RESUME OF LITERATURE

PHARMACOLOGY OF AUTONOMIC GANGLIA :

"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". (Perry, 1957).

Eccles (1935 a) 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. There is summation both spatial and temporal, facilitation and occlusion; almost all the Sherringtonian principles of integrative action, we have. But since Eccles first demonstrated the phenomena, they had been neglected for relatively long period of time.

Instead we all have "shocked" the ganglion with maximal electrically-induced volleys in the presynaptic nerves at frequencies chosen only to suit ourselves. Most of the work of this nature had been done in the superior cervical ganglion of cat. Transmissions of the nerve impulses were based on the assumption that all the preganglionic fibres are cholinergic and all synapse in the ganglion.

Perry (1957) remarked that neither of the assumptions is necessarily correct. It is just possible that non-cholinergic presynaptic fibres exist - if they do, they are small minority of the total population of presynaptic fibres; there is a certain number of so-called through and through fibres most of which are postsynaptic fibres arising, from cells proximal to the ganglion being studied.

1. Phases of ganglionic transmission :

Application of a maximal electrical stimulus to the presynaptic nerve supplying the ganglion, induces 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 cell is variable and, even more important, befause 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. 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 synapse (Fig. 1). There is not the least similarity of the ganglion cell to the model, as the nerve terminals

LEGEND FOR FIG. 1

Diagrammatic representation of ganglionic synapse. The phases of transmission are represented spatially along the horizontal scale (The numerals represent the phases). (From Br. Med. Bull. 13, 220, 1957).

ramify over the entire surface of the cells and one presynaptic axon will terminate on numerous presynaptic axons. Nevertheless for our artificial maximal stimulus the model will serve a useful purpose. The sequence of events initiated by the maximal presynaptic volley can be classified into seven distinct phases. Perry (1957) represented these phases in what he termed as oversimplified manner. The seven phases may be described as follows :

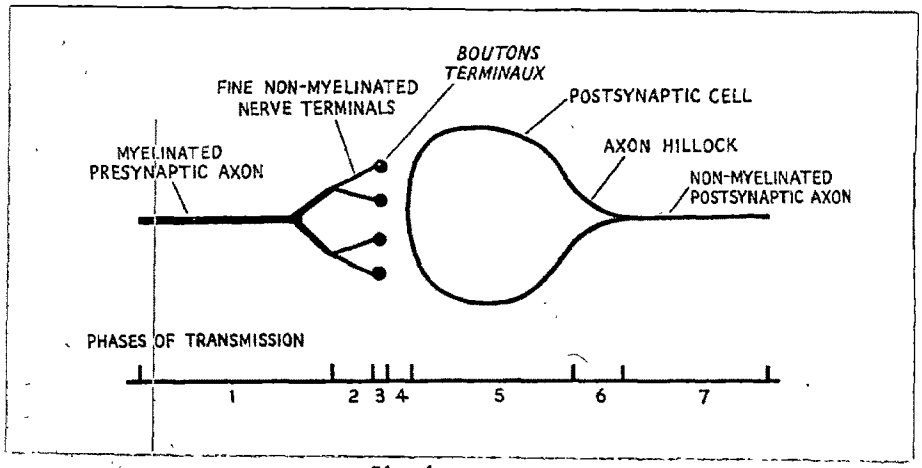

Fig. 1

Phase 1 : Saltatory conduction in the myelinated presynaptic fibre.

Phase 2 : Conduction in the non-myelinated presynaptic terminals.

Phase 3 : Release of acetylcholine at boutons terminaux.

Phase 4 : Acetylcholine crosses the synaptic gap : occupies receptors on the cell membrane; and eliminated.

Phase 5 : Local response of cell to acetylcholine.

Phase 6 : Initiation of propagated action potential.

Phase 7 : Conduction in the non-myelinated postsynaptic axon.

Factors affecting transmission :

Table 1 summarizes the ways in which the function of each phase can be tested experimentally. It is also possible 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.

Table 1

Experimental tests of the function of phases of transmission

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 eserinized 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.

Factors affecting phase 1: Local anaesthetics such as cocaine are powerful agents for artificially interrupting saltatory conduction; and in the analysis of a new drug to possess true ganglion-blocking

potency, it is necessary to exclude such activity before presuming it to possess true ganglion blocking potency.

Factor affecting phase 2 : As discussed phases 2 and 3 cannot be separated. Action of the toxin of Cl. botulinium deserves mention. Ambache (1949, 1951) showed that the toxin possessed a characteristic action in blocking transmission at cholinergic junctions i.e. at ganglion synapses and at motor and plates. Presynaptic nerve stimulation fails to excite the end organ but acetylcholine would excite the postsynaptic cell; he further showed that phase 1 was not affected by the toxin. Burgen et al., (1949) showed directly that the toxin prevented the release of acetylcholine. They concluded that the block is due to an irreversible fixation of toxin on the fine non-myelinated fibres. Brooks (1954) supported this contention of Castillo & Katz (1955) who showed that the action occurred at the sites of acetylcholine release, the tips of the nerve fibres i.e. by an action on phase 3.

Factors affecting phase 3 : The precise mechanism of the release of acetylcholine at the boutons terminaux remains obscure. Birks & MacIntosh (1957) discussed the problem of storage and release of acetylcholine. Perry (1953) showed that over a wide range of frequencies of stimulation, the output fell exponentially to a final constant rate per minute. This indicates that at a high frequency a single stimulus released less acetylcholine than did a single stimulus at a low frequency; and Perry argued that the explanation lay in the exhaustion of the stock of 'available' acetylcholine and that the synthesis failed to keep pace with

release. The presence of anticholinesterase accelerated this exhaustion of the stock, since it prevented the re-synthesis of choline to acetylcholine in the presynaptic terminals.

Other factors play a large part in determining the release of acetylcholine and it is necessary to examine some of them.

i. Inorganic cations. (See Table 2).

Table 2

Effect of cations on release of Acetylcholine

Cations		Acetylcholine release	Notes
Increased	Decreased		
K	-	Increased	Spontaneous release occurs.
-	K	Normal	-
Ca	-	Increased	No spontaneous release, but increase in release during stimulation.
-	Ca	Decreased	-
Mg	-	Decreased	-
-	Na	Normal	-
K	Ca	Normal	Increased K has no effect in the absence of Ca.
Mg Ca	-	Normal	-
-	K Ca	Normal	Decreased Ca has no effect in the absence of K

ii. pH & temperature : MacIntosh & Emmelin (1956) showed that variation in pH resulting from changing from phosphate-buffered to bicarbonate Lock'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 normal, as judged by the contraction of the nictitating membrane. Brown concluded that this was clear indication of the high "safety factor" in transmission to which he had previously called attention. Clearly minor modifications of the amount of acetylcholine release would not, in such circumstances, have any profound effect on the transmission. It is important to note that maximal presynaptic stimulation is not a normal physiological event. Kostial & Vouk (1956) stimulating in the same way but at a frequency of 2 per sec instead of the 10 per sec 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 & 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

stores are depleted.

Factors affecting phase 4 : Phase 4 is divided into three separate components which we are called 4a, the transient free existence of acetylcholine in the synaptic gaps; phase 4b - the stage also transient - during which acetylcholine is attached to the receptor sites; and phase 4c the phase of elimination of the acetylcholine.

i. Phase 4a. The phase, arising as a result of nerve stimulation is analogous to the injection of acetylcholine into artery supplying the ganglion. MacIntosh & Emmelin (1956) 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 & 1:250; but as MacIntosh & Emmelin pointed 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 than 1/10 of the volume. Perry (1957) argued that the discrepancy is more apparent than real. No such factor is known save cholinesterase which affects all the stages of phase 4.

ii. Phase 4b. Attachment of acetylcholine to "receptor sites" is influenced by a large number of drugs described as "competitive" ganglion-blocking agents (Paton & Perry, 1953) which compete with acetylcholine to occupy the receptor and are discussed later at greater length.

iii. Phase 4c. Very rapid elimination of acetylcholine after its

attachment to the receptors is a vital part of efficient ganglionic transmission and enzyme cholinesterase is reputed for these effects. The other view of MacIntosh & Emmelin (1956) was 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 acetylcholine. If acetylcholine were to persist for long in high concentration it would rapidly lead to failure of transmission. Paton & Perry (1953) showed that injected acetylcholine produced a long lasting depolarization of the cell membrane during which transmission i.e. phase 5 is blocked. Thus, the efficient elimination of acetylcholine is essential; in fact, elimination is extremely efficient whether the major role is played by cholinesterase or not.

Factors affecting phase 5 : Nature of local response: If in the local response all electrical changes which are not propagated are included, but which spread only decrementally, then there are several separate components involved. Fig. 2 shows a diagrammatic

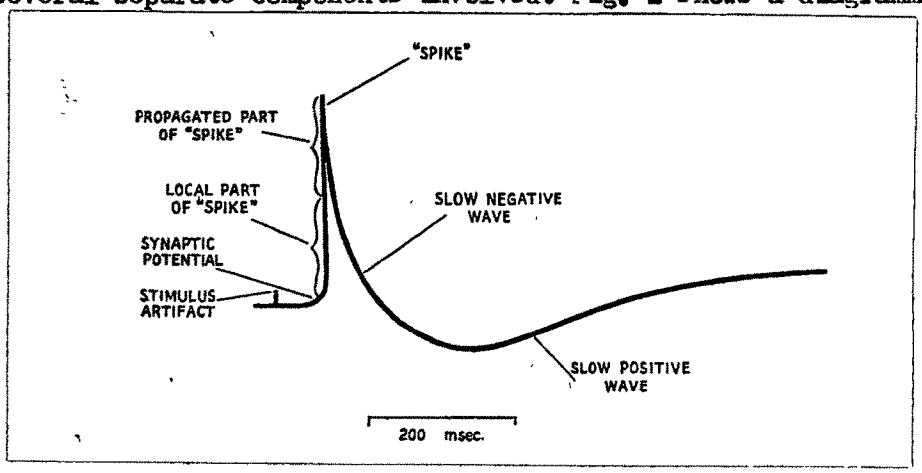

Fig. 2

Diagrammatic representation of ganglionic action potential. (From Br. Med. Bull. 13, 223, 1957).

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 axons (Eccles, 1935b;Paton & Perry, 1953).

i. "synaptic" potentials. Eccles (1937) described the detonator response of ganglion cells, but the evidence for such responses was more an inference than the clear-cut demonstration. Eccles (1952) further showed in the same ganglia from rabbits, but excised and maintained in vitro, 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 1956 she obtained similar records with dihydro-beta-erythroidine: but failed to detect any synaptic potential when using nicotine as a 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 the ganglion. Acetylcholine produced a long-lasting depolarization of the ganglion cells, a depolarization which often exceeded in magnitude the initial "spike" potential. Even if we accept the "spike" as a propagated action potential, it is not surprising that the depolarization should apparently exceed it in magnitude; the "spike" will be greatly reduced in size owing to both temporal and spatial dispersion, and to the shunting action of the tissues surrounding the cells; and more prolonged changes of potential

will not be so greatly attenuated. The depolarizing action of acetylcholine did not affect the presynaptic fibres, and the effect on the postsynaptic fibres was restricted to that which would be expected from the decremental spread of a change occuring locally in the cells. These results were confirmed by Pascoe (1956) who did not, obtain a depolarization by acetylcholine greater than some 50% of the "spike" potential. There seems then to be little doubt that acetylcholine does produce a long-lasting negativity of the ganglion cells. This change is 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. 3). Eccles (1935c) had previously shown that these slow waves were also local responses of the ganglion cell.

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 ganglia: so that block of the all-or-nothing response in a varying number of individual units also appears as a graded phenomenon.

i. Depolarizing blocking drugs. A group of substances, described

LEGEND FOR FIG. 3

Diagrammatic representation of the effect of acetylcholine on ganglionic action potential. (From Br. Med. Bull. 13, 224, 1957).

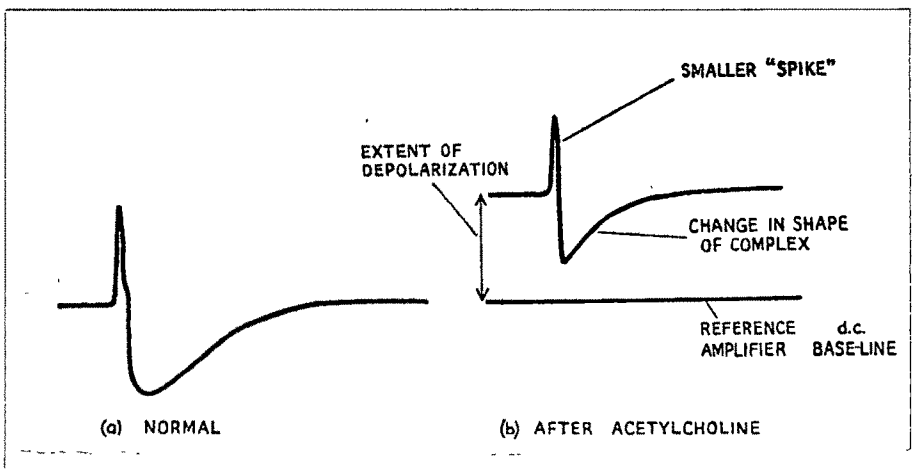
SMALLER "SPIKE"
EXTENT OF DEPOLARIZATION
CHANGE IN SHAPE OF COMPLEX
REFERENCE AMPLIFIER
d.c. BASE-LINE
(a) NORMAL
(b) AFTER ACETYLCHOLINE

Fig 3

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, indicating that the activation of the end organ is there, 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 agent affecting phase 4. The depolarizing action of nicotine and tetramethylammonium was confirmed by Pascoe (1956).

ii. Axotomy. Transmission fails in a ganglion after section of the postsynaptic axon (Brown & Pascoe, 1954). Acetylcholine release occurs normally and block is not due to accumulation of acetylcholine (McLennan, 1954). The failure is 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.

iii. Denervation. The extraordinary increase in the sensitivity of denervated tissues to the normal transmitter is well known and thus 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 (Perry & Reinert, 1954a, b) and these authors believe that denervation induces some fundamental change in the metabolism of the ganglion cells (Perry & Reinert, 1955), possibly by inhibition of aerobic glycolysis, and that a reduction of the extracellular potassium concentration may be the initial cause of this change.

Factors affecting phase 6. Factors potentiating submaximal stimulation can do so only by increasing the number of post-synaptic 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.

Trendelenburg (1961 a,b) did not feel any necessity to challenge the classical concept that the release of acetylcholine is the central event in ganglionic transmission, although there is a good possibility that detailed study with improved methods of the mechanism of synaptic transmission will lead to some modification of the classical theory. Till 1961 a, the following suggestions have been made :

a) an inhibitory system arising from the C.N.S. may act on the presynaptic nerve terminals.

b) presynaptic nerve terminal may be an important site for the action of drugs believed to have an action on the postsynaptic structures only. Similar ideas discussed in connection with motor nerve endings, are now recognized as an important site of drug action.

c) ganglion cell may be affected or even stimulated by a variety of substances which differ from acetylcholine and the well known group of nicotine-like substances and the non-nicotine-like substances.

d) peripheral ganglion may serve functions which extend considerably beyond those of a simple relay station; they may, for instance, be acted upon by sensory structures.

The isolated and perfused superior cervical ganglion of the cat, a classical preparation for the study of autonomic ganglia does have disadvantages and limitations. They have been discussed by Paton (1954), Ambache (1954) and Perry & Reinert (1954 a,b). Additional disadvantage of the perfused preparations is their low sensitivity to certain substances; the perfused ganglia are hundred times less sensitive to histamine and 5-HT than the non-perfused preparations (Trendelenburg, 1955).

The toughness of the tissue surrounding ganglion cell precludes the use of certain electrophysiological methods as routine procedures in the analysis of the action of drugs on ganglionic transmission. The preganglionic fibre of the superior cervical ganglion for instance, consists of four different groups, possessing different conduction velocities, S_1 to S_4 (Eccles,1935 a, b,c). The fastest, S_1 innervates structures in the orbit; S_2 fibres carry vasomotor impulses. These observations were confirmed by Folkow et al, (1958) who also identified the very slow conducting fibres as sympathetic vasodilators. Trendelenburg (1961 a) remarked that no systematic studies have been made of the stimulating or

depressing actions of drugs on these functionally different groups of fibres. In the evaluation of ganglion blocking substance it is customary to use either the nictitating membrane (i.e. the S_1 fibres) or the "postganglionic action potentials" (presumably the S_2 fibres, since these contribute most to the size of the action potential). It is unknown, however, whether the different groups of fibres are equally sensitive to ganglion blocking agents; occasionally reported "resistant" pathways may find their explanation in such quantitative differences of sensitivity.

Douglas & Ritchie (1956) reported the occurence of an accessory cervical ganglion in the rabbit, and Boyd (1957) discussed the occurence of intermediate sympathetic ganglia. which may escape sympathectomy. Chronic preganglionic denervation of the superior cervical ganglia increases its sensitivity to acetylcholine by a factor of three, and completely changes the characteristics of its responses to hexamethonium and azamethonium (Perry & Reinert 1954b). Perfusion of the normal ganglia with low potassium perfusate results in similar changes, whereas perfusion of the denervated ganglia with high potassium perfusate or with glutamate restores it to the normal. The analysis of the effects of various amino acid and determination of the intracellular potassium concentrations showed that this "effect of denervation" is attributable to changes in the oxidative metabolism of denervated ganglion cells rather then to their decreased intracellular concentration of potassium ions (Gertner & Reinert 1957). Homologous reinnervation (i.e. reinnervation of the superior cervical ganglia by cholinergic fibres of the

glossopharyngeal, the phrenic, or the vagus nerves) is easily established. Heterogenous reinnervation also seems possible contrary to earlier postulates. DeCastro (1951) reported that ascending fibre of the vagus are able to make the functional contact with ganglion as indicated by a response of the nictitating membrane to stimulation of the afferent fibres of the vagus. This interesting synapse has not been studied pharmacologically. Sensory vagal fibre can be assumed to liberate the sensory transmitter substance. Holton (1959) confirmed earlier observation that antidromic stimulation of sensory nerves of the rabbit ear caused the appearance of ATP in the perfusate. Murray & Thompson (1957) studied in great detail the occurence and importance of collateral sprouting after partial denervation of superior cervical ganglia.

Zakusov & Ul'ianova (1958) observed that distension of the bladder or of part of the intestine causes a relaxation of another part of the intestine. The viscero-visceral reflex persists after total destruction of the central nervous system and after chronic preganglionic denervation; it is abolished by ganglionectomy or by ganglion blocking substances.

Riker & Szreniawski (1959) found that acetylcholine and tetramethylammonium, when injected into the blood supply of the superior cervical ganglia elicit an antidromic preganglionic activity in addition to the well known postganglionic effect. Intermittent spontaneous antidromic activity in preganglionic fibre has also been demonstrated in superior cervical ganglia of the rat after infection with pseudorabies virus, the activity

being increased by acetylcholine and physostigmine and inhibited by d-tubocurarine (Dempsher & Ricker, 1957). This activity is increased by topical application of cocaine or by section of the preganglionic fibre and reduced by GABA, epinephrine and norepinephrine (Dempsher & Zabara, 1960). From such observation it has been postulated that acetylcholine has a presynaptic site of action and that an inhibitory system originates in the central nervous system and acts on the preganglionic nerve terminal.

Factor I of Florey & McLennan (1958) does not affect synaptic transmission through the superior cervical ganglia of the cat, but other ganglia of the cat and of the rabbit are blocked (Honour & McLennan, 1960). GABA, gamma-amino-butyrylcholine, beta-guanidinopropionic acid and gamma-guanidinobutyric acid are ineffective.

MICROSCOPIC ANATOMY :

In the superior cervical ganglion of the cat, rabbit and rat, the overwhelming majority of synapses result from the extensive contacts made between the very fine (0.1 to 0.3 μ in diameter) terminals of the preganglionic nerve and the dendritic processes of the ganglion cell body (Causey & Barton, 1958; Elfvin, 1963; Taxi, 1961). The preganglionic fibres course with the dendrites, often winding about the dendritic process and forming synapses at several sites. At these points of synaptic contact, the nerve terminals appear to be dilated. In the superior cervical ganglia of these species, few synaptic contacts have been found between the nerve terminals and the soma of the cells.

By contrast, the dominant synaptic relationship in the superior cervical ganglia of the frog is made up of contacts between the nerve endings and the somatic portions of the cell (DeRobertis & Bennett, 1955; Hunt & Nelson, 1965; Pick, 1963). In the frog, each ganglion cell receives its innervation from a single preganglionic fibre that makes multiple contacts on the cell body. As a result of paucity of dendritic processes, the ganglion cells appear to be unipolar. It is also of some interest that many synaptic contacts are found on the axon hillock.

The synaptic cleft in the ganglion of the mammal and frog is approximately 100 Å wide. In many of the synaptic regions, the presence of electronopaque material has been detected in the cleft. In this connection, it should be noted also that the cytoplasm of the satellite cells (Schwann cells) invests the presynaptic nerve endings and the perikaryon of the ganglion cells. It is somewhat surprising that the dense synaptic material and the location of the Schwann cells apparently do not impede the access of the drugs to the synapses. That this is so, is indicated by the immediate response of the ganglia to applied agents. On the other hand, the differential sensitivity to drugs of the several cell groups in the superior cervical ganglia of the cat (Mainland & Shaw, 1952) and the rat (Hertzler, 1961) may possibly be due to variations in the pattern of the investments by the satellite cells of the ganglion cells.

The occurence of a number of vesicular organelles in the preganglionic nerve endings represents one of the most consistent ultrastructural features of the sympathetic ganglion.

One type of vesicle has diameters ranging from 200 to 400 Å, has centres of modest electronopacity, and is found mainly in the region of the synapse (DeRobertis & Bennett, 1955; Elfvin, 1963). These have been termed the "synaptic vesicles" and are believed to be the containers of the transmitter substance, acetylcholine. The second commonly found vesicular element of the nerve terminal is much larger (500 to 1000 Å) and possesses a centre of high electron opacity. For this reason the larger vesicles are termed "densecore vesicles" because of their similarity to the "densecore vesicle" of the adrenal medulla (Sjostrand & Wetzstein, 1956) and postganglionic sympathetic nerves (DeRobertis & Delraldi, 1961; Richardson, 1962). It has been suggested that the dense core vesicles contain catecholamine (Pick, 1963). In view of the considerable circumstantial evidence for a functional role for catecholamines in the ganglionic transmission, it is unfortunate that it has not been possible to establish the identity of the material in the dense-core vesicles of the preganglionic nerve terminals.

The dendritic processes of the ganglion cell of the superior cervical ganglia of the cat undergo extensive branching and are elaborate in their structural features as those of the preganglionic nerve endings. In addition to the large numbers of synaptic contacts made with the preganglionic terminal arborization, the dendrites form many close appositions with each other (dendrodendritic junctions) and with the perikarya of the ganglion cells (dendro-somatic junctions). Elfvin (1963) remarked that the well-defined structural organisation of some

of these contacts indicates that they may be more than a fortituous arrangement resulting from the close packing of a large number of the cells in a small volume. Their existence suggests an anatomical basis for functional interactions among ganglion cells.

In addition to the variety of junctional arrangements occurring in the superior cervical ganglion that might contribute to the differential responsiveness of the ganglion cells to a broad spectrum of cholinomimetic and sympathomimetic agents, the ganglion cells themselves exhibit marked variation in form and size . Attempts have been made to classify ganglion cells on the basis of features such as size, morphological appearance and of the dendrites, or the arrangement of the Nissl substance. The cells of the superior cervical ganglion range in size from 20 to 60 μ. Approximately one half of the cells are said to be in the lower middle of the range (DeCastro, 1932) with the remainder devided almost equally between the larger and smaller cells. Hillarp (1960) has commented critically on the possibility that the three cell groups observed with the electrophysiologic techniques (Bishop & Heinbecker, 1932; Eccles, 1935 c) might correspond to this rather arbitrary anatomical classification. It has been noted above that the pattern of the dendrite processes of the ganglion cells in the mammalian ganglia is extremely complex. For most ganglion cells, the dominant dendritic form consists of long,extensively branching processes. Only a very small number of ganglion cells with short dendritic processes have been noted. There is no evidence to relate either cell size or the configuration

assumed by the dendritic processes to the function or the pharmacology of the cell.

In contrast to the synaptic contact of the ganglion cells considered so far, the synapses of the ciliary ganglion of the chick have the unique ultrastructural feature of a single, cup-like nerve ending enveloping a considerable portion of the ganglion cell (DeLorenzo, 1960). On the basis of this morphological characteristic DeLorenzo (1960) predicted that transmission would occur by means of electrical coupling . Martin & Pilar (1963, 1964) subsequently presented electrophysiological evidence of simultaneous "electrical"; "chemical" transmission. The presence in the nerve endings of "synaptic vesicles" provides a morphological correlate for the chemical form of transmission. Since classical ganglion blocking drugs interfere with the chemical component in the transmission processes, the transmitted substance is believed to be acetylcholine.

HISTOCHEMISTRY :

Acetylcholinesterase : Because of the cholinergic nature of transmission in autonomic ganglia, the enzyme acetylcholinesterase has been the centre of interest for many years. Using a histochemical technique for acetylcholinesterase, Koelle (1950, 1951, 1955) was the first to demonstrate that the enzyme is irregularly distributed in the ganglion cells of the sympathetic ganglion. While most of the cells exhibit little enzymatic activity, a small population of cells has a histochemically demonstrable heavy enzyme content.

Among several thousand cells in the stellate and superior cervical ganglion of the cat, 80 to 85% of the cells possessed little or no enzymatic activity and 0.5 to 7% of the cells possessed marked activity, the remainder showed an intermediate activity (Holmstedt & Sjoqvist, 1959). Sjoqvist (1963) concluded from his statistical study of the correspondance between histochemical staining of the enzyme in ganglion cells and the outflow from the sympathetic ganglion neurons subserving cholinergic functions (e.g. sweat secretion) that cells with moderate to high levels of enzymatic activity were from cholinergic neurons while those with weak activity were from adrenergic neurons e.g. the 7th lumber ganglia contain the highest percentage of heavily stained ganglion cells (10.8% of 28.9×10^{-3} cell counted) and the highest number of cells sending fibres to the eccrine sweat glands.

Conversely, the coeliac and inferier mesentric ganglia are essentially devoid of any cells with acetylcholinesterase activity and with cholinergic functions. There is little reason to doubt the cholinergic nature of the heavily stained ganglion cells. Consistent with this conclusion is the observation that almost all the cells of the parasympathetic ganglia (e.g. ciliary) are derived from cholinergic neurons and demonstrate high cholinesterase activity (Kewitz & Reinert, 1952). On the other hand, the meaning of the presence of small amount of the enzyme in the adrenergic cell is open to conjecture. It has been suggested (Eccles, 1964) that the presence in adrenergic neurons of small amount of acetylcholinesterase is compatible with the proposition that acetylcholine acts as an intermediate

in the release of noradrenaline from adrenergic nerve endings. This suggestion is based on the assumption that the presence of acetylcholinesterase is a reflection of the presence of acetylcholine. In any event, the unequal distribution of the enzyme in cells of the sympathetic ganglion reflects biochemical heterogeneity in autonomic ganglia.

One important difference between the cells of sympathetic ganglia showing high levels of enzyme and those of the parasympathetic ganglia requires comment. In the former, the enzyme is located inside a barrier to water soluble drugs; in the latter, the enzyme is found on both sides of the barrier (Koelle & Koelle, 1959). These authors further pointed out that the internal localization of the esterase makes it unlikely that the enzyme participates in the transmission process and consequently, unlikely that the inactivation of the intracellularly localized enzyme by drugs would have much effect on transmission in sympathetic ganglia.

METABOLISM OF ACETYLCHOLINE :

The studies of the formation, storage and release of acetylcholine in the cholinergic neurones are not as precise as the more elegant method used to study the metabolism of catecholamine in adrenergic neurones. Almost all the studies of the metabolism of acetylcholine in autonomic ganglia have been performed on the perfused superior cervical ganglia of cat.

Synthesis and storage :

The most remarkable feature of the sympathetic ganglion is the ability of the synthesizing mechanisms in the nerve terminals to maintain the concentration of acetylcholine in the ganglia at or near normal levels during intensive and prolonged activity (Birks & MacIntosh, 1961; Matthews, 1963). As long as, the perfusion fluid contains the ingredients (primary choline) required to support the synthetic processes, the total amount of acetylcholine present in the ganglion is unchanged by prolonged intensive stimulation of the preganglionic nerve. The striking synthetic capabilities of these cholinergic nerve terminals is illustrated by the facts that an amount of acetylcholine equivalent to the total resting content (300 µg per ganglion) can be recovered from the perfusion fluid during 10 min of preganglionic stimulation at the rate of 20 c.p.s.

By the use of the drugs that interfere with the synthesis of acetylcholine and of drugs that prevent the enzymatic hydrolysis of the ester, it has been possible to develop some concept about the intra-ganglionic distribution of acetylcholine and the availability of the transmitter for synaptic activity. Ganglia perfused with media lacking choline or containing the hemicholinium compound (HC-3) that prevents the synthesis of acetylcholine are unable to maintain normal concentrations of acetylcholine during intensive, repetitive stimulation. Under these conditions, the ganglia lose 85% of the acetylcholine content approximately. Birks & MacIntosh (1961) concluded that

the storage forms of acetylcholine can be classified as "depot" and "stationary". The "depot" form represents the component available for release by the incoming nerve impulse. The "stationary" form is the component remaining after treatment of the ganglia with HC-3. They suggested further that the "stationary" pool of acetylcholine is probably confined to the preganglionic axons (as distinct from nerve terminals) that penetrates into the ganglion proper.

A further classification of the "depot" pool of acetylcholine into "readily" and "less readily" releasable components is based on changes in ganglionic content of acetylcholine produced by anticholinesterase agents. When the resting ganglia are exposed to large doses of anticholinesterase agents and their content of acetylcholine determined, the concentration of the transmitter in the ganglia rises to value considerably above those of the concentrations of ganglia. One of the most interesting features of acetylcholine metabolism revealed by this experiment is the fact that no acetylcholine appeared in the perfusion fluid at the time when the ganglionic concentrations of transmitter was markedly elevated. This observation and the observations that the amount of acetylcholine released by nerve stimulation is the same in the ganglia treated with anticholinesterase agents as that of the normal ganglia led Birks & MacIntosh (1961) to postulate that newly formed acetylcholine is not available for participation in the transmission process. The fact that the content of acetylcholine falls to the normal

values, when the anticholinesterase agents are removed indicates that the newly formed acetylcholine is subjected to hydrolysis. Thus the curious situation exists in which acetylcholine is formed rapidly and in large amounts is retained by the ganglia, is not subjected to release by the nerve action potential, but is hydrolysed by cholinesterase enzyme. This collection of circumstances makes it tempting to suggest that the newly formed acetylcholine exists primarily in the cytoplasm of the nerve endings and as a corollary, that only the acetylcholine confined to the synaptic vesicles participates as a transmitter substance.

The excitatory transmitters other than acetylcholine also present in sympathetic ganglia as variety of pharmacologically active choline esters can be isolated from neural tissues. In one attempt, no evidence was obtained with physical and chemical techniques of the presence of any ester of choline other than acetylcholine (Friesen et al, 1965). In a second study using similar techniques, it was reported that most of acetylcholine-like activity extractable from the ganglia was in the form of a co-enzyme A ester of betaine (Hosein & Proulx, 1965).

Release of acetylcholine :

1. Neurogenic transmitter release: The process liberating acetylcholine from the nerve terminals by the action potential is one of the most fascinating and least understood of all the steps in synaptic transmission. Drug-induced alteration of conduction in the nerve terminals would result in some alteration

of transmitter output. It is presumably by an impairment of conduction in the fine terminals that the local anaesthetics reduce the output of acetylcholine in response to preganglionic stimulation (Harvey, 1939). Certain quaternary ammonium ions (i.e. tetraethyl-ammonium) enhanced the amount of acetylcholine released from sympathetic ganglia by nerve stimulation (Douglas, 1961) and at the other cholinergic junctions (Collier & Exley, 1963; Koketsu, 1958), most likely by prolonging the duration of the nerve action potential. Modification of the ionic composition of the fluid bathing the nerve terminals should markedly alter the output of acetylcholine from the terminal either by producing changes in nerve conduction or by direct action on the process of transmitter release. Lipicky et al,(1963), using isolated rabbit ganglia depolarised by potassium chloride found that a slightly less than a one-to-one molar exchange occurred between the influx of radio-active calcium and the release of acetylcholine. It has been suggested that the role of calcium, and the other divalent ions in the release mechanism does not involve neuronal mechanism at the membrane but most likely at the storage site of the transmitter. Katz and Miledi (1965), supported this view as the effects of calcium ions on the relase of acetylcholine from the motor nerve terminals.

2.Drug evoked release of acetylcholine : Carbaminoylcholine (carbachol) causes the release of acetylcholine from the nerve endings into the perfusion fluid of superior cervical ganglia of cats(McKinistry et al, 1963). Koelle (1962, 1965) studied the release of acetylcholine by preganglionic nerve stimulation and

that evoked by carbachol and proposed the following points of differences:

(1) Doses of the hexamethonium having no effect on the liberation of acetylcholine by nerve stimulation reduced markedly the ability of carbachol to cause the release of acetylcholine. It is interesting to note that high doses of mecamylamine (which like hexamethonium blocks transmission at a postganglionic site) are without effect on either form of stimulation.

(2) The addition of strychnine to the perfusion stream abolishes the responses to nerve stimulation, but has no effect on the responses to injected carbachol.

(3) Increasing the concentration of calcium in the perfusion stream enhances the output of the transmitter induced by nerve stimulation, but abolishes that induced by carbachol.

(4) Removal of the calcium from the perfusion medium blocks the response to nerve stimulation, but has no appreciable effect on the responsiveness of the nerve terminals to carbachol. These results suggest that while the action potential effects the release of acetylcholine indirectly by mechanisms considered above, carbachol may actually penetrate the nerve terminals and exchange with the endogenous acetylcholine as first proposed by Renshaw et al, (1938).

Thus carbachol is the only cholinomimetic compound known to consistently evoke the release of large amount of

acetylcholine from the nerve endings. The "dimethyl" and "monomethyl" analogues of carbachol have been tested (McKinistry, 1965). When compared with carbachol both the compounds are more potent ganglionic stimulants, but possess only minimal activity for evoking the release of acetylcholine.

Koelle (1962, 1965) has proposed on several occasions that the ability of carbachol to cause the release of acetylcholine is a pharmacological manifestation of a physiologically occuring positive feed-back mechanism in the terminal that allows for reinforcement of the transmission process by the release of acetylcholine.

3. Miniature synaptic potentials: It has been possible with the intracellular recording technique, to demonstrate spontaneously occurring miniature potentials in frog sympathetic ganglia (Blackman et al, 1963 a; Hunt & Nelson, 1965; Nishi & Koketsu, 1960). Since these potentials were reduced by the application of d-tubocurarine and are analogous to those seen at the neuromuscular junction (Fatt & Katz, 1952) they are presumably due to acetylcholine liberated from nerve endings. In support of this contention is the observation that 24 to 36 hr following transection of the preganglionic trunk, there is initially an increase and then a complete disappearance of these potentials. Unlike the miniature potentials of the denervated frog neuromuscular junction, the potential of the denervated ganglia do not return unless reinnervation has been established.

POSTSYNAPTIC CHOLINOCEPTIVE SITES :

A. Trans-synaptic activation : The magnitude of the resting membrane potentials or latency of depolarisation and the generation of impulses in sympathetic ganglia conform to the pattern of events observed at other excitatory sites. It has been demonstrated by the microelectrode recording technique that preganglionic stimulation produces the classical pattern of a slowly occurring, decrementing, excitatory synaptic potential giving rise to an action potential in isolated sympathetic ganglia of the rabbit and frog (Blackman et al, 1963b; McKinistry, 1965; Riker, 1965). When ganglia are exposed to an appropriate concentration of a classical ganglionic blocking compound i.e. hexamethonium, there is no change in the resting membrane potential or in the latency of depolarisation produced by preganglionic stimulation; however, preganglionic stimulation produces only the excitatory synaptic potentials (Eccles, 1963). These experiments provide good electrophysiological corroboration of the notion that hexamethonium - sensitive cholinoceptive sites are the primary postsynaptic component in the sequence of events leading to impulse transmission in the sympathetic ganglion. Koppanyi (1932) showed that pilocarpine causes the stimulation of superior cervical ganglion of the cat that can be prevented by atropine. Trans-synaptic activation of more than one type of ganglionic cholinoceptive sites has been demonstrated (Eccles & Libet, 1961). These authors showed that several components of the complex surface potential evoked by preganglionic stimulation of partially curarized

superior cervical ganglion of the rabbit are sensitive to blockade by various antagonists of the actions of acetylcholine.

In rabbits, preganglionic stimulation of partially curarized superior cervical ganglia evoked a long-lasting waveform that can be recorded from the surface of the ganglia and is characterised by a triphasic sequence of negative (N), positive (P), and the late occurring negative (LN) potentials (Eccles, 1952). Increasing the concentration of d-tubocurarine or dihydro-beta-erythroidine hydrobromide in the bath decreases the amplitude of the initial negative potential and increases the amplitude of the late negative potential. Small amounts of atropine selectively abolish the positive and late negative potential. Since all the potentials disappear when the ganglia are exposed to botulinum toxin, a substance known to impair the release of acetylcholine from the nerve endings, it is quite likely that acetylcholine is the mediator involved in the generation of each phase of the complex potential. The positive wave was attributed to catecholamines released by acetylcholine. Following model of ganglionic transmission was suggested by Eccles & Libet (1961). Acetylcholine subsequent to its release from the nerve ending, is able to cause ganglionic depolarisation by acting upon curare-sensitive sites (N potential) or atropine-sensitive sites (LN potential). In addition, hyperpolarisation (P potential) of the ganglia results from the activation by acetylcholine of atropine sensitive sites on chromaffin cells causing the release of the catecholamine, that in turn, acts upon the ganglion cell.

The second example of trans-synaptic activation of atropine-sensitive ganglionic cholinoceptive sites is the demonstration in the cat that treatment of the superior cervical ganglia with anticholinestrase agents evokes postganglionic firing that is unaffected by doses of hexamethonium sufficient to block transmission produced by nerve stimulation.

Thirdly, repetitive stimulation of the preganglionic nerve activates atropine-sensitive cholinoceptive sites (Libet, 1964; Takeshige & Volle, 1964). The persistent depolarisation of the ganglia that occurs following intense preganglionic activity (Takeshige & Volle, 1964) can be prevented by small doses of atropine.

B. Drug Evoked Activation: In addition to the several anticholinestrase agents (Takeshige & Volle, 1964; Volle, 1962 and Volle & Koelle, 1961), ganglionic stimulation that can be blocked by prior administration of atropine has been demonstrated in the superior cervical ganglia of cat for pilocarpine (Ambache, 1949; Koppanyi, 1932; Marrazi, 1939; Root, 1951), muscarine (Ambache, 1955; Gyermek, 1963; Jones, 1963; Konzett & Waser, 1956), acetyl-beta-methylcholine (Pappano & Volle, 1962; Takeshige et al, 1963), 4-(m-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium (Jones, 1963; Roszkowski, 1961), N-benzyl-3-pyrrolidyl acetate methobromide (Franko et al, 1963; Jones, 1963), and oxotremorine, (DeGroat & Volle, 1963). The ganglionic firing produced by each of these compounds is either unaffected or enhanced by traditional ganglion blocking compounds. In addition to the fact that the ganglionic responses

to these stimulating agents are blocked by doses of atropine having little effect on impulse transmission, the fact that the blockade of the responses to the drug by atropine persists for several hours, suggests that ganglionic atropine-sensitive sites have characteristics common to those of the peripheral parasympathetic neuro-effector junctions. Acetylcholine is the only substance with demonstrated ability to activate both types of ganglionic excitatory receptors(Gebber & Volle, 1965; Takeshige & Volle, 1962). In untreated ganglia, the postganglionic response to acetylcholine is characterized by a single burst of firing that is immediate in onset and sensitive to blockade by hexamethonium. However, after treatment of the ganglia with small doses of anticholinestrase drugs, repetitive preganglionic stimulation, or repeated injections of potassium ions, the response to acetylcholine is composed of two periods of firing : an early response sensitive to blockade by hexamethonium and a late response, prevented by small doses of atropine (Takeshige & Volle, 1962). In ganglia conditioned by the above procedures, the threshold dose of acetylcholine required for activation of the atropine sensitive sites (late discharge) is lower than that required for activation of the hexamethonium sensitive early firing.

The potentials recorded from the surface of the superior cervical ganglion of the cat following the administration of acetylcholine are strikingly similar to those evoked by nerve stimulation in the curarized rabbit ganglia.

DRUG ANTAGONISM :

Clark & Raventos (1937) described three types of drug antagonism i.e. physiological, chemical and specific.

The physiological or independent antagonism results when two drugs have action on the two different responsive systems, but of such a nature that the manifested effects are antagonistic. For example, carbachol causes a contraction of rat uterus; this effect is antagonised by adrenaline.

The chemical antagonism does not involve the receptor mechanisms and results when an inactive or substantially less active compound is formed due to chemical interaction between two drugs. The toxification of sulphonamides by acetylation in the liver is an example.

The specific antagonism is a more important variety for the pharmacologists because of its greater prevalence and usefulness.

The specific antagonism might be competitive or non-competitive in nature. If one states in general terms that the agonist on combination with a receptor activates events or reactions of undetermined length and nature to lead to a manifest response, then it becomes evident that the presence of another drug can reduce the response to the agonist in question. Such an "antagonist" may interfere with the primary combination of the agonist with the specific receptor and exhibit a high specificity of action. This constitutes the

"competitive" type of antagonism. If however, the antagonist acts at some point between the receptor - agonist interaction and its ultimate manifest response, and thus reduce the latter, it exemplifies the"non-competitive" type of antagonism, because the competition of two drugs to occupy the same receptors does not occur.

There is good evidence that the reaction between the drug molecules and receptors is a bimolecular reaction (Ariens & van Rossum, 1957).:

$$(R) + (A) \underset{K_2}{\overset{K_1}{\rightleftharpoons}} (RA)$$

Where R is the concentration of free receptors, RA the concentration of occupied receptors and A the concentration of drug A. There is no estimate of the total amount of receptors in a tissue so that we can calculate the fraction of receptors RA / r occupied by the drug;

$$(RA) / r = 1 / (1 + KA / A)$$

Where r is the total number of receptors and KA is the dissociation constant of drug A. The affinity of a drug A is defined as the reciprocal of the dissociation constant. The logarithm of the affinity equals the negative logarithm of KA.

As a consequence of receptor occupation by an agonistic drug a stimulus is generated (Ariens, 1964 ,; Stephenson, 1956) ;

$$S_A / S_m = \alpha (RA) / r$$

where S_A is the numerical value for the stimulus generated by a certain dose of drug A, while S_m is the maximal possible stimulus to be obtained by the particular tissue. The proportionality constant, α has been called the "intrinsic activity" (Ariens, 1954; Ariens et al, 1956).

The intrinsic activity determines whether the drug can generate a stimulus and therefore, whether a drug can be "active" or not. The affinity and the intrinsic activity are determined by the molecular properties of both receptor and drug.

Competitive antagonism :

The efficiency of a drug acting as competitive antagonist will depend on the relationship between affinity and intrinsic activity of the compound. Burgi's law indicates that in a homologous series the antagonistic character increases with decreasing intrinsic activity, assuming the affinity to remain fairly constant; and a compound with zero intrinsic activity is in fact a competitive antagonist. Competition for the receptor will usually decrease the frequency of the best interaction and with decreasing intrinsic activity of one of the components, the combined action will more and more take the form of a competitive antagonism.

Most compounds acting in this fashion appear to be in mass action equilibrium with the receptors and the blockade produced is a measure of competition between the agonist and the antagonist for receptor occupancy. Such agents are referred to as classical competitive antagonists.

Ariens et al, (1956) reported that if one agonist can give the maximal effects by acting on particular receptors and another can give only submaximal effect by acting on the same receptors, then the latter by competing for the receptors can act as an antagonist of the former. The other drug has been called as a "partial agonist". Stephenson (1956) demonstrated that progressive changes in the structure of an agonist produce a series of derivatives with a progressive change from agonistic to competitive antagonistic properties.

Recent studies have demonstrated that certain blocking agents react with the receptors or some adjacent group of receptors to form a relatively stable chemical bond. This reaction precludes further mass action "competition" and effectively reduces the number of available receptors (Nickerson, 1956). Such agents are best described as non-equilibrium antagonists e.g. organophosphorus anticholinesterase agents (DFP, TEPP), beta-haloalkylamine adrenergic blocking agents (phenoxybenzamine and its congeners). The blockade produced has been referred to also as "irreversible competitive" (Furchgott, 1954) and "unsurmountable" (Gaddum et al, 1955). However, the blockade is not strictly

irreversible and the term unsurmountable is appropriate only when the antagonist is used in sufficiently large doses to prevent a maximal response even in presence of massive amount of agonist.

Tests for competitive antagonism :

(A) Clark & Raventos (1937) suggested a method of estimating the activity of drug antagonists in terms of "the concentration which is altered by a selected proportion e.g. 10 fold the concentration of an active drug needed to produce a selected effect. The negative logarithm of this (molar) concentration has been termed pA_x where x is the proportion selected(Schild, 1947).

The pA_x is a measure which is particularly suitable for determining the activity of antagonists which do not alter the slope of the log dose effect curve of the agonist. Difficulties arise, however, with the antagonists which affect this slope. pA_x values may then be determined when the effect of the agonist is half the maximal(Schild, 1949).

One of the two tests for competitive antagonism is based on a comparison of equiactive doses. This type of test involves no assumption about the manner in which the receptor and physiological effect are related. It assumes only that equal effect involves equal number of receptors. There are two stages to the test. First a series of log-dose-effect curves are plotted, one without antagonist and the others with different concentrations of the antagonists. If these curves are parallel they afford presumptive evidence of competitive antagonism (Schild, 1957).

Next, the equation given by Gaddum (1943) for this case may be written as follows :

$$\frac{y}{1-y} = K_1 A = \frac{K_1 Ax}{K_2 B^n + 1}$$

where y is the fraction of the active receptors and K_1, K_2 and n are constants. A and A_x are the concentrations of agonist causing the response corresponding to y in the absence and presence respectively of the antagonist in concentration B

Eliminating K_1 A and taking logarithm

$$\log (x - 1) = n \log B + \log K_2$$

since $pA_x = -\log B$,

$$\log (x - 1) = \log K_2 - n \, pA_x$$

The plot of log (x - 1) against pA_x (-log B) thus gives a straight line with slope -n. The line intersects the pA_x axis at a point corresponding to pA_2

when $n = 1$,

$pA_2 = \log K_2$

and $pA_2 - pA_{10} = 0.95$

This equation provides a useful test of competitive antagonism which is independent of y. This relation is readily verified experimentally.

However, certain aspects of the relationship between the log dose of agonist and the effect, as well as the effect of antagonist on such a relationship should receive greater attention. These aspects are concerned with certain sources of error and may invalidate the results.

1. During the course of the experiment, there might be a gradual alteration in the sensitivity of the test preparation to the agonists.

i) certain agonists might exhibit "tachyphylaxis" with some preparations, a phenomenon which is related both to the agonist concerned and the test preparation under study. Increasing the interval between successive additions of the agonist and using agonists in submaximal concentration may at times overcome this difficulty.

ii) while using the agonists in maximally effective concentrations the phenomena of (a) desensitization to agonist and (b) autoinhibition may be encountered.

(a) Desensitization to agonists : On continued exposure to a depolarising dose of acetylcholine, repolarization can occur in muscle end plate despite continued presence of the agonist. This phenomenon of receptor "desensitization" was first studied by Thesleff (1955) for acetylcholine acting on frog sartorius muscle. Another example is the second phase of blockade of autonomic ganglia by nicotine. Paton (1961) investigated the partial desensitization of guinea pig ileum to large doses of acetylcholine and histamine. Whatever may be the innate cause of this phenomenon, it might result in a profound change in sensitivity of the preparation for the agonist which in turn might complicate subsequent investigation of antagonism.

(b) Autoinhibition : The importance of phenomenon of autoinhibition as a complicating factor in the studies on drug antagonism has been recognized in recent years. Intermediate derivatives in some of the

homologous series had the properties of agonists or partial agonists exhibiting 'autoinhibition' (Ariens, 1964). With increasing concentration of such derivatives, there was first an increase in response and then a decrease so that the resulting log-dose-effect curves became bell shaped.

This was thought to be due to the action of agents as noncompetitive antagonists at a second site of action, counteracting their own effect at the receptor sites. Similar observations have been made with certain cholinergic ganglionic stimulants including nicotine and dimethylphenyl piperazinium.

That autoinhibition might be due to a successful competitive antagonism by the compound under question in high doses, has also been recognised by Bijlsma et al, (1961).

It becomes imperative, therefore, that this phenomenon should be considered in studies on antagonism, if the dose-effect relationship becomes bell shaped, especially when higher dose of the agonists are used.

2. The recording condition will sometimes modify the slope of the dose-effect-curve. Paton (1961) found that curves for histamine and acetylcholine on guinea pig ileum were close to theoretical rectangular hyperbolas when recording was with an auxotonic lever (one in which shortening and tension both increase with increasing contraction); or an isometric transducer. But with an isotonic lever there was an initial "foot" to the curves followed by a slope which was much steeper than in theoretical curves. In

contrast to this finding, Rocha e Silva (1959) found excellent agreement between experimental and theoretical curves for histamine on guinea pig ileum with an isotonic lever, both in the absence and presence of antihistaminics. The reason for this discrepancy is not clear.

3. The shape of actual dose-effect-curves of agonists varies considerably (Segre, 1957). In the usual plot of effect agsinst log dose, the maximum slope is frequently much greater than that predicted in classical receptor theory which has E proportional to RA. Correspondingly a plot of 1/E against 1/A (Lineweaver-Burk plot 1934) does not usually give straight lines but upward curving lines as 1/A increases.

A 'foot' on a dose-effect-curve often indicates a threshold phenomenon. If one extrapolates the curve back to the effect axis, it intersects this axis below the dose axis. It has been suggested that the distance of the inter-section below the dose axis is a measure of threshold stimulus, a stimulus which must be exceeded before any effect is seen (Kirschner & Stone, 1951).

4. Failure of the antagonists to reach equilibrium also constitutes a possible source of error. This could be avoided by studying the effect of increasing the duration of exposure of the preparation to the antagonist on the degree of antagonism obtained, when the dose of antagonists is kept constant. It is preferable to fix this duration in such a fashion that further increment in duration of exposure does not increase the antagonistic effect obtained.

To add to the significance and reliability of the results, testing of competitive antagonist should be explored over a wide range of concentrations.

(B) If a competitive antagonist produces the same pA_x in different biological test preparations it can be assumed that it reacts with closely similar receptors. If this assumption holds true then it can be deduced that if the different preparations with closely similar receptors produce the same pA_x value then it is competitive antagonism. Agonists are as a rule more variable than antagonist e.g. the guinea pig ileum is much more sensitive to histamine than the tracheal chain but the pA_x values of antagonists in the two preparations are the same (Schild, 1957). Stephenson (1956) and Ariens (1954) have suggested that the activity of an agonist depends on atleast two factors; its affinity for the receptors and the contribution made by the drug receptor complex to the physiological effect whereas the activity of a competitive antagonist depends only on its affinity for the receptors.

(C) Receptor protection : If competitive blockade involves the occupation of the specific agonist receptors by the antagonist, the presence of the agonist in high concentrations will protect the specific receptors from the blockade and this can be verified after removal of both the agonists and the unreacted antagonist. The specificity of such "receptor protection" tests is indicated by the fact, that where an antagonist is effective against responses to several types of stimulants such as adrenergic, cholinergic, histamine

etc. the presence of agonist during exposure to the blocking agent inhibits the blockade of responses to only one specific type of agonist (Furchgott, 1954).

Differentiation between classical competitive and non-equilibrium (irreversible competitive) antagonists :

No single clear cut test is available to differentiate classical competitive from non-equilibrium blockade of tissue responses. However, certain tests may be suggestive of the type of antagonism.

(1) Straight-line relationship in plots of agonist versus antagonist is characteristic of classical competitive agents, whereas, lines describing the action of non-equilibrium agents curve towards the agonist axis (Nickerson, 1957).

(2) Development of the blockade produced by classical competitive agent ceases and is reversed as soon as active drug is removed from the surrounding medium (biophase) whereas formation of a stable drug receptor complex from active non-equilibrium inhibitor present in biophase may continue for some time after washing. It may be mentioned, that some classical competitive antagonist such as atropine and ergotamine produce relatively prolonged blockade. Nachmansohm et al,(1947) and Nickerson (1949) have demonstrated that the reactions of all known non-equilibrium inhibitors with receptors appear to occury in two steps, the first reversible "absorption" and the second a more stable chemical reaction. From this it can be concluded, that rate of dissipation of classical competitive agents

when washed out of tissues is a continuous function of the concentration gradient, whereas the dissipation of non-equilibrium blocking agents is discontinuous.

(3) The cumulative effect of several short exposures to a competitive blocking agent should be less than that produced by a single exposure of equal total duration whereas cumulative effect of short exposures to a non-equilibrium agent should be greater than the effect of the single longer treatment, although experimental verification of this assumption has been limited to relatively small number of compounds. Comparison of the effects of single and repeated exposures has provided a clear differentiation of blockade produced by dibenzyline from that due to several competitive inhibitors (Nickerson, 1957).

Tests for noncompetitive antagonism :

In this case, where the antagonist reacts with some site other than the site on the receptor with which the agonist reacts, the following equation has been proposed by Schild (1954):

$$y = \frac{K_1 A}{K_1 A_1^{+1}} = \frac{K_1 A_x}{K_1 A_x + 1} \times \frac{1}{K'_2 B + 1}$$

where y is the fraction of 'active' receptors (i.e. receptors combined with agonist), K_1 and K_2 are constants, A & A_x are the concentrations of agonist causing the response corresponding to y in the absence, and in the presence respectively of the antagonist in concentration B.

In this type of antagonism, the log dose-response curves are not parallel, but become progressively flatter and their maxima declines. Both the pA_x values and the pA_x differences now depend upon y (the number of receptors activated). For the calculation of K'_2 from the above equation, it is necessary to know the value of y; however, when y is unknown, K'_2 can be calculated as

$$K'_2 = \frac{x_1 \cdot x_2 (B_2 - B_1) + x_1 \cdot B_1 - x_2 \cdot B_2}{B_1 \cdot B_2 (x_2 - x_1)}$$

where x_1, and x_2 are the dose ratios corresponding to antagonist concentrations B_1 and B_2.