó (1950) have observed it in the deeply curarized cervical ganglia of turtles, but gave no data on its size or time course. Observations on the isolated stellate ganglion of the cat showed a complete absence of this late negativity (LN wave). In fact the few experiments done showed that the responses of the isolated cat stellate were the same as reported in the blood-perfused state (Bronk et al., 1938; Bronk, 1939; Eccles, 1943,1944). Neither have these large LN waves been seen in the isolated ganglia of the frog or lizard. Thus rabbit ganglia seem to give responses that differ from those of other ganglia.

One of the first objections that could be raised is that use of platinum wires could lead to artefacts. However, recording with Ag-AgCl electrodes (Fig. 11), the same potentials were observed as thirty minutes earlier with platinum ones. The electrodes were made of silver wire with silver chloride deposited electrolytically. After this the electrodes
were dipped in Agar and cotton wicks were tied to their ends to make contact with the isolated preparation. This precaution was taken to avoid direct contact of the silver with the ganglion which might lead to damage of the peripheral ganglion cells. It should be noted that Larrabee and Posternak (1952) employed platinum electrodes to record the potentials of the excised rat superior cervical ganglion.

Fig. 12 shows the effect of progressively increasing dosage of d-Tubocurarine on the ganglionic afterpotentials following a brief preganglionic tetanus. The continuous line shows the afterpotential in the absence of d-Tubocurarine, there being a P wave and no trace of an LN wave. A d-Tubocurarine solution as low as $1.6 \times 10^{-6}$M significantly changed this afterpotential by causing the rather large P wave to reverse to a small LN wave at one second after the tetanus. Further increase in d-Tubocurarine (to $3.2 \times 10^{-6}$M, $1.6 \times 10^{-5}$M and $3.2 \times 10^{-5}$M) increased the LN wave progressively and caused it to begin earlier and reach an earlier summit. The smaller size of the P wave seems to be attributable to the earlier onset of the LN wave, but the progressive decrease in the synaptic potential (cf. Fig. 9b, i and ii) presumably also entailed a diminution of the P wave.

The relative contributions of the low threshold and higher threshold or Sb ganglion cells to the LN wave was determined by plotting the size of synaptic potential, i.e. the response to a single volley, against the size of the LN wave produced by a repetitive stimulus. Fig. 13 shows that the fast preganglionic fibres have a greater effect than the slower fibres on the mechanism which generates LN waves, since a very weak preganglionic stimulus giving a synaptic potential 10% of the maximum, possible will lead to the production of an LN wave nearly 50% of the maximum.
In order to complete the effects of tubocurarine a further action should be noted which is illustrated in a later section, that on the effect of proctigine on these potentials. In Fig. 22, it is seen that an increase in the d-Tubocurarine concentration from $1.6 \times 10^{-5}$ to $8 \times 10^{-5}$ M caused the initial negativity to reverse to a large positivity during the tetanus, an effect which was not at all apparent with a just blocking concentration of d-Tubocurarine. A similar effect has been reported for d-Tubocurarine with turtle ganglia by Laporte and Lorente de Nó (1950). This positivity was later diminished by the development of the late negativity. At the end of the stimulation this LN wave developed rapidly and reached its summit $1.3$ sec after the end of the tetanus. From this summit it decayed slowly, its total duration being usually about $5.5$ sec (extremes from $5$ to $7$ sec). It may be noted in passing, that these potential changes were reversible. Soaking out in Krebs solution caused almost complete recovery to the original controls.

6. A curarine extracted from the bark of Strychnos toxifera (King)

In Fig. 14 the response to a single preganglionic volley is seen at different levels of curarization. In (i) the curarization was inadequate to give complete blockage. A just-blocking dose ($1.5 \times 10^{-4}$ M) produced a synaptic potential (ii), which was reduced (iii) by further increase in concentration to $3 \times 10^{-4}$ M.

In Fig. 14 (iv), though the response to the first volley of a tetanus was a synaptic potential, e.g. Fig. 14, ii, the responses to all following volleys were spike discharges. This repetitive discharge from the ganglion cells may probably be accounted for by the fact that there was a much more sustained negative plateau than with tubocurarine. Possibly this high plateau may be attributable to the small size of the P
wave, for the after-positivity was very small after the tetanus. Increasing the dose to $3 \times 10^{-4}$ M and increasing the frequency led to a higher voltage for the sustained negativity during the tetanus (v), which subsequently declined towards the baseline, but the late negativity supervened before any after-positivity occurred. Further increase to $4 \times 10^{-4}$ M merely caused a depression of all potentials during and after the tetanus (vi).

This curare thus produced ganglionic blockage in the same manner as tubocurarine, i.e. by reducing the effective depolarizing action of acetylcholine so that the acetylcholine released from the preganglionic terminals by a single volley was only able to set up a partial depolarization of the post-synaptic membrane. The principal difference seemed to be attributable to the smaller size of the P wave, and the consequent predominance of the IN wave both during and after the tetanus.

C. C-toxiferin II chloride (Wieland)

This was the least potent of all the ganglion-blockers. Even at the highest concentration reached ($6.45 \times 10^{-4}$ M) there was incomplete blockage of synaptic transmission (Fig. 15, i and ii). On tetanization of the preganglionic trunk there were spike discharges initially from a ganglion soaked in $3.25 \times 10^{-4}$ M, though these soon declined to synaptic potentials superimposed on a negative plateau (Fig. 15, iii). The sequence at the end of the stimulation resembled that of some other curares, e.g. the curarine from Strychnos toxifera. The negative plateau decayed towards the baseline but reversal by the late negativity prevented an actual positivity from developing. An increase in the concentration to $6.45 \times 10^{-4}$ M produced an increase in both the negativity during the tetanus and the subsequent IN wave. This increased negativity coupled with a decrease in the small positively directed dip after the tetanus.
indicate that further increase in concentration of this curare to the blocking dose would not have changed the response to the prominent positivity that characteristically obtains with high concentration of tubocurarine. Thus this curare resembles the curarine from Strychnos toxifera and pentamethonium iodide rather than tubocurarine.

D. Dihydro-β-erythroidine

Dihydro-β-erythroidine's usefulness as a ganglion blocking agent was based on the discovery that ganglia soaked in concentrations of this drug showed very large LN waves and fairly well developed P waves. This coupled with the fact that ganglia completely recovered from high concentrations within an hour makes it a more useful ganglion blocking agent than Tubocurarine. In a just-blocking concentration of dihydro-β-erythroidine ganglionic transmission is blocked, but a presynaptic volley still sets up a synaptic potential (Fig. 16a and b i), followed by a P and LN wave (Fig. 16b ii and c i). Repetitive stimulation produces a series of synaptic potentials which sum to a plateau during the tetanus. Following the end of the tetanus is a P wave which is unable to reach any size before it is submerged by the large LN wave, seen in Fig. 16c. Compared to Tubocurarine there is not a large development of the LN wave after the contributions of the first few preganglionic impulses. This is clearly seen in the Figure 16c, ii and iv, where there is little difference in size between the LN wave following the tetani with durations of 0.5 secs and 5.0 sec (cf. Fig. 21). In this respect dihydro-β-erythroidine resembles di-hydro toxiferin I since they both lead to the appearance of a large dominant LN wave preventing any development of a P wave. In the following sections dihydro-β-erythroidine was used as a ganglion blocking agent in preference to Tubocurarine as the large LN waves permit the use of a very low amplification and drift can be re-
duced. Tubocurarine and erythroidine were employed on those occasions when alternative ganglion blocking agents were required to show that the reactions or lack of them, were not a special property of dihydro-β-erythroidine but were characteristic of all ganglion blockers.

**Dihydro-β-erythroidine on blood-circulated ganglia**

Injection of 1 mg/Kg into a rabbit greatly reduced the response to a single preganglionic volley (Fig. 17 ii). Only a few cells were sufficiently depolarized to generate an impulse (cf. the spike response of Fig. 17 i and ii). On repetitive stimulation (iii) all responses following the second were greatly depressed. When the afterpotentials were recorded, it was observed that the P wave following the termination of the tetanus, did not differ in duration from the P wave of the control though it was reduced to 50% in size (Fig. 17 iv). In this experiment it was observed repeatedly that little or no LN wave followed the P wave, as is typically shown in Fig. 17, iv. However a very small LN wave has been observed with blood circulated curarized ganglion (cf. R. Eccles, 1952b). The only significant difference between circulated and isolated ganglion was the appearance of the LN wave. Since the LN wave is such a slow prolonged response the most probable explanation is that the transmitter substance causing the LN wave diffuses out into the bloodstream so fast in the circulated ganglion that it is unable to produce an LN wave. It is interesting that the only occasion that an LN wave was recorded in a circulated ganglion occurred when the circulation had been greatly depressed by the ganglionic-blocking action of the drug, the blood pressure having fallen to a very low level.

**E. Hexamethonium iodide (C₆) and Pentamethonium iodide (C₅)**

These drugs have been widely accepted in clinical practice as the most satisfactory drugs for relief of high blood pressure. It is
believed that they act by blocking the sympathetic outflow to the blood vessels (Grob and Harvey, 1950). Rabbits have been found to be very insensitive to pentamethonium or hexamethonium and an injection of 20-25 mgms in a one and a half to two kilo animal has been required before blockage of transmission occurred (Fig. 18). In blood-circulated ganglia C6 first blocked the ability of ganglion cells to respond to repetitive stimuli before it produced a great reduction in the response to a single preganglionic volley (Fig. 18).

In the isolated ganglia a concentration of $2.1 \times 10^{-4} \text{M}$ C$_6$ failed to prevent all spike discharges in response to a single preganglionic volley (Fig. 19 i). On repetitive stimulation there was a facilitation of the second response but the following responses were nearly pure synaptic potentials (ii). These synaptic potentials built up to a negative plateau during a tetanus wave, which was followed by typical P and LN waves such as were seen after any curarizing agent at its just-blocking concentration (iii). Hexamethonium was of the same order of potency as pentamethonium, a concentration of $1.04 \times 10^{-4} \text{M}$ being required for nearly complete block, Table 4.

Fig. 20 shows that the frog superior cervical ganglion was even less sensitive than the rabbit as a concentration of $2.1 \times 10^{-4} \text{M}$ C$_6$ has only produced a 50% reduction in the first action potential with a slight reduction of the later ganglionic discharges.

The actions of pentamethonium and hexamethonium are then similar to other ganglion blocking agents as Paton and Perry (1953) have already reported.

F. Discussion

The electrical response of the isolated ganglion to a single preganglionic volley can be analysed into at least three constituents
which appear in overlapping sequence (Fig. 9b, Fig. 16 a and b): a brief initial negative wave (the N wave), of a true synaptic potential; a positive potential (P wave); and a late negativity (LN wave). No trace of the LN wave has been observed in the uncurarized ganglion, and the P wave then has a more prolonged time course, which suggests that in the curarized ganglion the P wave is terminated by the concurrent development of the LN wave, as is shown in the diagram (Fig. 12). Since the duration of the LN wave is about 3 seconds, it may be assumed that its later part is uncomplicated by superposition of the P wave.

The interpretation of the potentials recorded during and after repetitive stimulation will be based on the hypothesis that the recorded response is a combination of the three potentials, N, P and LN. Some indication of the manner in which they are combined is given by recording tetani with a wide range of durations, as is shown in the superimposed tracings of Fig. 21. The initial negativity and the sudden decay of negativity (or increase in positivity) at the end of the tetanus (cf. Figs. 15 and 16 c) are both indications of the size of the N wave, which has a sudden onset and a relatively quick decay. As identified in this way, the N wave was reduced by tubocurarine and summed very little during a tetanus (cf. Figs. 10, 14, 19) and not at all cumulatively after the first few impulses. It is not so easy to distinguish between the P and the LN waves, as these both run very slow time courses. However, the P wave will initially be dominant because its onset is earlier and its increase more rapid than those of LN. Thus the initial negativity built up by N wave summation is rapidly reversed by the cumulative P effect and this positivity in turn is deflected towaed negativity by the later developing LN wave. With prolonged tetani an approximate balance of the P and LN waves is often struck (cf. Figs. 16c and 21).
At the end of the tetanus the relatively quick decay of the N wave leaves a potential determined by the relative effects of the opposed P and LN waves. The P wave then decays more rapidly, hence LN progressively dominates, the large post-tetanic LN wave being so produced. It was thought that since the positive afterpotential, P wave, in uncurarized ganglia had a short duration it could be inferred that there would be no residuum of a P wave by the time the LN wave reaches its summit, the subsequent course being attributable to the LN wave alone.

The appearance of the LN wave only in poorly circulated or isolated ganglia at least indicates the need of a slow rate of diffusion for its production. The possibility arises that the LN wave is due to some deterioration of these ganglia. Since the ganglionic responses were similar to those of curarized blood-perfused ganglia without any signs of attendant depolarization or hyperpolarization, and also since these ganglia recovered after soaking in Krebs solution, it is improbable that "deterioration" is an important factor in LN wave production. The introduction of a transmitter substance necessitates a further explanation of the absence of LN waves except in the presence of ganglion-blocking drugs.

According to Laporte and Lorente de Nó (1950) "the positive phase of the post-synaptic potential of deeply curarized ganglion may be interpreted as an anelectrotonus impressed on the ganglion cells by the presynaptic impulses" and the late negativity (LN wave, R. Eccles, 1952) is the "analogue of the post-anodal overshooting of the electrotonus". They further suggest that tubocurarine acts by depressing and blocking the propagation of impulses in the preganglionic terminals. A possible explanation of the production of anelectrotonus by preganglionic impulses could be that, if blockage occurred in the presynaptic fibres close to
the synapses, then an anelectrotonus would be generated under the synaptic knobs by the current that there flowed into the ganglion cells; but there would be a compensating diffuse catelectrotonus where this current flowed out across the post-synaptic membrane. It is thus difficult to see how a net balance of anelectrotonus could be produced, such as would be required to give the observed P wave with high tubocurarine dosage (cf. Fig. 12). Also the fields of current generated under these conditions would be very weak in density at the postganglionic membrane and it is very unlikely that the large positive potential observed could be due to such an anelectrotonus. The present experiments cannot exclude such a blocking action by tubocurarine, though it is important to realize that there is no experimental evidence for such an effect at the low concentration at which tubocurarine blocks synaptic transmission in mammalian sympathetic ganglia. On the other hand there is the impressive array of experimental evidence indicating that acetylcholine is the transmitter at the ganglionic synapse, (Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934; MacIntosh, 1938; Feldberg, 1950), and that curare blocks transmission by competitive action on "receptors" on the postganglionic membrane just as occurs in neuro-muscular transmission (Brown and Feldberg, 1936; Acheson, 1948). This effect seems adequate to explain the action of low concentration of tubocurarine without the postulated blockage of impulses in the preganglionic terminals.

As described in the later sections, the effects of different substances on these potentials were investigated in an attempt to determine the manner in which they are produced. Anti-cholinesterasees are of importance since acetylcholine presumably plays some part in producing these potentials (Eccles, 1943, 1944; Feldberg, 1950). There is also evidence that adrenaline may play a part in ganglionic transmission.
Potassium is also probably related to these potentials, for it is liberated on excitation of ganglia (Vogt, 1936).

Finally, strychnine, which possesses an ability to block the receptors for the inhibitory substance on motoneurones (Bradley, Easton and Eccles, 1953), was employed in the hope that perhaps there might be several transmitter mechanisms operating in deeply curarized ganglia, one of which might have been strychnine-sensitive.
VII. THE EFFECT OF DRUGS ON CURARIZED GANGLIA

In the preceding sections the potentials which were produced by repetitive stimulation of the preganglionic nerve of curarized ganglia have been described. It has been suggested that there are three constituents of the complex potential form a discussion of these three potentials were superimposed on each other forming a very complex pattern of negativity, positivity, and a later negativity. However, according to the blocking agent used, the recorded potential altered, e.g. in the presence of dihydro-β-erythroidine, the N and LN waves dominated the picture so that the P wave appeared merely as a transient depression of the dominant negativity. In an attempt to analyse the mechanisms responsible for the potentials, isolated ganglia were curarized and were subjected to the action of various other substances. The usual blocking drugs were Tubocurarine and dihydro-β-erythroidine, which were available in large supply and which in high concentrations led to the production of large P and LN waves.

A. The effect of anticholinesterases in curarized ganglia

I. In deeply curarized ganglia

The response to a single volley. After soaking in Tubocurarine (8 X 10⁻⁵ M) a single preganglionic volley evoked a small negative synaptic potential followed by P and LN waves (Fig. 9b ii). After the addition of prostigmine (3 X 10⁻⁶ M) there appeared to be a great increase in the P wave (Fig. 9b iii), while only a small late component of the LN wave survived. The whole change in wave form might be attributed simply to a greatly increased P wave, but it seems more probable that a reduction of the LN wave disclosed the large P wave that had previously been submerged beneath it.

The response to repetitive stimulation. In Fig. 22b i the lowest concentrations of prostigmine (3 X 10⁻⁷ M) caused a large increase in
the size of the positivity during stimulation. Subsequent stimulation to the reversal to the LN wave was greatly delayed and it was reduced in size. Tenfold increase in concentration (3 x 10^{-6} M) resulted in further increase in the positivity during and after the stimulation and even later reversal to the small residuum of LN wave (Fig. 22b ii). In Fig. 22b (iii), 6 x 10^{-6} M prostigmine had caused a slight depression of the positivity during the period of tetanic stimulation and a complete suppression of the LN wave. This depression of the P wave was even more pronounced on higher dosage of prostigmine (3 x 10^{-5} M) (Fig. 22b iv). The depression of the positivity may be due to blockage in the fine pre-ganglionic endings, which have been found to be affected by prostigmine at concentrations of 6.6 x 10^{-5} M, but not by the lower doses employed in Fig. 22b (ii) (see Section V), or alternatively it could be due to a blocking action of eserine on the post-synaptic membrane. Virtually identical results have been obtained in all experiments with prostigmine.

It seems unlikely that this is a special action of prostigmine. All anticholinesterases tested (D.F.P., prostigmine, eserine, TEPP and Nu 2126) have given identical results in the presence of high curare concentrations (Figs. 32 and 33). The large LN wave has disappeared and all that is left of the potentials following a tetanus is the long P wave. This increase in P wave seems to be due to the anticholinesterase action of these drugs since it has been shown that curare does not modify the anticholinesterase action of eserine (Eccles, Katz and Kuffler, 1942). Nor is there any signs of ganglion-blocking action at these low concentrations of anticholinesterase (see Section IX and also Eccles, 1944; Paton and Perry, 1953).

Since this increase in P wave by an anticholinesterase has been shown to be independent of the anticholinesterase used, it seemed
important to show that any curarized agent behaves in a manner comparable
to that of tubocurarine. In following sections the effects of other
curarizing agents will be briefly described to illustrate the fact that
this disappearance of LN and associated increase in P wave is not a
special action of Tubocurarine on the addition of anticholinesterase.

c-dihydro-toxiferin I chloride (Wieland)

Synaptic potentials up to 28% of the spike height in the uncurarized
ganglia have been obtained after soaking in a just-blocking dose
(3.5 X 10^-5 M). Subsequent to the synaptic potential there was a small
P wave and then an LN wave (Fig. 23a i). The P wave was regularly ob-
served to be smaller than with a comparable dose of tubocurarine. At a
concentration of 7 X 10^-5 M there was a large diminution of the synaptic
potential to only 4% of the initial spike height, while the LN wave was
only halved (Fig. 23a ii).

With repetitive stimulation, after soaking in a just-blocking dose
(3.5 X 10^-5 M) (Fig. 23b i), the synaptic potentials summed to reach a
negative plateau. At the end of stimulation there was a small decay of
the negative plateau and then the LN wave supervened, there being little
sign of any P wave which was only a small dip in a large negative wave.
An increase to 7 X 10^-5 M concentration (ii) led to the development of a
large negativity even during the tetanus. The slight decay of the nega-
tivity observed just after the commencement of the stimulation was probably
due to the positive after-potential of a few cells that fired impulses.
Following this slight reduction the negativity continued to build up, but
this increase to a negative plateau ended abruptly at the cessation of
stimulation. The LN wave was again apparent after a small brief decline
which was probably attributable to the decay of the N wave, and to the
appearance of a small P wave.
Addition of prostigmine \((3 \times 10^{-6} \text{ M})\) to the solution of toxiferin \((7 \times 10^{-5} \text{ M})\) caused a positivity to develop during the tetanus (Fig. 23b ii). At the end of the period of stimulation there was an initial increase in the positivity and this then slowly decayed to return to the original baseline i.e. a large \(P\) wave had appeared and the \(LN\) wave had been completely suppressed.

In contrast to Tubocurarine this curare did not produce a large positivity during the tetanus; however a large \(P\) wave appeared on the addition of prostigmine. Addition of an anticholinesterase, prostigmine, has had the same effect when the ganglion-blocking drug was c-dihydrotoxiferin I or tubocurarine.

**Dihydro-B-erythroidine**

Even in very high concentrations (Fig. 16c), dihydro-B-erythroidine did not cause the appearance of a large positivity, \(P\) wave, to develop during a tetanus like tubocurarine (Fig. 22a iii); nor did it resemble c-dihydrotoxiferin I, since it produced a large \(P\) wave after the period of stimulation. Addition of an anticholinesterase to ganglia deeply curarized by dihydro-B-erythroidine produced the usual potential alterations, i.e. a large increase in \(P\) wave completely obliterating all signs of the \(LN\) wave and reducing the size and duration of the \(N\) wave (Fig. 24, also Figs. 32, 33). An increase in the dihydro-B-erythroidine concentration from \(4.3 \times 10^{-5} \text{ M}\) to \(1 \times 10^{-4} \text{ M}\) reduced the size of the \(P\) wave and slightly shortened its time of decay (Fig. 24).

From these experiments, rabbit ganglia deeply curarized by a blocking drug, the three components of the recorded potentials, \(N\), \(P\) and \(LN\) waves, are observed to be altered by the addition of anticholinesterases. The \(N\) wave is reduced in size and its time course is shortened with a concomitant earlier appearance of the \(P\) wave. This increase in size and
duration of $P$ wave prevents the development of the $IN$ wave.

II. Responses of ganglia soaked in a just-blocking concentration of a ganglion-blocking drug.

In the above section the effect of anticholinesterases were shown when ganglia were deeply curarized so that there were no complications due to the superposition of spike discharges. In a just-blocking concentration (Fig. 25) there was always facilitation of the second synaptic potential leading to the generation of impulses in a few cells.

After soaking in a erythroidine $4 \times 10^{-5}$ M a single preganglionic volley set up a large synaptic potential with a small spike discharge superimposed. Fig. 25 illustrates the large negative plateau - $N$ wave - which developed during a tetanus. A large positive wave appeared at the end of the stimulation, which would probably be compounded of several components, the positive component of the spike, a true positive after-potential arising in the many cells which discharged impulses, and a $P$ wave arising in the cells that failed to discharge impulses. An $IN$ wave appeared about two seconds after cessation of the stimulation. Addition of $3 \times 10^{-5}$ M Prostigmine produced a slight increase in the size of the composite wave, and at the same time the $IN$ wave disappeared. The quicker return to the resting level was probably brought about by the cumulative action of acetylcholine molecules, liberated by activity in the preganglionic terminals and still unhydrolysed. Higher concentrations of Prostigmine ($3 \times 10^{-5}$ M) reduced the size of the composite positive wave and further decreased its duration.

C. The Effect of Nicotine

Nicotine, like acetylcholine, depolarizes the post-synaptic membrane (Lundberg, 1953; Paton and Perry, 1953). Depolarization by acetylcholine
at the neuro-muscular junction is due to a great increase in permeability of the membrane, i.e. to an increased ionic conductance (Fatt and Katz, 1951). It would be expected therefore that the time constant of the endplate membrane would consequently be shortened. This has not been experimentally tested for Ach, because it is destroyed by the local concentration of cholinesterase. However nicotine also acts in the same way as a depolarizing agent at the neuromuscular junction and the time constant of decay of the epp shows the expected shortening (Eccles, personal observations).

In Fig. 26, 6 X 10^{-7}M nicotine slightly reduced the synaptic potential whose rate of rise was not altered (cf. Fig. 26, a, i and ii). However the time of decay was shortened from 98 msec to 55 msec for decay to 1/e. Increase in the nicotine concentration to 6 X 10^{-6}M led to the production of spike discharges in response to preganglionic stimulation (iii). Presumably the ganglionic membrane was depolarized to such a degree that a very small depolarization now caused the generation of impulses. Spike discharge was suppressed by doubling the concentration of dihydro-6-erythroidine (iv). The time course of the synaptic potential has been greatly shortened (time for decay 1/e, 20 msec) (cf. i and iv). From another ganglion this reduction of the duration of the synaptic potential was recorded on progressively increasing the concentration of nicotine (Fig. 26b). It should be noted that an increase in the con-
centration of a ganglion-blocking drug does not alter the time course of the synaptic potential only its size (cf. Eccles, 1943). Nicotine in the concentrations of $3 \times 10^{-6}$ M and $6 \times 10^{-6}$ M produced a reduction in time to decay to $1/e$ from 80 msec (control size) to 43 msec and 19 msec respectively. Fig. 26c illustrates the reversibility of the nicotine action, since a complete recovery to control size occurred after soaking in ganglion-blocking agent alone (cf. i and ii). Following this recovery, addition of eserine, $2.5 \times 10^{-6}$ M, did not alter the time course or size of the synaptic potential (cf. Eccles, 1944).

In Fig. 27, $6 \times 10^{-6}$ M nicotine has greatly reduced the N, P and LN waves, yet the form of these potentials was virtually unaltered. After recovery (iv) it was observed that eserine ($2.5 \times 10^{-6}$ M) produced the typical anticholinesterase effect: the large increase in the P wave with disappearance of the LN wave (v).

The effects produced by nicotine can all be attributed to an increased ionic conductance of the post-synaptic membrane. This would cause both the shortened time constant of the membrane and its depolarization. The depolarization would account also for the initial stimulating action of nicotine on ganglion cells and their eventual depression, i.e. the nicotine effect resembles $C_{10}$ at the endplate. Furthermore the depolarization and shortened time constant can be shown to account for the various other effects produced by nicotine (cf. Section X).

C. The effect of potassium ions

Potassium ions are known to have a non-specific effect on the post-synaptic membrane in sympathetic ganglia. Increase in the external potassium concentration led to an increase in the response to perfused Ach (Feldberg and Vartiainen, 1954b, and Bronk, 1939) and also an increase in sensitivity to preganglionic stimulation (Brown and Feldberg, 1936b).
In heavy doses, however, potassium liberated Ach from the preganglionic terminals and the ganglion cells initially responded with a rapid asynchronous and spontaneous discharge (Feldberg and Väisänen, 1934; Brown and Feldberg, 1935, 1936b) before there appeared a heavy depression of the responses to preganglionic stimulation or perfused Ach (Brown and Feldberg, 1936b, c and d). Low potassium in the perfusate reduced the ganglionic response to perfused Ach (Bronk, 1939).

In this section the effects of potassium were investigated on the ganglionic responses to stimulation of curarized ganglia whereas in Section VIII, the responses of uncurarized ganglia to external potassium alterations will be described.

Decrease in potassium concentration in the external medium. In Fig. 28 the effects of a long tetanus (about 0.9 sec) are compared with those of brief tetanus (about 0.1 sec) at different concentrations of potassium. At the normal level (6.2 mM) the long and short tetani were followed by P and LN waves (i), which had the size and time relationship characteristic of ganglia soaking in that concentration of tubocurarine (8 x 10^{-5} M). Reduction of the potassium to 3.6 mM (ii) caused an increase in the P wave and a very slight diminution in the absolute height of the LN wave. Both these effects were accentuated by a further fall in potassium content to 2.4 mM (iii), the LN wave being now greatly reduced. Finally the potassium content was diminished to 1.2 mM. After soaking for ten minutes the P wave was approximately halved, while the LN wave was still further reduced (iv). After one and a half hours further soaking in this solution there was almost complete suppression of the after-positivity though the LN phase showed no further change (v). After returning to 6.2 mM potassium solution the responses (vi) one hour later had almost completely recovered to normal. Thus the effect of a decreased
potassium concentration was initially a slight decrease of the LN wave, while with a large reduction in potassium the LN wave appeared to be a quarter of its original size (Fig. 28 i). The P wave was initially increased but later decreased probably due to preganglionic blockage.

Increase in potassium concentration. Fig. 29 shows the response to external repetitive stimulation on increasing the potassium. The continuous line gives the control which followed the normal sequence of events as recorded in the normal potassium concentration (6.2 mM) in the presence of a high dose of tubocurarine (8 X 10^{-5} M). Soaking for 30 minutes in 11.8 mM potassium led to a reduction both of the positivity which developed during the tetanus and of the LN wave, which also had a decreased rate of rise and decay. A further increase in the potassium content (to 16 mM) caused little change in the positivity during the tetanus, but further reduced the LN wave. On returning to 6.2 mM potassium for 60 min. the response could virtually be superimposed on the initial control. Thus the effect of raising the potassium would appear to be a diminution of both the P and LN waves, the effect on the LN wave being slight compared to the 50% decrease in P wave. More prolonged soaking in 16 mM caused progressive depression of the whole response, an effect presumably attributable to preganglionic blockage.

D. The effect of adrenaline and its analogues on the after-potentials

The effect of addition of various adrenaline analogues on curarized ganglia were investigated to find if such drugs could alter the size and time course of these late P and LN waves. Among the possibilities for the mechanisms whereby these P and LN might be produced was the one that perhaps adrenaline or an adrenaline-like substance, liberated from the preganglionic terminals in response to stimulation, produced a hyperpolarization (P wave) or depolarization (LN wave) of the post-
synaptic membrane. There are several references in the literature to the presence of amine oxidase and adrenaline or nor-adrenaline in sympathetic ganglia (Lissak, 1939; Raab and Humphreys, 1947). The addition of low concentrations of adrenaline produced a facilitation of the ganglionic response to ganglionic stimulation (Bulbring and Burn, 1942b; Bulbring, 1944). However adrenaline and other sympathomimetic amines were stated to possess only an inhibitory action on synaptic transmission (Marrazzi, 1939; Marrazzi and Marrazzi, 1947). This inhibitory action was observed by Bulbring (1944) as occurring only in high concentrations of adrenaline. The effect of sympathomimetic amines were tried on P and LN waves to see whether they would indicate any adrenaline mechanism responsible for the generation of P and LN waves.

In Fig. 30, nor-adrenaline in a concentration of $6 \times 10^{-5}$ M has slightly reduced the size of the P wave. There was no alteration in the size of LN wave and the increase in duration appearing two seconds after the peak is not likely to be significant. The response to a lower concentration of nor-adrenaline is not illustrated as it was exactly superimposable on the response following a period of soaking in nor-adrenaline. Thus $6 \times 10^{-6}$ M, nor-adrenaline had no effect on the P and LN waves whether it was present in a low concentration or in a concentration ten times greater.

In the figure following (Fig. 31) it can be seen that an hour's soaking in phenylephrine hydrochloride ($2 \times 10^{-4}$ M) has left the LN wave virtually unaltered but a very small decrease in the P wave was observed. An increase in the phenylephrine concentration to $4.6 \times 10^{-4}$ M produced a 20\% reduction in the size of the LN wave without any alteration of the P wave. This reduction may be due to a blocking action of the phenylephrine.
on the preganglionic terminals, rather than to an increase in P wave or inhibition of an LN mechanism, since it is known that in the uncurarized ganglion there is a 40-45% reduction in spike response size when phenylephrine is present in a concentration of $3.2 \times 10^{-4} \text{M}$. This postulate of preganglionic blockage is strengthened by the observation that there is no concomitant increase in size or time course of the P wave or decrease in the time course of the LN wave.

From this series of experiments, then, there is no evidence of the facilitatory action of adrenaline (Bulbring, 1944) or the inhibitory action as described by Marrazzi and Marrazzi (1947). Since none of the sympathomimetic amines affected the P and LN waves it seems very unlikely that adrenaline or a related substance could be the transmitter responsible for production of the LN or P waves.

**The effect of strychnine**

In the spinal cord, Bradley and Eccles (1953) and Bradley, Easton and Eccles (1953) have shown that strychnine depresses the direct inhibitory on motoneurones. It could do this by competing with the inhibitory terminal endings, by competing with the inhibitory transmitter for the same receptors on the post-synaptic membrane, or by preventing the outward ionic flux of cations i.e. the hyperpolarization which was produced by the inhibitory transmitter (Brock, Coombs and Eccles, 1952). However later work (Eccles, Fatt and Koketsu, 1954) proved that strychnine competes for the same receptors on the post-synaptic membrane as strychnine in the same way that curare reduces the endplate sensitivity to Ach (Brown, Dale and Feldberg, 1936; Acheson, 1948). In the isolated sympathetic ganglia a hyperpolarization, the P wave, was observed in deeply curarized ganglia when there were no spikes generated by the ganglion cells and it could not be a true positive after-potential.
Furthermore under such conditions only a very small N wave preceded the P wave. Possibly, therefore, the P wave is directly produced by a substance released from the preganglionic terminals just as occurs with inhibitory action in the spinal cord. This suggestion could be directly tested by investigation of the effect of addition of strychnine.

In Fig. 32, addition of $1 \times 10^{-5}$M strychnine produced a slight reduction in the size of the LN wave. The times of rise to the peak, and decay are both slightly increased. Even a concentration of $2.5 \times 10^{-5}$M strychnine has produced only a slight reduction of the LN wave. The time of rise is identical with the LN wave observed before addition of strychnine. The early negative potentials during the tetanus - N waves - are identical both before and after strychnine. The usual large P wave is obtained on addition of an anticholinesterase, DFP. The N wave was greatly decreased in size and duration by $5 \times 10^{-6}$M DFP. The only trace of this in the records, is the more positive potential apparent at the end of the tetanus.

Fig. 33 illustrates that strychnine has little effect on the P wave. Incidentally it may be noted that an increase in the dihydro-β-erythroidine concentration from $1.7 \times 10^{-4}$M to $3 \times 10^{-4}$M produced a reduction in the P wave with little alteration in the size of the LN wave. Addition of eserine produced a rapid decay of the N wave so that a large positivity develops even during the tetanus. $2.4 \times 10^{-5}$M eserine led to such a large potential alteration that, at the end of the stimulation, the P wave appeared at $370 \mu V$ in the downward or positive direction instead of at $18 \mu V$ in the upward or negative direction. $1 \times 10^{-5}$M strychnine has produced a slight increase in the P wave though the rates of development and decay of this hyperpolarization remained unaltered. Further increase in the strychnine concentration to $2.5 \times 10^{-5}$M produced a slight depress-
ion of the P wave relative to the response recorded before the addition of strychnine. These two figures are typical of several experiments. After careful measurements it was felt that the small changes in the time course or size of the LN and P wave cannot be considered significant. The only conclusion is that these results do not rule out the possibility of an inhibitory transmitter, but make it seem very unlikely that it is the same inhibitory transmitter as in the spinal cord (Bradley, Easton and Eccles, 1953; Eccles, Patt and Koketsu, 1954).

The effect of veratrine

Since veratrine greatly prolongs the negative afterpotential of a nerve, which was first shown by Graham and Gasser (1931), and this prolonged negativity has been recorded in all nerves studied (Cowan, 1937; Grundfest and Gasser, 1936, 1938). Similarly application of veratrine to single muscle fibres of the frog led to the appearance of a prolonged negative afterpotential (Kuffler, 1945). It has been shown that veratrine has no effect on the end plate potential (epp) of normal and curarized amphibian muscle in concentrations which greatly prolonged the negative afterpotential (Eccles et al., 1942; Kuffler, 1945).

From Fig. 34a it can be seen that the addition of $1.3 \times 10^{-6}$ M veratrine has increased the time constant from 70 to 85 msec for decay to $1/e$. There is a slight but not significant increase in the time constants on addition of veratrine. In the series partly illustrated the time constants were 76, 85, 85 msec for concentrations of $1.3 \times 10^{-7}$ M, control $7 \times 10^{-7}$ M and $1.3 \times 10^{-6}$ M veratrine respectively, while the time constant equalled 70 msec. In Fig. 34b it can be seen that veratrine has had no effect on the N, P and LN waves. The ganglionic response to preganglionic stimulation is unaltered by a concentration of $1.3 \times 10^{-6}$ M veratrine (cf. i and iii).
It can be concluded that veratrine in concentrations that greatly altered the negative afterpotential of uncurarized ganglia (Section XII) had no effect on the ganglionic potentials of deeply curarized ganglia following preganglionic stimulation. This confirms an earlier report of unpublished observations (Eccles, 1944) on the negative effects following the application of veratrine to curarized ganglia.
DISCUSSION

The synaptic potential

When transmission through a ganglion is blocked by any of the ganglion-blocking agents, a single volley in the preganglionic fibres causes a depolarization of the post-synaptic membrane. This depolarization or synaptic potential may be considered analogous to the endplate potential (epp) set up by motor nerve impulses in curarized muscle, and presumably is likewise composed of small heavily depolarized areas on the post-synaptic membrane with catelectrotonic surrounds (cf. Eccles, Katz and Kuffler, 1942; Fatt and Katz, 1951). At the critical level for ganglionic blockage by any of these agents there are no significant differences in the heights of the synaptic potentials or in their time courses. Thus the ganglion-blocking agents would appear to act by depressing the depolarizing action of the preganglionic impulses and not appreciably by depressing the generation of impulses by the depolarized post-synaptic membrane. In this respect also the ganglion is analogous to the neuro-muscular junction (Kuffler, 1942, 1943, 1945).

A comparison of the synaptic potentials from the superior cervical ganglion and the stellate ganglion (see Table 2) shows that the time course is much slower in all respects in the isolated preparations. Two factors contribute to this temporal discrepancy. In the rabbit's superior cervical ganglion there is an especially large group of slow fibres (the Sb group) which would add their contribution to the synaptic potential later than the Sa complex (Eccles, R.M., 1952a), thus delaying the time to summit as well as the rate of decay.

The size of the synaptic potential relative to the spike height generated by the unblocked ganglion is found to be far higher in the isolated superior cervical ganglia than in the stellate. This may
### TABLE 2

Comparative table of various junctional potentials

<table>
<thead>
<tr>
<th></th>
<th>Time to summit msec.</th>
<th>Origin to half decay msec.</th>
<th>Time Constant of decay msec.</th>
<th>Size relative to spike height at just-blocking dose per-cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior cervical ganglion</td>
<td>25-35</td>
<td>90-110</td>
<td>80-100</td>
<td>22-29</td>
</tr>
<tr>
<td>Stellate ganglion (Eccles, 1943)</td>
<td>10-15</td>
<td>60-90</td>
<td>60-95</td>
<td>5-12</td>
</tr>
<tr>
<td>Frog e.p.p. Kuffler (1942)</td>
<td>1.0</td>
<td>3.5</td>
<td>Ca.10</td>
<td>30-35</td>
</tr>
<tr>
<td>Mammalian e.p.p. using surface electrodes Liley &amp; North (1953)</td>
<td>0.85</td>
<td>2.45</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>Mammalian motoneuronal synaptic potential (intracellular electrodes) (Brooks, Coombs &amp; Eccles, 1951,1952)</td>
<td>0.7</td>
<td>3.0</td>
<td>3.6</td>
<td>0-15</td>
</tr>
</tbody>
</table>
partly be attributable to a removal of all connective tissue from the ganglion and the postganglionic trunk in these present experiments. In addition the potentials have been recorded with the "active" lead on the ganglion, not merely on the origin of the postganglionic trunk as in the stellate ganglion preparations, whose synaptic potentials would thus be recorded after some electrotonic decrement.

An attempt was made using microelectrodes to obtain direct measurements of the synaptic potential and of its size relative to the spike potential of the ganglion cell (cf. Section XV). In the few cells that were recorded intracellularly the spike did not arise until the synaptic potential had attained about 30% of the control spike size, which is in good agreement with the extracellular observations.

When curarization was incomplete, a small spike was observed superimposed on the synaptic potential e.g. Fig. 9a (ii). In the presence of even less of the blocking agent, the spike rose earlier from the synaptic potential. The earlier evidence (Eccles, 1943) that the synaptic potential is responsible for the initiation of impulses in non-curarized ganglia has thus received additional strong support.

**Special actions of different curares**

In Fig. 35 it can be seen that the effectiveness of tubocurarine as a ganglion-blocking agent showed considerable variation (just-blocking dose 1.6 to 2.5 X 10^-5 M). This may be attributed to two causes: a variation between preparations and some deterioration of tubocurarine solutions that were several weeks old. Relative to the tubocurarine concentration for blockage (1.6 to 2.5 X 10^-5 M), the just effective doses were 3.9 X 10^-5 M, 7.9 X 10^-5 M, 7 X 10^-5 M, 1.5 X 10^-4 M and greater than 6.5 X 10^-4 M, for dihydro-β-erythroidine, erythroidine, C-dihydro-
toxiferin I, curarine (S toxifera) and C-toxiferin II respectively. The concentrations of other ganglion-blocking drugs which were never used in very high blocking concentrations are given in Table 4.

Comparison of the effects of ganglion-blocking agents on the ganglionic response to repetitive preganglionic volleys indicates little difference between these drugs. There was always a facilitation of the second response and occasionally of the third as well, while all the rest of the responses were synaptic potentials (Figs. 10, 19). In the few experiments on circulated ganglia certain drugs, C5 and C6, blocked nearly all negative potentials i.e. spike and synaptic potentials, except the first two (Fig. 18). On the other hand with other drugs, e.g. tubocurarine and dihydro-S-erythroidine, the synaptic potentials were observed to show a greater potentiation of the second response of a repetitive series than was seen in isolated preparations (cf. Figs. 10, 17).

The toxiferins and erythroidines differed greatly in their effects on the P and LN waves, there being approximately an inverse relationship as shown in Table 3. The dominance of the LN wave is illustrated in Fig. 23b for dihydro-toxiferin I and at the other end of the scale, the P wave dominance is shown in Figs. 12 and 22 for tubocurarine. The same relative effects on P and LN waves were observed over the whole range of concentrations. Increasing dosage of all ganglion-blocking drugs diminished the size of the N wave, whether this was measured by the initial synaptic potential or by the size of the deflection in a positive direction immediately at the end of the tetanus. At high concentrations of tubocurarine ($3.2 \times 10^{-5}$M) the P wave became dominant during the tetanus. This effect was not observed with the toxiferins or the erythroidines. In particular C-dihydrotoxiferin I greatly increased the LN wave. This action may be limited to a potentiation of LN without any alteration in the P wave.
which is merely submerged by the cumulative negativity, or alternatively the P wave may be depressed as well. However, when the LN wave is removed by anticholinesterases (Fig. 33b iii) the P wave does not appear to be appreciably depressed, an observation which favours the first alternative. Paton and Perry (1951) tested the potency of some toxiferines as ganglion-blockers using the cat's superior cervical ganglion, but the toxiferines they tested were different compounds from those employed in this paper.

Further analysis was attempted by comparing the effects produced by concentrations of tubocurarine ranging from $1.6 \times 10^{-6}$ M to $1.6 \times 10^{-4}$ M. With increasing tubocurarine concentration there was after a tetanus a diminution of the positive deflection that followed the rapid decay of the N wave (cf. Fig. 12), and also a progressively earlier rise of the LN wave to an earlier summit (Fig. 12). One explanation of the series of curves illustrated in Fig. 12 would be that the LN wave is normally submerged by the large P wave following the tetanus, and that progressive diminution of the latter by tubocurarine was responsible for the emergence of the LN wave. However such an explanation has difficulty in accounting for the appearance of LN waves as large as 0.1 mV at low tubocurarine concentrations, when at the same time the positivity after the tetanus was not diminished in size, but even at times increased (cf. $1.6 \times 10^{-6}$ M-tubocurarine in Fig. 12). A more probable explanation is that, besides depressing the depolarizing action of the liberated acetylcholine (Eccles, 1943, 1944; Feldberg, 1950), the curares also specifically increase the depolarizing action of some substance liberated by preganglionic stimulation and which by its cumulative effect gives the LN wave. Further discussion will be deferred until the striking changes produced by anticholinesterases have been discussed.
### TABLE 3

**Effects of various curares**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration for just-blocking dose (M)</th>
<th>Relative sizes of waves at high concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Tubocurarine</td>
<td>$2.5 \times 10^{-5}$</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Curarine from Strychnos toxifera</td>
<td>$1.5 \times 10^{-4}$</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>c-Toxiferin II</td>
<td>$6.5 \times 10^{-4}$</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>c-Dihydrotoxiferin I</td>
<td>$7 \times 10^{-5}$</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>$3.9 \times 10^{-5}$</td>
<td>+ + + + +</td>
</tr>
</tbody>
</table>

### TABLE 4

**Effects of other ganglion-blocking agents**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration for just-blocking dose (M)</th>
<th>Relative size of waves at just-blocking concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
<td>$2.1 \times 10^{-4}$</td>
<td>+</td>
</tr>
<tr>
<td>C6</td>
<td>$1.0 \times 10^{-4}$</td>
<td>+</td>
</tr>
<tr>
<td>Ro 2-4658</td>
<td>$7 \times 10^{-5}$</td>
<td>+</td>
</tr>
<tr>
<td>β-erythroidine</td>
<td>$7.9 \times 10^{-5}$</td>
<td>+</td>
</tr>
<tr>
<td>dihydro-β-erythroidine</td>
<td>$3.9 \times 10^{-5}$</td>
<td>+</td>
</tr>
</tbody>
</table>
The effect of addition of anticholinesterases

In the curarized animal (Eccles, 1944) the injection of an anticholinesterase (eserine) caused a lengthening of the slow decay of the N wave so that the P wave was completely suppressed. This work has been confirmed in the isolated ganglion preparations with low curare concentrations (Fig. 25). At the relatively low erythroidine concentration of \(4 \times 10^{-5} \text{M}\) a small spike discharge is superimposed on the synaptic potential. Addition of the anticholinesterase, in this case prostigmine, produced a slight increase in the positive wave which is probably compounded of a positive component of the spike, the true positive afterpotential and the P wave. This increase in the positive deflection may be due to a slight depolarization of the ganglion cells. The mechanism responsible for the production of the LN wave seems to be unable to operate under these conditions. On the other hand the anticholinesterases or Ach may inhibit the LN wave production. An increase in the prostigmine to \(3 \times 10^{-5} \text{M}\) produced, as in the uncurarized ganglion, a further lengthening of the negative wave or depolarization which has been satisfactorily attributed to the unhydrolysed Ach liberated from preganglionic terminals during their period of repetitive stimulation (Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934b; and Eccles, 1944).

However the addition of prostigmine to a ganglion, deeply curarized by any curare, caused an increase in the positivity developed during the tetanus, and after the tetanus a slower development of dominance by the LN wave, which finally disappeared altogether at high levels of anticholinesterase (cf. Figs. 22, 23 and 33). An increase is observed in the P wave which is probably coupled with a depression of the N and LN waves (Fig. 22). The depression of the N wave by the anticholinesterase is
indicated by the reduction both of the initial negativity and of the sudden decay of negativity at the end of the tetanus (Fig. 22bi).

It is difficult to offer a satisfactory explanation of the two widely differing actions of an anticholinesterase: (i) in the presence of a just-blocking solution of any of the curares, it causes a slowing of the decay of the N wave and probably a decrease of the LN wave; (ii) on the other hand, in the presence of high doses of the curares, it causes a decrease of the N wave, an increase in the P wave and decrease and finally suppression of the LN wave. As a general rule it can be said that in the presence of tubocurarine, an anticholinesterase produces the second action when the transmission across the synapse is blocked to such a degree that spike discharges are absent from all responses to repetitive stimulation of the preganglionic nerve. In the presence of tubocurarine the action of an anticholinesterase, therefore, depends on the size of the N wave. This rule is more or less applicable to all curarizing agents though it was noticed that, if the ganglion blocking agent employed was dihydro-β-erythro-oidine, the second action of anticholinesterase was present even when a small spike was superimposed on the second response.

The only conclusion that can be drawn from the results is that anticholinesterases have two different actions in the presence of curarizing agents though the reasons for this diversity of action have not been discovered. Presumably the actions are relatable to specific actions of acetylcholine, which accumulates in the presence of anticholinesterases. Understanding of these postulated differences in acetylcholine behaviour will not be possible until an intracellular electrode is used to record the membrane potentials of ganglion cells. Then the direct application of Ach will be possible, and its effects both on resting potentials as
well as on potentials initiated by preganglionic activity at all levels of curarization may be studied.

The effect of addition of nicotine

The increase in ionic permeability of the ganglionic membrane by nicotine led to a reduction in the time for decay to $1/e$, i.e., the time constant of the membrane. Even a concentration as low as $6 \times 10^{-7}$ M nicotine approximately halved the time constant from 98 to 55 msec (Fig. 26a). Further increase in the nicotine concentration reduced the size of the synaptic potential as well as the time course of the synaptic potential. At these low concentrations of nicotine the P wave following a tetanus was slightly increased but the IN wave appeared to be unaltered. This increase in the positive potential is typical of the increase in the positive afterpotential of uncurarized ganglia when the membrane resting potential is moved further from the potassium equilibrium potential (Section VIII). In Fig. 27 ii and iii it can be observed that a very high concentration of nicotine greatly reduced the size of the N, P and IN waves. There was no large increase of the P wave such as occurred in the presence of anticholinesterases (cf. Fig. 27 iii and v). It can then be concluded that depolarization of the membrane e.g. by nicotine, will produce a slight increase in the P wave. The large P wave recorded in the presence of anticholinesterases must however be explained on the basis that it is due to unhydrolysed Ach or even to some action of the anticholinesterases themselves but not entirely to a depolarization of the post-synaptic membrane.

Effect of external potassium concentration

The effect of potassium on the P wave is characteristic of a potential dependent on the ratio of the intracellular and extracellular potassium concentrations. At a low extracellular potassium level, (Section VIII),
the difference between the resting potential and the potassium equilibrium potential is increased. It can be observed from Figs. 28 and 29 that the size of the P wave, like the positive afterpotential to be discussed (Section VIII), is increased at low external potassium and decreased at high external potassium levels. Thus the changes in P wave are explicable by a consideration of the resting potential and the relative potassium concentrations inside and outside. This effect of potassium on the P wave leads to the conclusion that it is due to an increase in potassium permeability. Exactly how this is produced is unknown. It could be due to an unknown transmitter.

Addition of nor-adrenaline and other sympathomemetic amines

In the concentrations employed which are either present physiologically or present when there are noticeable pharmacological reactions to the injected drug, no effects are observed on the potentials of curarized ganglia. The slight alteration in time course of the LN wave (Fig. 30), could hardly be considered significant. The reduction in the size of the P and LN waves when the phenylephrine concentration was raised to \(4.4 \times 10^{-6}\) M is explained by the depressant action of phenylephrine which is observed at these concentrations (Section XI). It seems improbable that adrenaline has an inhibitory action in the sympathetic ganglia unless it is present in a far higher concentration than that which occurs physiologically in the blood.

Addition of strychnine

In Fig. 32 and 33 strychnine had no significant effect on either the P or LN waves. The slight alterations observed were likely to be due to drift rather than to a real effect of strychnine. This lack of any strychnine action excludes the possibility that the P wave is due to an
inhibitory transmitter similar to the strychnine-sensitive transmitter in the spinal cord (Bradley, Easton and Eccles, 1953). Since all types of inhibition in the spinal cord are similarly depressed by strychnine, it seems likely that there is only one inhibitory transmitter in the central nervous system (Coombs, Eccles and Fatt, unpublished observations). Experiments such as those illustrated in Figs. 32 and 33 show that the central inhibitory substance is not the transmitter giving the P wave of ganglia.

Veratrine. Veratrine slightly lengthened the time constant of the synaptic potential from 70 to 86 msec but this was not considered significant. Veratrine did not alter either the size or time course of the P and LN waves. Therefore it was concluded that neither the N or LN waves were produced by a similar process as the negative afterpotentials of uncurarized ganglia which were greatly prolonged by veratrine (Section XII).

Possible mechanisms for the production of N, P and LN waves

When transmission of impulses through a ganglion is blocked by a curarizing agent, three potentials are recorded from ganglion cells following stimulation of the preganglionic nerve, the synaptic potential, the P wave and the LN wave. The experimental evidence has shown that these are three independent responses of the post-synaptic membrane, for the N and the LN wave may be specifically removed by drugs without affecting the P wave. These three waves should therefore be discussed independently.

The synaptic potential. Since many of the steric receptors for Ach on the post-synaptic membrane would be occupied by the ganglion-blocking drug, the Ach liberated from the preganglionic terminals will bind with only a few receptors and hence produce a small depolarization - a synaptic potential which does not develop into a propagated impulse. Since this synaptic potential is still present when the ganglion cells are heavily
depolarized by nicotine, it is probable that the equilibrium potential for the synaptic potential approximates to zero in conformity with the epp (Fatt and Katz, 1951) and the synaptic potential of motoneurones (Coombs, Eccles and Fatt, 1953, and unpublished observations). Presumably, as with the endplate and motoneuronal membranes, permeability is greatly increased to ions; in particular to potassium, chloride and sodium ions. The LN wave. Similarly the LN wave seems to possess a low equilibrium potential since depolarization by nicotine did not abolish it. The LN wave is blocked by all anticholinesterases and the potency of the various anticholinesterases in this respect parallels their anticholinesterase activity. Hence it is postulated that the LN wave is due to an action of Ach on steric receptors that have the cholinesterase configuration, for only under such conditions would they be susceptible to block by anticholinesterases in the manner found experimentally. It is further postulated that these receptor areas are located more peripherally (relative to the synaptic knob) on the post-synaptic membrane in order to explain the slow development of the LN wave, which would thus be attributed to the diffusion time of Ach. Also the slow build-up of the LN wave following a preganglionic tetanus would be explicable on this postulate. Zupancic (1953) has already proposed a related hypothesis, namely that cholinesterase acts as the whole steric receptor mechanism for Ach, i.e. for the synaptic potential, but this is an improbable hypothesis, since a greater depressant effect of anticholinesterases on the synaptic potential would be expected, and furthermore this depressant action should parallel the anticholinesterase activity. It is possible that the non-specific cholinesterase could form the postulated receptor areas for the LN wave, for it is largely postganglionic, at least it is not destroyed on preganglionic degeneration (Sawyer and Hollinshead,
1945). However this suggestion makes it difficult to explain the long duration of the LN wave. If these areas are formed by non-specific cholinesterase, then this enzyme would have to be bound to the post-ganglionic surface so that it is relatively inactive in splitting Ach, and yet can be blocked by low concentration of an anticholinesterase. An alternative explanation of the LN wave is that it is produced by the action on the post-synaptic membrane of the choline arising from hydrolysis of Ach. However choline is an excitatory agent in sympathetic ganglia (Feldberg and Vartiainen, 1934) so that this explanation seems unlikely.

The P wave. When the P wave occurs in the absence of ganglionic impulses, it probably contains no component that can properly be called a positive after-potential. Under such conditions it presumably is generated directly by some synaptic transmitter. This component of the P wave may be called the positive post-synaptic potential. Variations in the external concentrations of potassium have been explained above by postulating that this potential is produced by an increased potassium permeability. It is unlikely that Ach is the transmitter because neither type of compound sterically related to Ach, i.e. the ganglion-blocking agents and the anti-cholinesterases, acts as a potent depressor of the positive post-synaptic potential (cf. Figs. 22, 23, 32, 33). The positive postsynaptic potential seems to be analogous to the inhibitory post-synaptic potential (IPSP) of motoneurones, which is produced by a brief high permeability of the post-synaptic membrane to small ions, in particular to potassium and chloride ions, but not to the larger sodium ions (Coombes, Eccles and Fatt, 1954). However it is unlikely that the actual transmitter is the same as the inhibitory transmitter of the spinal cord, since it was not blocked
by strychnine which exerts a specific depressant action on all types of 
IPSP in the spinal cord (Coombs, Eccles and Fatt, 1954). This hypothesis 
that the positive post-synaptic potential is produced by an inhibitory 
transmitter substance is supported by the experimental evidence of Laporte 
and Lorente de Nó (1950a) which led them to postulate that the P wave of 
curarized turtle ganglia was produced by an inhibitory transmitter.
However, despite the evidence provided for a true inhibitory action of 
preganglionic impulses on ganglion cells (Alvarez-Buylla, 1948; Lorente 
de Nó and Laporte, 1950a), the existence of such fibres is still not 
established (cf. Job and Lundberg, 1953). Alternatively, the post-synaptic 
positive potential may merely be as a consequence of the removal of the 
LN wave unmasking the post-synaptic positive potential. It need not 
indicate, as was suggested earlier (R. Eccles, 1952b), that the post-
synaptic positive potential is produced by acetylcholine.

These postulates have been incorporated into a diagram (Fig. 37). 
The post-synaptic areas responsible for the synaptic potential and post-
synaptic positive wave are shown forming a central mosaic while the LN 
areas surround them. Furthermore the effectiveness of different ganglion-
blocking drugs are explicable on the basis that some block the positive 
post-synaptic potential receptors as well as the receptors responsible 
for the synaptic potential. These drugs give rise to a ganglionic response 
with large LN waves and insignificant P waves e.g. c-dihydro-toxiferin I, 
whereas other drugs do not block the positive post-synaptic receptors 
e.g. tubocurarine. Similarly the various actions of the anticholinesterase 
can be explained e.g. Nu 1250 is particularly effective in blocking the 
synaptic potential receptors.
TABLE 5

The depressant effects on the potentials of curarized ganglia by different drugs explained by the above postulates.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Synaptic potential</th>
<th>IN wave</th>
<th>Positive postsynaptic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curarizing agents</td>
<td>+ to + +</td>
<td>0 to +</td>
<td>0 to + +</td>
</tr>
<tr>
<td>Anticholinesterase</td>
<td>0 to +</td>
<td>+ +</td>
<td>No depression even possibly an increase</td>
</tr>
</tbody>
</table>
THE EFFECT OF CHANGES IN THE EXTERNAL POTASSIUM RATION ON GANGLIONIC TRANSMISSION

Feldberg and Vartiainen (1934) observed that injection of potassium chloride into perfused superior cervical ganglia resulted in several actions. At low concentrations the ganglion cells became more excitable and spontaneously discharged impulses, whereas high concentrations of potassium led to a complete blockage of transmission. When acetylcholine was postulated as the transmitter substance that acts across sympathetic synapses (Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934), the excitatory action of potassium required further investigation. However careful analysis of the effect of potassium both on the contraction of the end organ, the nictitating membrane, and on the acetylcholine output showed that though potassium caused the liberation of acetylcholine from the preganglionic terminals, it also increased the excitability of the ganglion cells (Brown and Feldberg, 1936b). The effects of potassium and acetylcholine on the post-synaptic membrane were differentiated by curare which can be assumed to combine with the receptor areas on the post-synaptic membrane of muscle fibres (Dale, Feldberg and Vogt, 1936). Addition of curare to perfused ganglia greatly decreased the sensitivity of the ganglion cells to perfused acetylcholine without altering the stimulant action of potassium (Brown and Feldberg, 1936a). Changes in external potassium have also been shown to alter the positive potentials following a ganglionic response in amphibian ganglia (Saunders and Sinclair, 1949).

Fig. 38 shows the effect of alterations in the external potassium concentration on the ganglionic response to a single preganglionic volley. This ganglion had been excised for twenty six hours. Under such conditions
the large positive after-potential made it a favourable preparation for illustrating the effect of low potassium. Apart from the increase in the positive after-potential there had been no appreciable alteration in the ganglionic response even after this prolonged soaking (i). Increasing the external potassium concentration to 15 mM produced a slight increase in spike height (about 4%). The positive component of the spike, i.e. the positive phase between the Sa response and the combined Sb response and N waves, was increased. The positive after-potential, on the other hand, became very much smaller. A decrease in the external potassium concentration to zero initially did not alter the height of the Sa response though a large positive after-potential became apparent (Fig. 38 iii). A further twenty minutes immersion in this potassium-free solution reduced the spike response to 65% of the control. Nevertheless the size and time course of the positive after-potential (iv) is still as large as the positive after-potential following the control responses (i and v).

The effects of potassium alterations on responses to repetitive stimulation are illustrated on a freshly excised ganglion in Fig. 39. An increase in the external potassium to 15 mM has left the spike responses unaltered though the positive component of the spikes has become less negative and the positive after-potential has been reduced in size but not in time course. On the other hand, soaking in a potassium-free Ringer-Locke solution produced a decrease in the spike height with little change in the positive after-potential (cf. iii with i and iv). Return to a normal potassium solution (5 mM) produced a complete recovery to the original response (cf. iv with i).

From both figures the following observations can be made. An increase to three times normal produced a slight increase in spike height, which
probably is due to more cells firing, and an increase in the positive component of the spike; yet at the same time there was a decrease in the positive after-potential. Reduction in the external potassium led to a reduced spike response, an increase in positive after-potential but no alteration in the positive component of the spike.

**Discussion on membrane potentials**

Boyle and Conway (1941) first explained their observations of potassium and chloride movements across the muscle membrane by the theory that the distributions of potassium and chloride, both permeable ions, would be on a Gibbs-Donnan ratio, since the cell membrane was assumed to be impermeable to sodium and also to the large intracellular anions.

\[
\frac{(K^+)_i}{(K^+)_o} = \frac{(Cl^-)_o}{(Cl^-)_i} = r \text{ (the Gibbs-Donnan ratio)}
\]

i.e. \((K^+)_i(Cl^-)_i = (K^+)_o(Cl^-)_o\)

where \(K_i, K_o, Cl_i\) and \(Cl_o\) are the activities of these ions inside and outside.

From this the equilibrium potentials of these ions under conditions of a steady state, are given by the following equations.

\[
E = \frac{RT \log e}{F} \left(\frac{(Cl^-)_o}{(Cl^-)_i}\right) = \frac{RT \log e}{F} \left(\frac{(K^+)_i}{(K^+)_o}\right)
\]

For frog muscle the intracellular potassium = 125 mM/l

the extracellular potassium = 2.5 mM/l

\[
E_K = \frac{RT \log e}{F} \left(\frac{(K^+)_i}{(K^+)_o}\right) \approx 58 \log_{10} \left(\frac{(K^+)_i}{(K^+)_o}\right) = 98 mV
\]

Ling and Gerard (1949) using microelectrodes recorded resting potentials in the blood circulated frog's sartorius of 85 mV to which must be added about 10 mV for junction potentials giving 95 mV, which is very
close to the value of 98 mV calculated above. Isolated sartorii preparations gave resting potential values in reasonable agreement with this, from 85-95 mV (Nastuk and Hodgkin, 1950; Fatt and Katz, 1951; Desmedt, 1953). It seems then that amphibian striated muscle could be considered fairly near to a steady state.

However this is not true for the isolated axons of the cephalopods. Even in a short period of twenty minutes Steinbach and Spiegelman (1943) and Keynes and Lewis (1951) showed an appreciable net uptake of sodium ions from the surrounding medium and a net loss of potassium. Radio-tracer technique has revealed that membranes hitherto regarded as impermeable to sodium are in fact permeable but to a much lesser extent than to potassium ions (Keynes, 1951; Hodgkin, 1951). The steady state of nerves and muscle must then be maintained by a constant pumping out of the sodium ions which have diffused in, or whose entry was brought about by the sodium-carrier operating during the action potential. Such a pump will require energy derived from cellular metabolism.

However isolated rabbit ganglia will still respond to a preganglionic stimulus forty-eight hours after excision, with no appreciable alteration in the spike discharge, and there can have been little net gain of sodium, i.e. it can be assumed that the excised ganglion is virtually in a steady state and that it resembles excised frog muscle rather than cephalopod axon. The resting potential would be predominantly determined by the potassium concentrations, excess sodium being removed by the sodium pump.

\[ E = \text{theoretical potassium potential or the potassium equilibrium potential} \]

\[ = 62.5 \log_{10}(\frac{[K^+])}{[K^+]_0} \]

The external potassium in the Ringer = 5 mM

The internal potassium was taken as 140 mM
Therefore the potassium equilibrium potential = 90.5 mV.

This intracellular potassium value of 140 mM is the value calculated for intracellular potassium of motoneurones (Coombs, Eccles and Fatt, 1954).

However the resting potential as recorded in the motoneurones of the cat's spinal cord averages about 70 mV, which is about 20 mV below the potassium equilibrium potential (Coombs, Eccles and Fatt, 1954). On the few occasions in which I penetrated ganglion cells with a microelectrode the resting potentials were never greater than 80 mV (Section XV). Therefore the calculated value of 90.5 mV (or potassium equilibrium potential) is higher than the resting potential for ganglion cells as well as motoneurones. The low value of resting potential compared with the potassium equilibrium potential is probably due to the coupling of K⁺ influx and Na⁺-outflux (Hodgkin and Keynes, 1953; Keynes, 1954).

Since the potassium flux is not entirely independent of a pump mechanism the resting potential will not equal the potassium equilibrium potential.

A decrease in the external potassium concentration would lead to a large increase in the potassium equilibrium potential, which would be followed by a lesser increase in the resting potential (cf. Cole and Curtis, 1942; Ling and Gerard, 1950; Huxley and Stampfli, 1951), i.e. a greater difference between the resting potential and the potassium equilibrium potential. This caused little alteration in the positive component of the spike which probably is analogous to the positive phase of the action potential in isolated cephalopod axons (Hodgkin and Katz, 1949) and also to the positive component of the spike in motoneurones (Eccles, 1952; Coombs, Eccles and Fatt, 1954). In squid axons and mammalian motoneurones it has been demonstrated that a
reduction in resting potential, whether by deterioration or experimentally produced, can cause an increase in the size of the positive component. Similarly in sympathetic ganglia, as measured with external electrodes, there was an increase in the positive component of the spike twenty four hours after excision which probably resulted from a lowering of resting potential by deterioration. Furthermore, it will be seen later (Section XV) that the positive component of the spike progressively increased in the single ganglion cell as depolarization developed following insertion of a microelectrode. The responses in Fig. 30 showed no increase in the positive component of the spike in potassium-free solution and yet there was an increase on trebling the potassium concentration, which is the reverse of the expected change (cf. Hodgkin and Katz, 1949). The size of the positive component of the spike, when recording with external electrodes probably depends on several factors; the number of ganglion cells discharging impulses, the asynchronism of these discharges, the developing negative after-potential as well as its relationship to the resting potentials of ganglion cells. It is quite likely that the depolarization by potassium has altered the characteristic time course of the negative after-potential and hence caused the apparent increase in the positive component of the spike.

The positive after-potential was increased by the reduction of potassium ions in the environment. During the positive after-potential permeability to potassium is increased as there is experimental evidence of the potassium carrier operating, at least in the motoneurone of the cat's spinal cord (Coombs, Eccles and Fatt, 1954). Therefore the dependence of the positive after-potential of ganglion cells on the potassium concentrations is not an unexpected phenomenon. When ganglia are soaked
in solutions containing 15 mM of potassium, the positive after-potential was reduced relative to the control responses. An increase in external potassium to 15 mM means a reduction of the potassium equilibrium potential to 60.5 mV and the resting potential should be slightly less. The difference between resting potential and potassium equilibrium potential would be much less than normal and therefore very little potential change can be produced by an increased potassium permeability. These experiments illustrate that the relationship between the potassium concentration and positive after-potential is explicable on the theory if there is an increase in the potassium permeability for a short period after the generation of an impulse. These changes in the positive potentials are similar to the changes reported earlier for frog ganglia (Saunders and Sinclair, 1949). A lowering of the external potassium concentration resulted in a decrease in $P_1$ voltage (this potential is similar to the positive component of the spike) and an increase in $P_2$ (i.e., positive after-potential) and, on the other hand, an increase in potassium decreased the $P_2$ component and increased the $P_1$ potential.

Since the recovery from the peak of an action potential depends on potassium, it might be expected that alterations in external potassium could modify the spike discharge of ganglion cells. According to Brown and Feldberg (1936b) perfusion of a sympathetic ganglion with a potassium-rich solution led to a steady output of Ach for 20-60 secs. However, the ability of potassium to cause contraction of the nictitating membrane in denervated ganglia was taken as an indication that the effects of potassium were not entirely the result of Ach liberated from the preganglionic terminals. With high external potassium, the ganglion cells will be depolarized and hence be more excitable and a larger spike discharge may
be produced. However this increase in spike discharge can only occur if some ganglion cells were not responding to maximal stimulation in the normal potassium concentration and if the ganglion cells have not been too heavily depolarized by the high concentration of potassium. A larger spike discharge, (Fig. 38) was the usual observation until the potassium concentration was increased above 20 mM when heavy depolarization produced blockage. This is in accordance with the excitability changes in ganglion cells reported by Bronk (1939) and Brown and Feldberg (1936b). The effects of low external potassium were not so consistent. Usually there was a slight decrease of the spike, which may be a result of the hyperpolarization of the membrane of ganglion cells and their consequent depression. Often the effects on the spike discharge were negligible, but Bronk's findings (1939) of an increased ganglionic spike under these conditions were not confirmed. Throughout the experiment the thresholds were tested and kept maximal for the second group of high threshold fibres where these existed. No direct evidence could be gained from the present mode of recording on changes in transmission into the preganglionic terminals. On theoretical grounds it might be expected that a higher external K\(^+\) concentration would lead to a partial depolarization of the preganglionic terminals so that invasion of them by a preganglionic volley would be easier than when the potassium concentration was 5 mM. Similarly a reduction in external potassium could lead to a blockage in the preganglionic terminals. This would then produce a smaller preganglionic volley and therefore a decrease in the ganglionic response. There is experimental evidence that no change occurs in the output of acetylcholine from the preganglionic terminals even when synaptic transmission was completely blocked by potassium (Brown
and Feldberg, 1936b). Hence it may be assumed that a high concentration of potassium does not affect preganglionic terminals for some time after the appearance of complete paralysis (Brown and Feldberg, 1936b).

For the purpose of this discussion it was assumed that in the few minutes required for equilibration changes in external potassium had had no effect on the internal concentration of this ion. At one and a half minutes the full effect of the changed potassium had not developed, presumably because diffusion equilibrium had not yet been reached for the environment of the innermost ganglion cell. After a few minutes the responses remained constant for an hour.

The only conclusion from the above experiment was that the dependence of the positive after-potential on the external potassium concentration was in good agreement with the hypothesis that it was produced by an increased potassium conductance of the ganglionic cell membrane. The number of ganglion cells responding to a preganglionic stimulus varied according to the resting potential of the cells themselves. A very high level of potassium, greater than four times normal, produced a heavy depolarization preventing the generation of impulses. Similarly a low potassium level probably hyperpolarizes the membrane making the cells less excitable, but however, a slight increase in potassium could lead to an increased excitability. It was noticed that isolated ganglia preparations on the second day always gave spike discharges which were followed by much larger positive components of the spike and also positive after-potentials than when first excised. This is to be expected when ganglion cells are slightly depolarized - in this case by deterioration. The resting potential has decreased relative to the potassium equilibrium potential.
ANTICHOLINESTERASES

Introduction

It is now generally accepted that acetylcholine is the chemical transmitter substance in sympathetic ganglia. Stimulation of the preganglionic nerves of the superior cervical ganglion caused the release of a substance pharmacologically identifiable with acetylcholine when the ganglion was perfused with eserinized blood or Locke's solution (Feldberg and Gaddum, 1933, 1934; Feldberg and Vartiainen, 1934a and b). This acetylcholine was shown to be liberated from the preganglionic terminals because stimulation of ganglion cells either by antidromic invasion or by nicotine failed to produce more than a trace of Ach (Feldberg and Vartiainen, 1934b; Macintosh, 1938d). The amount of acetylcholine liberated during repetitive stimulation fell rapidly in the first few seconds to a constant level which was maintained throughout the period of stimulation (Macintosh, 1938d; Emmelin and Macintosh, 1948).

In his experiments Macintosh (1938b) showed that, if the ganglion was kept in good condition with a plentiful supply of oxygen and glucose, the amount of acetylcholine for a given stimulation remained constant. It has generally been stated (but not by Lorente de Nó, 1938) that ganglia do not produce a resting discharge of acetylcholine from the preganglionic nerve terminals (Feldberg and Vartiainen, 1934b). Emmelin and Macintosh (1948) showed that, if great precautions were taken not to handle the ganglion, no Ach would be found in the perfusate from a resting ganglion provided that the preganglionic nerve had been sectioned two hours earlier. They suggested that injury to the preganglionic fibres or to the ganglion could lead to the production of injury currents in the preganglionic fibres and thence to the generation of impulses. This injury might
account for a small variable output of acetylcholine found in the perfusate of many of these cat stellate ganglia. MacIntosh (1938d) recorded an output of acetylcholine in the resting state that was 5% of the maximum during repetitive stimulation.

Ganglion cells discharged impulses when acetylcholine was added to the perfusate (Feldberg and Vartiainen, 1934b). Govaerts (1936) showed that this stimulating effect of acetylcholine was obtained even after degeneration of the preganglionic fibres. Very high concentrations of acetylcholine in the perfusate depressed the excitability of ganglion cells to preganglionic stimuli or perfused acetylcholine (Feldberg and Vartiainen, 1934a and b). Depression of the ganglion cell discharge by potassium, nicotine, curarine or eserine did not alter the acetylcholine output from the preganglionic terminals (Brown and Feldberg, 1936b).

Further evidence for acetylcholine as chemical transmitter was derived from histological studies and degeneration experiments. During preganglionic degeneration the acetylcholine content of ganglia was shown to decrease rapidly (Brown and Feldberg, 1936b; MacIntosh, 1938a). This reduction in acetylcholine concentration was reflected in a decrease in spike height until synaptic transmission was blocked (Coppée and Bacq, 1938). On histological examination the failure of synaptic transmission occurred when the preganglionic terminals started to disintegrate (Gibson, 1940), so the degeneration experiments do not provide as much support for the chemical theory of transmission as formerly believed, since under these conditions electrical transmission, if it existed, would also decrease (Eccles, 1936).

The action of the anticholinesterases, prostigmine and eserine, caused a certain amount of confusion for some workers (Roepke, 1937)
referred to their action as being like that of non-competitive inhibitors of cholinesterases, while other evidence seemed to indicate that eserine and prostigmine were competitive inhibitors (Eadie, 1942). In a detailed study of inhibitors Straus and Goldstein (1943) showed that inhibition of enzymes could be divided into three types. Eserine and prostigmine were shown to be in group C, since they reversibly compete with acetylcholine for the active receptor groups on the cholinesterases molecules (Goldstein, 1948). Many substances referred to as anticholinesterases often possess other properties e.g. the potent blocking action of Nu1250 (Eccles and MacFarlane, 1949).

The addition of anticholinesterases to the perfusate of ganglion cells had several actions. In small quantities eserine was shown to sensitize the ganglion to perfused acetylcholine (Feldberg and Gaddum, 1934); and also to potentiate the response of the nictitating membrane (which itself could not have been directly affected) to a submaximal preganglionic tetanus (Feldberg and Vartiainen, 1935a and b). However this sensitization of the ganglion cell by eserine was thought to be non-specific since there was an increase in the stimulating action of potassium, nicotine, choline and hordenine methiodide (Feldberg and Vartiainen, 1934b). In eserinized ganglia Feldberg and Vartiainen (1934b) did not find any alteration or lengthening of the facilitation curve. However Rosenbleuth and Simeone (1938a and b) showed a facilitation of the second of two preganglionic volleys in the presence of eserine. This was explicable as a persistence of the Ach liberated by the first preganglionic volley causing a subthreshold depolarization of the ganglion cells which were more easily caused to discharge on the arrival of the second volley. Eccles (1944) has shown that in the
presence of eserine there was very little alteration in the synaptic potential of the curarized cat's stellate, there being at most only a slight lengthening of the time course of the synaptic potential. Following a repetitive stimulation of the preganglionic nerve it was found that the positive after-potential had been replaced by a long-lasting negativity which persisted for several seconds. It was postulated that the large amount of Ach liberated by the stimulation was still unhydrolysed and produced this depolarization of the membrane which coincided with a period of increased excitability of the ganglion cells and the appearance of spontaneous activity. Holaday, Kamijo and Koelle (1954) described an increase in the facilitation curve, up to 30%, when DFP was added to the perfusate of cat's stellate ganglia.

In the present series of experiments on isolated rabbit ganglia the effects of different anticholinesterases were compared. A DC amplifier was used to record more accurately the time course of these after-potentials over several seconds. A thorough investigation has already been done on the effects of eserine on the curarized and the uncurarized ganglion (Eccles, 1944). The results in these circulated ganglia were variable and difficult to interpret. Presumably this is due to the effect of several conflicting factors, a positive after-potential of the ganglion cells, a negativity or depolarization by the unhydrolysed Ach, and afterdischarges. Presumably the ganglionic response to preganglionic stimulation in the presence of anticholinesterase will depend on the quantity of Ach in the vicinity of the ganglion cells. The prolonged negativities are explicable as a depolarization by Ach since diffusion of Ach will be slight in isolated ganglia or those with impaired circulation.
The series to be described is a comparison of other anticholinesterases against prostigmine or eserine. In several cases it was possible to soak the ganglia in one anticholinesterase, and after a period of several hours in Krebs solution try the effects of another anticholinesterase. The effects of DFP were found to be almost completely irreversible even after three days, but TEPP produced a more reversible inhibition of cholinesterase.

Results

Addition of $3.4 \times 10^{-7}$ M Nu2126 did not alter the size of the spike response of Fig. 40(i and ii). However the after-potentials have been greatly altered in size. The positive component of the spike has become more positive and there is, at the same time, an increase in the positive after-potential. The increase in the concentrations of Nu2126 to $3.4 \times 10^{-5}$ has decreased the spike response, i.e. probably fewer cells were firing impulses. An increase in the depolarization of ganglion cells in the presence of anticholinesterase was indicated by the large positive component of the spike (cf. iii and ii). The positive after-potential has been shortened, an effect which is probably attributed to the depolarization effect of the acetylcholine liberated by the stimulation. An hour's soaking in Krebs solution (iv) did not produce complete recovery since the spike response was smaller than the control (i) and the positive component and positive after-potential were larger than in the control response, suggesting that there was a persisting residual depolarization of the ganglion cells.

In Fig. 41, the depression of the ganglionic spike response was compared with different anticholinesterase concentrations, the values for both graphs being based on those obtained in several experiments.
Nu 2126 has produced much less depression of the spike size than comparable concentrations of Nu 1250. The reduction in the spike to approximately 70% of the control size in solutions of $3.4 \times 10^{-5}$M Nu 2126 is comparable to the effects of prostigmine and eserine in similar molar concentrations. Nu 1250, on the other hand, possessed a more potent blocking action as shown by the depression to 30% by a concentration of $2.5 \times 10^{-5}$M. On a few occasions a facilitation of spike size was observed when the anticholinesterase was in a very low concentration. Presumably not all the ganglion cells were responding to the maximal control preganglionic volley and these neurones in the subliminal fringe were excited after the accumulation of acetylcholine had caused a slight depolarization (cf. Feldberg and Vartiainen, 1934b).

There were several differences between the control response to a short preganglionic tetanus and the response following an hour's soaking in prostigmine $3.3 \times 10^{-6}$M (Fig. 42). During the tetanus itself the spikes were reduced in size and the negative plateau from which the spike responses arose was noticeably lower. At the end of the stimulation the positive after-potential led on to a large negative plateau which presumably is due to the depolarizing action of the Ach which had accumulated during the tetanus. Following a longer duration of tetanus (vi) this negativity or depolarization due to Ach was increased and the positive after-potential accordingly reduced, both in size and duration. In the slower record of this longer tetanus (viii), there was little decay in this depolarization even after seven seconds. The shorter tetanus (iv) led to the production of a smaller depolarization which decayed slowly during the eight seconds it was observed. The large negative plateau in viii was not as great as in vi because the response to $2.4 \times 10^{-6}$M
eserine and not $3.3 \times 10^{-6} \text{M}$ prostigmine is illustrated, in preference to the prostigmine response which was marred by a drifting baseline. Prostigmine produced a higher depolarization plateau than either eserine or Nu 2126 and, since the height of the plateau is an indication of the amount of Ach unhydrolysed, $3.3 \times 10^{-6} \text{M}$ prostigmine is a slightly more powerful anticholinesterase than either $2.4 \times 10^{-6} \text{M}$ eserine or $3.4 \times 10^{-6} \text{M}$ Nu 2126, at least for these rabbit superior cervical ganglia. Though the height of the depolarization plateau may differ between anticholinesterases, its long duration is characteristic of the ganglionic responses in the presence of all types of anticholinesterase in low concentrations. For example the effects of Nu 2126, DFP and TEPP were identical with those illustrated.

**Discussion**

The chemical theory of ganglionic transmission can be divided by artificial boundaries into five events;

1) Activity in the preganglionic terminals causes the liberation of Ach

2) The Ach so released diffuses to the post-synaptic membrane where there are certain specialized receptors.

3) The Ach-receptor reaction causes an increased ionic permeability and consequent depolarization of the surface of the ganglion cells into which the surrounding areas of the cell and the axon acting as sources depolarize.

4) Sufficient depolarization of the membrane leads to the generation of a spike potential, and lastly

5) The removal of the Ach by diffusion and by enzymatic hydrolysis by cholinesterase.

Anticholinesterases all have in common an ability to inhibit, some
reversibly others irreversibly, the specific and non-specific cholinesterases. The position or site of the cholinesterases is given as close to, and possibly even inside, the preganglionic endings (Koelle, 1950, 1951; Koelle and Gilman, 1949). The location of the enzyme, cholinesterase, means that Ach may be hydrolysed by the cholinesterase at three places. The Ach molecules may be broken down before being released from the preganglionic terminals; the cholinesterase could reduce the amount of Ach diffusing to the post-synaptic membrane from the preganglionic source; and the enzyme, by its destruction of Ach could remove Ach from the vicinity of the ganglion cells i.e. after the Ach has depolarized the membrane. Though Nachmansohn (1950) and Feldberg (1950) state that eserine, unlike the quaternary ammonium anticholinesterase prostigmine, can penetrate the nerve membrane, it is doubtful whether eserine increases the output of Ach. Experimental evidence shows that in the presence of eserine a second preganglionic tetanus produces less Ach (Perry, 1953), as if eserine obstructed the release of Ach. However it was postulated by Perry (1953) that during the first tetanus the presence of eserine prevented the hydrolysis of Ach to choline. This choline was lost to the ganglion and was not available for the resynthesis of Ach; hence there was a reduction in the stock of "available" acetylcholine in the preganglionic terminals (Perry, 1953). This theory is supported by the evidence that reduction in the amount of Ach released by a second tetanus only occurred if eserine was present in the perfusate during the first period of stimulation.

In accounting for the effects of potassium ions (i.e. Section VIII), it was argued that depolarization of the membrane without alteration in the potassium equilibrium potential would be accompanied by an increase
in the positive potentials. Thus it seems likely that the increase in the positive component of the spike and the positive after-potential indicates a depolarization of the ganglion cells even by this low concentration of anticholinesterase. This could be due either to a post-synaptic depolarization by Nu 2126, or to the presence of some other depolarizing agent. The ganglia are usually removed from the bathing solution into the moist atmosphere for 1-2 minutes before the records are taken in order to permit the amplifier to stabilize. It is suggested that during that time sufficient Ach leaks from the preganglionic terminal to depolarize the ganglionic terminals. It could not have arisen from stimulation as these records are the first or second stimuli, because there was a long soaking time of at least 40 minutes since the last tetanus. Previously it has been stated that acetylcholine is not liberated in the resting state of perfused ganglia, such as occurs in frog muscle (Fatt and Katz, 1952, 1953). In the earliest samples of an eserinized perfusate MacIntosh (1938d) found an activity which was "doubtless due to Ach". He postulated that this activity came from the injured nerve endings either of vagal or sympathetic origin. However this activity never exceeded 5% of the Ach liberated by preganglionic stimulation and hence was too "small and evanescent" to complicate his results. In our experiments the isolated rabbit ganglia were in a moist atmosphere of oxygen during the recording, hence any substance such as acetylcholine liberated in the ganglion would not be removed by diffusion, which may explain why this slight resting depolarization by anticholinesterases is more pronounced in isolated preparations (cf. Holaday, Kamijo and Koelle, 1954). However Feldberg and Vartiainen (1934a and b) observed that eserine even in low concentrations potentiated the responses of
ganglion cells to the stimulating action of potassium, choline and nicotine. Under these conditions of depolarization the amount of any stimulating drug causing the generation of impulses is less, i.e. there would be an increase in response to any stimulating drug. This explanation attributes eserine sensitization to a specific action of eserine on a cholinesterase rather than to a non-specific action of eserine on the post-synaptic membrane. It seems that this increase in the positivity is characteristic of all anticholinesterases, since this increase in the positive component of the spike was observed with all anticholinesterases used, and also an increase in positive potentials was noted by Holaday, Kamijo and Koelle (1954) when sympathetic ganglia were acted on by the anticholinesterase. It should be pointed out that Holaday et al. (1954) have stated that their results "are similar to those described by R. Eccles (1952b) and Laporte and Lorente de Nó (1950) as occurring typically following anticholinesterases". This must be a misunderstanding, since both these papers are concerned with ganglionic potentials in the presence of ganglion blocking drugs and not with anticholinesterases.

Increase in the anticholinesterase concentration slightly increased the positive component of the spike but there was depression of the positive after-potential (Fig. 40 ii and iii) which also is produced by an increased flux of potassium ions, (Section VIII). Presumably the large depolarizing action of the acetylcholine counteracted this hyperpolarizing action of the potassium flux.

During a long preganglionic tetanus the action potentials of a normal ganglion indicate that the concentration of acetylcholine does not increase in the vicinity of the ganglion cells. Ach is either destroyed by the cholinesterase or diffuses away from the immediate environment of the ganglionic membrane into the large extracellular space. Removal of
the cholinesterase either reversibly with eserine or prostigmine, or by
destruction with DFP and TEPP, results in a reduction in the rate of hydro-
lysis of the Ach. Thus large quantities of Ach, previously destroyed,
would be expected to accumulate near ganglion cells. Its presence is
known from the following observations: 1) a large prolonged negativity
existing for seconds (Fig. 42); 2) a subsequent test volley fails to
generate a ganglionic spike potential for several seconds, see Section
cells XIV; 3) there is an asynchronous discharge of ganglion i.e. an after-
discharge, which was observed in the isolated ganglion just as with the
circulated ganglion (cf. Eccles, 1944 Fig. 11 and 14). These results
on the action of anticholinesterase and their interpretation differ in
only one respect from the previous description of anticholinesterase
activity in the ganglion in the whole animal with blood supply intact.
This exception is the suggestion that there is a very small resting dis-
charge of Ach from the preganglionic animals which, on addition of an
anticholinesterase, produces a slight depolarization of the ganglionic
membrane. Perhaps this suggestion does not come into conflict with the
earlier ideas of acetylcholine and its effects, since MacIntosh (1938d)
found a small discharge of Ach in ganglia which had been handled and
during excision ganglia must inevitably be handled more than when left
in situ.
Nicotine has been used for the identification of synapses in the sympathetic ganglion (e.g. Lloyd, 1937) ever since the demonstration that nicotine's paralytic effect occurred only at sympathetic synapses (Langley and Dickinson, 1893). Langley and Dickinson referred to a junctional origin of the paralysis, a conclusion that was generally accepted. However Chauchard (1947) believed that very high doses of nicotine blocked preganglionic terminals and not the post-synaptic membrane, but this was in concentrations of nicotine 10,000 times greater than the stimulating dose (Eccles, 1935a and c). Feldberg and Vartiainen (1934b) showed that in low concentrations nicotine had a stimulating action on the ganglion cells and that in high doses it prevented the ganglion cells responding to either preganglionic stimulation or perfused Ach. Since nicotine produced "no output of acetylcholine from the ganglion" (Feldberg and Vartiainen, 1934b) and did not interfere with the liberation of Ach from the preganglionic terminals during stimulation (Feldberg and Vartiainen, 1934b), it was concluded that nicotine blocked not by an action on the preganglionic terminals but rather by a direct action on the ganglion cells first exciting and later depressing. This stimulating effect of nicotine was shown to be due to a depolarization of the ganglion cells (Paton and Perry, 1953; Lundberg and Thesleff, 1953).

Results: The following experiment, typical of all the experiments, illustrates the effect of nicotine of known concentration outside the ganglion cells. At least forty-five minutes were allowed for diffusion so as to be certain that effects were maximal, but no alterations in the potentials were observed after twenty minutes.

In Fig. 43a and b, the effect of nicotine is observed to be like
that of other depolarizing agents. Nicotine $6 \times 10^{-7}$M did not alter the size of the ganglionic response, hence it may be presumed that the number of ganglion cells discharging in response to a single preganglionic volley was unchanged. However there was a great increase in the positive component of the spike and in the size of the later positive after-potential which appeared about ten milliseconds after the positive component of the spike. The first indication of the positive after-potential was the beginning of a slow decay of the positive potential following the rapid positive component of the spike. Further increase in the nicotine concentration to $1.2 \times 10^{-6}$M produced a slight decrease in the spike response, as well as an increase in the positive component of the spike. The positive after-potential has been depressed as shown by the increase in the rate of decay of the positivity following the ganglionic spike response. It would seem that this increase in nicotine concentration by a factor of two has increased the depolarization of the ganglion cells and reduced the negative after-potential as indicated by the larger positive component of the spike. The reduction in the ganglionic response shows that this heavy depolarization has prevented some cells from firing impulses in response to stimulation. $6 \times 10^{-6}$M nicotine produced a blockage so complete that only a very small spike response remains (Fig. 43 iv). However an hour's soaking has almost restored the ganglion response to control size (v). There was a slight decrease in the ganglionic spike response but the positive component of the spike and the positive after-potential have returned to the control size (cf. Fig. 43 v and i), an indication that there was no residual depolarization.

In Fig. 43b, the responses to tetani at these concentrations of
nicotine are illustrated. In Fig. 43b i it may be noted that the positive after-potential was composed of two components, an early quick decaying phase and a later slower-decaying component corresponding to the \( P_2 \) wave of Eccles (1944). On the addition of nicotine, \( 6 \times 10^{-7} \text{M} \), there was a large deflection in the positive direction developing even during the tetanus. Following the tetanus the after-potential decayed along a time course very little different from the control (i). The only difference after soaking in nicotine \( 1.2 \times 10^{-6} \text{M} \) was an earlier development of this positive deflection during the tetanus. The ganglion cells were still able to follow repetitive stimulation. Except for a few cells, \( 6 \times 10^{-6} \text{M} \) nicotine completely blocked synaptic transmission. The reversible action of nicotine is illustrated by the complete recovery after an hour's soaking in Krebs solution. The only distinction that could be made between Fig. 43b(v) and the control Fig. 43b(i) is an increase in the positive after-potential. This may be due to a slight depolarization still existing from the nicotine or, more probably, due to a slight increase in the positive after-potential which was usually observed during the first hours after excision.

Discussion

The experiments described above illustrate the depolarization produced by concentrations of nicotine, \( 6 \times 10^{-7} - 1.2 \times 10^{-6} \text{M} \). This is in complete agreement with the increase in negativity i.e. depolarization of the ganglion cells after a nicotine injection (Paton and Perry, 1951, 1953; Lundberg and Thesleff, 1953). The results illustrated in Fig. 43 are similar to those obtained on application of nicotine to the cat superior cervical ganglion (Eccles, 1935a and c). It can be seen from Fig. 43 that a low concentration of nicotine had greatly diminished the
negative after-potential and as a result the positive component of the spike was increased and the peak of the positive after-potential appeared earlier. Increase in the nicotine to $1.2 \times 10^{-6}$ M now diminished the size and time course of the positive after-potential. This could be explained as a depolarization of the cell membrane by nicotine which would lead to an increase in ionic permeability and hence a reduction in time constant of the membrane. Therefore slow negative potentials decayling exponentially e.g. the synaptic potential (Section VII B) will possess a much shorter time of recovery.

At higher concentrations, $6 \times 10^{-6}$ M, nicotine there is almost complete failure of transmission yet no sign of depolarization i.e. a large positive component following the spike (Fig. 43b iv). The response may be a small spike discharge of a few cells more resistant to nicotine, as its time course is too fast for it to be a synaptic potential generated by many cells. However it may be that these responses were synaptic potentials, since it was observed that the addition of $6 \times 10^{-6}$ M nicotine to curarized ganglia reduced the time constant from 85 to 19 msec (Section VII B). "This association of block without depolarization with a resistance to a depolarizing drug during the block probably implies a blocking action by nicotine of the competitive type" - according to Paton and Perry (1953). It might be expected that, under these circumstances, the ganglionic response to stimulation should resemble those in the presence of other competitive inhibitors e.g. curare. This however was not seen (Fig. 43a iv). There is reason for believing that the block by nicotine was accompanied by depolarization, as small potentials resembling synaptic potentials in their size but not time course always appeared in high concentrations of nicotine. Perhaps in very high concentrations
nicotine may have a slight inhibitory action on the preganglionic terminals either preventing Ach release or blocking conduction in the fine nerve terminals (cf. Lundberg and Thesleff, 1953). There is evidence against this postulate, for Feldberg and Vartiainen (1934b) found no alteration in the Ach liberated in ganglia where transmission was completely blocked by nicotine. From the ganglionic response to stimulation in high concentrations of nicotine it seems as if the second blocking action of nicotine is accompanied by a slight depolarization, which is contrary to previous reports (Paton and Perry, 1951, 1953; Lundberg, 1951; Lundberg and Thesleff, 1953).
There has been a controversy in regard to the effects of adrenaline on transmission across synapses. Bulbring and Burn (1942b) found that low concentrations of adrenaline had a very small facilitatory effect but Marrazzi and Marrazzi (1947) only found inhibition such as Bulbring (1944) observed with high concentrations of adrenaline. Marrazzi (1939) suggested that adrenaline depressed the excitability of the post-synaptic membrane since it did not affect the conduction in the through-fibres of the inferior mesenteric ganglion. On preganglionic stimulation Bulbring (1944) reported that the perfusate contained an active principle which was not Ach or K⁺, but which resembled adrenaline in its effect on the cat's blood pressure or frog heart preparation. This section describes the effects observed on the responses of isolated ganglia by the sympathomimetic drugs, nor-adrenaline, phenylephrine, methyl amphetamine and pholedrine.

### Results

<table>
<thead>
<tr>
<th>Sympathomimetic Amine</th>
<th>Concentration required for the reduction of response (Column 3)</th>
<th>Size of response expressed as a % of control size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nor-adrenaline</td>
<td>$6 \times 10^{-5}$</td>
<td>80</td>
</tr>
<tr>
<td>Phenylephrine HCl</td>
<td>$3 \times 10^{-4}$</td>
<td>60</td>
</tr>
<tr>
<td>Methyl amphetamine</td>
<td>$1.7 \times 10^{-4}$</td>
<td>10</td>
</tr>
<tr>
<td>Pholedrine</td>
<td>$3.8 \times 10^{-4}$</td>
<td>30</td>
</tr>
</tbody>
</table>

In Table 6 the effects of different sympathomimetic drugs are compared for their ability to depress synaptic transmission. The response i.e. spike and synaptic potential, was never greater than 100 % control, i.e. facilitated, even with low concentrations of these sympathomimetic.
amines. Nor-adrenaline in a concentration of $6 \times 10^{-5}$ M has had little depressant effect. Methyl amphetamine almost completely blocked transmission. However complete recovery to control size was obtained by soaking in Krebs solution overnight. Only 60% of the spike remained after soaking in $3 \times 10^{-4}$ M phenylephrine hydrochloride, which was shown earlier (Fig. 31) in higher concentrations to produce an 80% reduction in LN wave of curarized ganglia. Another amine, pholedrine, $3.3 \times 10^{-4}$ M has produced a reduction to 32% control size, which is very little larger than the synaptic potential that is observed with complete spike blockage (usually 20-25% of control). This action of pholedrine is illustrated in Fig. 44 (ii), where a small spike response was superimposed on the synaptic potential. The after-potentials following repetitive stimulation show no trace of the late negativity, the LN wave. There was a large facilitated spike on the second response and spikes were probably superimposed on the subsequent synaptic potentials. The positive after-potential was slightly smaller and had a shorter duration than the control positive after-potential (cf. Fig. 44 iv and v). Presumably this can be attributed to fewer ganglion cells developing the large positive after-potential following a spike discharge. It may be noted that there seems no indication in this ganglion of the late component of the positive after-potential, the $P_2$ component of Eccles (1944).

Adrenaline was tested several times in these isolated sympathetic ganglia but no significant results were obtained. However adrenaline would have been oxidised by oxygen since 95% oxygen is used to oxygenate the Krebs solution and also by the amine oxidase which is known to be present in large amounts in sympathetic ganglia (Lissak, 1939; Raab and Humphreys, 1947). Therefore the more stable sympathomimetic amines were preferred.
Discussion

From these results it seems that sympathomimetic amines only have a depressant action on transmission across sympathetic synapses. There are two explanations for the failure to observe the facilitatory action of Burn and Bulbring (1942a and b) and Bulbring (1944). Firstly maximal stimuli were employed throughout these experiments, whereas Bulbring (1944) remarked that facilitation "is only observed when stimulation is submaximal, and applied at a rate below 8 a second". Secondly the concentration of sympathomimetic amines were 100 times greater than her solution of adrenaline, 1 in 100 or 1 in 200 million. In 1947 Marrazzi and Marrazzi criticized Bulbring's Figure 2 on the grounds that a very small dose of adrenaline 0.01µg there produced depression rather than the expected facilitation. However in the text Bulbring had stated that adrenaline in a concentration of 0.01 µg "once caused a depression followed by an augmentation" of the responses of the nictitating membrane. The figure must have been given to illustrate the occasional discrepancies in these experiments probably due, as is suggested, to a difference in sensitivity of the animals rather than as an indication that facilitation was obtained in the majority of experiments.

High concentrations of adrenaline were observed to have the depressant action which was described by Bulbring (1944) and by Marrazzi and Marrazzi (1947) and which was thought by them to resemble that of a curarizing agent. However the after-potentials revealed no trace of an IN wave (Fig. 44). This dissimilarity from curarizing drugs seems also to be suggested by the observation of Marrazzi and Marrazzi (1947) that an increase of the preganglionic stimulus caused some relief of the adrenaline depression. Nor is the action of adrenaline similar to an anticholin-
esterase, since there is none of the signs of the depolarization produced by the presence of unhydrolysed Ach (Section IX). As these authors point out their results make it improbable that the post-synaptic membrane is made more sensitive to Ach, which was suggested for muscle (Bulbring and Burn, 1942a). There is a possibility that adrenaline hyperpolarizes the post-synaptic membrane so that a greater depolarization than usual is required before the initiation of a spike discharge. But with such a hyperpolarization a much smaller positive after-potential would be expected, for the positive after-potential depends on the difference between the resting potential and the potassium equilibrium potential (Section VIII), so a decrease in the difference should lead to a decrease in the actual size of the positive after-potential. However the positive after-potential is hardly altered by $10^{-4} M$ pholedrine (Fig. 44, v) hence it does not seem likely that the ganglion cells had been hyperpolarized. 

Lundberg (1952) measured the demarcation potential between the ganglion and the postganglionic trunk, and usually found no alteration in the potential on close arterial injection of adrenaline or nor-adrenaline, whereas nicotine and acetylcholine (cf. Paton and Perry, 1953) caused the ganglion cells to become negative relative to the post-ganglionic trunk, i.e. nicotine and acetylcholine were shown to produce depolarization. Occasionally Lundberg (1952) observed a hyperpolarization but he concludes that "this small hyperpolarization was not the main cause of the block."

There is a large facilitation of a second response following in the wake of an earlier response (Fig. 44) which resembles the facilitation in response size observed by Marrazi and Marrazi (1947). This large facilitation of the second response was also reported by Lundberg (1952) who noted that the facilitation of the second response is quantitatively
always greater than occurs during partial blockage by tubocurarine, i.e. the blocking effect of adrenaline is not quite like that of tubocurarine. Both Marrazzi's and Lundberg's observations would be explained if adrenaline either blocked preganglionic impulses in the fine preganglionic terminals or depressed the liberation of Ach by them. This could be tested by measuring the Ach liberated by a standard preganglionic tetanus and comparing it with the control observed in the absence of adrenaline. There seems to be no references in the literature to such an experiment. It is possible that adrenaline either by hyperpolarization or reduction in safety factor prevents the invasion of as many preganglionic terminals as usual. Yet a second impulse down the preganglionic fibres would find a slight residual depolarization due to the first volley, sufficient to allow it to invade terminals which were not invaded by the first impulse.

Bessard and Posternak (1952) suggest that "l'adrenaline intervient peut-être dans la régulation de l'activité ganglionnaire mais ne paraît pas jouer un rôle déterminant dans la transmission synaptique proprement dite". No experimental support is given for this general statement. Marrazzi (1939) put forward the theory that adrenaline inhibition at the synapse prevents the over-production of adrenaline from the postganglionic endings and can be considered a homeostatic mechanism. However Adrian, Bronk and Phillips (1932) found complete cessation of activity in the sympathetic nerves, following an injection of adrenaline, and suggested that it was a reflex effect reducing the vasomotor tone. A rise in arterial blood pressure increased the rate of discharge of units in the carotid sinus nerve (Bronk and Stella, 1932). This resulted in a reflex action reducing the vasomotor tone of the blood vessels which is brought about by a decrease in the rate of discharge of impulses in sympathetic fibres.
It is not easy to find any explanation of Larrabee and Posternak's experiments where inhibition of the synaptic transmission occurred on occlusion of the aorta (Larrabee and Posternak, 1948). It seems doubtful from the present experiments whether adrenaline or nor-adrenaline liberated from the adrenal medulla would appear in the blood stream in sufficient quantities to depress significantly synaptic transmission through sympathetic ganglia. However Lundberg (1952) found that nor-adrenaline had only a fourth of the depressant action of adrenaline. Adrenaline even in the amounts expected physiologically could inhibit transmission (Lundberg, 1951, 1952). Using dihydroergotamine to reduce the sensitivity of ganglion cells to adrenaline Lundberg was able to prove that adrenaline and nor-adrenaline liberated from preganglionic sources (Bulbring, 1944) did not inhibit the normal ganglion response to preganglionic stimulation, i.e. no preganglionic source of inhibition by adrenaline.

It is suggested that the most probable explanation of the depressant action of sympathomimetic amines is that they cause either blockage of conduction into the preganglionic terminals, or inhibition of the Ach release mechanism. It is possible that in addition there is some competition for the post-synaptic receptors for Ach, i.e. a slight curare action.
Veratrine is known to possess the remarkable property of increasing the negative after-potentials of nerve and muscle responses to stimulation (see Krayer and Acheson, 1946, for detailed references). In sympathetic ganglia Lloyd (1939) found that veratrine could increase the size of the negative after-potential to 75% of the spike height. This large negative after-potential had a long time for decay, usually lasting one second. Besides this lengthening of the negative after-potential Rosenblueth and del Pozo (1942) often observed a facilitation of the spike height in response to a single preganglionic stimulus and an increase in the positive after-potential. Other effects described were repetitive firing of the ganglion cells to a single preganglionic volley, a failure to follow repetitive stimuli and the blockage of conduction sometimes pre-ganglionically but often postganglionically. When applied locally to curarized ganglia, veratrine did not seem to alter the synaptic potential or the facilitation curve (Eccles, 1944, unpublished observations). This lengthening of the negative after-potential in uncurarized ganglia is similar to the veratrine effect on muscle, where it increases the negative after-potential without altering the end-plate potential (epp) in normal or curarized muscle (Eccles et al., 1942; Kuffler, 1945). The present experiments were designed as controls for the work described earlier on synaptic potentials. Since veratrine had no effect on the potentials of curarized ganglia, the drug was tested in similar concentration on uncurarized ganglia.

**Results:**

The veratrine used in this investigation was known to be impure, containing at least two of the veratrine alkaloids one of which was
protoveratrine, the most powerful veratrum alkaloid known (Krayer, Moe and Mendez, 1944).

When present in a concentration of $1.3 \times 10^{-7} \text{M}$ veratrine had no apparent effect on the response to a single preganglionic volley. There was no alteration in spike size nor in the time course of the spike. There seemed to be a slight depression of the positive after-potential. A tenfold increase to $1.3 \times 10^{-6} \text{M}$ greatly lengthened the negative after-potential which was clearly seen in the slow record (Fig. 45a iv). The effects of veratrine were more noticeable on the responses to repetitive stimulation. In Fig. 45b ii, veratrine greatly diminished the size and duration of the fast positive after-potential ($P_1$ observed by Eccles, 1944) without any change in the later positive component, $P_2$. Increase in veratrine to $1.3 \times 10^{-6} \text{M}$ reduced the $P_1$ component by the appearance of a large negative after-potential which was superimposed on the positive after-potential. The size of the earlier component of the positive after-potential ($P_1$) has been greatly reduced yet this was not accompanied by depression of the $P_2$ component (Fig. 45b iii). Occasionally an increase in the spike response as well as an increase in the positive after-potential were observed. In Fig. 45, veratrine was added to a ganglion which had nearly recovered from a ganglion-blocking agent. The negative after-potential appeared as a hump in the middle of the positive after-potential and a further increase in veratrine concentration led to the appearance of a pronounced negative wave during the positive after-potential whose rate of recovery was faster so that the total duration of the positive after-potential was no longer than that observed in the presence of $1.3 \times 10^{-6} \text{M}$ veratrine (cf. Fig. 46 ii and iii). Incidentally it can be noticed that the spike potential was increased by addition of veratrine.
Discussion:

Veratrine has greatly increased the size and the duration of the negative after-potential as has already been described for nerve fibres (Cowan, 1933; Gasser and Grundfest, 1936), muscle (Kuffler, 1945) and the blood-circulated cervical ganglia (Lloyd, 1939; Rosenblueth and del Pozo, 1942). However there were no indications of the extremely long negativities lasting for several seconds after the ganglionic response to a single preganglionic volley (Lloyd, 1939; Rosenblueth and del Pozo, 1942).

Veratrine's action in increasing the size and duration of the negative potential is a specific action on the negative after-potential since it was shown earlier (Section VII F) that veratrine in identical concentrations did not alter, in curarized ganglia, the time course of the synaptic potential nor the size and duration of the P and LN waves. The probable explanation is that veratrine alters the mechanism responsible for the generation of the negative after-potential. This process may be a residual sodium-carrier action following the propagated response. This hypothesis, however, does not explain the action of veratrine on the positive after-potentials which were usually reduced yet occasionally were increased. It seemed essential that a pure alkaloid, preferably protoveratrine, should be employed in any further attempts at elucidating the full action of veratrine on ganglionic transmission. In summarizing the effects the earlier observations are included in the following table.

TABLE 7

<table>
<thead>
<tr>
<th>The effect of veratrine on superior cervical ganglia</th>
<th>Rosenblueth &amp; del Pozo</th>
<th>This work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demarkation potential</td>
<td>Depolarization</td>
<td>slight depolarization</td>
</tr>
<tr>
<td>Spike potential (small dose)</td>
<td>increased</td>
<td>increased(sometimes)</td>
</tr>
<tr>
<td>Spike potential (large dose)</td>
<td>?</td>
<td>decreased</td>
</tr>
<tr>
<td>Negative after-potential</td>
<td>Greatly increased</td>
<td>Greatly increased</td>
</tr>
<tr>
<td>Positive after-potential</td>
<td>sometimes increased</td>
<td>increased sometimes</td>
</tr>
</tbody>
</table>
It can be observed that in the presence of veratrine the responses of the isolated rabbit superior cervical ganglia differed little in their response to preganglionic stimulation from the blood-circulated ganglia of the cat (Lloyd, 1939; Rosenblueth and del Pozo, 1942).

STRYCHNINE

In contrast to the excitatory action of Strychnine on spinal cord reflexes, only depressant effects have been recorded on transmission through sympathetic ganglia (Feldberg and Vartiainen, 1934b; Lanari and Luco, 1939). The depressant action was thought to be comparable to the action of atropine which blocked "the response to preganglionic stimulation or to stimulant alkaloids." Lanari and Luco compared the action of strychnine to that of curare since prostigmine exerted a decurarizing action similar to that found when curare blocked transmission. In Section VII E strychnine was employed on the curarized ganglia to show that none of the potentials could have arisen from a similar inhibitory transmitter similar to that causing inhibition in the spinal cord (Eccles, Fatt and Koketsu, 1954). The following experiments were carried out to see whether strychnine itself possessed a curarizing action such as described by Feldberg and Vartiainen (1934b) and Lanari and Luco (1939).

Results:

On addition of 2.5 X 10^{-5} M strychnine the spike response was reduced by more than 50% (cf. Fig. 47a and ii). On repetitive stimulation facilitation of the second response occurred as well as a reduction in the P wave by the appearance of an LN wave. Doubling the concentration of the strychnine caused in response to a single preganglionic volley the appearance of a synaptic potential uncomplicated by superimposed spike discharges. The resemblance of strychnine to tubocurarine is even more pronounced when the response to repetitive stimulation is observed.
The synaptic potentials built up to a large negative peak which decreased during the stimulation and was followed at the end of the stimulation by a small $P$ wave which was submerged beneath the large LN wave. Soaking out in Krebs solution showed that the effects of strychnine were nearly completely reversible (Fig. 47a iv and b iv). The spike response had not fully recovered to the control size (Fig. 47a i and iv) and there was still a small LN wave following a tetanus (Fig. 47b i and iv). It should be noted that this isolated ganglion was more sensitive to strychnine than most ganglia since nearly complete removal of the spike response was more characteristic of a concentration of $5 \times 10^{-5}$ M strychnine than the weaker solution of $2.5 \times 10^{-5}$ M strychnine (Fig. 47a ii).

Discussion:

Strychnine has been shown to closely resemble curare in its action. It must therefore compete for the Ach-receptors on the post-synaptic membrane so that the ganglion cells became less sensitive to Ach whether this Ach was derived from preganglionic sources or was added to the perfusion fluid (Feldberg and Vartiainen, 1934b; Lanari and Luco, 1939). Since in high concentrations of strychnine the same type of response occurred as an addition of curare, there is no further discussion required on these potentials already described in detail (Section VI). It should be noted, however, that even at very low concentrations of strychnine, no facilitation of the spike response was ever observed. The action of strychnine was then similar to earlier reports except in one respect. Nachman- sohn (1938c) found an inhibition by strychnine of cholinesterases. This anticholinesterase action has not been confirmed since the LN wave (Fig. 47b ii and iii) was apparent as a large negativity, rising early from a positive wave. It is known that even very low concentrations of anticholinesterases lengthened the $P$ wave by inhibition of the LN wave.
(Fig. 22 and Section VII A). A possible explanation is that the rabbit's cholinesterases differed from those used by Nachmensohn (1938, a and b). It can be concluded from Fig. 47 that strychnine blocks transmission across ganglionic synapses in a similar manner to that of other ganglion-blocking agents.
Larrabee and Bronk (1947) showed that a preganglionic tetanus was followed by a prolonged potentiation (several minutes) of the ganglionic discharge evoked by single testing volleys. By stimulating only one of the preganglionic trunks to the stellate ganglion they further showed that the potentiation was restricted to test volleys in the tetanized preganglionic fibres, the excitability of the ganglion cells being depressed when tested by other preganglionic volleys and even by perfused acetylcholine. They concluded, therefore, that the post-tetanic potentiation was probably attributable to an enhanced stimulating action of the tetanized presynaptic fibres, there being an increased output of acetylcholine and/or a larger action potential. These suggestions were not tested experimentally. Lloyd (1949), in a later investigation on an analogous potentiation of monosynaptic reflexes, recorded a phase of increased presynaptic action potentials, which corresponded so closely in time course to the observed post-tetanic potentiation that he postulated a causal relationship. Further investigation (Eccles and Rall, 1951), however, has shown that this correspondence in time course was fortuitous, occurring only for the durations of conditioning tetani employed by Lloyd. In these ganglia preparations the potential changes after a preganglionic tetanus were followed for several minutes. The interpretation of the ganglionic response to a preganglionic test volley gives some indication of the excitability of the ganglion cells. Throughout this work on post-tetanic potentiation the conditioning tetanus was kept constant at 64 volleys per second for 15 seconds, and both conditioning and test volleys were applied through the same electrodes to the whole of the preganglionic nerve.
Normal ganglia

a) Isolated ganglia. The potentiation of the ganglionic spike potential (Fig. 48b) was observed to follow a time course comparable with that described by Larrabee and Bronk (1947, Fig. 6) after a comparable stimulation. It has been further observed that an initial depression of the positive component of the spike was followed by a return to normal size before being potentiated three minutes after the end of the tetanus. There is evidence (Eccles, 1935b, 1936a) that the positive after-potential of a testing volley is depressed during a pre-existent positive after-potential; hence the initial post-tetanic phase of depression is satisfactorily explained by the prolonged positive after-potential following repetitive preganglionic activation (Bronk et al., 1938). The increase in the response of ganglion cells in the early post-tetanic period may likewise be explicable as an increase in spike size normally seen when a second response falls in the positive after-potential of an earlier volley. The subsequent enhancement of both the positive component of the spike and the positive after-potential (Fig. 48b, filled circles) is possibly attributable to a late development of a post-tetanic negativity. In curarized ganglia it has been shown (Eccles, R.M., 1952b) that the P wave terminates in a late negative wave (the LN wave), which, however, with the relatively brief tetani there investigated, ran a much briefer time course than is here postulated for a late negative wave in normal ganglia. However, there is not much evidence for this late negativity (LN wave) in uncurarized ganglia.

b) Ganglia in situ. Repetitive stimulation of the preganglionic fibres led to certain changes in the response to testing preganglionic volleys. A preganglionic stimulus in the initial stages of recovery from the conditioning tetanus produced a potentiated ganglionic spike
associated with a smaller positive component of the spike and an
increase in the negative after-potential (cf. Fig. 49 i and ii). The
increase in the spike response and negative after-potential as well as
the depression of the positive after-potential indicates the presence of
a pre-existent positive after-potential. One minute after cessation of
the conditioning stimulation the test volley elicited a ganglionic
response which was almost identical to the control (cf. Fig. 49 i and iii).
However a test volley three minutes after the end of the conditioning
tetanus revealed a response with an increase in the positive component
of the spike, and the positive after-potential but which differed from
the response of isolated ganglia because it was not accompanied by a
reduction in the spike.

The initial depression of the positive component of the spike and
the positive after-potential are characteristic of all ganglia, whether
they be in blood-circulated, perfused or isolated state (Bronk, 1939;
Bronk and Larrabee, 1947; Paton and Perry, 1953). The later potentiation
of the positive component of the spike is not observed in Bronk's records
but this is to be expected as no sign of late negativity was ever seen
in isolated cat's stellate (Section XVI) but it was this late increase in
the positive component of the spike that was seen in ganglia in situ.

Curarized ganglia

When the tubocurarine was just above blocking concentration, the
testing preganglionic volley evoked a large ganglionic spike during the
post-tetanic potentiation (Fig. 50a ii, iii). The time course of potentia-
tion may be shown by plotting as ordinates the sizes of the negative
ganglionic potential, i.e. spike action potential plus synaptic potential
(Fig. 50b, open circles). The large ganglionic spike was followed by a
greatly increased P wave (cf. Fig. 50a), which ran a time course comparable with the negative ganglionic potential (filled circles, Fig. 50b). Presumably this increased P wave is largely secondary to the potentiated ganglionic spike, and its time course would thus give a more accurate record of the post-tetanic potentiation of the ganglionic spike than the negative potential, which has relatively a much larger component from the synaptic potential. However both curves of Fig. 50b show a much slower time course of post-tetanic potentiation, which differs characteristically from that for the ganglionic spike in the un-curarized ganglion (Fig. 48b). For example the summit and time to half decay occurred at about 25 and 75 seconds in Fig. 50b, as against 5 and 35 seconds in Fig. 48b (open circles). A similar slowing of time course of post-tetanic potentiation was observed by Larrabee and Bronk, (1947, Fig. 6) when they increased the duration of the conditioning tetanus.

With tubocurarine in the concentrations the control testing volley set up a P wave with little or no preceding N wave, (cf. Laporte and Lorente de Nó, 1950a; Eccles, R.M., 1952a). With post-tetanic potentiation this P wave was greatly potentiated, and a small initial N wave also appeared (Fig. 51a). The time course of post-tetanic potentiation of this P wave (Fig. 51b) corresponded approximately with that observed in less deeply curarized ganglia (Fig. 50b).

The effect of addition of anticholinesterases

In the presence of anticholinesterases large amounts of unhydrolysed acetylcholine accumulate, following a tetanus, in the vicinity of the preganglionic terminals and thereby cause a heavy depolarization of the ganglion cells (Section IX). Prostigmine in a concentration of $1.7 \times 10^{-7}$M had no effect on the excitability of the ganglion cells as measured by test volleys. Doubling the concentration of prostigmine ($3.3 \times 10^{-7}$M)
caused during the post-tetanic period, a balance to be attained between the ganglionic depression by the unhydrolysed Ach and the presynaptic potentiating factor. Thus no change occurred in the size of the ganglionic response to a test volley. With further increments in the prostigmine concentration to $1.7 \times 10^{-5}$ M, $3.3 \times 10^{-6}$ M, $6.6 \times 10^{-6}$ M there was a heavy depression of the test response until at the highest concentration no ganglionic response to a test volley occurred for three minutes after the end of the conditioning tetanus. Presumably most of the Ach liberated from the preganglionic terminals had remained unhydrolysed since all the cholinesterase had been effectively inhibited by the prostigmine. There can be no ganglionic response until the Ach concentration has been reduced by diffusion. In conclusion, it can be observed that the depression of transmission through ganglia by anticholinesterases can be explained as a result of the accumulation of Ach. Removal of the Ach by soaking in Krebs would lead to complete recovery in a few minutes. Even when these ganglia were left in a moist atmosphere of oxygen the ganglionic response recovered slowly, e.g. the excitability curve following a conditioning tetanus in the presence of $6.6 \times 10^{-6}$ M prostigmine.

Conclusions

There is no evidence that preganglionic action potentials were potentiated during post-tetanic potentiation but this conclusion applies rather to the preganglionic trunk since impulses in the preganglionic terminals were not effectively recorded (Fig. 5). However, when this recording from terminals was done with monosynaptic reflexes in the spinal cord (Eccles and Rall, 1951), no presynaptic correlate of post-tetanic potentiation was observed. It was therefore suggested that post-tetanic potentiation was not primarily attributable to an enhancement of the presynaptic
action potential (cf. Lloyd, 1949), but possibly was caused by a swelling of the presynaptic fibres induced by repetitive stimulation, an effect actually observed by Hill (1950) in giant nerve fibres. The same effect would explain ganglionic post-tetanic potentiation and also the post-tetanic potentiation of neuro-muscular transmission (Boyd, 1932; Rosenthal and Morison, 1937; Brown and von Euler, 1938; Feng, 1941; Liley and North, 1953). Presumably preganglionic impulses would liberate an increased amount of acetylcholine from the swollen presynaptic fibres. The initial depression, in the first second following the tetanus, of the orthodromic testing volley (Job and Lundberg, 1953) can probably be explained as due to a heavy reduction in the Ach store of the preganglionic fibres and the lost Ach must be replaced before potentiation of the test volley can occur. The action of anticholinesterases in increasing and prolonging the P wave of deeply curarized ganglia suggested that high concentration of tubocurarine produced a reversal of acetylcholine action from depolarization to polarization (Eccles, P.M., 1952b). Hence an increased liberation of acetylcholine during post-tetanic potentiation would also explain the potentiation of the P waves in Fig. 51. Another explanation could be that the large LN wave on which the test volley is superimposed would greatly increase any positive wave that appears.

The excitability curves (Fig. 52) following addition of anticholinesterases demonstrate that Ach in high concentrations completely prevents ganglion cells from responding to stimulation. This reduction in spike height has been shown to occur simultaneously with the heavy depolarization produced by intravenous Ach (Paton and Perry, 1953). However Paton and Perry were unable to find any simple relationship between the depolarization and the reduction in the ganglionic response.
In the last few years the problem of recording from a single cell apart from other cellular tissue has been approached from several angles. Large cells may exist isolated from others of their kind, e.g. the large plant cell, Nitella. It is even possible to dissect single nerve fibres free from the animal body with little apparent sign of injury, e.g. the large nerve fibres found in the annelids and the cephalopods. The electrical activity of giant squid axons can be recorded with external leads or with microelectrodes inserted at one of the cut ends of the axon (Cole and Curtis, 1942; Hodgkin et al., see Hodgkin, 1951). This dissection of the axon to obtain a preparation free from surrounding tissue has been applied to only one vertebrate junctional region, when Kuffler dissected out the single nerve muscle junction in amphibian muscle (1942). However the long and tedious dissection technique cannot be applied to other vertebrate junctional regions. A much more favourable and valuable method was to record nervous activity with microelectrodes. The earliest microelectrodes of steel were 20μ or more in diameter and obviously could only record activity in the vicinity of the cells. King and Gerard (1949) introduced the technique of recording cellular activity with glass microelectrodes with diameter of less than 1μ and filled originally with isotonic KC1 but later with 3M KC1 in order to lower the resistance and reduce the liquid junction potentials (Nastuk and Hodgkin, 1950). These 3M KC1 microelectrodes had an electrical resistance of 5 - 30 MΩ. Since nerve and muscle fibres possess a high membrane resistance, the potential measured across the membrane by an internal electrode will have arisen almost entirely from activity of
that particular cell or fibre and not from activity in neighbouring cells. Thus it is possible to measure the membrane potential under various conditions without the necessity of dissecting the cell, e.g. the work on frog muscle and the neuromuscular junction (Ling and Gerard, 1949; Nastuk and Hodgkin, 1950; Fatt and Katz, 1950, 1951), and it is even possible to record the electrical responses in cells which lie some millimetres below the surface e.g. the motoneurones of the spinal cord (Brock, Coombs and Eccles, 1951, 1952).

When microelectrodes were first tried on the superior cervical ganglion cells, it was soon realized that it was not often possible to have ideal conditions. The criteria for microelectrodes were very rigid, they must be about 0.5μ in diameter at the tip, they must possess an electrical resistance of 15-30 MΩ when filled with 3MKCl, and lastly they must be fairly robust so as to withstand the stress of penetrating through connective tissue without breaking. In penetrating the ganglion a considerable resistance was encountered due to the large amount of connective tissue. Some of this forms a thick sheath around the ganglion and this can be dissected off, but connective tissue is dispersed right through the ganglion and more especially around each ganglion cell where it forms a thick capsule. It seemed that it was this capsule that formed the greatest barrier to penetration of the ganglion cells themselves. The basis for this assumption was the electrical changes noted whilst penetrating a ganglion. Immediately prior to the penetration of a ganglion cell the baseline was very unsteady as if the microelectrode had suddenly had its electrical resistance increased. Following this fluctuation there was sometimes recorded a resting potential of 60-70 mV, which steadily decreased. The ganglion cell often spontaneously dis-
charged impulses, presumably due to the injury currents produced by the damage to the cell, wrought by the penetrating electrode. However, in a few experiments, it was possible to penetrate cells successfully and record action potentials from them for up to ten minutes before the resting potential decreased. For this work young rabbits were chosen so as to reduce the amount of connective tissue present in the ganglion.

In Fig. 53 the potentials are shown from a typical ganglion cell in response to single preganglionic volleys. The spike potential arose from a synaptic potential which was approximately 30% of the spike size (i). During the deterioration of the cell the resting potential was decreasing and the spike potential was also getting smaller (cf. i and iv), until the cell failed to respond. Though the afterpotentials (i and ii) were complicated by the superposition of extraneous synaptic potentials, it can clearly be seen that the response to a preganglionic volley consisted of a pre-potential which was approximately 20 mV, from which the spike arose at a particular threshold. The spike was followed by a quick positive phase, the positive component of the spike, and a slow positive wave, the true positive afterpotential. The positive component of the spike was probably greater than normal because the ganglion cells presumably would have been somewhat depolarized.

Another large ganglionic response to preganglionic stimulation is illustrated in Fig. 54 a. The spike discharge was nearly 100 mV, the largest ever recorded. Here again the spike potential took off from a point of inflexion. It is interesting to note that the ratio of synaptic potential to spike discharge was approximately 1:3 whereas in the motorneurones of the cat's ventral horn the pre-potential ratio to spike is about 1:8 on orthodromic stimulation but 1:3 on antidromic stimulation.
(Brook, Coombs and Eccles, 1951, 1952). The long duration of the spike (Fig. 54) and the long negative afterpotential may be accounted for by the fact that this cell was at approximately 28°C instead of the more usual 37°C. The synaptic potential of a heavily depolarized cell (Fig. 54b) had an extremely rapid time to decay to 1/e (6.5 msec), which was not surprising since addition of 6 X 10^-6 M nicotine to a curarized ganglion reduced the time of decay to 1/e from 80 to 19 msec when recorded with external leads (Fig. 26).

In another cell (of the same ganglion preparation) a single preganglionic volley set up a synaptic potential which occasionally fires a spike. It will be noticed that the synaptic potential was just below threshold for generation of impulses in iii.

From these few intracellular records it can be noted that the ganglionic spike is preceded by a synaptic potential which at a particular threshold gives rise to a spike discharge. The resting potentials are known to be small and the ganglion cells were all depolarized, which is probably the reason for the large positive component of the spike. The spike discharges at 37°C are usually 3-4 msec in duration but at lower temperatures are increased, 10 msec at 28°C. These experiments have not been very rewarding and only demonstrate that ganglion cells resemble other excitable tissues that have been studied, since spike discharges are preceded by a synaptic potential, generated by a presynaptic volley.
CONCLUSIONS AND SUMMARY

The generally accepted explanation of synaptic transmission in the sympathetic ganglion may be stated as follows. A nerve impulse invading the preganglionic terminals liberates Ach, which in part is destroyed by cholinesterase or diffuses into the blood stream and is lost to the ganglion, but part of the Ach comes into contact with the post-synaptic membrane. On this membrane are situated receptor areas specifically combining with Ach, which in some way causes a leakage of the resting charge on the membrane. Consequently a slight depolarization of the ganglion cell is produced by the Ach liberated from each preganglionic terminal. Summation with the depolarizations produced by other post-synaptic areas on the ganglion cell may reach the threshold level for generation of an impulse by that cell. At this level there is a sudden increase in sodium permeability which leads to a reversal of the membrane potential, i.e. to the impulse. The experimental work described in this thesis is not in conflict with this general hypothesis of synaptic transmission, but it does require further developments of this hypothesis.

Isolated rabbit superior cervical ganglia have been used throughout this work. Under strictly controlled physiological conditions these isolated ganglia survive for 48 hours after excision and their responses which resemble those of blood-circulated ganglia show little deterioration in this time. The advantages of knowing the exact concentrations of drugs, ions, glucose and oxygen in the surrounding medium with isolated preparations are offset by the complication that any substance liberated during activity will remain in a higher concentration around the ganglion cells than in the blood-circulated or perfused ganglia. On the other hand the isolated ganglion is much more convenient for electrical recording, and particularly intracellular recording.
Insertion of microelectrodes into ganglion cells showed that
resting potentials were never greater than 70-90 mV and that the impulse
was always preceded by a prepotential which could be as great as 30 mV
before firing a spike. This resting potential is virtually identical
with the values for mammalian motoneurones (Brock, Coombs and Eccles,
1951, 1952), which are the only other mammalian nerve cells so far investi-
gated. However the values for the threshold level of the pre-potential
for synaptic stimulation are two to three times higher than for moto-
neurones, though they are in very good agreement with the values for the
threshold level of the epp at the frog neuromuscular junction (Fatt and
Katz, 1951). The low value for mammalian motoneurones has also been
observed for stimulation by square pulses (Coombs, Eccles and Fatt, 1954),
hence there appears to be a genuine difference between the levels of
depolarization required to generate an impulse with the mammalian moto-
neurones on the one hand and the sympathetic ganglion cell and the frog's
neuromuscular junction of the other. When the ganglionic responses were
recorded with external leads, a positive phase was often observed, the
positive component of the spike. This could be due to a diphasic recording
of the ganglionic spike since it is difficult to obtain a completely
inactive killed end with C fibres. However, this positive component of
the spike was also observed with intracellular recording from an individual
ganglion cell. This positive potential appears to be similar to the positi-
ve phase of the cephalopod axons (Hodgkin and Katz, 1949) and the
positive potentials observed in depolarized motoneurones (Coombs, Eccles
and Fatt, 1954), and is easily distinguished from the true positive after-
potential by its earlier appearance and faster time course (Sections IX and
X). Unfortunately the microelectrode technique has proved to be technic-
ally very difficult on account of the connective tissue capsule around
each cell, hence no studies on changes in external ionic composition or on the effect of drugs have been carried out with intracellular recording.

The prepotential or the synaptic potential is a depolarization of the post-synaptic membrane by Ach. The curarizing agents reduce the size of the depolarization since they compete with Ach for the receptors on the post-synaptic membrane specific for Ach. The time constant of decay of the synaptic potential normally was 85-95 msec, but this was greatly decreased where the ganglion cells were depolarized by nicotine, which reduced the time constant to as low as 19 msec (Section VII B) or by injury produced by a penetrating microelectrode when a time constant of 6.5 msec was recorded (Section XV). The very brief synaptic potentials recorded under these conditions indicate that the active depolarizing action of the Ach is only a few milliseconds in duration, the slow decay of the normal synaptic potential being attributable to a long time constant of the membrane and not to a continuation of the depolarizing action of acetylcholine. Addition of anticholinesterases to the uncurarized ganglia caused the appearance of a prolonged negativity, due to the great prolongation of the depolarization by Ach (Section IX). It can be concluded that the depolarization of the post-synaptic membrane subsequent to the release of Ach from the preganglionic terminals is the post-synaptic event responsible for the generation of impulses.

Following the spike potential there is in the uncurarized ganglia a negative potential which is probably composed of two potentials; the true negative after-potential of the cells which have generated spike discharges and the synaptic potential of all cells. Veratrine can be employed in the analysis of this potential since it has been shown
(Sections VII, XII) that veratrine has no effect on the synaptic potential whilst greatly prolonging the negative after-potential. Little is known about the ionic mechanism generating negative after-potential, which may be due to a small amount of residual sodium carrier activity, a chloride efflux or a general increase in permeability of all ions.

The negative after-potential is followed in the normal ganglion by a positive after-potential, while in a curarized ganglion the synaptic potential is also followed by a positive wave, the P wave, with the same time course as the positive after-potential. It must not be assumed that this P wave of deeply curarized ganglia and the positive after-potential are produced by identical mechanisms, though there is evidence that both are produced by a phase of increased potassium permeability (Sections VII C, VIII). The P wave of normal uncurarized ganglia is probably a composite wave form, composed of the true positive after-potentials of the ganglion cells which fired a spike discharge and a P wave from the cells only subliminally excited, such as is observed in curarized ganglia. Antidromic stimulation could probably lead to a differentiation between these two positive potentials, for it would presumably evoke only the positive after-potential. The P wave, of deeply curarized ganglia, is unlikely to be produced by some reaction of Ach with receptors on the post-synaptic membrane. Its apparent increase in size and duration on addition of an anticholinesterase seems to be secondary to the removal of the IN wave. When ganglia are deeply curarized so that the synaptic potential is greatly depressed and when the IN wave is removed by an anticholinesterase (Section VII A), virtually a pure P wave is produced by a preganglionic volley as seen in Fig. 16c (i). The summation of P waves by repetitive stimulation can be clearly observed in Fig. 16c (ii).
It is postulated that the P wave is produced by some specific transmitter substance which acts on the post-synaptic membrane by increasing the potassium permeability. Possibly the action resembles that of the central inhibitory transmitter which opens up the post-synaptic membrane to all small ions (Coombs, Eccles and Fatt, 1954). However the ineffectiveness of strychnine as a blocking agent indicates that the transmitter substance is not identical with the central inhibitory transmitter.

In the curarized ganglion the P wave is followed by a prolonged negativity, the IN wave. This IN wave may even build up during a tetanus (Figs. 21, 22, 23b) though it attains its maximum several seconds after the end of the tetanus (Figs. 30, 32, 33). The most remarkable fact is that anticholinesterases depress the IN wave mechanism. In Section vii C, it was suggested that the postganglionic receptors, responsible for the production of IN waves, may possess a type of cholinesterase configuration (Fig. 37) so that a specific combination could occur with acetylcholine and affect the membrane just as with the synaptic potential so giving the late negativity. This hypothesis certainly explains the depression by anticholinesterases of the IN wave (Figs. 22, 23b, 32, 33), since anticholinesterases would combine specifically with the cholinesterase-like receptors and thereby block these receptors in the same manner as the curarizing drugs block the Ach receptors responsible for the synaptic potential.

In conclusion the modified hypothesis of transmission through sympathetic ganglia can be stated in the following manner. The depolarizing action of Ach leads to a post-synaptic potential which builds up to a particular threshold before firing a spike. This spike discharge is followed by negative and positive after-potentials. In the presence of
a ganglion-blocking drug the post-synaptic potential is unable to reach the threshold for firing a spike and it decrements exponentially with the time constant of the ganglionic membrane (about 90 usec.). This synaptic potential is followed by P and LN waves. The former has been postulated to be produced by a transmitter substance other than acetylcholine while the latter seems to be due to a more peripheral action of acetylcholine on cholinesterase-like receptors. The two postulated actions of Ach, i.e. the initial depolarization and the LN wave can be diminished by curarizing drugs and anticholinesterases respectively leaving virtually an uncomplicated P wave. It seems likely that the synaptic potential and LN wave both resemble the epp and the synaptic potential of motoneurones in being produced by an increased permeability to all ions. On the other hand the positive after-potential and the P wave are probably phases of increased permeability to potassium ions alone.

Some of the problems that have not been answered may be now considered. (i) Why do anticholinesterases which greatly prolong the Ach depolarization of the post-synaptic membrane of uncurarized ganglia have little effect on the Ach depolarization i.e. the synaptic potential, of curarized ganglia? This is a similar problem to that of transmission at the neuro-muscular junction where it was found (Fatt & Katz, 1951) that anticholinesterases prolonged the depolarizing action of Ach in the uncurarized muscle more than in the curarized muscle. (ii) The negative after-potential of a normal ganglion is probably due to a prolongation of the increase of ionic permeability during the spike. However this does not explain why the negative after-potential is lengthened by veratrine while the synaptic potential is unaltered. (iii) The P wave of curarized ganglia has been
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postulated to be produced by a transmitter other than Ach, yet the nature and origin of the transmitter is unknown. Apparently it is neither adrenaline nor the central inhibitory transmitter. It could arise from a specific group of inhibitory fibres but further investigation on this problem is required. (iv) The whole of the ionic mechanism occurring during the P wave is not understood, whether it is entirely due to an increase in potassium permeability or the permeability to all small ions as with the IPSP of the spinal cord. (v) There is a possibility that the IN wave is normally present in uncurarized ganglia but is submerged under the P2 wave. (vi) Finally there is the problem of generation of the IN wave. If it is due to a reaction between cholinesterase-like receptors and Ach, why is the Ach action so prolonged? Does the binding of the enzyme to the membrane inactivate its hydrolysing power?
I wish to thank Professor J.C. Eccles for acting as supervisor of this research. I am also indebted to Professor A.L. Hodgkin for the equipment that enabled me to use microelectrodes during my stay in Cambridge. For the equipment which has been employed for most of the experiments described in this thesis, I thank Mr. J.S. Coombs, Mr. G.J. Winsbury and Mr. H.C. Daynes. In the preparation of this thesis I have to thank Miss R. Burkitt, Mr. A. Chapman and Mr. V. Paral for their technical assistance.
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Fig. 1. Photograph of chamber used for soaking and recording. Note ganglion in position on electrodes.

Fig. 2. Schematic drawing of ganglion preparation showing to left, stimulating leads on preganglionic trunk, then earthed plate, and the five positions of recording electrodes as in text.
Fig. 3. Action potentials set up by single preganglionic volleys recorded with R3 R5 leads (cf. Fig. 2). In (a) (i), (ii) and (iii), preganglionic stimulus progressively increased to give maximum Sb spike response. Time scale 14/sec. Potential scale 0.5 mV.

Fig. 3b. Responses of three ganglia to maximum stimulation. Upper time scale, 100 msec. for i and ii; lower time scale, 100/sec. Potential scales, 0.5 mV.
Fig. 4. Action potentials with R3R5 leads set up by repetitive preganglionic volleys at (i) 32 (ii) 56 (iii) 125/sec; (v) 40/sec. Time scale (iv), 10/sec for (i), (ii), (iii); (vi), 1/sec for (v). Potential scale, 1.0 mV.

Fig. 5. Action potentials with R3R5 leads (cf. Fig. 2) set up by single preganglionic stimuli, maximal for both the fast and slow fibres. (i) is control response, (ii) the response 5 sec. after a tetanus (60/sec for 15 sec.). Time scale, 130/sec. Potential scale, 0.5 mV.

Fig. 6. Action potential with R3R5 leads. Time scale 130/sec. Potential scale, 1.0 mV.
Fig. 7. The ganglionic responses recorded with blood-supply intact.

(a) Responses to single preganglionic volleys at different sweep speeds. Time scale, 100/sec for ii alone. Potential scale, 1.0 mV.

(b) Responses to repetitive preganglionic volleys in a ganglion, with blood supply intact, at (i) 32 (ii) 50 (iii) 100/sec. Time scale, 10/sec. Potential scale, 1.0 mV.
Fig. 8. Preganglionic recording (R1 R2 leads) of the preganglionic volley (i) control, (ii) after soaking in 8.7 x 10^{-5} M Tubocurarine, (iii) 4.3 x 10^{-5} M eserine, (iv) 5 x 10^{-5} M strychnine, (v) control, (vi) 2.5 x 10^{-5} M nicotine.

Time scale, 100/sec. Potential scale, 0.25 mV.
Fig. 9a. Ganglionic action potentials set up by single preganglionic volleys: (i) before curarization; (ii) partial curarization (d-tubocurarine $1.6 \times 10^{-5}$M); (iii) complete curarization ($3 \times 10^{-2}$M). Time scale, 14/sec. Potential scale for (i) 0.5 mV, for (ii) and (iii) 0.2 mV.

Fig. 9b. Ganglionic action potentials set up by single preganglionic volleys: (a) synaptic potential followed by P and LN waves (d-tubocurarine $2.5 \times 10^{-5}$M); (b) $8 \times 10^{-7}$M d-tubocurarine; (c) addition of $3 \times 10^{-5}$M prostigmine to (b). Time scale, 1/sec. Potential scale, 0.1 mV.

Fig. 10. The effect of just-blocking tubocurarine ($1.6 \times 10^{-5}$M) on repetitive preganglionic stimulation at (i) 20, (ii) 35, (iii) 60/sec. Time scale, 14/sec. Potential scale, 0.2 mV.
Fig. 11. The effect of $1 \times 10^{-4}$M dihydro-β-erythroidine on the ganglionic responses to preganglionic stimulation recorded with silver electrodes (filled circles) or platinum electrodes (broken line). Duration of tetanus between the arrow and the beginning of the graph.
Fig. 12. The effect of increasing tubocurarine on the ganglionic after-potentials following preganglionic stimulation at 20/sec for a period indicated by the two arrows. Continuous line, before curarization; open circles, $1.6 \times 10^{-6}$M tubocurarine; line broken by filled circles, $3.2 \times 10^{-6}$M; line broken by open circles, $1.6 \times 10^{-5}$M tubocurarine; filled circles, $3.2 \times 10^{-5}$M.

Fig. 13. The size of the synaptic potential uncomplicated by any spike discharges is plotted against the height of the LN wave on increasing the strength of the preganglionic volley from threshold to maximum for sympathetic ganglion cells. Concentration of dihydro-β-erythroidine, $4.3 \times 10^{-5}$M.
Fig. 14. The effects produced by various concentrations of the curarine from Strychnos toxifera on ganglionic response to single preganglionic volleys. (i) $7.6 \times 10^{-4}$ M; (ii) $1.5 \times 10^{-4}$ M; (iii) $3 \times 10^{-4}$ M; (iv) repetitive preganglionic stimulation 20/sec, curarine concentration $1.5 \times 10^{-4}$ M; (v) and (vi) 60/sec stimulation at $3 \times 10^{-4}$ M and $4 \times 10^{-4}$ M curarine respectively. Time scales, 14/sec. Potential scales, 0.1 mV.

Fig. 15. Ganglionic responses to single (i) and (ii) or repetitive preganglionic volleys in the presence of c-toxiferin III: (i) and (iii) $3.23 \times 10^{-4}$ M; (ii) and (iv) $6.45 \times 10^{-4}$ M. Time scale for (i) and (ii), 14/sec; for (iii) and (iv) in seconds. Potential scale, 0.2 mV for all records.
Fig. 16a. Ganglionic responses to preganglionic volleys in the presence of dihydro-β-erythroidine.

(a) Response to a single volley. Concentration of drug, $2 \times 10^{-4}$ M. Time, 100 msec. Potential scale, 0.5 mV.

(b) Response to a single volley at two sweep speeds. Concentration, $1 \times 10^{-} M$. Time scales, 10/sec for (i) and 1/sec for (ii). Potential scale, 0.25 mV.

(c) Response to single and repetitive stimulation after soaking in $3 \times 10^{-7}$ M dihydro-β-erythroidine. Time scale, 1/sec. Potential scales 0.25 mV for (i) and 0.25 mV for (ii), (iii) and (iv).
Fig. 17. The ganglionic responses to preganglionic stimulation when the blood supply was intact. (i) Control; (ii), (iii) and (iv) responses after injection of 1 mg/Kg of dihydro-β-erythroidine. Time scale below (iv) for (iv) alone 1/sec, time scale for other records, 10/sec. Potential scale, 1.0 mV.

Fig. 18. Ganglionic responses to preganglionic stimulation, 25/sec when the blood supply was intact. (i) Control; (ii) after injection of 5 mg C6 ace/Kg, (iii) after a further 10 mgs of C6/Kg. Potential scales, 1.0 mV for (ii) and (iii).
Fig. 19. The ganglionic responses to preganglionic stimulation in the presence of $2.1 \times 10^{-4} \text{ M} C_6$. (i) a single preganglionic volley; (ii) and (iii) at 40/sec. Time scales, upper is 10/sec for (ii); lower, 1/sec for (iii). Potential scale, 0.5 mV.

Fig. 20. The ganglionic response to single preganglionic volleys in the frog superior cervical ganglia. (i) after soaking in $2.1 \times 10^{-4} \text{ M} C_6$; (ii) after 20 minutes soaking out from (i).
Fig. 21. The effect of duration of repetitive preganglionic stimulation at 20/sec on the time courses of the ganglionic potentials during and after the tetanus. First arrow marks the start of each tetanus, and the subsequent arrows signal the end of the four tetani, being identified by the appropriate symbol. The slight positive dip at the end of the tetanus was neglected. Concentration of d-tubocurarine, $8 \times 10^{-3}$M.

Fig. 22. The effect of d-tubocurarine followed by prostigmine on the ganglionic potentials recorded on stimulation of the preganglionic fibres at 20/sec. (a) d-tubocurarine concentration $1.6 \times 10^{-4}$M; (b) d-tubocurarine, $8 \times 10^{-5}$M; (c), (d) and (e) on addition of prostigmine, $3 \times 10^{-7}$M, $3 \times 10^{-6}$M, $6 \times 10^{-6}$M respectively.
Fig. 23a. Ganglionic potentials set up by single preganglionic volleys with varying dose of c-dihydrotoxiferin I: (i) $3.5 \times 10^{-5}$ M; (ii) $7 \times 10^{-5}$ M. Note that initial brief spike-like wave is synaptic potential with following VN wave. Time scale, 1/sec. Potential scales, 0.5 mV for (i), 0.05 mV for (ii).

Fig. 23b. Ganglionic potentials set up by repetitive preganglionic volleys at 20/sec, in presence of c-dihydrotoxiferin I at concentrations: (i) $3.5 \times 10^{-5}$ M; (ii) $7 \times 10^{-5}$ M; (iii) prostigmine $3 \times 10^{-6}$ M added to (ii). Time scale in seconds. Potential scales, 0.5 mV for (i), 0.5 mV for (ii) and (iii).
Fig. 24. Ganglionic potentials to repetitive preganglionic stimulation at 40/sec. Small broken line after soaking in 4.3 X 10^{-5}M dihydro-β-erythroidine; filled circles after addition of 2.4 X 10^{-6}M eserine; large broken line after increasing the concentration of dihydro-β-erythroidine to 1 X 10^{-4}M, the eserine concentration being unaltered. Tetanus duration from arrow to beginning of curve.

Fig. 25. Ganglionic potentials set up by repetitive preganglionic stimulation at 40/sec. (i) in the presence of 4 X 10^{-5}M β-erythroidine, (ii) and (iii) addition of prostigmine 3 X 10^{-6} M, and 3 X 10^{-5}M respectively to (i) Time scale, in seconds. Potential scale 0.5 mV.
Fig. 26. The effect of nicotine on synaptic potentials.

(a) After soaking in (i) $7 \times 10^{-5}$M dihydro-β-erythroidine. (ii) and (iii) on addition of $6 \times 10^{-6}$M, $6 \times 10^{-6}$M nicotine to (i), (iv) $1.4 \times 10^{-5}$M dihydro-β-erythroidine and $6 \times 10^{-6}$M nicotine.

(b) After soaking in (i) $1 \times 10^{-4}$M dihydro-β-erythroidine; the same ganglion in $2 \times 10^{-4}$M dihydro-β-erythroidine (ii) and (iii) on addition of $3 \times 10^{-6}$M and $6 \times 10^{-6}$M nicotine respectively.

(c) In another ganglion on soaking in
(i) $1.2 \times 10^{-4}$M dihydro-β-erythroidine; (ii) addition of $5 \times 10^{-6}$M nicotine to (i); (iii) soaking in $1.2 \times 10^{-4}$M dihydro-β-erythroidine; (iv) addition of $2.5 \times 10^{-6}$M eserine to (iii). Time scale for all records, 100/sec. Potential scales, 0.2 mV.
Fig. 27. The effect of nicotine on the ganglionic potentials of a curarized ganglion following repetitive stimulation at 40/sec. (i) $1.2 \times 10^{-6}$ M dihydro-β-erythroidine, (ii) on addition of $6 \times 10^{-6}$ M nicotine to (i); (iv) after soaking in $1.2 \times 10^{-4}$ M dihydro-β-erythroidine alone; (v) on addition of $2.5 \times 10^{-6}$ M eserine to (iv). (iii) a record from another ganglion after soaking in $1.2 \times 10^{-4}$ M dihydro-β-erythroidine and $6 \times 10^{-6}$ M nicotine to show a more prominent IN wave. Time scale, 1/sec. Potential scale, 0.2 mV.
Fig. 28. Ganglionic potentials set up by preganglionic stimulation at 20/sec, for about 0.9 sec in (a) and about 0.1 sec in (b). Tubocurarine concentration 8 X 10^{-5} M throughout. (i) External potassium 6.2 mM; (ii) and (iii) external potassium reduced to 3.6 and 2.4 mM respectively; (iv) 10 min and (v) 90 min soaking in external potassium 1.2 mM; (vi) after 1 hour in 6.2 mM potassium. Time scale, 1/sec. Potential scale, 0.1 mV.

Fig. 29. Ganglionic action potentials set up by preganglionic stimulation at 20/sec in presence of 8 X 10^{-5} M tubocurarine and continuous line 6.2 mM K^+; filled circles, 11.8 mM K^+; circles with crosses, 16 mM K^+. Tetanus duration indicated from first arrow to one of the other three arrows, it being increased inadvertently during the series.
Fig. 30. The effect produced by nor-adrenaline on the ganglionic potentials of curarized ganglia. The broken line indicates the potential following the end of the tetanus in the presence of $2 \times 10^{-4}$ M dihydro-$\beta$-erythroidine; filled circles after the addition of $6 \times 10^{-5}$ M nor-adrenaline.

Fig. 31. The effect produced by phenylephrine hydrochloride on the ganglionic potentials of curarized ganglia. Line broken infrequently by dashes records potentials in $2 \times 10^{-4}$ M dihydro-$\beta$-erythroidine; filled circles and smaller broken line the effect of addition to above of phenylephrine $2 \times 10^{-4}$ M and $4.6 \times 10^{-4}$ M respectively.
Fig. 32. Ganglionic responses to a preganglionic repetitive stimulus at 40/sec which ran between arrow and beginning of curve; line broken very frequently is the record after soaking in $3 \times 10^{-4}$M dihydro-$\beta$-erythroidine; curve composed of large dashes and curve of filled circles after addition of $1 \times 10^{-5}$M and $2.5 \times 10^{-5}$M strychnine respectively; large positive curve on addition of $5 \times 10^{-5}$M DFP to the latter.
Fig. 33. Ganglionic responses to repetitive preganglionic stimulation at 40/sec. Beginning of tetanus marked by arrow, the end by the beginning of the curve. Broken line upwards, $2 \times 10^{-4}$M dihydro-β-erythroidine; curve with double circles, $3 \times 10^{-4}$M dihydro-β-erythroidine; small broken line on addition of $2.4 \times 10^{-5}$M eserine; the line of filled circles and line broken by dots on the addition of strychnine $1 \times 10^{-5}$M, $2.5 \times 10^{-5}$M respectively.
Fig. 3c. Ganglionic responses to preganglionic stimulation are shown in (a) to single volleys in (b) to repetitive stimulation at 40/sec. (i) illustrates the responses after soaking in $1.4 \times 10^{-4}$M dihydro-2-erythroidine and (ii) and (iii) on addition of veratrine in concentrations of $1.3 \times 10^{-4}$M and $1.3 \times 10^{-5}$M respectively. Time scales, 100/sec for (a), 1/sec for (b). Potential scales, 0.2 mV for (a) 0.5 mV for (b).
Fig. 35. The relative sizes of the initial negative potentials (spike and/or synaptic potential) set up in the ganglion by single preganglionic volleys are plotted as percentage of the control normal spike potential. Abscissae are the negative logarithms of the corresponding molar concentrations of d-tubocurarine. Pooled results of ten experiments. Potentials below 25% are pure synaptic potentials; with larger potentials, spike are superimposed.

Fig. 36. Comparison of the four curarines plotted as in Fig. 36. Circles with crosses, c-toxiferin II; open circles, curarine from Strychnos toxifera; filled circles, c-dihydro-toxiferin I. Values from two, two and four experiments respectively. Broken line gives d-tubocurarine chloride curve of Fig. 35. Dihydro-3-erythroidine gave an identical curve to tubocurarine and was therefore not add...
Fig. 37. This is a schematic drawing of a preganglionic termination and its relationship to the post-synaptic membrane. The specific cholinesterase is mainly present in the preganglionic terminal itself though probably some of it is distributed in the extracellular space, i.e. the crosses here signify the presence of both cholinesterases. The post-synaptic membrane is composed of different receptor areas. Those labelled SP and P are responsible for the synaptic potential and P wave respectively. The more peripheral areas which may contain non-specific cholinesterase receptors are denoted by LN since it is postulated that they produce the LN wave.
Fig. 38. The ganglionic response to a single preganglionic volley on altering the extracellular potassium concentration (i) 26 hours soaking in normal K⁺ (5 mM) (ii) 8 mins. in 15 mM K⁺ (iii) 6 mins. in 0 mM K⁺ (iv) a further 20 mins. in 0 mM K⁺ (v) after soaking 30 min. in normal K⁺ (5 mM). Time scale, 10/sec. Potential scale, 0.5 mV.

Fig. 39. The ganglionic responses to repetitive stimulation, at 40/sec, on varying the potassium concentration. (i) two hours after excision and soaking in 5 mM K⁺ (ii) 19 min. soaking in 15 mM K⁺ (iii) 22 min. soaking in 0 mM K⁺ (iv) 30 min. soaking in the control solution 5 mM K⁺. Time scale, 10/sec. Potential scale, 0.5 mV.
Fig. 40. The ganglionic response to a single preganglionic volley recorded after soaking in an anticholinesterase, Nu 2126. (i) control shortly after excision (ii) after soaking in $3.4 \times 10^{-7}$M (iii) $3.4 \times 10^{-5}$M (iv) Control after one hour's soaking in Krebs solution. Time scale, 10/sec. Potential scale, 1 mV.

Fig. 41.

(a) The relative sizes of the ganglionic response to a single preganglionic volley are plotted as percentages of the control normal spike potential. Abscissae are the negative logarithms of the Nu 1250 concentrations. Results are taken from three experiments.

(b) Comparison of the ganglionic responses to single preganglionic volleys are plotted against the concentration of another anticholinesterase Nu 2126. Results are taken from three experiments (one partly illustrated in Fig. 40).
Fig. 42. Ganglionic responses to repetitive stimulation at 40/sec. (i) and (v) control tetani; (ii) and (vi) after addition of $3.3 \times 10^{-5}$M prostigmine. (iv) and (viii) are records of the same tetani taken at a slower speed (vii equalling 1/sec). (viii) The response of the same ganglion to $2.4 \times 10^{-5}$M eserine. Time scales, (vii) 1/sec for (iv) and (viii); (iii) 10/sec for other records. Potential scale, 1 mV.
Fig. 43. The effect of nicotine on the ganglion responses to single preganglionic volleys (a) or repetitive volleys at 40/sec (b). (i) control responses; (ii), (iii) and (iv) after soaking in nicotine $6 \times 10^{-7}$M, $1.2 \times 10^{-6}$M, and $6 \times 10^{-6}$M respectively (v) recovery after soaking one hour in Krebs solution. Time scales, 10/sec for (a), 1/sec. for (b). Potential scale 1 mV. Arrow on a(ii) marks the beginning of the positive after-potential.
Fig. 44. The ganglionic responses to preganglionic stimulation are illustrated before (i) and (iv) and after (ii) and (v) the addition of $3.8 \times 10^{-5}$M pholadine. Note the spike heights during the tetanus are missing as they were too faint on the trace (iv). Time scale 10/sec for (a), 1/sec for (b). Potential scale, 0.5 mV.

Fig. 45. The ganglionic responses to preganglionic stimulation on addition of veratrine. (a) single volleys (b) repetitive stimulation at 40/sec. (i) Control (ii) and (iii) after soaking in veratrine, $1.3 \times 10^{-7}$M, $1.3 \times 10^{-5}$M respectively. (iv) is the response above (iii) at the slow speed of (b).
Time scales, 10/sec for (a); 1/sec for (b) and (a iv). Potential scale, 1 mV.
Fig. 46. The ganglionic response of another ganglion to repetitive stimulation at 40/sec. (i) control, (ii) and (iii) after addition of 1.3 \times 10^{-7} M, 1.3 \times 10^{-5} M veratrine respectively. Time scale, 1/sec. Potential scale, 1 mV.
Fig. 47. Ganglionic responses to single and repetitive preganglionic stimulation (40/sec) after addition of strychnine. (i) control, (ii) and (iii) after strychnine, 2.5 X 10^{-5}M and 5 X 10^{-5}M, (iv) control on recovery time scales, 10/sec for (a); 1/sec for (b). Potential scales, 1 mV for (i) and (ii); 0.2 mV for (iii); and 1 mV for (iv).
Fig. 48.
(a) Action potentials set up by single preganglionic volleys: (i) Control, (ii), (iii) and (iv) 5, 60, 120 secs. after a conditioning preganglionic tetanus, 6/sec for 15 sec. Time scale, 10/sec. Potential scale, 1 mV.

(b) Open circles plot ganglionic spike height as percentage of control. Time scale measured from onset of conditioning tetanus, hence first point is at 5 sec. post-tetanically. Filled circles similarly plot size of P wave as percentage of control, but value plotted downwards (see scale). Broken lines show control levels.
Fig. 49. Action potentials set up by single preganglionic volleys in a ganglion with blood supply intact. (i) control (taken at the wrong sweep speed), (ii), (iii) and (iv) at 5, 60, 120 minutes after a conditioning volley, 64/sec. for 15 sec. Time scale for (ii), (iii) and (iv), 10/sec. Potential scale, 1 mV.
Fig. 50. As in Fig. 48a, but for a ganglion that had been soaked in $5.2 \times 10^{-5}$ M $\alpha$-tubocurarine, (i) being control and (ii) and (iii) the responses at 5 and 75 sec. post-tetanically. Time scale, 14/sec. Potential scale, 0.1 mV.

(b) Plotting as in Fig. 48b. Note that ordinate scale for filled circles gives multiples of control P wave, not percentages.

Fig. 51. (a) As in Fig. 50a, but for a ganglion in $1.6 \times 10^{-4}$ M $\alpha$-tubocurarine, (i) being control and (ii), (iii) and (iv) 5, 45 and 75 sec. post-tetanically. Time scale, 14/sec. Potential scale, 0.1 mV.

(b) Plotting of P wave as in Fig. 50b, but as percentage of control value.
Fig. 52. The height of responses to single preganglionic volleys following a conditioning tetanus (64/sec for 15 sec) are plotted against time on a logarithmic scale. On the x axis the arrow denotes the end of the tetanus and the seconds after the end of the tetanus are marked. The filled-in circles are the control curve; The open circles $1.6 \times 10^{-7}$M prostigmine; $\Theta$ after soaking in $3.3 \times 10^{-7}$M; $\bigcirc$ in $1.6 \times 10^{-6}$M; $\bigotimes$ in $3.3 \times 10^{-5}$M and $\bigcirc$ in $1.6 \times 10^{-5}$M prostigmine.
**Fig. 53.** Responses of a ganglion cell to a single preganglionic volley recorded intracellularly with a microelectrode. (i), (ii), (iii) and (iv) are recorded as the cell deteriorates. Time scale, 50/sec. Potential scale, 40 mV.

**Fig. 54a** (a) The response of another ganglion cell to a single preganglionic volley but the temperature of the Krebs solution was only 28°C. Duration of sweep, 50 msec. Potential scale, 60 mV.

**Fig. 54b(i)** (b) Response to a single preganglionic volley of a heavily depolarized ganglion cell. Occasionally an impulse is fired (ii) and (iii). Time scale, 50/sec. Potential scale, 40 mV.