THE USE AND LIMITATIONS OF ATROPINE FOR PHARMACOLOGICAL STUDIES ON AUTONOMIC EFFECTORS

N. AMBACHE

Ophthalmological Research Unit, Medical Research Council, Institute of Ophthalmology, Judd Street, London, W.C. 1

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In this review it is intended to consider a few examples of the use and limitations of atropine in elucidating certain aspects of neuro-humoral physiology. The property of blocking the effects of certain parasympathetic nerves, and of cholinomimetic drugs, is shared by various isomers of atropine, by fragments and derivatives of the atropine molecule, and by related alkaloids occurring in solanaceous plants. The actions of all these can be grouped together as atropine-like, without further consideration of their individual differences, a subject beyond the scope of this article. Full particulars of the botanical distribution of these various alkaloids, of their complex chemical constitution and structure-action relationships can be found in various textbooks (28, 72, 96), in the reviews by Cushny (36), Henderson and Roepke (71), and Nyman (106); and in the paper by Lands (88).

Medicinal trials of solanaceous plant products can be traced back to antiquity, beginning perhaps with the references in the Ebers papyrus, about 1550 B.C., to hyoscyamus (known as pśd). This was used for expelling pains caused by roundworm or tapeworm (43, p. 35), and also for the expulsion of "magic in the belly" (p. 45). At the time of writing, some 3500 years later, the reader must needs be left in doubt as to whether the relief afforded to the inhabitants of Ancient Egypt was really due to suppression of peristalsis by the pśd, or to its anodyne properties. Hyoscyamus was one of the plants listed in the Greek

herbal of Dioscorides (64) and its mydriatic properties were known to Galen well before their exploitation for cosmetic purposes in mediaeval times, and their later therapeutic application in ophthalmic surgery towards the end of the 18th century (77).

The present subject can be said to begin in 1833 with the isolation of the alkaloid by Mein (101) and by Geiger and Hesse (60).[1] It was not, however, till 1869–1870 that the vagolytic action of atropine, discovered some 3 years earlier by von Bezold and Bloebaum (26), was studied in detail by Schmiedeberg (119) on the frog heart. Cardiac slowing and arrest were produced by electrical stimulation either of the vagus nerve or of the sinus venosus region in the heart; the inhibition was always abolished by atropine, as was that produced by small doses of nicotine (cf. Kottegoda, 87). With electrodes on the sinus venosus it is probable that Schmiedeberg was in fact exciting neurones which are now called "postganglionic", and the atropine-block which he demonstrated can therefore be located with confidence "at" or beyond the endings of these "final" neurones. In the same year Adamück (2), extending some earlier work by Bernstein and Dogiel (25), performed corresponding experiments which now serve to locate the mydriatic action of atropine "at" or beyond the postganglionic endings of the short ciliary nerves (see also 120). Both these results, on the heart and on the eye, remain valid to this day, although they were in fact obtained some years before Langley and Gaskell had laid the precise foundations of our present knowledge of the pre- and postganglionic relays in the autonomic system. However, it does seem that in the second half of the 19th century ideas about the functions of peripheral ganglia were already astir in the minds of several investigators, which perhaps influenced, if only subconsciously, the course of subsequent research. In the work on the eye the presence of a visible swelling at the ciliary ganglion, known already to contain ganglion-cells, may have prompted Adamück to place his electrodes on the ganglion itself. In Schmiedeberg's paper there is clear evidence that he was fully aware of the presence of peripheral ganglia in the wall of the heart. Both Remak's (110, Plate XII) and Bidder's (27) ganglia

[1] The formula for atropine $C_{17}H_{23}O_3N$ is that assigned to it by Liebig. The reader is referred to the chapter by Holmes in (96) for the other important landmarks in the chemistry of the tropane alkaloids, notably: (a) The hydrolysis of the ester group in atropine (*dl*-tropyltropine) with the production of an inactive base, tropine, and *dl*-tropic acid, found in 1863 to 1866 by Pfeiffer, by Kraut, and by Lossen. (b) Ladenburg's formula for *l*-hyoscyamine in 1880, and the isomerism of atropine and *l*-hyoscyamine, shown by him in 1881. (c) Fractional crystallisation methods for separating atropine, *l*-hyoscyamine and *l*-scopolamine, by Schmitt and Schütte in 1891 and by O. Hesse in 1892. (d) The observation by various authors (Will, 1888; Schmidt, 1888; Gadamer, 1901) that a number of chemical procedures convert *l*-hyoscyamine to atropine by racemisation. (e) The suggestion that the plant product does not contain atropine but chiefly *l*-hyoscyamine from which atropine is then formed by racemisation during the process of chemical isolation (Gadamer, 1901). (f) The syntheses of tropine by Willstätter in 1901 and 1921, and by Robinson in 1917; and various syntheses of tropic acid.

A comparison of the pharmacological activity of the two optical isomers of hyoscyamine was made by Cushny (37) who found that the laevo-isomer was 40 times as powerful as the dextro-rotatory form in antagonising pilocarpine salivation.

had been described long before Schmiedeberg's time. Bidder refers to them as nerve centres and appended to his paper a figure (Plate VI) which is highly suggestive of their anatomical connection with the vagi. They appear to have been correctly associated in Schmiedeberg's mind with the inhibitory function of the vagus, since he refers to them as "Hemmungsganglien".

The remaining links in our present understanding of the parasympatholytic properties of atropine were provided by the work of Dale (39), who showed that the alkaloid antagonised the peripheral actions of acetylcholine, and by Loewi and Navratil (94), who demonstrated clearly that it did not prevent the liberation of acetylcholine by the vagal nerve endings in the heart. The atropine-block can therefore be located distally to the nerve endings and is currently explained as due to antagonism by the alkaloid, at the level of the effector cells, to nervously released, or to artificially applied, acetylcholine.

I. SUSCEPTIBILITY TO ATROPINE AS A CRITERION OF CHOLINERGIC TRANSMISSION

Antagonism at effectors to acetylcholine released by the arrival of nervous impulses explains the paralysis by atropine of the following autonomic nervous functions: (1) pupillary constriction; accommodation (134); (2) lacrimation, salivation and sweating; (3) cardiac inhibition; (4) constriction and secretion of the bronchi; (5) contraction and secretion of various parts of the alimentary canal, with some exceptions given below; (6) certain vasodilator effects. Some of these atropine-sensitive effects are considered in greater detail in the section on atropine of Henderson and Roepke's review (71).

In instances of this kind, the abolition of a nerve effect by atropine can be taken as strong evidence that the endings of that nerve are cholinergic. However, the converse is not always true, since there exist a number of tissues in which 'specific' doses of atropine antagonise acetylcholine but not the effect of the nerve, although from other evidence the nerve fibres concerned are presumed to be cholinergic. Among the better known examples of this type of phenomenon are the following: 1) the vesico-motor effect of the pelvic nerve (69, 89); 2) the peristaltic reflex and the intestinomotor action of the vagus in some animals (22, 126); 3) the motor effect on the uterus of the cholinergic postganglionic fibres in the hypogastric nerves of the dog (121); 4) the vasodilator action of the chorda tympani (68).

II. ATROPINE-RESISTANT NERVE EFFECTS

These atropine-resistant anomalies have posed a problem in humoral physiology which has been discussed in some detail by Dale and Gaddum (40). As suggested by them, the evidence provided by eserine potentiation may, in such instances, carry greater weight than the absence of antagonism by atropine. Dale and Gaddum put forward the following hypothesis to explain the non-antagonism of atropine. "Where ... (atropine) ... still suppresses the effects of choline esters artificially applied, but fails to paralyse the similar nervous effects, it is still possible that the latter might be due to the liberation of a choline ester,

but in a relation of so much greater intimacy with the receptive mechanism that atropine cannot prevent its access thereto. We know nothing of the mechanism of the atropine paralysis, but for purely diagrammatic purposes we may regard it as creating a barrier which a choline ester cannot pass. If such an ester is liberated at the parasympathetic nerve endings to act as transmitter of the effects of the nerve impulses the latter will be paralysed, completely or partially, or not at all, by atropine, according as the liberation takes place wholly without, partially within, or wholly within the barrier". We shall return to this concept, which may be termed the "proximity theory", when discussing the action of nicotine, as it is believed that the theory receives some support from recent experimental findings on isolated tissues. There are instances, however, where alternative explanations have to be considered, as for example the possibility of a mixed postganglionic innervation. For instance, the bladder in some species is able to contract in response not only to acetylcholine, but also to adrenaline (62). Supposing for a moment that the pelvic nerve were to supply a mixture of cholinergic and adrenergic postganglionic fibres to the bladder, then eserine would potentiate the response to the cholinergic innervation, but atropine-resistance would occur because the adrenergic nerve supply was not blocked.

Thus, although susceptibility of a nerve effect to atropine is suggestive of a cholinergic transmission, insusceptibility does not necessarily exclude it. This is pertinent to the use of the alkaloid for the classification of drug actions at isolated peripheral effector tissues. It would, for instance, be useful to know before undertaking any investigation on the interaction with atropine of ganglion-stimulating drugs, whether the response to motor nerve stimulation of the particular effector under examination is in fact blocked by atropine. Unfortunately information on this point is often patchy and sometimes contradictory, perhaps because of differences in experimental conditions, and in methods of anaesthesia; possibly, also, because of other variables which will be mentioned. The review will attempt to summarise any relevant information of this kind and to classify it according to species. But its main concern is with the subject of atropine-resistance and the ways in which this can arise, of which the following are known: 1) Destruction of atropine by an esterase. 2) 'True' atropine-resistance in cholinergic neuro-effector systems, due perhaps to 'proximity'. 3) Secondary formation of atropine-resistant pharmacological agents, *e.g.*, bradykinin in the salivary glands. 4) Non-cholinergic transmission. It will be shown that in some situations uncertainty remains as to which of these mechanisms is at play.

III. THE MUSCLES OF THE ALIMENTARY TRACT[2]

A. Atropine and Vagal Stimulation; Species Differences

1. Dog. One of the first investigators to examine the action of atropine on the gastro-intestinal tract was Jacobj (84). In dogs he found atropine-resistance, as

[2] In this section stomach and small intestines will be considered together. Many of the papers reviewed below do not mention whether atropine was administered as the base or as a salt (usually the sulphate). This would affect the dosages given by the negligible factor of 578/694, allowing for one molecule of water of crystallisation.

did also Bayliss and Starling in their later experiments (22). Bayliss and Starling recorded from the duodenum and from the ileum (22, Figs. 25 and 26) and showed that 4 mg. of atropine partially antagonised the contractions elicited by vagal stimulation but did not abolish them. On repeating the stimulus 5 or 6 times contractions were obtained after an initial phase of inhibition. These motor responses were still present even after the administration of 30 mg. of atropine (22, p. 138). In Cushny's experiments (35), 2 mg. of atropine abolished pilocarpine-contractions but not vagal responses, and the same result was obtained later by Henderson (69) with 10 to 30 mg.

2. Cat. On the other hand, in the same paper (69) Henderson reported that in cats the motor response of the intestine to vagal stimulation was practically abolished by 10 mg. of atropine, without affecting "peristalsis" (possibly meaning pendulum movements). In the stomach it appears to be easier to block vagal responses with atropine; this is evident both in the investigations of McSwiney and Robson (100) on cats' isolated vagus-stomach preparations and also in Harrison and McSwiney's (67) *in vivo* experiments on spinal cats. Quoting from the latter authors: "there is no detectable difference between the threshold doses of atropine necessary for abolishing the vagus effects on the stomach and the heart". The effective dose of atropine in their experiments was 0.025 to 1 mg. per cat.

It is therefore clear from these various sources that vagal responses, although atropine-resistant in dogs, can be blocked by atropine both in the stomach and, perhaps less easily, in the intestines of most cats. With regard to rabbits, guinea-pigs, and rats, there appears to be considerably less information, which is unfortunate because the isolated intestines of these species provide the most commonly used test-objects in pharmacological laboratories.

3. Guinea-pig. Apart from the paper by Straub and Stefánsson (123) there have not been many observations on vagal susceptibility to atropine in guinea-pigs. But this paper shows convincingly that stimulation of the vagus *in vivo* evokes a contraction response in the small intestine, which is termed 'peristaltic'; this is easily blocked by atropine, even in doses as small as 10 μg. per kg. Assuming an even distribution of the drug this would represent a concentration of 10^{-8}, which is in reasonable agreement with the concentration of atropine required to block acetylcholine-contractions on guinea-pig ilea *in vitro*.

Variable effects of atropine on the response to mesenteric periarterial stimulation have been described by Munro (104), as well as a transient extinction by the alkaloid of the intestinal contractions elicited by direct, coaxial, electrical stimulation of the nervous structures in the gut wall. However, Munro's results were obtained on the terminal portion of the ileum, which appears to differ in several respects from the rest of the small intestine proximal to it. For instance, this part of the ileum resembles the nictitating membrane, the spleen, and some uteri in its ability to respond by contraction to adrenaline as well as to acetylcholine. A result different from Munro's was obtained by Paton (107) in similar experiments on guinea-pig preparations taken from the ileum, but presumably not from the terminal portion of it. Direct, coaxial, electrical stimulation with

brief pulses elicited rapid twitches which were shown to be due to stimulation of postganglionic neurones in the preparation. These were abolished by atropine sulphate 10^{-8}.

4. Rabbit. Information on rabbits is also relatively scanty; there are only Trendelenburg's reference to a dissertation by Hagen (66), two contradictory experiments by Jacobj (84) in which the minimum dose of atropine sulphate was 25 mg., some experiments by Langley (88a) with 15–60 mg. doses and a non-committal statement by Meltzer and Auer (103).

In this species the problem of atropine susceptibility is complicated by an additional factor of considerable importance, namely the presence of an atropinesterase in the serum of some individuals. Evidence of atropine destruction by a thermolabile catalyst in the blood of some animals was first obtained by Cloetta in 1908 (33a) and by Fleischmann in 1910 (58). This early work was of a qualitative nature and depended, for the detection of atropine, on biological assays. More accurate quantitative measurements of atropinesterase activity followed later, when a manometric technique, similar to that used for cholinesterase determinations, was adopted for the estimation of atropinesterase (14a, 24, 61). Atropine sulphate is incubated with serum in a Warburg vessel containing a buffer and $NaHCO_3$, and filled with 95 per cent N_2 and 5 per cent CO_2; the rate of atropine hydrolysis is given by the evolution of CO_2. Using this technique, Bernheim and Bernheim (24) found the enzyme in only ⅔ of their rabbits and Ammon and Savelsberg (14a) in 9 out of 35 animals of random origin. It is therefore not every rabbit that can browse on Deadly Nightshade with impunity. Both Bernheim and Bernheim and Ammon and Savelsberg reported that the esteratic destruction of atropine was inhibited by eserine; using a biological method of assay, Lévy and Michel (92a) were unable to confirm this. Further data on atropinesterase have been published by Glick and Glaubach (61) who found the enzyme in one out of every four rabbits; it was present in the serum but not in the erythrocytes (see also 92a), and was distinct from cholinesterase. It was also detected in various tissues (iris, liver, intestinal mucosa, etc.), but in animals devoid of the enzyme in their blood there was no demonstrable activity in any of the organs tested. The enzyme was capable of hydrolysing *l*-hyoscyamine, homatropine (the mandelic ester of tropine) and scopolamine (the tropic ester of scopine), the latter observation disagreeing with an earlier result of Bernheim and Bernheim (24). A similar enzyme may be present in the liver of other species. Thus in guinea-pigs the liver can split homatropine but not atropine; the serum is entirely inactive.

Since the hydrolysis products of atropine are relatively, if not completely, inert pharmacologically, the existence of this enzyme could clearly lead to transience in atropine susceptibility in individual experiments. In fact a transience of atropine block has been observed in some rabbits by Ambache and Lippold (12) in a study of the bradycardia which is produced by centripetal injections of tetanus toxin into the cut central end of the vagus nerve on one side. The toxin produces a tetanic hyperactivity of the cardio-inhibitory centre, which discharges down the intact vagus nerve on the side opposite to the injection. A

'vagal tetanus' ensues, which is tantamount to applying continuous stimulation to one vagus nerve, except that this is achieved by pharmacological means instead of electrically. The resulting bradycardia is abolished by vagotomy, and by depressing the cardio-inhibitory centre with pentobarbital sodium. It can also be abolished by blocking the vagal endings at the periphery with atropine (sulphate). However, in some rabbits the removal of tetanic bradycardia by atropine is transitory and, as reported by Ambache and Lippold (12, Table 5), in such animals the administration of atropine has to be renewed at intervals, presumably because the alkaloid undergoes destruction by serum atropinesterase.

It is therefore clear that for some physiological experiments it might be necessary to know more about the animal in use on each occasion, and it may even be advisable for certain types of work to select rabbits devoid of atropinesterase activity. To this end the interesting results of Sawin and Glick (116), who studied the genetics of this trait in rabbits, may be of some assistance. The ability to hydrolyse atropine (and, incidentally, mono-acetylmorphine, probably by the same enzyme system (135), but see Ellis, 47a) is inherited through a gene (A_s) borne in the same chromosome as the gene (E) for extension of black pigment in the coat. The A_s gene is incompletely dominant, homozygotes producing the enzyme more effectively than heterozygotes. The enzyme is absent at birth and appears in the serum at the age of one month. It is found in higher concentrations in females, and in a larger proportion of females than of males. Some idea of the variations which are likely to be encountered in atropinesterase activity can be gained from the following figures. In four rabbits homozygous for the presence of this enzyme the serum contained an average of 2710 units/ml. (range 2320–3480), whilst in 25 heterozygotes the average was 1070 units/ml. (range 520–1740), the unit being the quantity of enzyme which will hydrolyse an amount of atropine equivalent to 1 cmm. of CO_2 in 5 hr. at 30°C, at a substrate concentration of 0.25 per cent. In more recent experiments by Hobbiger and Lessin (79), performed at 37°C with a substrate concentration of 10^{-2} M, the rate of destruction of atropine sulphate by 14 different rabbit sera, all active, averaged 0.09 mg./min./ml. serum (range 0.04–0.23 mg./min./ml.).

It should therefore be possible, by selecting and inbreeding a colony of high titre rabbits, to obtain the enzyme in sufficient quantity for purification. Such a product might be of therapeutic value in the treatment of acute atropine poisoning in human beings; although this condition is rare, it is reported from time to time (29).

When, to offset the destruction of atropine by enzymic hydrolysis, or for any other reason, it becomes necessary to use tremendous doses of the alkaloid, the possibility of side effects may arise. For instance it has been shown by Ellis (47) that, in concentrations of 1.4×10^{-3} M, atropine inhibited 87 per cent of the hydrolysis of benzoylcholine by rabbit serum. Todrick (125) has reported 50 per cent inhibition of the pseudo-cholinesterase in the rat's intestinal mucosa by atropine sulphate in concentrations of 6×10^{-4}. Also, atropine stimulates the central nervous system; in large doses it releases histamine (Schachter, 117) and also antagonises histamine (Schild, 118). Atropine is therefore useful for the

analysis of cholinergic phenomena only when this is possible with small doses of the alkaloid.

Bearing these points in mind, the problem of vagal susceptibility to atropine in rabbits, on which data are relatively scarce, has been re-examined. In an experiment by Ambache and Lippold (128) it was noticed that the intravenous administration of 0.25 mg./kg. of atropine sulphate did not affect gastric contractions elicited by vagal stimulation; this dose of atropine is usually sufficient to paralyse gastric contractions in cats. The reason for this discrepancy has recently been clarified in this laboratory (79) in some experiments conducted on rabbits of which the serum atropinesterase levels had been previously determined manometrically. It was found that in all of 5 rabbits devoid of the esterase there was a lasting block of vagal stomach contractions by atropine sulphate (0.25–1 mg./kg.). In 5 other experiments in which transience of gastric-block occurred this was correlated without exception with the presence of atropinesterase in the serum of the animals. These two contrasting types of results are illustrated together in Fig. 1 of reference 79. In the "non-esteratic" rabbit (A in 79 fig. 1) the atropinesterase activity of the serum was zero; a single dose of 0.25 mg./kg. i.v. atropine sulphate virtually abolished the response to vagal stimulation without significant recovery during the next 2 hours. On the other hand, in the "esteratic" rabbit B the first dose of atropine sulphate, 0.25 mg./kg., hardly affected the gastric response; the second dose, 1 mg./kg., reduced it at once to less than 3 per cent, but with rapid recovery to 72 per cent in 22 min. followed by a second phase of much slower recovery.[3] It is interesting to note that *in vitro* the level of serum atropinesterase was, in this particular rabbit, equivalent to a destruction rate of only 0.09 mg. atropine sulphate/min./ml. serum, *i.e.*, less than half the maximum value recorded in this series. Clearly, the atropinesterase content of the serum would influence not only the rate of recovery in the "transience" phenomenon but also the dose-level at which atropine would begin to be effective in any given rabbit.

In summary, there now seems to be less justification for the view expressed by Trendelenburg (126, p. 100) that the therapeutic action of atropine on the alimentary tract rests upon insecure experimental foundations. The apparent confusion of results may only be due to the factors mentioned already or to others as yet to be elucidated. These include: 1) species differences; 2) genetically determined individual variations within species; and possibly 3) regional differences between various parts of the digestive tube. When approaching the problem of the clinical value of atropine as an intestinal sedative, the possibility needs to be examined that a proportion of human beings may display refractoriness to atropine even if the majority respond to this drug. In particular it would perhaps be worth-while to explore further the alleged connection between pigmentation

[3] The incomplete recovery may be due to the persistence of *d*-hyoscyamine, which does not seem to be rapidly destroyed by the enzyme, since in manometric experiments hydrolysis of racemic atropine does not proceed beyond 50 per cent (78). Thus each mg. of atropine, by virtue of the ½ mg. of *d*-hyoscyamine in it, would, after destruction of the ½ mg. of *l*-hyoscyamine, exert 1/41st. of its original pharmacological activity.

and insensitivity to atropine. It is frequently reported that patients with heavily pigmented irides are relatively refractory to the mydriatic action of atropine. A search for atropinesterase in their serum and, *post mortem* or at operation, in the tissues of the iris might be of interest.

B. The Effect of Atropine upon Peristalsis

The word "peristalsis" is here intended to mean the reflex response of the intestine to distension or to other stimuli, which was first observed *in vivo* by Bayliss and Starling (22) and later studied *in vitro* by Trendelenburg (126).

It is of course not known whether the final motor neurones which excite the muscle fibres in the motor phase of this reflex are identical with the postganglionic neurones of the vagus nerve. Histological information regarding their identity or distinctness would be invaluable but is still awaited, although it is nearly a hundred years since the enteric plexuses were discovered, Meissner's in 1857 (102) and Auerbach's in 1862 (16, 17, 18, Pl. IX, Fig. 3). If there is identity, in other words, if a final common path exists, then this should be reflected by an equal susceptibility to atropine of peristalsis as of vagal contractions in the intestines of the same animal. One would then expect, from the evidence given in the foregoing section, that peristalsis might be abolished by atropine more easily in guinea-pigs and in cats than in dogs and rabbits. According to Trendelenburg (126) clear cut results could be obtained only on the guinea-pig gut, where atropine (1/500,000 to 1/100,000 of the sulphate) abolished the peristaltic reflex, apparently without exception. By contrast, on isolated rabbits' intestines the action of atropine (1/50,000 to 1/20,000) was unpredictable. Peristalsis was sometimes augmented, sometimes depressed, often only temporarily as in his Fig. 17. It is clear, however, that peristalsis was atropine-resistant in a proportion of Trendelenburg's rabbits. The effect of atropine on tonus and on rhythmic longitudinal muscle contractions was also variable, but will not be considered here since both these processes may be influenced by non-nervous or locally diffusing acetylcholine, and thus throw little light on the pharmacology of the motor nerve endings.

In dogs Bayliss and Starling reported the persistence, *in vivo*, of peristaltic reflexes after atropine. Trendelenburg obtained the opposite result on the gut of young puppies *in vitro*. However, his tracing (126, Fig. 18) is not convincing; although it does show relaxation by atropine of the longitudinal muscle and a decrease in its pendulum movements, no conclusion can be reached on peristalsis.

C. Atropine and the Stimulation of Motor Neurones by Nicotine; the Longitudinal Muscle Layer

Reasons have been given elsewhere (8, 11) for the assumption, which will be made here, that the longitudinal contraction of the intestine elicited by low concentrations of nicotine (10^{-6} to 10^{-5}) is due to stimulation of the parasympathetic motor neurones imbedded in the gut wall. To describe this action the term 'neuronal' was suggested previously, as it covers the possibility that stimulation may take place at the nerve endings as well as at ganglion cell-bodies. It is neces-

sary, however, to bear in mind the further possibility that nicotine would stimulate not only those ganglion cells which subserve the function of final motor neurones, but also any other local intercalary neurones which might impinge upon these final neurones. Such a concept is still based on somewhat insecure histological foundations, although it follows a suggestion which was first put forward in 1922 in a diagram published by Langley (90). The following considerations add weight to this opinion.

Recent fibre counts by Evans and Murray (52) after supranodose vagotomy in rabbits have revealed the fact that the sum of the preganglionic fibres in the two abdominal vagi does not exceed 3000; in cats, it is about 3500 (105). On the other hand, the number of nerve cells in the enteric plexuses runs into millions. In the guinea-pig's intestine it can be calculated from Irwin's and from Matsuo's figures (83, 97) that there are about 1,000,000 ganglion cells in Auerbach's plexus alone. For the cat's small intestine the total number of nerve cells in Auerbach's and in Meissner's plexus is given as 20 million by Sauer and Rumble (115). In the absence of intercalated neurones each preganglionic fibre would need to establish connection with some 6000 ganglion cells. Langley's awareness of such a disproportion led him to postulate the existence of a 'third' neurone in the vagal pathway, intercalated between the preganglionic fibres and the final motor neurones. These intercalated neurones he called 'vagus cells', and in his diagram each vagus cell is shown connecting one preganglionic fibre to several terminal neurones. In support of Langley's hypothesis Babkin (20) has drawn attention to the greater abundance of Dogiel's type II cells in the nerve plexuses of the small intestine, where the vagal supply is much diminished, than in the stomach; the possible function of these type II cells as associative neurones is discussed. The final motor neurones are assumed to be the Dogiel type I cells, as thought by Lawrentjew (92), although Hill (74) and McSwiney (99) have held the contrary opinion. Whichever view is correct, there appears to be a distinct difference between the constitution of the enteric plexus (a) in the oesophagus and stomach, with a preponderance of type I cells, and (b) in the small intestine, where the proportion of type II cells increases from 30 per cent of the myenteric cell total in the duodenum to 50 per cent in the lower ileum. These facts may have a considerable bearing upon regional differences not only in physiological properties but also in pharmacological reactions to ganglion-stimulating drugs, for instance their atropine-resistance. Lastly, it is of course conceivable that with the dwindling of the vagal nerve supply to the lower reaches of the small intestine the ganglion cells become increasingly autonomous. In other words, the final motor neurones might be less and less 'vagal', and more and more 'peristaltic', perhaps in correspondence with the change-over from type I to type II.

Whether the contraction elicited by nicotine is due to stimulation of final motor neurones only, or of intercalated neurones as well, this response should exhibit the same degree of susceptibility to atropine as either the vagal, or the peristaltic, response. Some correlation can be found, as the following observations indicate.

1. Cat. In vitro experiments conducted in this laboratory (10, 14) showed that

atropine (sulphate) 10^{-7} abolished or reversed, the contractions of the longitudinal muscle in response to nicotine both in dissected stomach strips and in 7 whole segments of kittens' ileum. In an eighth segment (128) the effect of atropine (up to 3×10^{-5}) was transient. These results, on the whole, agree with the evidence given above that atropine blocks vagal responses in cats. They imply that in the longitudinal muscle of this species all the final motor neurones, whether 'vagal' or 'peristaltic', are usually cholinergic. Nicotine-atropine interactions have not been studied in the circular layer.

2. Guinea-pig. As in cats nicotine-contractions of the longitudinal muscle are easily abolished by atropine in isolated preparations suspended *in vitro* (14, 48, 50, 111, 127). Whether this is equally true of the terminal portion of the ileum has not yet been investigated. In the experiments of Ambache and Robertson (14), who avoided the terminal region, nicotine-contractions could be abolished by 0.5 to 1×10^{-7} of atropine (sulphate) in three out of four preparations, as also in six others examined by Robertson (111). These results on nicotine-atropine interactions are in good agreement with the fact that both vagal contractions and peristalsis are easily abolished by atropine in this species.

The influence of pH on the effectiveness of atropine block in the guinea-pig's ileum has been described by Dahlbom *et al.* (38). An acid shift in Tyrode-pH towards neutrality produced a thousandfold increase in the power of atropine to antagonise barium-induced contractions, which are now known to be partly ganglionic in origin. The same effect of pH is mentioned in the paper by Rocha e Silva *et al.* (114) in connection with the atropine antagonism to serotonin-contractions. In view of the interesting comparisons which have been made between serotonin- and nicotine-contractions (59, 111, 112, 114) it would be valuable to know whether the nicotine-atropine antagonism is likewise affected by pH. The findings of Dahlbom *et al.* provide an instructive example of how organ-bath conditions may influence a pharmacological result, and they may also supply the explanation of Emmelin and Feldberg's (50) earlier failure to block barium-contractions regularly with atropine.

Attention has also been drawn recently by Parkes (106a) to genetic factors as a source of variation in pharmacological experiments on the guinea-pig ileum. During an investigation of the spasmolytic action of various thioesters of benzilic acid, great differences were encountered in their ability to antagonise barium and nicotine; this was particularly noticeable with Ro 3-0226.[4] These differences were later traced to the use of animals from different sources. Further experiments were therefore conducted on preparations from four different guinea-pig strains. In one of these strains Ro 3-0226 was much less active than in the rest; moreover, in the same strain hexamethonium (1/150,000) was inactive against nicotine although it was active at a concentration of 10^{-6} in another strain.

3. Rabbit; atropine-resistance of nicotine contractions. There have been several reports from different laboratories that nicotine-contractions are frequently atropine-resistant in preparations of rabbits' ileum (10, 49, 51). In the presence

[4] Diethylaminoethyl thioester of benzilic acid hydrochloride.

of atropine sulphate 10^{-7} the nicotine-contractions sometimes remain unaltered, or are slightly reduced, even after previously matched responses to equipotent doses of acetylcholine or of muscarine have been completely blocked. As the concentration of atropine is raised there may be some further decrease in the height of the nicotine response, although in some preparations even $1–4 \times 10^{-6}$ may not produce any further change. Weak contractions to nicotine still occurred in the presence of atropine concentrations as high as 2×10^{-5} in Ellis and Rasmussen's experiments, and 10^{-4} in those of Ambache and Edwards (10, Fig. 4, I).

This matter was re-examined later by Ambache and Robertson (14) who obtained a result opposite to the above in some of their experiments, and came to the conclusion that the occurrence of atropine-resistance of nicotine-contractions in rabbits' intestines was subject to individual variation. Although it is more usual to record atropine-fastness in this species, a number of rabbit preparations may resemble the guinea-pig ileum in that nicotine-contractions display a greater susceptibility to atropine; both these results are in agreement with those of Trendelenburg on peristalsis. Thus in two out of five preparations nicotine-contractions could be abolished by $0.5–2 \times 10^{-7}$ of atropine (sulphate). In the remaining three the nicotine responses were atropine-resistant. In two of these 10^{-7} of atropine reduced nicotine responses, but only to 50 and 60 per cent, respectively, of their initial heights, although responses to equipotent doses of muscarine were completely abolished. When the concentration of atropine was increased to 10^{-6} the remnant of the nicotine contraction fell from 50 to 40 per cent in one of these preparations, whilst in the other it was still 80 per cent.

The incidence of this type of atropine-resistance could not be ascribed to any difference in organ-bath conditions which, in all these rabbit experiments, were identical with those adopted in the guinea-pig and kitten series, referred to above, in which atropine susceptibility was the rule.

To what extent the presence of atropinesterase in intestinal preparations from rabbits may contribute to the occurrence of atropine-resistance in them remains a matter for further investigation. It might repay future workers in this field to breed colonies of atropinesterase-free rabbits by mating 'non-esteratic' individuals. Suspended intestinal preparations from 'esteratic' rabbits might contain the esterase by retention of serum in their capillaries and the enzyme has also been found in the mucosa of such rabbits (61); whether it is also present in the muscular layers is not known. It is probable that mucosal destruction of atropine is eliminated if the lumen of the intestinal preparation is closed by end-ligatures, as in the experiments of Ambache and Edwards (10). It would, nevertheless, be useful to re-examine this question on preparations of the longitudinal muscle layer, previously rid of serum by brief perfusion and separated from the other layers of the intestine, including the mucosa, by the method described by Ambache (9). Pharmacological results obtained on such preparations should be correlated with biochemical determinations of their atropinesterase content. Until this is done the view may be taken, provisionally, that the atropine-resistance of nicotine-contractions cannot be accounted for by alkaloidal destruction, for the

following reasons. The dose of atropine in the experiments referred to above (10, 14) was renewed frequently, and preceded the test dose of nicotine, or of muscarine, by a fixed interval, which in the experiments of Ambache and Robertson (14, Fig. 11) was one minute. The response to the equipotent dose of muscarine was well and truly blocked at the end of this brief time-interval (see Fig. 11 at E, reference 14); there was thus no evidence of any significant atropine destruction in so short a time. Lastly, atropine-resistance of nicotine-contractions has occurred, though less strikingly, in intestinal preparations taken from rabbits known to be devoid of atropinesterase in their serum (128).

Summary: In those preparations in which atropine blocks nicotine the motor neurones can be presumed to be entirely of the cholinergic variety. When atropine-resistance of nicotine-contractions is found two possibilities can be invoked. 1) The motor neurones are in fact cholinergic, as in other species and in other individuals of the same species, but their action is not blocked by atropine because of 'proximity' of the nerve endings, a view favoured by evidence given below. 2) A proportion of the motor neurones are non-cholinergic. In this connection it is interesting to note that in Ellis and Rasmussen's experiments nicotine-contractions could be abolished by a combination of atropine with 10^{-5} of dihydroergotamine, but not by either alkaloid alone. Nevertheless, since the block occurred only with excessive doses of dihydroergotamine, these authors concluded that adrenergic neurones played no part in the motor response to nicotine in this species. Such a possibility is also rendered unlikely by the fact that the rabbit's ileum, including the terminal portion, is relaxed by sympathomimetic amines.

D. The Other Muscular Layers of the Intestine

Atropine-resistant ganglionic responses have been described in the circular layer of the rabbit's intestine by Vogt (129).

Some interesting observations on the *muscularis mucosae* of dogs have been reported by King and Robinson (85). Contractions of this muscle in response to vagal stimulation persisted after the administration of sufficient atropine to paralyse the inhibitory effect on the heart. *In vitro* the muscle contracted in response to adrenaline as well as to acetylcholine. Nicotine-contractions persisted in the presence of ergotamine, or of atropine, administered singly, but were abolished by the combination of these two antagonists; the concentration of atropine was not stated but was presumably adequate to block equipotent doses of acetylcholine. Similar results have been obtained with nicotine on strips of *muscularis mucosae* from human stomachs by Walder (133).

To explain their findings King and Robinson postulated a mixed postganglionic innervation to the *muscularis mucosae*, consisting of adrenergic as well as of cholinergic motor neurones. Although their interpretation may be correct, yet this type of evidence is weakened by the fact that the combination of dihydroergotamine with atropine gives the same result on preparations of rabbit ileum (49), where adrenaline is inhibitory.

E. The Evidence Provided by Botulinum Toxin Regarding the Neurology of the Intestine

The symptoms of botulism are those of cholinergic nerve paralysis; and in its parasympatholytic action the intoxication bears superficial resemblances to atropine poisoning. Although the intimate nature of the lesion produced by the toxin is still open to further inquiry (3, 4, 5, 30, 31, 65) the end result of it is the prevention of acetylcholine-release at the nerve endings. On the other hand effector cells do not appear to be damaged by this toxin. Thus, even after complete paralysis of their nerve supply, not only skeletal and smooth muscle, but also heart- and gland-cells continue to respond normally to appropriate electrical or pharmacological stimuli.

In the gastro-intestinal tract the toxin is known to obliterate vagal responses and also peristalsis; it produces severe constipation. These effects could be due to paralysis either of pre- or of postganglionic terminals, or of both. It was therefore of interest to study the action of the toxin on the 'final motor neurone' system in the gut. These studies have been carried out with toxins of types A and D, which, though distinct immunologically, are very similar, pharmacologically, in their power to destroy cholinergic nerve functions.

In the rabbit's intestine botulinum toxin extinguishes the motor response to nicotine without fail, whether administered *in vivo* subperitoneally before excision of the preparation (type A toxin; 6) or *in vitro* (type D; 11). Treatment with type D toxin *in vitro* also abolishes the nicotine-contractions of the guinea-pig's ileum and of circular muscle preparations from cats' intestines. The toxin thus appears to paralyse the final motor neurones in all three species with equal facility. The results with toxin in guinea-pigs and in cats reinforce the evidence provided by atropine, and the equal susceptibility to toxin of the rabbit gut suggests that the motor neurones in it are also cholinergic.

The more recent of these experiments have demonstrated the advantages of type D toxin for pharmacological work of this kind, where irreversible functional 'denervation' of the cholinergic endings in an isolated autonomic effector is desired. The following considerations favour its use. 1) Pure type D toxin is 20,000 times as lethal to mice as pure type A (109). 2) The paralytic action of type D on intestinal segments can be obtained *in vitro* after short exposures to the toxin of 5–20 min. The concentration of toxin in mouse LD_{50}/ml. of bath fluid has been, in these experiments with type D, 50 to 1000 times that used by Burgen *et al.* (31) to paralyse guinea-pig phrenic-diaphragm preparations with type A. 3) In experiments of this kind the danger always exists of swallowing inhaled droplets of toxic spray from the bath-fluid. With type D toxin this should not matter; there is considerable evidence that, except for ruminants, this toxin is, unlike A and B, innocuous by mouth to most laboratory animals, including monkeys (63), and also to men (122). Type D botulism is unknown in man. 4) The advantage of being able to poison the preparation *in vitro* instead of *in vivo* is as follows. Although it is possible to produce functional 'denervation' of skeletal muscles *in vivo* by parenteral injections of toxin (type A), it is difficult to paralyse the gut completely in rabbits unless toxin is injected directly into an intestinal segment.

Such a procedure is, however, not feasible in animals with thin guts, *e.g.*, in guinea-pigs.

1. Distinction between botulinum toxin and atropine. Fallacious comparisons can be found in the older literature (41) between the action at parasympathetic nerve endings of botulinum toxin and that of atropine. The difference in their mode of action was brought out in experiments on the sphincter pupillae (3, 4). After intraocular inoculation of type A toxin the sphincter muscle remained immobile during stimulation of the oculomotor nerve, but was still capable of constriction in response to acetylcholine. In all the subsequent experiments on the intestine, although the motor response to nicotine and to other ganglion-stimulants was abolished, there was no evidence of any atropine-like effect either with toxin A or with D. The paralysed intestines responded to acetylcholine, muscarine, 2268F,[5] and 5-methylfurmethide, all of which are easily blocked by atropine (6, 11, 14).

There is therefore as great a difference between botulinic paralysis at parasympathetic nerve endings and atropine block as there exists between the paralytic actions of botulinum toxin and curare at the motor nerve endings in skeletal muscle. The latter distinction from curare is evident in work carried out simultaneously in several laboratories, using different *in vivo* and *in vitro* techniques and a variety of skeletal muscles from different animals, including the extrinsic muscles of the eye (3, 4, 31, 65).

2. Limitations of the evidence obtained with botulinum toxin. The evidence given above is limited by uncertainties about the specificity of botulinum toxin. If the hypothetical assumption is made that there exists in the rabbit gut an unrecognised type of motor nerve ending capable of releasing some unknown, atropine-fast, humoral transmitter, *e.g.*, substance P, then we are left in ignorance as to whether or not botulinum toxin would be able to paralyse the "P-ergic", as well as the cholinergic, endings. However, since it is known that cholinergic nerve endings are particularly sensitive to this toxin, and until a similar action of the toxin is discovered on these other, hypothetical, transmitter-systems, it appears justified to continue with the assumption that the toxin in appropriate dosages is specific.

F. Supporting Evidence for the Predominance of Cholinergic Motor Neurones in the Gut

1. Use of anticholinesterases. Anticholinesterases would potentiate the effectiveness of acetylcholine, when released by neuronal stimulation. It is possible to show that nicotine-contractions are potentiated by eserine in the rabbit gut; but this is difficult to do because of the persistent unsteadiness of the baseline when eserine is present in the organ-bath. More convincing evidence of the potentiation of nicotine by an anticholinesterase can be obtained by using BW 284C51,[6] a compound synthesized by Copp (34). This drug is of particular interest because of its high specificity towards 'true' cholinesterase as shown in studies on blood esterases by Austin and Berry (19). Further evidence of this specificity has been

[5] α,β-Ethylal-γ-trimethylammoniumpropanediol.

[6] 1,5-Bis-(*p*-allyldimethylammoniumphenyl)pentan-3-one dibromide.

obtained on the enzymes of the rabbit's small intestine by Hobbiger (78); 10^{-5} M of BW 284C51 inhibited 95 per cent of the 'true' cholinesterase activity with less than 5 per cent inhibition of the 'pseudo' cholinesterase. In pharmacological experiments on isolated rabbit ilea Robertson (112) observed a reversible potentiation of nicotine-contractions by BW 284C51. When BW 284C51 (10^{-6} M to 10^{-5} M) was introduced into the organ-bath the gut contracted almost immediately; but after several minutes, for reasons which are not yet clear, it relaxed to its original length and the baseline then remained steady despite the continued presence of the anticholinesterase in the bath, thus providing more favourable conditions for quantitative comparisons of drug actions than prevail with eserine. At this stage nicotine-contractions were much enhanced, often to a greater extent than equivalent acetylcholine responses; but this difference may only have been due to an atropine-like side action of BW284C51. Such an action was noticed by Tedeschi (124) in his experiments on isolated hearts and also by Ambache and Lessin (11) on the gut, where it was found that BW284C51 antagonised the response to muscarine. Other anticholinesterases may also possess atropine-like properties (124). The initial spasm produced by BW284C51 appears to be due to activation of nervous structures in the gut wall, because it is not seen if the preparation is treated with botulinum toxin (11).

The potentiation by BW284C51 of nicotine-contractions, which are the result of neuronal stimulation by a drug, has an interesting parallel in some experiments by Paton (107), in which the twitch response of guinea-pig ileum preparations to coaxial electrical stimulation, a response believed to be due to excitation of postganglionic motor neurones, was also found to be potentiated by very low concentrations (2×10^{-8} M) of BW284C51.

As mentioned by Dale and Gaddum (40), potentiation by anticholinesterases may, in some instances, yield information denied by the use of atropine. Even so, the use of anticholinesterases may be of limited value in the gut. Enhancement of vagal or peristaltic responses is not necessarily an indication that the final motor neurones are cholinergic, since the enhancement could be due to potentiation at the pre-to-postganglionic synapses. Potentiation of nicotine responses is perhaps more informative, though not completely decisive, because of the possibility, already raised, that nicotine may be exciting chains of local neurones in the gut and not just the final motor neurones. This may also apply to electrical stimulation in the presence of anticholinesterases. In other words, whether one is stimulating intestinal ganglia with nicotine or electrically, it is just possible that the potentiation produced by an anticholinesterase might be due in part to a greater recruitment of final motor neurones by associative neurones.

2. Ganglion-stimulants other than nicotine. Studies with other ganglion-stimulants have given the same results as with nicotine. For instance, Euler (51) has described the atropine-resistance of piperidine responses on the rabbit ileum, recording longitudinally. In experiments on the circular coat, also of rabbits, Vogt (129) observed ganglion stimulation with sodium lactate and hypertonic sodium chloride; the responses to such stimulation were relatively atropine-resistant.

Two new powerful ganglion-stimulants have been used in this laboratory (11, 14) in parallel with nicotine, in many of the experiments outlined above, namely 1) *m*-bromophenyl choline ether, synthesized by Hey (73) and 2) 1,1-dimethyl-4-phenylpiperazinium iodide (32). These two compounds behaved in every respect like nicotine. The contractions elicited by them were (a) atropine-fast in rabbits' but not in guinea-pigs' and cats' intestines, (b) abolished by botulinum toxin, and (c) potentiated by BW284C51.

3. The action of Darmstoff. Darmstoff is an acidic substance detected by Vogt (130, 131) in extracts and washings of frogs' and rabbits' intestines. Vogt has purified this substance and listed some of its properties (131). Its present interest lies in the fact that it is able to make the atropinised rabbit gut contract, and it has therefore been suggested as a possible "alternative transmitter" of atropine-resistant vagal effects. However, the following observations make it unlikely that Darmstoff could function as a postganglionic transmitter, at least in the rabbit's intestines: (a) Darmstoff-contractions are enhanced by BW-284C51; (b) they are abolished or reversed by botulinum toxin. It seems unlikely that a substance could function as a postganglionic transmitter when the muscle fibres appear to be unresponsive to it, as shown after 'denervation' by toxin. Although Darmstoff-contractions (like serotonin-contractions) cannot, in the normal preparation, be blocked by hexamethonium, they appear nevertheless to be 'neuronal' in origin; and their atropine-fastness seems to be in line with that of the other neuronal stimulants enumerated above.

Vogt (132) believes that Darmstoff may act by facilitating the spread of rhythmic "myogenic impulses" in the rabbit gut. It is difficult to reconcile this view with the observation that, after treatment with botulinum toxin, Darmstoff-contractions are abolished even when pendulum movements are unaltered.

A further point worth mentioning is Fischer and Vogt's finding that crude preparations of Substance P are contaminated with Darmstoff (57, 130). Therefore, some aspects of the pharmacology of Substance P may need checking. For instance, there have been reports that "Substance P"-contractions are abolished, in the rabbit's ileum, by short periods of cooling (also sometimes by botulinum toxin). It remains to be seen whether this change is not in fact due to loss of response to the Darmstoff which is present in Substance P as a contaminant; in other words, it is possible that some preparations of the rabbit's ileum may be more sensitive to the Darmstoff than to Substance P proper, and that so-called "Substance P"-contractions may in fact be Darmstoff responses.

G. The Classification of Intestinomotor Drugs by Means of Atropine

From the examples given above it is evident that the analysis of drug actions by means of atropine may be misleading, unless certain precautions are adopted. The first is not to rely on a single test object. This has been stressed by Ellis (48) in a study on piperidine. The action of piperidine, as of nicotine, can be blocked by atropine on the guinea-pig's intestine (93) and might therefore be erroneously classified as 'muscarinic'. But, as Ellis has shown, in confirmation of Euler's (51) earlier conclusions, concurrent tests on the rabbit gut reveal that piperidine-contractions are atropine-fast and therefore classifiable with nicotine.

This example illustrates the value of using two test objects; but in view of the fact that rabbit preparations are now known to vary, it is advisable to use atropinesterase-free rabbits and to confirm the atropine-resistance of nicotine-contractions on each preparation before tests are begun with unknown substances. Moreover, when 'nicotinic' activity is suspected it is useful to obtain confirmatory evidence with botulinum toxin and with BW284C51.

An instance of a drug which may act by different mechanisms according to the species from which the test-object is taken is provided by histamine. In rabbit as in guinea-pig gut the response to histamine is atropine-resistant; but it appears to be so for different reasons in the two preparations. In the guinea-pig gut it is due to the failure of atropine to block histamine receptors except in high concentrations; botulinum toxin also does not alter histamine responses. But in rabbits the atropine-fastness of histamine resembles that of neuronal stimulants in that histamine responses are potentiated by BW284C51 and are reduced or abolished by botulinum toxin (11). The classification of this and of other intestinomotor drugs, including barium and potassium chlorides, is discussed more fully elsewhere (11).

IV. THE MUSCLES OF THE BLADDER

The complexities of bladder innervation and of species differences in the response to certain drugs, for instance to adrenaline, are very extensively dealt with in the review by Gruber (62) to which the reader is referred. Before discussing the action of atropine it is necessary to consider briefly the reactions of the bladder to some autonomic drugs.

In this field pharmacological experimentation appears to have been confined mainly to the bladders of those species in common laboratory use. The stimulus of re-immersion into some of the older literature may remedy this regrettable tendency. Particularly thought-provoking are the two classical papers by Elliott (45, 46) written at the dawn of the neuro-humoral era. His diagram of the various types of bladder innervation (46, Fig. 11), which is reproduced in Figure 1, may serve as a useful pharmacological guide, for Elliott also showed that the effect of hypogastric stimulation is in every species mimicked by the action of adrenaline (see also 62, Table 4). We may then, extending the meaning of Elliott's diagram, assume the "broken signs inside the muscle wall" to represent adrenaline receptors. The unbroken signs outside it would represent acetylcholine receptors, though here the pharmacological evidence is admittedly incomplete, as acetylcholine has not been tried in all the species that Elliott examined. It is evident, however, that there could exist an interesting pharmacological duality in the detrusor muscles of ferrets and frogs (and in male goats), since these muscles may contract not only in response to acetylcholine but also to adrenaline. Elliott thought it possible that in these animals the muscle innervated by the hypogastric nerves is a sheet separate from that innervated by the pelvic nerves, being an upward extension from the urethra and trigone areas, where motor receptors to adrenaline occur in all mammals. It would be useful to re-examine this suggestion of Elliott's as it remains uncertain to this day whether the

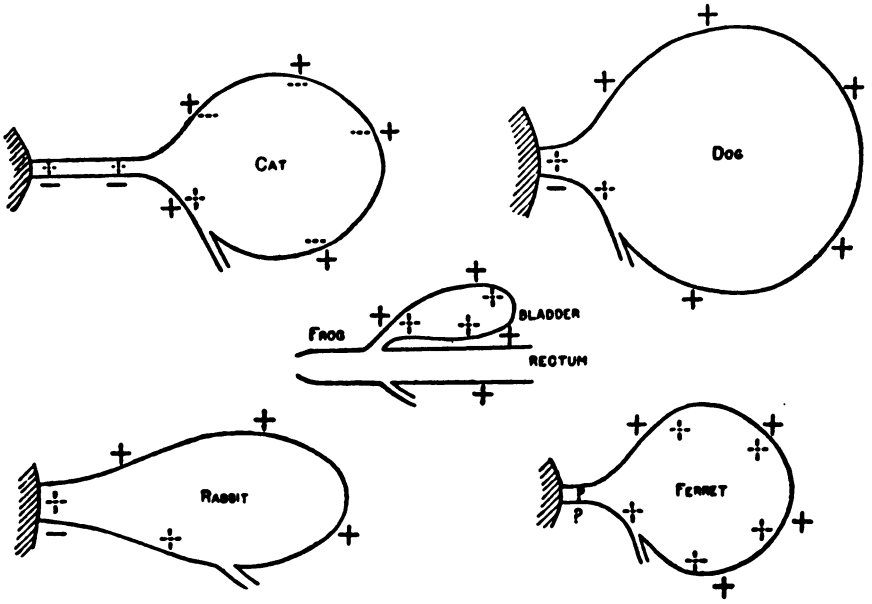

FIG. 1. Diagram of the innervation of various types of bladder and urethra. Level of entrance of ureters is shown, and the position of the genital ducts is indicated by the shaded mass of the prostate overlying the urethra. The distribution of the sacral nerves is given by the signs outside of the muscle, that of the hypogastrics by the broken signs inside the muscle wall (Elliott, 46).

adrenaline- and the acetylcholine-receptors are present together in the same muscle fibres, as is believed to be the case in the cat's nictitating membrane, or whether they are distributed in two distinct muscle sheets. The problem does not arise in the cat's bladder, as the detrusor muscle *in vitro* contracts in response to acetylcholine and relaxes in response to adrenaline; there is thus the usual reciprocity of pharmacological reaction which is found in other viscera. These facts are relevant to the discussion of atropine-resistance.

The bladder presents the advantage over other viscera that most of its parasympathetic ganglia can be located as visible swellings either on the peritoneal surface of the organ, or at some little distance from it in the pelvic ganglia (89, Fig. 29; 91); in the dog the ganglia are found 0.5 cm. away from the bladder (70). Langley (89) showed that the bladder contracted when nicotine was administered, whether parenterally or by direct application to the ganglia of the pelvic plexus. It is of interest to recall his observation that excess of nicotine (35 mg. per cat) paralysed the response to preganglionic, but not that to postganglionic, stimulation; in other words, excessive nicotine does not appear to depress the musculature of the bladder unduly.

The nature of the parasympathetic postganglionic neurones in the bladder remains unsolved. Several authors are agreed on the atropine-resistance of detrusor contractions elicited by parasympathetic stimulation (69, 70, 89); their experiments were performed on rabbits, cats and dogs. In the most recent

investigation of Edge (44) 1 mg. of atropine sulphate per kg. actually potentiated the response to pelvic nerve stimulation in cats. The following observations serve to locate the atropine-fastness at or beyond the parasympathetic postganglionic endings. Langley reported that stimulation of the postganglionic fibres was still effective after 10 mg. of atropine sulphate and in Henderson's experiments, also on cats, even 100 mg. of atropine failed to block nicotine-contractions *in vivo*; the atropine-fastness of nicotine-contractions has been confirmed in this laboratory on the cat's bladder *in vitro* (128). Both in Henderson and Roepke's experiments on dogs (70) and in the most recent experiments of Edge on cats, evidence was presented that after atropine the bladder became insensitive to small doses of acetylcholine but responded to larger doses, presumably by ganglion stimulation since the effect could be abolished by hexamethonium (44).

Edge's experiments provide further evidence relevant to this discussion. Firstly, adrenaline and noradrenaline produced only relaxation of the cat's bladder isolated *in vitro; in vivo*, other effects were observed due probably to vasoconstriction. But the results *in vitro* seem to exclude the possibility of adrenergic postganglionic motor neurones in the parasympathetic supply, since adrenaline was not motor to the detrusor muscle. This view is strengthened by the observation of Edge that the parenteral administration of dihydroergotamine abolished hypogastric contractions but did not affect the response to sacral root stimulation. It is not known whether the parasympathetic response persists in the combined presence of atropine and dihydroergotamine.

Other available facts on the neuro-humoral physiology of the bladder do not prove the nature of the parasympathetic postganglionic neurones. For instance, the acetylcholine released by pelvic nerve stimulation in Henderson and Roepke's (70) experiments could have originated entirely from preganglionic endings. It would be useful to know whether acetylcholine appears in bladder perfusates after postganglionic or nicotine stimulation; it would be desirable, also, to have data on the acetylcholine content of the postganglionic nerve strands lying on the bladder wall.

Again, in the experiments of Dickson and Shevky (42) paralysis of the pelvic nerve by botulinum toxin is described, but the location of this paralysis was left in doubt and could have been either pre- or postganglionic. The same doubts apply to the few existing facts about the action of anticholinesterases. In Edge's experiments responses to pelvic nerve stimulation were reduced by eserine, but those to sacral root stimulation were potentiated. As in the gut, it would be desirable to ascertain whether or not bladder responses to postganglionic or to nicotine stimulation are potentiated by anticholinesterases, and abolished by botulinum toxin.

V. SALIVARY GLANDS

It has been known for some considerable time that the secretomotor and the vasodilator effects of the chorda tympani are unequally susceptible to atropine block. As shown by Heidenhain in 1872 (68), even when salivary secretion is

completely paralysed by atropine there is still an increase in the blood flow through the salivary gland during stimulation of the chorda. This phenomenon has recently been re-examined by Hilton and Lewis (75, 76, 76a). They have found that the administration of botulinum toxin into the submandibular gland of cats abolished both the secretory and the vasodilator effects of parasympathetic stimulation. After paralysis of the chorda by the toxin the effector cells responded as usual to pilocarpine and also to sympathetic nerve stimulation. This suggests that the constituent fibres of the chorda tympani are all of the cholinergic variety. It raises the question whether or not the atropine-resistance of the vasodilator response is due to 'proximity' of the nerve endings. Such a view assumes the existence of special vasodilator fibres to the gland, but this has been questioned by Hilton and Lewis. Their evidence suggests that this particular phenomenon differs from any of the examples considered above; it seems to be explicable on an entirely different principle, namely the elaboration, by an enzyme from the gland, of a vasodilator substance the action of which is not affected by atropine. That the gland does in fact produce a stable vasodilator agent was shown after arterial injection of acetylcholine. Even if the artery to the salivary gland was occluded for 1 min., starting 5 sec. after the acetylcholine injection, vasodilatation occurred on re-establishing the circulation, although during the 5 sec. the injected acetylcholine had passed through the gland. Likewise if the chorda tympani was stimulated during the first 15 sec. of a 1 min. period of circulatory arrest, vasodilatation occurred at the cessation of occlusion. Perfusate collected from the gland during stimulation of the chorda contained a stable substance, the vasodilator activity of which was not blocked either by atropine or by mepyramine.

Further experiments have led Hilton and Lewis (76, 76a) to the conclusion that the formation of this substance was in fact secondary to the release, during activity, of a proteolytic enzyme from the cells of the salivary gland. Perfusates from activated glands, tested on isolated intestinal smooth muscles, were pharmacologically inert. If, however, such perfusates were incubated for a time with plasma, they became pharmacologically active as a result of the formation of a "slow" smooth muscle contracting substance, thought to be the same as the vasodilator agent. Thus perfusates from activated glands, and also the saliva itself, appear to contain an enzyme capable of producing an active substance from plasma. This substance is probably a polypeptide, in view of certain pharmacological and physico-chemical resemblances to bradykinin. The enzymatic formation of bradykinin from plasma globulins was discovered by Rocha e Silva *et al.* (113), who first obtained this polypeptide by incubating plasma with *Bothrops* snake venom. It is interesting to note that a proteolytic enzyme is present not only in the saliva of some snakes but also in that of certain mammals, thus providing partial explanation of the atropine-resistant vasodilatation. However, it is still necessary to explain how atropine discriminates between two secretomotor actions of acetylcholine on the salivary gland cells. The first, which leads to salivary flow, is blocked. The second, which initiates the extrusion of the enzyme to form bradykinin, is not; nor is, apparently, the

increase in metabolic activity of the gland prevented by atropine, as was shown by Barcroft (21).

VI. ANTIDROMIC PHENOMENA

Two phenomena which occur in rabbits after antidromic stimulation of sensory nerves have recently attracted the attention of physiologists. Both effects persist after atropine. Unlike most of the instances cited above the evidence from other sources is in agreement with that provided by atropine, and neither process would appear to depend upon cholinergic transmission.

1. Trigeminal pupillary constriction. In 1824 Magendie (95) made the observation that intracranial section of the fifth nerve in rabbits was followed by a prolonged constriction of the homolateral pupil. The investigation of this phenomenon was taken a stage further by Claude Bernard (23) who showed that the trigeminal constriction occurred even if the oculomotor nerve was cut and also after conjunctival instillation of "belladonna". The phenomenon has been studied recently in greater detail by Maurice (98). The pupillary response can be elicited by mechanical or electrical stimulation of the fifth nerve in rabbits, but not in cats or dogs. It occurs even after degeneration of the sympathetic supply to the orbit. If the trigeminal is sectioned pupillary constriction can be elicited again by stimulation of the peripheral, but not of the central, end of the nerve; but after degeneration of the nerve the response cannot be elicited. Maurice has drawn attention to the following differences between oculomotor and trigeminal pupillary constriction: (a) the trigeminal effect is slower in onset; (b) recovery from maximal constriction is usually very much slower after trigeminal than after oculomotor stimulation (½ to 2 hr. as against 1 min.); (c) atropine sulphate blocks the oculomotor but not the trigeminal effect.

The long duration of the trigeminal constriction makes it appear highly unlikely that the phenomenon could be cholinergic, since there is sufficient cholinesterase in the rabbit's iris to nullify within 1 min. the cholinergic effect of previous oculomotor stimulation.

Irin. It is, therefore, of interest that it has been possible to extract from freshly excised (constricted) irides and from trigeminal nerves of rabbits a pharmacologically active substance which is not antagonised by atropine (9a). This substance, 'irin', is soluble in 95 per cent acetone and in chloroform. The most sensitive test-object for its detection has proved to be the atropinised rat colon. This preparation responds to 0.5–1 mg. of iris extract, in a 5 ml. organ-bath, or to 5–10 μg. of a more purified 'crude irin'. This substance can be differentiated from: (a) 5-hydroxytryptamine by lysergic acid diethylamide; (b) histamine by the fact that the colon preparation is insensitive to this drug; (c) from bradykinin, which relaxes the rat colon; moreover, irin is not destroyed after 2 hours' incubation with heparinised rabbit blood; (d) from substance P by its extractability in 20 volumes of acetone; and from other polypeptides by the fact that it is not destroyed by chymotrypsin; (e) from adenosine triphosphate and from the substance of Major, Nanninga and Weber by its ability to

contract rabbit and guinea-pig ileum preparations in the presence of atropine and mepyramine.

Further work has shown that irin behaves like an acid (128). In 8 paper electrophoreses conducted at pH's 5.2–9.1 it has migrated towards the anode. Moreover, when partitioned between equal volumes of water and of chloroform irin is found mainly in the watery phase if the latter is alkaline; but if the watery phase is acid most of the irin moves into the chloroform.

2. Antidromic vasodilatation in the rabbit's ear. The characteristics of the vasodilator response in the rabbit's ear evoked by antidromic stimulation of the great auricular nerve have been described by Holton and Perry (82). Armin and Grant (15) have shown that acetylcholine dilates the central artery of the rabbit's ear, and that atropine prevents this dilatation. On the other hand, the antidromic vasodilatation in Holton and Perry's experiments was not affected by atropine. In this case atropine-resistance is a true indication that the phenomenon is non-cholinergic. There is other evidence from which this can be deduced, namely (a) the prolonged duration of the vasodilator response (30 minutes after 25 stimuli); and (b) its *reduction* by anticholinesterases (80). In both these respects the antidromic vasodilator response resembles that produced by adenosine triphosphate (ATP) and in their most recent paper Holton and Holton (81) have presented evidence of a release of ATP in the rabbit's ear during antidromic stimulation.

VII. SKELETAL MUSCLE AND GANGLIA

It is usual to distinguish the 'nicotinic' from the 'muscarinic' actions of acetylcholine by their difference in atropine susceptibility, but there are certain observations which suggest that this may be only a matter of degree. Thus Abdon (1) has reported that the action of acetylcholine on frog skeletal muscle is antagonised by atropine sulphate provided the muscle is soaked for 1 hour in the drug. The antagonism between atropine and acetylcholine was studied earlier by Clark (33) on the frog's rectus abdominis and by Dale and Gaddum (40) on isolated strips of denervated cat's diaphragm. In Abdon's experiments on the rabbit's gastrocnemius muscle 'quick' contractions elicited by close arterial injections of acetylcholine could be abolished by atropine, although the alkaloid did not affect motor nerve twitches. Since it is necessary to use larger amounts of acetylcholine for the elicitation of 'nicotinic' than of 'muscarinic' effects, Abdon points out that it is not surprising that larger amounts of atropine are required for competitive blocking of the nicotinic effects.

Although it has been generally considered that atropine is inert at autonomic ganglia, there is now considerable evidence to the contrary. This question has recently been examined on normal ganglia with intact circulation by Fink and Cervoni (56). The ganglion-blocking potency of atropine was determined by comparing the reduction in the contractions of the cat's nictitating membrane evoked by pre- and by postganglionic stimulation. Intravenous administration of 3.9 mg. of atropine per kg. produced a 50 per cent depression in the 'preganglionic' response, which was attributable to an interference with synaptic

transmission. The ganglion-blocking power of atropine was only 1/5th that of tetraethylammonium, whilst that of the quaternary methylatropine was 8 times greater.

On perfused superior cervical ganglia variable results have been obtained with atropine. In the experiments of Feldberg and Vartiainen (55), the response to preganglionic stimulation or to injected acetylcholine was abolished only when the dose of atropine was raised to 100 μg. On the other hand Konzett and Rothlin (86) were able to extinguish the ganglionic response to 10 and 20 μg. of acetylcholine with as little as 0.3 μg. of atropine sulphate. However, their experiments were performed on chronically denervated ganglia, and evidence is accumulating that after preganglionic denervation the superior cervical ganglion cells may undergo changes which increase their similarity, pharmacologically, to the 'muscarinic' receptors in smooth muscles and glands. It is, for instance, no longer possible to block the acetylcholine response of such ganglia even with large doses of hexamethonium, or with other non-depolarising ganglion-blocking substances (13, 108). Moreover, the response to muscarine-like drugs appears to be more regularly obtainable on denervated than on normal ganglia and is easily blocked by atropine, as is the response to acetylcholine itself (in about 50 per cent of experiments) (13). Even in normal perfused ganglia it is possible to block the responses to 2268F and to muscarine-like drugs with 0.2 to 1 μg. of atropine (sulphate), without affecting acetylcholine responses or synaptic transmission (8, 13). Larger amounts of atropine (1 to 10 μg.) may reduce acetylcholine responses without altering synaptic transmission, whilst 100 μg. abolish both.

There is another nicotinic action of acetylcholine which appears to be atropine-sensitive. Under certain conditions the effect on the adrenal medulla of splanchnic nerve stimulation can be abolished by small doses of atropine, as can the effect of injected acetylcholine (54).

Lastly, effects of acetylcholine on the central nervous system are sometimes classified as 'nicotinic', yet numerous examples of their suppression by atropine are found in the literature. Although the evidence of different authors is often conflicting (53), it is clear that atropine not only antagonises some of the actions of acetylcholine on various parts of the central nervous system, but also interferes with certain reflex responses and with other types of central nervous activity.

VIII. SUMMARY

The well known fact that atropine susceptibility is not an infallible criterion of cholinergic transmission is discussed in some detail. Various examples of atropine-resistant nerve effects have been considered, and the following types of atropine-resistance can be distinguished:

1) Apparent resistance which is due to destruction of atropine in the animal. This occurs in a proportion of rabbits, in which atropinesterase is present in the blood and in some tissues. The effect of atropine is then very brief and may erroneously be thought to be absent.

2) Atropine-resistance in cholinergic neuro-effector systems, as for example in

intestinal preparations from some species, and possibly in the bladder. In the intestine corroborative evidence provided by anticholinesterases and by botulinum toxin strongly suggests that the nerve endings concerned are cholinergic, and therefore supports the theory put forward by Dale and Gaddum that this type of atropine-resistance is due to "proximity" of the nerve endings to the effector cells. It is pointed out that the classification of intestinomotor drugs by means of atropine is influenced by these considerations.

3) The secondary formation of an atropine-resistant pharmacological agent, *e.g.*, of bradykinin in the salivary glands. Acetylcholine or chorda stimulation lead to the extrusion of a proteolytic enzyme capable of forming bradykinin from serum proteins.

4) Atropine-resistance due to non-cholinergic transmission. Two examples of this are considered: (a) antidromic trigeminal pupillary constriction; and (b) antidromic vasodilatation in the rabbit's ear. Confirmatory evidence from other sources indicates the non-cholinergic nature of these effects.

5) Several nicotinic actions of acetylcholine are relatively resistant to atropine, but can nevertheless be blocked by the alkaloid in appropriate concentrations.

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