Section of Anæsthetics

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Problems of Developing Muscle-Relaxants in the Laboratory

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PROPERTIES REQUIRED OF RELAXANTS FOR CLINICAL USE

FOUR relaxants, which act by blocking the nerve-muscle junction, are already used clinically in this country. We cannot tell whether new relaxants are required, nor what properties would be particularly desirable in them, until we have answered the following questions:

(i) What properties does the clinician need?

(ii) How far do existing relaxants fulfil his requirements?

In Table I, I have tried to summarize the properties that are particularly important clinically.

TABLE I.—PROPERTIES REQUIRED OF MUSCLE-RELAXANTS FOR CLINICAL USE

(1) *Specificity of muscle-relaxant action.*
Adequate muscle-relaxation at doses not producing side-effects, such as:
  (*a*) Release of histamine
  (*b*) Block of sympathetic ganglia
  (*c*) Block of parasympathetic ganglia

(2) *Effects on respiration.*
  (*a*) Sparing: e.g. lower abdominal surgery, explorations requiring anæsthesia
  (*b*) Arresting: e.g. thoracic surgery

(3) *Duration of muscle-relaxant action.*
  (*a*) Short: e.g. intubation, explorations
  (*b*) Medium: e.g. shorter operations
  (*c*) Long: e.g. longer operations
  (*d*) Very long: e.g. spastic paralysis

(4) *Antagonism by reasonably safe substance.*

(5) *Relation to anæsthetics.*
  (*a*) Lack of serious antagonism between anæsthetic and relaxant
  (*b*) Suitability for mixture with intravenous anæsthetic

This table is largely based on discussions with members of this Section. They have made it clear that a property which suits one purpose may not necessarily suit another. For

example, a drug which relaxes muscles for only a few minutes might be best for intubation, while another substance, an equipotent dose of which lasts several times as long, would be more suitable for an abdominal operation.

The properties required of a relaxant for a particular operation will depend, too, on the background of anæsthetic and surgical technique to be used. Where, for instance, it is expected to ventilate the lungs artificially, the relaxant need not spare natural respiration.

It seems to be clear from Table I that a successful muscle-relaxant must combine a whole set of desirable properties. It is worth while attempting to outline very briefly how far existing muscle-relaxants exhibit some of these properties in man.

Gallamine triethiodide (Flaxedil) does not appear to block sympathetic ganglia at effective doses; but it does block parasympathetic ganglia, giving rise to the tachycardia recently described by Drs. Wylie and Doughty (1951). The figures published by these authors and by Unna *et al.* (1950*b*) suggest that gallamine spares respiration in man to a greater extent than tubocurarine or decamethonium.

Tubocurarine chloride is liable to produce each of the three side-effects given in Table I (*see* e.g. Grob, Lilienthal and Harvey, 1947). It acts for longer than other muscle-relaxants in equipotent doses.

Dimethyltubocurarine iodide has not yet been reported to produce in man side-effects similar to those of tubocurarine. According to Unna *et al.* (1950*a*) this compound spares respiration in volunteers more than the other three established relaxants.

Decamethonium iodide is free, at effective doses, of the side-effects which may accompany the other relaxants. An effective dose acts for a relatively short time. Decamethonium iodide is not antagonized by neostigmine; and it appears to be agreed that its antagonist—pentamethonium iodide—is not a safe drug to administer after surgical operations (*see* e.g. Davison, 1950). Unlike curare derivatives decamethonium iodide antagonizes ether (*see* Paton and Zaimis, 1950).

From the above summaries of their properties in man, it would seem that these four compounds represent a fairly satisfactory set for clinical use. However, as far as purely clinical needs go, niches would seem to exist for:

(i) A really short-acting relaxant, without the side-effects described, with sparing of respiration and preferably antagonized by neostigmine.

(ii) A relaxant acting for as long or longer than tubocurarine, but without liability to its side-effects, and antagonized by neostigmine.

Assessment of Muscle-relaxants in the Laboratory

I have put clinical requirements and their present state of fulfilment first because they are, in my opinion, the starting point from which one should attempt to develop in the laboratory new muscle-relaxant drugs. Our next problem is—what experiments in animals or on isolated animal tissues will tell us how far a new substance is likely to fulfil the needs of clinicians? The best way to answer this question is perhaps to consider first the established muscle-relaxants which I have already mentioned. We can then compare the results obtained with these drugs in animals with those obtained in man, both in volunteers and in patients. It will be most convenient to follow out this comparison under the heads given in Table I.

(1) *Specific Relaxant Activity*

From the point of view of its specific relaxant activity, a good compound is one in which the paralysing dose is many times lower than the dose producing any undesirable side-effects.

If we want to try and assess this relationship in laboratory experiments, then we must measure both the dose which relaxes muscles and the doses which produce the various side-effects given in Table I. Each of these measurements presents its own problems. How are we to measure muscle-relaxant activity? And, what species of animals should we use?

(*a*) *Animal species to be used.*—I would like to limit the discussion of animal species to mammals. It is true that muscle-relaxant activity has been assessed in birds and amphibia, but I think it is *a priori* less likely that vertebrates of another class will indicate the probable activity of compounds in man.

In order to show how results may vary from species to species, I have given in Fig. 1 the relaxant activities of the four clinical drugs in relation to tubocurarine in 5 mammals. These figures are based on the publications of Collier and Hall, 1950; Collier *et al.*, 1948; Mushin *et al.*, 1949; Paton and Zaimis, 1949; Unna *et al.*, 1950*a*, 1950*b*; Wien, 1948.

Fig. 1 shows that the order of activities varies widely from mammal to mammal. The only animal which even places the drugs in the same order of activity as in man is the cat.

The rabbit does no more than put them in the same relation to tubocurarine as they stand in man. The rat and mouse are unduly insensitive to the synthetic compounds.

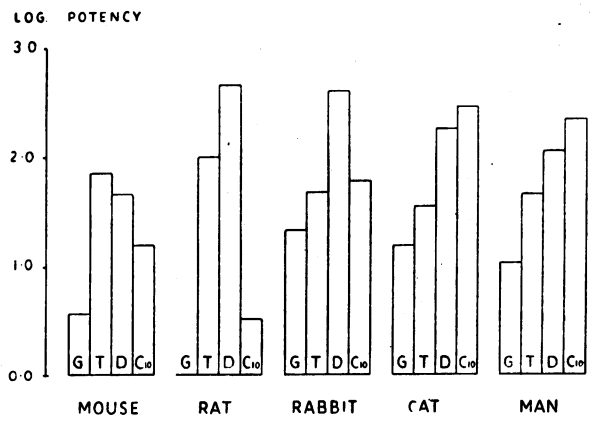

FIG. 1.—Log. potency of muscle-relaxants in various mammals in relation to tubocurarine in the rat (= 2·0). G = Gallamine triethiodide. T = Tubocurarine chloride. D = Dimethyltubocurarine iodide. C_{10} = Decamethonium iodide. The log. potency of gallamine triethiodide in the rat = 0·0 if that of tubocurarine = 2·0 (*see* Wien, 1948).

I think Fig. 1 shows that no laboratory animal can give an accurate forecast of the activity of a new compound in man. We certainly ought to make tests in the cat and rabbit, and, for good measure and because of its value in anæsthetic experiments, in the mouse.

(*b*) *Type of test to be used.*—Our next question is—what muscle or group of muscles ought we to use in our tests? Since generally, though not always, surgeons require relaxation of the abdominal body-wall, it might be thought that the musculature of this region would be the most desirable for tests in animals. Owing to technical difficulties, these muscles are not generally used in the laboratory. Longo and Bovet (1949) have, however, described a method of measuring intra-abdominal pressure in the rabbit; and they have compared by this means gallamine, tubocurarine and other drugs.

Most of the simpler and quicker tests at present in use depend on observing the failure of a group of muscles which usually work in co-ordination. Responses of this type which have been used include:

(i) Loss of righting reflex (Collier *et al.*, 1949*a*).

(ii) Loss of ability to hold on to a rotating surface or inclined plane (Skinner and Young, 1947; Allmark and Bachinski, 1949; Collier *et al.*, 1949*b*).

(iii) Head-drop in the rabbit (Dutta and MacIntosh, 1949).

Tests of types (i) and (ii) give a quantal response; and results can be expressed in terms of the 50% effective dose, or ED50. The head-drop test of Dutta and MacIntosh, which involves slow intravenous infusion of a drug solution, is in the nature of a titration; the end-point being the drop of the rabbit's head.

In my opinion tests of this type are exceedingly useful. They are rapid and simple, because they only involve intravenous injection, and they give a good idea of the capacity of a drug to relax important groups of skeletal muscles. But, of course, an animal may respond to other drugs than muscle-relaxants by losing its righting reflex or falling off a drum. So for this reason, and for others, results obtained with these simple tests must be checked in another way.

Preparations useful for this purpose can be made with the *tibialis*, *soleus* or other muscles of the cat. Since these muscles are not separated from the whole animal, and the blood flows to and from them normally, they can be used to show for how long a drug acts, and to compare the effect with that on respiration.

It is interesting that experiments with *soleus* and *tibialis*, performed by Paton and Zaimis (1950), show that the two muscles react differently to relaxants. *Tibialis* is relatively more sensitive to decamethonium than is *soleus*; but it is not more sensitive to tubocurarine. It is the *tibialis* of the cat which puts the drugs in the same order as does the hand-grip test in human volunteers. If the cat's *soleus* had been used, the relative potency of these drugs in the cat would have been different. By the same token, we do not know, as Drs. Paton and Zaimis have pointed out, how far the activities of these drugs in reducing the hand-grip in conscious volunteers correspond with their relative abilities to relax the abdominal muscles

of people under anæsthesia. I think we may agree with these authors that we can tell the relative action of different relaxants on such muscles only by observations made in clinical practice.

(*c*) *Release of histamine.*—The release of histamine is one of the most important side-effects we are likely to meet. How are we to detect and measure this release? At least two methods have been applied to muscle-relaxants in the laboratory. One is that of Rocha e Silva and Schild (1949), who were able to assay the histamine released when a solution of tubocurarine was shaken up with isolated rat's diaphragm.

Dr. Wien (Mushin *et al.*, 1949) used this method for estimating how far gallamine releases histamine. He concluded that it releases one-fifth to one-half as much histamine as does tubocurarine. If this figure were applicable to man, we might expect equipotent doses of the two relaxants to release about the same amount of histamine, since the synthetic compound has about one-fifth of the activity of the curare derivative. Various authors have reported evidence of histamine release by tubocurarine in man, but I do not know to what extent gallamine may have the same effect.

An alternative method of studying histamine release has been devised by MacIntosh and Paton (1949). In the cat a small intravenous dose of a compound which releases histamine will produce a rapid fall in blood pressure which lasts a short time. This fall resembles that due to injection of a little histamine, except that it does not start till about 20–25 seconds after the injection. The delay is presumably caused by the actual process of release. A fall in blood pressure of this type may be seen after injections of *d*-tubocurarine.

Since curarizing drugs are also liable to lower the blood pressure by blocking sympathetic ganglia, it is necessary, before using this fall as an index of histamine release, to block autonomic ganglia with a suitable agent.

By using this test MacIntosh and Paton showed that many organic bases including tubocurarine, dimethyltubocurarine and, to a much less extent, dodecamethonium iodide, are liable to release histamine. Some of their results are expressed in Table II, together with some results obtained by Depierre (1947).

TABLE II.—COMPARISON OF HYPOTENSIVE AND PARALYSING DOSES OF MUSCLE-RELAXANTS IN CAT AND DOG. DOSES IN MICROGRAMS PER KILOGRAM, INTRAVENOUS

	Cat		Dog		Ratio of hypotensive to paralysing dose
Relaxant	Paralysing dose	Hypotensive dose*	Paralysing dose	Hypotensive dose	
Tubocurarine Chloride ..	300	600	200	1,000	2 (cat) 5 (dog)
Dimethyltubocurarine iodide	50	600	—	—	12
Gallamine triethiodide ..	—	—	1,000–2,000	50,000	25–50
Decamethonium iodide ..	30	> 4,000	—	—	> 133

* After ganglia blocked.

Obviously the delayed depressor response provides a means of comparing in the cat or dog the doses which block the nerve-muscle junction with those which release histamine. I must leave the anæsthetists to decide how far the results agree with those in man.

(*d*) *Sympathetic block.*—The method which has been successfully used for studying sympathetic block involves recording the tension of the nictitating membrane of the cat's eyelid in response to repeated electrical stimulation of the sympathetic in the neck. This stimulation reaches the membrane through relays of ganglion cells. If these are blocked the tension slackens.

It is obvious, in view of the liability of muscle-relaxants to block autonomic ganglia, that a test of this type ought to be included in the overhaul of new muscle-relaxants.

(*e*) *Parasympathetic block.*—For the same reason we ought to investigate in muscle-relaxants their ability to block parasympathetic ganglia. Jacob and Depierre (1950) have used the response of the dog's heart to stimulation of the vagus to study this type of ganglionic block. And they found that gallamine blocks the dog's vagus at much lower doses than it blocks sympathetic ganglia. This finding satisfactorily accounts for the ability of gallamine to quicken the heart-rate in man, recently described by Drs. Wylie and Doughty.

An alternative preparation for studying parasympathetic block is that of the guinea-pig or rabbit ileum. Feldberg and Lin (1949) have shown that tubocurarine abolishes the reflex response of the ileum to increase in internal pressure, and Paton and Zaimis (1949) have shown the same effect with compounds like C6. Feldberg (1951) has also shown the ability

of tubocurarine and C6 to reduce the response of the ileum to nicotine. This preparation may therefore be used for study of parasympathetic block.

(2) *Effect on Respiration*

Can we foresee from experiments in animals how far a muscle-relaxant is likely to spare respiration in man?

TABLE III.—SPARING OF RESPIRATION BY MUSCLE-RELAXANTS. IN ANIMALS, VALUES ARE RATIOS OF DOSE ARRESTING RESPIRATION TO DOSE EFFECTING PARALYSIS. IN MAN, VALUES GIVE PER CENT OF NORMAL VITAL CAPACITY AT DOSES EQUALLY ACTIVE ON HAND GRIP.

(The figures in brackets give the order in which the drugs spare respiration)

Relaxant	Mouse	Rat	Rabbit	Cat	Monkey	Man
Tubocurarine chloride ..	2·5	1·8	1·5 (3)	1·7	1·5	69 (3)
Dimethyltubocurarine iodide ..	1·7	3·1	3·0 (1)	—	—	84 (1)
Gallamine triethiodide ..	—	—	1·7 (2)	1·9	—	80 (2)
Decamethonium iodide ..	1·2	1·2	1·0 (4)	2·45	2·6	39 (4)

In Table III I have put together comparisons of the dose which causes a certain degree of motor paralysis with that arresting respiration in various animals. In the rabbit, for instance, the ED50 or the head-drop dose is compared with the LD50. In the cat the reduction of *gastrocnemius* twitch is measured against reduction of respiratory volume. These figures are derived from the following authors: Collier *et al.*, 1948; Mushin *et al.*, 1949; Paton and Zaimis, 1948; Unna *et al.*, 1950*a*, *b*. Table II shows the same puzzling reversals in different species. There is no doubt that decamethonium spares respiration in the cat. It is equally clear that dimethyltubocurarine spares respiration better than decamethonium in the rabbit. The figures for man are those of Unna *et al.*, who compare effects on hand-grip and vital capacity. In this comparison man and the rabbit put the drugs in the same order; and so the rabbit happens to give a surer guide than the cat.

(3) *Duration of Action*

At equipotent doses, each muscle-relaxant has its own characteristic duration of action. Can we foresee from experiments in animals what the duration of action in man is likely to be? The usual difficulties apply.

TABLE IV.—DURATIONS OF ACTION OF EQUIPOTENT DOSES OF MUSCLE-RELAXANTS IN VARIOUS SPECIES IN RELATION TO TUBOCURARINE (= 100)

Relaxant	Mouse	Rat	Rabbit	Cat	Monkey	Man
Tubocurarine chloride ..	100	100	100	100	100	100
Dimethyltubocurarine iodide	100	1,100	200–300	>100	—	83
Gallamine triethiodide ..	—	—	—	67	—	70
Decamethonium iodide ..	—	—	—	<100	200–300	79

In Table IV I have collected data on the duration of equipotent doses of our four muscle-relaxants in various mammals. It is as puzzling as the others. Decamethonium iodide is longer than tubocurarine in the monkey, shorter in the cat (Paton and Zaimis, 1948). Dimethyltubocurarine is much longer than tubocurarine in the rat and longer in the rabbit and cat (*see* Collier *et al.*, 1948; Swanson *et al.*, 1949), but shorter in man. It is clear that each species has its own reaction to each muscle-relaxant and none of the animals in Table IV foreshadows correctly the order of duration of these four compounds in man.

(4) *Antagonism*

Any method of assaying muscle-relaxant activity in the whole animal appears to provide a means of estimating antagonism by other compounds. The LD50, the ED50 for loss of righting reflex or falling off a drum, the head-drop dose, the response of *tibialis* in the chloralosed cat and so on can readily be used (*see* e.g. Chase *et al.*, 1947).

If we are content to envisage neostigmine as an antagonist, then tests of antagonism need only use this substance.

A further question that arises is—how far is the antagonist liable to have unwanted side-effects? But I must leave both this and the question of developing new antagonists.

(5) *Administration with Anæsthetics*

The mouse loses its righting reflex after suitable doses of anæsthetics as well as of muscle-relaxants. By estimating the ED50 for loss of the righting reflex in the mouse after giving an anæsthetic and a muscle-relaxant separately and together, the relationship of the two drugs may be studied.

THE DEVELOPMENT OF NEW MUSCLE-RELAXANTS

From the foregoing I think it is clear that, while we must carry out in the laboratory tests of the types I have outlined, we must be exceedingly cautious in applying the results

to man. We must bear in mind the general rules of specificity, which may be stated thus: *Each chemical species has its own characteristic effects in living organisms; and each biological species has its own characteristic reactions to a chemical species. Specific differences may be quantitative or qualitative.*

The development of new muscle-relaxants is a process of approximation. First, we must assess the salient features of molecular structure likely to lead to muscle-relaxant properties. Then we must design compounds of a suitable type, make them, and assess their properties. It will then no doubt be necessary to modify the compounds in order to improve these properties. As this is a long task it must be done with fairly rapid biological tests. When compounds of high activity have been produced they must be carefully examined by the series of tests I have already described.

Molecular form and neuromuscular block.—To begin with—what molecular forms are likely to possess powerful muscle-relaxant action? This is such a wide question that I must immediately narrow it down to—what molecular forms are likely to oppose acetylcholine in a reversible way at the nerve-muscle junction?

Applying the well-established principle that substances of similar molecular form may oppose one another, we can see from the formula of acetylcholine that substances possessing quaternary nitrogen atoms may well antagonize it. This is a fact established many years ago by Crum Brown and Fraser, long before the advent of the acetylcholine theory of nerve-muscle transmission.

If we imagine that, on muscle fibres, and on other structures sensitive to acetylcholine, there exist receptors which take up this compound, then we may suppose that antagonists of acetylcholine might temporarily occupy these receptors at the expense of the natural compound. We might expect that a molecule with two quaternary nitrogens, provided their distance apart corresponds with the distance apart of the receptors, would adhere more strongly to the receptors than a molecule with one quaternary nitrogen. And, generally speaking, it is true that the blocking activity of a compound is increased by addition of a second quaternary nitrogen.

I have mentioned the distance apart of our imagined receptors. If this is regular, then we may expect an optimum distance for our quaternary nitrogen atoms. Such an optimum exists and, in fact, is now known to be round about the distance occupied by 10 carbon atoms in a methylene chain. It is well known then that quaternary nitrogen atoms placed at a distance of 10 carbon atoms apart, as in decamethonium iodide, are likely to have strong neuromuscular blocking activities. These considerations apply to blocking agents both of curare and C10 type. But while curare appears merely to block the access of the acetylcholine to the receptors, decamethonium carries the process a stage further, fires off the contraction process and depolarizes the end-plate for some time. Acetylcholine and hence neostigmine therefore, while antagonizing curare, tend to potentiate decamethonium (*see* Paton and Zaimis, 1949, 1950).

The fact that decamethonium iodide is not antagonized by neostigmine, owing to its mode of action, is a disadvantage of this otherwise excellent compound. The question arises—how can we produce molecules having equivalent potency to decamethonium iodide, but antagonized by neostigmine?

I should like to illustrate from work in our laboratories (Collier and Taylor, 1949; Taylor and Collier, 1950, 1951) one of the ways in which this problem may be tackled. In tubocurarine and its dimethyl ether the quaternary nitrogen atoms are placed in *iso*-quinoline rings, linked together by two chains (*see* Fig. 2 (*a*)). In decamethonium salts (Fig. 2 (*b*) the quaternary nitrogens are linked by a simple decamethylene chain. In our attempt to solve the problem posed above, Taylor prepared compounds in which

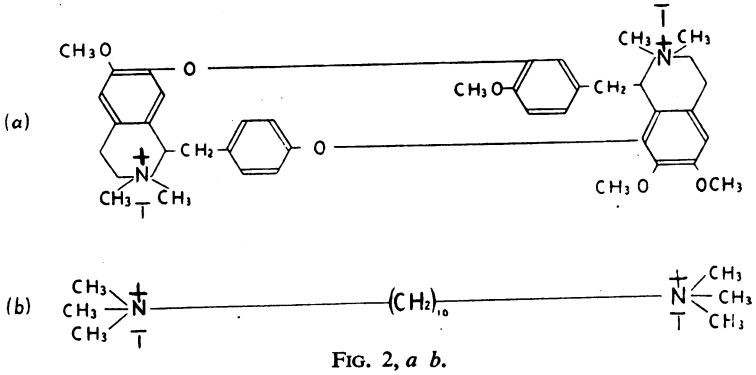

FIG. 2, *a b*.

quaternary nitrogen atoms, placed in *iso*quinoline, quinoline or other heterocyclic rings, were linked together by a decamethylene chain attached to the two nitrogens (Taylor, 1951).

The first compounds of this series he prepared—decamethylene-*bis*-quinolinium iodide and the corresponding *iso*quinoline derivative—both showed some ability to paralyse mice and rabbits. By a series of modifications of these compounds it was possible to produce substances of very high paralysant activity. Increase in activity was brought about by modifying the compounds in two directions: (1) by reduction of the heterocyclic rings; and (2) by introducing methoxy groups in the 6, 7 and/or 8 positions.

By reducing the quinoline compound to the 1.2.3.4-tetrahydroquinoline derivative, activity in the rabbit is increased about sixfold. Further reduction of the tetrahydro derivative to the *cis* or *trans* decahydroquinoline compound increased activity in the rabbit by about a further seven times. It is interesting that there is no appreciable difference between the activities of the *cis* and *trans* forms.

Similar results were obtained with the *iso*quinoline compounds. Reduction of the parent compound to the 1.2.3.4-tetrahydro derivative trebled paralysant activity in the rabbit. Taking the process of reduction further, the *cis* decahydro*iso*quinoline derivative was more than three times as active as the tetrahydro compound.

The second method by which we increased activity was by introducing methoxy groups in the 6, 7 and/or 8 positions. Introducing a methoxy group in the 6 position on the 1.2.3.4-tetrahydro*iso*quinoline compound increased activity in the rabbit about sevenfold. The 6.7-dimethoxy compound (No. 14, Fig. 2 (*c*)) and 6.7.8-trimethoxy derivative (No. 15, Fig. 2 (*d*)) were still more active.

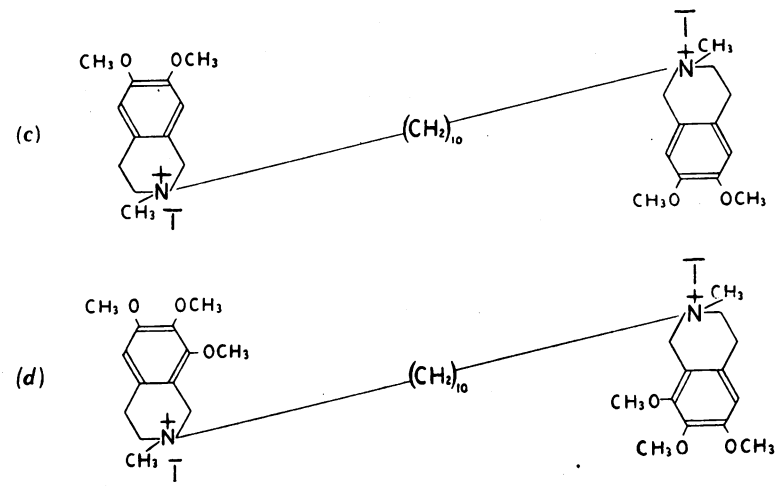

FIG. 2, *c*, *d*.

It will be seen from Fig. 2, in which the most active compounds are illustrated, that the process was one of making the end-groups resemble those of dimethyltubocurarine more closely. We have carried this process still further in Compound 20 (Fig. 2 (*e*)). It will be

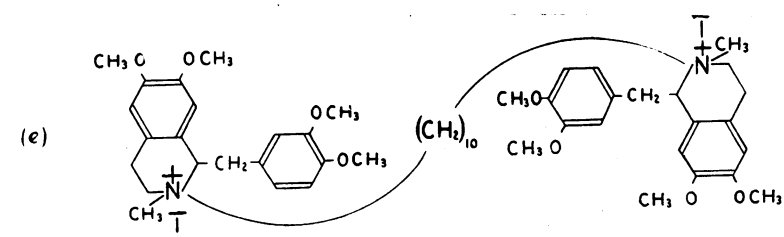

FIG. 2, *e*.

FIG. 2.—Synthetic compounds related to dimethyltubocurarine and decamethonium. (*a*) Dimethyltubocurarine iodide. (*b*) Decamethonium iodide. (*c*) Decamethylene-*aω-bis* [6.7-dimethoxy-1.2.3.4-tetrahydro*iso*quinolinium methiodide] (Compound 14). (*d*) Decamethylene-*aω-bis* [6.7.8-trimethoxy-1.2.3.4-tetrahydro*iso*quinolinium methiodide] (Compound 15). (*e*) Decamethylene-*aω-bis* [1.(3′ 4′-dimethoxybenzyl)-6.7-dimethoxy-1.2.3.4-tetrahydro*iso*quinolinium methiodide] (Compound 20).

seen that the end-groups of this compound resemble those of dimethyltubocurarine very closely indeed. In the cat, Compound 20 is the most active of all the synthetic compounds of this series that we have tried.

As might be expected, all of the compounds of this series which have been examined for this property are antagonized by neostigmine.

The development of compounds of this type raises further problems. Some of these problems arise because these compounds may antagonize acetylcholine not only at the nerve-muscle junction, but also in autonomic ganglia, or in relation to the enzyme which destroys acetylcholine. In short, these compounds may have ganglionic blocking or anti-cholinesterase activity. The latter type of activity has been demonstrated, for example, in polymethylene compounds bearing quaternary nitrogen atoms by Barlow and Ing (1948).

A second problem arises from the fact that many long chain organic bases, such as diamines and diamidines, liberate histamine, as MacIntosh and Paton have shown. In the diamines, liberation of histamine is greatest where there are 10 or 11 carbon atoms in the polymethylene chain.

We have attempted, in the case of the two most active compounds obtained—Numbers 15 and 20—to investigate a number of these questions in the laboratory by some of the methods described above. Some of the results of our work so far are summarized in Table V.

TABLE V.—PROPERTIES OF COMPOUNDS 15 AND 20 IN RELATION TO D-TUBOCURARINE (=100)

	Potency				Ratio LD50 to ED50	Duration	Total hypotensive activity	Antagonism neostigmine	Man	
Relaxant	Mouse	Rat	Rabbit	Cat	Rabbit	Cat	Cat		Potency	Histamine release†
Tubocurarine	100	100	100	100	100	100	100	+	100	100
Compound 15	23	5	600	*circa* 133	*circa* 120	*circa* 50	30	+	37*	200–1,200
Compound 20	23	4	360	„ 260	„ 90	„ 150	12	+	—	15–60

*Figure obtained by Dr. R. I. Bodman and colleagues.
†Ranges obtained by skin tests in two volunteers.

It will be seen from this table that both compounds are more active in the cat and rabbit and less active in the mouse and rat than tubocurarine. Compound 15 spares respiration in the rabbit more and Compound 20 less than tubocurarine. In the cat a dose of Compound 15 lasts about half as long as an equipotent dose of tubocurarine. On the other hand, the duration of action of Compound 20 in this animal is 50% longer than that of tubocurarine.

We have administered Compound 15 to a group of rabbits daily on five successive days without obtaining evidence of alterations in the animals' sensitiveness to the drug. We have also administered this compound to mice together with thiopentone or ether. As with tubocurarine and dimethyltubocurarine, the action of Compound 15 on the righting reflex is potentiated by ether. Thiopentone, on the other hand, has no obvious effect on the response of mice to Compound 15, as measured by loss of the righting reflex.

When Compound 15 is administered intravenously to the cat it lowers the blood pressure temporarily, after a short latent period. This depression is smaller than that produced by an equal dose of tubocurarine. Compound 20 also exhibits depressor activity in the cat, but it is less active in this respect than Compound 15.

My colleague, Miss B. M. Macauley, has investigated the release of histamine by Compounds 15 and 20, using the technique of Rocha e Silva and Schild. By exposing excised rats' diaphragms to 0·05% solutions of the drugs for thirty minutes, she finds that Compounds 15 and 20 liberate about the same amount of histamine as tubocurarine. Owing to certain technical limitations, however, the method does not appear to discriminate well with these compounds.

Dr. Bodman and his colleagues (1951) have administered Compound 15 to volunteers. They find that it possesses about one-third of the activity of tubocurarine in man. It thus shows the cat to be an unreliable guide to curarizing activity in man. The main side-effect of Compound 15, which appears to be the liberation of histamine, is important. At the dose giving 57% reduction of handgrip power (260 μg. per kg.) Dr. Bodman found side-effects, such as flushing, "pins and needles" and subsequent frontal headache to be severe. He confirmed this conclusion by injecting Compound 15 intradermally, when a marked wheal and flare similar to that given by histamine was produced. Considerably lower doses of Compound 15 than of tubocurarine were required to produce this response.

In view of Dr. Bodman's findings, and since Professor Bain (1949) has shown that the areas of wheals produced by intradermal injection of histamine in man bear a linear relation to the logarithm of the dose, we have attempted to compare the histamine-releasing potencies in man of Compounds 15 and 20 with that of tubocurarine by intradermal injection of graded doses in two volunteers. The results, which are expressed in Table V, confirm Dr. Bodman's conclusion regarding Compound 15, and indicate that Compound 20, on the other hand, is considerably less active than tubocurarine in this test.

Compound 20 has not yet been administered to man except in skin tests. In view of the difficulties I have described in the interpretation of animal experiments, I will not permit myself any forecasts of its possible activity or side-effects.

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Dr. R. I. Bodman: The results of clinical trials of "Compound 15 (A & H)" carried out by us in conscious volunteers may be seen in the Table I. Nine trials were done with doses varying from 1 to 20 mg. In each case the drug was injected into a volunteer whose hand-grip power and maximum inspiration were measured at intervals of two minutes. Significant reduction of power was seen only in the ninth case, in which over 50% reduction was brought about by a dose of 0·26 mg. per kg. body-weight of "Compound 15": an equivalent reduction might be expected from a dose of about 0·50 mg. per kg. of gallamine triethiodide ("Flaxedil"). In other words "Compound 15" has a potency about twice that of gallamine. Its duration of action is of the order of half that of gallamine.

The side-effects, which varied in severity from case to case, were "pins and needles", with flushing of the face and upper part of the body, accompanied by palpitations and tachycardia; these were followed by a frontal headache. The character of these effects and their successive onset led us to believe they were due to release of histamine; they became so severe that we decided it would be unwise to proceed with experiments on volunteers and unjustifiable to give the drug to anæsthetized patients.

TABLE I

RESULTS OF CLINICAL TRIAL OF "COMPOUND 15" IN CONSCIOUS VOLUNTEERS

Subject					Response			Side-effects			
No.	Volunteer	Wt. (kg.)	Dose (mg.)	Dose/wt. (μg./kg.)	Double vision	Reduction in hand grip	Reduction in max. inspn.	Pins and needles	Flushing	Palpitations	Headache
1	A	77	1	13	0	0	0	0	0	0	0
2	B	64	2	32	?	0	0	0	0	0	0
3	C	74	4	54	?	0	0	+	+	0	0
4	D	72	6	83	+	0	0	++	++	+	++
5	A	77	8	104	+	0	0	+	+	0	0
6	E	89	10	112	+	0	0	0	0	0	0
7	E	89	14	157	+	0	0	0	0	0	0
8	F	68	12	175	+	0	0	+++	+++	+	0
9	A	77	20	260	+	57%	0	+++	+++	+++	+++

Dr. Ronald Woolmer asked what reactions, other than hypotension, were used as evidence of histamine release, since hypotension itself might be attributable to a ganglionic blocking effect. Secondly, it was known that adrenaline acted to some extent as an antagonist to the depolarizing muscle relaxants, and he thought that the possibility of a high blood adrenaline in conscious volunteers should be taken into account, if the drugs to be tested were also affected by adrenaline.

Dr. A. H. Galley said that he noted that Dr. Collier discarded synthetic muscle relaxants which reduced blood pressure by means of histamine release ("histamine flush"). He wondered, however, whether it were possible to build up molecules containing one pair of quaternary groups separated by a ten carbon atom chain and another pair separated by a chain of only five (or six) carbons, thus:

$$\begin{array}{l} CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.N.(CH_3)_3.I \quad \alpha \\ | \\ CH_2.CH_2.CH_2.N.(CH_3)_3.I \quad \beta \\ | \\ CH_2.CH_2.N.(CH_3)_3.I \quad \gamma \end{array}$$

Hypothetical molecule containing three quaternary ammonium groups (α, β and γ) in which β and γ are separated from α by a chain of ten carbon atoms, whilst they are separated from each other by a chain of only five carbon atoms

Such a substance (assuming that some molecules did not neutralize the curarizing effect of others) might make it possible to obtain muscular relaxation and a bloodless surgical field (paralysis of sympathetic ganglia) with one and the same injection.

Dr. Collier (in reply): It is useful to have Dr. Bodman's results before us in detail to remind us how far we succeeded and how far we failed in foretelling human reactions to Compound 15 from animal experiments. We correctly inferred that in man Compound 15 would prove to be a muscle-relaxant of high potency and of short duration of action, which might release some histamine. We were incorrect however in thinking, from experiments in the cat and rabbit, that Compound 15 would be more active than tubocurarine in man and that full relaxant activity would be manifested by doses which did not release histamine.

As Dr. Woolmer says, it is necessary to block sympathetic ganglia in the cat before estimating release of histamine. But, without blocking ganglia, if a dose of drug causes no fall in blood pressure, it seems reasonable to conclude that no histamine has been released. Tests have shown that in the cat, even after blocking the ganglia, Compound 15 has less hypotensive activity than tubocurarine. Since this is the reverse of the situation in man, I believe that the human skin test described above is a better test for our purpose.

I agree with Dr. Woolmer that adrenaline may interfere with results obtained with muscle-relaxants in volunteers.

In reply to Dr. Galley's interesting suggestion, I think it would be possible to make branched molecules on the principles he proposes; and it is certainly possible that the effects might be the expected ones.