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Cyclodextrins and the emergence of sugammadex

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Summary

Residual paralysis, with its subsequent postoperative pulmonary sequelae, is one of the major complications of anaesthesia, and was recognised shortly after the introduction of neuromuscular blocking drugs into routine clinical practice. Although its incidence decreased with the introduction of intermediate duration drugs, and further diminished with routine neuromuscular monitoring and reversal with cholinesterase inhibitors, residual paralysis still remained a problem. In the search for alternatives to stop the effect of neuromuscular blocking drugs and to match their duration of action to clinical need, chelation of the non-depolarising neuromuscular blocking drugs was considered. It was recognised that cyclodextrins could encapsulate steroidal molecules and thereby inactivate the aminosteroidal neuromuscular blocking drugs. In order to improve the binding of rocuronium to the cyclodextrin and to increase the compound's water solubility, the molecule was modified. This led to the development of sugammadex (Org 25969), a modified γ-cyclodextrin. The modification of the molecule and the initial in vitro studies that led to *in vivo* and later human studies of this conceptually new drug for anaesthesia are described.

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Ever since the introduction of curare-like drugs in the routine clinical practice of anaesthesia by Griffith and Johnson, it was noticed that curare influences pulmonary function and causes morbidity and mortality. In 1954, Beecher and Todd [1] published a controversial paper showing that the mortality amongst patients in whom curare was used was five to six times higher than in those not given curare. As can be read from their tables, but which was apparently not realised by the authors, this was especially so in the non-depolarising drug group. The authors did not recognise the reason for the increased mortality, but were postulating an inherent toxic curare effect. The conclusions of the paper were heavily disputed by anaesthetists and others.

In 1959, Bunker et al. [2] demonstrated that there was no increased mortality after the use of suxamethonium, although they found a 16% pulmonary area collapse in the neuromuscular blocking drug (NMB) group and 10% in the non-NMB group. After this publication the discussion about the long duration of the NMBs and their postoperative residual effects continued. To overcome the disadvantageous long duration, ways of antagonising neuromuscular blockade were considered. This led to the use of the cholinesterase inhibitors neostigmine, pyridostigmine and edrophonium for this purpose. Although the incidence of residual paralysis was decreased by use of the cholinesterase inhibitors, it was never completely prevented. Besides, the cholinesterase inhibitors had many adverse effects. In 1949, Macintosh [3] had already described a patient dying immediately after the administration of atropine and neostigmine for reversal of tubocurarine. Studies with 4-aminopyridine, which stimulates the release of acetylcholine, were giving disappointing results [4]. Continuous research in the field led to the development of shorter-duration non-depolarising NMBs vecuronium, rocuronium, atracurium, and mivacurium. However, a useful, ultra-short-duration NMB has so far not been found. Although morbidity and mortality decreased with the development of intermediate-duration NMBs, it was not completely avoided as was demonstrated by Berg et al. [5].

Many more studies have been published on residual curarisation as is mentioned by others in this supplement. Arbous et al. [6] demonstrated that routine reversal decreased given postoperative mortality. Development of new clean cholinesterase inhibitors did not lead to compounds without side effects; in addition, their onset of action remained relatively slow and their

time to peak effect long. So other pathways had to be explored.

Chemical antagonism

Already in 1924 Petroff in Russia had noticed that congo red inactivates crude curare in dogs. He suggested a mechanism involving binding of curare to the colloidal congo red micelle. This was supported by the observation that a precipitate is formed when congo red and crude curare are mixed in vitro. In 1948, Kensler [7] studied the interaction of congo red with tubocurarine in more detail. He confirmed the observation both in vitro and in vivo, and proved that it was not due to cholinesterase inhibition. He also showed that the reversal was absolutely dependent upon an interaction between congo red and curare in a molar 1 : 1 relationship, the binding being between the sulphuric acid groups of congo red and the nitrogen groups of tubocurarine. Brücke et al. [8] demonstrated in 1955 that the azo-dye congo red also reverses decamethonium-induced blockade both in vitro and in vivo. They also studied germanine (suramin, also an azo-dye) and found that it had antagonistic properties but was less potent than congo red. Later it was demonstrated that it is not binding of germanine with curare that causes reversal, but an action promoting relatively short lasting release of acetylcholine [9]. This presumably results from an interaction with prejunctional calcium channels [10].

In 1953, Gaddum [11] mentioned so-called antagonism by neutralisation, namely two drugs combining with one another to form an inactive compound. In 1956, Ariens et al. [12] also mentioned chemical interaction: 'The interaction between a substance A and one receptor system, while in the same medium substance B reacts with A. Neither B nor the reaction product AB reacts with the receptor system'. They gave the interaction between decamethonium, as agonist, and germanine, as antagonist, as an example. Removal of NMBs from the receptor was mentioned as a possible way of reversing paralysis in 1961 by Linssen [13] in his PhD thesis in Nijmegen. He described in vitro and in vivo studies with germanine, antagonising gallamine, d-tubocurarine and suxamethonium. He wrote: 'By chemical antagonism or antagonism by neutralisation is an antagonism meant in which a pharmacological active substance is removed from the (*receptor*) environment via another, possible pharmacological inactive, substance. The effective concentration of the pharmacological active substance in the biophase is thereby decreased'. And further: 'In case of chemical antagonism is the activity of the antagonist not determined by its affinity for certain specific receptors, but by the affinity between the pharmacological active substance, the agonist, and the antagonist'.

The antagonisms that he described are types of chelation. The substance administered to bind the active compound is called the host, the active compound is called the guest; the combination the host–guest complex. The mechanism of action of chemical antagonism implies that neither the host nor the host–guest complex has an intrinsic activity, thus they are pharmacologically inactive. Chemical antagonism (chelation, complexation or encapsulation) has a long history in clinical use for the reversal of the action of heparin by protamine.

The birth of an idea

In the search for new methods of reversal of non-depolarising neuromuscular block at Organon Laboratories in Newhouse, Scotland (now part of Schering Plough), studies were started with new anticholinesterase inhibitors [14, 15]. These were disappointing because the new compounds retained the muscarinic side effects observed with the older traditional cholinesterase inhibitors. The work progressed to the potential reversal of NMBs by chemical chelation with anionic cyclophanes [16]. This was based on the knowledge that both quaternary ammonium guests and steroidal guests form complexes with cyclophanes. Three compounds were synthesised and studied in vitro in the chick biventer cervicis preparation, reversing the NMBs pancuronium and gallamine. The compounds proved to be less potent in vitro in their reversal activity than the traditionally used cholinesterase inhibitor neostigmine, although with one of the compounds almost complete reversal could be achieved when administered in a high concentration of 2.3 mM. However, the cavity of such small host-molecules vary considerably and their water solubility is relatively low. It was anticipated that this might cause problems for the reliability and safety of reversal.

Dr Anton Bom, also at the Organon Laboratories at Newhouse, was searching for a new solvent for rocuronium bromide. At some point he worked with cyclodextrins and detected that, besides the excellent solubility of the cyclodextrins, there was a decreased potency and a change in the pharmacodynamics of rocuronium [17]. He interpreted these changes as being due to a decreased release of rocuronium because of a more permanent binding to the cyclodextrin. He also realised that cyclodextrins have a better defined lipophilic cavity and are more soluble in water than the cyclophanes that were tested previously at Newhouse. This started the search for more specific binding of rocuronium to cyclodextrins to produce host–guest complexes. It was known from publications on research into chemical steroid receptors that the α-cyclodextrin cavity is too small to hold a

steroid nucleus, so it was speculated that investigations could be limited to the β and γ-series [18].

The history of cyclodextrins

Cyclodextrins were first described in France by Villiers in 1891 as 'cellulosine' [19]. He detected both the α and β-cyclodextrins as a product of digestion of starch by *Bacillus amylobacter*. In 1903, the Austrian microbiologist Schardinger [20] suggested that the term 'crystalline dextrin' was a better name for the compounds which he isolated from material produced by *Bacillus macerans*, and which seemed to be identical to the compounds isolated by Villiers. Later he changed the names into α-dextrin and β-dextrin and laid the foundation for cyclodextrin chemistry. This resulted in the name 'Schardinger-dextrins' for the compounds.

In 1935 γ-cyclodextrin was discovered by Freudenberg and Jacobi [21]. Freudenberg also discovered in 1936 that the cyclodextrins have a ring structure of α(1 → 4)-linked glucose units with a central cavity [22]. In 1948, Freudenberg and Cramer discovered the γ-cyclodextrins. It was Saenger [23], in 1980, who discovered that the amylase called cyclodextrin glucosyl transferase (GCTase) could detach a turn of the polysaccharide helix and link the two ends of the fragment to give cyclic dextrin. CGTase is produced by micro-organisms of the genus *Micrococcus* and *Klebsiella*. After this discovery of the synthesis pathway mass production of cyclodextrins could start. The compounds were used as solubilisers and, in 1953, Freudenberg et al. [24] obtained the first patent on the use of cyclodextrins for drug formulation. Many lipophilic drugs are currently complexed with cyclodextrins to increase their water solubility, i.e. prostaglandins, dexamethasone, nitrogycerin, cephalosporins, diphenhydramine, chlordiazepoxide, piroxicam, itraconazole, chloramphenicol, cisapride, nicotine, dextromethorphan, omeprazole, diclofenac, etc. However, the most intensive use of cyclodextrins is in the food and cosmetic industries where they are used as solubilisers and stabilisers [25]. It is estimated that the daily intake with food of γ-cyclodextrin is 4 g per person per day with a maximum of 8.8 g per person per day [26]. That such amounts do not lead to adverse effects is an indication that the cyclodextrins are safe and have a low toxicity.

The structure and use of the cyclodextrins in anaesthesia

Cyclodextrins are cyclic oligosaccharides consisting of six or more α-1,4-linked D-glucopyranose units, containing a large number of hydroxyl groups (Fig. 1). Their cyclic structure makes a cavity with a water-soluble hydrophilic exterior and a hydrophobic interior. They are known to be able to form inclusion complexes with many hydrophobic organic molecules, and have good biological tolerance. The number of glucopyranose molecules determine the size of the cavity. When six glucopyranose units are incorporated we call them α-cyclodextrins, with seven molecules β-cyclodextrins and with eight molecules γ-cyclodextrins. Fewer glucopyranose molecules then six cannot form cyclodextrins because of steric hindrances. Cyclodextrins with nine or more glucopyranose molecules are frequently unstable and upon synthesis contain many impurities. In addition, their cavity is collapsed and can only contain smaller molecules. The α-cyclodextrins have a cavity opening with a diameter of 4.7–5.3 Å, the β-cyclodextrins 6.0–6.5 Å, and the γ-cyclodextrins 7.5–8.3 Å. The size of the cavity diameter limits the size of the molecules that can be encapsulated. The depth of the cavity largely determines the binding strength. The encapsulation process is extremely fast at rates close to diffusion. In humans the cyclodextrins are not metabolised due to a lack of the required enzymes, but they are excreted via the kidneys.

Some β-cyclodextrin derivatives that encapsulate drug molecules in a reversible manner have been studied as carriers for anaesthetic drugs like etomidate (in 2-hydroxypropyl-beta-cyclodextrin), propofol (in sulfobutyl ether-β-cyclodextrin), sufentanil (in hydroxypropyl-beta-cyclodextrin and ropivacaine [27–29]. If the encapsulation is made irreversible then the cyclodextrins can be theoretically used for detoxification or reversal of drug effects.

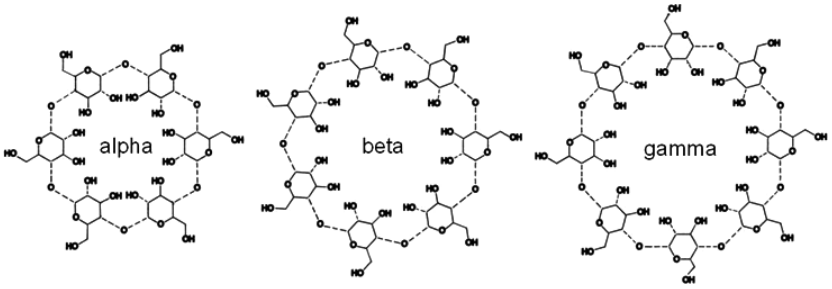

Figure 1 Structure of cyclodextrins.

The emergence of sugammadex

After Anton Bom had developed the idea of using cyclodextrins to encapsulate rocuronium, studies were started. Because of publications it was known that both β- and γ-cyclodextrins could encapsulate steroidal structures. The water solubility of the β-cyclodextrins, however, is markedly less than that of the γ-cyclodextrins which could also fit the rocuronium molecule more easily because of its larger cavity. It was found that the binding was relatively weak, and strong binding was considered crucial to prevent liberation of rocuronium resulting in recurarisation. Studies with modified cyclodextrins were performed on rocuronium-induced neuromuscular block in the mouse phrenic nerve hemi-diaphragm preparation. The reversal potencies clearly depended on the cavity size, with the γ-cyclodextrins having the highest potency. Studies using nuclear magnetic resonance, microcalorimetry, and crystallography techniques revealed that natural cyclodextrins have a relatively short cavity that only could host the A, B and C rings of the steroid nucleus.

Thus elongation of the cavity was thought to be a solution to strengthen the binding force. Side chains were attached to the eight glucose monomers at the 6-position, extending the depth of the cavity. Binding force was further enhanced by attaching a negative charge (an anionic carboxylic function) to the end of these side-chains. This creates electrostatic interaction with the positively charged nitrogen of rocuronium and increases binding. Attaching these side-chains elongated the cavity from a depth of 7.9 to 11–12 Å and a diameter of 7.5–8.3 Å. Studies confirmed that, by this elongation of the cavity, the whole rocuronium molecule was encapsulated. A series of 41 per-6-thiolated cyclodextrins was developed that made complexes with rocuronium bromide and caused reversal of neuromuscular blockade in in vitro mouse nerve-hemi-diaphragm preparations [30]. Further modifications with 4-carboxybenzyl moieties were made on the γ-cyclodextrins [31]. It appeared that the derivatives with negatively charged carboxyls at the rim of the cyclodextrin cavity, had a higher potency because of electrostatic interaction with the quaternary nitrogen of rocuronium. A number of compounds were further studied both in vitro and in vivo. From this modulated series of γ-cyclodextrin derivatives, Org 25969 (Octakis-(6-deoxy-6-S-mercaptoproprionyl-γCD sodium salt) was selected for its high affinity. Org 25969 (see Fig. 2) received the generic name sugammadex and is the first selective and efficient NMB-binding agent.

The affinity of the cyclodextrins for the steroidal NMBs was determined using micro-calorimetry. Both vecuronium and rocuronium demonstrated with nuclear magnetic resonance spectroscopy using single crystal X-ray analysis a 1 : 1 binding to sugammadex (Org 25969) [32–34]. It was found that all four rings of the steroid nucleus were in close contact with the lipophilic wall of the sugammadex cavity. The positively charged nitrogen in the D-ring is surrounded by the carboxylic function of the thiopropanic acid substituents in the sugammadex molecule (see Fig. 3) [35]. The association constants were determined to be respectively 10 megamol^{-1} for vecuronium and 25 megamol^{-1} for rocuronium. In mouse phrenic nerve hemi-diaphragm preparations, sugammadex caused a dose-dependent reversal of a 4 μM rocuronium-induced complete block. Sugammadex 3.9 μM caused 50% reversal in a mean (SD) time of 120 (17) s, and a dose of 11 μM in 27 (5) s [36]. In mouse nerve-hemi-diaphragm studies, 4.0 μM rocuronium, 2.0 μM pancuronium, and 1.0 μM vecuronium concentrations were needed to cause complete blockade of contractions; 50% recovery of these blocks were obtained with 1.22 μM and 0.52 μM sugammadex respectively for rocuronium and vecuronium, while pancuronium was only reversed by 60%.

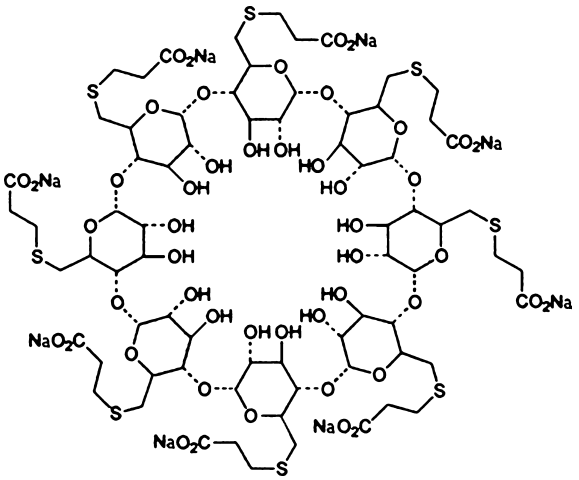

Figure 2 Structure of sugammadex.

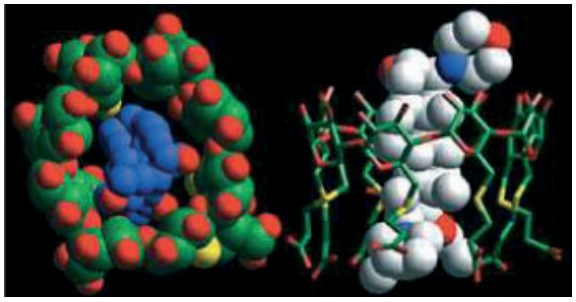

Figure 3 The relation between rocuronium and sugammadex.

This indicates that rocuronium is indeed more effectively reversed than the other aminosteroids. Studies were also performed with atracurium, mivacurium and suxamethonium; they appeared not to be reversed [37]. To explain the binding of the different aminosteroids with their different geometry, studies were performed using nucleomagnetic resonance, isothermal titration calorimetry, fluorescence in solution, and X-ray crystallography. They showed that the modified γ-cyclodextrin and the aminosteroids can change their conformation, allowing adaptation of their structures to fit the different steroid molecules and their chair and twist-boat conformations [38].

Studies were performed regarding the possibility that sugammadex binds other drugs or endogenous substances. However, many anaesthesia-related drugs needed sugammadex concentrations 120–700 times higher than those required for the antagonism of rocuronium and vecuronium [39]. Sugammadex is thus highly selective for aminosteroidal neuromuscular blocking agents.

Toxicological studies with intravenous administration of γ-cyclodextrins were performed in rats [40]. They showed that daily intravenous administration of 120–200 $mg.kg^{-1}$ were tolerated without adverse effects. With dose levels of 600–630 $mg.kg^{-1}$ transient changes were observed that were considered as biochemical responses without toxicological relevance. Sugammadex also underwent toxicological screening and no relevant adverse effects were observed.

The mechanism of action of sugammadex in reversing neuromuscular blockade

Sugammadex is a highly selective binding agent for aminosteroidal NMBs. It encapsulates the complete steroid skeleton of the aminosteroidal NMB molecule in its elongated cavity, while the negatively charged carboxyl-groups bind to the positively charged nitrogen in the D-ring. This makes solid binding of the NMB molecule to the sugammadex molecule, decreasing the free NMB concentration in the central compartment. The penetration of the hydrophobic part of the NMB molecule into the cavity causes dehydration of the guest molecule and indicates that van der Waals forces and hydrophobic interactions are involved in the binding. Due to the binding of NMB a concentration gradient develops, that moves NMB from the biophase toward the central compartment by diffusion. This causes liberation of acetylcholine receptors to which acetylcholine can then bind again. The whole process is rapid because the encapsulation speed is similar to the speed of diffusion of the rocuronium molecules. As a result neuromuscular blockade will recover and the total rocuronium plasma concentration will increase. That such an increase in plasma rocuronium concentration parallels the recovery of muscle contraction was demonstrated in in vivo studies [41]. The rocuronium–sugammadex complex is rapidly excreted via the kidneys, as was confirmed in studies in the guinea pig [42]. The excretion proved to be similar to creatinine clearance indicating that it is mainly via glomerular filtration. Furthermore, sugammadex has a small volume of distribution and a short elimination half-life. In the same investigation it was demonstrated that sugammadex enhances the urinary excretion of rocuronium. Because sugammadex does not affect the acetylcholine receptor or the amount of acetylcholine present at the nicotinic or muscarinic receptor, is it not to be expected that there will be adverse effects related to these receptors from its administration.

Conclusion

Sugammadex emerged from the search for a safer reversal agent for the non-depolarising NMBs. This modified γ-cyclodextrin binds steroidal non-depolarising NMBs rapidly and 'permanently'. Because its effect is based on a chemical interaction with the NMB molecule in which no receptors are involved, adverse effects are not to be expected. Furthermore, in many other human applications, the γ-cyclodextrins have been proven to be without allergic or toxicological effects, as confirmed in toxicology studies with sugammadex. Based on the in vitro studies, sugammadex was further developed in in vivo animal and human studies as described in this supplement.

Conflicts of interest

LHDJB is a member of the scientific advisory board on anaesthesia to Schering-Plough (formely Organon), and has received honoraria for lectures.

References

1 Beecher HK, Todd DP. A study of the deaths associated with anesthesia and surgery. *Annals of Surgery* 1954; **140**: 2–34.
2 Bunker JP, Bendixen HH, Sykes MK, Todd DP, Surtees AD. A comparison of ether anesthesia with thiopental–nitrous oxide–suxamethonium for upper abdominal surgery. *Anesthesiology* 1959; **20**: 745–52.
3 Macintosh RR. Death following injection of neostigmine. *British Medical Journal* 1949; **21**: 852.
4 Miller RD, Booij LH, Agoston S, Crul JF. 4-Aminopyridine potentiates neostigmine and pyridostigmine in man. *Anesthesiology* 1979; **50**: 416–20.
5 Berg H, Viby-Mogensen J, Roed J, et al. Residual neuromuscular block is a risk factor for postoperative pulmonary

complications. A prospective randomized, and blinded study of postoperative pulmonary complications after atracurium, vecuronium, and pancuronium. *Acta Anaesthesiologica Scandinavica* 1997; **41**: 1095–103.
6 Arbous MS, Meursiong AEE, van Kleef JW, et al. Impact of anesthesia management characteristics on severe morbidity and mortality. *Anesthesiology* 2005; **102**: 257–68.
7 Kensler CJ. The antagonism of curare by congo red and related compounds. *Journal of Pharmacology and Experimental Therapeutics* 1948; **95**: 28–44.
8 Brücke F, Kobinger W, Kraupp O. Über den Antagonismus von Kongorot und anderen Substanzen gegenüber quartaren Ammoniumbasen. *Archiv für Experimentelle Pathologie und Pharmakologie* 1955; **224**: 123–36.
9 Henning RH, Nelemans A, Scaf AHJ, van Eekeren J, Agoston S, den Hertog A. Suramin reverses non-depolarising neuromuscular blockade in rat diaphragm. *European Journal of Pharmacology* 1992; **216**: 73–9.
10 Henning RH, Rowan EG, Braga MFM, Nelemans A, Harvey AL. The prejunctional inhibitory effect of suramin on neuromuscular transmission in vitro. *European Journal of Pharmacology* 1996; **301**: 91–7.
11 Gaddum JH. Theories of drug antagonism. *Pharmacological Reviews* 1957; **9**: 211–8.
12 Ariens EJ, van Rossum JM, Simonis AM. A theoretical basis of molecular pharmacology. Part I: Interactions of one or two molecules with one receptor system. *Arzneimittel-Forschung* 1956; **9**: 282–93.
13 Linssen GH. *Curariform Drugs. The Action of the Different Types and Their Combinations on the Neuromuscular Transmission*. PhD Thesis, Nijmegen, The Netherlands: Radboud University (Katholieke Universiteit), 1961: 104–13.
14 Grove SJ, Kaur J, Muir AW, Pow E, Tarver GJ, Zhang MQ. Oxyaniliniums as acetylcholinesterase inhibitors for the reversal of neuromuscular block. *Bioorganic and Medicinal Chemistry Letters* 2002; **12** (2): 193–6.
15 Clark JK, Cowley P, Muir AW, et al. Quaternary salts of E2020 analogues as acetylcholinesterase inhibitors for the reversal of neuromuscular block. *Bioorganic and Medicinal Chemistry Letters* 2002; **12**: 2565–8.
16 Cameron KS, Fielding L, Mason R, et al. Anionic cyclophanes as potential reversal agents of muscle relaxants by chemical chelation. *Bioorganic and Medicinal Chemistry Letters* 2002; **12**: 753–5.
17 Bom A, Epemolu O, Hope F, Rutherford S, Thomson K. Selective relaxant binding agents for reversal of neuromuscular blockade. *Current Opinion in Pharmacology* 2007; **7**: 298–302.
18 Wallimann P, Marti T, Fürer A, Diederich F. Steroids in molecular recognition. *Chemical Reviews* 1997; **97**: 1567–608.
19 Villiers A. Sur la fermentation de la fécule par l'action du ferment butyrique. *Compte Rendu de l Académie de Sciences* 1891; **112**: 536–8.
20 Schardinger F. Über Thermophile Bakterien aus verschiedenen PEISEN UND Milch, sowie über einige Umsetzungsprodukte derselben in kohlenhydrathaltigen Nährlösungen, darunter krystallisierte Polysaccharide (Dextrine) aus Stärke. *Zeitschrrift für Untersuchuing vo. Nahrungs und Genussmittel* 1903; **6**: 865–80.
21 Freudenberg K, Jacobi R. Über Schardinger Dextrine aus Stärke. *Liebigs Annalen der Chemie* 1935; **518**: 102–8.
22 Freudenberg K, Meyer-Delius M. Über die Schardingerdextrine aus Stärke. *Berichte der Chemie* 1938; **71**: 1596–600.
23 Saenger W. Cyclodextrin inclusion compounds in research and industry. *Angewandte Chemie International. Edition* 1980; **19**: 344–62.
24 Freudenberg K, Cramer F, Plieningen H. *German Patent 895.769*, 1953.
25 Szejtli J. Past, present, and future of cyclodextrin research. *Pure Applied Chemistry* 2004; **76**: 1825–45.
26 Munro IC, Newberne PM, Young VR, Bär A. Safety assessment of γ-cyclodextrin. *Regulatory Toxicology Pharmacology* 2004; **39**: S3–13.
27 Doenicke A, Roizen MF, Neubauer AE, Kugler A, Hoernecke R, Beger-Hintzen H. A comparison of two formulations for etomidate, 2-hydroxypropyl-beta-cyclodextrin (HOPCD) and propylene glycol. *Anesthesia Analgesia* 1994; **79**: 923–39.
28 Egan TD, Kern SE, Johnson KB, Pace NL. The pharmacokinetics and pharmacodynamics of propofol in a modified cyclodextrin formulation (Captisol) versus propofol in a lipid formulation (Diprivan): an electroencephalographic and hemodynamic study in a porcine model. *Anesthesia Analgesia* 2003; **97**: 72–9.
29 Meert TF, Mesens J, Verheyen P, Noorduin H. Hydroxypropyl-β-cyclodextrin can modulate the activity of spinally administered sufentanil. *European Journal of Anaesthesioloy* 1992; **9**: 399–409.
30 Adam JM, Bennett DJ, Bom A, et al. Cyclodextrin-derived host molecules as reversal agents for the neuromuscular blocker rocuronium bromide: synthesis and structure–activity relationships. *Journal of Medicinal Chemistry* 2002; **45**: 1806–16.
31 Tarver GJ, Grove SJA, Buchanan K, et al. 2-O-substituted cyclodextrins as reversal agents for the neuromuscular blocker rocuronium bromide. *Bioorganic and Medicinal Chemistry* 2002; **10**: 1819–27.
32 Cameron KS, Fielding L. NMR diffusion coefficient study of steroid–cyclodextrin inclusion complexes. *Magnetic Resonance in Chemistry* 2002; **40**: S106–9.
33 Cameron KS, Fletcher D, Fielding L. An NMR study of cyclodextrin complexes of the steroidal neuromuscular blocker drug rocuronium bromide. *Magnetic Resonance in Chemistry* 2002; **40**: 251–60.
34 Cameron KS, Fletcher D, Fielding L, Clark JK, Zhang M-Q, Orbons LPM. Chemical chelation as a novel method of NMB reversal characterization of the org 25969 NMB complex. *European Journal of Anaesthesiology* 2001; **18** (Suppl. 23): 99.
35 Bom A, Bradley M, Cameron K, et al. A novel concept of reversing neuromuscular block: chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. *Angewandte Chemie International Edition* 2002; **41**: 265–70.
36 Bom A, Thomson KI. Effect of high doses of sugammadex on mouse hemi-diaphragm contractions during TOF stimulation. *Anesthesiology* 2006; **105**: A110 (Abstract).

37 Miller S, Bom A. Org 25969 causes selective reversal of neuromuscular blockade induced by steroidal NMBs in the mouse hemi-diaphragm preparation. *European Journal of Anaesthesiology* 2001; **18** (Suppl. 21): 100–1.
38 Cooper A, Nutley M, MacLean EJ, et al. Mutual induced fit in cyclodextrin–rocuronium complexes. *Organic and Biomolecular Chemistry* 2005; **3**: 1863–71.
39 Zhang M-Q. Drug specific cyclodextrins: the future of rapid neuromuscular block reversal? *Drugs Future* 2003; **28**: 347–54.
40 Donaubauer HH, Fuchs H, Langer KH, Bär A. Subchronic intravenous toxicity studies with γ-cyclodextrin rats. *Regulatory Toxicology and Pharmacology* 1998; **27**: 189–98.
41 Epemolu O, Bom A, Hope F, Mason R. Reversal of neuromuscular blockade and simultaneous increase in plasma rocuronium concentration after the intravenous infusion of the novel reversal agent Org 25989. *Anesthesiology* 2003; **99**: 632–7.
42 Epemolu O, Mayer I, Hope F, Scullion P, Desmond P. Liquid chromatography/mass spectrometric bioanalysis of a modified γ-cyclodextrin (Org 25969) and rocuronium bromide (Org 9426) in guinea pig plasma and urine: its application to determine the plasma pharmacokinetics of Org 25969. *Rapid Communications in Mass Spectrometry* 2002; **16**: 1946–52.