Curare-like action of polymethylene bis-quaternary ammonium salts

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Commentary by

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H.R. (Raymond) Ing was a chemist who developed a unique understanding of pharmacology, which arose from working on alkaloid chemistry. To the chemist he is Ing of the Ing and Manske reaction (for the preparation of primary amines) and responsible for working out the structure of the alkaloid cytisine. To the pharmacologist he is the inventor of Lachesine, the master of "onium" salts and the first editor of the British Journal of Pharmacology. He followed on from Paul Ehrlich in trying to relate the pharmacological activities of drugs to their chemical properties and at Oxford in 1945 he and Professor J.H. Burn set up the first course in Chemical Pharmacology (as a supplementary subject for chemistry students).

Ing was particularly struck by Crum Brown and Fraser's work at the end of the 19th century which showed that the quaternary salts of many diverse alkaloids all produced paralysis in frogs by an action similar to that which Claude Bernard had observed for various "curare" preparations. This action is shown by simpler molecules, e.g. tetramethyl ammonium iodide, tetramethyl arsonium iodide, trimethyl sulphonium iodide, and Ing coined the phrase "onium" salts to describe such compounds, which are permanent ions, in a survey of their "curariform" activity (1936).

As Ing explains, this paper on bis-onium salts is a logical development from King's report (1936) that (+)tubocurarine chloride appeared to contain two quaternary nitrogen atoms. During the war Ing had been busy with work on substitutes for atropine and on antimalarial drugs but in 1946 he was able to return to onium salts. By this time there was clinical interest in relaxants to supplement anaesthetics and to prevent fractures during electro- convulsive therapy and Edith Bülbring (1946) had invented the rat phrenic nerve-diaphragm preparation, the first mammalian preparation for testing neuromuscular blocking activity in vitro. It seemed a very suitable research project for a chemistry student who had just completed the supplementary course in "Chemical Pharmacology" and could be expected to make and test the compounds as a part II project, starting in September 1946.

The synthetic route involved reducing dibasic esters to polymethylene glycols, which were converted to polymethylene dibromides and condensed with the wide range of tertiary amines indicated. Hydride reducing agents had not been invented so the method of Bouveault and Blanc was used to convert the esters to the glycols by pouring redistilled ester in specially dried ethanol down a 3-foot copper condenser (borrowed from the Dyson Perrins laboratory next door) on to sodium heated in an oil bath at 120°C. The yields were not good and hexamethylene glycol cannot be made at all in this way (cyclohexane derivatives are produced) so this work did NOT include hexamethonium. An advantage of this synthetic route is that the compounds made were not restricted to the "methonium" series. There was particular interest in triethylammonium compounds because tetraethyl- ammonium was inactive on the rat-diaphragm, though it was known to block autonomic ganglia. A disadvantage of the synthetic route is that it produced bromides, many of which were hydrated and hygroscopic and "their analysis has presented some difficulty". The sample of BTM 2 is actually tetramethyl- ammonium bromide and it appears twice as active as this because it was (wrongly) given almost twice the molecular weight.

When reading this paper it should be realised that the compounds were expected to be acting like "curare" and that it should be a simple matter to test the new compounds using the assay method

described by Chou (1947): at that time pure (+)tubocurarine chloride was very scarce and solutions of curare alkaloids (such as Intocostrin) were standardised by bioassay. It is easy to see now why (+)tubocurarine chloride was not a suitable standard for comparison and the methods section in this paper may seem unduly laboured but the use of the probit of the percentage inhibition to obtain a straight line relation to log. dose is worth noting. The anticholinesterase activity of some of the compounds was entirely unexpected but was appreciated by Dr Blaschko, who arranged for Pamela Holton and Pamela Kordik to make the tests which gave the results shown in Table III.

By the summer 1947 there were enough results to make a respectable part II thesis but the real importance of the compounds was not appreciated until later, when Nora Zaimis told Raymond at a meeting of the Physiological Society about results she and Bill Paton had obtained in experiments on cat muscle. Accordingly Professor Burn arranged for Dr. N.K. Dutta to help with the experiments using the rabbit head-drop preparation, which confirmed the high activity of the compounds. The work was continued until December 1947, mostly this involved making and testing missing members of series, when it was clear that further developments must occur elsewhere.

With the BTM (methonium) compounds it is usually accepted that there is a maximum in neuromuscular blocking activity with 10 methylene groups and in this paper Ing wrote "It may not be entirely fanciful to point out that in the tubocurarine structure I (King, 1948) the shortest route between two nitrogen atoms traverses 10 atoms..." The recent appearance of King's paper, which identified the free phenolic groups in (+)-tubocurarine chloride, probably prompted this remark (though the distance is just the same in the dimethyl ether whose structure was already known) but it conflicts with the results obtained with the bis-triethyl- ammonium compounds. On the rat diaphragm the most active compound was actually BTE 13 for which the head-drop dose in rabbits (not included in Table II) was $642 \pm 120 \mu g/kg$, comparable with BTM 11. Professor Linus Pauling was probably, without knowing it, responsible for the idea that bis-onium salts might be active because they are attached to two points on the receptor. As Eastman Kodak visiting Professor at Oxford in 1947 he gave some lectures, in one of which he mentioned the dissociation of dibasic acids and there seemed to be a parallel.

Of course the ideas are nearly all wrong and this paper shows clearly that although you need theories to plan experiments, the theories are expendable and it is the experimental results which matter. The paper also clearly shows the value of collaboration. Professor Burn had gathered round him a group of scientists from widely differing backgrounds who combined to make his department a marvellous place in which to work. Their readiness to help should be apparent from this paper, though not everyone is thanked officially. All the results in Table III were obtained by Pamela Holton and Pamela Kordik, working with Dr. Blaschko, and I can only believe that Raymond thought of them so much as part of the family (even with rationing the department met for lunch in the library) that he felt that formal thanks were inappropriate. More work was done later by Dr Blaschko's group, in fact, on the anticholinesterase activity of polymethylene bis- quinolinium bromides (Barlow & Himms, 1955).

This paper also shows that you don't find what you don't look for. The activity of BTE 3, BTE 5 and BTE 10 on the superior cervical ganglion of the cat had been reported by Chou & Elio (1947), because they were developments from tetra-ethyl ammonium salts but there were never any plans to look for ganglion-blocking activity in the methonium compounds.

The activity of succinyl choline (Bovet *et al.*, 1949) reinforced the idea of the importance of the 10 atom chain between onium atoms for neuromuscular blocking activity and a similar maximum is seen with polymethylene bis-acetoxyethyl dimethyl ammonium salts, though this includes effects on frog heart and cat blood-pressure as well as on frog rectus muscle (Barlow, 1955: these compounds contain two molecules of acetylcholine linked by a polymethylene chain attached to the nitrogen atom). The two-point attachment idea is probably wrong, however, even if it is memorable. With bis-onium salts containing up to 21 methylene groups (Barlow & Zoller, 1964), activity in the BTE series at the neuromuscular junction and at ganglia increases up to compounds with 15 to 17 methylene groups and with the BTM series a similar maximum occurs with 18 groups. These compounds are much more active than decamethonium or hexamethonium though how the block is produced is not clear.

Note: the symbol d was formerly used to indicate a dextro- rotatory isomer: in the 1950s it was replaced by (+) to avoid confusion with D, indicating configuration.

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