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STUDIES IN SYNAPTIC

TRANSMISSION

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF  
SCIENCE IN PURE SCIENCE  
OF THE  
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BY

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1958



DECLARATION

I hereby declare that this thesis

- (a) is based upon original research work which I have personally carried out, often in collaboration with my colleagues, to whom appropriate reference has been made in the text and on page (b)
- (b) has been composed by me; and
- (c) has not been submitted nor accepted for any other degree.

All references cited in this thesis have been consulted by me personally.

March 1958

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In none of the work reported in this thesis was I working under supervision as a junior colleague; in those cases marked with an asterisk the work was done by a research team, each member of which played an equal role in the investigation; in the remaining cases, in addition to taking part in the practical experimentation, I was personally responsible for the general direction of the work.

L I S T   O F   C O N T E N T S

	<u>Page</u>
<u>GENERAL INTRODUCTION</u> .....	1
<u>PART I - STUDIES ON SYMPATHETIC GANGLIA</u> .....	10
CHAPTER I - <u>Introduction</u> .....	11
CHAPTER II - <u>Review of the literature</u> ...	14
(1) Phases of Ganglionic Transmission.	14
(2) Factors affecting Transmission....	18
(3) Complicating factors in cholinergic transmission.....	35
CHAPTER III - <u>Release of Acetylcholine</u> ..	39
Introduction .....	39
Methods .....	41
Results .....	42
Discussion .....	54
CHAPTER IV - <u>Ganglion cell responses</u> ...	61
Introduction .....	61
Methods .....	63
Results .....	66
Discussion .....	72

	<u>Page</u>
CHAPTER V - <u>Block of transmission</u> .....	75
Introduction .....	75
A. Analysis of block by ganglion- cell recording .....	76
Methods .....	76
Results .....	76
Discussion .....	78
B. The effects of muscarine .....	82
Introduction .....	82
Methods .....	85
Results .....	86
Discussion .....	94
CHAPTER VI - <u>Effects of Denervation</u> .....	98
Introduction .....	98
Methods .....	100
Results .....	101
Discussion .....	109
CHAPTER VII - <u>Ganglion Metabolism</u> .....	114
Introduction .....	114
Methods .....	116
Results .....	117
Discussion .....	121

	<u>Page</u>
<u>PART II - STUDIES ON PARASYMPATHETIC GANGLIA ..</u>	127
CHAPTER VIII - <u>Introduction</u> .....	128
CHAPTER IX - <u>Ganglion cell responses</u> ...	131
Methods .....	132
Results .....	135
Discussion .....	141
CHAPTER X - <u>Block of Transmission</u> ....	147
Methods .....	149
Results .....	150
Discussion .....	160
<u>PART III - STUDIES ON THE SPINAL CORD</u> .....	165
CHAPTER XI - <u>Introduction and Review of the Literature</u> .....	166
CHAPTER XII - <u>Acetylcholine and Spinal Cord Activity</u> .....	171
Introduction .....	171
Methods .....	172
Results .....	176
Discussion .....	182
CHAPTER XIII - <u>Antidromic Vasodilatation</u> .....	186
Introduction .....	186
Methods .....	189
Results .....	191
Discussion .....	197
<u>REFERENCES</u> .....	(1) - (xvi)

GENERAL  
INTRODUCTION

Probably the greatest single contribution to public health that can at present be envisaged, is a practicable curative treatment for one or more of the common types of psychosis. At present nearly 50% of the hospital beds in this country are occupied by the mentally sick; and recent estimates suggest that nearly 1 in 10 of the adult population is hospitalised at one time or another during life for treatment of a mental disorder. Such figures make the problems of infectious disease, of malignant disease, and even of degenerative disease pale into insignificance.

At present the treatment of the mentally sick takes two main forms - first, the empiric treatment of the acutely psychotic by heroic measures such as electro-shock convulsive therapy or prefrontal leucotomy, and secondly, the individual treatment of the neurotic or border-line psychotic by psycho-analytical methods. That the heroic measures are frequently attended by partial success is undisputed; that they are of widespread applicability is doubtful; that they are based upon a rational appreciation of the essential lesion they are designed to cure is certainly untrue.

Psycho-analytical methods represented the first really rational approach to the problem of the treatment of

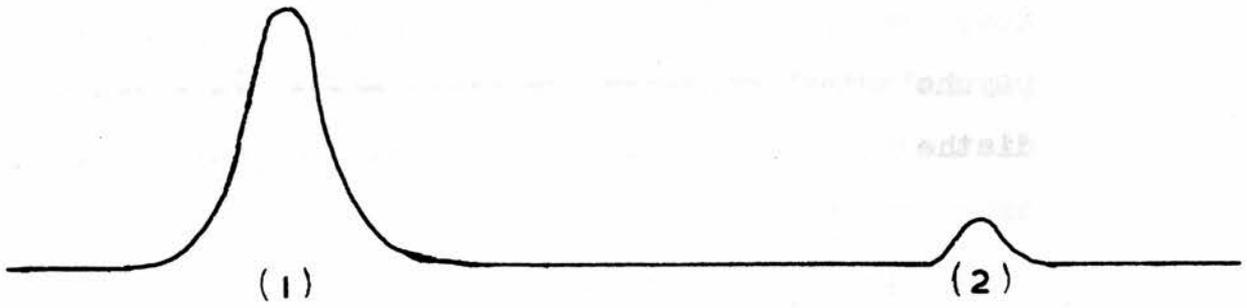
mental disease. That they often achieve striking success is indubitable; but the success is dearly won in terms of the expenditure of time on the part of highly skilled and intensively trained staff. The treatment is, furthermore, often inapplicable to the severely psychotic patient. In the treatment of the neuroses alone it is doubtful whether it would ever be practicable to train sufficient analysts to tackle more than a fraction of the total sufferers.

The psycho-analytical approach to neurosis is highly individualistic. It is an attempt to discover the emotional cause of the disorder, an emotional cause which may well be deeply subconscious, and to treat by explanation, persuading the patient to compensate on the conscious level for the emotional cause. Thus the emotional cause is regarded as peculiar to the individual, and must therefore be sought afresh in each patient. That there may be common patterns of such emotional causes is evident, but it is equally evident that no two individuals can ever have a complete series of identical emotional experiences.

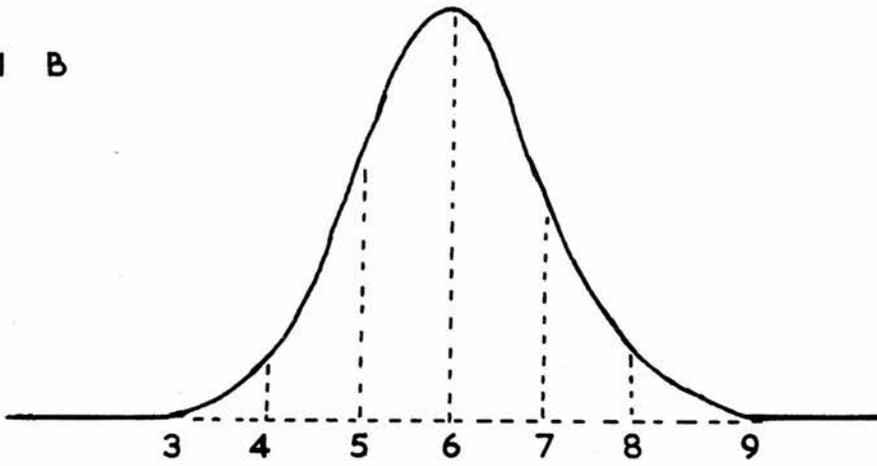
Is there then any common factor underlying the various emotional causes of neurosis? Is there a basis for explaining why an experience shared by two individuals will be an adequate emotional cause for the development of neurosis

Fig 1

Model A



Model B



in one and not in the other; and is this basis solely determined by the previous emotional history of the two subjects or is it partly determined by somatic rather than by psychological factors? In other words, is there a physical diathesis which predisposes to neurosis and psychosis on exposure to emotional stress?

It has frequently been remarked that the neurotic and the psychotic exhibit behavioural tendencies which are merely exaggerations of the normal parameters of behaviour. If it were possible accurately to measure a parameter of behaviour and consequently to plot its frequency distribution in the population, one might get any of an infinite variety of patterns. Of these let us consider two, neither being inherently improbable. Both of these hypothetical frequency distributions ~~are~~ illustrated in Fig. 1.

We might regard these distributions as illustrative of the degree of introversion of a population of individuals. In Model A we have at (1) a normally distributed population of non-psychotic individuals more or less introverted, and at (2) a normally distributed group (much smaller in total size) of more or less extremely introverted individuals. Such a model would represent the view that schizophrenic withdrawal from reality is an abnormality rather than an exaggeration of a normal characteristic. Model B on the other hand, seeks to

represent the introversion of schizophrenia as the exaggerated tail end of a distribution of degrees of introversion found in so-called normal subjects - i.e. as the exaggeration that is always found in the minority in respect of any variable in biology that is normally distributed. According to such a view schizophrenia would be regarded as an exaggeration of a normal characteristic rather than as an abnormality.

Thus, in a similar frequency distribution of the parameter height, the normal population includes a few individuals who are unusual, but not abnormal, in being either very short or very tall. But, whereas a very short man is not abnormal in this sense, an achondroplastic dwarf of the same stature is, in fact, abnormal and would not be expected to fall into the usual pattern of distribution of heights. Thus, the achondroplasics might be expected to be, as a group, as differentiated from the main distribution as (2) is from (1) in Model A. Which then is the correct model for the distribution of introversion as an individual parameter?

Let us assume for the moment that Model B is correct. There are a number of interesting consequences:

1. an individual falling, in degree of introversion, between 3 and 4 or between 8 and 9, is regarded as psychotic;
2. an individual falling between 4 and 5 or between

7 and 8 is regarded as neurotic;

3. an emotional stress which causes a shift to the right in the degree of introversion will, in the case of
  - (a) an individual between 5 and 6 cause no detectable abnormality;
  - (b) an individual between 6 and 7 cause a neurosis;
  - (c) an individual between 7 and 8 change neurosis to psychosis;
4. any treatment which causes a shift to the left in the degree of introversion will reverse these changes;
5. if the position of an individual on the scale is initially determined by somatic factors, and is later only modified by emotional factors, then it is conceivable that, knowing the nature of the somatic factors, a general form of treatment could be developed which would be virtually independent of the particular emotional stresses which triggered the psychosis in the susceptible individual.

In short, if there were a somatic factor or factors, humoral, nervous or metabolic, which determined the effect of emotional stresses, the treatment of mental disease might cease to be dependent upon the highly individual approach of psycho-

analysis. It is only upon such a hope that one can predict any dramatic improvement in the treatment of mental disease in general. Perhaps it is significant that the recent introduction of the group of drugs known as the tranquillisers exhibits, for the first time, the fact that one shot in the dark can affect radically a widely diverse collection of syndromes of mental disease. The fact that these drugs act in a way which is not understood at all and produce changes which cannot as yet be quantitatively assessed, means that they must be used with caution, not that their importance in the historical sequence of the therapy of mental disease should be underestimated.

The search for a somatic basis underlying mental disease can properly be started only when the normal physiology of the central nervous system is understood. The study of the physiology of the central nervous system is beset by difficulties such as the inaccessibility of the tissues, their extreme susceptibility to damage by physical trauma or altered metabolic conditions, and the complexity of their anatomical and histological pattern. Nevertheless, considerable progress has been made in recent years and the rate of progress is continuously accelerating. Thus, whereas only 20 years ago it seemed a forlorn hope that any significant insight into the physiology of the central nervous system could be gained in the

<sup>2</sup>  
for~~see~~able future, it is now not uncommon to meet the view that enough is now known to enable a start to be made in the rational study of factors which interfere with normal function.

This radical change of outlook has been brought about through a number of significant advances:

1. the refinement of electrophysiological methods of study of small groups of cells or fibres, with focal recording, and of individual cells, with intracellular recording with microelectrodes;
2. the development of histochemistry, i.e. the use of staining methods specific for individual chemical substances, particularly enzymes. This method has, for instance, made it possible to localise with extreme accuracy the sites at which true cholinesterase is present;
3. the development of methods of studying chemically materials of biological origin, especially polypeptides and proteins. Among these methods are chromatography, electrophoresis and counter-current analysis. Many of the active substances found in nervous tissue are polypeptide in nature and it is only recently that it was possible to contemplate their chemical analysis at all;

4. the introduction of new methods of application of drugs to the central nervous system. One of the main problems in the pharmacology of the central nervous system was the so-called "blood-brain barrier". The use of methods such as the injection of drugs directly into the cerebral ventricles of conscious animals has greatly increased our knowledge of recent years - although it is fair to say that we still do not know how to interpret that knowledge;
5. the increasing study of drugs in respect of their action on conditioned responses and on patterns of behaviour has indicated that there are whole fields of pharmacological activity which the more common but less exotic methods of test fail to detect. This fact has been clearly evident in the history of the development of the tranquillisers.

The outlook is thus much more hopeful; and the physiologist may now be pardoned if he considers that, from his continuing researches into normal central nervous system function, may spring a rational approach to the study of somatic factors playing a part in influencing mental health. This must provide, indeed, much of the drive and incentive to the continued prosecution of his work.

PART I

STUDIES ON SYMPATHETIC GANGLIA

CHAPTER I

INTRODUCTION

Apart from the interest inherent in the autonomic system itself, there is an overriding fascination afforded by the existence, in a sympathetic ganglion, of a microcosmic nervous system. Here nature provides us with an aggregate of neurones, readily accessible to experiment, readily isolated from the influences of other active neurones, sometimes even fitted with modern plumbing in the form of a vascular system that can, with relative ease, be segregated from the general circulation. What more can the physiologist ask of nature?

It is disappointing to find how little advantage has been taken of the opportunity thus afforded. Eccles (1935a) showed us, almost at the start, what was available. Many of the subtle interactions between neurones which must form the basis for the integrative and modulatory activities of the brain are also exhibited in the microcosm of the ganglion. Here we have summation, both spatial and temporal; here we have facilitation and occlusion; here we have almost all the Sherringtonian principles of integrative action. But since

Eccles first demonstrated these phenomena, they have been shamefully neglected. Neither physiologists nor pharmacologists have probed any further.

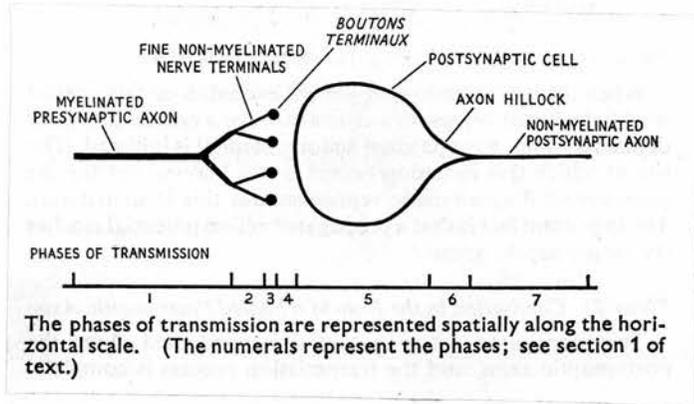
Instead we have all "shocked" the ganglion with maximal electrically-induced volleys in the presynaptic nerves at frequencies chosen only to suit ourselves. We have learned a great deal about how the ganglion cells react to such heroic treatment. It is all the more important to state that we are not yet ready to make inferences about the normal functioning of the microcosm of a ganglion; and, far less, are we ready to make inferences about the normal functioning of the macrocosm of the brain.

The superior cervical ganglion of the cat has, for reasons of experimental simplicity, been the preparation of choice in most of the studies of ganglion physiology. I have tried, in Chapter II, to describe the normal events taking place in the ganglion during the transmission of nerve impulses. The description is based on the assumptions that all the presynaptic fibres are cholinergic and that all synapse in the ganglion. Neither assumption is necessarily correct. It is just possible that non-cholinergic presynaptic fibres exist - <sup>but if</sup> ~~they~~ they do, they are a small minority of the total population of presynaptic fibres; there is certainly a number

of so-called through-and-through fibres, most of which are postsynaptic fibres arising from cells proximal to the ganglion being studied.

I have consequently described, in Chapter II, an uncomplicated picture of normal cholinergic transmission, but I have attempted, after describing it, to summarize the various anomalies in this picture.

FIG. 2



CHAPTER II

REVIEW OF THE LITERATURE

1. Phases of Ganglionic Transmission

When we apply a maximal electrical stimulus to the presynaptic nerve supplying the ganglion, we induce all the nerve fibres in the nerve to fire simultaneously. This in turn leads to the discharge of all the cells in the ganglion, although this discharge is not quite synchronous. It is not synchronous because the distance between the point of stimulation on the presynaptic nerve and the individual ganglion<sup>cell</sup> is variable; and, even more important, because the size of the presynaptic fibres and their conduction velocity is also variable. The asynchrony is not, however, sufficiently great to prevent the recording from the postsynaptic nerve of a relatively uncomplicated action potential - i.e., of an approximately synchronous discharge of all the axons in the postsynaptic nerve. Nevertheless, the slight asynchrony is important to a proper understanding of the electrical events in the ganglion.

When the ganglion is stimulated in this way, it is convenient to try to follow the events during transmission in terms of a simplified model of the synapse (Fig. 2). The

ganglion is not in the least like the model, for nerve terminals ramify over the entire surface of the cells, and one presynaptic axon will terminate on numerous ~~post~~<sup>cells.</sup>synaptic axons. Nevertheless, for our artificial maximal stimulus the model will serve a useful purpose.

On this model the events initiated by the maximal presynaptic volley can be classified into seven distinct phases. I have represented these phases spatially along a horizontal scale, which again is an over-simplification of the true picture.

#### Phase 1: Saltatory Conduction in the Myelinated Presynaptic Fibre

The process of saltatory conduction in myelinated axons has received a great deal of elegant study in recent years, and a detailed picture of the mechanisms involved is emerging. There is no reason to think that presynaptic fibres vary in any essential way from other axons of similar size in respect of the nature of the propagated action potential and there is no need to discuss the mechanisms here.

#### Phase 2: Conduction in the Non-Myelinated Presynaptic Terminals

It is generally believed that the propagated action

potential is conducted along the branching terminals - which are not myelinated - of the presynaptic axons. During this phase of the passage of the impulse it may be subject to influences which have no role in modifying conduction in the myelinated axon proper.

Phase 3: Release of Acetylcholine at boutons terminaux

When the action potential has traversed the fine terminals of the presynaptic axon it causes, on reaching the boutons terminaux, the release of a quantum of acetylcholine. It has been estimated that a single maximal volley causes the release, in the superior cervical ganglion, of the cat, of about 0.1  $\mu$ g. of acetylcholine (Feldberg & Vartiainen, 1934; Perry, 1953).

Phase 4: Acetylcholine Crosses the Synaptic Gap; Occupies Receptors on the Cell Membrane; and is Eliminated

For a brief fraction of time the acetylcholine released is present free and crosses the synaptic gaps. It then, so it is presumed, forms some sort of attachment to the membrane of the ganglion cell, fixing on to the so-called receptor sites. Having occupied these receptors, the acetylcholine is then rapidly eliminated. It may be eliminated in a number of ways, from physical diffusion to specific hydrolysis by cholin-

esterase. It is certainly subject to hydrolysis from the moment it is released from a protected or "bound" form in the boutons terminaux.

#### Phase 5: Local Response of Cell to Acetylcholine

While the acetylcholine is attached to the receptor sites on the cell membrane and before it is eliminated, it produces a change which results in a local depolarization of the cell membrane. This depolarization is not propagated and spreads decrementally from the area immediately beneath the boutons terminaux. When all the boutons are discharged by a maximal volley, the local response presumably occurs simultaneously all over the cell surface (some 70% being covered by boutons (de Castro, 1942)), the local response of the cell being the sum of all local responses under individual boutons.

#### Phase 6: Initiation of Propagated Action Potential

When the local response of the cell exceeds a certain critical level (which may represent a critical area or a critical degree of depolarization), a propagated action potential is initiated. The site at which this initiation occurs is not known, but for the purposes of diagrammatic

# TABLE I

Phase of transmission	Test to ensure this phase is functioning normally (Each test applies only if the preceding tests listed in this table have been carried out successfully.)
1	Stimulate presynaptic nerve and record action potential at a point distal on the same nerve.
2 and 3	Stimulate presynaptic nerve to eserized perfused ganglion and test effluent for presence of acetylcholine. (It is not yet possible to differentiate with certainty between Phases 2 and 3.)
4 and 5	Stimulate presynaptic nerve and record local potential of ganglion cell. (It is not yet possible to differentiate between Phases 4 and 5.)
6	Stimulate presynaptic nerve and record propagated action potential in postsynaptic nerve.
7	Stimulate postsynaptic nerve and record action potential at a point distal on the same nerve.

representation this is immaterial. The important fact is that a propagated action potential reaches the postsynaptic axon.

#### Phase 7: Conduction in the Non-Myelinated Postsynaptic Axon

The propagated action potential is conducted along the postsynaptic axon, and the transmission process is complete.

### 2. Factors Affecting Transmission

It is interesting to examine the factors affecting transmission in the light of the various phases of transmission just described. Before doing so, it is desirable to summarize the ways in which the function of each phase can be tested experimentally. This is shown in Table I. It must also be remembered that a factor may affect any one phase of transmission, not only directly but also indirectly, by interfering with a function which subserves that particular phase. Thus depression of the release of acetylcholine may be direct or may be indirect as, for example, the result of a depression of acetylcholine synthesis; or initiation of the propagated action potential may be prevented by direct action on the cell membrane or indirectly by depression of the cell metabolism.

### Factors Affecting Phase 1

There is no point in discussing here the many factors known to affect saltatory conduction, since this has recently been reviewed by Stämpfli (1954). It is, however, worth mentioning that, pharmacologically, local anaesthetics such as cocaine are powerful agents for artificially interrupting saltatory conduction; and there is frequently a need, in the analysis of a new drug, to exclude such activity before presuming it to possess true ganglion-blocking potency.

### Factors Affecting Phase 2

It is difficult to separate by any experimental technique the effects on transmission occurring at Phase 2 from those occurring at Phase 3. Nevertheless, the action of the toxin of Cl. botulinum deserves mention at this point. Ambache (1949, 1951) showed that the toxin possessed a characteristic action in blocking transmission at cholinergic junctions, namely, at motor end-plates and at ganglion synapses. He showed that presynaptic stimulation failed to excite the end-organ, but that acetylcholine would excite the postsynaptic cell; he furthermore showed that Phase 1 was not affected by the toxin. He therefore inferred that block occurred at Phase 2 or at Phase 3. Burgen, Dickens & Zatman (1949) showed directly that the toxin prevented the release of acetylcholine

at the neuromuscular junction. They discussed three possible mechanisms, namely, block of Phase 2, block of Phase 3, or indirect block of Phase 3 due to depression of acetylcholine synthesis, and came to the conclusion that Phase 2 block, due to an irreversible fixation of the toxin on the fine non-myelinated fibres, was the most probable explanation. This view received some support from Brooks (1954), who was able to release acetylcholine by massive repetitive stimulation even after blocking with botulinum toxin. On the other hand, Brooks (1956), arguing by analogy from the work of Castillo & Katz (1955), put forward the considered view that the action occurred at the sites of acetylcholine release, the tips of the nerve fibres, i.e. by an action on Phase 3. Thus the difficulty of differentiating between actions at the two sites still defeats any attempt to define with certainty the site of action of botulinum toxin.

### Factors Affecting Phase 3

The precise mechanism of the release of acetylcholine at the boutons terminaux remains obscure, in spite of the very large amount of work done. Birks & MacIntosh (1957) discussed the general problems of the storage and release of acetylcholine. The release of acetylcholine during prolonged

stimulation has been the subject of one part of my research (see Chapter III).

Many other factors play a part in determining the release of acetylcholine and it is necessary to examine some of them at this point.

(i) Inorganic cations. Brown & Feldberg (1936) showed that perfusion of the ganglion with Locke's solution containing a raised proportion of potassium resulted in a prolonged release of acetylcholine. It has been a favourite hypothesis that this finding is an illustration of the normal mechanism of acetylcholine release, since the potassium must leak out of the nerve terminals during passage of the action potential. Harvey & MacIntosh (1940) showed, however, that this release by potassium would not occur in the absence of calcium ions. Recently, Fatt & Katz (1952) suggested that the release of acetylcholine at the neuromuscular junction might occur, in consequence of the entry of sodium ions, by a cation exchange mechanism. Hutter & Kostial (1955) showed, however, that reduction of the sodium-ion concentration does not affect the release of acetylcholine at the ganglion until it reaches a level which prevents saltatory conduction in the presynaptic axons (Phase 1). Other experiments on the role of calcium and magnesium ions were reported by Hutter & Kostial (1954). The

TABLE II

Cations		Acetylcholine release	Notes
Increased	Decreased		
K — Ca	— K —	increased normal increased	Spontaneous release occurs — No spontaneous release, but increase in release during stimulation
— Mg —	Ca — Na	decreased decreased normal	— — —
K Mg + Ca —	Ca — K + Ca	normal normal normal	Increased K has no effect in the absence of Ca — Decreased Ca has no effect in the absence of K

effect of these various cations on acetylcholine release is summarized in Table II . It is apparent that release of acetylcholine is dependent upon a proper balance between these cations; but the precise nature of the control is not yet clear.

(ii) pH and temperature. MacIntosh & Emmelin (1956) showed that the variation in pH resulting from changing from phosphate-buffered to bicarbonate-buffered Locke's solution did not affect the acetylcholine release from the ganglion. In 1954 Brown showed that the release of acetylcholine was susceptible to fluctuations in temperature. Reducing the temperature from 39°C. to 20°C. reduced the acetylcholine output to approximately one-tenth, although transmission continued apparently normally, as judged by the contraction of the nictitating membrane. Brown concluded that this was a clear indication of the high "safety factor" in transmission to which he had previously called attention (Brown & Feldberg, 1936). Clearly minor modifications of the amount of acetylcholine released would not, in such circumstances, have any profound effect on transmission. Yet it is important to recall, once again, that maximal presynaptic stimulation is not a normal physiological event. In this connection it is interesting that Kostial & Vouk (1956), stimulating in the

same way but at a frequency of 2 per second instead of the 10 per second used by Brown, failed to find any modification of the amount of acetylcholine released on changing the temperature from 39°C. to 20°C. Kostial and Vouk inferred that it was the synthesis of acetylcholine rather than its release which was sensitive to temperature.

(iii) Factors affecting acetylcholine synthesis. The release of acetylcholine in normal fashion is obviously dependent upon the pre-existence of an adequate stock of "available" acetylcholine. This stock is maintained enzymically by choline acetylase. Factors affecting synthesis, however, affect transmission only indirectly and usually only after a relatively long latency, during which the stock is depleted.

#### Factors Affecting Phase 4

It will be convenient to regard Phase 4 as having three separate components which we shall call a, b and c. Phase 4a, represents the transient free existence of acetylcholine in the synaptic gaps; Phase 4b the stage - also transient - during which the acetylcholine is attached to the receptor sites; and Phase 4c the phase of elimination of the acetylcholine.

(i) Phase 4a. It is probable that this represents a

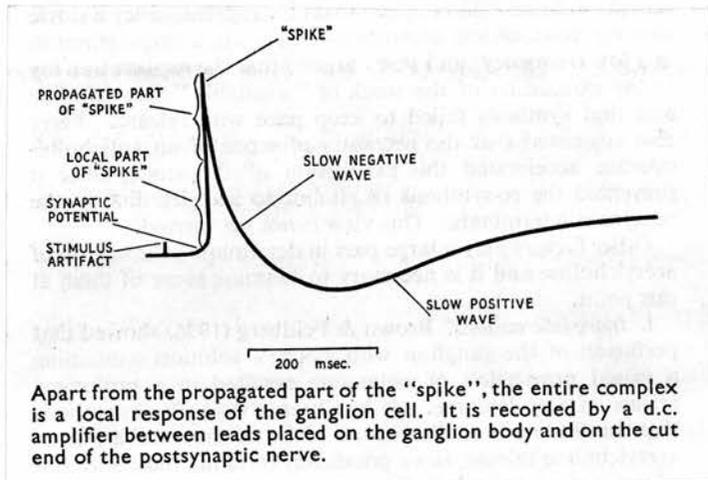
theoretical rather than a real stage of transmission. Nevertheless, this stage, transient as it is, represents the situation arising as a result of nerve stimulation, which is analogous to the injection of acetylcholine into the arterial supply to a ganglion. MacIntosh & Emmelin (1956) have made an interesting comparison of the relative doses required to produce ganglionic stimulation in each case. The ratio between these doses lies between 1:10 and 1:250, but, as MacIntosh and Emmelin point out, the acetylcholine released by stimulation is distributed, not throughout the whole volume of the ganglion, but only in the "effective synaptic space". It is difficult to imagine that this space occupies 1/250 the volume of the ganglion, far less 1/10 of the volume. Consequently, the discrepancy is more apparent than real. To affect this stage of transmission would require a factor which altered the amount or the nature of the acetylcholine during its free existence. No such factor is known, save cholinesterase. Since cholinesterase acts throughout all stages of Phase 4, we will consider it later.

(ii) Phase 4b. Attachment of acetylcholine to "receptor sites" is, by repute, influenced by a large number of drugs, including all those usually described as "competitive" ganglion-blocking drugs. Such drugs are supposed to act by competition with acetylcholine for the receptors. The evidence

in favour of this concept is not good for the ganglion; but it is fairly good for the same drugs acting on the neuromuscular junction, so that argument by analogy has some validity. The evidence depends upon the fact that constant proportions of inhibitor and activator exert a constant effect. Such relationships hold very accurately with enzyme inhibitors in vitro and approximately with competitive blocking drugs at the neuromuscular junction. Results suggestive of a similar type of action at the ganglion have been obtained. (Paton & Perry, 1953). Work on this problem is described in Chapter V.

(iii) Phase 4c. Very rapid elimination of acetylcholine after its attachment to the receptors is a vital part of efficient ganglionic transmission. It is generally attributed mainly to the action of the enzyme cholinesterase, which is known to be present in the ganglion (Sawyer & Hollinshead, 1945). Yet anticholinesterases do not so strikingly potentiate the actions of acetylcholine in the ganglion as they do at the neuromuscular junction (Eccles, 1944). Although cholinesterase undoubtedly plays a part, there is probably another mechanism involved and the nature of this has been reviewed by MacIntosh & Emmelin (1956). They suggest that the most likely mechanism is the physical removal of the acetylcholine by simple diffusion out of the limited "effective synaptic space", possibly reinforced by an increased mobility of the acetyl-

FIG. 3



choline ion.

If acetylcholine were to persist for long in high concentration it would rapidly lead to a failure of transmission. This is clearly indicated by the experiments described in Chapter IV. Thus, efficient elimination of acetylcholine is essential, and, in fact, elimination is extremely efficient whether the major role is played by cholinesterase or not. The safety factor of this phase is probably much higher than that for Phase 3.

#### Factors Affecting Phase 5

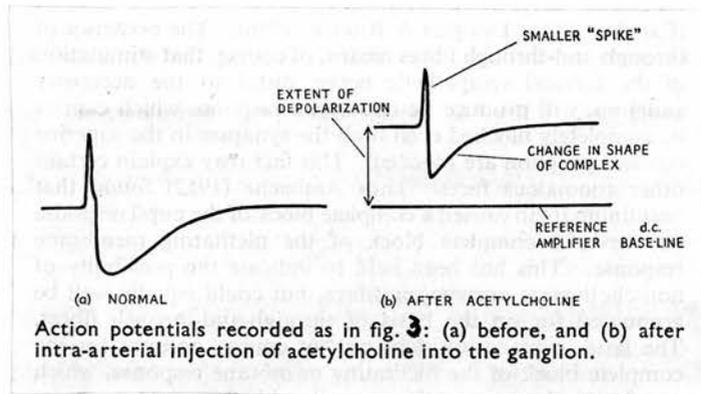
Nature of local response. Before discussing factors which affect the local response, it is necessary to determine the nature of the response itself. The local response of the ganglion cell is a phenomenon which is, as yet, still not fully understood. If by local response we include all electrical changes which are not propagated but which spread only decrementally, then there are several separate components involved. Fig. 3 shows a diagrammatic representation of the ganglionic potential complex. Apart from some 50% of the initial "spike", all the components of this complex decay exponentially, with approximately the same space constant, as they spread along the postsynaptic axon (Eccles, 1935b;

Paton & Perry, 1953).

(i) "Synaptic" potentials. Eccles (1937) first described what he called the detonator response of ganglion cells, but the evidence for such responses was more an inference than a clear-cut demonstration. It is extremely difficult to spot this phase in Fig. 3, in normal recordings from the ganglion. Indeed Paton & Perry (unpublished data, 1952) failed to find convincing evidence for a synaptic potential of this nature even by using blocking drugs such as tubocurarine, which is the classical tool for showing the end-plate potential of muscle. They worked on the cat's superior cervical ganglion in situ. R. Eccles (1952a, 1952b), in the same ganglion from rabbits, but excised and maintained in vitro, demonstrated, using curare, changes in the complex which, she believed, indicated that the propagated "spike" had been abolished and that the remaining potential was a pure synaptic potential. In a further paper (R. Eccles, 1956) she obtained similar records with dihydro- $\beta$ -erythroidine; but failed to detect any synaptic potential when using nicotine as the blocking agent.

(ii) Action of injected acetylcholine. Paton & Perry (1953) gave intra-arterial injections of acetylcholine and recorded the changes in the resting and evoked potentials of

FIG. 4



the ganglion (see Chapter IV). Their results were confirmed by Pascoe (1956). There seems, then to be little doubt that acetylcholine does produce a long-lasting negativity of the ganglion cells. This change is, however, also accompanied by striking alterations in the shape of the evoked potential complex. The spike height is reduced and the slow waves are greatly modified in a way which can be explained (Paton & Perry, 1953) on the assumption that the time constant of the slow negative wave is reduced almost to vanishing point (Fig. 4). Eccles (1935b) had previously shown that these slow waves were also local responses of the ganglion cell.

(iii) Intracellular records. R. Eccles (1955) succeeded in recording from ganglion cells with intracellular microelectrodes. Prodromal stimulation evoked a complex described by Eccles as consisting of a synaptic potential (15-30 mV), which triggered a "spike" potential (10-100 mV). Slow "after potentials" were occasionally seen but were often obscured because of the cell injury caused by the micro-electrode insertion. Antidromic stimulation never evoked any slow negative potential (R. Eccles, 1956), and it was suggested that part at least of this wave was due to the continuing synaptic potential.

It might be useful, in trying to visualize events at

the ganglion cell, to consider the model of the neuromuscular junction, about which much more is known. In doing this it is, of course, essential to remember that the spatial relations at the two sites are entirely different. In the muscle cell there is a real differentiation between the membrane of the end-plate and the membrane of the remaining cell surface, so that the end-plate membrane subserves the fundamental processes underlying a propagated response. What is the position in the ganglion cell? Is there a similar differentiation of the membrane or do the molecular configurations subserving the two functions co-exist in a lattice structure in the same membrane?

If there is a differentiation analogous to the situation in muscle, it is very difficult to imagine that it is between membrane underlying the boutons terminaux and other membrane, since de Castro (1942) found that some 70% of the cell surface was covered by boutons. The only real basis for differentiation would lie in regarding the entire cell soma as being analogous to the end-plate, with the corollary that the propagated potential was initiated only in the axon. This concept is not at variance with any of the known facts. Furthermore, it receives some support from the failure of anti-dromic stimulation to invade the soma of the cell. This failure is illustrated by the finding of R. Eccles (1956) that

intracellular electrodes failed to record any negative after-potential when antidromic stimuli were applied. It also would explain why Emmelin, MacIntosh & Perry (1949) were unable to provoke potassium loss from the ganglion by antidromic stimulation, although such loss did occur on prodromic stimulation. It is, of course, true that failure to excite by antidromic volleys might be attributable to a "safety factor" due to the sudden spatial enlargement of the postsynaptic axon at the axon hillock, so that evidence of this kind is by no means conclusive.

On the other hand the membrane of the soma may be undifferentiated except at the molecular level, and the functions of local and propagated response may co-exist. In this case it is necessary to assume that the local response is actively maintained for a relatively long time, i.e. that acetylcholine continues to exert its action throughout the whole period of "spike" potential and continues thereafter to provide the cause of the negative after-potential. Once again there is no reason why this should not be so. Thus it is apparent that the precise nature of the local response is still somewhat obscure.

Factors affecting the local response. The local response is a graded response and is thus open to all sorts of

modulating stimuli. The propagated response is an all-or-nothing reaction. In consequence, it can be blocked but not otherwise modified. Most pharmacological analysis has, of course, been based on the records made of end-organ responses - in other words on the changes induced in the whole ganglion - so that block of the all-or-nothing response in a varying number of individual units also appears as a graded phenomenon. Thus the detailed mechanisms of action are often unknown, and either Phase 5 or Phase 6 may be involved.

(i) Depolarizing blocking drugs. A group of substances, described by Paton & Perry (1953) as depolarizing blocking drugs, affect the local response of the ganglion cell to acetylcholine by reducing the normal resting potential of ganglion cells for a prolonged period of time. During this time further depolarization cannot occur and consequently transmission is blocked. The initial depolarization produced by these drugs causes repetitive firing of the ganglion cells, indicated by activation of the end-organ, but this phase may be followed by inexcitability of the ganglion cells. Nicotine and tetramethylammonium both have an action of this kind. It is not known whether these drugs also compete with acetylcholine for receptors, or whether this action on the membrane is produced at some other site or by some other mechanism. If they compete with acetylcholine they could also, of course, be

regarded as agents affecting Phase 4. The depolarizing action of nicotine and tetramethylammonium was confirmed by Pascoe (1956). (See Chapter V. [redacted]).

(ii) Drugs modifying ganglionic transmission.

Adrenaline (Marrazzi, 1939), histamine (Konzett, 1952) and cardiac glycosides (Konzett & Rothlin, 1952) will all, under suitable conditions, potentiate the action of acetylcholine or the effects of submaximal stimulation of the ganglion. It seems probable that such actions must take place at Phase 5.

Adrenaline also possesses a blocking action (Bülbring, 1944), part of which is due to depression of acetylcholine release (Paton & Thompson, 1953). The sensitizing effect of cardiac glycosides is discussed <sup>by Perry & Rainart (1954)</sup> [redacted]. Trendelenburg (1954, 1955) has found that the potentiating action of histamine is not blocked by competitive blocking drugs (Phase 4) and is therefore, presumably, an effect on a later phase of transmission (Phase 5).

(iii) Axotomy. Transmission fails in a ganglion after section of the postsynaptic axon (Brown & Pascoe, 1954). Acetylcholine release occurs normally (Brown & Pascoe, 1954) and block is not due to accumulation of acetylcholine (McLennan, 1954). The failure is therefore probably in Phase 5, and Brown & Pascoe (1954) were indeed able to show that the cells

of the axotomized ganglion are less sensitive than usual to acetylcholine. The explanation is not clear.

(iv) Denervation. The extraordinary increase in the sensitivity of denervated tissues to the normal transmitter is well known and this increase does occur - although not to a very large degree - in the ganglion (Cannon & Rosenblueth, 1949). There is again little doubt that this phenomenon affects Phase 5, but the mechanism is equally obscure. Thus the presence of an intact presynaptic nerve exerts a remarkable modulating influence on the postsynaptic membrane, and no reason is yet known. Some of the effects of denervation can be strikingly mimicked by reducing the extracellular potassium concentration (see Chapters V and VI).

#### Factors Affecting Phase 6

Factors potentiating submaximal stimulation can do so only by increasing the number of postsynaptic units firing. This can be accomplished by increasing the local effect of the acetylcholine or by reducing the threshold of the mechanism for propagation of the response so that the cells fire at a smaller value of the local response. In the one case we would classify the action as affecting Phase 5, and in the other as affecting Phase 6. The distinction can be made only by recording the local response itself and, since this is seldom

undertaken, we must remain in doubt about the precise nature of these changes. On the other hand, the initiation of the propagated action potential will be dependent upon the maintenance of normal conditions at the cell membrane. This maintenance is in turn dependent upon normal cell metabolism. Both the metabolic and cell membrane conditions necessary for the initiation of a propagated response may be different from those necessary for a local response. Consequently, an entirely separate set of factors may be operative in affecting the two phases. Failure to differentiate at the present moment does not imply that differentiation is unimportant. Indeed, proper differentiation might aid considerably in solving some of the outstanding physiological problems. At the moment, however, all those factors described as affecting Phase 5 may equally well affect Phase 6.

#### Factors Affecting Phase 7

In general, transmission in the postsynaptic axon will be subject to the same sort of effects as is transmission in the presynaptic axon (Phase 1), although it must be remembered that the postsynaptic axon is non-medullated.

### 3. Complicating Factors in Cholinergic Transmission

#### Other Nerve Pathways

Douglas & Ritchie (1956) made a detailed study of the conduction of impulses through the rabbit's superior cervical ganglion, and found incontrovertible evidence of the existence of an accessory cervical ganglion lying proximal to the superior cervical ganglion. The postsynaptic fibres from this accessory ganglion run straight through the superior cervical ganglion. Furthermore, single presynaptic axons may innervate cells in both ganglia. There appears to be no functional distinction between cells of the two ganglia (Langley, 1894; Douglas & Ritchie, 1956). The existence of through-and-through fibres means, of course, that stimulation of the cervical sympathetic nerve, distal to the accessory ganglion, will produce an end-organ response which cannot be completely blocked even if all the synapses in the superior cervical ganglion are blocked. This fact may explain certain other anomalous facts. Thus Ambache (1952) found that botulinum toxin caused a complete block of the pupil response but never a complete block of the nictitating membrane response. This has been held to indicate the possibility of non-cholinergic presynaptic fibres, but could equally well be

accounted for on the basis of through-and-through fibres. The latter explanation does not, of course, account for the complete block of the nictitating membrane response, which can be produced by other ganglion-blocking agents under exactly the same conditions.

In the inferior mesenteric ganglion of the cat (Job & Lundberg, 1952) and of the rabbit (Brown & Pascoe, 1952; McLennan & Pascoe, 1954) evidence has been found for the existence of afferent fibres from the periphery (the intestine) which will excite presumably as part of a reflex activity the ganglion cells. No such evidence has as yet been found in the cervical sympathetic ganglia (Douglas & Ritchie, 1956). Nevertheless, the existence of reflex excitation of ganglion cells is a further complication to the picture of normal transmission. Whether such reflex excitation is also cholinergic is not known.

#### Other Transmitter Substances

Mention has been made of the finding of Ambache (1952) that botulinum toxin failed to block completely the transmission of impulses to the nictitating membrane. Such a finding obviously raises the possibility of non-cholinergic synapses in the ganglion, although the nature of such an

alternative transmission mechanism is wholly unknown. Kewitz & Reinert (1953) showed that, under certain circumstances, a ganglion-blocking drug could inhibit the action of acetylcholine and fail to block electrical stimulation. Perry & Reinert (1954b) showed that, when the extracellular potassium concentration was reduced, hexamethonium would block transmission but failed to block injected acetylcholine. This anomaly can be explained in a number of ways without the necessity of concluding that non-cholinergic synapses exist; but, of course, their existence would also provide an adequate explanation of the phenomena.

The action of sympathin has also been regarded as suggestive that adrenergic synapses may be found in the ganglion, and Bülbbring (1944) was able to detect minute amounts of sympathin in the effluent from a perfused ganglion during presynaptic stimulation. It is quite possible that this sympathin derives not from adrenergic presynaptic fibres but from the activation of short postsynaptic fibres innervating the blood vessels of the ganglion, since postsynaptic sympathetic fibres are normally adrenergic.

There is, therefore, still doubt as to the uniformity of the fibres in the preganglionic nerve, but so far there has been no convincing demonstration of non-cholinergic fibres,

although their existence cannot be excluded; the existence of through-and-through fibres and, in certain sites, of afferent reflex fibres in ganglia is undoubted.

CHAPTER III

RELEASE OF ACETYLCHOLINE

INTRODUCTION

The release of acetylcholine at sympathetic ganglion synapses during preganglionic stimulation was first demonstrated in 1934 by Feldberg and Gaddum on the perfused superior cervical ganglion of the cat. Since then a number of workers, notably Feldberg & Vartiainen (1934), Brown & Feldberg (1936), and MacIntosh (1938), have confirmed and extended the observations. None of these workers, however, made a detailed quantitative study of the amounts of acetylcholine released under different experimental conditions; they confined their observations mainly to demonstration of the fact that, in the cat's eserinated perfused superior cervical ganglion, the output of acetylcholine during preganglion stimulation began relatively high and fell in an approximately exponential fashion finally to reach a fairly steady level.

Furthermore, since all these experiments were carried out in the presence of eserine, it was always tacitly assumed first that the output of acetylcholine would be the same

whether the released acetylcholine was destroyed or not, and second, that eserine itself had no effect on the output of acetylcholine.

I studied the output of acetylcholine, during long periods of preganglionic stimulation at different frequencies, in an attempt to interpret the results on a theoretical pattern; this pattern is based on two assumptions, both of which seem reasonable ones, namely, that the rate of synthesis of acetylcholine is constant, and that the amount of acetylcholine liberated by a single preganglionic volley is a constant fraction of the stock of acetylcholine available for release at the time.

I also studied whether eserine influences in any way the release of acetylcholine during prolonged preganglionic stimulation. This problem was tackled in two ways. In the first place, the effect of eserine on the recovery of the acetylcholine release during a period of rest after prolonged stimulation was studied; in the second place, a comparison was made during preganglionic stimulation between the output of acetylcholine in the presence of eserine and the output of choline in the absence of eserine. It has been shown in these experiments that eserine appears to exert a considerable influence on the apparent release of acetylcholine.

### METHODS

Cats were anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (80 mg./kg.). The superior cervical ganglion was prepared for perfusion by the method described by Kibjakow (1933) with the modifications suggested by Feldberg & Gaddum (1934). In addition, the perfusion fluid, instead of being warmed in an electrically heated cannula, was warmed by passing the inflow tube through the cat's oesophagus from below upwards, so that the tip emerged at the point where the common carotic artery was cannulated; and the perfusion fluid consisted of Locke's solution containing twice the usual amount of glucose (making a final concentration of 2 g./l. of glucose. These modifications were suggested to me by Dr. F.C. MacIntosh, who had used them in previous unpublished experiments. When necessary, eserine was added to the perfusion fluid to make a final concentration of 1 in 100,000.

The preganglionic cervical sympathetic trunk was stimulated with square waves of 0.5 m.sec. duration, the stimulus strength being supramaximal for the contraction of the nictitating membrane. Except where specifically stated otherwise, the frequency of stimulation used was 10 shocks per

# TABLE III

TABLE III Recovery of choline in acetylation process

Sample no.	Weight of choline chloride (mg)	Time of acetylation (min)	Weight of acetylcholine recovered, assayed on frog rectus (mg)
1	19.6	180	20
2	17.0	20	16.6
3	19.8	5	20

Acetylcholine was assayed on the blood pressure of the eviscerated chloralosed cat as described in detail by MacIntosh & Perry (1950).

sec.

In some experiments the choline output of the uneserinized perfused ganglion was measured. For this purpose the choline in the perfusate was acetylated, as first described by Guggenheim & Loeffler (1916), and was then assayed as acetylcholine. For the details of the method of acetylation the description of Fletcher, Best & Solandt (1935) was followed with the minor change that, as clear perfusates instead of tissue extracts were being acetylated, it was possible to reduce the time necessary for full acetylation from 2 hours to 5 min. This was checked in a control experiment, the results of which are shown in Table III.

Acetylcholine was assayed on the blood pressure of the eviscerated chloralosed cat. This method was described in detail by MacIntosh & Perry (1950).

### RESULTS

The output of acetylcholine during stimulation of the eserininized perfused ganglion has been shown to fall exponentially (Feldberg & Vartiainen, 1934; Brown & Feldberg, 1936; MacIntosh, 1938). At the start of stimulation, when the output is falling rapidly, the shorter the period of collection, the higher are the initial concentrations of acetylcholine, and

FIG. 5

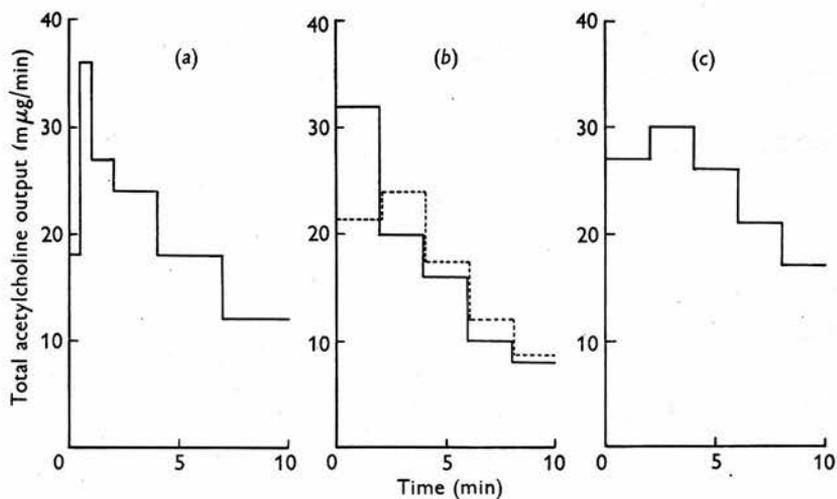


Fig. 5. Histograms of output of acetylcholine from perfused eseriniz superior cervical ganglion. (a) Effect of reducing periods of collection of output to 30 sec (flow rate 0.8 ml. per min). (b) Effect of slowing rate of flow; continuous lines: outputs per 2 min period; dotted lines: theoretical outputs per 2 min period if one-third of total output from ganglion in each period is carried into next sample owing to slow flow. (c) Outputs per 2 min period with flow of 0.3 ml. per min. (For details see text.)

this is true until the period of collection is so short that the acetylcholine liberated is not carried out into the collecting vessel within the period, but owing to the limited rate of flow, is carried over into the following sample. When the initial collection periods were reduced to 30 secs. (Fig. 5(a)) the output rose in the second period before starting to fall exponentially; this result is similar to one reported by MacIntosh (1938). In Fig. 5(b) the output of acetylcholine per 2 min. sample in one experiment is shown (solid lines). If, owing to a slow flow of the perfusion fluid, one third of the amount of acetylcholine liberated in each period had been carried over into the following period, the result would be the outputs of acetylcholine illustrated graphically (dotted lines). Thus, in this case also - i.e. where the flow is very slow - the result is a rise in output in the second, and occasionally even into the third periods; an experiment in which this actually was observed in 2 min. periods of collection is shown in Fig. 5(c).

In order to avoid these initial rises in output, collection periods of 2 min. duration were usually used, and this was found to be successful in its object, provided that the rate of flow was of the order of 0.5 ml. per min. at least.

Rate of stimulation. The output of acetylcholine was

FIG. 6

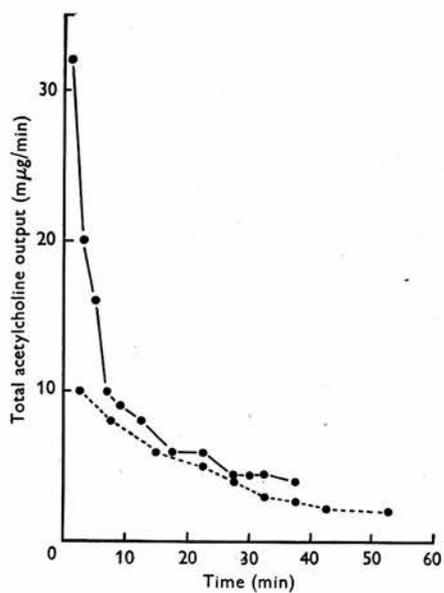


Fig. 6. Graph of output of acetylcholine from perfused eseriniz superior cervical ganglion. Continuous line: experiment with periods of collection of 2 min. Dotted line: experiment from Brown & Feldberg (1936) with periods of collection of 5 min.

FIG. 7

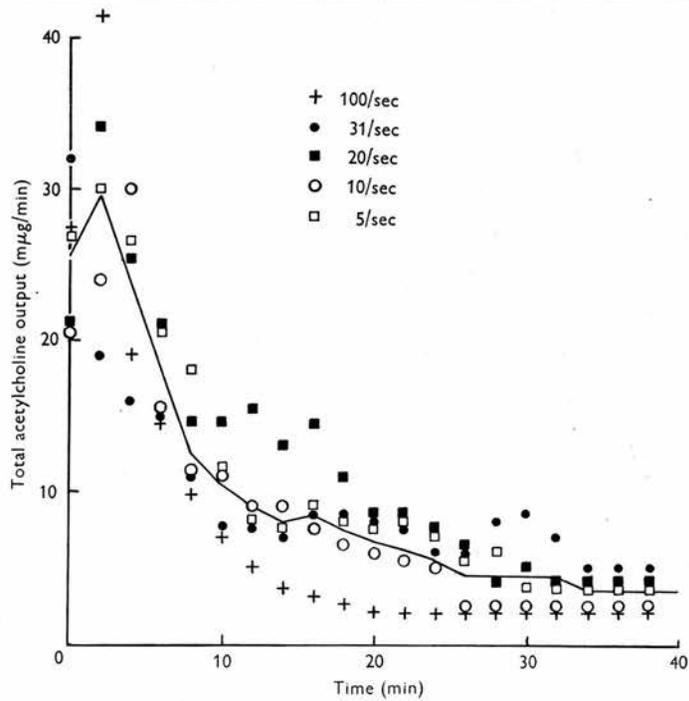


Fig. 7. Graph of output of acetylcholine from perfused eserized superior cervical ganglion at various rates of stimulation. Continuous line: mean of all experiments.

followed during 40 min. stimulation at frequencies of 5, 10, 20, 30 and 100 per sec., samples being collected over 2 min. periods. Fig. 6 shows the exponential fall in output observed in one experiment at a frequency of 10 per sec., and a similar experiment by Brown & Feldberg (1936) at a frequency of 15 per sec. and collected for periods of 5 min.

The outputs of acetylcholine for different rates of stimulation, obtained in different experiments, are shown in Fig. 7. The curves obtained are substantially the same in each case; the output of acetylcholine per min. falls at about the same rate and to about the same final steady level of 4  $\mu$ g. per min. whatever the rate of stimulation. There is thus no evidence of any greater reduction in the acetylcholine output at the more rapid rates of stimulation. Therefore, the amount of acetylcholine liberated per volley must be considerably reduced for the more rapid rates of stimulation; and this reduction must occur from the same initial output level for the first volley, since the ganglion cannot anticipate the rate of stimulation from the initial shock. Thus the rate of deterioration in output per volley must be much more rapid for the higher frequencies of stimulation. The amounts of acetylcholine liberated per volley for different stimulus frequencies are plotted on a logarithmic scale against time in

FIG. 8

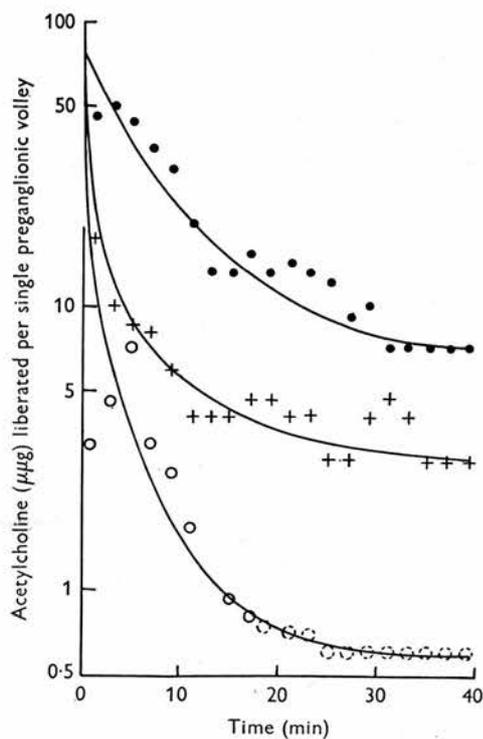


Fig. 8. Graph of output of acetylcholine from perfused eserinizied superior cervical ganglion at stimulation frequencies of (●—●), 10 per sec; (+—+), 31 per sec; and (○—○), 100 per sec. The dotted circles represent values estimated in an assay where the total amount of acetylcholine was so small that the error of the estimate was very large.

FIG. 9

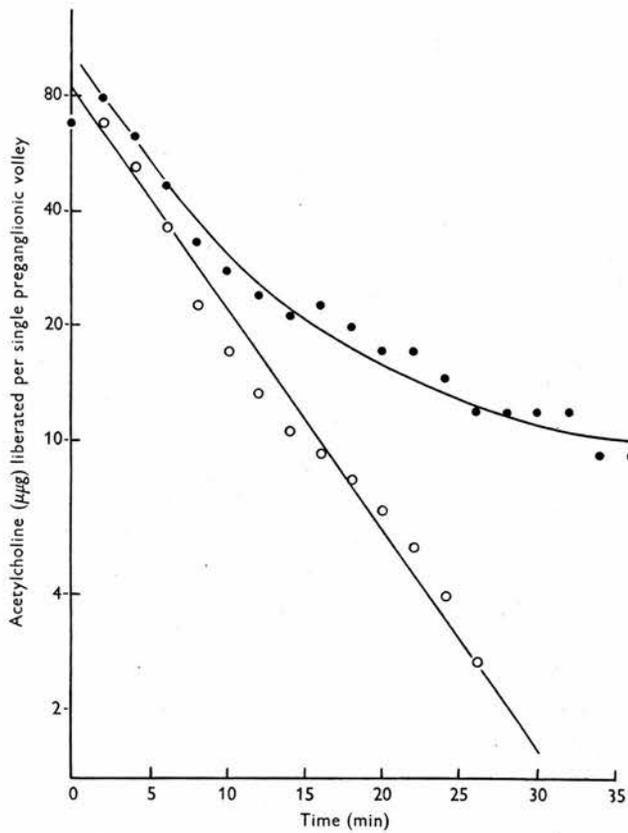


Fig. 4. Graph of output of acetylcholine from perfused eserized superior cervical ganglion stimulated at 10 per sec. (●—●), observed output of acetylcholine; (○—○), output of acetylcholine calculated on the basis that no synthesis occurs.

Fig. 8 .

The fact that the final steady level of output of acetylcholine of about 4  $\mu$ g. per min. is constant for all rates of stimulation could be taken to imply that, under the conditions of eserinizied perfusion, this is the rate at which the ganglion can synthesize acetylcholine; while the initial high rates of output could be held to imply the release of some available stock of acetylcholine held in the ganglion. If these premises were correct, the release of acetylcholine might take place according to some definite pattern.

For instance, if we assume that any one preganglionic volley will liberate a constant fraction ( $\alpha$ ) of the total acetylcholine available in the ganglion at the moment, and that the rate of synthesis of the ganglion (K) is a constant independent of the rate of stimulation, then the stock of acetylcholine available for release after a definite time (t) may be written as:

$$y_t = y_0 e^{-\alpha t} + \frac{K}{\alpha} (1 - e^{-\alpha t})$$

where  $y_0$  is the initial stock of acetylcholine in the ganglion and available for release. In Fig. 9 the results of one experiment, at a stimulus frequency of 10 per sec., are shown to fit this hypothesis without serious discrepancy. The

equation of the fitted curve is, of course,

$$\alpha y_t = K(1 - e^{-\alpha t}) + \alpha y_0 e^{-\alpha t}$$

since  $\alpha y$  is the amount liberated per volley. Fitted values for  $\alpha$  and  $K$  in this case of

$$\alpha = 0.0005$$

$$K = 2.0 \text{ } \mu\text{g. per min.}$$

were used: a  $\chi^2$  test between the observed and fitted values of  $\alpha y_t$  gives  $\chi^2 = 4.40$  on 9 degrees of freedom, so that the fit is satisfactory at the  $P = 0.05$  level of probability.

The values of  $y_0$ , i.e. the original stock of acetylcholine available in the ganglion estimated by this method, fall between 200-250  $\mu\text{g.}$ , so that with  $\alpha = 0.0005$  the initial output per volley falls in the range ~~200-250~~ <sup>100-125</sup>  $\mu\text{g.}$  This figure is independent of this rate of stimulation, and is in good agreement with the figures given by Feldberg and Vartiainen, who stimulated at a rate of approximately 2 per sec. for very short periods and obtained outputs of 66 and 100  $\mu\text{g.}$  per volley in two experiments.

If this theoretical explanation of the output per volley has any truth, we would not expect that the outputs per min. at different rates of stimulation would follow exactly

TABLE IV

TABLE IV Effect of rest periods on output of acetylcholine

Expt.	Observed output of acetylcholine in $m\mu g$			Calculated acetylcholine in $m\mu g$		Observed maximum output of acetylcholine after rest period ( $m\mu g/min$ ) (7)	Time in min of rest period		
	Total in 40 min (2)	Maxi- mum/min (3)	Final/ min (4)	Synthesized in 40 min (5)	Lost from initial stock (6)		Calculated as		Given in each expt. (10)
							Necessary to replace stock (8)	Necessary to give observed max. out- put/min after rest (9)	
1	437	32	4	160	277	6	69	12	5
2	406	41	3	120	286	9	25	20	25
3	408	32	4	160	248	—	62	—	—
4	613	34	6	240	373	24	62	47	35
5	351	30	3	120	231	6	77	16	20

the same pattern; but they would fall into patterns so similar that the differences between them would not be much greater than those observed, which are well within the experimental errors of the method.

Recovery of acetylcholine output during rest periods.

It was found that a period of rest of 15-20 min. between two periods of prolonged stimulation did not restore the rate of output of acetylcholine in the second period to its initial level in the first period. The results of five such experiments are illustrated in Table IV. In each experiment a 40 min. period of stimulation was followed by a rest period of varying duration, and stimulation was then restarted at the same frequency. The acetylcholine output was determined on 2 min. samples both before and after the rest period. The maximal outputs of acetylcholine per min. during the first and the second period of stimulation are given in columns 3 and 7. Usually the maximal rate occurred in the first sample, except when the rate of flow was very slow. In column 10 the duration of the period of rest between the two periods of stimulation is given in minutes. It will be seen that, even after 35 min. rest, the maximum output had risen <sup>to</sup> only 24  $\mu\text{g./min.}$  although the maximal output in the first period of stimulation was 34  $\mu\text{g./min.}$ , i.e. to 70% of the original value. To some extent this difference may be attributable to a general deterioration in the condition

of the preparation, since after 2 hrs. perfusion with Locke's solution, the ganglion has usually become oedematous.

Neglecting this factor, however, and knowing the amount of acetylcholine released during the first 40 min. period of stimulation, we can calculate the amount of acetylcholine produced during this period by the assumed constant rate of synthesis; namely by multiplying the final steady level of output of acetylcholine per min. reached during the first stimulation period by 40. The final steady rate of output per min. is shown in column 4, and the total synthesis in the first 40 min. period of stimulation in column 5. By subtracting this value from the total output of acetylcholine during the first stimulation period (column 2), we get an estimate (column 6) of the loss of acetylcholine from the stock originally present in the ganglion. The mean value from the five experiments for this loss is 283  $\mu$ g., and it may well be significant that this figure closely approximates to the total amount of extractable acetylcholine in the ganglion, which, according to Brown & Feldberg (1936) averages 250  $\mu$ g. Their statement that this figure was exceeded by the total amount of acetylcholine liberated during the period of exponential fall is also compatible with the present finding.

Further, if we assume that the constant rate of

synthesis which accompanies stimulation is maintained but not increased during the rest period, we can calculate the total synthesis that should have occurred during rest; and we can, by interpolation on a graph such as that shown in Fig. 9, arrive at an estimate of the time of rest which would theoretically be necessary to restore the output to the level observed at the start of the second period of stimulation. For instance, the figure 12, given for the first experiment in column 9 of Table , was obtained in the following way. The initial stock of acetylcholine available for liberation was 277  $\mu\text{g.}$ , and 65  $\mu\text{g.}$  were liberated and ~~8  $\mu\text{g.}$  were liberated and~~ 8  $\mu\text{g.}$  synthesized in the first 2 min.; so that after 2 min. stimulation 220  $\mu\text{g.}$  were available for release. In the second 2 min. period X  $\mu\text{g.}$  were found to be released and therefore, had X  $\mu\text{g.}$  been released at the start of the second stimulation period, the stock of acetylcholine at that time would, by inference, be 220  $\mu\text{g.}$  Thus, at a rate of synthesis of 4  $\mu\text{g.}$  per min., it would take  $220/4$  min., i.e. 56 min. rest to attain the observed maximum output of X  $\mu\text{g.}$  per 2 min. By a continuation of this process, the time necessary can be calculated for any value of the maximum output in the second period of stimulation. The times calculated in this way for each experiment (column 9) and the periods of rest actually

given (column 10) agree fairly well.

There are several reservations to be made before drawing any conclusion from these findings. First, the experimental error in determining the rate of synthesis is large, since the assay of the very small amounts of acetylcholine in the later samples of a perfusion is difficult and has wide limits of error. In the second place, the times calculated as necessary to replace the whole of the lost stock of acetylcholine in all five experiments are of the order of  $1-1\frac{1}{2}$  hr., as seen in column 8. However, Brown & Feldberg (1936), on removing the ganglion shortly after prolonged stimulation, found no apparent reduction of the extractable acetylcholine, and MacIntosh (1938) was able to demonstrate a loss of up to 50%, only by taking extreme measures to prevent synthesis occurring during the very short time between the end of stimulation and the extraction of the ganglion.

Effect of stimulation in the absence of eserine on subsequent stimulation in the presence of eserine

It has been shown that, when eserine is present throughout, the acetylcholine output in a second period of stimulation after a period of rest of 10 min. starts at a greatly reduced level. This 'fatigue' of the ganglion was not found to occur when the first period of stimulation took place

FIG. 10

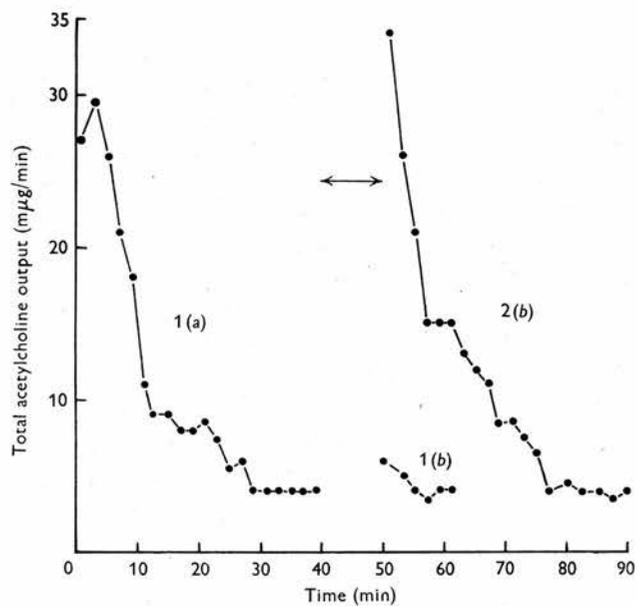


Fig. 10. The effect of eserine on recovery of superior cervical ganglion from stimulation at 10 per sec. 1 (a) and (b) eserinizd throughout. 2 (b) eserinizd during 10 min rest after 40 min stimulation of uneserinizd perfused ganglion. Rest period: 10 min.

in the absence of eserine. If the initial stimulation was carried out with the normal blood supply to the ganglion left intact, or during perfusion with non-eserinized Locke's solution, the acetylcholine output of the second period of stimulation during perfusion with eserinated Locke's solution showed no such reduction. In all these experiments the rate of stimulation was 10 per sec., the length of the first stimulation period was 40 min., and of the period of rest was 10 min. A typical experiment is illustrated in Fig. 10. Experiment 1(a) and (b) shows the outputs of acetylcholine during the first and second period of stimulation respectively, when the ganglion was perfused throughout with eserinated Locke's solution. Experiment 2(b) shows the output of acetylcholine in the second period of stimulation, when the ganglion was perfused with eserinated Locke's solution. In Experiment 2, however, the first stimulation period was carried out in the absence of eserine, and thus no figures are available for the acetylcholine output at this stage. Experiment 2(a) was an initial stimulation period performed during perfusion without eserine, and during the period of rest the perfusion fluid was changed to eserinated Locke's solution. In another experiment an initial stimulation was performed with the blood supply to the ganglion still intact, cannulation being carried out during

FIG. 11

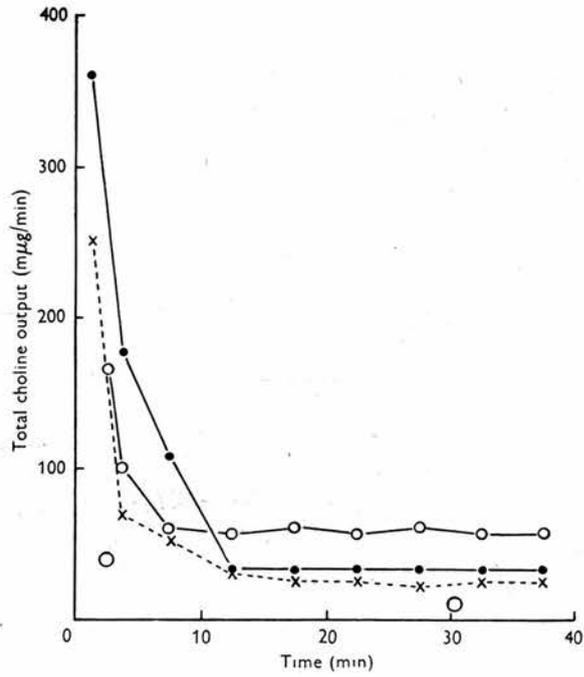


Fig. 11. Graph of output of choline from the unstimulated perfused superior cervical ganglion. Three experiments.

the 10 min. rest. The outputs in the second period of stimulation were very similar to those of Experiment 2(b).

In order to discover whether eserine alone, in the absence of stimulation, would cause a reduction in the output of acetylcholine once stimulation was started, the ganglion was perfused with eserine for 40 min. before stimulating; no reduction in the output was detectable under these conditions. Moreover, the time between starting the perfusion and starting the stimulation was varied from experiment to experiment and no correlation between this delay and the output of acetylcholine was demonstrable.

#### Output of choline from the unstimulated perfused ganglion

When determining the output of choline by acetylating the perfusate, the figures obtained naturally include any acetylcholine present. But, since no acetylcholine is released from the unstimulated ganglion, the values can be taken as reliable figures for choline output.

The observation of MacIntosh (personal communication) that perfusate obtained from a perfused, unstimulated ganglion contains considerable quantities of choline was confirmed. At the start of perfusion the choline output was high and varied from 100 to 400  $\mu$ g. of choline per min. (Fig. 11). This big variation is attributable to the time taken between the

beginning of the perfusion and the collection of the first sample of effluent; if there is any delay in cannulation of the vein, the initial large concentrations of choline pass back into the animal's venous blood. In all experiments the output of choline fell sharply and became steady within 15-30 min. at a level varying from 20 to 60  $\mu$ g. per min. in different experiments, but in any one experiment being quite well defined within narrow limits. The choline in the perfusate may be derived not only from the sympathetic ganglion but also from the stump of the vagus nerve and the nodose ganglion, and parts of the glossopharyngeal and accessory nerves which are included in the perfused tissue. It seems probable that the initial high choline levels are due to a washing out of choline from these structures. The fact that the output of choline becomes steady is more difficult to explain, but may be due to continuous metabolic changes in the perfused tissues; in any case it enables measurements to be made of the effect of stimulation on the choline output.

#### Output of choline from the stimulated perfused ganglion

In order to measure directly the output of acetylcholine in the absence of eserine, it is necessary to assume that the extra choline liberated by stimulation in the absence of eserine derives solely from the destroyed acetylcholine.

FIG. 12

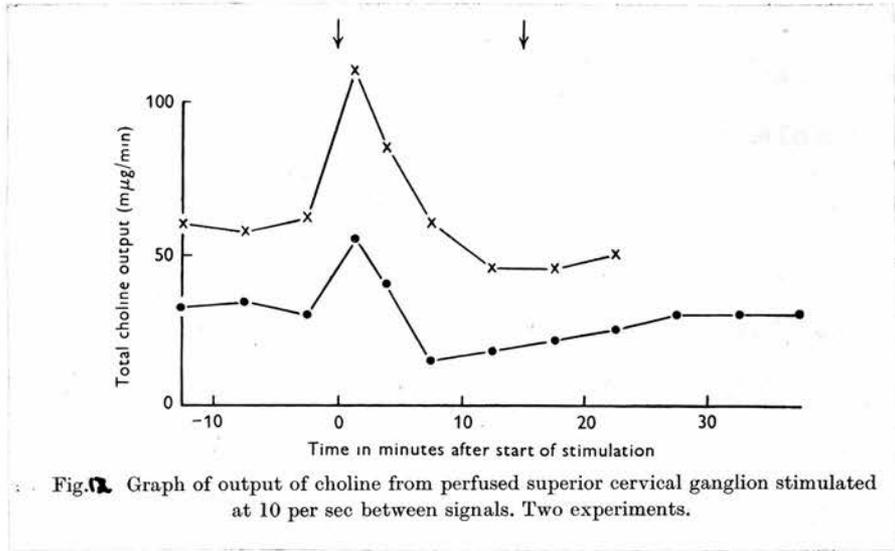


Fig. 12. Graph of output of choline from perfused superior cervical ganglion stimulated at 10 per sec between signals. Two experiments.

Stimulation invariably increases the output of choline by about 20-50  $\mu$ g. per min. (Fig. 12). This corresponds to the amount of acetylcholine found during the initial stimulation of an eserinated preparation. MacIntosh (personal communication) was also able to obtain a release of choline of this order of magnitude during stimulation. The increased output of choline, however, is maintained for a few minutes only, and after about 5 min. the choline output falls below the original resting level, even if stimulation is maintained. The fall below the resting level was slight, but was regularly obtained. At the end of a stimulation period the output may rise again to attain its original resting level.

### DISCUSSION

The results obtained confirm the findings of Brown & Feldberg (1936) that the output of acetylcholine from the stimulated, eserinated, perfused ganglion falls approximately exponentially. The course of this fall has been traced in rather more detail, since the periods of collection of samples were reduced from 5 or 10 to 2 min. This has enabled the early part of the time-course of the output to be followed sufficiently closely to estimate the initial level of output

at the first preganglionic volley. It was found that the first volley would be expected to liberate 100-125  $\mu$ g. of acetylcholine, and this figure is in good agreement with that suggested by Feldberg & Vartiainen (1934) on different grounds.

The hypothesis has been advanced that each preganglionic volley liberates a constant fraction of the available stock of acetylcholine, and that there is a fixed rate of synthesis of acetylcholine which is constant whether or not the ganglion is stimulated preganglionically and whatever the frequency of stimulation may be. Although at frequencies of 30 and 100 per sec. the preganglionic terminals may fail to respond adequately all the results obtained at a variety of frequencies of stimulation fit this hypothesis. Moreover, the recovery in output during periods of rest following prolonged stimulation was found to occur at a rate which also fits in with the assumed constant rate of synthesis - e.g. after 40 min. stimulation, full recovery in output to the original level did not occur even when the rest period given was 35 min.

But there is one fact that is not in agreement with the hypothesis in its present form; both Brown & Feldberg (1936) and MacIntosh (1938) found that the replacement of any loss which might have occurred during prolonged stimulation in the extractable acetylcholine of the ganglion was very rapid indeed.

In fact it required considerable care in extracting the ganglion as quickly as possible after stimulation in order to observe any loss at all. This finding implies a very much more rapid rate of synthesis, at least during periods of rest, than the present hypothesis indicates, and the discrepancy cannot easily be explained. The possibility exists that the synthesis of acetylcholine occurring during rest at a very rapid rate does not provide 'available' acetylcholine in the sense that it cannot be at once released by further stimulation.

The failure of the ganglion to return to its original level of output of acetylcholine, even after long periods of rest, might be attributable to a general deterioration in the preparation over such a long period of perfusion, or to a non-specific effect of eserine (e.g. Holton & Perry, 1951). But eserine alone does not produce such an effect. Perfusion with eserine in the absence of stimulation does not reduce the amount of acetylcholine liberated by stimulation. It is the two factors eserine and stimulation, together, and not either alone, which causes the reduction in output. Dr. W. Feldberg has suggested that in the presence of eserine, the released acetylcholine persists near the nerve terminals for a longer period and that the acetylcholine itself might block subsequent impulses in the nerve terminals. It proved

impossible, however, to demonstrate any reduction in the amount of acetylcholine liberated by stimulation from a ganglion which had previously perfused with Locke's solution containing eserine  $10^{-5}$  and acetylcholine  $10^{-6}$  for 40 min. in the absence of stimulation. It therefore seems unlikely that this explanation is the correct one.

The experiments on the choline output of the ganglion are of theoretical interest. If the amount of choline released had remained high throughout the period of stimulation, it would have been reasonable to infer that this choline represented the destroyed acetylcholine released by stimulation. Therefore, in the absence of eserine, the release of acetylcholine during stimulation would presumably also have remained high, and the fact that it fell off in an exponential fashion in the presence of eserine would suggest that eserine exerted an inhibitory effect on the synthesis of acetylcholine.

If, on the other hand, stimulation had not increased the choline output at all, then the fact that in the presence of eserine increased amounts of acetylcholine were released during stimulation would suggest that in the absence of eserine the released acetylcholine was not only immediately hydrolyzed by the cholinesterase, but that the choline thus formed was at once resynthesized to a 'bound' form of acetylcholine.

In fact, the output of choline actually observed lay

midway between these two hypothetical results. There is a transient increase in the output of choline during the first 5 min. stimulation, and thereafter there is no evidence of any additional release of choline in spite of continued stimulation. It might be assumed that the choline released during the first 5 min. of stimulation is derived from structures in the perfused tissue other than the superior cervical ganglion, and in that case, the second of the hypothetical explanations would be the correct one; namely, that, in the absence of eserine, the output of acetylcholine remains consistently high, but that it is immediately hydrolysed and the choline used in the resynthesis of a 'bound' form of acetylcholine. This is correct fits in with the hypothesis originally advanced to explain the findings in the presence of eserine, since the available stock of acetylcholine in the ganglion would hardly be depleted during stimulation, and each preganglionic volley, liberating a constant fraction of the stock, would release almost the same total amount of acetylcholine. There would, of course, probably be a limit to this in so far as the enzymic destruction and resynthesis might not be sufficiently rapid to cope with the released acetylcholine during very fast rates of stimulation; in such cases there might still be a fall in the output of acetylcholine even in the absence of eserine.

All the known facts about acetylcholine release from the ganglion can be accounted for by the following tentative hypothesis, which is based on a differentiation of the total acetylcholine in the ganglion into two components, an 'available' stock which can be released by stimulation and a total extractable stock which cannot be released by stimulation but which can be extracted by the usual procedures. Such a differentiation has already been proposed, notably by Abdon & Hammarskjöld (1944). A single preganglionic volley liberates a constant fraction of the stock of 'available' acetylcholine. In the absence of eserine the acetylcholine is immediately hydrolyzed and the choline is used in the resynthesis of 'available' acetylcholine. Continuous stimulation is thus accompanied by continuous release of the same amount of acetylcholine per volley (except perhaps at very high rates of stimulation) since the stock of 'available' acetylcholine is little depleted. In the presence of eserine the hydrolysis of the liberated acetylcholine is prevented and consequently resynthesis cannot take place. Continuous stimulation thus leads to a depletion of the stock of 'available' acetylcholine. Replacement of the stock of 'available' acetylcholine is a slow process and occurs at a constant rate of about 4  $\mu$ g. per min., which corresponds to the rate of liberation of

acetylcholine during continuous stimulation after the 'available' stock has been used up. Synthesis of the total extractable acetylcholine in the ganglion is, however, a very rapid process and it is thus very difficult to demonstrate a reduction in the amount of extractable acetylcholine. The synthesized acetylcholine, however, is converted to 'available' acetylcholine only at a slow rate, as quoted, namely, 4  $\mu$ g. per min. For acetylcholine to become 'available' either a further chemical change or a change in its location may be required.

C H A P T E R    I V

GANGLION CELL RESPONSES

(Work done in collaboration with Dr. W.D.M. Paton)

INTRODUCTION

It is generally accepted that synaptic transmission in the superior cervical ganglion and at the neuromuscular junction is chemically mediated, and that acetylcholine is the transmitter responsible. The neuromuscular junction has been the more closely studied, and direct evidence has been obtained (Kuffler, 1943) that acetylcholine, applied directly, will produce a local electrical change at the end-plate sufficient to initiate a propagated impulse in the muscle fibre. Moreover, Burns & Paton (1949) showed that acetylcholine's ability to depolarize the end-plate was shared by certain of the neuromuscular blocking agents (e.g. decamethonium) but not by others (e.g. d-tubocurarine).

Direct evidence of this type has not hitherto been available for the ganglionic synapse and, although Brink,

Bronk & Larrabee (1946) have shown that intra-arterial perfusion with acetylcholine will produce a train of impulses in the postganglionic fibres, there has been no evidence of a local electrical response of the ganglion cell to injected acetylcholine comparable to that described by Kuffler at the end-plate. Eccles (1935<sup>b</sup>) described slow potential waves localized to the ganglion in response to preganglionic nerve volleys; he showed that nicotine affected these slow waves, but did not apply any such tests to acetylcholine.

Emmelin, MacIntosh & Perry (1949) attempted to show depolarization of the ganglion cell membrane, using the exchange of radio-potassium ( $^{42}\text{K}$ ) across the cell membranes as a measure of depolarization. They found that preganglionic stimulation significantly increased the uptake of radio-potassium by the perfused ganglion. They were unable, however, to show that antidromic stimulation of the postganglionic fibres produced the same effect, so that their experiments were inconclusive, since the effect observed might conceivably have been due to the stimulation of short postganglionic fibres supplying the blood vessels of the ganglion, or to potassium exchange in the stimulated preganglionic nerve terminations. It has since been shown (R. Eccles, 1956) that it is very difficult to excite the soma of ganglion cells by antidromic

stimulation and this is consequently the probable explanation of the failure of Emmelin, MacIntosh & Perry (1949).

The following experiments show a local response of the ganglion to injected acetylcholine, and in these experiments remarkable changes in the shape of the action potential complex, caused by acetylcholine-like drugs, led us to investigate the relation between the action potential complex and the cell membrane depolarization.

#### METHODS

Cats were anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (80 mg./kg.). The superior cervical ganglion, usually the right, was prepared as for ganglion perfusion by the method suggested by Kibjakow (1933) but the blood supply was left intact. To minimize the mass of inert tissue under the recording leads, the last stage of the preparation consisted of a dissection of the vagus nerve and nodose ganglion away from the cervical sympathetic trunk and superior cervical ganglion. Bleeding at this point is inevitable, since the blood supply to the nodose ganglion passes through the capsule of the superior cervical ganglion; but if care is

FIG. 13

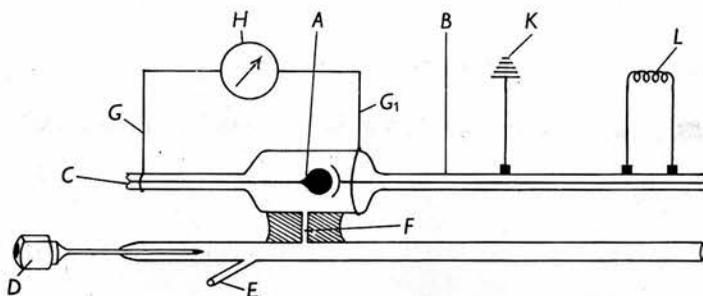


Fig. 13 Recording and injection technique for cat's superior cervical ganglion. *A*, superior cervical ganglion cell; *B*, preganglionic cervical sympathetic trunk; *C*, cut tied end of postganglionic trunk; *D*, needle cannula in distal end of external carotid artery; all other branches of the common carotid are tied except *E*, occipital artery, and *F*, arterial branches supplying the ganglion; *G*, recording electrodes; *H*, d.c. amplifier; *K*, earth lead; *L*, stimulating electrodes.

taken, the bleeding is transient and the preparation does not suffer, and can be used for many hours.

Platinum wire stimulating electrodes were placed on the cut preganglionic cervical sympathetic trunk, and square wave stimuli of 0.5 m.sec. duration were applied at varying frequencies. Non-polarizable (Ag - AgCl) recording electrodes were used and contact with the ganglion itself and with the cut postganglionic trunk was made by thin silk threads soaked in agar - 0.9% NaCl. The arrangement of the electrodes is illustrated diagrammatically in Fig. 64. One lead was looped round the body of the ganglion, the other placed at the point where the postganglionic trunk had been tied and cut.

Retrograde intra-arterial injections were usually used, to avoid the systemic effects of ganglion stimulating drugs, since the vigorous carotid pulsation resulting from intravenous injections interfered seriously with the electrical recording. All the branches of the common carotid artery were tied, except those supplying the ganglion, and a needle cannula was tied into the cut stump of the external carotid artery which was occluded proximal to the cannula by a "bulldog" clamp operated by remote control. In most experiments the occipital artery was left intact, to permit a free flow of blood past the ganglion, which otherwise lay at the end of a cul-de-sac in the arterial

circulation. This procedure appeared to prolong the life of the ganglion.

Action potentials from the recording leads in response to single maximal preganglionic volleys were fed into cathode followers, passed through a D.C. amplifier and recorded on one beam of a cathode ray oscillograph, the second beam being used solely as the marker of an arbitrary baseline. Thus the distance between the two beams provided a measure of the steady potential difference between the ganglion and the cut postganglionic trunk, and slow changes of potential could be recorded as an increase or decrease in this distance. In all records a negativity of the ganglion relative to the cut postganglionic trunk is recorded as an upward deflection, whether transiently due to an action potential, or as a relatively prolonged rise in base line due to a steady depolarization.

In two experiments the spatial distribution of potential along the ganglion and its postganglionic trunk relative to the cut end of the postganglionic trunk was recorded, using the "space-base" described by Burns & Paton (1950). With this technique, one electrode is made to traverse the ganglion and its postganglionic trunk, this movement being recorded horizontally on the cathode ray screen; the other electrode is fixed at the cut end of the trunk, and the potential

FIG. 14

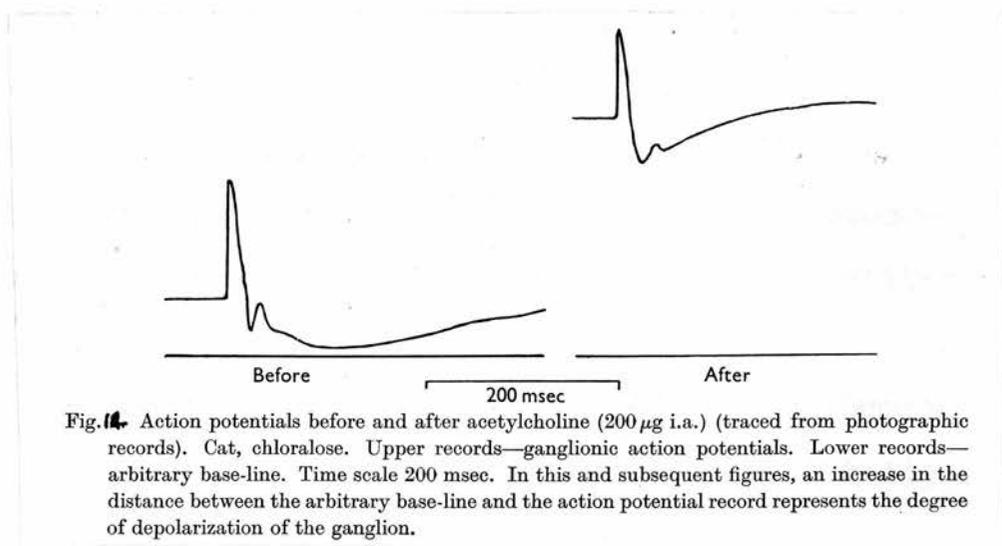


Fig. 14. Action potentials before and after acetylcholine ( $200 \mu\text{g}$  i.a.) (traced from photographic records). Cat, chloralose. Upper records—ganglionic action potentials. Lower records—arbitrary base-line. Time scale 200 msec. In this and subsequent figures, an increase in the distance between the arbitrary base-line and the action potential record represents the degree of depolarization of the ganglion.

difference between these electrodes is recorded as a vertical deflection. In one experiment, the superior cervical ganglion with both its preganglionic and postganglionic trunks was dissected free and immersed in oxygenated Locke's solution at a temperature of 37°C.; in the other experiment, the stellate ganglion was isolated in the same way, since it offers a longer postganglionic nerve from which to record. In order to make a recording, the saline bath was removed from round the ganglion for a few seconds.

## RESULTS

### Effects of injected acetylcholine

Small doses of acetylcholine (1-10 µg. intra-arterially; 200 µg. intravenously) have as their only apparent effect a transient depolarization of the ganglion. The action potential is not greatly affected, although there are actually definite changes, if not in the spike height of the action potential at least in the accompanying slow waves. Larger doses of acetylcholine (200 µg./i.a.) increase both the extent and the duration of the depolarization, and this is then accompanied by a partial or complete abolition of the action potential (Fig. (4)).

FIG. 15

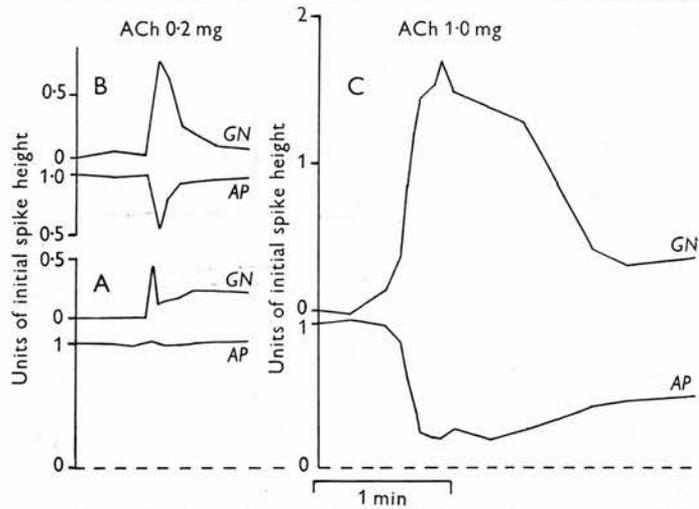


Fig. 15 The effect of acetylcholine on the spike height of the ganglionic action and the depolarization of the ganglion cells. *GN*, ganglionic negativity in terms of initial spike height; *AP*, spike height of action potential. *A*, effect of 200  $\mu$ g acetylcholine i.v.; *B*, effect of same dose of acetylcholine after 200  $\mu$ g eserine i.v.; *C*, effect of 1.0 mg acetylcholine i.v.

FIG. 16

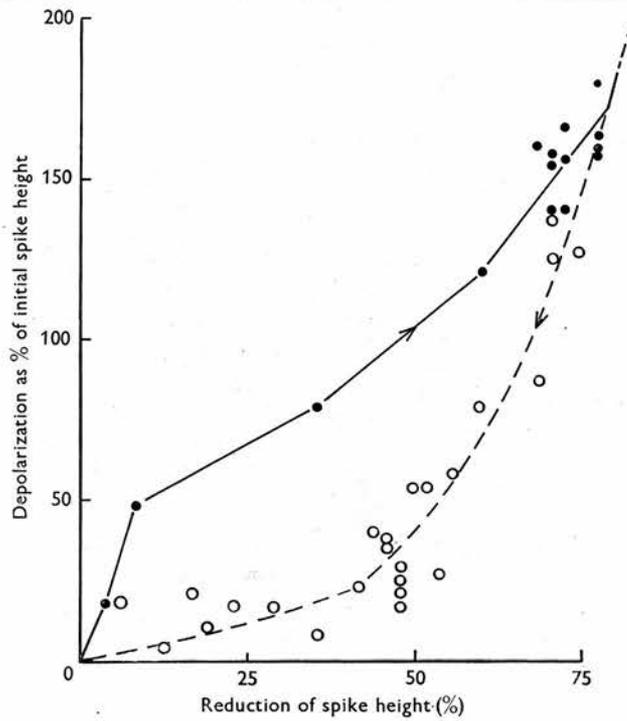
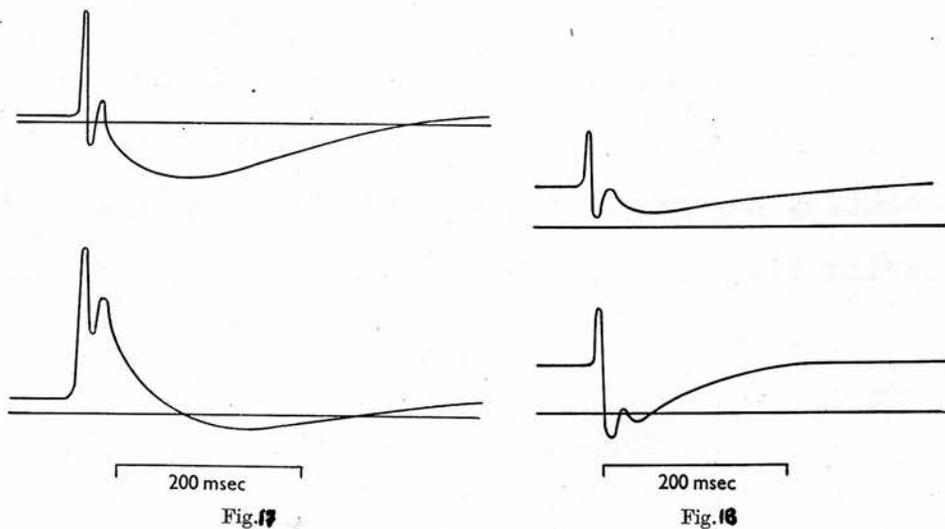


Fig.16 Relationship between depolarization and reduction of spike height of action potential after acetylcholine. Abscissa: depolarization of the ganglion as percentage of initial spike height. Ordinate: percentage reduction of spike height. Closed circles, during onset of block; open circles, during recovery from block.

Where the action potential is only partially abolished there is, in addition to a reduction of the spike height, an obvious distortion in shape, which seems to be due mainly to an effect on the after-positivity. Both the depolarization and the reduction of the action potential may be slightly potentiated by eserine in doses of approximately 200  $\mu$ g. intravenously. These actions are illustrated graphically in Fig. 15. It will be seen that 0.2 mg. of acetylcholine intravenously produced a slight depolarization but no reduction in spike height; in the presence of eserine the same dose produces a larger depolarization accompanied, in this case, by a reduction in spike height. When a dose of 1.0 mg. acetylcholine is injected intravenously, even in the absence of eserine, there is almost complete abolition of the spike with slow recovery during 3-5 min. (Fig. 15). Simultaneously, there is a depolarization of more than twice the initial spike height.

A study of the onset of, and recovery from, depolarization and block of the spike height following acetylcholine (Fig. 16) shows no simple relationship between the two phenomena. The degree of depolarization for a given degree of reduction of the spike potential is much greater during the onset of the effect than during the recovery from acetylcholine. This may be put in another way; the reduction of spike height is greater for a given degree of depolarization the longer the

FIGS. 17 + 18



**Fig. 17.** Action potentials before and after tetanic stimulation. Cat, chloralose. Upper record, tracing of normal action potential. Lower record, tracing of action potential immediately after tetanic stimulation at 76/sec for 10 sec.

**Fig. 18.** Action potentials before and after tetanic stimulation in the presence of eserine (200  $\mu$ g i.v.). Cat, chloralose. Upper record, tracing of action potential before tetanic stimulation. Lower record, tracing of action potential after tetanic stimulation at 76/sec for 10 sec. Time scale 200 msec.

depolarization has been present.

Larger doses of eserine, without the administration of acetylcholine, will abolish the action potential without producing any depolarization of the ganglion. This type of eserine block is seen only after single shocks, since in these circumstances tetani release sufficient acetylcholine to produce a depolarization.

#### The effects of a tetanus

If a tetanus at a frequency of 76 per sec is applied to the preganglionic trunk, a depolarization of the ganglion occurs which outlasts the period of tetanization and of post-tetanic increase of the spike height by 5-10 min. This depolarization is slower in development of its peak size than that observed after intra-arterial injections of acetylcholine, possibly because of a concurrent but shorter-lasting after-positivity resulting from the tetanus. At the same time, the shape of the action potential is greatly altered, although in a way quite different from the change produced by acetylcholine (Fig. 17).

The depolarization evoked by a tetanus is still observed in the presence of eserine, and with sufficient eserine the spike height may be reduced considerably by the tetanus. The action potential again changes in shape (Fig. 18), but now in the same way as it does after acetylcholine.

FIG. 19

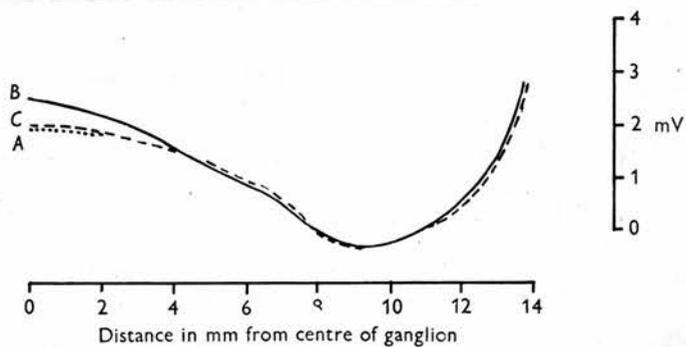


Fig. 19. Records of potential of isolated stellate ganglion and postganglionic trunk relative to cut end of postganglionic trunk using 'space-base'. Abscissa: distance from the centre of the ganglion along the post-ganglionic trunk. *A*, before the addition of tetramethylammonium (TMA) to the bath to make a final concentration of  $1 \times 10^{-4}$  in *B*, during the action of TMA. *C*, after the removal of the TMA.

Decremental spread of ganglion negativity

The fact that the ganglionic body becomes negative to the postganglionic trunk after treatment with the depolarizing drugs, suggests that the depolarization is a local response of the ganglion cells.

Depolarization of the ganglion cells was produced by acetylcholine ( $10^{-5}$ ) in one superior cervical ganglion and by tetramethylammonium iodide ( $10^{-4}$ ) which produces an effect similar to but more long-lasting than that produced by acetylcholine, in one stellate ganglion. Space base records were taken before the addition of these drugs to the bath, during their action, and after washing the ganglion, and Fig. 19 shows tracings of the results obtained on the stellate ganglion. Even before the addition of tetramethylammonium the body of the ganglion was partially depolarized, possibly because the cells in the centre of the ganglion had died from asphyxia as would be expected in a tissue 2-3 mm thick (Hill, 1929). Despite this, tetramethylammonium nevertheless produced a further depolarization of 600-700  $\mu$ V at the centre of the ganglion which disappeared on removal of the drug. Fig. 19 also shows how this additional depolarization diminishes with distance from the centre of the ganglion, falling to one-third of its maximum value approximately 1.8 mm from the centre of the ganglion. Acetylcholine produced on the superior cervical ganglion a

similar but smaller localized ganglion depolarization. The ganglion negativity, therefore, which results from the action of the depolarizing drugs, is a local depolarization of the ganglion cell spreading decrementally a short distance along the postganglionic trunk.

#### Changes in shape of the action potential complex

Eccles (1935) suggested that the latter part of the action potential complex consisted of two separate components, a negative wave (N) and a positive wave (P), both decaying approximately exponentially, the P wave more slowly than the N wave. In order to express the changes in the shape of the action potential in a quantitative way, the records were analysed graphically and the complex could be satisfactorily fitted by the sum of two such exponential curves, although, no doubt, other mathematical functions fitting equally well could be devised. This analysis provides a short and convenient description of the shape of a given action potential in terms of four parameters (two initial magnitudes,  $N_0$  and  $P_0$ ; and two time constants,  $t_n$  and  $t_p$ ). Although only the latter part of the action potential (later than the  $S_3$  and  $S_4$  spikes) was used for this analysis, it was noted that summing the two waves obtained back to zero time reproduced a 'spike' as well as the 'slow waves'.

Although this analysis has a potential value in providing a short means of description, its theoretical value is doubtful, since all records were taken with external ganglion electrodes. Nevertheless, certain facts about the parameters, consistently observed, are worth mentioning.

(1) The mean values for the normal action potential were  $t_n = 33$  msec. (range 26-38),  $t_p = 86$  msec. (range 62-112),  $N_o = 2.8 \times$  initial spike height (range 1.3-3.7),  $P_o = 1.6 \times$  initial spike height (range 0.6-2.4).

(2) The maximal depolarization of a ganglion which could be produced, was approximately equal to the initial value of  $N_o$ , and was much greater than the initial spike height.

(3) With partial depolarization,  $N_o$  was reduced by an amount approximately equal to the degree of depolarization.

(4) The change in shape after acetylcholine and the depolarizing blocking drugs was largely due to a reduction of  $t_n$  which might become vanishingly small. On the other hand,  $t_p$  was not affected. This change in  $t_n$  was completely absent after competitive blocking drugs.

(5) Immediately after a tetanus,  $P_o$  was greatly reduced, without change in the other parameters.

The fact that these parameters can, to some extent, be varied independently, e.g. by a tetanus or by depolarization, lends some support to the idea originally put forward by

Eccles (1935<sup>b</sup>), that there are at least two independent processes taking place during the action potential.

### DISCUSSION

The observation that injected acetylcholine will produce a depolarization of the cells of the superior cervical ganglion fills one of the main remaining gaps in the picture of the mechanism whereby acetylcholine mediates the transmission of the nervous impulse from the preganglionic to the postganglionic trunk.

It has been shown that the depolarization is localized to the ganglion and spreads only decrementally down the postganglionic trunk. In this respect it resembles the slow afterpotentials described and analysed by Eccles (1935<sup>b</sup>). Thus, acetylcholine at the ganglion, as at the neuromuscular junction, can cause a localized depolarization of the cell membrane, which is then capable of exciting the discharge in the postsynaptic fibre already described (for the ganglion) by Brink et al. (1946).

The electrical effects of an injection of acetylcholine are considerably more prolonged than is the response to a single preganglionic volley, where the acetylcholine is released locally

at the nerve terminals and does not flood the whole ganglion. As Brink et al. showed, perfusion with acetylcholine does not cause a single propagated postganglionic spike, but rather a train of such impulses; this corresponds well with the depolarization which we have observed. But even with injected acetylcholine, the transience of the depolarization compared with that produced by other drugs, and the considerable prolongation by eserine, show that the cholinesterase activity of the ganglion is considerable.

With large doses of acetylcholine, or smaller doses in the presence of a little eserine, the spike potential to pre-ganglionic shocks dwindles or disappears entirely. Although the exact relationship between the height of the spike potential and the degree of block (as defined by contraction of the nictitating membrane), has not been established there is presumably some continuous relation between the two, and obviously complete absence of spike implies complete block. In general, the depression of the spike parallels the depolarization in its active course; but a closer comparison shows that the reduction in spike is greater, for a given degree of depolarization, the longer the depolarization has lasted. There are at least two possible explanations for this observation. First, it may be that prolonged depolarization of the ganglion cell membrane leads to a change in its electrical response to

acetylcholine, due (one might imagine) to an alteration in the distribution of ions on either side of the membrane.

Alternatively, the situation may be comparable to that of the neuromuscular junction, for which it has been shown (Burns & Paton, 1951) that persistence of the localized depolarization of the end-plate region by specific depolarizing drugs leads to a spread of the depolarization by discharge of the adjacent membrane. At this site, therefore, the area depolarized some time after the injection of decamethonium may be considerably wider than that immediately after the injection, although the peak height of depolarization is smaller, and the resulting inexcitability of the adjacent membrane leads to a rise of the propagation threshold with lapse of time. If such an explanation is to be applied to the ganglion, it implies that there is a differentiation of the structures within the ganglion as a whole into specifically reactive membrane (the site of a local depolarization) and adjacent membrane excited by electrotonic spread; and that even under the propagated ganglion spike there is a considerable element of local slow non-propagated response. This conclusion had already been reached by Eccles (1935<sup>6</sup>) who states that 'the N wave is considerably developed under the S<sub>2</sub> summit'.

CHAPTER V

BLOCK OF TRANSMISSION

INTRODUCTION

The study of drugs which block ganglionic transmission is normally done by measuring the response of an end-organ innervated by the post-synaptic nerve, e.g. by measuring the contraction of the nictitating membrane of the cat in response to stimulation of the presynaptic cervical sympathetic nerve. The method of direct recordings from the ganglion cells described in Chapter IV, is even more informative, since it can be used to elucidate in more detail the precise mode of action of drugs blocking transmission. In this Chapter studies of this kind, carried out in collaboration with Dr. W.D.M. Paton, are described; and a further study of the effects of muscarine, carried out in collaboration with Dr. N. Ambache and Dr. P.A. Robertson, is also reported.

# TABLE V

TABLE V Modes of action of various drugs blocking transmission at ganglion synapse and motor end-plate

Action	Ganglion	Motor end-plate
Depolarization	Acetylcholine Nicotine Tetramethylammonium (TMA)	Acetylcholine Nicotine Tetramethylammonium (TMA) Decamethonium
Competition with acetylcholine	D-Tubocurarine Pentamethonium Hexamethonium Tetraethylammonium (TEA) Decamethonium (in large doses)	D-Tubocurarine Pentamethonium } (in large Hexamethonium } doses)

FIG. 20

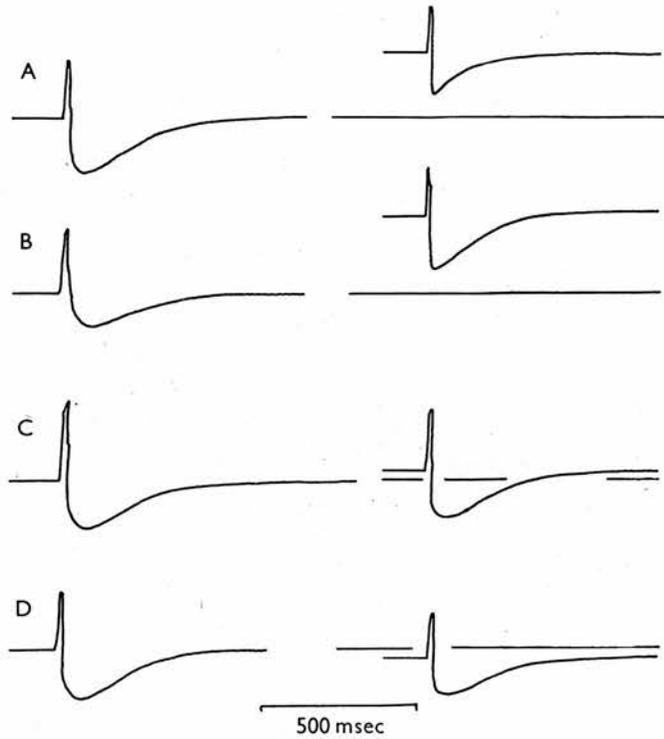


Fig 20. Effect of ganglionic blocking drugs on action potential. Cat, chloralose. Tracings in left-hand column were obtained before and those in right-hand column after the injection of drugs. A, nicotine 50  $\mu$ g; B, tetramethylammonium 50  $\mu$ g; C, tetraethylammonium 50  $\mu$ g; D, pentamethonium 50  $\mu$ g.

Analysis of Block by Ganglion-cell

Recording

METHODS

The methods used were identical with those described in Chapter IV. Doses of tubocurarine chloride, toxiferine I chloride, penta, hexa and decamethonium iodides, nicotine tartrate, tetraethyl and tetramethylammonium iodides are given in terms of these salts. Doses of all drugs were usually given in a volume of 0.2 ml. when injected intra-arterially.

RESULTS

The effects of a number of ganglionic blocking drugs are summarized in Table V, which shows that these drugs can be clearly differentiated into those which depolarize the ganglion and those which do not.

In Fig. 20 some tracings from these experiments are shown. In this figure all the doses given were such that the depression of the action potential is small; nevertheless, the depolarization produced by acetylcholine, nicotine and tetramethylammonium is, in all cases, about 1-1½ times the initial spike height. It is apparent, too, that these substances

FIG. 21

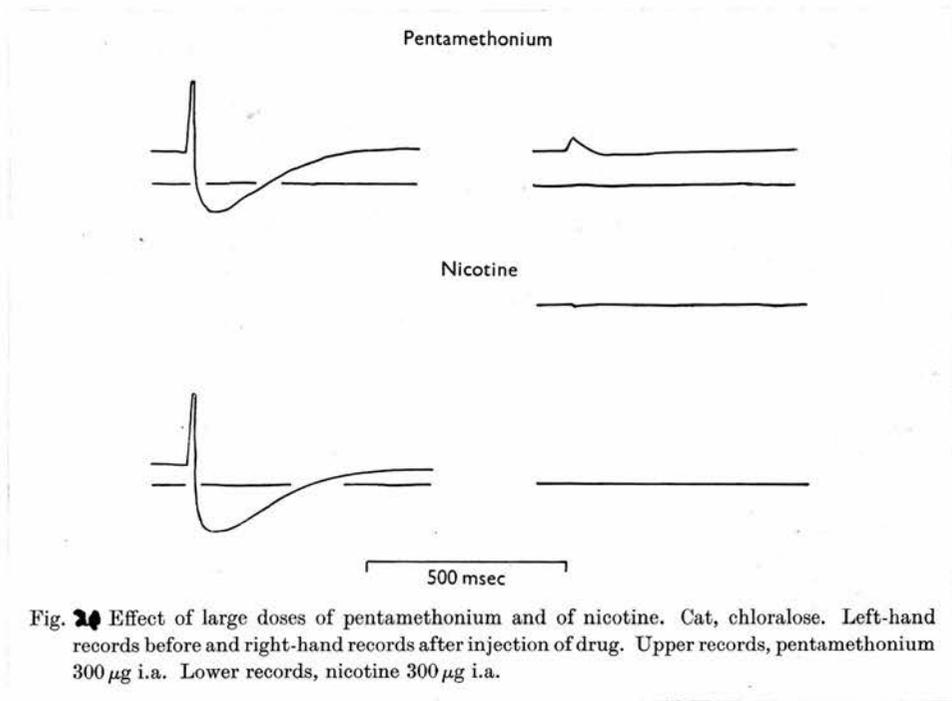


Fig. 20 Effect of large doses of pentamethonium and of nicotine. Cat, chloralose. Left-hand records before and right-hand records after injection of drug. Upper records, pentamethonium 300  $\mu$ g i.a. Lower records, nicotine 300  $\mu$ g i.a.

FIG. 22

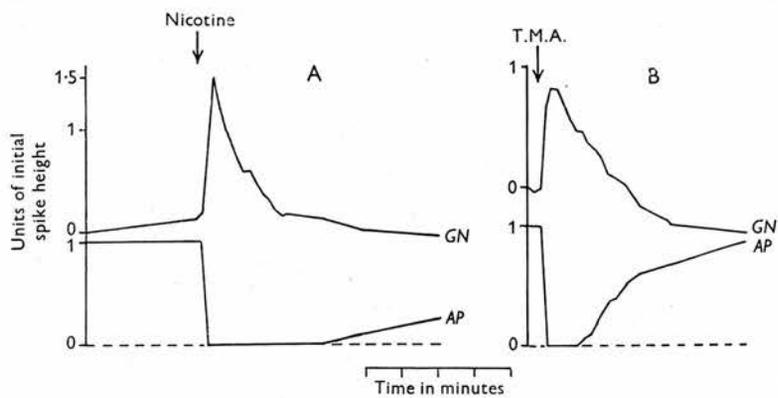


Fig. 22. The effect of nicotine and of tetramethylammonium. (Conventions as in Fig. 15.) A, effect of nicotine  $300 \mu\text{g}$  i.a.; B, effect of tetramethylammonium  $300 \mu\text{g}$  i.a.

produce the same typical distortion in the shape of the action potential complex, a distortion quite absent with the non-depolarizing drugs. The effect of larger doses of nicotine and pentamethonium is shown in Fig. 21. In this case the abolition of the action potential is almost complete; with pentamethonium there is no evidence at all of depolarization, whereas with nicotine the degree of depolarization is nearly three times the initial spike height.

#### Further analysis of the effects of nicotine

The depolarization produced by nicotine was always much more transient than that with acetylcholine or tetramethylammonium, compared with the duration of reduction of spike height produced by it. In the experiment illustrated in Fig. 22A, the ganglion negativity had disappeared 3 min. after the injection, although the spike did not reappear for 10 min., nor return to normal for 30 min. Fig. 22B shows a comparable experiment with tetramethylammonium, in which the recovery from depolarization is much slower and almost parallels that of the spike height.

These observations suggested that nicotine might possess a mixed action, partly acetylcholine-like, and partly by antagonizing the effects of acetylcholine. If this were the case, one would expect that a subsequent identical dose of

FIG. 23

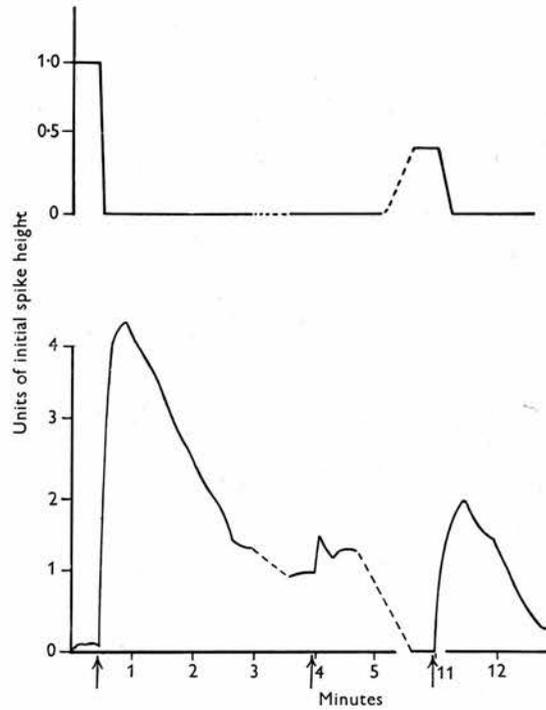


Fig. 23. The effect of repeated doses of nicotine. Upper record, spike height of action potential in terms of initial spike height. Lower record, depolarization in terms of initial spike height. At arrows  $200 \mu\text{g}$  nicotine i.a. Continuous lines represent continuous recording with stimuli once every 3 sec; dotted lines represent absence of recording. Time scale in minutes.

nicotine, given soon after the disappearance of the ganglion depolarization but before full recovery of the spike potential, would produce a smaller depolarization than normally. Fig. 23 shows that this is true. The second dose of nicotine produced only one-eighth or less of the original depolarization, which had almost completely passed off. As the spike potential returned towards its normal height, the depolarization produced by a third identical dose of nicotine also increased; in Fig. 23 where the spike had returned to 50% of normal, the depolarization was 50% of its original magnitude.

#### DISCUSSION

The classification of ganglion blocking drugs according to whether or not they depolarize the ganglion (Table V) corresponds to their pharmacological properties, in that those which depolarize also have as their first action a stimulation of the ganglion, whereas those lacking such stimulant action uniformly fail to depolarize. The first group will be referred to as depolarizing, and the second group as competitive blocking agents. (We are not here concerned with drugs such as local anaesthetics which interfere with ganglionic transmission by preventing the release of acetylcholine). It follows that depolarization of the ganglion (of any origin) may give rise to a train of impulses in the postganglionic trunk, before block

supervenes due to the persistence or spread of the depolarization. This classification is precisely analogous to that at the neuromuscular junction (Burns & Paton, 1951; Zaimis, 1951), where the depolarizing drugs are also those causing signs of stimulation (twitch), contracture and repetitive discharge.

Apart from acetylcholine, the only two drugs amongst those tested which depolarized were tetramethylammonium and nicotine, of which tetramethylammonium appeared to resemble acetylcholine in everything except its speed of destruction. With nicotine, however, the depolarization was always transient, although the reduction in spike and indeed the block might be prolonged. During the period of block, in the absence of depolarization, a further identical dose of nicotine produced only a small fraction of the original depolarization. This association of block without depolarization with resistance to a depolarizing drug during the block probably implies a blocking action by nicotine of the competitive type. It appears, therefore, that nicotine has two distinct actions, an initial depolarization accompanied by excitation and then block, which passes over into a typical competitive block. This may explain why it is traditionally recommended that to produce block with nicotine the doses should be divided and slowly worked up, thereby securing the competitive block with a minimum of stimulation.

Among the competitive blocking drugs investigated were decamethonium and eserine. A particularly interesting comparison is afforded by the actions of decamethonium at the two synapses. That the cell membranes at these two sites are closely related physiologically follows from the fact that at both the normal transmitter, acetylcholine, produces as its first effect a local depolarization. Yet whereas at one site, the neuromuscular junction, decamethonium almost rivals acetylcholine in its depolarizing activity, at the ganglion synapse it is not only almost inactive but, when given in relatively large doses it actually produces block not by depolarizing the membrane but by the alternative method of competition with acetylcholine. This illustrates the fact that, in spite of the similarity in the two membranes in respect of their reaction to acetylcholine, there must be distinct physical differences between them; a fact which might well go undiscovered but for pharmacological distinctions of this sort. This appears to be the only known case where a drug will produce block at both synapses, but by different mechanisms.

The effects of eserine are usually overshadowed by its powerful antipesterase activity. But other actions have been described, such as the non-specific increased excitability it

produces in the ganglion (Feldberg & Vartiainen, 1934), its blocking action in relatively small doses on antidromic vasodilatation (Holton & Perry, 1951), its curare-like action at the end-plate and in very large doses (Eccles & MacFarlane, 1949; Fatt, 1950), its depressant action on nerve conduction (Bullock, Nachmansohn & Rothenberg, 1946). To these actions can now be added ganglionic block which (in the absence of pre-ganglionic excitation) is not accompanied by depolarization. This is probably a true competitive block and not, like procaine, a block of the preganglionic nerve terminals, since Feldberg & Vartiainen (1934) showed that during perfusion with high concentrations of eserine the ganglion was not responsive to injected acetylcholine.

There is usually little practical difficulty at present in allotting a given compound to one or the other class of blocking agents, since injection of any depolarizing blocking agent causes (by ganglionic excitation) a vigorous contracture of the unexcited nictitating membrane, whereas none of the competitive blocking agents do this. But the action of nicotine, in which a depolarizing is succeeded by a competing action, already indicates that this simple test may not reveal the complete picture. It is, further, possible that a drug might produce a depolarization of such slow onset that

excitatory effects were trivial, although the block was due to this depolarization. For the time being, therefore, it appears that an electrical record of the type described is essential to the complete study of the mode of any paralysis of ganglionic transmission.

## B. The Effect of Muscarine

### INTRODUCTION

In 1914 Dale described the actions of acetylcholine as being of two distinct types, namely, muscarinic and nicotinic. He defined the muscarinic action as "the action which true muscarine exhibits in its pure form, uncomplicated by the nicotine action". The muscarine effects of choline esters were "purely peripheral in their origin, unaffected by nicotine in large doses, but readily abolished by small doses of atropine". This useful classification has been widely used ever since. With increasing knowledge of the physiology of the autonomic nervous system, it is now possible to define nicotinic effects as the effects of acetylcholine and other drugs at ganglionic synapses of both sympathetic and parasympathetic ganglia, and also at neuromuscular junctions; and muscarine actions as

actions on the effector cells innervated by postganglionic cholinergic fibres, mostly in parasympathetic nerves.

Reasons have been given previously (Ambache, 1949) for suspecting that natural muscarine may have actions other than those hitherto strictly defined as "muscarinic". During an investigation of the pharmacology of the synthetic compound 2268F (acetal of 2:3-dihydroxypropyl trimethylammonium iodide) thought to be isomeric with muscarine, it was found that, although endowed with intense muscarinic activity this compound also exhibited nicotinic effects on striated muscle and sympathetic ganglia. The ganglionic effect was shown to be antagonized by atropine, and the suggestion was made that in experiments on the blood pressure of atropinized cats this type of antagonism might well prevent the detection of the nicotinic effects of 2268F and of similar compounds (as in the experiments of Fourneau, Bovet, Bovet & Montezin, 1944) and possibly also of muscarine itself. An analogous situation was described later by Ing, Kordik, and Tudor Williams (1952) for furmethide (furfuryl trimethylammonium iodide) and for its 5-methyl derivative. Both these compounds have powerful muscarinic actions; they also display some degree of nicotinic activity on the perfused superior cervical ganglion. Yet, even in large doses, neither produces a rise in blood pressure in the atropinized cat. Root's (1951) experiments with pilocarpine

also suggest that a pressor action of this substance may have been masked by atropine. Furthermore, the ganglion-blocking action of atropine may antagonize even the effect of acetylcholine, as found in denervated ganglia by Konzett & Rothlin (1949). Several examples of this type of ganglionic action of atropine have been tabulated in a previous paper (Ambache, 1954). We are thus faced, firstly, with a considerable body of evidence suggesting that atropine may block not only muscarinic effects but also certain nicotinic effects of muscarine-like substances, and, secondly, with the possibility that muscarine itself may have certain nicotine-like actions.

The effect of a sample of chromatographically purified, crystalline, muscarine chloride on perfused preparations of the cat's superior cervical ganglion was therefore examined. Waser (1955) found another sample of muscarine chloride, prepared by Eugster & Waser (1954) that had no pressor, "nicotinic" effect on the cat's blood pressure, but his experiments were conducted in the presence of atropine. When, however, Konzett & Waser (1956) examined the activity of Eugster & Waser's sample of muscarine on perfused preparations of cats' cervical ganglia, they obtained results which were identical with those reported here, and also showed that a subthreshold dose of

muscarine may potentiate responses to acetylcholine and to preganglionic stimulation.

### METHODS

Muscarine. A sample of highly purified crystalline muscarine chloride, prepared from Amanita muscaria at the Wellcome Research Laboratories, was sent to us through the courtesy of Dr. S. Wilkinson and Dr. J.W. Trevan. When tested on guineapig ileum preparations, this sample was active at a threshold concentration of  $3 \times 10^{-9}$  (Ambache & Lessin, 1953; 1955).

Perfusion of the Superior Cervical Ganglion. Cats were anaesthetized with 40 mg./kg. pentobarbitone sodium intraperitoneally or, in a few experiments, with ether followed by intravenous chloralose (80 mg./kg.). The right superior cervical ganglion was prepared by the method described by Kibjakow (1933), with modifications suggested by Feldberg & Gaddum (1934), and was perfused with aerated Locke's solution containing 2 g./l. of glucose and filtered through sintered glass (No. 4 Jena). The Locke's solution was warmed by passage through a plastic tube lodged in the oesophagus, as suggested by MacIntosh (personal communication). Otherwise, apparatus and method were the same as used by Ambache (1949), except that the linen filter in the

FIG. 24

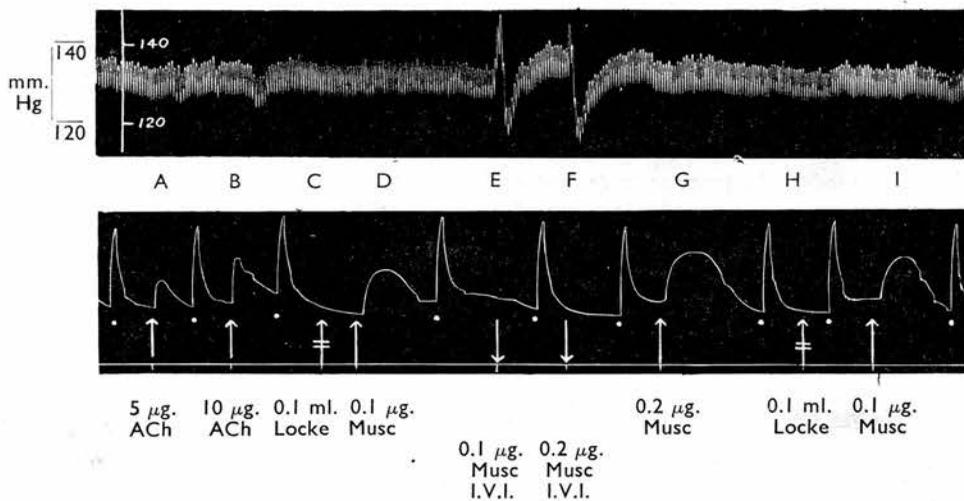


FIG. 24—Cat, perfusion of innervated superior cervical ganglion. Effect of pure muscarine. Above, femoral blood pressure; below, contractions, upwards, of the nictitating membrane. At the dots, maximal preganglionic stimulation applied for 5 sec. Muscarine: 0.1  $\mu$ g. at D and I, and 0.2  $\mu$ g. at G, administered to the perfused ganglion; and 0.1  $\mu$ g. intravenously at E and 0.2  $\mu$ g. at F (downward arrows). Acetylcholine at A and B, and Locke solution at C and H, into the perfusion stream.

perfusion circuit was replaced by a small sintered glass filter. Blood pressure was recorded from a femoral artery. Intravenous injections were made into the contralateral femoral vein.

Denervated Ganglia. In an initial aseptic operation under pentobarbitone sodium anaesthesia the right, or sometimes both, superior cervical ganglia were decentralized by avulsion of about 1 in. of the vagosympathetic trunk low in the neck. The denervated ganglia were perfused after an interval varying from 11 days to 5 months.

### RESULTS

Action of Muscarine on Normal Ganglia. As is well known, very small doses of muscarine, given intravenously, cause a large fall in blood pressure in animals which have received no atropine. Muscarine was therefore administered to the ganglion by injection into the perfusion fluid. Injection of muscarine in this way into normal ganglia was followed by a contraction of the nictitating membrane in 14 out of 16 experiments. The effective dose of muscarine varied considerably. Thus in the fourteen experiments in which muscarine produced a contraction, the doses were: in eight, 20-187  $\mu\text{g.}$ ; in three, 1.6-4  $\mu\text{g.}$ ; and in another three, 0.1  $\mu\text{g.}$  An experiment on one of the most sensitive preparations is illustrated in Fig. 24.

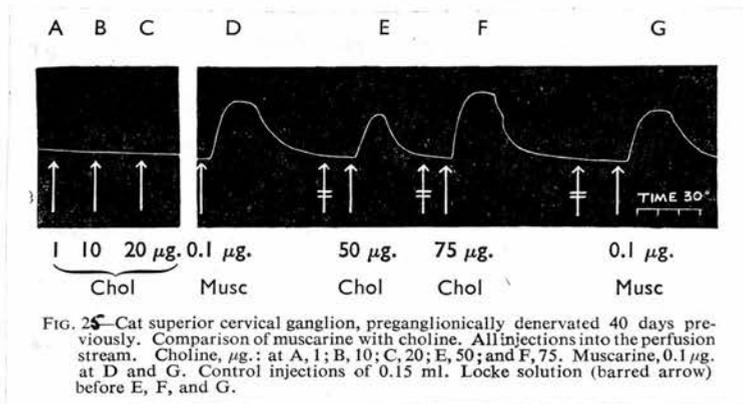
In this experiment the femoral blood pressure was also recorded. Muscarine in a dose of 0.1  $\mu$ g. produced a contraction of the nictitating membrane, which was roughly equal in size to that produced by the injection of 10  $\mu$ g. of acetylcholine, but which developed more slowly, after a longer latency (9 sec.), and which was of longer duration. In this experiment the ganglion was rather less sensitive at the start of perfusion, requiring doses of 0.1-1  $\mu$ g. of muscarine for contractions to be elicited.

This increasing sensitivity during prolonged perfusion was noticed in several other experiments. In one cat 20  $\mu$ g. of muscarine was quite without effect at an early stage of the perfusion, although later on a threshold response was obtained with 0.2  $\mu$ g. This gradual sensitization of the ganglion may be due to a cumulative effect of muscarine. In the 2 experiments in which we failed to get any response to muscarine, the drug was given only at the start of the perfusion and the dose was not increased above 20  $\mu$ g. owing to the scarcity of material. From Fig. 24 it is apparent that the potency of muscarine relative to acetylcholine was of the order of 100:1, but values of this ratio varying from 200:1 to 1:20 were observed. The varying ratio could, however, almost wholly be accounted for by variations in the sensitivity to muscarine. Thus the ratio not only varied from cat to cat but also tended to increase as the perfusion proceeded in any one cat.

Control injections of Locke solution did not produce stimulation in any of the experiments.

Action of Muscarine on Denervated Ganglia. Muscarine stimulated all of 13 ganglia which had been decentralized for periods long enough to allow degeneration of their preganglionic nerve supply. This showed that the ganglionic effect of muscarine could not be attributed to indirect stimulation of preganglionic nerve fibres and endings. As a group, the denervated ganglia appeared to be more consistent in responding to low doses of muscarine than the normal ganglia, of which only a small proportion had responded to as little as 0.1  $\mu\text{g}$ . muscarine. In fact all the denervated ganglia responded to small doses of muscarine - five to 0.2-0.5  $\mu\text{g}$ .; seven to 2-4  $\mu\text{g}$ .; and one to 12.5  $\mu\text{g}$ . Thus in the denervated ganglia the threshold dose for stimulation was rarely greater than 3  $\mu\text{g}$ . It appears, therefore, that the occasional relatively insensitive normal ganglion may be rendered sensitive by denervation. Little increase in the sensitivity to acetylcholine after denervation was observed, and, on the average, the dose required for stimulation was some one-third to one-half of that required in normal ganglia, there being a considerable overlap in the effective doses in the two groups.

FIG. 25



Comparison with Choline. The most common pharmacologically active impurity in extracts of Amanita muscaria is choline; in fact such extracts may contain 20 times as much choline as muscarine (King, 1922). Although the specimen of muscarine used was believed to be pure, the effects of choline injected in the same way as the muscarine were tested (Fig. 25). It will be seen that 75  $\mu$ g. choline was required to produce a contraction equivalent in size to that produced by 0.1  $\mu$ g. muscarine. Thus, the effect of the crystalline material could not be due to traces of choline in it, since, even if it had consisted entirely of choline and had contained no muscarine at all, it would then have had no effect. In other experiments muscarine was 200-500 times as active as choline.

Site of Action of Muscarine. The contractions of the nictitating membrane produced by the injection of small doses of muscarine into the perfusion stream of the isolated superior cervical ganglion were most likely to have been due to a stimulant action of muscarine on the ganglion cells. Nevertheless, the following additional evidence was obtained to show that its site of action was truly ganglionic.

It is known that the cat's nictitating membrane receives both adrenergic and cholinergic fibres from the

postganglionic cervical sympathetic nerve (Bacq & Fredericq, 1935); and, indeed, the smooth muscle in the membrane responds to acetylcholine, an effect which is classifiable as muscarinic. If, therefore, muscarine were to escape out of the perfused ganglion and to reach the nictitating membrane itself, it might produce a local contraction unrelated to any ganglion-stimulant effect. In a number of experiments it seems to be virtually impossible to achieve complete isolation of the perfused tissue from the general circulation. In about 10% of cats this is due to patency of the internal carotid artery, a condition described by Davis & Story (1943). The existence of a patent internal carotid artery can be detected by momentarily lowering the perfusion pressure to zero and observing whether there is a reflux of arterial blood into the perfusion cannula. When reflux occurred, the internal carotid artery was looked for and tied. Even in the absence of a patent internal carotid, and of reflux, the ganglionic perfusate is always slightly tinged with blood when the perfusion pressure is below a certain critical level, indicating that there must be small vascular connexions between the ganglion and the general circulation. These, almost certainly, are situated in the postganglionic trunk, which has to be left intact. On raising the perfusion pressure above the critical level, the venous effluent becomes clear, but the possibility then exists

FIG. 26

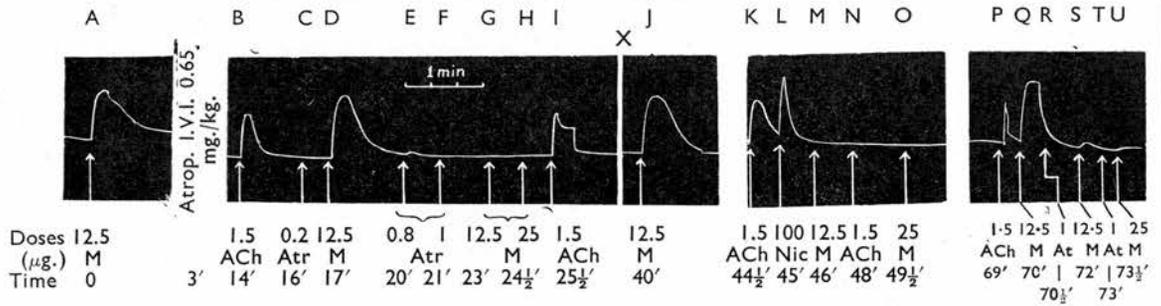


FIG. 26—Cat, perfusion of superior cervical ganglion, 142 days denervated. Ganglionic responses to muscarine 12.5  $\mu\text{g.}$  at A, D, G, J, M, Q, and S, and 25  $\mu\text{g.}$  at H, O, and U; to acetylcholine 1.5  $\mu\text{g.}$  at B, I, K, N, and P, all injected into the perfusion system. Atropine 0.65 mg./kg. administered intravenously to the cat 11 min. before B. Atropine administered to the perfused ganglion: 0.2  $\mu\text{g.}$  at C, 0.8  $\mu\text{g.}$  at E, and 1  $\mu\text{g.}$  at F, R, and T. At L, 100  $\mu\text{g.}$  nicotine to perfusion. Drum stopped at X. Timing of doses given below in min.

FIG. 27

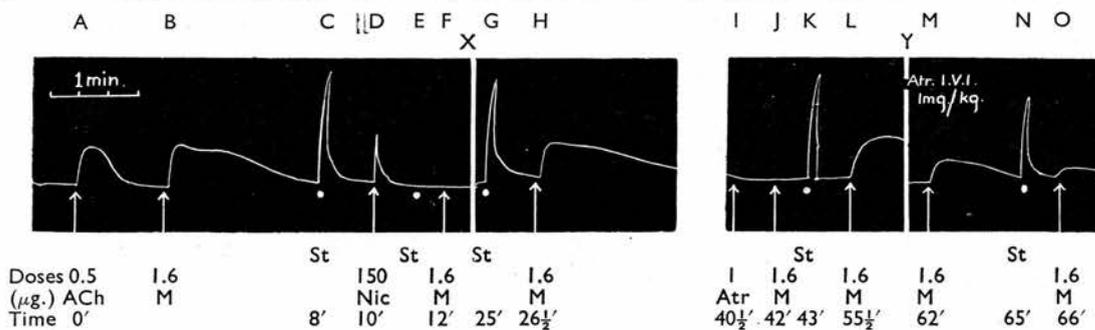


FIG. 27—Cat, perfusion of normal superior cervical ganglion. Injections into the perfusion stream: at A, acetylcholine 0.5  $\mu$ g.; muscarine 1.6  $\mu$ g. at B, F, H, J, L, M, and O. At the white dots maximal preganglionic stimulation for 5 sec. At D, 150  $\mu$ g. nicotine, and at I, 1  $\mu$ g. atropine, administered to the ganglion. Atropine 1 mg./kg. intravenously before M (at Y). Drum stopped at X and Y; timing of doses given below in min.

of some perfusate entering the general circulation. Some idea of the extent of this leakage into the general circulation may be gained by taking concurrent records of the blood pressure. In a few experiments, a fall in blood pressure occurred after injections of muscarine or of acetylcholine into the perfusion fluid; but in 5 of the experiments this was completely absent, and in general it was negligible. Thus, Fig. 24 illustrates that the administration to the ganglion of 0.2  $\mu$ g. muscarine, which elicited a large contraction of the nictitating membrane, was almost without effect on the blood pressure, whereas 0.1  $\mu$ g. muscarine, when injected intravenously, produced a considerable depressor effect and very little action on the nictitating membrane.

Further evidence for the ganglionic site of action of the muscarine in these experiments was obtained from the following study of its inter-action with other drugs.

Antagonism of the Ganglionic Action of Muscarine by Atropine Administered to the Ganglia. Atropine in very small doses blocked the stimulant action of muscarine on the ganglion. This effect was reversible and was obtained both in normal and in denervated ganglia, as illustrated in Figs. 26 and 27. In the experiment of Fig. 27, on a normal ganglion, after a dose of 1  $\mu$ g. atropine was administered into the perfusion fluid at I, the response to a previously effective

dose of muscarine (1.6  $\mu$ g.) was completely abolished (J). This dose of atropine had no effect on the response to preganglionic stimulation (K), and its blocking effect to muscarine itself had completely passed off in 15 min. (L). A dose of 1 mg./kg. of atropine given intravenously (before M) produced, in this experiment, some depression of the response to muscarine and to preganglionic stimulation. This was attributed to some reflux, in this experiment, of atropine from the general circulation into the perfused ganglion, since in several other experiments similar doses of atropine given intravenously had no such depressant action on the ganglionic response to muscarine, as can be seen in the experiment of Fig. 26. These observations again excluded an action of muscarine on the nictitating membrane since the ganglionic effect of muscarine was obtained when the rest of the cat was atropinized, including its nictitating membranes.

Small doses of atropine did not block the effects of injected acetylcholine to the same extent as they blocked the effect of muscarine (Fig. 26); nevertheless, slightly larger doses (1-10  $\mu$ g.) did block injected acetylcholine and still larger doses (100  $\mu$ g.) could block the effects of preganglionic stimulation as well. (Fig. 27)

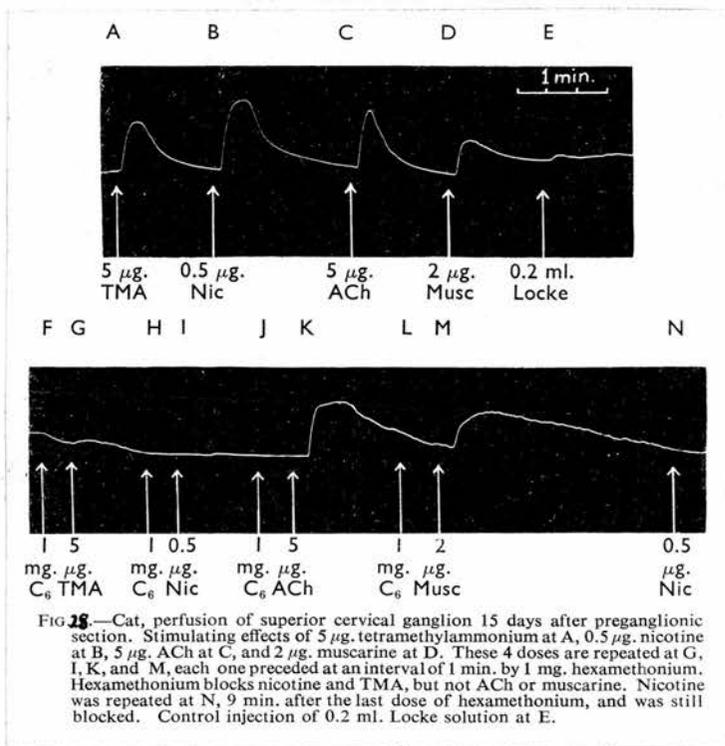
Suppression of Muscarine Responses by Ganglion-  
blocking Drugs

Normal Ganglia. Fig. 27, D-F, shows that when transmission in the ganglion had been completely blocked by 50-100  $\mu$ g. nicotine, the stimulant effect of muscarine was also totally abolished, but recovered later when normal transmission returned. Similar effects were produced by tetramethylammonium (TMA) when this was substituted for nicotine in other experiments.

On the innervated ganglia 0.1-1 mg. hexamethonium blocked preganglionic stimulation, acetylcholine and muscarine.

Denervated Ganglia. Preganglionic denervation produced an interesting change in the susceptibility of ganglia to certain blocking agents. Drugs which block by depolarizing ganglion cells (Paton & Perry, 1953), such as nicotine and TMA, never failed to block the ganglionic actions of muscarine or of acetylcholine. On the other hand, hexamethonium and tetraethylammonium (TEA), which are non-depolarizing competitive blocking agents on normal ganglia, were no longer effective in blocking either acetylcholine or muscarine. Whereas in normal ganglia, 0.1-1 mg. hexamethonium produced total block, in

FIG. 28



denervated ganglia, doses of 10 mg. were completely ineffective. On the other hand, in denervated ganglia, relatively small doses (0.5-1 mg.) of hexamethonium and TEA still blocked the stimulant effects of nicotine, of the 3-bromophenyl ether of choline, and of TMA completely. Some of these findings are shown in Fig. 28, which illustrates the block produced by successive doses of hexamethonium to TMA and nicotine but not to acetylcholine or muscarine.

#### DISCUSSION

Muscarine, like acetylcholine, is capable of stimulating the ganglion cells of the cat's superior cervical ganglion. The fact that muscarine itself has such an action suggests to us that it might be preferable, in future, to use the term "parasympathomimetic" when referring to actions hitherto known as "muscarine".

The ganglionic stimulant action of muscarine is blocked by a tropine even in very small doses. Likewise, atropine has some effect, even in normal ganglia, in depressing the responses to acetylcholine. The doses of atropine required are not large and the action must be regarded as a specific one. Moreover, the ganglion-stimulant effects of muscarine are also blocked,

in normal ganglia, by all the usual ganglion-blocking agents, both depolarizing and competitive.

The fact that, in normal and denervated ganglia, muscarine stimulation is abolished by nicotine is strong supporting evidence that the effect of the drug is truly ganglionic. This is also borne out by the results with other ganglion-blocking drugs. However, after denervation a remarkable change was observed, for which no simple explanation is at present forthcoming.

Hexamethonium, which before denervation completely blocked the actions of all the ganglion-stimulating drugs, failed after denervation to block acetylcholine and muscarine, although still fully effective against nicotine and TMA. This finding seems to argue that the changes in the cell membrane after denervation are such that the receptors for acetylcholine and muscarine are no longer affected by hexamethonium, while those for nicotine and TMA remain susceptible to this drug. If this is the correct interpretation, it implies that the membrane of the denervated ganglion cell is differentiated and contains at least two types of receptor. Paton & Perry (1953) discussed the possibility that the ganglion cell membrane is differentiated into specifically reactive patches of membrane which are the site of a local depolarization, and adjacent parts of the membrane

excited by electrotonic spread. It is conceivable that a similar differentiation may explain the present drug effects.

Župančić has propounded the hypothesis (see references in Župančić & Majcen, 1956) that the receptors for acetylcholine are very similar to, if not identical with, cholinesterase (ChE). According to this hypothesis, the receptor protein at sites where acetylcholine exerts a nicotinic action would resemble aceto-ChE, as both are known to be depressed by an excess of substrate. At "muscarinic" sites the receptor would resemble butyro-ChE, as neither is inhibited by substrate excess. According to Župančić, some of the changes in the pharmacology of skeletal muscle after denervation are explicable by assuming an alteration in the receptor protein towards the butyro-ChE type, and it is conceivable that a similar change may occur in denervated ganglia. In histochemical studies on various ganglia in cats, including the superior cervical, Koelle (1950, 1951) reported that, after preganglionic denervation, aceto-ChE had almost completely disappeared, except in occasional ganglion cells. On the other hand, the butyro-ChE remained abundantly visible throughout the histological sections. Were the muscarinic site of action in denervated ganglion cells predominantly of the butyro-ChE type, hexamethonium would not block muscarine

or acetylcholine, since it does not block the action of these drugs at the "muscarinic receptors" in smooth muscle, which are also of the butyro-ChE type. Moreover, Županić has shown that atropine can block the butyro-ChE but not the aceto-ChE; this would also fit with the results we obtained on the ganglia. Excess of nicotine on the other hand, appears to render the ganglion cells inexcitable to all drugs, whatever their receptor sites.

Muscarine was shown by Dale & Gasser (1926) to be devoid of nicotinic action at the neuromuscular junction. We have in a few unpublished experiments confirmed this, using the frog's rectus abdominis, the leech dorsal muscle, and the pigeon's iris; Dr. E. Zaimis (personal communication) has also confirmed it in an experiment on a normal cat's tibialis anticus. Thus muscarine appears to belong to a group of drugs all of which possess an action at ganglionic synapses but not at normal neuromuscular junctions. The other members of this group are arecoline (Dale & Gasser, 1926; Feldberg & Vartiainen, 1934), pilocarpine and acetyl- $\beta$ -methyl choline (unpublished observations).

## CHAPTER VI

### EFFECTS OF DENERVATION

#### INTRODUCTION

That previous section of the preganglionic nerve alters the reactions of ganglion cells has long been recognized. Cannon & Rosenblueth (1949) described the increased sensitivity of denervated ganglion cells to acetylcholine. Electrical stimulation of the distal end of the cut preganglionic nerve is usually completely ineffective 72 hr. after section, and this failure in transmission is accompanied by other changes. Thus MacIntosh (1938) showed that the acetylcholine content of the ganglion fell to 20% of normal 70 hr. after section of the preganglionic fibres. Feldberg (1943) and Banister & Scrase (1950- described the reduced power of such a denervated ganglion to synthesize acetylcholine and ascribed this to a concomitant reduction of its choline acetylase content; while Sawyer & Hollinshead (1945) showed that the true cholinesterase content of the ganglion also fell after denervation, although somewhat more slowly,

reaching 20% of normal only some 200 hr. after section of the preganglionic fibres.

As described in Chapter V, hexamethonium failed to block the stimulant action of acetylcholine in a denervated ganglion. This failure could not be ascribed simply to an increased excitability of the cells to acetylcholine, nor to any of the other known biochemical changes resulting from denervation, and its explanation remained obscure.

Both Kewitz & Reinert (1953) and Ambache (personal communication) have shown that, in innervated ganglia, under certain circumstances, ganglion-blocking drugs might block the effects either of injected acetylcholine or of preganglionic stimulation, but not of both; and it was conceivable that these phenomena were related to the analogous one in denervated preparations. The reactions of innervated ganglion cells in extracellular fluids containing varying concentrations of potassium, the effects on denervated ganglia of glutamate ions, which are known (Davies & Krebs, 1952) to promote the inwards transfer of potassium ions into various tissue cells against the ionic gradient, were therefore studied. This work was done in collaboration with Dr. H. Reinert.

METHODS

Cats were anaesthetized with ethyl chloride and ether followed by intravenous chloralose (80 mg./kg.). The superior cervical ganglion was perfused by the method introduced by Kibjakow (1933) with some modifications suggested by Perry (1953). The perfusion fluid was Locke's solution containing twice the usual amount of glucose thus making a final concentration of 200 mg./100 ml. Normal Locke's solution contains 20 mg./100 ml. potassium chloride; any variation in this amount is specifically noted in the text. In certain experiments, also noted in the text, sodium L-glutamate was added to the Locke's solution. The concentration was adjusted so that one molecule of glutamate was available for every potassium ion in the solution. In Locke's solution this is achieved by using a concentration of glutamate of approximately 50 mg./100 ml.

Denervated ganglia were prepared 3-9 weeks before use, by anaesthetizing the cats with ethyl chloride and ether and, with the usual aseptic precautions, removing about 2 cm. of the appropriate cervical sympathetic trunk.

Contractions of the nictitating membrane were recorded with a frontal writing-point lever in the usual way. Precautions to ensure that the drugs injected into the perfusion

FIG. 29

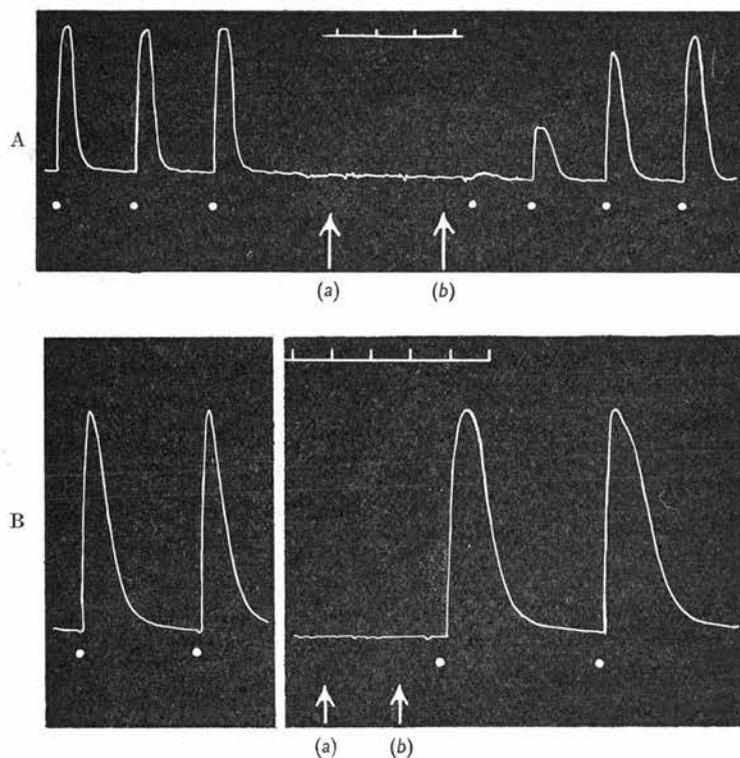


Fig. 29 (A) Cat, chloralose, innervated superior cervical ganglion perfused with Locke's solution. Contractions of nictitating membrane. Injections into perfusion stream. At signals acetylcholine  $20 \mu\text{g}$ . At (a) Locke's solution  $0.2 \text{ ml}$ . At (b) pendiomid  $20 \mu\text{g}$ . Time trace, 30 sec (B) Cat, chloralose, perfused denervated superior cervical ganglion. Contractions of nictitating membrane. Injections into perfusion stream. At signals acetylcholine  $5 \mu\text{g}$ . At (a) Locke's solution  $0.2 \text{ ml}$ . At (b) pendiomid  $40 \mu\text{g}$ . Time trace, 30 sec.

stream acted only on the ganglion and not directly on the muscle of the nictitating membrane or elsewhere were taken as described in Chapter V. All drugs were given in volumes of 0.2-0.5 ml. of saline (0.9% NaCl) solution. Doses are in terms of acetylcholine and tubocurarine chlorides, tetramethylammonium (TMA) and tetraethylammonium (TEA) iodides, penta-, hexa- and decamethonium bromides, pendiomid dibromide, and nicotine tartrate.

## RESULTS

### Action of ganglion-blocking drugs on denervated ganglia

#### Methonium compounds and pendiomid

Fig. shows the action of pendiomid in normal and denervated ganglia on the responses to injections of acetylcholine into the perfusion stream. Whereas in the innervated preparation 20  $\mu$ g. pendiomid is sufficiently complete to block the action of 20  $\mu$ g. acetylcholine (Fig. 29A), in the denervated preparation 40  $\mu$ g. pendiomid exerts no blocking action but instead appears to potentiate the effect of 5  $\mu$ g. acetylcholine (Fig. 29B); the potentiation is exhibited largely

FIG. 30

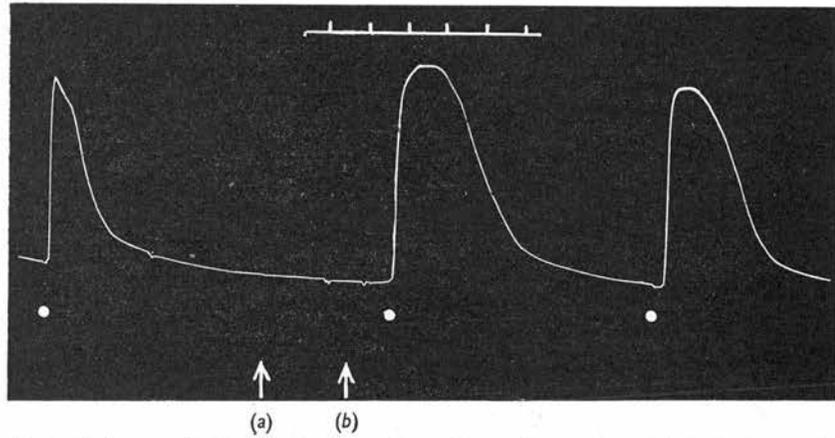


Fig. 30 Technique as in Fig. 27 Denervated ganglion. At signals acetylcholine  $5 \mu\text{g}$ . At (a) Locke's solution  $0.2 \text{ ml}$ . At (b) hexamethonium  $100 \mu\text{g}$ . Time trace 30 sec.

FIG. 31

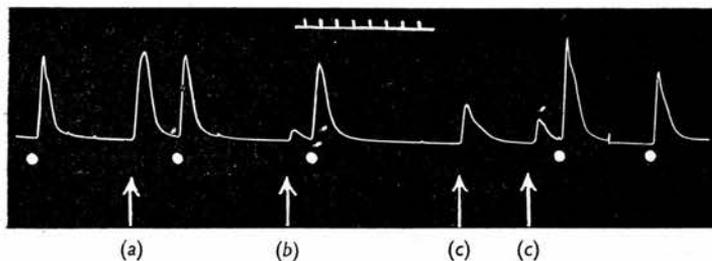


Fig. 31. Technique as in Fig. 29 Denervated ganglion. At signals acetylcholine 5  $\mu\text{g}$ . At (a) hexamethonium 100  $\mu\text{g}$ . At (b) pentamethonium 100  $\mu\text{g}$ . At (c) decamethonium 100  $\mu\text{g}$ . Time trace, min.

FIG. 32

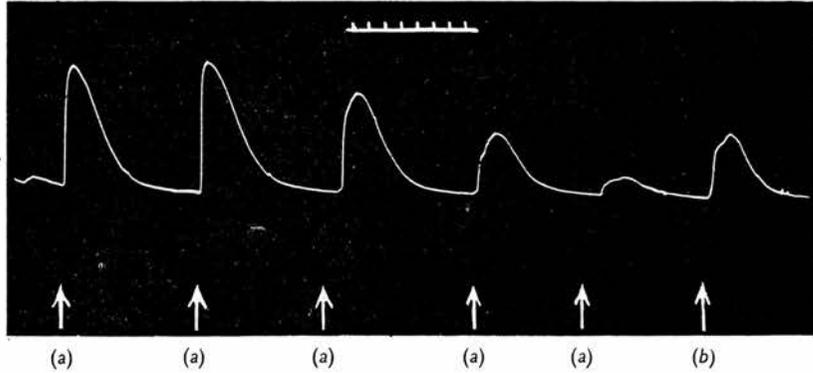
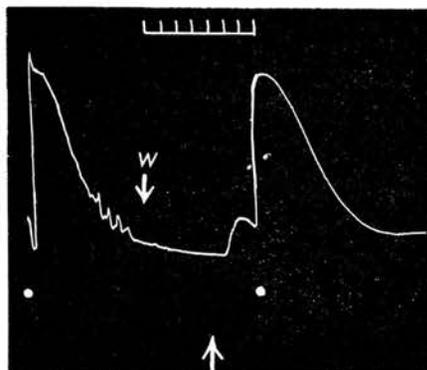


Fig 32. Technique as in Fig. 19 Denervated ganglion. Pentamethonium 200  $\mu$ g at (a), 400  $\mu$ g at (b). Time trace, min.

FIG. 33



(a)

Fig. 33 Technique as in Fig. 29 Denervated ganglion. At signals acetylcholine  $10 \mu\text{g}$ . At W Locke's solution  $0.2 \text{ ml}$ . At (a) pentamethonium  $100 \mu\text{g}$ . Time trace, min.

as an increased duration of the contraction and not so much as an increase in shortening. In other experiments, however, increased shortening was also observed, but the characteristic increase in duration was invariably obtained. These results with pendiomid are similar to those reported for hexamethonium (Fig. 30)(see Chapter V); similar results have also been obtained using pentamethonium and decamethonium. Moreover, in many experiments (8 out of 21), these drugs themselves caused stimulation of the ganglion cells as judged by contraction of the nictitating membrane. In Fig. 30 is shown one experiment with three of the methonium compounds. This stimulant action of the methonium drugs is subject to tachyphylaxis; in Fig. 31, a series of six injections of 200  $\mu$ g. of pentamethonium are shown to exert decreasing stimulant effects.

It seemed possible that, although such a stimulant action of the methonium compounds was not followed by block to acetylcholine, the ganglion cells might be unresponsive to acetylcholine during the stimulant action of the methonium compound, but, as shown in Fig. 33 for pentamethonium, there was no sign of block at any time.

The absence of stimulant effects of the methonium drugs in thirteen of twenty-one denervated preparations might have been due to differences in the time interval between denervation

# TABLE VI

TABLE VI Relationship between length of time since denervation of the ganglion and appearance of stimulant effect of methonium compounds

Time interval since denervation (in days)	Proportion of ganglia showing stimulant action of methonium drugs
20-29	3/7
30-39	5/8
40-49	0/2
50+	0/4
	8/21

FIG. 34

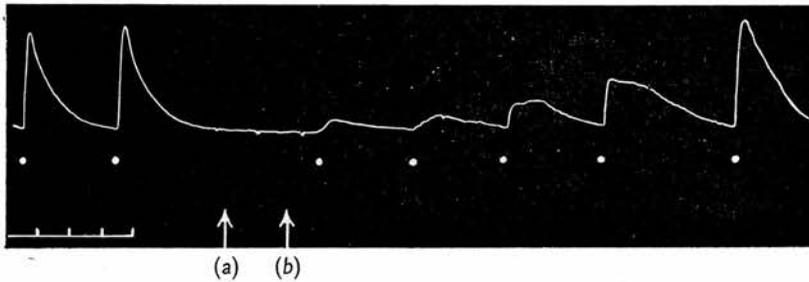


Fig. 34 Technique as in Fig. 19 Denervated ganglion. At signals acetylcholine  $5 \mu\text{g}$ .  
At (a) Locke's solution  $0.2 \text{ ml}$ . At (b) tubocurarine  $200 \mu\text{g}$ . Time trace, 30 sec.

and experiment.

Table VI shows the experiments grouped according to this interval, and the proportion in each such group which showed the stimulant effect, and there would appear to be a tendency for the stimulant effect to be more commonly found before the end of the fifth week than later. Two other factors are involved however; first, that in all twenty-one experiments the drugs failed to block and, indeed, potentiated the effect of injected acetylcholine, which indicates that not all the effects of denervation show this tendency to recover after the fifth week; and secondly, that in a few cases increase of the dose of the methonium drug did finally produce a stimulant effect. Thus, in one experiment no stimulant action of pentamethonium was observed until 1 mg. was given. It may well be, therefore, that the presence or absence of stimulation represents only a quantitative difference between the preparations.

#### Tubocurarine and TEA

The effects of tubocurarine and of TEA on denervated ganglia were also studied. These compounds block the action of acetylcholine, as they do in innervated preparations. A typical result is shown for tubocurarine (Fig. 34), 200  $\mu$ g. causing complete block of the stimulant action of 5  $\mu$ g. of

FIG. 35

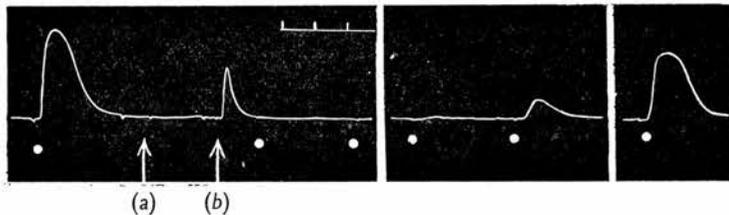


Fig. 35 Technique as in Fig. 29 Denervated ganglion. At signals acetylcholine  $10 \mu\text{g}$ .  
At (a) Locke's solution  $0.2 \text{ ml}$ . At (b) TMA  $200 \mu\text{g}$ . Time trace, 30 sec.

TABLE VII

TABLE VII Reactions of normal and denervated ganglia to various ganglion-blocking compounds

Compound	Normal ganglion		Denervated ganglion	
	Stimulation by compound	Block to injected acetylcholine	Stimulation by compound	Block to injected acetylcholine
Acetylcholine	+	+*	+	+*
Nicotine	+	+	+	+
TMA	+	+	+	+
Tubocurarine	-	+	-	+
TEA	-	+	-	+
Pentamethonium	-	+	+	-
Hexamethonium	-	+	+	-
Decamethonium	-	+*	+	-
Pendiomid	-	+	+	-

\* Large doses

FIG. 36

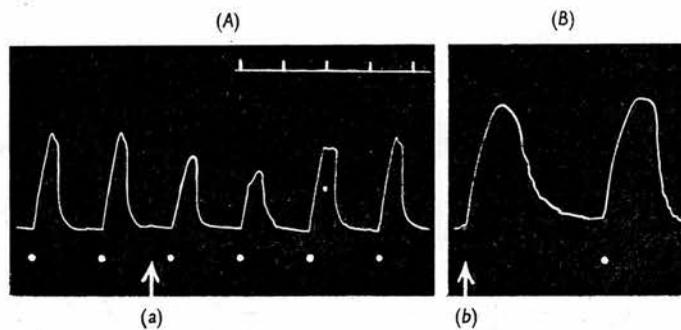


Fig. 36 Cat, chloralose, innervated superior cervical ganglion. Contractions of nictitating membrane. Injection into perfusion stream. Ganglion perfused with (A) Locke's solution (B) Locke's solution containing no KCl. At signals acetylcholine 20  $\mu$ g. Hexamethonium 200  $\mu$ g at (a), 100  $\mu$ g at (b). Time trace, 30 sec.

acetylcholine in a denervated ganglion.

### Nicotine and TMA

Furthermore, nicotine and TMA, which are depolarizing ganglion-blocking drugs (Paton & Perry, 1953), exhibit in denervated preparations the same initial stimulation of the ganglion cells followed by block of the action of acetylcholine as they do in innervated preparations. Thus, in Fig. 35 200  $\mu$ g. of TMA itself causes contraction of the nictitating membrane, while subsequent injections of acetylcholine are no longer effective. The results obtained with all the ganglion-blocking drugs investigated are summarized in Table VII in which the drugs are arranged according to the classification proposed in Chapter V.

### The effect of varying the extracellular potassium concentration on the action of ganglion-blocking drugs

If the innervated ganglion is perfused with Locke's solution containing no potassium, there is a dramatic change in the response of the preparation to the methonium compounds. Thus Fig. 36 shows normal blocking action of 200  $\mu$ g. hexamethonium to injection of acetylcholine in the innervated

FIG. 37

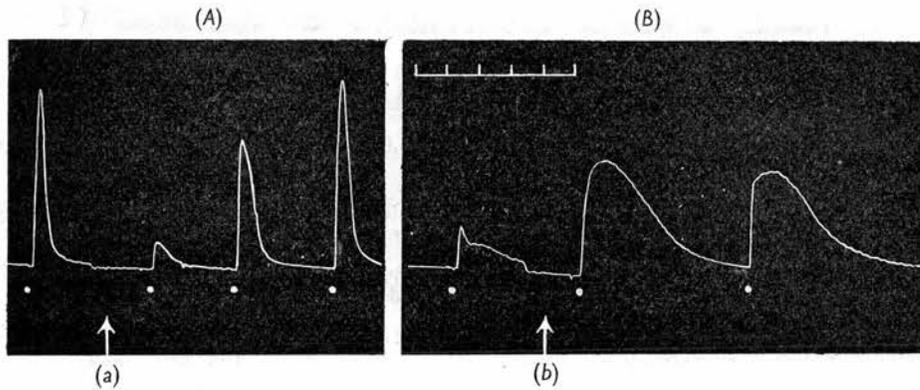


Fig. 37 Technique as in Fig. 36 Ganglion perfused with (A) Locke's solution, (B) Locke's solution containing no KCl. At signals acetylcholine 10  $\mu$ g. Pendiomid 10  $\mu$ g at (a), 200  $\mu$ g at (b). Time trace, 30 sec.

preparation perfused with normal Locke's solution; when the potassium is removed from the perfusion fluid 100  $\mu$ g. hexamethonium (half the previous dose) causes, in the same experiment, a large contraction of the nictitating membrane. This stimulant action is not followed by block to injected acetylcholine, but, on the contrary, by a potentiation of the acetylcholine effect; furthermore, this effect shows the same characteristic increase in duration as well as in tension of the contraction as was observed in denervated preparations. Similar results were obtained with pentamethonium, decamethonium and with pendiomid, although the last of these occasionally showed no stimulant action of its own but only the potentiating effect on subsequent injections of acetylcholine (Fig. 37). The very large potentiating effect of pendiomid is specially clear in this experiment.

Removal of potassium from the perfusion fluid had no such effect on the action of TEA and tubocurarine; nor on that of any of the depolarizing blocking drugs. There is, therefore, a very close parallel between these reactions and the reactions of denervated preparations.

In experiments in which the ganglion was perfused with Locke's solution containing varying amounts of potassium chloride, it was found that the reduction of the potassium

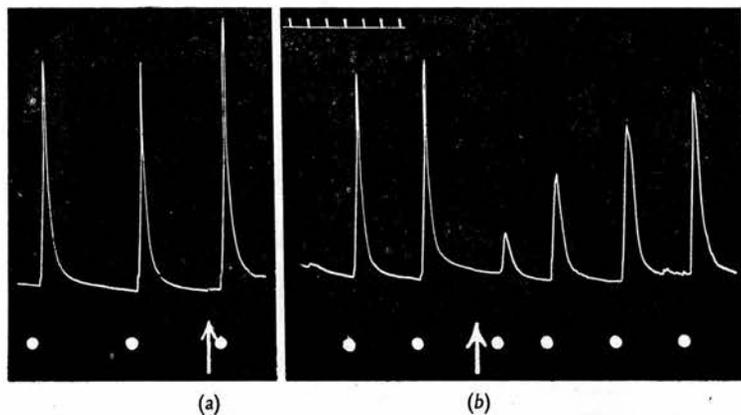


Fig. 38. Cat, chloralose, innervated superior cervical ganglion perfused with Locke's solution containing 10 mg/100 ml. KCl. Contractions of nictitating membrane. Injections into perfusion stream. At signals acetylcholine 10  $\mu$ g. At (a) decamethonium 150  $\mu$ g. At (b) pentamethonium 100  $\mu$ g. Time trace, min.

TABLE VIII

TABLE VIII Relationship between concentration of KCl in Locke's solution perfusing the ganglion and presence or absence of blocking action of methonium compounds to injected acetylcholine

Compound	KCl concentration (mg/100 ml.) in Locke's solution perfusing the ganglion				
	0	2.5	5	10	20 (Normal)
Decamethonium	-	-	-	-	+
Pentamethonium	-	-	-	+	+
Hexamethonium	-	-	+	+	+
Pendiomid	-	-	+	+	+

+ = drug blocks injected acetylcholine.

- = drug fails to block injected acetylcholine.

FIG. 39

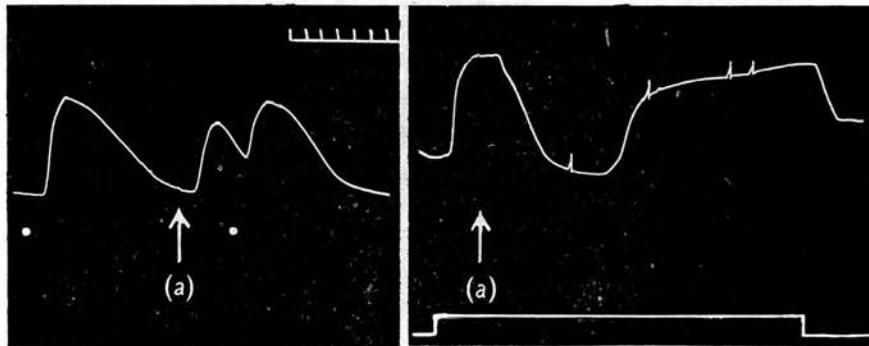


Fig. 39. Cat, chloralose, innervated superior cervical ganglion perfused with Locke's solution containing 2.5 mg/100 ml. KCl. Contractions of nictitating membrane. Injection into perfusion stream. Preganglionic cervical sympathetic trunk stimulated during period shown on lower trace with submaximal square pulses of 0.5 msec duration at a frequency of 10/sec. At signals acetylcholine 20  $\mu$ g. At (a) hexamethonium 50  $\mu$ g. Time trace, min.

concentration necessary to convert the response to a methonium drug to a stimulant one is variable, possibly owing to difference in the doses necessary to produce stimulation; but the reduction necessary to prevent the blocking action of the drug is remarkably constant. Thus all the drugs block injected acetylcholine when the Locke's solution contains 20 mg./100 ml. potassium chloride. All fail to block when the concentration is reduced to 2.5 mg./100 ml. Between these extremes the reduction necessary for any particular compound is correlated with the ganglion-blocking potency. Thus, in Fig. 38, the concentration of potassium chloride was 10 mg./100 ml. and while decamethonium fails to block, pentamethonium is still fully active in blocking the effect of injected acetylcholine. The results of these experiments are summarized in Table VIII.

In further experiments it was shown that although the methonium compounds failed to block the action of injected acetylcholine under these circumstances, they remained effective in blocking preganglionic stimulation (Fig. 39). In this experiment, in which the Locke's solution contained 2.5 mg./100 ml. potassium chloride, 50  $\mu$ g. hexamethonium produced a contraction of the nictitating membrane and failed to block injected acetylcholine. Yet some 10 min. later, during continuous preganglionic stimulation, the same dose of hexa-

FIG. 40

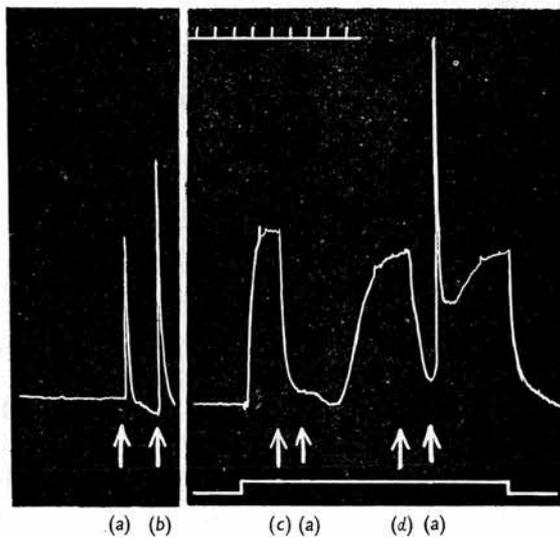


Fig. 40 Technique as in Fig. 39. Preganglionic stimulation during signal. At (a) acetylcholine 10  $\mu\text{g}$ . At (b) acetylcholine 20  $\mu\text{g}$ . At (c) pentamethonium 100  $\mu\text{g}$ . At (d) decamethonium 100  $\mu\text{g}$ . Time trace, min.

methonium caused complete block of transmission.

This differentiation was always present, so that the methonium compounds blocked the effects of preganglionic stimulation whatever the concentration of potassium chloride in the Locke's solution; yet variation of this concentration altered the effect of the methonium compounds on injected acetylcholine. Thus, in Fig. 40, 100  $\mu$ g. of pentamethonium blocked transmission during continuous preganglionic stimulation and also blocked injected acetylcholine (10  $\mu$ g.); but the concentration of potassium (2.5 mg./100 ml.) was such that, although decamethonium also blocked transmission during continuous preganglionic stimulation, it failed to block acetylcholine (10  $\mu$ g.) injected at the height of the transmission block.

The effect of extracellular potassium concentration on denervated ganglia

The parallelism between the reactions of denervated ganglia perfused with normal Locke's solution, and of innervated ganglia perfused with Locke's solution containing a reduced potassium concentration, led to the study, on denervated preparations of the effects of (a) excess of potassium, and (b) glutamate ions in the perfusion fluid.

When denervated ganglia were perfused with Locke's

FIG. 41

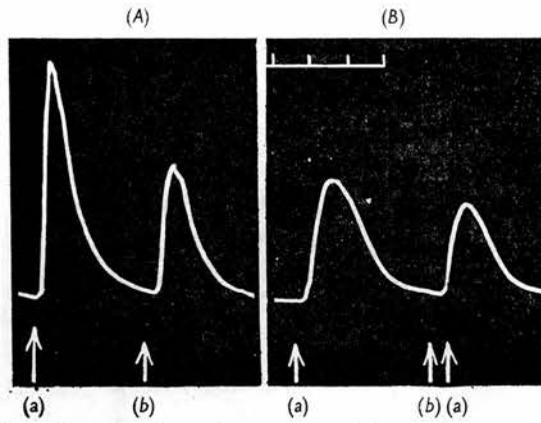


Fig. 41. Cat, chloralose, denervated superior cervical ganglion perfused with (A) Locke's solution and (B) Locke's solution containing 100 mg/100 ml. KCl. Contractions of nictitating membrane. Injection into perfusion stream. At (a) acetylcholine 1  $\mu$ g. At (b) pendiomid 50  $\mu$ g. Time trace, 30 sec.

FIG. 42

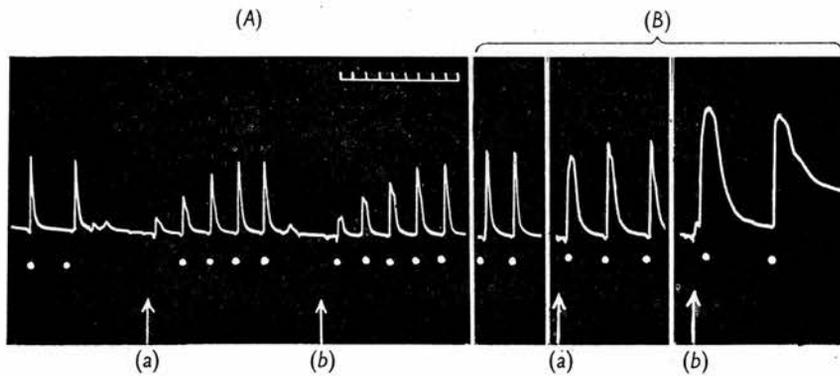


Fig. 42. Cat, chloralose, denervated superior cervical ganglion perfused with (A) Locke's solution containing 40 mg/100 ml. KCl and L-sodium glutamate 100 mg/100 ml. and (B) Locke's solution. Contraction of nictitating membrane. Injections into perfusion stream. At signals acetylcholine 10  $\mu$ g. At (a) pendiomid 100  $\mu$ g. At (b) hexamethonium 100  $\mu$ g. Time trace, min.

solution containing an increased concentration of potassium (100 mg./100 ml.), this concentration of potassium itself produced some ganglionic stimulation. Even in such an unsatisfactory preparation, however, the excess potassium reduced the stimulant action of acetylcholine in both degree and duration, abolished the stimulant effect of pendiomid (previously present) and may have restored to some extent its blocking action to injected acetylcholine (Fig.41).

Because of the difficulties of using such high concentrations of potassium denervated preparations were perfused with Locke's solution containing 100 mg./100 ml. L-glutamate and only twice the usual potassium chloride concentration (40 mg./100 ml.), and a typical result is shown in Fig. 42. In this experiment the denervated ganglion, when perfused with this solution, gave responses like those obtained in an innervated ganglion; thus pendiomid and hexamethonium under these conditions exerted their usual blocking action to injected acetylcholine. Moreover, the responses to acetylcholine itself were typical of those obtained in innervated preparations. Later, the same preparation, perfused with normal Locke's solution, shows that pendiomid and hexamethonium have lost their blocking action, and that hexamethonium has now a potentiating effect on subsequent injections of acetylcholine and itself stimulates the ganglion cells; finally, the responses

to acetylcholine show the increased duration and shortening characteristic of denervated preparations.

## DISCUSSION

### Role of potassium in the action of the methonium compounds

The results on innervated preparations show that when the extracellular potassium concentration ( $K_o$ ) is reduced, the methonium compounds no longer block injected acetylcholine but potentiate its effects and, indeed, may themselves stimulate the ganglion cells. When  $K_o$  is normal, these compounds block transmission without themselves producing any depolarization of the cell membrane; yet when  $K_o$  is reduced, and the methonium compound causes stimulation of the ganglion cells, there must be an accompanying depolarization. There is, therefore, a critical level of  $K_o$  at which a methonium compound develops the power of depolarizing the ganglion cells. Furthermore, this critical level is correlated to the ganglion-blocking activity of the compound (Table VII); thus, the stronger the ganglion-blocking activity of the compound when  $K_o$  is normal, the more must  $K_o$  be reduced before this blocking activity is lost and the

compound acquires a potentiating or stimulant action.

This is of particular interest in the case of the compound decamethonium, which blocks the ganglionic synapse in the absence of any depolarization (Paton & Perry, 1953) but blocks the neuromuscular junction by producing a prolonged depolarization (Burns & Paton, 1951), although at both sites the membrane is specifically reactive to acetylcholine. Loewi (1954) and Perry (1954) pointed out that such variations in the reactions of two cell membranes to a particular drug might depend not so much on differences between the receptors at the two sites as on differences in the 'environmental states' of the two cell membranes. The fact that decamethonium does depolarize the ganglion cell membrane when  $K_o$  is low but not when  $K_o$  is normal lends support to this view.

Another interesting finding in innervated ganglia is that although, when  $K_o$  is reduced, the methonium compounds fail to block injected acetylcholine, whatever the dose, they are still fully active in blocking the effects of preganglionic stimulation. This finding is difficult to explain if we accept acetylcholine as the chemical transmitter at ganglionic synapses. Stimulation of the ganglion is known to cause a release of potassium itself (Vogt, 1936; Emmelin, MacIntosh & Perry, 1949). This local release of potassium during preganglionic stimulation

whether from pre- or post-synaptic elements, will tend to restore  $K_o$  to normal and hence to permit the methonium compounds to exert their normal blocking action. But, as shown in Fig. 40, even if such a local increase of  $K_o$  does occur during stimulation, it does not restore the blocking action of the methonium compounds to injected acetylcholine. It is also possible to explain this discrepancy on the basis of a differentiation of the cell membrane into areas under the boutons terminaux specifically reactive to acetylcholine and other areas not so specialized in function. Such a differentiation was postulated in Chapter V, to explain the dual action of nicotine. On the other hand, it is an unattractive concept since De Castro (1942) showed that over 70% of the ganglion cell membrane is covered by boutons terminaux. Finally, the reduced  $K_o$  may affect the release of acetylcholine from the presynaptic terminals; increased  $K_o$  is known to release this acetylcholine (Brown & Feldberg, 1936). Yet this, too, seems an improbable reason for the observed discrepancy, which appears to be a qualitative rather than merely a quantitative one.

#### Effects of denervation

When  $K_o$  is normal, the denervated ganglion reacts to the methonium compounds in a way similar to that in which the innervated ganglion reacts when  $K_o$  is low. This parallelism is very close. In both cases the effects of injected acetylcholine

are potentiated in duration and degree; and in both the methonium compounds do not block but potentiate the effects of injected acetylcholine, and may themselves stimulate the ganglion cells. In this connexion the results with sodium glutamate are of particular interest. Glutamate ions are known (Davies & Krebs, 1952) to cause an inwards transfer of potassium into liver, brain, kidney and retinal cells across the cell membrane against the ionic gradient. Similar concentrations of sodium glutamate in the fluid perfusing denervated ganglia produced (Fig. 41) a reversal of those effects of denervation just described. Thus certain responses of an innervated ganglion can be converted to those of a denervated ganglion by reducing  $K_o$ ; and the same responses of a denervated ganglion can be converted to those of an innervated ganglion by increasing  $K_o$  and by providing glutamate ions extracellularly.

It is tempting to speculate whether the effects of denervation may depend upon a resultant change in  $K_o$ . Such a change would necessarily be a localized one, since no change can be visualized in the potassium concentration of the extracellular fluids of the body in general. A local fall of  $K_o$  in the ganglion is conceivable if a continual leakage of potassium from pre-synaptic terminals in the ganglion occurs in the innervated preparation and consequently maintains a

higher local  $K_o$  than that in a denervated preparation where no such leakage is possible, but this is the only basis that we can visualize for such a change.

On the other hand, a change in the intracellular potassium concentration ( $K_1$ ) after denervation might produce similar results. Denervated ganglion cells discharge spontaneously (McLennan & Pascoe, 1954) and the denervated ganglion contains less potassium than does the innervated (Vogt, 1936). These findings might be expected to lead to a reduction of  $K_1$  after denervation; but a reduction of  $K_1$  might be expected to have opposite effects to a reduction in  $K_o$ , and consequently would not easily explain our results. Furthermore, changes in either  $K_1$  or  $K_o$  would have effects on the resting potential of the ganglion cells and an answer to this question, as to many others, must await the intracellular recording of ganglion cell potentials. Dale (1920) described the different modes of action of relatively complicated molecules as being 'as mysterious as the physiological contrast between sodium and potassium'. It is possible that many of the actions of such complicated molecules as the methonium compounds depend ultimately upon their interference with the normal actions of these simple ions.

CHAPTER VII

GANGLION METABOLISM

INTRODUCTION

After chronic preganglionic denervation of the superior cervical ganglion of the cat, methonium compounds no longer block but potentiate the effects of injected acetylcholine and, in some 50% of experiments, themselves cause stimulation of the ganglion cells, as judged by contraction of the nictitating membrane. Furthermore, in normal ganglia, perfused with modified Locke's solution containing a reduced concentration of potassium, the same changes in response to the methonium compounds occur, although even in such conditions these drugs still block preganglionic stimulation (Chapter VI).

It was suggested that the methonium compounds could only block transmission when the extracellular potassium ion concentration was normal; and their success in blocking preganglionic stimulation during perfusion with potassium-free Locke's solution was attributed to a local release of potassium from the activated preganglionic nerve endings. That potassium ions appeared to play an important role in these reactions was

further indicated by the findings that L-glutamate, which has been shown by Krebs & Eggleston (1949) and Davies & Krebs (1952) to promote theinwards transfer of potassium ions into many cells against the ionic gradient, when added to the fluid perfusing a denervated ganglion reversed the effects of denervation (Chapter VI). In this Chapter further experiments, carried out in order to study in more detail the role of potassium ions in these reactions, are described.

Krebs and his co-workers found that, of the amino-acids which they studied, only L-glutamate and L-aspartate had a 'potassium carrier-function'. The action of a number of amino-acids other than L-glutamate, including D-glutamate, L- and D-aspartate, L- and D-alanine,  $\beta$ -alanine, L-arginine and L-lysine, in both normal and chronically denervated ganglia, has therefore been studied.

There is a great deal of evidence that potassium ions are important for the maintenance of oxidative metabolism (Krebs, 1935; Weil-Malherbe, 1938; Shanes, 1951; Pressman & Lardy, 1952; von Korff, MacPherson & Glaman, 1954). Intracellular potassium is lost under anaerobic conditions from nerve (Shanes, 1951), from denervated ganglia (Gertner & Reinert, unpublished observations) and from denervated muscle (Humoller, Griswold & McIntyre, 1950a). In denervated muscle there is

inhibition of glycogen synthesis and decrease of the resting potential (Ware, Bennett & McIntyre, 1954), reduction of phosphorylation (Varga, Kostya, Szabo, Aszodi & Kesztyüs, 1950) and decrease of ATP and creatine phosphate (Levine, Hechter & Soskin, 1941; Humoller, Griswold & McIntyre, 1950b).

Some of the effects observed in the ganglion may thus be due to the action of potassium on oxidative metabolism rather than to its action on the cell membrane; and the experiments reported here are discussed with this possibility in mind. These experiments were done in collaboration with Dr. H. Reinert.

#### METHODS

The methods used were identical with those described in Chapter VI.

All amino-acids used were brought to pH 7.4 with either hydrochloride acid or sodium hydroxide and were thus added to the Locke's solution either as sodium salts or as chlorides. Except when otherwise stated, they were used at a final concentration in the Locke's solution of 100 mg./100 ml.

FIG. 43

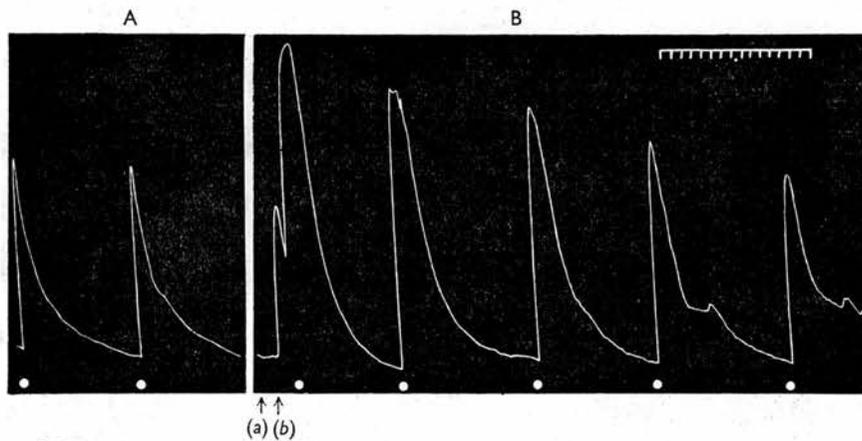


Fig. 43 Cat, urethane, denervated superior cervical ganglion, perfused with Locke's solution. Contractions of the nictitating membrane. Injections into perfusion stream. At signals acetylcholine  $10\mu\text{g}$ . Between A and B cannula washed with several injections of 0.2 ml. of Locke's solution, until no further response obtained. At (a) 0.2 ml. Locke's solution; at (b) hexamethonium  $250\mu\text{g}$ . Time trace 30 sec.

## RESULTS

### Effect of Denervation

A dose of 100  $\mu$ g. of hexamethonium always blocks the action both of injected acetylcholine and of preganglionic stimulation in a normal ganglion. In a denervated ganglion, however, hexamethonium in doses up to 5 mg. or more has no blocking effect on injected acetylcholine. Hexamethonium (250  $\mu$ g.) has been used routinely as a test dose (Fig. 43). When this dose does not block but instead potentiates the effect of injected acetylcholine in a denervated ganglion, this is referred to as the 'effect of denervation'; should some modification of the Locke's solution cause the ganglion to react to the same dose of hexamethonium by block of the action of injected acetylcholine, this is referred to as 'reversal of the effect of denervation'.

It should be noted that, together with the reaction of the denervated ganglion to hexamethonium, another typical change resulting from denervation is observed, namely, the alteration in the response to acetylcholine from a small, relatively short contraction of the nictitating membrane to a larger and longer lasting effect, i.e. sensitization to the transmitter as described by Cannon & Rosenblueth (1949).

FIG. 44

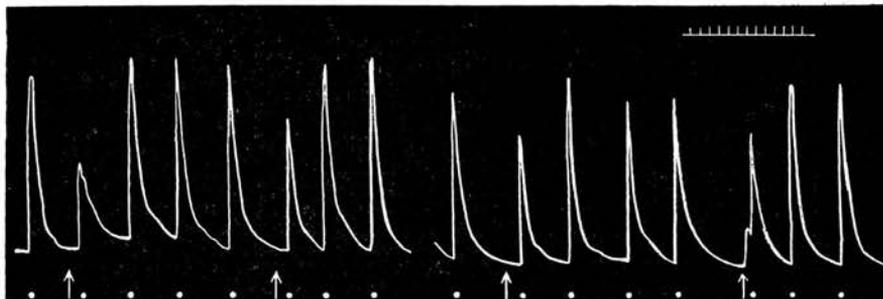


Fig. 44 Technique as in Fig. 43 Denervated ganglion perfused with Locke's solution containing L-sodium aspartate 100 mg/100 ml. At signals acetylcholine  $10\mu\text{g}$ . At arrows hexamethonium  $250\mu\text{g}$ . Time trace 30 sec.

The effect of denervation is observed whether the ganglion is perfused with Locke's solution or left with an intact blood circulation, the drugs being given by intra-arterial injection retrogradely into the ligated stump of the external carotid or lingual artery.

#### Effects of amino-acids on denervated ganglia

As described in Chapter VI, L-glutamate at a concentration of 40 mg./100 ml. in the perfusing Locke's solution reverses the effect of denervation. This can also be accomplished by using, instead of L-glutamate, L-aspartate (Fig. 44). It should be noted that the ganglion was perfused with the amino-acid for 30 min. before testing with a methonium compound. Once reversal of the effect of denervation was obtained with L-glutamate or L-aspartate, it was long-lasting, and block by hexamethonium could be elicited over and over again (Fig. 44). Indeed this reversal was still obtained for some 30-90 min. after removing these amino-acids from the perfusion fluid, after which the hexamethonium ceased to block and the effect of denervation was once more observed. This long-lasting reversal was only obtained when L-glutamate or L-aspartate was used.

Each one of the other amino-acids tried produced a

FIG. 45

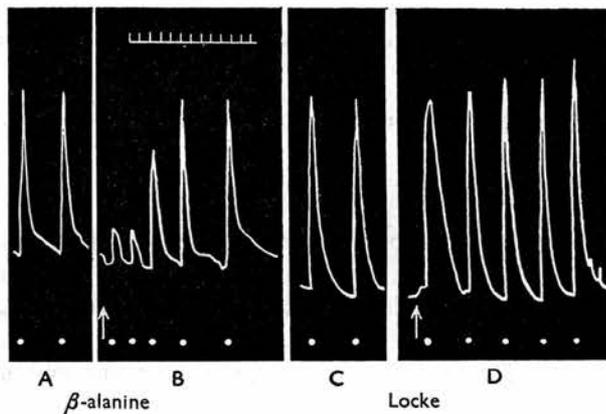


Fig. 45 Technique as in Fig. 43 Denervated ganglion perfused in A and B with Locke's solution containing  $\beta$ -alanine 100/mg 100 ml. and in C and D with Locke's solution. Between A and B and between C and D cannula washed as in Fig. 43 30 min between B and C. At signals acetylcholine  $10\mu\text{g}$ . At arrows hexamethonium  $250\mu\text{g}$ . Time trace 30 sec.

reversal, but only a transient one. Thus, for example, the first dose of hexamethonium, given 30 min, after the start of perfusion with  $\beta$ -alanine, blocked injected acetylcholine (Fig. 45), but when the amino-acid was removed from the perfusion fluid after the first dose of hexamethonium had produced block, there was no continuation of the reversal, and the effect of denervation was immediately restored (Fig. 45). Furthermore, even during continued perfusion of this amino-acid, the reversal passed off and the effect of denervation was observed with the second or third dose of hexamethonium.

It was extremely difficult to reverse the effect of denervation if the ganglion had been previously perfused with normal Locke's solution. It was only possible to do this at all if the amino-acid had been added not more than 15 min. after the start of perfusion. Thus, experiments were normally begun by perfusing with the amino-acid, when reversal was readily obtained, and then changed to perfusion with normal Locke's solution, thereby obtaining the effect of denervation.

#### Influence of glucose on denervated ganglia

The amino-acids produce reversal of the effect of denervation in the presence of glucose. When glucose was removed from the Locke's solution, they were wholly ineffective

FIGS. 46 + 47

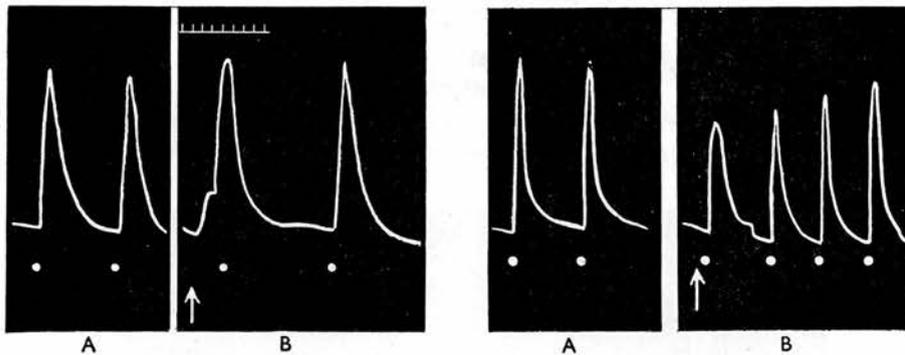


Fig. 46

Fig. 47

Fig. 46 Technique as in Fig. 43 Denervated ganglion perfused with Locke's solution containing L-sodium glutamate 100 mg/100 ml. and no glucose. Between A and B several injections of Locke's solution 0.2 ml. until no further response obtained. At signals acetylcholine 10  $\mu$ g. At arrow hexamethonium 250  $\mu$ g. Time trace 30 sec.

Fig. 47 Technique as in Fig. 43 Denervated ganglion perfused with Locke's solution containing L-sodium glutamate 200 mg/ml. and no glucose. Between A and B several injections of Locke's solution 0.2 ml. At signals acetylcholine 10  $\mu$ g. At arrow hexamethonium 250  $\mu$ g.

FIG. 48

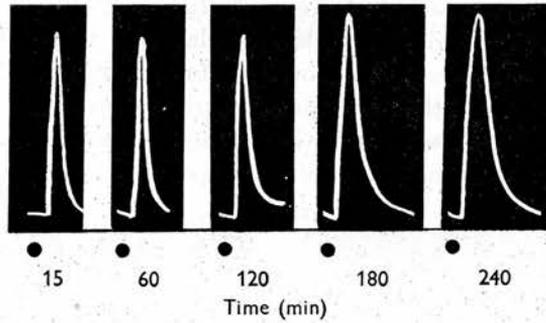


Fig 48. Technique as in Fig. 43 Denervated ganglion perfused with Locke's solution containing no glucose. At signals  $5 \mu\text{g}$  acetylcholine. Time (min) of each injection after start of perfusion.

FIG. 49

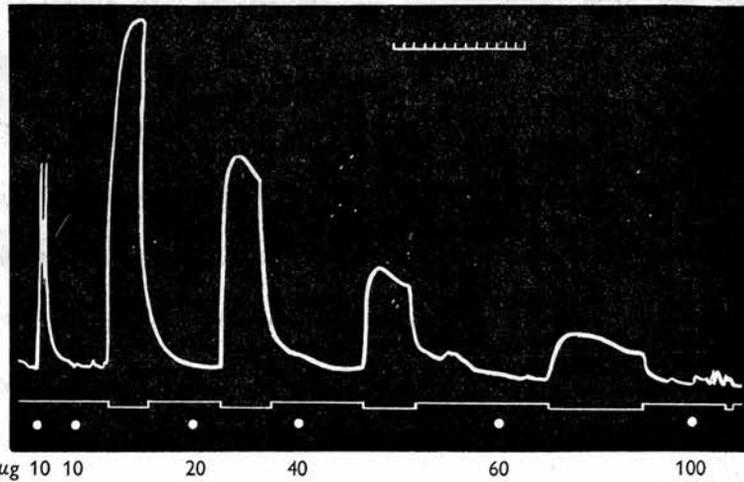


Fig 49. Technique as in Fig. 43 Normal ganglion perfused with Locke's solution containing no glucose. Supramaximal preganglionic stimulation 2 c/s during periods indicated on lower trace. At signals acetylcholine-dose ( $\mu\text{g}$ ) as stated. Time trace 30 sec.

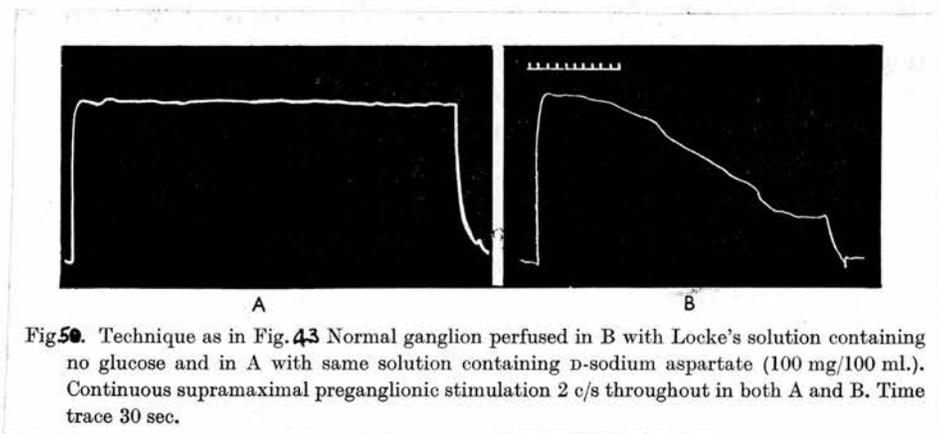
at the usual concentration of 100 mg./100 ml. In Fig.46, L-glutamate, 100 mg./100 ml., failed entirely to reverse the effect of denervation. It was found that glucose could be replaced by the addition of acetate or pyruvate to the Locke's solution, or by perfusion with blood. Furthermore, increasing the concentration of L-glutamate or L-aspartate to 200-300 mg./100 ml. did produce reversal even in the absence of glucose (Fig.47). Nevertheless, these increased concentrations of amino-acids without glucose were never as effective in producing reversal as lower concentrations in the presence of glucose.

Pyruvate, acetate or glucose alone did not reverse the effect of denervation. In the absence of glucose, the denervated ganglion continued to react to injected acetylcholine for many hours without showing any sign of fatigue (Fig.48).

#### Influence of glucose on normal ganglia

When the normal ganglion was perfused in the absence of glucose, it rapidly became unresponsive both to preganglionic stimulation and to injected acetylcholine (Fig.49). Kahlson & MacIntosh (1939) also described this fatigue to preganglionic stimulation and explained it on the basis of a failure of the ganglion to synthesize acetylcholine. On the other hand, they

FIG. 50



stated that the ganglion continued to respond to injected acetylcholine; the results reported here do not confirm this. Provided that the absence of glucose was not maintained for too long, the fatigue produced was reversible by the restoration of glucose.

#### Effect of amino-acids on normal ganglia

Fatigue of the ganglion resulting from the absence of glucose was prevented by acetate or pyruvate, or by a concentration of 100 mg./100 ml. of any of the amino-acids used, whether D or L-form. The prevention of fatigue was judged by the maintenance of contraction of the nictitating membrane in response to continuous maximal preganglionic stimulation, and a typical result is shown in Fig. 50 for D-aspartate.

#### DISCUSSION

It appears that the metabolism of the ganglion is greatly altered by preganglionic denervation. Thus, the denervated ganglion continues to react to injected acetylcholine for several hours without any apparent fatigue in the total

absence of glucose, although under similar conditions we have shown that the normal ganglion becomes wholly inexcitable both to electrical stimulation (cf. Kahlson & MacIntosh, 1939) and to chemical stimulation (cf. Kewitz & Reinert, 1954).

The fact that denervated ganglia do not become fatigued when glucose is absent from the perfusion fluid suggests that the energy for continued function is not obtained from the metabolism of extrinsic glucose. The failure of amino-acids to reverse the effect of denervation in the absence of glucose may be due to one or both of two factors: (a) it may be that the reversal involves a restoration of aerobic glucolysis and that it consequently cannot occur in the absence of glucose; (b) it may be that in the presence of glucose the cells take up a sufficient amount of amino-acids to use as a substrate for oxidative metabolism. Thus, Stern, Eggleston, Hems & Krebs (1949) showed that in the absence of oxidizable substrate, the active uptake of amino-acids was decreased.

If the action of the amino-acids is to restore aerobic glycolysis we are thus faced with the problem of how this effect is brought about and whether it is linked to the 'potassium carrier-function' of the amino-acids. After denervation there is a loss of intracellular potassium from the ganglion cells (Gertner & Reinert, unpublished observations), and this change

must presumably also follow perfusion of the normal ganglion with solutions of low potassium concentration. Thus the amino-acids may reverse the effect of denervation by promoting the inward transfer of potassium ions into the ganglion cells. The position is complicated, however, by the fact that those amino-acids which lack the 'potassium carrier-function' in vitro can also, at least in part, reverse the effect of denervation.

The linkage of acetate with coenzyme A is catalysed by the acetate activating enzyme (AAE). This enzyme is inhibited by sodium ions and activated by potassium ions (v. Korff, 1953). Thus a fall in the intracellular potassium-ion concentration leads to an inhibition of aerobic glycolysis at this point, and the energy resulting from aerobic glycolysis is not available. Restoration of the normal intracellular potassium ion concentration reactivates the AAE: thus acetyl-CoA can be formed and aerobic glycolysis can proceed. This reactivation of the AAE in denervated ganglion cells may be one explanation of the action of the amino acids.

The other factor which might be involved is the increased uptake of the amino-acids themselves by the cells and their subsequent use as substrates for oxidative metabolism. The oxidative metabolic pathway involved would have to be one which by-passed the AAE. It may be a pathway through the fatty acid cycle after oxidative deamination (e.g. lysine) or after

transamination to the corresponding keto-acids either directly through the Krebs cycle (e.g. glutamate and aspartate) or via pyruvate (e.g. alanine). The entrance of the amino-acids via these mechanisms into the Krebs cycle would thus explain their function in providing for the generation of the energy of respiration chain and oxidative phosphorylation even when the AAE is inhibited by lack of potassium ions. Further evidence of this is found in the maintenance of function of a normal ganglion perfused with any of these amino-acids in the absence of glucose where the amino-acids must themselves be acting as substrates for energy production.

If this hypothesis is true, it goes some way towards elucidating the type of ganglion block produced by the methonium compounds since they only produce block when the ganglion cells are metabolizing aerobically, and they may therefore be presumed to act by inhibiting this metabolic pathway. This may be of great importance to our understanding of synaptic block, for it indicates an interruption of cellular function and of the consequent generation of the post-synaptic impulse rather than a membrane or surface phenomenon. It is noteworthy that at the boutons terminaux not only is there a differentiation of the cell membrane, but there is also a pronounced localization of mitochondria and microsomes (Estable, Reissig & de Robertis, 1954); (Gibson & Purkis, 1953;

De Castro, 1942).

On the other hand, these results and their hypothetical explanation take no account of the stimulating effect which the methonium compounds themselves often exert on denervated ganglia. The nature of this stimulating action is unknown, but may well be due to a totally different mechanism from the blocking action. It seems probable that the stimulating action derives from an action on the cell membrane rather than from an action on the cell metabolism. Access of these drugs to the metabolic centres inside the cell must depend upon passage across the cell membrane, and during this phase of entry into the cell the stimulant action may occur and its occurrence may depend upon the ionic gradients locally present at the time.

The hypothesis put forward also helps to explain many other findings reported in the literature. Thus, Brock, Druckrey & Herken (1939) first showed that oxidative metabolism in vitro in tissue slices of salivary glands could only be maintained in the presence of both glucose and potassium. Krebs (1935) and Weil-Malherbe (1938) found that glycolysis in vitro was inhibited by glutamate or by potassium. Feldberg & Hebb (1946) showed that acetylcholine synthesis was increased by glutamate. Dialysed choline acetylase preparations are reactivated by glutamate and potassium (Nachmansohn & John, 1945). Feldberg (1943) found that the in vitro choline acetylase activity of

of ganglia decreased after denervation. Potassium is essential for the acetylation of choline in the presence of citrate or acetate (Balfour & Hebb, 1952) and causes an increased formation of acetylcholine in brain slices (Mann, Tennenbaum & Quastel, 1939).

The difficulty found in reversing the effect of denervation by amino-acids in a ganglion which has already been perfused with Locke's solution for more than a few minutes has been mentioned. Raaflaub (1953) found that when mitochondria were kept in 'physiological' saline solutions, they 'leaked' enzymes, and this leakage could be prevented by adding an energy source such as ATP, which also prevented the decrease in oxygen consumption of the mitochondria. In a denervated ganglion where no aerobic glycolysis is occurring, less ATP is produced, and in its absence we may expect a leakage of enzymes during perfusion with Locke's solution. The early addition of amino-acids may prevent this loss and enable the reversal effect to be obtained.

PART II

STUDIES ON PARASYMPATHETIC GANGLIA

CHAPTER VIII

INTRODUCTION

It has usually been assumed, since the suggestion made by Dale (1933), that transmission at the parasympathetic ganglionic synapse is cholinergic, but proof of the validity of the assumption is difficult for two reasons. In the first place, most parasympathetic ganglion cells are diffusely scattered throughout effector tissues and are not, as are sympathetic ganglion cells, congregated in anatomically recognizable ganglia; only three recognizable ganglia of the parasympathetic system are known, the ciliary, otic and sphenopalatine ganglia. In the second place, the post-synaptic, parasympathetic neurone is itself cholinergic, and when, as is usual, there is a diffuse scattering of ganglion cells, it is difficult to discover whether acetylcholine is released and stimulates at the ganglionic synapse or at the postganglionic-effector junction, or at both. Thus there is a volume of evidence, dating back to the classical experiments of Loewi (1921), that preganglionic, parasympathetic stimulation leads to the release of acetylcholine in such tissues as the heart and the intestine; but this acetylcholine could

have been derived from either of the two junctional regions.

In the work of Emmelin & Muren (1950) some evidence is provided that acetylcholine is released at both synapses. They showed that in a perfused salivary gland, a ganglionic blocking drug (curare), given in doses sufficient to prevent salivary secretion on preganglionic stimulation, did not prevent the release of acetylcholine, although considerably reducing the total amount released; they concluded from these observations that the preganglionic nerve endings were releasing acetylcholine in spite of the ganglion block, inferring therefrom that transmission at the ganglionic synapse was cholinergic.

Whitteridge (1937) described the action potential complex of the ciliary ganglion which showed a resemblance to that of sympathetic ganglia, save that its duration was of the order of 150 msec. as compared with some 500 msec. in the superior cervical ganglion. Suden, Hart & Marazzi (1952) found that the ciliary ganglion responded to adrenaline in the same way as did sympathetic ganglia.

These studies, while they are all suggestive, do not offer any real proof of the cholinergic nature of the presynaptic parasympathetic fibres. More direct studies, carried out with Dr. J. Talesnik, are described in Chapter IX.

A further interest in transmission, in parasympathetic ganglia derives from the fact that, if both the sympathetic and parasympathetic presynaptic nerves are cholinergic, then the use of ganglion-blocking drugs in clinical medicine is bound to be limited since they are likely to attack both systems indiscriminately. The search for drugs which will block selectively the sympathetic system is thus likely to be considerably affected by any knowledge gained of the physiology of the ganglia. A series of experiments, carried out in collaboration with Dr. C.W.M. Wilson, on the blocking action of drugs on the two systems, is described in Chapter X.

CHAPTER IX

GANGLION CELL RESPONSES

In this chapter are described two ways in which the problem of parasympathetic ganglionic transmission has been approached. First, using the ciliary ganglion preparation described by Whitteridge (1937), an attempt has been made to study the changes in the electrical response of the ganglion following the arterial injection of acetylcholine and hence to determine the sensitivity of the postsynaptic neurones to acetylcholine. Secondly, the general pattern of reaction to a number of ganglionic blocking drugs has been investigated both in the ciliary ganglion and in the isolated cat's heart. An analysis of this sort may be of considerable use in determining the nature of a transmission process, as shown by Paton & Perry (1953) for a sympathetic (superior cervical) ganglion.

METHODS

Experiments on the ciliary ganglion

The exposure of the ciliary ganglion was based on the description given by Whitteridge (1937). Cats were anaesthetized with ethyl chloride and ether. In some experiments chloralose (80 mg./kg.) was given before decerebration; in others the decerebration was done under ether. After decerebration the orbital plate was removed and the ganglion exposed between the lateral and superior rectus muscles, which were retracted with suitably placed threads. The optic nerve was not cut nor was the eyeball removed, as suggested by Whitteridge (1937), since it was found that, given good retraction, these procedures were unnecessary. The eyeball was pulled forwards by means of a stitch through the cornea. The short ciliary nerve was picked up on the optic nerve, ligated, cut and cleaned and the third cranial (oculomotor) nerve divided at its origin from the brain stem. All other branches of the ciliary ganglion were cut.

Injections were made retrogradely into the lingual artery of the same side through a needle cannula, leaving the carotid circulation intact. This is a similar injection

technique to that recently described by Suden, Hart & Marrazzi (1952), who injected into the external carotid artery. The cannula was kept closed between injections by a metal tap. The volume injected was usually 0.5 ml.

The recording electrodes used were of the type described by Paton & Perry (1953). One electrode was placed on a cut end of the postganglion, short ciliary nerve; the other loop was passed round the body of the ganglion, nearer to the preganglionic than to the postganglionic pole. The oculomotor nerve was stimulated through platinum electrodes, at a frequency of 1 per 2 sec. The recording electrodes led, through cathode followers, to a d.c. amplifier and the ganglionic action potentials were recorded on one beam of a cathode-ray oscilloscope, the other beam of which was earthed and used as an arbitrary base-line. Thus slow changes of potential of the ganglion relative to the cut end of the postganglionic trunk could be recorded as a deflexion of the whole beam relative to the fixed earthed beam, while shorter changes, e.g. the spike of the action potential, could be recorded as transient deflexions. In all our records an upward deflexion represents an increased negativity of the ganglion relative to the cut end of the postganglionic trunk.

In other experiments cats from which the superior

cervical ganglion had been removed under ether anaesthesia and with aseptic precautions 10 days previously were used. The cat was prepared for the experiment in the usual way, but no electrical records were taken, and the eyeball was immobilized by suturing it to a metal ring, and changes of pupil size in two diameters, magnified roughly x2 by a camera lens, were measured directly on a ground glass screen over which was fixed a transparent millimetre grid. Stimuli were applied at a frequency of 10 per sec. for periods of 5 sec. to the preganglionic (oculomotor) nerve and to the postganglionic (short ciliary) nerve alternately. Drugs were given either intra-arterially into the lingual artery, or intravenously into the femoral vein.

#### Experiments on the isolated cat's heart

The usual Langendorff preparation of the isolated cat's heart perfused through its coronary vessels was used, lengths of both vagi also being dissected out. Perfusion was with Tyrode's solution at constant pressure and temperature. Drugs were introduced in two ways, either by injection of a small volume in Tyrode's solution into the perfusion stream or by switching the reservoir fluid to a second reservoir containing a dilute solution of the drug in the Tyrode's solution.

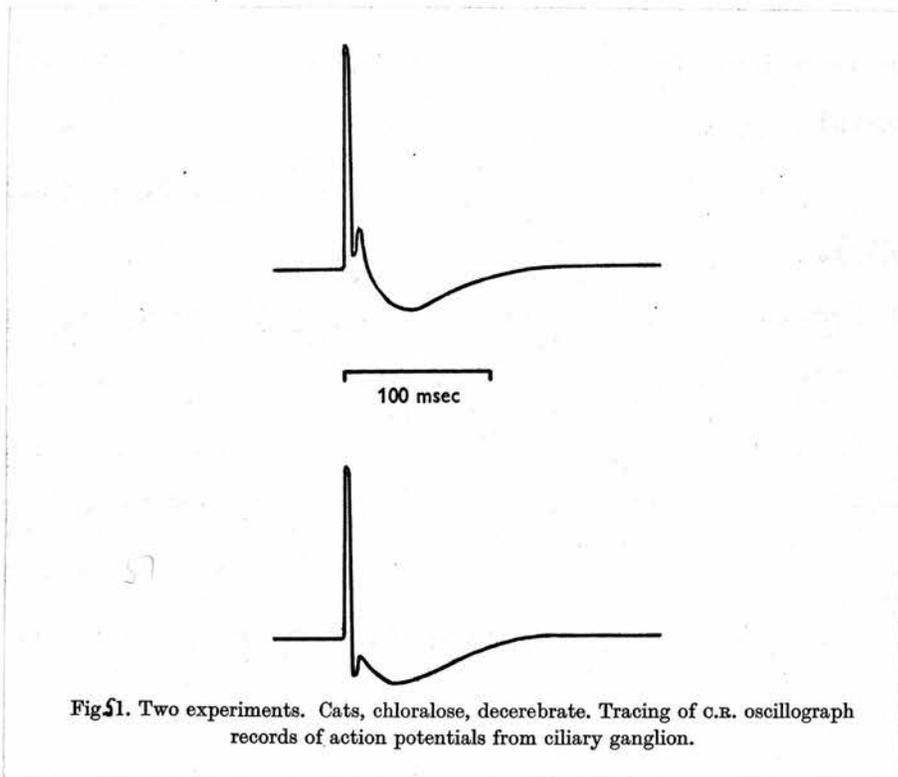
Stimulation of the vagi was carried out through platinum electrodes at a frequency of 20 per sec. Records of the ventricular heart beat were taken on a kymograph.

Doses quoted are in terms of acetylcholine chloride, nicotine hydrogen tartrate, tetraethyl- and tetramethyl-ammonium iodides, and hexamethonium and decamethonium bromides.

## RESULTS

### Normal action potentials of the ciliary ganglion

Whitteridge (1937) described in some detail the normal action potential of the ciliary ganglion. He found that the complex consisted of a rapid negative spike lasting some 5-10 msec., followed by a slow positive wave which lasted some 125-150 msec. He also observed a second spike component which followed the main spike and was usually smaller. This he attributed to a group of fibres with slower time relationships. Whitteridge referred to these components of the action potential as the  $T_1$  spike (the main component), and the  $T_N$  spikes of which the  $T_2$  spike was the most constant. These



components are similar to those described by Eccles (1935) for the superior cervical ganglion, as the  $S_2$  and  $S_3$  spikes respectively.

Our records are similar to those described by Whitteridge (1937). The duration of the complex varied from about 120 to 160 msec., and the  $T_1$  spike from 5 to 10 msec. Typical records are illustrated in Fig. 5! . In the upper record the  $T_2$  spike occurs before the  $T_1$  spike is complete, whereas in the lower record, as is more usual, the  $T_2$  spike does not begin until the slow positive wave following the  $T_1$  spike has begun. The positive waves have maximum values of 16 and 24% of their initial spike heights respectively. These are typical values. Whitteridge (1937) found a maximum of 30%, but his published records show an average of about 12%, for this value.

The slow positive wave is only found with a ganglionic electrode, but when this electrode was placed near the post-ganglionic pole of the ganglion, only a postganglionic spike lasting about 25 msec. was obtained. The largest ganglionic potentials were obtained with the ganglion lead near the pre-ganglionic pole, suggesting that most of the cells are situated in this region. Christensen (1936), in his anatomical description of the ganglion, made no reference to this, however. The whole action potential complex of the ciliary ganglion lasts

FIG. 52

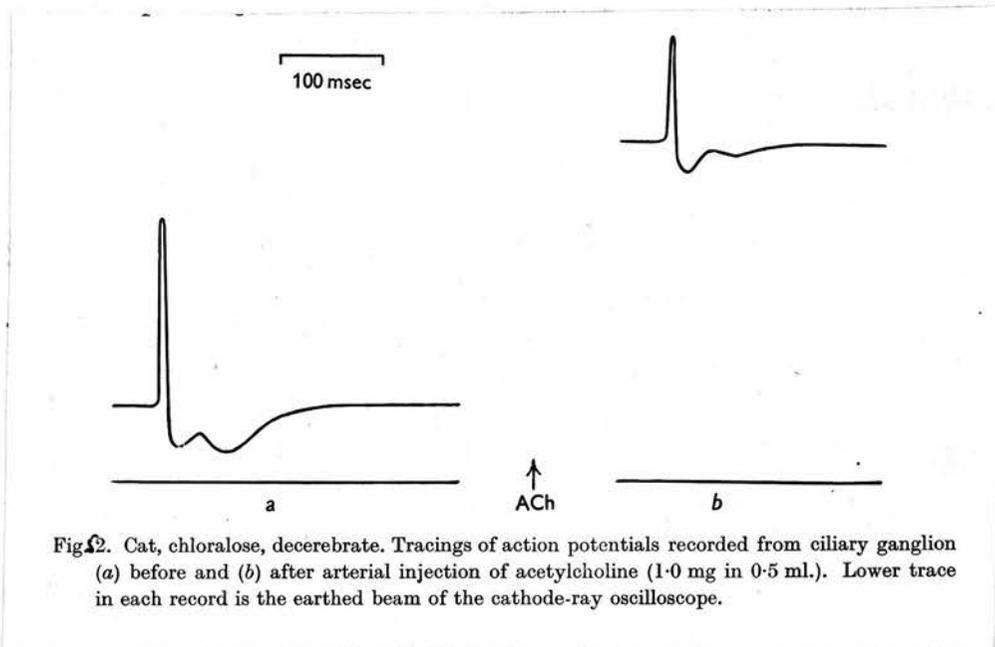


Fig. 52. Cat, chloralose, decerebrate. Tracings of action potentials recorded from ciliary ganglion (a) before and (b) after arterial injection of acetylcholine (1.0 mg in 0.5 ml.). Lower trace in each record is the earthed beam of the cathode-ray oscilloscope.

only about 150 msec. and is thus much shorter than that in the superior cervical ganglion which lasts 600-700 msec.; otherwise the shape of the complex is similar, although the after-positivity is relatively smaller.

#### Acetylcholine on the ciliary ganglion

Arterial injections of acetylcholine were without effect unless large doses were given. This is presumably due to the fact that injections into the lingual artery are not 'close arterial' in the proper sense of the term, so that not only does most of the injected material pass to structures other than the ganglion, but a large proportion of the dose is probably destroyed before it reaches the ganglion. Doses of the order of 0.2-1.0 mg. were necessary to produce effects. At this dose level there was an immediate depolarization of the ganglion cells, so that the ganglion became negative to the postganglionic trunk. This effect lasted for up to 3 min., and was accompanied by a partial block of the action potential spike and by a characteristic change in the shape of the action potential complex. Fig. 52 shows records taken before and after the injection of 1 mg. of acetylcholine, and shows that the spike was reduced by nearly 50%, while the ganglion was

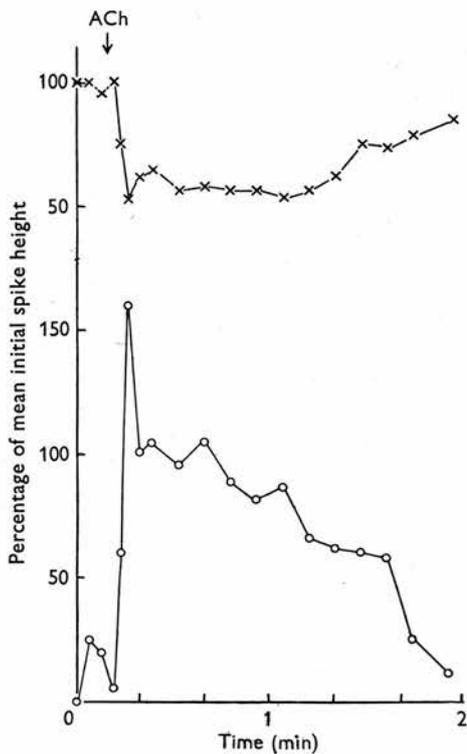


Fig. 53.

Fig. 53. The effect of acetylcholine on height of action potential spike ( $\times - \times$ ) and on negativity of ganglion with reference to cut end of postganglionic nerve ( $\circ - \circ$ ).

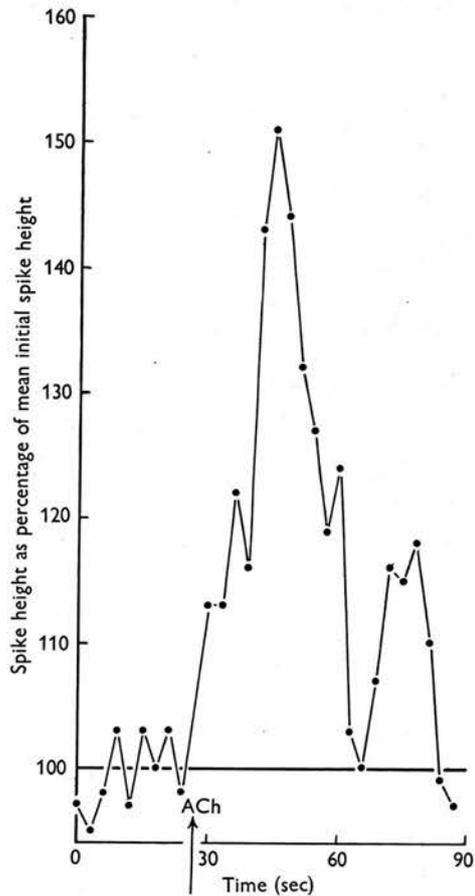


Fig. 54.

Fig. 54. The effect of acetylcholine on height of spike of action potential recorded from short ciliary nerve in response to submaximal stimulation of oculomotor nerve.

depolarized by an amount corresponding to about 130% of the initial spike height. The characteristic change in shape of the action potential is clearly seen. This change in shape is essentially due to the fact that the point of maximum after-positivity occurs much earlier than before so that, neglecting the  $T_2$  spike, the positive after-potential comes to have no appearance of being complicated by a negative after-potential, and its decay becomes more obviously exponential throughout. In Fig. 53 the effect of 1.0 mg. acetylcholine on the spike height and the depolarization of the ganglion is shown graphically. In this experiment the spike was again reduced by about 50% and the initial depolarization was as much as 150% of the initial spike height.

The change in shape of the action potential complex after acetylcholine closely resembles that described in the superior cervical ganglion by Paton & Perry (1953), who suggested that the change could be attributed to a specific reduction of the time constant of the slow negative component of the complex.

In one experiment we recorded postganglionic spikes in response to submaximal preganglionic stimulation; under these conditions an injection of 20  $\mu$ g. of acetylcholine produced a potentiation of the postganglionic spike (Fig. 54). In one experiment an attempt was made to record spontaneous discharge

FIG. 56

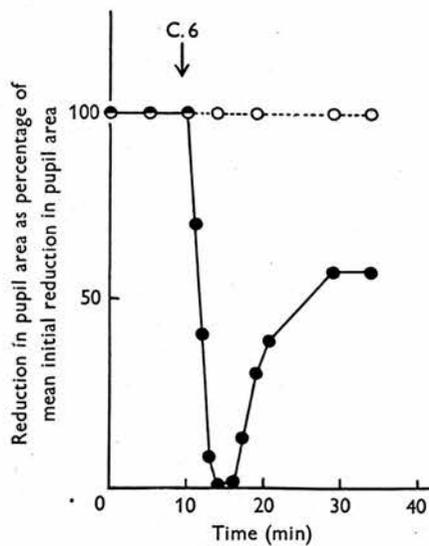


Fig. 56. The effect of hexamethonium, (C.6) (5 mg/kg) on response of pupil to preganglionic (●—●) and postganglionic (○ --- ○) stimulation.

of impulses in the postganglionic trunk after an injection of acetylcholine, but the result was inconclusive probably because of the relatively low amplification.

Ganglionic blocking drugs on the ciliary  
ganglion

Nicotine, in doses of 250  $\mu$ g. arterially, produced changes which were similar to those produced by acetylcholine (Fig. 55). Hexamethonium, in doses of 250  $\mu$ g. arterially, produced a block of 30% of the spike height without any depolarization, and in the absence of the striking change in shape that was characteristic of both acetylcholine and nicotine block (Fig. 55).

In the experiments in which the pupil size was recorded it was found that hexamethonium, in intravenous doses of 5 mg./kg., would produce complete block of preganglionic stimulation without in any way affecting postganglionic stimulation (Fig. 56). In this experiment preganglionic stimulation had produced a reduction in pupil size by a certain amount (arbitrarily taken as a 100% effect). Four minutes after hexamethonium the reduction in size was zero and even 25 min. later this reduction in size was only 60% of the original amount.

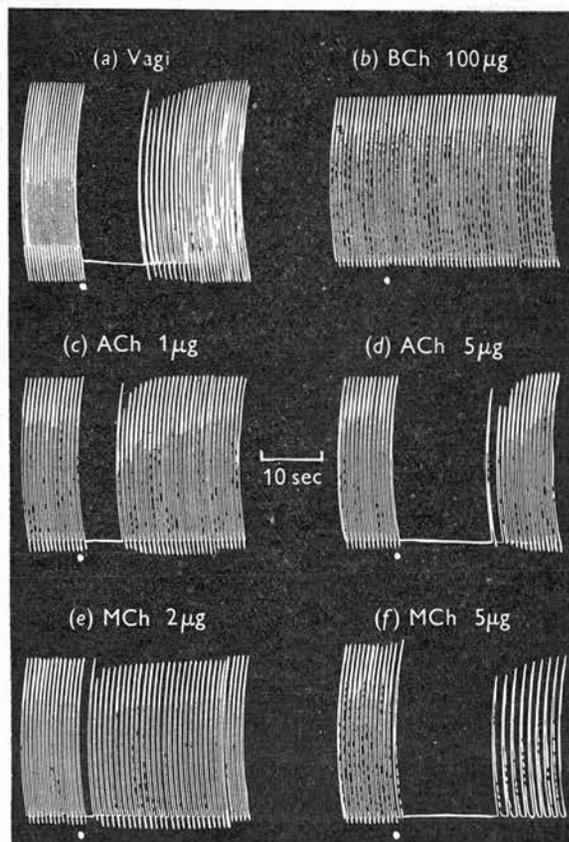


Fig. 57. Isolated cat's heart perfused through coronary arteries. Records of ventricular contractions. Stimulations and injections start at signals. In (a) stimulation of both vagi (20/sec, 0.5 msec, 5 V) for 10 sec; (b) benzoylcholine; (c) and (d) acetylcholine; (e) and (f) acetyl- $\beta$ -methylcholine.

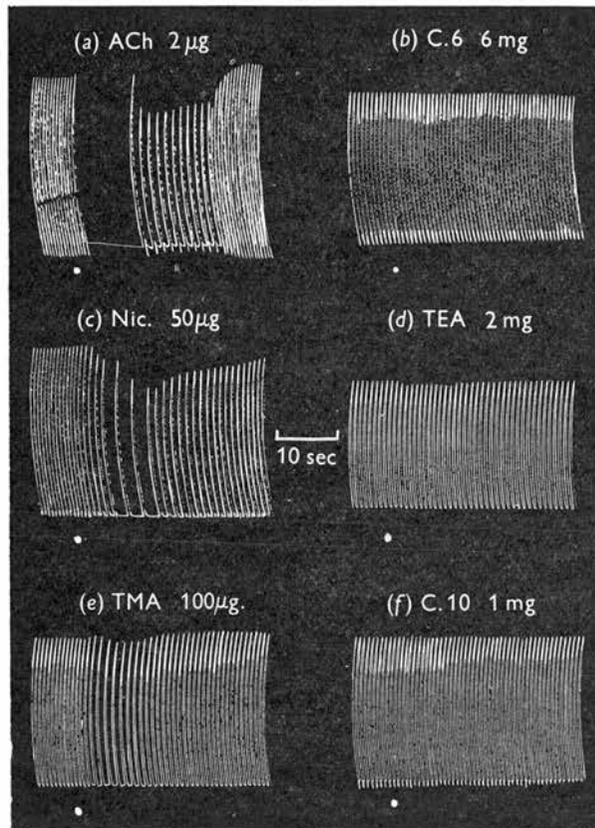


Fig. 58. Isolated cat's heart perfused through coronary arteries. Records of ventricular contractions. Injections at signals of (a) acetylcholine, (b) hexamethonium, (c) nicotine, (d) tetraethylammonium, (e) tetramethylammonium and (f) decamethonium.

Postganglionic stimulation remained equally effective throughout. In other experiments nicotine was given arterially in doses of 250  $\mu$ g.; its main effect was a constriction of the pupil, which thereafter did not respond to preganglionic stimulation, although postganglionic stimulation was still effective. This constriction was followed by a dilatation which was abolished by removal of both adrenal glands. After the short ciliary nerve had been cut, nicotine had no effect on the pupil.

Stimulation of the vagi produced an inhibition of the heart beat and the effect of a 10 sec. period of stimulation is illustrated in Fig. 57, which also shows the effect of small doses of acetylcholine and acetyl- $\beta$ -methylcholine. The graded nature of the response to small doses of these compounds is clearly shown. Benzoylcholine is inactive even in relatively large doses (100  $\mu$ g. in Fig. 57). Up to 3 mg. was given in other experiments. The effect of acetylcholine resembles the effect of vagal stimulation.

In Fig. 58, the effects of a number of known ganglionic blocking drugs is illustrated and it will be seen that nicotine (50  $\mu$ g.) and tetramethylammonium (100  $\mu$ g.) have the same inhibitory effect on the heart as do acetylcholine and vagal stimulation; while hexamethonium, tetraethylammonium and

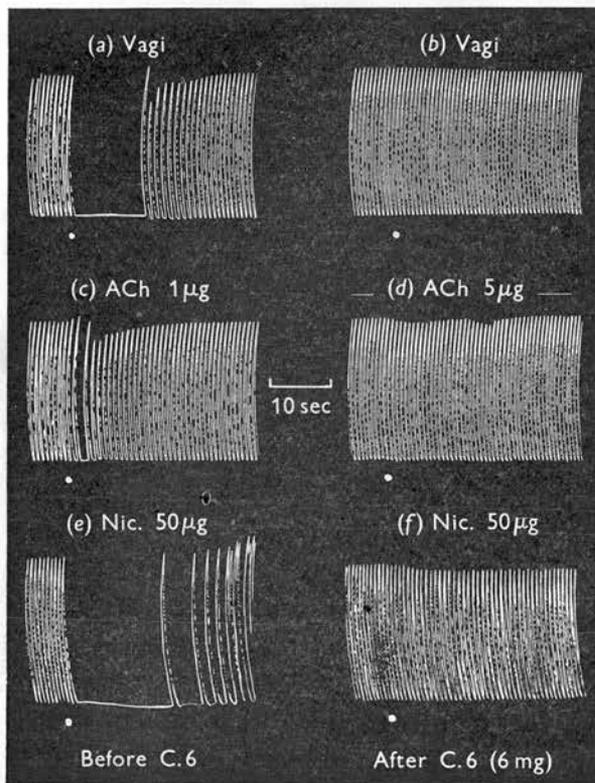


Fig 59. Isolated cat's heart perfused through coronary arteries. Records of ventricular contractions. Stimulation and injections start at signals. (a), (c) and (e) before, (b), (d) and (f) after hexamethonium 6 mg. In (a) and (b) stimulation of both vagi (20/sec, 0.5 msec, 5 V) for 10 sec; (c) and (d) acetylcholine, (e) and (f) nicotine.

FIG. 60

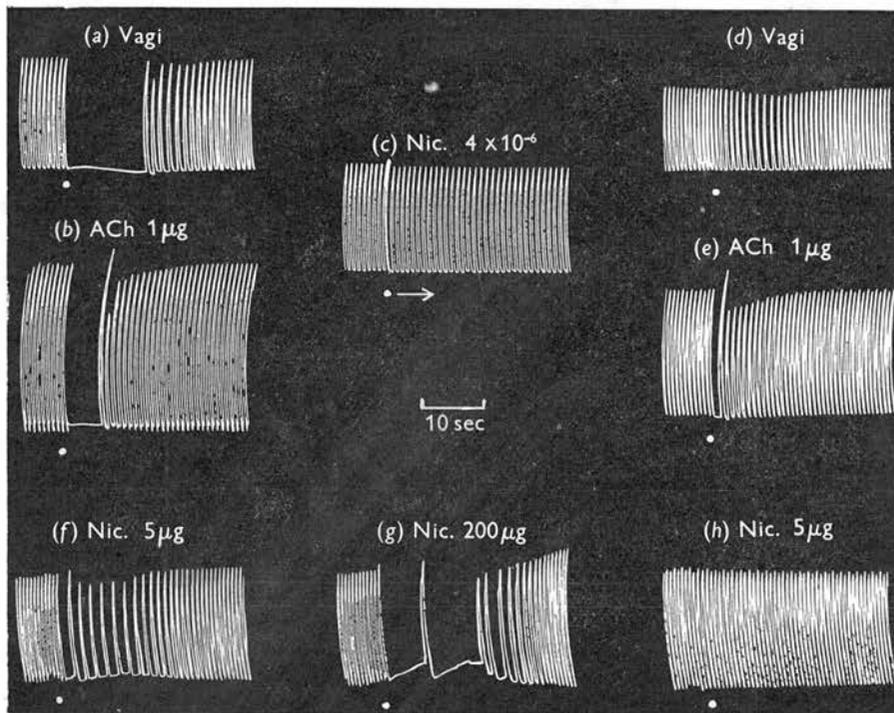


Fig. 60. Isolated cat's heart perfused through coronary arteries. Records of ventricular contractions. Stimulation and injections start at signals. In (a) and (d) stimulation of both vagi (20/sec, 0.5 msec, 5V) for 10 sec; (b) and (e) acetylcholine; (c) perfusion with nicotine ( $4 \times 10^{-6}$  solution) begun and maintained throughout (d) and (e); (f) 5 μg nicotine before and (h) 1 min after (g) 200 μg nicotine.

decamethonium, even in large doses, have no such inhibitory action.

A large dose (6 mg.) of hexamethonium, while producing no obvious effect by itself, blocks the inhibitory effects of vagal stimulation, of small doses of acetylcholine and of nicotine (Fig. 59). When nicotine is given by perfusing the heart with a dilute solution ( $4 \times 10^{-6}$ ), its inhibitory effect is not seen (Fig. 60), and during the perfusion the inhibitory effect of vagal stimulation and of acetylcholine is greatly reduced. Moreover, a very large dose of nicotine (200  $\mu$ g.), given by injection and not by perfusion, and having itself a large inhibitory effect, blocks the inhibitory effect of subsequent small doses of nicotine (Fig. 60).

### DISCUSSION

The results on the ciliary ganglion show clearly that the pattern of the responses to acetylcholine is closely similar to that described by Paton & Perry (1953) for the superior cervical ganglion. The cells of the ciliary ganglion are depolarized by acetylcholine; i.e. they react specifically to acetylcholine in the same way as the motor end-plate and the cells of the

superior cervical ganglion; moreover, the action potential complex is modified in shape in the same striking way as is that of the superior cervical ganglion. It is also worth noting that the recent experiments of Suden et al. (1952) have shown that the ciliary ganglion reacts to adrenaline in the same way as does the sympathetic ganglion, thus providing a further parallelism between the two types of synapse. Furthermore, small doses of acetylcholine potentiate the submaximal postganglionic spike, i.e. facilitate propagation at the ganglionic synapse. It would, of course, be desirable to show that acetylcholine was liberated in the ciliary ganglion by preganglionic stimulation. The difficulty of demonstrating this are, however, formidable, since not only has it proved impracticable to perfuse the ciliary ganglion in situ, but this ganglion also seems to be extremely sensitive to anoxia and will not easily survive isolation in a bath. The only evidence of release of acetylcholine at preganglionic nerve endings of the parasympathetic system has been that of Emmelin & Muren (1950) in the perfused salivary gland. Nevertheless, our results, incomplete as they are, provide strong supporting evidence for the cholinergic nature of transmission at the ciliary ganglionic synapse.

Two points of interest emerge from the experiments on the pupil. In the first place, hexamethonium has no effect on the

postganglionic-effector junction, even when it is given in doses sufficient completely to block ganglionic transmission; i.e. it has no atropine-like activity at all on the pupil. This finding is of great importance in interpreting other results. In the second place we found that nicotine has a stimulant effect on the ciliary ganglion which precedes its blocking action. This was not surprising in view of the fact that nicotine, like acetylcholine, produces a depolarization of the ganglion cells as well as producing block. Schofield (1952) had, however, reported that nicotine, painted on the ciliary ganglion, produced no constriction of the pupil. The discrepancy between our results and Schofield's can be explained by the different routes of administration of the nicotine. Paton & Perry (1953) showed that nicotine depolarized and blocked the superior cervical ganglion but that the depolarization passed off while the block still remained, and they concluded that the block by depolarization was followed by a 'competitive block' like that produced by hexamethonium. Thus, if the ciliary ganglion reacts in a similar way, it might be expected that nicotine painted on the ganglion would depolarize and stimulate the surface cells, but that they would soon enter the stage of block without depolarization, while the nicotine penetrated and started to depolarize and stimulate deeper cells. Thus the overall

picture might well be such that at any given moment the number of ganglion cells which are discharging is too small to produce any sign of overt stimulation, i.e. of constriction of the pupil, the final picture being one of block alone in the absence of stimulation. In our experiments, on the other hand, the nicotine, given arterially, would tend to produce the simultaneous discharge of many ganglion cells and thus an evident constriction of the pupil.

The experiments on the heart strongly suggest that the inhibitory action of small doses of acetylcholine is an effect not, as is generally assumed, on the cardiac tissue, but on the intracardial ganglion cells of the vagus. This view is based on the results obtained with ganglionic blocking substances. If these drugs act only on ganglion cells, the fact that they reduce or abolish the inhibitory effect of small doses of acetylcholine must be explained in this way. On the other hand, if they have, in addition, a blocking effect on the post-ganglionic-effector junction, i.e. an atropine-like action, this conclusion is not tenable. It seems unlikely that all the different ganglion blocking substances used should have such an additional action. On the pupil neither hexamethonium nor nicotine had such a peripheral blocking effect; moreover, both Ambache (1946) and Feldberg (1951) concluded that the blocking action of hexamethonium on the intestine was purely a ganglionic

effect. The finding that the cardiac inhibition produced by small doses of acetylcholine is reduced or abolished by ganglionic blocking substances is therefore most probably due to the fact that acetylcholine, in these doses, produces cardiac inhibition mainly by stimulation of the intracardiac ganglion cells of the vagus.

Nicotine has a similar cardiac inhibitory action so long as the intracardiac ganglion cells of the vagus are not blocked either by nicotine itself or by hexamethonium. The cardiac inhibitory effect of nicotine, however, appears to be entirely ganglionic, whereas larger doses of acetylcholine have, in addition, a peripheral cardiac inhibitory action which cannot be abolished by ganglionic blocking agents.

The conclusion that small doses of acetylcholine stimulate the vagal ganglion cells also suggests that the preganglionic fibres are cholinergic, because, if the ganglion cells are impinged upon by cholinergic fibres, one would expect them to be stimulated by acetylcholine and to be particularly sensitive to it.

There is, therefore, evidence that the post-synaptic cells of the parasympathetic system both in the ciliary ganglion and in the heart are sensitive to acetylcholine. Further evidence of the cholinergic nature of the transmission process

at both sites is to be found in the pattern of the reactions to a group of ganglion blocking substances. Paton & Perry (1953) were able to classify these substances into two groups. One group, typified by nicotine, stimulated the ganglion before blocking it, and blocked by depolarizing the ganglion cell membranes. The other group, typified by hexamethonium, did not stimulate the ganglion cells and blocked without producing any depolarization. These experiments were done on the cat's superior cervical ganglion, in which transmission is generally accepted to be cholinergic. We have been able to show, both in the ciliary ganglion and in the heart, where evidence for the cholinergic nature of the transmission process is incomplete, that the same classification of ganglion blocking drugs holds. In the ciliary ganglion 'depolarizing blocking drugs' did depolarize and 'competitive blocking drugs' did not; in the heart 'depolarizing blocking drugs' inhibited the heart and 'competitive blocking drugs' did not. Unless one postulates that the pattern of reaction to these drugs of a membrane which reacts specifically to a substance other than acetylcholine may be identical with that of a membrane which reacts specifically to acetylcholine, the conclusion that transmission at these parasympathetic ganglion synapses is cholinergic seems inescapable.

CHAPTER X

BLOCK OF TRANSMISSION

The use of ganglion-blocking drugs in clinical medicine is limited by the fact that they attack sympathetic and parasympathetic ganglia indiscriminately (Paton & Zaimis, 1952), and this has prompted the search for compounds which might exhibit a selective action on one or other of these autonomic pathways. One method of seeking selective blocking action lies in the study of the transmission process at the two sites to discover if any differences between them suggest a method of differential block. Both sites, are, however, cholinergic (Suden, Hart & Marrazzi, 1952; Perry & Talesnik, 1953). The latter workers showed that the ganglionic action potential of the ciliary ganglion is remarkably similar to that of the superior cervical ganglion save in its shorter duration, which may well be a characteristic not of the parasympathetic system but only of the ciliary ganglion from which neurones run to supply the pupillary muscles. Moreover, the ganglion-blocking compounds studied were similar in action at both sites; so little hope of determining a selectivity of action was offered by this method.

Another method of searching for such a selective action is the screening of large numbers of compounds for this effect on a few chosen ganglia of either system. Great difficulties in interpretation arise, however, because of the differing sensitivity not only of different animal species but of different end-organs within one particular species. Thus, to compare the effect of a drug on the nictitating membrane of the cat (sympathetic ganglion-block) with the effect on the guineapig ileum (parasympathetic ganglion-block) is meaningless. Even if the effects of a drug are tested in a single species, such as the cat, by recording comparatively block of nictitating membrane (sympathetic stimulation) and salivary secretion (parasympathetic stimulation), the extreme sensitivity of the latter to all blocking compounds (Paton & Perry, unpublished) makes comparisons very difficult.

The interpretation of such results might be easier if the effect of ganglion-blocking compounds was studied concurrently on the same end-organ. An end-organ with a dual innervation, both presynaptic nerve supplies being accessible for stimulation was therefore chosen; this chapter describes the results obtained in one such preparation, namely, the cat heart.

METHODS

Cats were anaesthetized with ethyl chloride and ether followed by intravenous chloralose (80 mg./kg.). Under artificial respiration the chest was opened widely on one side in order to expose the vagus and sympathetic nerves throughout their course to the heart; the pericardium was left intact. Care was taken to ensure that the blood supply to the thoracic sympathetic ganglion was not interrupted. Blood pressure was recorded through a cannula in the femoral artery. Pulse rate and pressure were recorded by transmission through a cannula in the carotid artery to a rubber diaphragm carrying a light balsawood lever which wrote on a kymograph.

Presynaptic parasympathetic fibres to the heart were stimulated in the cervical vagus nerve; presynaptic sympathetic fibres were stimulated in the thoracic sympathetic trunk between the 2nd and 3rd thoracic ganglia. A third stimulating electrode was placed on postsynaptic sympathetic fibres in the accelerator nerve. The nerves and vessels on the other side of the chest were left intact. Platinum-wire stimulating electrodes were used for all nerves, which were kept moist and were often covered with liquid paraffin. Square-wave pulses of 0.5 msec. duration, at a frequency of 17/sec., were

used for stimulation of each of the nerves. Suitable pulses were of 5-10 volts. The sympathetic and parasympathetic presynaptic nerves were stimulated alternately at 2 min. intervals for periods of 10 and 5 sec. respectively. Occasional periods of postsynaptic sympathetic stimulation for 10 sec. were interpolated. Recovery from the effects of stimulation for these periods was nearly always complete before the end of the 2 min. cycle. Drugs were administered intravenously into the femoral vein; hexamethonium and pentamethonium were used as the chlorides, azamethonium as the bromide ("Pendiomide"), and tetraethylammonium (TEA) and tetramethylammonium (TMA) as the bromides.

## RESULTS

### Effects of stimulation

#### Presynaptic sympathetic stimulation

Stimulation of the thoracic sympathetic chain between the 2nd and 3rd thoracic ganglia for 10 or 15 sec. produced an increase of blood pressure and acceleration of the heart rate. The onset of this effect was delayed and began some

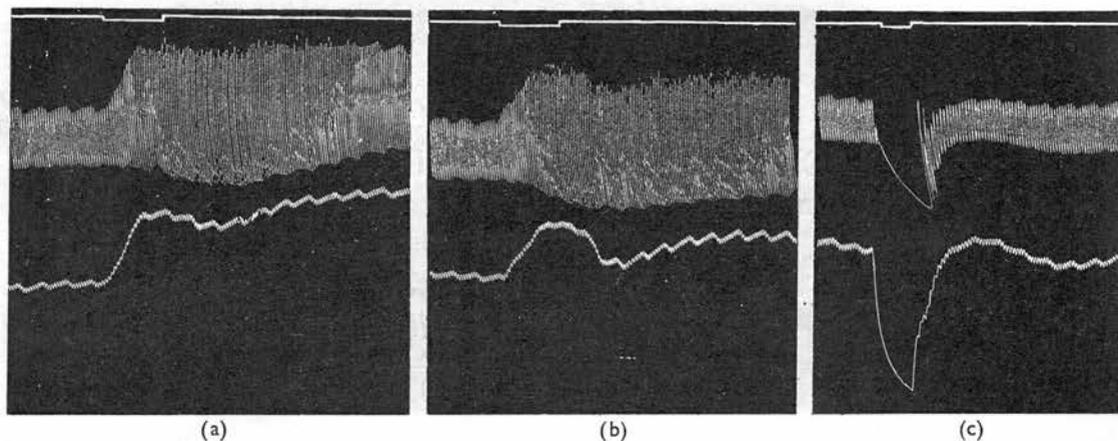


FIG. 61.—Cat, chloralose, artificial respiration. Top trace, periods of stimulation : middle trace, pulse rate and amplitude ; bottom trace, blood pressure. (a) 10 sec. stimulation of sympathetic trunk between 2nd and 3rd thoracic ganglia ; (b) 10 sec. stimulation of inferior accelerator nerve ; (c) 5 sec. stimulation of cervical vagus nerve.

3-4 sec. after the start of stimulation. A typical record is shown in Fig. 6(a). The blood pressure rose from 110 to 140 mm. Hg and the heart rate increased from 136 to 168 beats/min. There was also an increase in pulse amplitude. The effect lasted for about 60 sec., heart rate and blood pressure gradually returning to normal. Stimulation was repeated at 2 min. intervals for several hours and the same pattern of response obtained; the accelerator response was independent of the initial heart rate and blood pressure, but the pressor response was smaller if the initial blood pressure was high. Consequently, we used as the index of the normal response to stimulation the percentage increase in heart rate. This percentage increase was then taken as a 100% response, and responses after blocking drugs were expressed similarly. The initial heart rate was obtained by counting the heart beats during the 5 sec. just before stimulation, and the final rate by counting the beats during 5 sec. at the height of the response. Thus in Fig. 6(a) the initial heart rate was 136 and the final heart rate was 168, so that the percentage increase was  $32/136 \times 100 = 24\%$ . If after a dose of a ganglion-blocking drug the percentage increase in heart rate was found to be 12%, this is then taken to represent 50% inhibition of transmission.

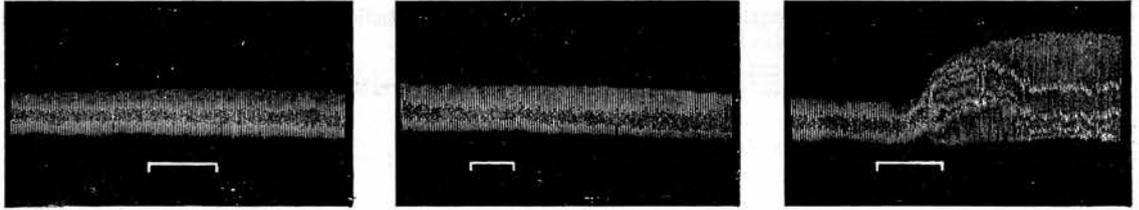
### Postsynaptic Sympathetic Stimulation

Stimulation of either or both accelerator nerves on the one side of the animal for 10 sec. led to the same typical response as that described above after stimulation of the presynaptic sympathetic nerves in the thoracic sympathetic trunk. The magnitude of the effect was similar with either the superior or inferior accelerator nerve, and concurrent stimulation of both did not materially increase the response. A typical record is shown in Fig. 6(b).

The responses were calibrated as described for the response to presynaptic stimulation. The response to postsynaptic sympathetic stimulation was unaffected by the doses of ganglion-blocking drugs used, and test periods of stimulation of this kind were interpolated in all experiments in order to ensure that the effects observed were not peripheral. Unfortunately, it was not possible to ensure that this is true of the parasympathetic innervation; but the results of Perry & Talesnik (1953) can be used as a basis for inferring that the two systems behave in similar fashion in this respect.

### Presynaptic Vagal (Parasympathetic) Stimulation

Stimulation of the vagus for 5 sec. produced cardiac arrest or very considerable slowing of the heart rate. A



(a)

(b)

(c)

FIG. 62.—Cat, chloralose, artificial respiration. Pulse rate and amplitude after pentamethonium 400 mg. intravenously in divided doses. At signals stimulation as in Fig. 61 of (a) thoracic sympathetic trunk, (b) vagus, (c) accelerator nerve.

typical record is shown in Fig. 61c.

The time of stimulation used was shorter than that used for the sympathetic nerves, since 10 sec. stimulation of the vagus produced an effect which lasted longer than the interval between successive periods of stimulation (i.e. longer than 2 min.). Longer intervals between successive periods of stimulation made it very difficult to follow the course of block produced by some of the drugs studied. The complete, or almost complete, cardiac arrest lasted throughout the 5 sec. period. Thereafter the heart rate often increased to a level at which it exceeded the normal rate for a short period. The responses were calibrated in the same way as that used for responses to sympathetic stimulation.

### Effects of Ganglion-blocking Drugs

#### "Competitive Blocking Drugs

In these experiments azamethonium, hexamethonium, pentamethonium, and tetraethylammonium (TEA) were used. All these drugs block the response to presynaptic sympathetic stimulation while leaving the effect of postsynaptic (accelerator nerve) sympathetic stimulation intact. Fig. 62 shows, for

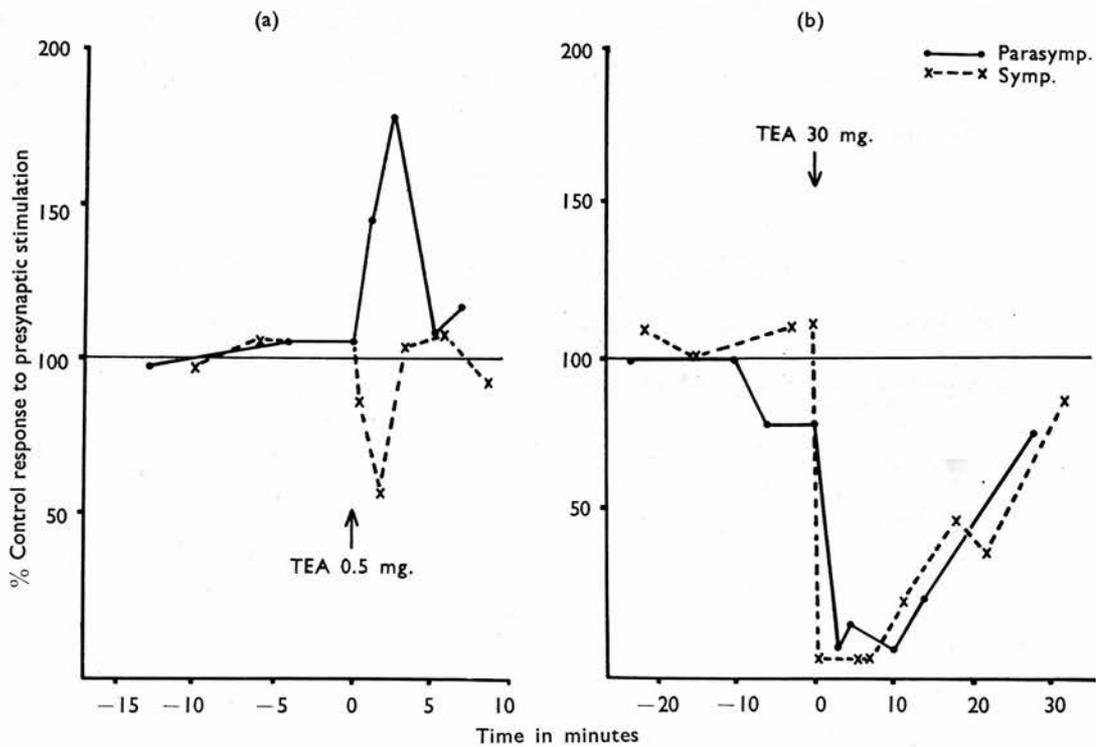


FIG. 63.—Graphs of time-course of block of presynaptic stimulation produced by TEA 0.5 mg. i.v. (a) and 30 mg. i.v. (b). Abscissa, time in min. before and after injection of drug. Ordinate, response of heart as % of control response. Solid line, response to parasympathetic stimulation. Dotted line, response to sympathetic stimulation.

example, a record obtained using pentamethonium. The block of presynaptic sympathetic responses is always accompanied by a block of the vagal stimulation.

Tetraethylammonium. The effects of TEA on sympathetic and parasympathetic ganglia appeared to be almost identical, although there was a slight apparent selectivity of action on the sympathetic cells. Fig. 63 shows the effects of graded doses of TEA on each system. Fig. 63a illustrates how 0.5 mg. TEA produced a transient block of sympathetic function of about 40%; there appears to have been no such block of vagal function and, in fact, the responses to vagal stimulation were increased, presumably due to the depression of sympathetic tone on the intact non-experimental side, since the two effects ran very parallel. When the dose of TEA was increased to 30 mg. (in the same experiment) there was almost complete block of both systems (Fig. 63b); the onset of sympathetic block was, however, rather more rapid although the duration of block and rate of recovery were similar for both systems. Doses of TEA intermediate between these two doses gave responses intermediate in both magnitude and duration. It will be seen that the block produced by a dose of 30 mg. TEA lasted for some 30 min.

We have attempted to relate the potencies of TEA on the

FIG. 64

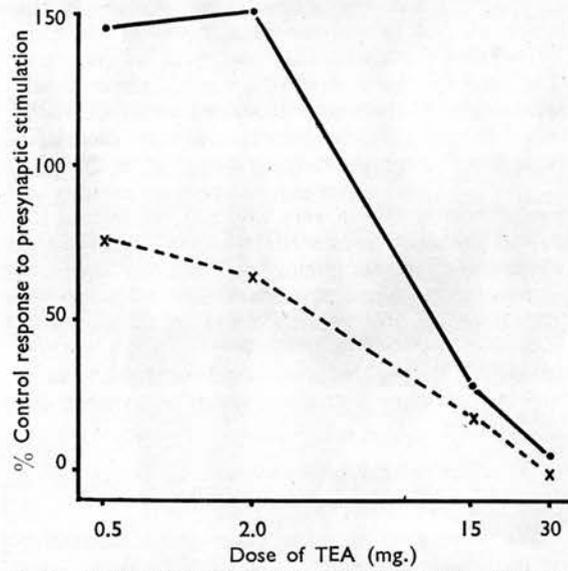
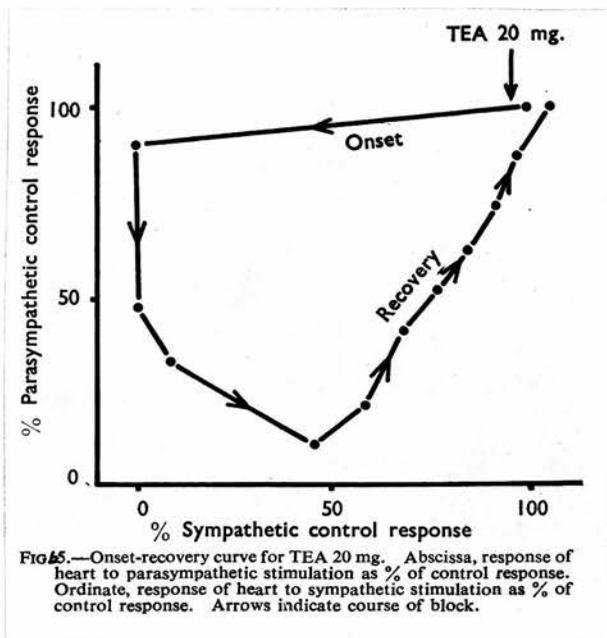


FIG. 64.—Graph of log dose-response lines of effect of TEA. Abscissa, dose of TEA in mg. Ordinate, response of heart as % of control response. Closed circles, response to parasympathetic stimulation. Crosses, response to sympathetic stimulation.

FIG. 65

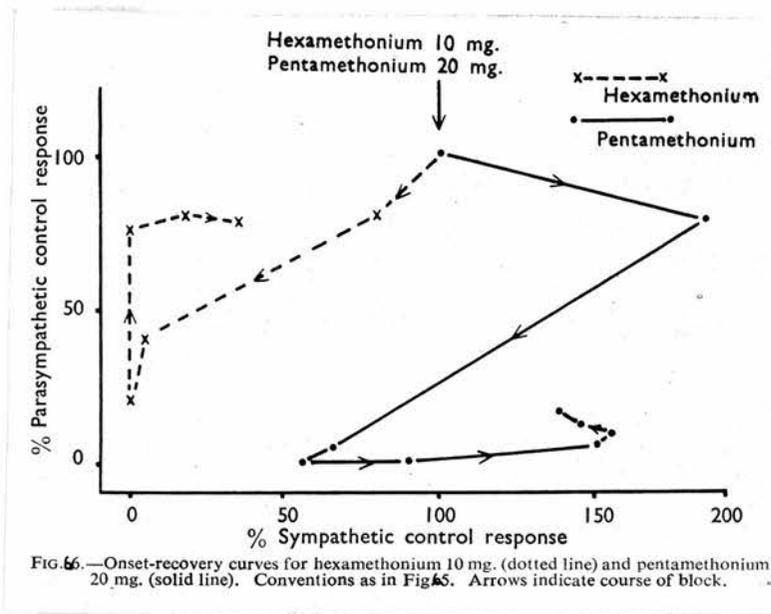


two systems by comparing the dose-response lines for each. The results of one such attempt is shown in Fig. 64 . It is obvious that no direct parallelism exists, since the two effects are closely interrelated that partial block of the sympathetic fibres is accompanied by a potentiation of the effect of vagal stimulation. Nevertheless, at doses which are sufficient to produce block of both, there appears to be no selective action on either system - at high doses the potency ratio being less than 1.5.

In view of the differences in rate of onset, duration and recovery of block, another method of illustrating these effects - similar to that used by Paton & Perry (1953) was used. This is shown for another experiment with TEA in Fig. 65 . The same type of reaction is seen, namely, a relatively rapid onset of sympathetic block, a slower development of vagal block - the later phases of which are accompanied by partial sympathetic recovery - and, finally, a steady recovery from both effects, reaching normal concurrently.

The effects of TEA are described in detail, since they provide the basis for describing the action of the other blocking drugs.

FIG. 66



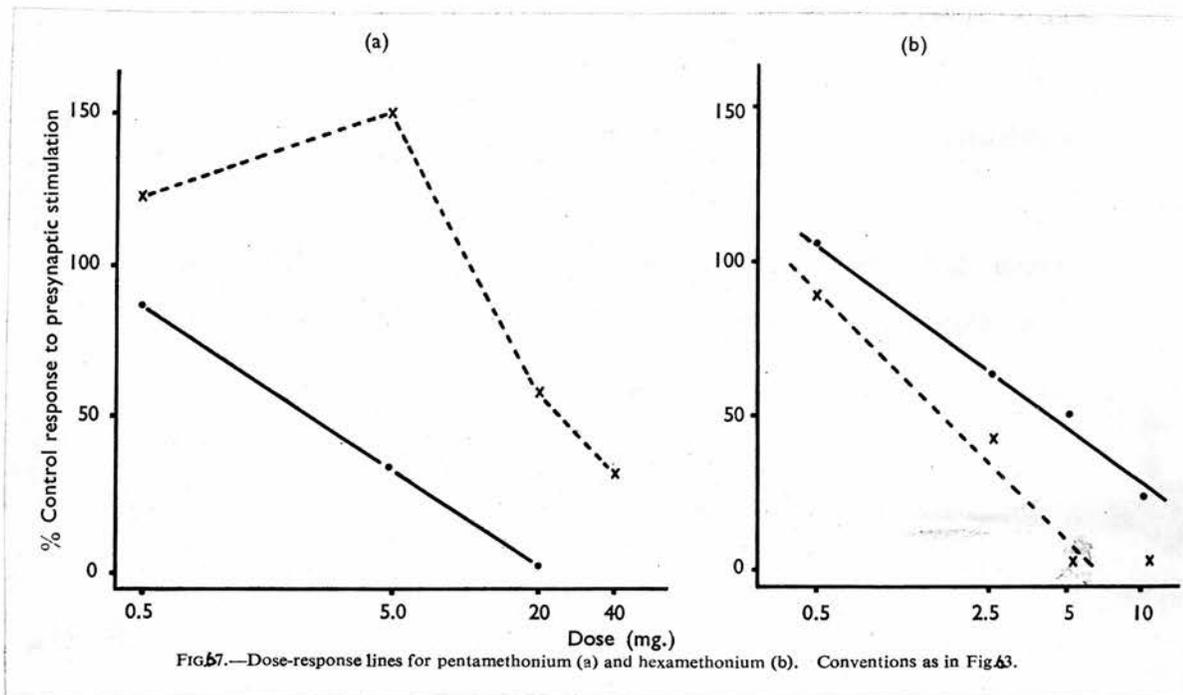
Azamethonium. The results which we obtained with azamethonium are almost identical with those already described for TEA. The onset of paralysis is rapid on the sympathetic ganglion, but the paralysis lasts slightly longer in the parasympathetic ganglia. With 5 mg. the block lasts some 15 min.; with 15 mg. the block is slightly greater, lasting 45 min.

Hexamethonium. Differing results were obtained with hexamethonium. In some experiments the parasympathetic block was slightly more severe; in others the reverse was true. However, the difference was never so great as to suggest a really selective action. The duration of action was also similar in both sympathetic and parasympathetic ganglia. One onset-recovery curve is shown in Fig. 66, where it is compared to a similar curve for pentamethonium. In this particular experiment the parasympathetic block was both less intense and less prolonged than the sympathetic block, although the rates of onset of block were similar.

Pentamethonium. This compound was the only one with which a consistent selectivity of effect on the cat heart was observed.

Fig. 67 shows the dose-response lines obtained in one

FIG. 67



experiment, and shows that pentamethonium was some 8 times as active in blocking the parasympathetic ganglia as it was in blocking the sympathetic ganglia. In a similar experiment another figure of about 12 was obtained. For comparison Fig. 67 shows similar dose-response lines for hexamethonium; in this case there appeared to be a slight selective action (say x3) on the sympathetic ganglia.

Moreover, the onset-recovery curves (Fig. 66) make the difference in the effects of these two closely related compounds very obvious indeed. Hexamethonium showed little selectivity either in degree or duration of the block, whereas pentamethonium produced a rapid onset of parasympathetic block accompanied at an early stage by potentiation of the effect of sympathetic stimulation, due presumably to release from parasympathetic tone on the other side of the animal. This was soon followed by partial block of the response to sympathetic stimulation which, however, was of lesser degree than the parasympathetic block. Furthermore, the partial sympathetic block disappeared completely, while the parasympathetic block remained complete. Thereafter there was a further potentiation of the sympathetic response, presumably due, once again, to the temporary absence of parasympathetic tone. Recovery from parasympathetic block was very slow and only the early phase is shown in Fig. 66 .

Since in all the experiments quoted only one drug was used it was necessary to confirm that the difference was not one caused by variation in the susceptibility of different cats; consequently, in one experiment the effects of two drugs was compared and the same differences were observed.

### "Depolarizing" Blocking Drugs

Tetramethylammonium. TMA produced first a marked slowing of the pulse rate and a fall in blood pressure, followed by a prolonged rise of blood pressure and increase of pulse rate above the initial resting levels. The latter effect might be attributed to a stimulation of the sympathetic supply which outlasted the parasympathetic stimulation; or to an effect of adrenaline released by TMA from the adrenals. This experiment was repeated after ligating the blood supply, of both adrenal glands, and the TMA then produced only a slowing of the pulse rate indicating that the later phase of increased pulse rate was in fact due to adrenaline release. Thus it appears that TMA is a more effective stimulant of the parasympathetic than of the sympathetic ganglion cells.

After its initial stimulant effect, TMA blocked the effect of stimulation of the sympathetic and parasympathetic nerves. The degree and time-course of the block of parasympathetic and of

TABLE IX

TABLE IX  
 MEAN RATIOS OF SYMPATHETIC/PARASYMPATHETIC  
 BLOCK, CALCULATED FROM DOSE-RESPONSE LINES AS  
 IN FIGS. 64 AND 67

Drug	No. of Expts.	Ratio
Tetraethylammonium ..	2	0.75
Tetramethylammonium ..	1	1.0
Azamethonium .. ..	2	0.75
Hexamethonium .. ..	3	0.6
Pentamethonium .. ..	2	10.0

sympathetic stimulation were almost identical. This block was relatively short-lived as compared with the block produced by the methonium drugs and closely resembled that produced by TEA.

After a dose of TMA it was necessary to test for block during the phase of increased pulse rate due to release of adrenaline. At this time there appeared to be block of both pre- and post-synaptic sympathetic stimulation. The apparent post-synaptic block was, however, less marked than the presynaptic block, because the pulse rate was already so fast that stimulation failed to produce the usual acceleration. After ligation of the blood supply to both adrenal glands, post-synaptic stimulation was fully effective after large doses of TMA which completely blocked presynaptic stimulation. Furthermore, if adrenaline was administered at this stage, the acceleration produced would again mask the effect of post-synaptic stimulation.

#### Relative Potencies of Blocking Drugs

The relative potencies of the different drugs on parasympathetic and sympathetic ganglia respectively, were calculated roughly by graphical methods.

DISCUSSION

The method of studying ganglion-blocking drugs which we have described represents the first attempt to compare concurrently the effects of such compounds on the ganglion cells of the sympathetic and parasympathetic supply to a single end-organ. Such studies should provide a more useful method of determining a selectivity of action on one or other part of the autonomic system than do the more commonly used methods of testing on more convenient but different end-organs in different species of animals. The relative effects of a drug on the ganglion cells of each system may, of course, depend not only upon inherent differences in sensitivity but also on the accessibility of the cells which are morphologically very differently arranged. Thus, administration of the drug by arterial injection close to the end-organ, might be expected to produce a selective action on the parasympathetic ganglion cells which are located in the end-organ itself, unlike the sympathetic ganglion cells which are collected in distinct ganglia and would be proximal to such an arterial injection. Intravenous injection of the drug at a distance from the end-

organ provided the best available means of minimizing such possible differences in accessibility, and consequently this technique was used throughout.

The selection of the heart as the end-organ for study was based on the relative ease of isolation of the presynaptic fibres of both systems supplying it. It is indeed probable that the clinical effects of ganglion-blocking drugs are attributable only to a small extent to their direct action on the autonomic supply to the heart. Further work on other end-organs is most desirable, and the present results merely indicate the possibilities inherent in techniques of this kind.

The results obtained on the rate of onset, duration and recovery from block of the two systems, underline the complexity of the autonomic control. Block of one system may result in potentiation of the effects of the other, possibly due to release of tone; and this potentiation may well complicate the pattern of any partial block of this second system which may be co-existent with the potentiation. In analysing these effects the onset-recovery curves, such as those illustrated in Figs. 65 and 66, have been most helpful

and go far towards elucidating the pattern of interaction. It is worth pointing out that the potentiation of the sympathetic response seen during parasympathetic block was also observed when the sympathetic stimulation was post-synaptic, indicating that this effect was a peripheral and not a ganglionic one.

As might have been expected from previous studies of the transmission process at both sites, most drugs studied showed little or no selectivity of action on the ganglion cells of one or other system. The single striking exception was pentamethonium, which did, apparently, exhibit a marked preferential block of the parasympathetic ganglion cells, being some 10 times more potent in blocking them than in blocking the sympathetic ganglion cells. This was, at first sight, a surprising finding in view of the fact that early work (Paton & Zaimis, 1951) showed pentamethonium to be much more active on the superior cervical ganglion than on the guineapig ileum and stressed the possible importance of this apparently selective action. Nevertheless, as Paton & Zaimis (1952) later pointed out, this relatively simple picture failed to survive when it became apparent that different end-organs

varied enormously in their sensitivity - as, for example, when Paton & Perry (unpublished) showed that the salivary secretion was blocked first of all by all the ganglion-blocking drugs tried. The present results indicate that, on the innervation of the heart at least, pentamethonium attacks the parasympathetic cells preferentially and not, as had at first appeared probable, the sympathetic cells. The 10-fold difference in sensitivity between the two systems, found with pentamethonium and not with hexamethonium, is not clearly reflected in the clinical activity of the two drugs. The main use of the drugs in clinical practice has been in the treatment of hypertension. What is required in such cases is a selective block of sympathetic ganglia; and pentamethonium is less effective than hexamethonium. It could be argued that this difference is due to the greater degree of block of parasympathetic ganglia produced by pentamethonium. The situation is obviously complex, and

further direct comparisons of the kind described above  
will be necessary to clarify it.

P A R T III

STUDIES ON THE SPINAL CORD

CHAPTER XI

INTRODUCTION AND REVIEW OF LITERATURE

The presence of acetylcholine in brain and spinal cord has long been recognised, and MacIntosh (1938) carried out detailed quantitative studies of its localization. Feldberg (1950) has interpreted these data and the similar distribution of cholinacetylase in terms of an alternation between cholinergic and noncholinergic neurones in the central nervous system. The basis of the hypothesis is that the dorsal roots and tracts are low (primary afferent), the lateral filament and spinocerebellar tracts are high (second afferent), the internal capsule is low (third afferent), the motor cortex is intermediate (a number of interneurones), the pyramids are low (upper motoneurones), and the motor roots and nerves are high (lower motoneurones) in acetylcholine and cholinacetylase content.

The distribution of cholinesterase in the central nervous system can be studied much more accurately than that of acetylcholine by using histochemical localization (Koelle, 1954; Burgen & Chipman, 1951). Pseudo cholinesterase (non-specific; butyryl) is located almost exclusively in the connective tissue

in the gliocytes and capillary walls; true cholinesterase (specific; acetyl) is differentially present in certain neurones and is located on the walls of cell, axon, and dendrites. Motoneurones, known to be cholinergic, contain large amounts of true cholinesterase, and similar quantities are found in tertiary afferents and in some correlation centres; on the other hand, primary afferents, considered on other grounds to be noncholinergic, contain very small amounts of true cholinesterase, as do other correlation centres. Secondary afferents contain intermediate amounts. Moderate amounts of the cholinesterase were found in the supraoptic nucleus, and other evidence has been adduced (Duke, et al. 1950) that acetylcholine acts there as a transmitter for the release of antidiuretic hormone. The pattern which Koelle describes thus agrees well with Feldberg's suggestion of an alternation of cholinergic-noncholinergic neurones. Support for Koelle's work is found in that of Cavanagh, et al. (1954), who claim that all pseudo cholinesterase in the central nervous system is located in glial cells; these workers also state that in peripheral nerve the pseudo cholinesterase is not associated with conduction and is probably a component of the cells of Schwann. Wolfgram (1954) showed that the same amounts of true and pseudo cholinesterase were present in both ventral and dorsal spinal

roots in cattle; cholinacetylase activity was, however, more than 20 times greater in ventral roots. This work could be interpreted as indicating that although cholinacetylase activity runs parallel with acetylcholine content (acetylcholine immigrating down the axon to the terminals), cholinesterase activity is specifically located pre- or post-synaptically near the site of release of acetylcholine, and consequently will not always be correlated with the acetylcholine content.

In examining transmission in the spinal cord, many workers have chosen to study monosynaptic reflexes in the belief that they were selecting the simplest possible transmission system available in the spinal cord. The simplicity of the system is, however, more apparent than real, since both components of the reflex are themselves subject to a complicated pattern of influences.

The nature of the reactions of motoneurones was greatly elucidated by the demonstration by Brock, et al. (1952, 1953), using intracellular recording, that stimulation of primary afferents from an agonist muscle produced depolarization, while stimulation of primary afferents from an antagonist muscle produced hyperpolarization of the cell membrane. The authors considered that this could only be explained by postulating

the release of both an excitatory and an inhibitory chemical transmitter at synaptic knobs on the motoneurone.

Before this demonstration, attention had been focussed upon the nature of excitation of the motoneurone. The nature of the excitatory chemical transmitter is still, however, completely obscure. All the evidence, and there is now much of it, is against acetylcholine. In the first place, there is the considerable evidence based on the noncholinergic nature of the primary sensory afferents; and in the second place, there is now a good deal of evidence that neither acetylcholine nor the anticholinesterases exert any direct action on the motoneurons. Even in 1948, Eccles was able to collect a formidable body of evidence of this kind. Holmstedt & Skoglund (1953) described the striking specific depression of the extensor reflex induced by an anticholinesterase (tabun), given by injection into the aorta close to the origin of the lumbar arteries, and Taverner (1954) has studied the actions of physostigmine given by the same route. My studies, carried out in collaboration with Drs. Gray and Feldberg, are reported in Chapter XII. Such effects could well be explained on the basis of a stimulation of cholinceptive interneurons and a resulting recruitment of inhibitory pathways.

Perhaps the most striking evidence against acetylcholine has, however, recently been obtained by Eccles, et al. (1954),

who showed that although acetylcholine exerted a stimulant effect on Renshaw cells in the ventral horn, it had no such effect on motoneurons as judged by intracellular recording. This work seems to have disposed finally of the possibility that acetylcholine may be an excitatory transmitter at synapses on motoneurons.

Ever since Dale (1935) suggested that the substance responsible for the vasodilatation, produced by antidromic stimulation at the peripheral end of a sensory nerve, might be the same as that released at the central end of such primary afferents, attempts have been made to follow up this attractive possibility of identifying a central sensory transmitter, which might also be the substance exciting motoneurons. Antidromic stimulation was said to release histamine (Kwiatkowski, 1943) and acetylcholine (Wybauw, 1938), but in work carried out with Mrs. Holton (see Chapter XIII) I was unable to confirm these claims when studying the phenomenon in the rabbit's ear.

CHAPTER XII

ACETYLCHOLINE AND SPINAL CORD ACTIVITY

INTRODUCTION

The role of acetylcholine in central synaptic transmission has for long been a vexed question, and one reason for the difficulty in settling it is the relative inaccessibility of the spinal cord to drugs. In the present experiments an analysis was made of the effects of acetylcholine given by close arterial injection, whereby momentarily the injected solution completely fills the vascular bed of the region being studied. Hitherto acetylcholine has either been injected into the blood stream at a more distant point, applied locally to the surface of the central nervous system, or injected intraventricularly (for references see Feldberg, 1945, 1950), or into the substance of the grey matter (Kennard, 1951).

In order to study some excitatory effects of drugs given intravascularly, for example acetylcholine at the neuromuscular junction, it is important to obtain a rapid rise in the concentration in the intercellular space. To obtain such a rapid rise it is necessary to establish a high concentration gradient across the vascular membrane. For a given dose of

drug this is most easily done by close arterial injection. In the spinal cord, where the 'blood-brain barrier' may accentuate the difficulty of building up the necessary intercellular concentration, there may be a special need for this technique. Further, when using acetylcholine, which is quickly hydrolysed enzymically in the blood, close arterial injections permit the study of its effects without previous administration of an anticholinesterase which may modify the response.

The arterial supply of the spinal cord runs on its ventral surface. This makes close arterial injections technically difficult, because for most of the length of the cord the bodies of the vertebrae present a formidable obstacle to a ventral approach. This difficulty was avoided by exposing the first cervical segment of the cord which, however, has very short rootlets from which we could obtain neither monosynaptic nor even monosegmental reflexes.

#### METHODS

The experiments were performed on cats anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (80 mg./kg.) or dial (Dial liquid 'Ciba' 0.4-0.8 ml./kg.). A short description of the exposure of the cord and the cannulation of the basilar artery has been given elsewhere (Feldberg, Gray & Perry, 1952).

The head and neck of the cat were held rigidly by transfixing the muscles and ligaments of the neck and jaw with knitting needles and clamping the needles in a rigid framework. The upper parts of the trachea and oesophagus were dissected out and removed, the tracheal cannula being inserted low. The prevertebral muscles were removed and the fine twigs of the first cervical nerves which supply them were carefully dissected out. Lengths of the second cervical nerves on both sides were prepared and cut peripherally. The third cervical nerves were divided to prevent neck movements during stimulation.

The atlanto-occipital membrane was opened by dividing it strand by strand with a curved triangular cutting needle. If bleeding occurred, the head was raised and this was usually sufficient to stop it; occasionally bleeding was severe and the carotid arteries were then clamped. This was avoided if possible as the preparation was not so healthy if the carotids were clamped at this stage. After opening the membrane, the occipital bone up to the bullae osseae, the arch of the atlas and the odontoid peg and upper part of the body of the axis were removed.

The edges of the wound were then stitched to a ring to form a paraffin bath, and the dura mater was opened and stitched back to expose the cord. A diagram of the exposed cord has been published (Feldberg et al. 1952). Under a dissecting microscope the basilar artery was freed from the arachnoid and pia mater.

Fine silk threads for tying in the cannula were passed beneath it and the larger branches tied.

By gentle retraction on a dentate ligament, the root of the first cervical nerve could be pulled upwards into view; it was then divided as far from the cord as possible. The rootlets, fanning out towards their origins from the cord, could then be brushed backwards and small strands prepared for recording.

The animal was then given 2 ml. of a 10% (w/v) heparin solution intravenously. The basilar artery was tied at the cephalic end and temporarily clamped with a fine pair of screw forceps at its caudal end, just at the origin of the vertebral arteries. A 26-gauge stainless steel cannula was used; to facilitate its insertion it was mounted on a long Perspex rod which acted as a light but rigid handle which could be manipulated from a distance while the cannula itself was being rigidly fixed to the frame. The blood supply to the cord does not always have the pattern shown in the diagram in our previous paper. When, for example, the basilar artery was a double trunk for part of its length, an effort was made to cannulate in such a way that the injection reached the anterior spinal artery via both vertebral arteries: e.g. by cannulating the united basilar artery at a higher level.

The animal was transferred to an electrically screened, heated box. One of the ventral rootlets was placed on two

recording electrodes which consisted of horse hairs mounted in agar-saline in which was embedded a chlorided silver wire. Potentials were amplified with an amplifier having a high impedance input and a frequency response which could be varied up to a maximum range of 0-25 kc/s, and were recorded by photographing two cathode-ray oscillographs. These were double-beam oscillographs and the potentials were recorded on one beam of each, the Y-plates of these two beams being connected in parallel. One oscillograph was used without a time base and was photographed on moving paper; a time mark, recorded on the moving paper, triggered the time base of the other oscillograph. In this way it was possible to synchronize the records obtained by the two methods and thus simultaneously to record spontaneous activity and the reflex response to stimulation. This stimulus was a short shock from a low impedance source applied through bright platinum electrodes to the trunk of C2.

During recording, the constantly collecting cerebrospinal fluid was removed by continuous suction and the paraffin in the bath was replenished by a slow drip.

When chloralose was used, the reflex spike was sometimes abolished by the intravenous injection of appropriate doses of pentobarbitone Na; whenever this was done it has been stated specifically in the text.

Control injections into the basilar artery were made with

fresh Locke's solution and the acetylcholine and other drugs were dissolved in the same solution. If not otherwise stated, the volume injected was 0.2 ml. and concentrations are given as g./ml. Doses of acetylcholine are given in terms of the hydrochloride, doses of eserine and atropine in terms of the sulphates.

## RESULTS

### Effects of acetylcholine

Close arterial injections of acetylcholine into the basilar artery produced regular changes in the spontaneous activity in the cord and in the polysynaptic reflex set up in the first cervical ventral root by stimulation of either the ipsilateral or contralateral second cervical nerve. The reflex changes were accompanied by changes in the ventral root potential, of which only the early phases have been studied. The dose of acetylcholine normally used was 0.2 ml. of a  $10^{-4}$  solution, but the changes were also observed with 0.2 ml. of  $10^{-1}$  acetylcholine.

### Spontaneous activity

An injection of acetylcholine produced in the first or

FIG. 68

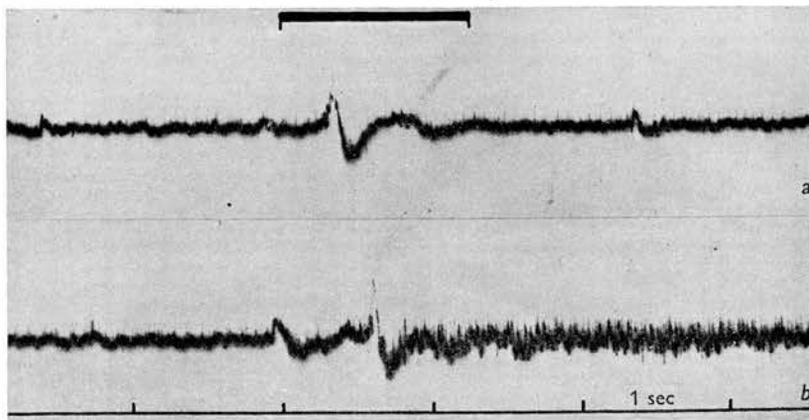


Fig. 68 Cat, chloralose. Records from ventral rootlet of C1. At signal, close arterial injections of (a) 0.2 ml. Locke's solution and (b) 0.2 ml. acetylcholine  $10^{-4}$ . Time, seconds. Amplifier frequency response 4 c/s to 2 kc/s.

FIG. 69

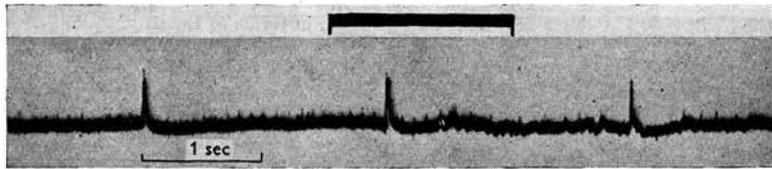
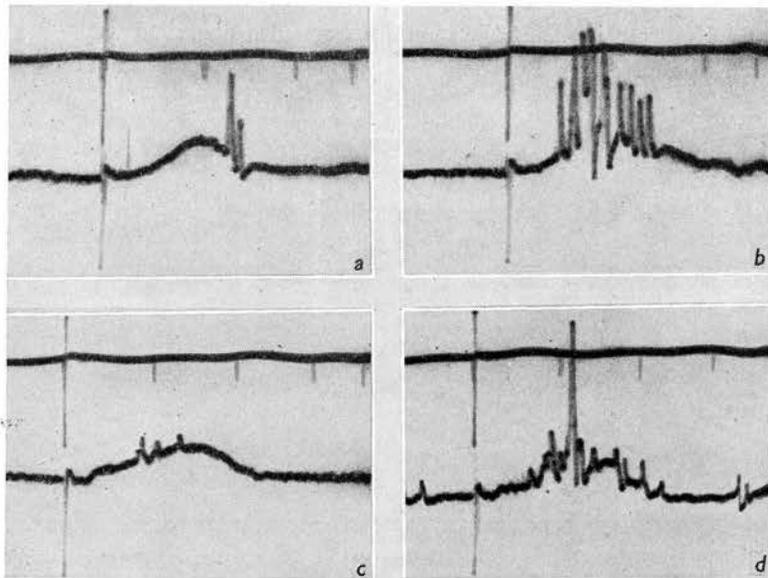


Fig. 69 Cat, chloralose, followed by pentobarbitone. Records from ventral rootlet of C1. At signal, close arterial injection of 0.2 ml. acetylcholine  $10^{-4}$ . Time, seconds. Amplifier frequency response 0.4 c/s to 2 kc/s.

FIG. 70



**Fig. 70** Cat, dial. Records of polysynaptic reflex from rootlet C1. (a) and (c) before, (b) and (d) 3 and 5 sec respectively after close arterial injection of 0.2 ml. acetylcholine  $10^{-4}$ . Between (b) and (c) the rootlet used for recording was changed and atropine ( $80 \mu\text{g}$  in 0.2 ml.) given arterially. Time, 10 msec. Amplifier response at (a) and (b) 0-2 kc/s, at (c) and (d) 4 c/s to 2 kc/s.

second cervical nerve an outburst of motor impulses which began within 0.5 sec. of the beginning of the injection and lasted, with steadily declining frequency, for periods varying from 2 to more than 40 sec. (Fig. 68). If the recording electrodes were on a ventral rootlet, the proximal electrode being on or near the cord surface, a fluctuation of the base-line was also seen to start about 0.5 sec. after the beginning of the injection (Fig. 68). The period of the more obvious fluctuations was of the order of 20-40 msec. The impulse discharge was most intense on the negative peaks (cord electrode negative) of the fluctuation. When a barbiturate was given in order to diminish or abolish the impulse discharges, an injection of acetylcholine was still followed by the base-line fluctuations. In the experiment illustrated in Fig. 69 this change is clearly seen. In this particular experiment such spontaneous discharge as remained after pentobarbitone was diminished by the injection of acetylcholine.

#### The ipsilateral polysynaptic reflex

When the main part of the second cervical nerve was stimulated with short shocks every 2 sec. and the polysynaptic reflex recorded from the ipsilateral first cervical nerve or ventral root, an injection of acetylcholine caused an increase in the area of the reflex. This is illustrated in Fig. 70, in which the reflex resulting from a supramaximal stimulus is seen on top of the ventral root potential recorded from a fine rootlet.

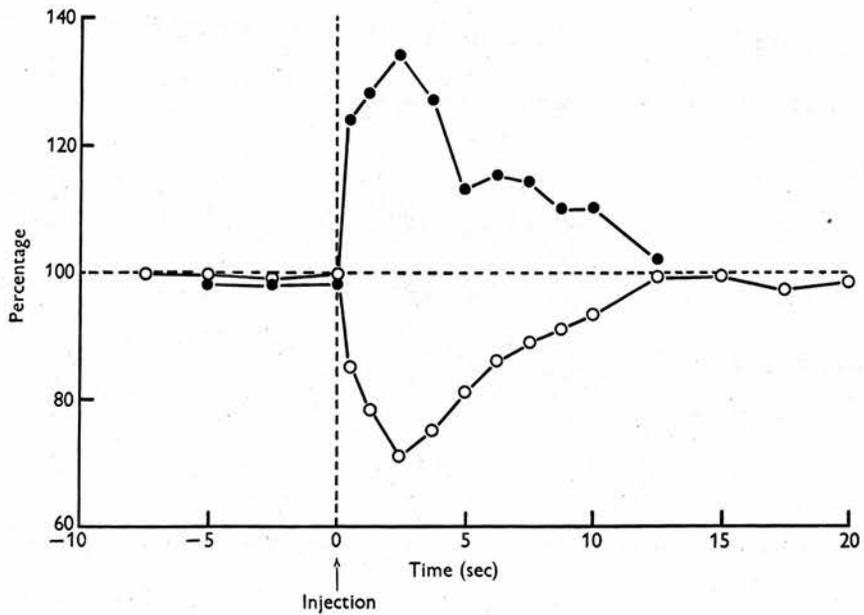


Fig. 71 Time course of changes produced by acetylcholine in area and latency of polysynaptic reflex. Records from small branch of C1. (Same experiment as Figs. 74 and 76) Ordinate: percentage change in area (●—●) and in latency (○—○) of reflex. Abscissae: time in seconds. At zero time close arterial injection of 0.2 ml. acetylcholine  $10^{-4}$ . (For details see text.)

In this experiment 80  $\mu$ g. atropine in 0.2 ml. was given by close arterial injection; it did not abolish the effect of acetylcholine (Fig. 7Bd).

The time course of the change can be seen in Fig. 7, in which the area of the reflex, given as a percentage of the mean pre-injection value, is plotted against time. The curve is the mean of three separate injections; the individual results were plotted and adjusted so that the beginnings of the injections were aligned and means were then taken at regular intervals along the abscissa. In this particular experiment the reflex was recorded diphasically, and in consequence there may be some distortion due to changes in synchronization. The change in reflex size was visible in records taken less than 0.5 sec. after the beginning of the injection, and reached a maximum in about 3 sec. The changes in area were larger with submaximal stimuli than with supramaximal ones.

It can also be seen from Figs. 7B and 7 that the interval between the stimulus and the beginning of the reflex discharge is shortened after the injection of acetylcholine and that the general time course of this change is similar to that of the change in area. The reduction in time was seen even in records taken within 0.5 sec. of the injection. The time was reduced to about 70% of its pre-injection value and this minimum occurred between 2.5 and 6 sec. after the injection began. Thus the

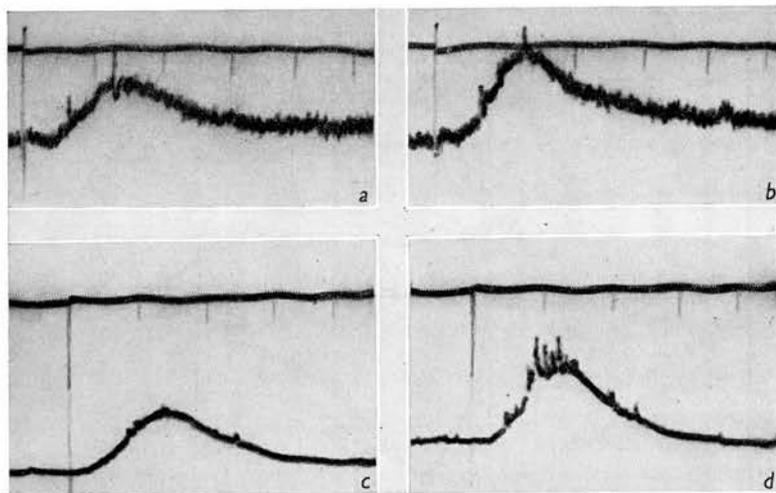
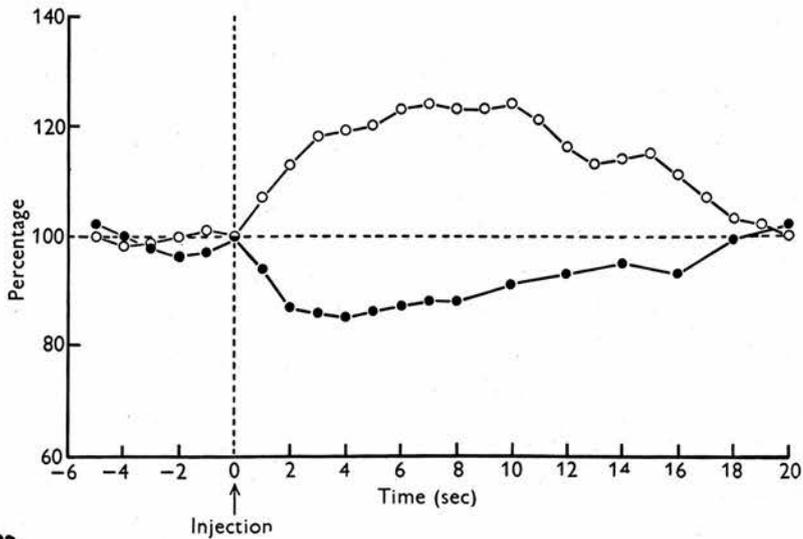


Fig. 72 Records from ventral rootlet of C1. Two experiments: (a) and (c) before, (b) and (d) 6 and 8 sec respectively after close arterial injection of 0.2 ml. acetylcholine  $10^{-4}$ . Records (a) and (b) cat, chloralose followed by pentobarbitone; amplifier frequency response 0.4 c/s to 2 kc/s. Records (c) and (d) cat, dial; amplifier frequency response 0-6 kc/s. Time, 10 msec.

FIG. 73



73  
 Fig. ■ Time course of changes produced by acetylcholine in amplitude and interval between stimulus and peak of root potential (polysynaptic reflex). Records from ventral rootlet C1. Ordinates: percentage change in amplitude (○—○) and interval (●—●). Abscissae: time in seconds. At zero time close arterial injection of 0.2 ml. acetylcholine  $10^{-4}$ . (For details see text.)

interval which was originally about 6 msec. was reduced to about 4 msec. when the effect was at a maximum. It is unlikely that the extra-cordal conduction time of the sensory fibres responsible for the bulk of the reflex was more than 0.5 msec., since the length of the second cervical nerve lying between the stimulating electrodes and the cord was about 15 mm.

#### Ventral root potential produced by ipsilateral stimulation

When the first phase of the ventral root potential was recorded from rootlets of the first cervical segment during stimulation of the ipsilateral second cervical nerve, an injection of acetylcholine was followed in most preparations by an increase in the amplitude of the ventral root potential; in one preparation, however, there was a decrease and, in another, a transient decrease preceded the usual increase. Examples of ventral root potentials before and after an injection of acetylcholine are shown in Fig. 72. The time course of the change in amplitude is shown in Fig. 73, which also illustrates the time course of the change in the interval between stimulus and peak of the root potential. The curves in Fig. 73 are the means of seven separate injections. The interval was reduced after the injection of acetylcholine to about 80% of its pre-injection level. In any given experiment, the time course of this change was practically identical with that of the change in reflex

latency. The absolute value of the time interval between the stimulus and the peak of the root potential was of the order of 11-13 msec. and the reduction in the interval from 2 to 4 msec.

#### Excitability and resting potential of the motor neurone

The excitability of the motor neurones can, to some extent, be gauged by the potential at which the reflex discharge begins. During recording from a large number of fibres, changes in synchronization can produce apparent changes in threshold, but it is fair to say that if acetylcholine increased the excitability of the motor neurones by reducing the membrane potential in the junctional region, there should be a systematic reduction in the level at which the root potential excites its first impulse and also an increase in the reflex discharge for a given size of root potential. The latter is difficult to measure, particularly as the occurrence of an impulse discharge may reduce the root potential (Brock, Coombs & Eccles, 1952).

The potential at which the first impulse arose was measured for several runs in each of four experiments, using ipsilateral stimulation. Acetylcholine always increased the variability of this threshold, and in most experiments there was a tendency for the threshold to be lowered. However, the variability after acetylcholine was so great that the reduction was not significant in any experiment.



Fig. 74 Cat, dial. Record from ventral rootlet C1. Effect of close arterial injection (at signal) of 0.2 ml. acetylcholine  $10^{-4}$  on resting potential. Root potentials elicited every 2 sec. Two of these (marked by arrows) are reproduced in Fig. 72c and d. Amplifier frequency response 0-6 kc/s.

FIG. 75

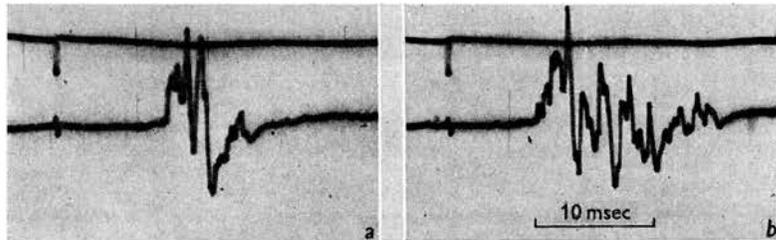


Fig. 75 Cat, chloralose. Records of polysynaptic reflex from ventral rootlet C1. (a) before and (b) 2 min after close arterial injection of 0.5 ml. eserine  $10^{-4}$ . Amplifier frequency response 0.4 c/s to 2 kc/s. Time, 10 msec.

FIG. 76

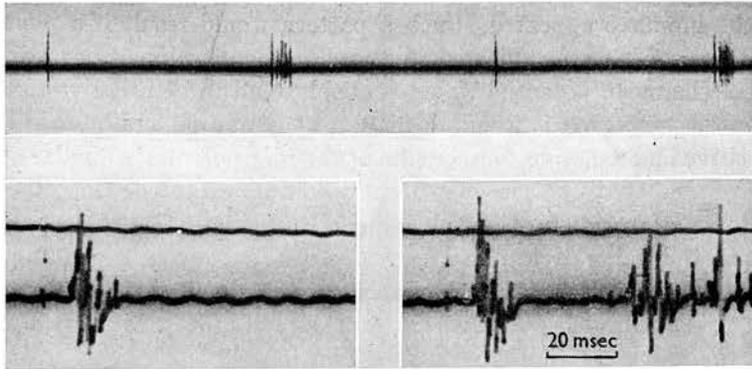


Fig. 76 Same experiment as Fig. 75. Upper record taken 2 min after eserine, showing reflex elicited every 2 sec followed twice by spontaneous outburst of activity. The first two reflexes are shown on lower record with a faster time scale (time, 20 msec).

Changes of membrane potential in the junctional region of the motor neurone can be recorded from electrodes on the motor root. In two experiments records were taken with the amplifier direct coupled. In one of these each injection of acetylcholine caused a potential change, the electrode nearer the cord going negative with an amplitude approximately one third of that of the root potential (Fig. 74). In the other experiment no change was detectable.

#### Effects of eserine and prostigmine

In a few experiments eserine or prostigmine (0.2-0.5 ml. of a  $10^{-4}$  solution) was injected into the basilar artery. No effects were seen unless the cord was stimulated either reflexly or by acetylcholine; in one experiment a spontaneous discharge began about 15 min. after the injection of eserine, but this may not have been due to the eserine.

In two experiments in which the reflex was recorded, eserine caused an increase in the area of the reflex which, unlike the increase produced by acetylcholine, was mainly due to a prolongation of the discharge (Fig. 75). In this experiment bursts of impulses occasionally followed the reflex discharge after a short interval (Fig. 76); this delayed response probably coincides with the peak of the second negative phase of the ventral root

potential (Barron & Matthews, 1938).

Eserine and prostigmine prolonged the effects of acetylcholine. In two experiments, after eserine and prostigmine, the stimulating effects of acetylcholine were unusually short, but following the outburst there was a long period during which the background discharge was abolished. In another experiment the area of the reflex increased for only a short period after the acetylcholine injection and thereafter it decreased to below pre-injection level.

### DISCUSSION

Our results show that, in the spinal cord, effects are produced by acetylcholine in concentrations which are of the same order of magnitude as those required to excite motor end-plates, post-ganglionic neurones and sensory pathways in the skin. Although the possibility that the observed effects are due to vascular changes has not been excluded, there is no evidence suggesting that this is so, and it seems more reasonable to attribute them to a direct action of acetylcholine on the nervous tissue.

Further, our results on the cervical cord suggest that acetylcholine acts predominantly on interneurones, although a

direct effect on the motor neurones cannot be excluded. The changes produced in the polysynaptic reflex can all be interpreted on this basis. The decrease in the latency of the reflex is of the order of 2 msec.; changes in conduction velocity could hardly account for this large reduction, which must therefore occur at synapses, perhaps by the by-passing of one or more of them. Facilitation at these synapses could result from direct depolarization of the post-synaptic region or from bombardment of the synapse by impulses excited by the acetylcholine. Furthermore, the peak of the ventral root potential occurs some 2-4 msec. earlier after acetylcholine; since Brock et al. (1952) have shown with an internal electrode that the rising time of the synaptic potential in response to a single presynaptic impulse is less than 1 msec., increasing the rate of rise of this potential would save little time. Changes in the cable properties of the motor neurone would also be insignificant. The decrease in time from the stimulus to the peak of the root potential can only be accounted for satisfactorily by assuming that impulses reach the motor neurone earlier. The main saving in time must therefore be at the synapses of interneurones.

The changes in the slow potentials occurring during a spontaneous discharge initiated by acetylcholine also support the view that acetylcholine acts predominantly on interneurones.

These changes consisted of a regular increase in the random fluctuations of the base-line and, in one experiment, of a steady negativity of the cord with respect to the ventral root. If the effect of acetylcholine were predominantly on the motor neurone, one would expect this steady negativity to occur regularly and to be proportional to the intensity of the spontaneous discharge. The most striking non-propagated change that began after acetylcholine was the base-line irregularities on the negative peaks of which the impulses appeared. Such a pattern would result if a population of motor neurones were subjected to a random bombardment of presynaptic impulses. The mean potential would probably tend to be negative to that in the quiescent state, but it would depend on the duration and intensity of the slow positive and negative components of the root potential. Similar changes in the excitability of the motor neurone would be expected; thus, the excitability after an injection of acetylcholine should fluctuate more than before, and the mean level of excitability might be expected to rise, depending on the duration and intensity of the two phases of the change in excitability that follows a reflex discharge (Bernhard & Therman, 1947). When we use the level of the root potential at which the first impulse appears as an index of the excitability of the motor neurone, our results are in

accord with these expectations.

All the evidence, therefore, suggests that the predominant effect of acetylcholine injected in the spinal cord is to excite some part of some interneurons, either by synaptic depolarization which could account directly for the synaptic facilitation and the spontaneous discharge, or by excitation of impulses at a non-synaptic point which would facilitate the interneuronal synapses by impulse bombardment.

CHAPTER XIII

ANTIDROMIC VASODILATATION

INTRODUCTION

Stimulation of the peripheral end of a cut sensory nerve results, in certain animals, in vasodilatation of the area of skin supplied by that nerve. This phenomenon was investigated by Bayliss (1901), who showed that it was due to impulses travelling towards the periphery along neurones belonging to the dorsal root system, and introduced the term antidromic vasodilatation to describe it. Since then it has been shown that antidromic vasodilatation can be obtained in man (Foerster, 1928), in the cat's paw (Langley, 1923), and in the rabbit's ear (Feldberg, 1926).

The experiments of Lewis & Marvin (1926) suggested that antidromic vasodilatation was due to liberation of a chemical transmitter at the peripheral ends of the sensory nerves, but about the nature of the transmitter little is known. Histamine,

acetylcholine and an unidentified vasodilator substance from posterior spinal roots have been suggested as possible transmitters, and our experiments were performed in order to find out which if any of these substances is responsible. Evidence is presented in this paper that the transmitter is neither acetylcholine nor histamine but the possibility that it is the posterior root substance has not been excluded.

Kwiatkowski (1943) reviewed the evidence concerning acetylcholine and histamine and suggested that histamine was the transmitter. Evidence in favour of acetylcholine was put forward by Wybauw (1936, 1938b). Some earlier observations of Kibjakow (1931), however, suggested that the transmitter was neither acetylcholine, because it was stable in blood, nor histamine, since it caused vasodilatation in the rabbit's ear in conditions where histamine produces constriction.

A third possibility which was first suggested by Dale (1935) is that the transmitter is identical with the central synaptic transmitter at sensory nerve endings, since it seems unreasonable to suppose that the same neurone should liberate two different transmitters. According to this hypothesis, sensory neurones, but not motor neurones, would be expected to contain the transmitter. Hellauer & Umrath (1948) produced some evidence in support of this; they found vasodilator activity in extracts of posterior but not of anterior spinal

roots. The activity of the extracts was reduced by incubation, but inactivation did not occur in the presence of strychnine. They suggest that this vasodilator substance is the central sensory transmitter; that strychnine inhibits its normal enzymic destruction; and that this is the explanation of strychnine convulsions. If this were so, we might expect strychnine to potentiate antidromic vasodilatation in analogy with the action of eserine on cholinergic nerve stimulation, provided that the enzyme is present at the peripheral ends of the neurone.

We have approached the problem of antidromic vasodilatation by measuring the effects of specific antagonists and synergists of acetylcholine and histamine, and the effect of strychnine upon it. In addition, the effects of injected acetylcholine and histamine on the vessels of the rabbit's ear were examined.

For the purpose of our experiments we needed a sensitive method by which antidromic vasodilatation could be obtained regularly and repeatedly and by which small changes in the degree of dilatation could be measured. Previous methods proved to be unsatisfactory. Thermal methods such as Wybauw (1938a) used have two disadvantages, namely the slow rate of response of the apparatus and the difficulty of maintaining an absolutely constant environmental temperature. We attempted to use the

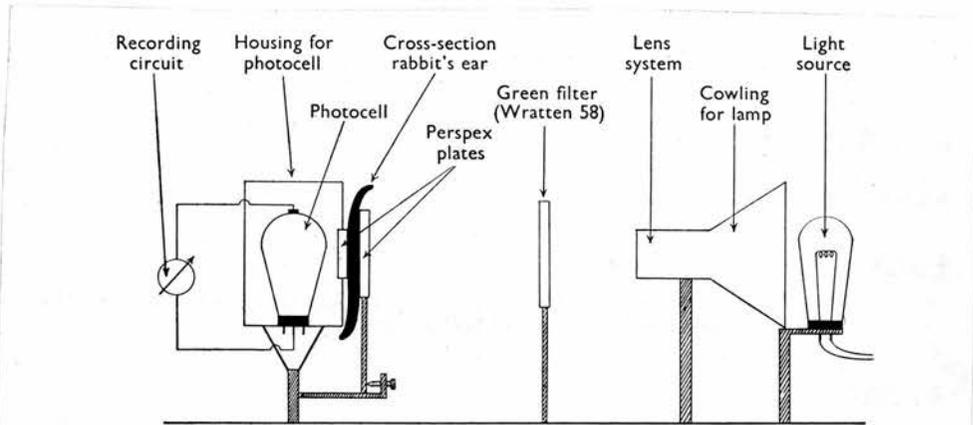


Fig. 77 Diagram of the recording apparatus.

Intra-arterial injections were made when necessary, through a needle cannula, into the facial artery. All other branches of the common carotid were tied, with the exception of the auricular artery. Intravenous injections were made into a cannula in the left external jugular vein.

In those experiments in which blood was collected from the stimulated ear, a two-way cannula, coated with silicone and with a side arm, was inserted between the right auricular vein and the right external jugular vein.

In most experiments the rabbit was given an intravenous injection of 5 ml. 1% heparin.

perfusion method of Gaddum & Kwiatkowski (1938) but were unable to obtain reproducible results; similar indecisive results were obtained using another perfusion technique suggested by Rischbieter (1913).

The classical method of observation of the changes in colour of the skin is the most satisfactory, but in its simple form the method is too subjective for quantitative work. We have modified this method by recording such changes with a photoelectric cell.

#### METHODS

The apparatus is illustrated in Fig. 77. A 100 W. projector bulb is used as the source of light, a parallel beam being obtained by the lens system; the beam is directed through a green filter (Wratten 58) and through the rabbit's ear on to the photoelectric cell (Cinema Television Ltd., type V.A. 26T). The green filter is interposed so that the light transmitted by it is strongly absorbed by both haemoglobin and oxyhaemoglobin. The Perspex plates hold the ear in position without constriction. The photoelectric cell itself is especially sensitive in the wave-band transmitted by the filter, thus ensuring maximal

contrast between vasoconstriction and vasodilatation.

The current passed by the photoelectric cell is directly proportional to the intensity of light entering it. The voltage across a resistance in series with the cell is picked up by a cathode follower and applied to a mirror galvanometer. Records are made from the galvanometer on a moving paper camera. The resting current through the galvanometer can be balanced out, so that the records obtained reflect only changes in the current passed, and the sensitivity of the recording apparatus is thus greatly increased.

The rabbits used were large albinos. The right external ear was sympathetically denervated by aseptic removal of the ipsilateral stellate and superior cervical ganglia at least 5 days before the experiment. The operation was carried out as described by Feldberg (1926), using phenobarbitone, and ethyl chloride followed by ether, as anaesthetics.

Records were always made from the right ear of the rabbit. For the experiment the animals were anaesthetized with urethane, given by slow intravenous injection of a 25% solution, until the flexor reflex disappeared. Further injections of urethane were given when necessary during the course of the experiment. Urethane causes a transient vasodilatation but does not otherwise influence the effects of antidromic stimulation.

The great auricular nerve was dissected and cut, and the

peripheral end inserted in a saline electrode (Porter & Allamon, 1936). The indifferent electrode (anode) was placed in either the mouth or the rectum.

Intra-arterial injections were made when necessary, through a needle cannula, into the facila artery. All other branches of the common carotid were tied, with the exception of the auricular artery. Intravenous injections were made into a cannula in the left external jugular vein.

In those experiments in which blood was collected from the stimulated ear, a two-way cannula, coated with silicone and with a side arm, was inserted between the right auricular vein and the right external jugular vein.

In most experiments the rabbit was given an intravenous injection of 5 ml. 1% heparin.

## RESULTS

### Antidromic stimulation

Our records show that, in the unstimulated preparation, a steady level of vasodilatation is maintained in the ear in the absence of sympathetic tone. There are constant slight irregularities of the baseline, which are probably due to

FIG. 78

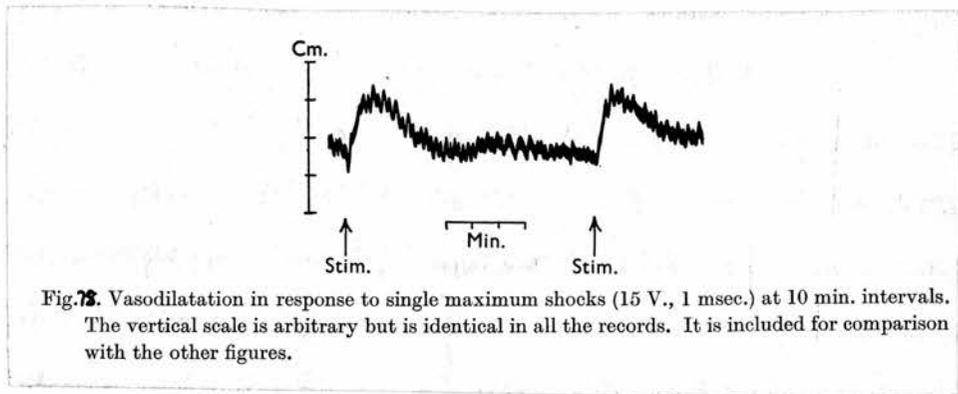


Fig. 78. Vasodilatation in response to single maximum shocks (15 V., 1 msec.) at 10 min. intervals. The vertical scale is arbitrary but is identical in all the records. It is included for comparison with the other figures.

spontaneous small fluctuations in the tone of the vessels.

A square wave stimulator was used to excite the peripheral end of the cut sensory nerve. The response became maximal for a given stimulus strength only when the duration was increased to 1.0 msec.. In most preparations the threshold for stimulus strength at this duration was about 5V, and a maximal response was usually obtained with 15-30 V. This seems to be a very high stimulus strength but, since in the saline electrode the cathode is not in direct contact with the nerve, it is impossible to say what fraction of this voltage was actually applied to the nerve.

Most preparations were sensitive to single shocks of 15-30 V. and 1.0 msec. duration, but in some rabbits single shocks produced a response which was not easily distinguishable from spontaneous baseline fluctuation, and short bursts of stimuli were used. Fig. 78 illustrates the response obtained to single shocks of 15 V. and 1.0 msec. duration. Upward deflexion of the record always represents vasodilatation.

After stimulation there is a latency of some 10 sec.; the response to a single shock takes about 1 min. to reach a maximum and is complete in 3-4 min. Repeated responses of similar magnitude and duration can readily be obtained, and Fig. 78 shows two such responses with an interval of 10 min. between them.

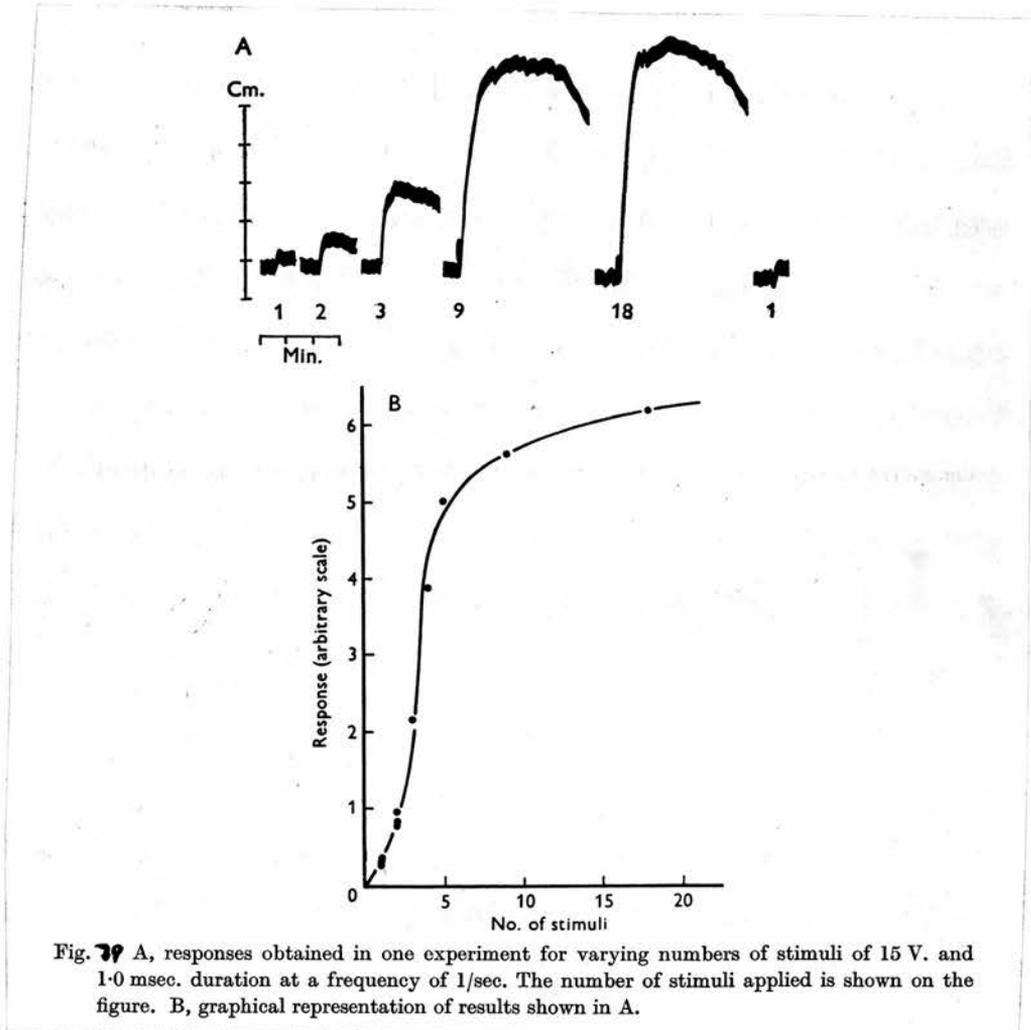


Fig. 79 A, responses obtained in one experiment for varying numbers of stimuli of 15 V. and 1.0 msec. duration at a frequency of 1/sec. The number of stimuli applied is shown on the figure. B, graphical representation of results shown in A.

Increasing the number of stimuli increases both the magnitude and the duration of the effect. After a burst of 25 stimuli at a frequency of 5/sec. the response was increased so that the record went off the photographic paper and the baseline was not regained until 30 min. or more later. A vasodilatation of this magnitude was visible on direct inspection. Fig. 79A shows the records obtained by increasing the number of stimuli given at a frequency of 1/sec.; and the results of this experiment are expressed graphically in Fig. 79 B

#### Acetylcholine

Intra-arterial injection of 0.1 or 0.2 ml. saline produces a transient, downward deflexion; this apparent constriction is due mainly to the dilution of the blood passing through the ear by a colourless fluid, but in some early experiments was also partly attributable to the occlusion of the common carotid artery during the injection; in the later experiments the artery was not occluded, in order to minimize the injection artefact. For this reason also, the volume of each injection was kept as small as possible - usually 0.1 ml.

Acetylcholine given by intra-arterial injection produces, after the constriction artefact, a transient vasodilatation.

FIG. 80

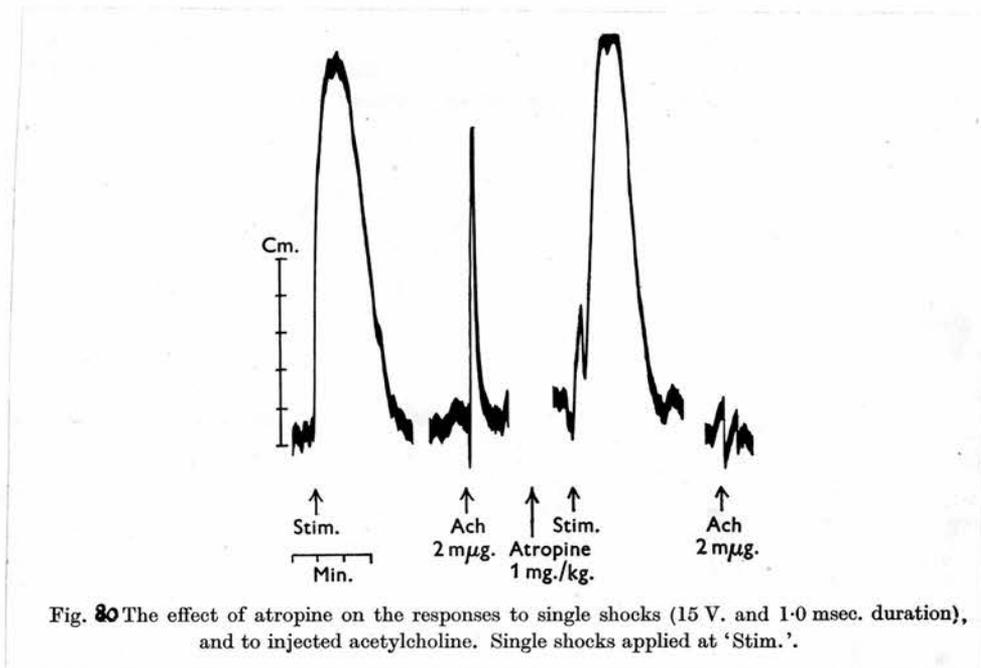


Fig. 80 The effect of atropine on the responses to single shocks (15 V. and 1.0 msec. duration), and to injected acetylcholine. Single shocks applied at 'Stim.'.

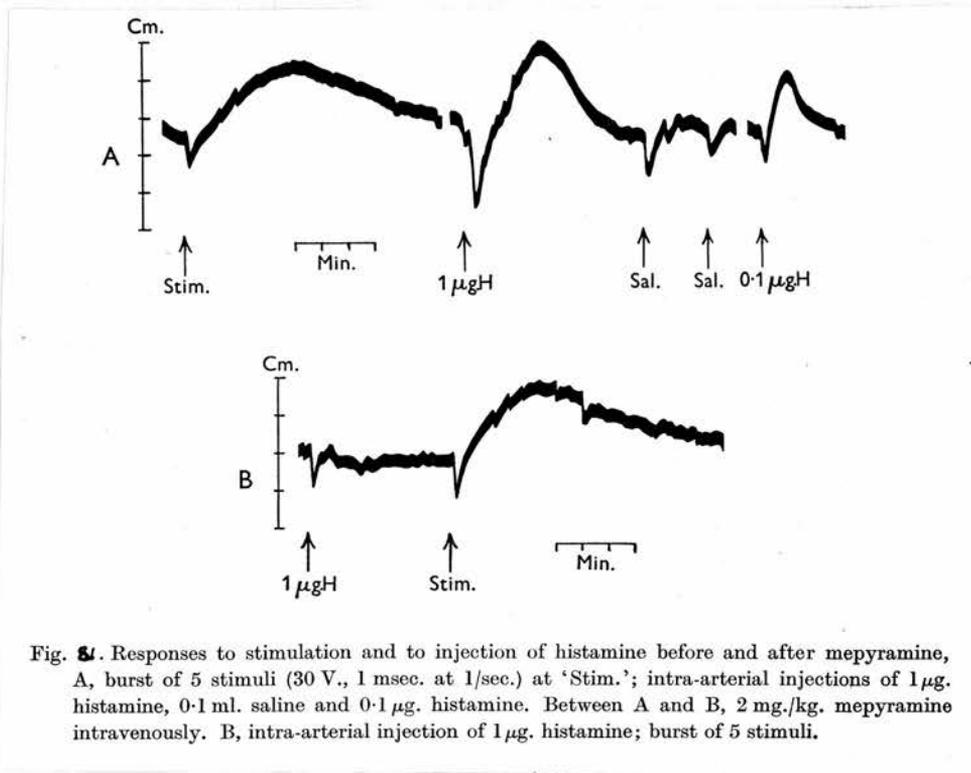
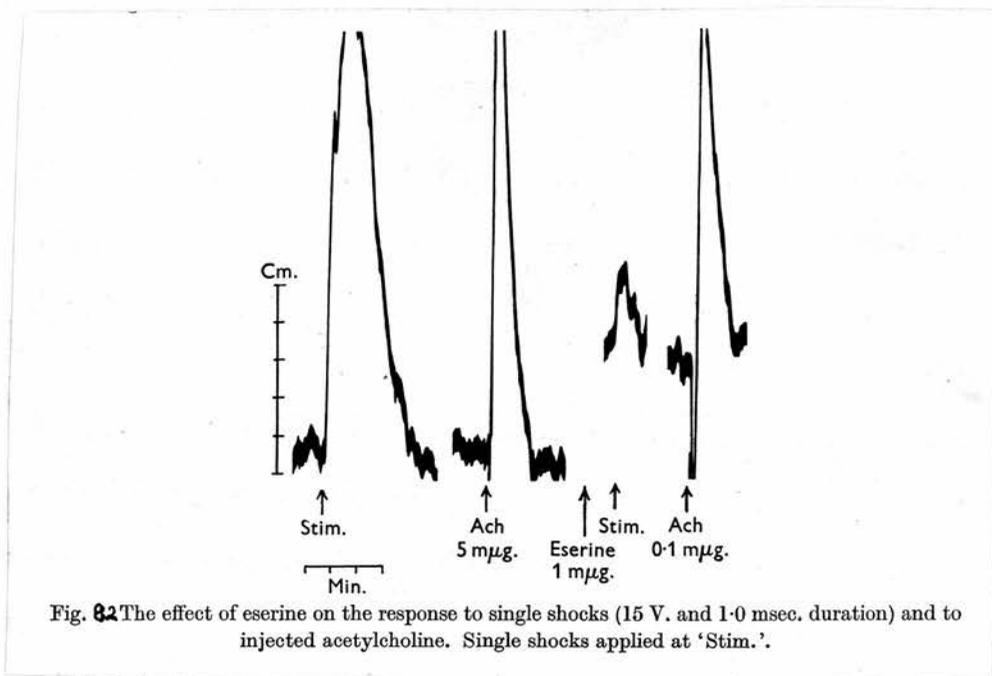


Fig. 81. Responses to stimulation and to injection of histamine before and after mepyramine, A, burst of 5 stimuli (30 V., 1 msec. at 1/sec.) at 'Stim.,'; intra-arterial injections of 1 μg. histamine, 0.1 ml. saline and 0.1 μg. histamine. Between A and B, 2 mg./kg. mepyramine intravenously. B, intra-arterial injection of 1 μg. histamine; burst of 5 stimuli.

The sensitivity of the preparations to acetylcholine varied considerably. In one experiment a measurable effect was obtained with 0.5 m $\mu$ g. acetylcholine, but usually the amount necessary to produce a satisfactory response was about 2 m $\mu$ g. while in a few animals as much as 5 or even 10 m $\mu$ g. was required. Fig. 80 shows one experiment in which the sensitivity was high, and in this case the response to a single shock was rather greater than the response to 2 m $\mu$ g. acetylcholine. This figure also illustrates the difference in the duration of the two effects. Although a single shock produces an effect lasting some 3-4 min., the effect of the injection of 2 m $\mu$ g. acetylcholine is over in less than 1 min.

### Histamine

Intra-arterial injection of 10  $\mu$ g. histamine produces a vasoconstriction. As the dose is decreased the constriction becomes less and is followed by a vasodilatation, and with even smaller doses (0.1  $\mu$ g.) the response becomes purely dilator. Fig. 81 shows records illustrating this. 1.0  $\mu$ g. histamine produces a biphasic response, while 0.1  $\mu$ g. produces a pure vasodilator response, the apparent constriction being no greater than the constriction artefact produced by the injection of saline alone.



Modification of the responses by  
drug action

The effects of atropine, eserine, mepyramine and strychnine on the responses to antidromic stimulation and to injected acetylcholine and histamine were investigated.

Atropine

Attempts to obtain temporary antagonism of acetylcholine by intra-arterial injection of small doses (1-100  $\mu$ g.) of atropine were unsuccessful; this may be because rabbit's blood contains an esterase which destroys atropine (Ellis, 1947). In order to abolish the effect of injected acetylcholine, large doses (1 mg./kg. or more) of atropine were given intravenously. In most experiments, this dose of atropine did not affect the response to nerve stimulation, as is shown in Fig. 20 ; but in one experiment the response was slightly diminished after atropine.

Eserine

After intravenous eserine (1 mg./kg.) the animal shows fibrillary twitching, and the response to injected acetylcholine is potentiated; Fig. 21 shows the response to 5 m  $\mu$ g. acetylcholine before eserine, and the response to 0.1 m  $\mu$ g.

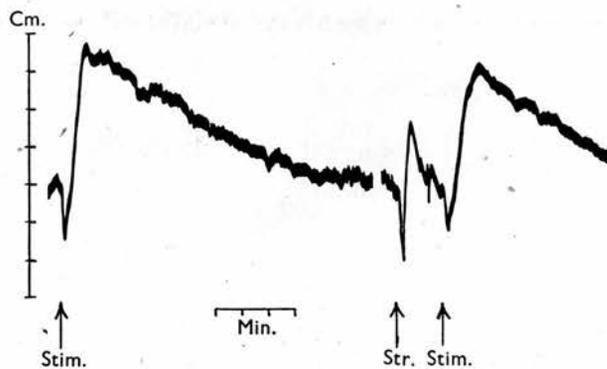


Fig. 83 Response to stimulation before and after intra-arterial injection of 0.4 mg. strychnine. The slight vasoconstriction after stimulation is probably due to incomplete removal of the sympathetic.

acetylcholine before eserine, and the response to 0.1 m $\mu$  g. acetylcholine after eserine, indicating that the sensitivity to acetylcholine is greatly enhanced. However, the response to stimulation is not potentiated after eserine, but is actually diminished (Fig. 82 ). This diminution is reversed by atropine.

### Mepyramine

After the intravenous injection of mepyramine (Necantergan) (2 mg./kg.) the response to injected histamine was abolished completely but the response to antidromic stimulation remained (Fig. 81 ).

### Strychnine

Strychnine, given by intra-arterial injection, produced a slight, transient vasodilatation but had no potentiating effect on the response to nerve stimulation (Fig. 83 ). The initial constrictor response seen on stimulation in this case is probably due to incomplete removal of the sympathetic fibres.

### Attempts to find the transmitter in venous blood from the ear

In a number of experiments we collected venous blood from the ear both during rest and during maximal antidromic

vasodilatation. Samples of blood were injected intra-arterially either into the same rabbit or into another rabbit. In some cases no effect was obtained with either sample; in others both samples contained small amounts of a vasodilator substance, probably histamine. In no experiment, however, were we able to find a greater vasodilator action with blood obtained during stimulation than with control blood. This was true even when the donor rabbit had received a large dose of eserine. In one experiment the ear of the recipient rabbit was sensorily denervated in an attempt to increase the sensitivity to the transmitter, but even with this refinement no transmitter was found in blood taken from the stimulated ear.

### DISCUSSION

The method which we have described of recording changes in the degree of vasodilatation in the rabbit's ear by using a photoelectric cell has several advantages. In the first place, measurements may be made without interfering with the normal circulation through the ear. In addition, the method is sensitive to small changes and there is no time-lag between the response in the ear and the response of the recording system.

The sensitivity of the method is such that reproducible effects could easily be obtained in response to single shocks applied to the peripheral end of the cut great auricular nerve of sympathetically denervated preparations. The time course of this response is highly characteristic. We always found a latency of about 10 sec. between the stimulus and the onset of the effect. This long latency is in agreement with previous observations on antidromic vasodilatation in the rabbit's ear and the cat's paw; as far as we know all other responses to nerve stimulation have much shorter latencies, even where chemical transmission is certainly involved. We have also confirmed previous observations, in that a volley of 25/sec. for 30 sec. produced a response which lasted for more than 30 min. Even more startling, however, is the fact that the response to a single shock lasts for 3-4 min.

These facts support the theory put forward by Lewis & Marvin (1926) that antidromic vasodilatation is brought about by the liberation of a chemical transmitter; but the long latency and duration of the effect make it unlikely that the transmitter is either acetylcholine or histamine, because both are freely diffusible and readily destroyed. We should expect their action to be exerted very soon after liberation, even though the site of action were some distance from the site of liberation, and to be over in a shorter time.

Further evidence against acetylcholine is provided by the fact that atropine does not abolish and eserine does not potentiate antidromic vasodilatation. The results with atropine are in accordance with those of Wybauw (1938b) who, nevertheless, maintained that the mechanism was cholinergic, for, as Dale (1929) pointed out, sensitivity to atropine is not a sine qua non; for example the parasympathetic vasodilatation in the salivary gland is relatively insensitive to atropine but is undoubtedly cholinergic. But even there the effect of nerve stimulation is potentiated by eserine, and Wybauw claimed that antidromic vasodilatation was potentiated by eserine. We have been unable to confirm this observation; in none of our experiments was there any sign of potentiation; in fact we found that the response to antidromic stimulation was reduced after eserine. In Wybauw's experiments, which were on the cat's paw, the skin temperature was taken as an indication of the state of the vessels. The intravenous injection of eserine itself caused a rise of temperature, probably through the dilatation of arterioles not under observation, and this itself may have modified the response to antidromic stimulation. In any case, Wybauw did not obtain eserine potentiation in every experiment.

The reduction of the response to antidromic stimulation by eserine was unexpected; it may be related to the finding

that high concentrations of anticholinesterases block nervous conduction in both cholinergic and non-cholinergic neurones (Bullock, Nachmansohn & Rothenberg, 1946). It is conceivable that the thinly myelinated terminal portions of the nerve fibres responsible for antidromic vasodilatation are blocked at the relatively low concentration of eserine that we were using. Recent evidence suggests that this blocking action is independent of inhibition of cholinesterase and is a direct action of the anticholinesterase drugs (Toman, Woodbury & Woodbury, 1947). If this were so, the action of atropine, which in our experiments restored antidromic vasodilatation, must be due to a direct antagonism of eserine, which is independent of its antagonism of cholinergic effects. That this action of eserine has so far been missed may be due to the fact that eserine is usually used in analysing cholinergic mechanisms where the augmentation resulting from the undestroyed acetylcholine might easily mask the reduction of nervous activity.

Evidence against acetylcholine was also put forward by Kibjakow (1931), who obtained an active substance which was stable in blood and so was unaffected by cholinesterase. In our experiments, in which we collected blood from the stimulated ear, vasodilator substances were not, however, present in greater quantities than in control blood, even when the donor rabbit was fully eserinizied. Although our findings are not

in agreement with those of Kibjakow, in our view they are a strong argument against acetylcholine being responsible for antidromic vasodilatation.

Our results also cannot be reconciled with the idea that histamine is the transmitter responsible for antidromic vasodilatation. In the past histamine has sometimes been rejected as a possible transmitter because, in the rabbit's ear which gives such a strong antidromic vasodilatation, histamine itself is usually vasoconstrictor. Our findings, however, like those of Feldberg (1927) and Lewis & Marvin (1926), show that histamine in small doses is vasodilator in the rabbit's ear with its natural circulation. Even in the perfused ear, Burn & Dutta (1948) have occasionally observed histamine vasodilatation. Nevertheless, mepyramine, in doses which abolished the histamine effects, both dilator and constrictor, left antidromic vasodilatation unaffected. It is known that mepyramine passes readily from the blood vessels into the skin, so that we must assume that the concentration was high enough to abolish the action of any histamine liberated outside the vessels. In addition, although mepyramine may not abolish all the effects of histamine, it does antagonize the effects of histamine on the blood vessels, both in perfused preparations and in the intact animal. Our results with mepyramine thus provide strong evidence that histamine is not the transmitter.

Our conclusion is in direct contradiction to that of Kwiatkowski (1943), who detected a histamine-like substance in the effluent from the perfused cat's paw in response to antidromic stimulation. We have been unable to obtain similar results on the rabbit's ear; and Kwiatkowski's observations therefore require confirmation before the histamine theory of antidromic vasodilatation in the cat's paw can be accepted. Two other facts make it unlikely that the nerves responsible for antidromic vasodilatation are histaminergic. According to Kwiatkowski himself (1943) histamine does not disappear from the sensory nerves of the rabbit's ear on degeneration; and histamine appears to inhibit transmission in sensory neurones (Chauchard, 1949).

Our results with strychnine do not support the conclusion of Hellauer & Umrath (1948). According to them the unidentified sensory transmitter is destroyed enzymically and this destruction is inhibited by strychnine. Our finding that strychnine does not potentiate or prolong the response to antidromic stimulation of the rabbit's ear makes it unlikely that the transmitter liberated at the peripheral end of sensory neurones is destroyed by a strychnine-sensitive enzyme. This, of course, does not exclude the possibility that the transmitter responsible for antidromic stimulation is identical with the central synaptic transmitter; it may be that the

enzyme responsible for its destruction is present in the spinal cord but not at the peripheral nerve endings.

Our attempts to identify the chemical transmitter responsible for antidromic vasodilatation have so far given negative results. We consider, however, that the evidence we have obtained excludes acetylcholine and histamine as possible transmitter substances, at least in the rabbit's ear. The fact that we have been unable to obtain the active principle in venous blood collected from the stimulated ear, together with the latency and long duration of the effect after stimulation, seems to us to indicate that it is not freely diffusible, or is not normally removed by the blood stream.

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