DICHOLINESTERS OF α,ω-DICARBOXYLIC ACIDS AND RELATED SUBSTANCES[1]

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TABLE OF CONTENTS

I. INTRODUCTION AND HISTORICAL DEVELOPMENT

Reference to the neuromuscular blocking action of some of the substances reviewed in the present article has been made in recent years on several occasions in surveys on quaternary ammonium compounds (310, 344). To avoid duplication, only those aspects of the neuromuscular blocking actions of cholinesters of dicarboxylic acids and related substances will be discussed here in full detail which have been either discovered in the period since the publication

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of the reviews referred to, or which are essential for the understanding of the complete pattern of activity of these compounds.

The interest in the pharmacodynamics of synthetic dicholinesters of dicarboxylic acids originated mainly from observations with polymethylene bisquaternary ammonium salts. It was first clearly recognised by Ing and Wright (215) that onium cations are on the whole exceptionally potent as neuromuscular blocking agents. With the idea in mind that the presence of the two quaternary nitrogen atoms in *d*-tubocurarine (dTC) is essential for its potency as a neuromuscular blocking drug, Barlow and Ing (20) synthesized bisquaternary compounds of the methonium series, which subsequently turned out to be either potent ganglionic or neuromuscular blocking drugs, depending on the number of methylene groups which separated the two quaternary nitrogen atoms. Cholinesters of α,ω-dicarboxylic acids are, however, also interesting for other reasons, apart from being bisquaternary compounds: the dicholinesters with a chain length of the acid of four or more C-atoms resemble acetylcholine (ACh) structurally in that they consist of two ACh moieties, which are separated by a certain number of methylene groups, starting with 0 in the case of succinyldicholine (SDCh). Such compounds therefore offer the advantage not only of being bisquaternary but, at the same time, being likely to interfere with, or imitate, actions of ACh in some way or other, following the "principle of similarity" which, in many instances, is found to hold true for the relation between metabolites and their specific antagonists. The ever-increasing number of synthetic cholinesters and similar derivatives of ACh derives its pharmacological interest from two further sources. First, there is an increasing amount of evidence that cholinesters other than ACh might be natural constituents of the animal body. Thus propionylcholine has been isolated from ox spleen (19) tissue and recently butyrylcholine was claimed to be present in brain extracts (205); this number may increase in the future. Second, by changing the arrangement of atoms within the cholinester molecule, some of the numerous and complex actions of the natural chemical transmitter ACh may be greatly enhanced, whereas others disappear more or less completely. Thus one obtains important chemical tools for the elucidation of physiological and pathological functions of the animal body, which are more specific than ACh itself.

At present the actions of ACh are usually classified after the nomenclature introduced by Dale (81) as muscarine-like or nicotine-like, since they resemble closely the pharmacological actions of these alkaloids. The muscarine-like properties of ACh especially, which either augment or diminish the function of smooth muscle fibres, heart muscle fibres or of numerous glandular epithelial cells, seem to be a well defined entity. They are all easily abolished by small amounts of atropine and they correspond closely to the effects reproduced by stimulation of the postganglionic cholinergic fibres of the autonomic nervous system. Derivatives of choline which can be said to have "muscarine-like actions" may be more or less effective than ACh, but they have all the functions mentioned above and usually in more or less the same relative strength as ACh itself. Therefore no substance could be incorporated in this group if, for instance, it had effects

on smooth muscles resembling those of muscarine, but no such effects on the salivary glands.

The situation seems to be somewhat different when "nicotine-like actions" are considered. These also can be described as either excitatory or inhibitory, but both effects are usually closely connected, paralysis following initial excitation. They are mainly exerted on structures connected in some way with the nervous system, such as synapses of the vegetative or central nervous system (including chromaffine cells which may be derived from vegetative ganglion cells), on peripheral receptors or, finally, on structures intercalated between the nervous tissue and the effector organ, such as the endplates of striated muscle fibres. ACh has all these properties but in greatly varying intensity. Thus exciting effects on vegetative ganglion cells or on striated muscle fibres are very evident, whereas the paralysing properties are much more pronounced, for instance, in nicotine itself. In recent years, mainly through the pioneer work of Bovet, derivatives of choline have been found in which the relative intensity of the different "nicotine-like effects" is entirely different from ACh; indeed, some effects may be lacking altogether and others may be enormously augmented. For instance, such a substance may cause a complete paralysis of striated muscle with hardly any previous stimulating effect and with hardly any effect on vegetative ganglion cells, or the exciting effects may be even more prominent than in nicotine or ACh, while the paralysing effects on muscle or on ganglion cells are less pronounced than in ACh. In some instances it is therefore questionable to what extent the term "nicotine-like" can still be reasonably applied. When paralysis of striated muscle is the main pharmacological property of such a substance, the word "curariform" may seem more appropriate for classification, but, as we shall see, the similarity with dTC may be doubtful.

Since many of these more or less "anomalous" derivatives of choline are found among the choline esters of α,ω-dicarboxylic acids, it seems worthwhile to review our knowledge of these substances. In doing this, it will also be necessary to mention substances which are related to the esters either as products of degradation, such as the corresponding monoesters, or by substitution either at the quaternary N-atom or in the aliphatic chain, although some of these products are no longer cholinesters at all.

It seems astonishing that apparently only three papers dealt with choline esters of dicarboxylic acids before the fundamental and systematic investigations of Bovet and his school. The first of these dates back as far as 1906, in which year Hunt and Taveau (212) synthesized SDCh and made a few pharmacological observations, missing, however, its specific action on striated muscle, because they used animals which were anaesthetized with urethane and were under the influence of preparations of curare. Another paper by these authors appeared in 1911 (213). The third paper, exactly thirty years later, was published by Glick (165), who was interested only in the enzymatic hydrolysis of SDCh by the cholinesterase of horse serum as compared with that of ACh. In the following review the different groups of choline esterases will be called either "acetylcholinesterase" (AChE) (corresponding to "true" cholinesterase) and "butyryl-

cholinesterase" (BChE) (instead of "pseudocholinesterase") after the nomenclature of Augustinsson (12, 13).

It is fascinating to follow step by step the series of publications from 1946 onward in which Bovet, starting from the constitution of dTC, finally arrived at the simple molecules of amino alcohol esters of simple aromatic and finally aliphatic dicarboxylic acids. Since the logical development of these "synthetic curares" has been admirably recorded by Bovet (38, 41) himself, there is no necessity to go into details here. However, even in 1951, in his review before the New York Academy of Sciences, Bovet (38) still held the view that throughout the simplifying chemical changes, the molecules of the different artificial curariform substances retained the basic pharmacological properties of the original alkaloid dTC, although he was fully aware of certain individual differences.

This is important, since Bovet's original investigations coincided with the discovery of decamethonium (C-10) by Barlow and Ing (20) and the elucidation of its pharmacological properties mainly by Paton and Zaimis (286). From this work it appeared that substances of an aliphatic type somewhat similar to SDCh and its congeners had an action resembling the depolarizing activity of ACh and, therefore, are fundamentally different from dTC which inhibits this process at the site of the motor endplate.

Recognizing the pharmacological similarity between the actions of C-10 and the SDCh type of drugs, Bovet (41) then proceeded to summarize these substances under the name of "leptocurares" which he contrasted with "pachycurares" of the dTC or gallamine type. This nomenclature was meant to indicate that the more linear structure and the less heavy substituents attached to the quaternary N-atoms of the molecules of the first group might be responsible for the somewhat different action. Nevertheless, Bovet (38) also stressed the chemical similarity of both groups. His nomenclature has not been widely accepted, but it can be said that the increasingly detailed knowledge which has accumulated since 1949, and the clear demonstration of the fact that intermediate pharmacological actions can be found in certain compounds related to SDCh, seem to support Bovet's (41) opinion, although in other cases the difference between dTC and the depolarizing substances remains clear-cut.

The fact that Bovet's extensive work first appeared in the Italian languags and in periodicals not readily available to all pharmacologists has probably been the reason for the publication, since 1950, of many independent papers on dicholinesters. The work by Castillo and de Beer (66), as well as that of Phillips (288) and of Walker (359), seems even to have been initiated by the papers on C-10 rather than by Bovet's work (42). These independent papers have the advantage that many facts about dicholinesters are by now well established. Further interest in the substances of this group was awakened by the introduction of SDCh into clinical work by H. Brücke *et al.* (57) and by Thesleff (346). Bovet (42) had previously suggested di-monoethyldimethylammoniumethyl succinate for this purpose and Valdoni investigated this substance clinically. Papers on the fate of SDCh in the animal body and its enzymatic degradation are now numerous.

A new and interesting line of research resulted from the analysis of the pharmacological actions of the cholinesters of dicarbamic acids first by Cheymol (75) and independently, but about one year later, by a group in the Vienna Pharmacological Institute (232, 237).

II. CHEMICAL AND PHYSICAL PROPERTIES

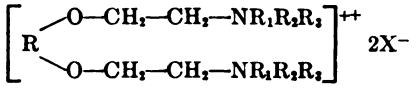
The different quaternary trialkylammoniumethyl esters of dicarboxylic acids to be described in this review were prepared principally as iodides, bromides and chlorides. A few, such as succinylmonocholine (SMCh) and SDCh have also been prepared as perchlorates (141, 365). All the halides are easily crystallizing white compounds and are readily soluble in water at neutral reaction. The melting points of the halides are listed in tables I, II and III. Exact data on solubility are lacking, but with the derivatives of dicarbamic acids, the higher homologues are less soluble than the lower ones. The reactions with alkaloid reagents are known only for SDCh and precipitates of low solubility have been described with the following reagents: tetraphenyl sodium boride (m.p. 214°C, molar solubility 5.4×10^{-5}, solubility product 6.3×10^{-13}) (318); ammonium reineckate (m.p. 194°C, molar solubility 3.2×10^{-5}, solubility product 1.3×10^{-13}) (318); chloroplatinate (165); and dipicrylamine (precipitation as "hexylate") (321).

Of great importance for the stability in aqueous solution of the compounds under observation, is the time course of the spontaneous hydrolysis of their esteratic bindings with dicarboxylic or dicarbamic acids. This non-enzymatic hydrolysis was first investigated by Glick for SDCh (165). According to this author, it is about seven times *higher* than that with BChE of horse serum, and it is about twice that of ACh if equal concentrations (1%) are used.

As Whittaker and Wijesundera (368) were able to show in subsequent investigations, the spontaneous hydrolysis is different from the enzymatic one: in the first case, after 20% hydrolysis the resulting monoester is further degraded to choline and to succinic acid, while the enzyme only splits one esteratic group to form the monoester until about 60% of the original substance has disappeared, before the latter is further changed. More detailed data on the kinetics and on the influences of pH and temperature on non-enzymatic hydrolysis of SDCh were published by Tammelin (342), Fraser (141) and Earles *et al.* (96). Using the photometric method of Hestrin (194), Tammelin (342) was able to prove that the non-enzymatic hydrolysis of SDCh follows a first order reaction. With the head drop method it could also be shown that a solution of SDCh (0.00266 molar) is hydrolyzed to half the initial value in thirty minutes at pH 7.3 and 75°C. The velocity constants of the hydrolysis of SDCh and SMCh in these experiments were 1.4 and 1.6 respectively. These experiments and others, carried out by Foldes *et al.* (122, 132) with heat-inactivated human plasma, show conclusively that the non-enzymatic hydrolysis of SDCh is higher than that of SMCh. Under the conditions mentioned above, Tammelin (342) could show that at 25°C no notable hydrolysis occurred; at 50°C, 10% was hydrolyzed within five hours and at 75°C about 50%. At 100°C practically

TABLE I

Derivatives of aliphatic dicarboxylic acids



No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
1	M 146	CO Carbonyldicholine	CH_3	CH_3	CH_3	Br I	263 233–237	Cat: 20 mg/kg, no action	−	−	−	+	
2	409 I.S.	CO— • CO— Oxalyldicholine	CH_3	CH_3	CH_3			HDD* 75 mg/kg					35, 41, 42, 144, 146, 149, 150
3		CO— • CO—	C_2H_5	C_2H_5	C_2H_5			HDD 57 mg/kg					42, 149, 150
4	326 I.S.	CO— • CH_2 • CO— Malonyldicholine	CH_3	CH_3	CH_3	I	250	HDD 2 mg/kg					35, 41, 42, 144, 146, 149, 150
5		CO— • CH_2 • CO—	C_2H_5	C_2H_5	C_2H_5	I	223						42, 149, 267

6	M 150	—OC · CH ·· CH · CO— Fumaryldicholine	CH_3	CH_3	CH_3	Cl I	246–248 253	HDD 0.5 mg/kg	+	–	–	–	41, 42, 146, 149, 150, 338
7		CO— · CH ·· CH · CO— Maleyldicholine	CH_3	CH_3	CH_3								165
8		—OC · CH ·· CH · CO—	CH_3	CH_3	C_2H_5	I	227	HDD 0.5 mg/kg					42, 149
9	M 115 370 I.S. 48.268	CO— · $(CH_2)_2$ · CO— Succinyldicholine	CH_3	CH_3	CH_3	Br I Cl + $2H_2O$	220 237 160–162.5	HDD 0.2 mg/kg	+	–	+	–	14, 38, 41, 42, 44, 66, 97, 126, 133, 155, 157, 159, 165, 166, 169, 170, 230, 231, 254, 288, 330, 342, 347, 354, 365, 366, 368

TABLE I—*Continued*

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
10	M 126 362 I.S.	CO— · $(CH_2)_2$ · CO—	CH_3	CH_3	C_2H_5	I Br	198 157-158	HDD 0.8 mg/kg	+	−			8, 28, 38, 41, 42, 44, 131, 146, 149, 150, 163, 182, 248, 254, 274, 288, 323, 356
11	M 131 369 I.S.	CO— · $(CH_2)_2$ · CO—	CH_3	C_2H_5	C_2H_5	I	142-144	HDD 15 mg/kg	+	+			7, 8, 38, 41, 42, 146, 149, 150
12	M 130 368 I.S.	CO— · $(CH_2)_2$ · CO—	C_2H_5	C_2H_5	C_2H_5	Br	204-205	HDD 12 mg/kg	−	+			7, 8, 38, 41, 42, 146, 149, 150, 163, 267
13		CO— · $(CH_2)_2$ · CO—	C_2H_5	C_2H_5	CH_2 · CH_2 · CH_3								288
14	M 151	CO— · $(CH_2)_4$ · CO—	CH_3	CH_3	CH_2 	Cl	183-186		−	+			

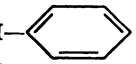
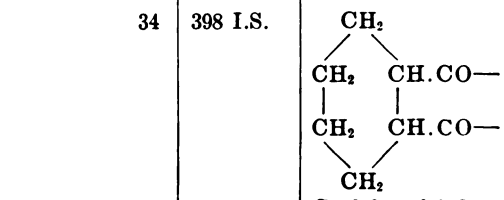
15	4667	CO— $(CH_2)_2$ CO—	CH_3	CH_3	CH_2  NO_2				−	+			297
16	G 9	CO— $(CH_2)_2$ CO—	C_2H_5	C_2H_5	CH_2 CO CH_3	Br	190–192	Cat: 10 mg/kg, no action	−	+	−	+	
17		CO— $(CH_2)_2$ CO—	CH_3	CH_2 CH_2 O	CH_2 CH_2	I	217						149
18	390 I.S.	CO— $CH.CH_3$ CH_2 CO—	CH_3	CH_3	CH_3	I	180	HDD 2 mg/kg	++	−			41, 42, 146, 149, 150
19		CO— CH—  CH_2 CO—	CH_3	CH_3	CH_3	I	250	HDD 8 mg/kg	+	−			342

TABLE I—*Continued*

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
20	M 123 406 I.S.	CO— $(CH_2)_3$ CO— Glutaryldicholine	CH_3	CH_3	CH_3	I	212–214	HDD 0.5 mg/kg	++	−	+	−	41, 42, 57, 146, 149, 150, 233, 288
21		CO— $(CH_2)_3$ CO—	CH_3	CH_3	C_2H_5								
22		CO— $(CH_2)_3$ CO—	C_2H_5	C_2H_5	C_2H_5	I	170	HDD 20 mg/kg	−	+			42, 149, 150, 267
23	M 111 357 I.S.	CO— $(CH_2)_4$ CO— Adipyldicholine	CH_3	CH_3	CH_3	I Br Cl	158–160 184–186 198–206	HDD 0.5 mg/kg	++	−	++	−	7, 8, 9, 14, 38, 41, 42, 44, 57, 146, 149, 150, 156, 157, 158, 159, 160, 161, 163, 180, 233, 273, 288
24	M 114 358 I.S.	CO— $(CH_2)_4$ CO—	CH_3	CH_3	C_2H_5	I Br	115–118 77–81	HDD 0.5 mg/kg	+	−	+	−	38, 42, 57, 149, 150, 157, 158, 159, 160, 163

25	M 124	CO— $(CH_2)_4$ CO—	CH_3	C_2H_5	C_2H_5	I Br	121–122 79–82 85–86	HDD 10 mg/kg	+	+	+	−	38, 42, 149, 150, 157, 163
26	M 106 B.T. 5201	CO— $(CH_2)_4$ CO—	C_2H_5	C_2H_5	C_2H_5	I Br	164–164.5 164.5–166	HDD 10 mg/kg	−	+	−	(+)	7, 8, 9, 38, 42, 149 150, 157, 158, 159, 160, 163, 320, 353
27	404 I.S.	CO— $(CH_2)_5$ CO— Pimelyldicholine	CH_3	CH_3	CH_3	I	160	HDD 3 mg/kg	+	−	++	−	41, 42, 146, 149, 150
28		CO— $(CH_2)_5$ CO—	C_2H_5	C_2H_5	C_2H_5	I	123		−	+	−	+	42, 149, 267
29	M 132	CO— $(CH_2)_6$ CO— Suberyldicholine	CH_3	CH_3	CH_3	Cl	200–203	Dog: 0.6 mg/kg 2 min.	+	−	+++	−	233
30	M 134	CO— $(CH_2)_6$ CO—	C_2H_5	C_2H_5	C_2H_5	Br I	162–164 146–147.5	Cat: 20 mg/kg no action	−	+	−	+	154, 229, 240, 338

TABLE I—*Continued*

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
31	G 5	CO— $(CH_2)_6$ CO—	C_2H_5	C_2H_5	CH_2 CO CH_3	Br	129–131.5	Cat: 20 mg/kg, no action	−	+	−	+	
32	G 14	CO— $(CH_2)_6$ CO—	C_2H_5	C_2H_5	CH_2 CH CH_2	I	104–107	Cat: 20 mg/kg, no action	−	+	−	+	
33	G 16	CO— $(CH_2)_6$ CO—	C_2H_5	C_2H_5	CH_2 CO O C_2H_5	Br	180–183	Cat: 20 mg/kg, 80% paralysis	−	+	−	+	
34	398 I.S.	 Cyclohexyl-1,2-dicarboxyldicholine	CH_3	CH_3	CH_3	I	217	HDD 25 mg/kg					36, 38, 41, 42, 144, 150

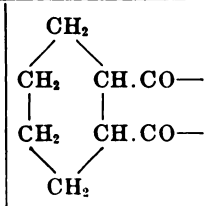
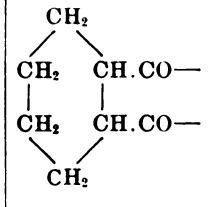
35			CH_3	CH_3	C_2H_5	I	140	HDD 15 mg/kg					42
36			C_2H_5	C_2H_5	C_2H_5			HDD 10 mg/kg					38, 41, 42, 144
37	M 136	CO— $(CH_2)_7$ CO— Azelayldicholine	CH_3	CH_3	CH_3	Br	172–173 170–173		+	−	+++	−	233
38	M 116 408 I.S.	CO— $(CH_2)_8$ CO— Sebacyldicholine	CH_3	CH_3	CH_3	I Cl	175–176 205–210	HDD 5 mg/kg	+	−	+++	−	41, 42, 146, 149, 150, 157, 158, 159, 160, 161, 233
39	M 140	CO— $(CH_2)_8$ CO—	C_2H_5	C_2H_5	C_2H_5	Br	152–157	Cat: 26 mg/kg, no action	−	+	−	+	42, 149, 154, 229, 240, 267, 338

TABLE I—*Concluded*

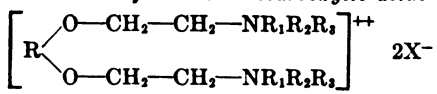
No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
40	G 8	CO— · $(CH_2)_8$ · CO—	C_2H_5	C_2H_5	CH_2 · CO · CH_3	Br	140–146 117–120	Cat: 20 mg/kg, no action	−	+	−	+	
41	G 7	CO— · $(CH_2)_8$ · CO—	CH_3	CH_3	CH_2 · CO · CH_3	Br	116–122	Cat: 10 mg/kg, 80% paralysis			−	+	
42	G 15	CO— · $(CH_2)_8$ · CO—	C_2H_5	C_2H_5	CH_2 · CO · O · C_2H_5	Br	147–152	Cat: 20 mg/kg, no action	−	+	−	(+)	
43	G 13	CO— · $(CH_2)_8$ · CO—	C_2H_5	C_2H_5	CH_2 · CH ·· CH_2	I	78–80		−	+	−	+	
44	G 4	CO— · $(CH_2)_8$ · CO—	CH_3	CH_3	CH_2 · CH_2 · CH_3	Br	69–74	Cat: 20 mg/kg, no action					

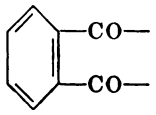
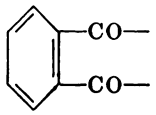
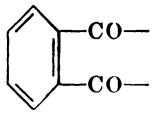
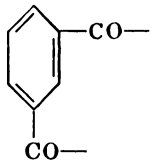
45	M 147	CO— $(CH_2)_8$ CO—	CH_2 CH_2 CH_2	CH_2 CH_2 CH_2	CH_2 CH_2 CH_3	I	92	Cat: 10 mg/kg, 50% paralysis	−	+	−	+	
46	G 18	CO— $(CH_2)_8$ CO—	CH_2 CH_2 O	CH_2 CH_2 CH_2	CH_2 CO CH_3	Br	184–188	Cat: 20 mg/kg, 30% paralysis	−	+	−	+	
47	MA 1	CO— $(CH_2)_{10}$ CO— α,ω-Decanedicarbonyldicholine	CH_3	CH_3	CH_3	Br	209–210	Dog: 1 mg/kg	+	−	+	−	232
48	G 17	CO— $(CH_2)_{10}$ CO—	C_2H_5	C_2H_5	CH_2 CO CH_3	Br	113–115	Cat: 20 mg/kg, no action	−	+	−	+	

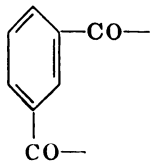
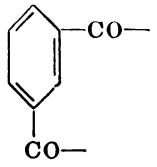
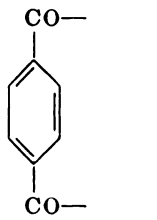
* HDD = Head drop dose for rabbits. I.S. = Code numbers of the "Istituto Superiore di Sanità Roma." M and G = Code numbers of the "Österreichische Stickstoffwerke A.G."

TABLE II

Derivatives of aromatic dicarboxylic acids



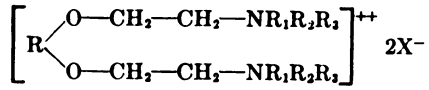
No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
49	330 I.S.	 Phthalyldicholine	CH_3	CH_3	CH_3	I	230	HDD* 25 mg/kg			+	−	36, 38, 40, 41, 44, 145, 150
50	306 I.S.		C_2H_5	C_2H_5	C_2H_5	I	168–170	HDD 7 mg/kg			−	+	38, 40, 41, 44, 45, 144, 145, 150, 250, 268
51	452 I.S.		C_2H_5	C_2H_5	$CH_2 \cdot CH_2 \cdot CH_3$	I							45
52	324 I.S.	 Isophthalyldicholine	CH_3	CH_3	CH_3	I	246–247	HDD 40 mg/kg			+	−	40, 41, 44, 145, 150

53			CH_3	CH_3	C_2H_5	I	215	HDD 25 mg/kg					145
54			C_2H_5	C_2H_5	C_2H_5	I	200	HDD 18 mg/kg			−	+	38, 40, 41, 44, 144, 145 150
55	320 I.S.	 Terephthalyldicholine	CH_3	CH_3	CH_3	I	281	HDD 7 mg/kg					40, 41, 44, 145, 148, 150, 254
56	302 I. S.	Terephthalyldicholine	C_2H_5	C_2H_5	C_2H_5	I	222	HDD 3 mg/kg		+			36, 38, 40, 41, 44, 45, 145, 150, 250, 267, 268
57	449 I.S.	Terephthalyldicholine	C_2H_5	C_2H_5	C_3H_7	I							45

* HDD = Head drop dose for rabbits. I.S. = Code number of the "Istituto Superiore di Sanità Roma."

TABLE III

Derivatives of dicarbamic acids



No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
58	771 I.S. BC 4 600 H.C.	NH.CO— NH.CO— Biscarbaminoylcholine	CH_3	CH_3	CH_3	I	232	HDD* 33.5 mg/kg					41, 70, 72, 74, 75, 150, 232, 237
59		N.CO— N.CO—	CH_3	CH_3	CH_3			HDD 60 mg/kg convulsions					41, 150
60	601 H.C.	NH.CO— CH_2 NH.CO— Methylenebiscarbaminoylcholine	CH_3	CH_3	CH_3			HDD					70, 72, 74, 75, 90, 91, 92
61	621 H.C.	NH.CO— CH.CH_3 NH.CO—	CH_3	CH_3	CH_3			HDD 8.4 mg/kg					70, 72, 74, 75, 90, 91, 92

62	622 H.C.	NH.CO— · CH.C_2H_5 · NH.CO—	CH_3	CH_3	CH_3			HDD 35 mg/kg					70, 72, 74, 75, 90, 91, 92
63	631 H.C.	NH.CO— · CH—C_6H_5 · NH.CO—	CH_3	CH_3	CH_3			HDD 2.6 mg/kg					70, 72, 74, 75, 90, 91, 92
64	784 I.S. BC 2 602 H.C.	NH.CO— · $(CH_2)_2$ · NH.CO— Dimethylenebis-carbaminoyl-choline	CH_3	CH_3	CH_3			HDD 1.5 mg/kg					38, 41, 70, 72, 74, 75, 90, 91, 92, 191, 232
65	623 H.C.	NH.CO— · CH.CH_3 · CH_2 · NH.CO—	CH_3	CH_3	CH_3			HDD 6.7 mg/kg					70, 72, 74, 75, 90, 91, 92
66	604 H.C. BC 12	NH.CO— · $(CH_2)_4$ · NH.CO— Tetramethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3	I	190–193	HDD 0.32 mg/kg Cat: 0.07 mg/kg 100% paralysis	+	(±)	+	−	70, 72, 74, 75, 90, 91, 92, 232

TABLE III—*Continued*

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
67	BC 24	NH.CO— $(CH_2)_4$ NH.CO—	CH_3	CH_3	C_2H_5	I	113–118	Cat: 0.03 mg/kg, 100% paralysis	+	+			
68	BC 27	NH.CO— $(CH_2)_4$ NH.CO—	C_2H_5	C_2H_5	C_2H_5	I	163–166	Cat: 2.5 mg/kg, 70% paralysis	−	+	−	+	
69	605 H.C.	NH.CO— $(CH_2)_5$ NH.CO— Pentamethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3			HDD 0.14 mg/kg					70, 72, 74, 75, 90, 91, 92
70	606 H.C. BC 16	NH.CO— $(CH_2)_6$ NH.CO— Hexamethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3	I Br Cl	172–173 175–178 187	HDD 0.034 mg/kg Cat: 0.007–0.01 mg/kg, 100% paralysis	+	(+)			52, 54, 56, 59, 70, 72, 73, 74, 75, 90, 91, 92, 192, 209, 210, 232, 237, 272
71	BC 28	NH.CO— $(CH_2)_6$ NH.CO—	CH_3	CH_3	C_2H_5	I		Cat: 0.004 mg/kg, 25% paralysis	+	(+)			

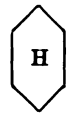
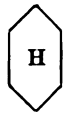
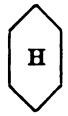
72	BC 17	NH.CO— · $(CH_2)_6$ · NH.CO—	C_2H_5	C_2H_5	C_2H_5	I		Cat: 10 mg/kg, 50% paralysis	−	+	−	+	74
73	BC 42	NH.CO— · $(CH_2)_6$ · NH.CO—	CH_3			I	195–198	Cat: 0.5 mg/kg, 100% paralysis	−	+	−	+	
74	BC 43	NH.CO— · $(CH_2)_6$ · NH.CO—	C_2H_5			I	189–194	Cat: 1 mg/kg, 100% paralysis	−	+	−	+	
75	BC 44	NH.CO— · $(CH_2)_6$ · NH.CO—	CH_2 · CH_2 O	CH_2 · CH_2	CH_2 · CO · O · C_2H_5	Br	112–116	Cat: 10 mg/kg, 100% paralysis	−	+	−	+	
76	BC 45	NH.CO— · $(CH_2)_6$ · NH.CO—	CH_2 · CH_2 O	CH_2 · CH_2	CH_2 · CO · CH_3	Br	115–120	Cat: 10 mg/kg, 100% paralysis	−	+	−	+	
77	BC 25	N(CH_3).CO— · $(CH_2)_6$ · N(CH_3).CO—	CH_3	CH_3	CH_3	I	165–170	Cat: 0.01 mg/kg, 100% paralysis					

TABLE III—*Continued*

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
78	607 H.C.	NH.CO— $(CH_2)_7$ NH.CO— Heptamethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3			HDD 0.029 mg/kg					74
79	BC 9	NH.CO— $(CH_2)_8$ NH.CO— Octamethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3	I Cl	125–127 217–220	Cat: 0.01 mg/kg, 100% paralysis	+	(+)	(+)	−	193, 209, 230, 231, 232, 234, 235, 339
80	BC 10	NH.CO— $(CH_2)_8$ NH.CO—	CH_3	CH_3	C_2H_5	Br	135–137	Cat: 0.02 mg/kg, 100% paralysis	+	(+)			
81	BC 11	NH.CO— $(CH_2)_8$ NH.CO—	C_2H_5	C_2H_5	C_2H_5	I	−	Cat: 2.5 mg/kg, 60% paralysis	−	+	−	+	

82	BC 29	NH.CO— (CH$_2$)$_8$ NH.CO—	C_2H_5	C_2H_5	CH_2 CO CH_3	Br	85–88	Cat: 5 mg/kg, 25% paralysis	−	+	−	+	
83	BC 26	NH.CO— (CH$_2$)$_8$ NH.CO—	CH_3	CH_3	CH_2 	Cl	161–168.5	Cat: 2 mg/kg, 40% paralysis	−	+			
84	610 H.C. BC 14	NH.CO— (CH$_2$)$_{10}$ NH.CO— Decamethylene-biscarbaminoyl-choline	CH_3	CH_3	CH_3	I	135–136.5	HDD 0.09 mg/kg Cat: 0.015–0.02 mg/kg, 100% paralysis					70, 72, 74, 75, 90, 91, 92, 232
85	BC 34	NH.CO— (CH$_2$)$_{10}$ NH.CO—	C_2H_5	C_2H_5	C_2H_5	I		Cat: 2.5 mg/kg, 100% paralysis	−	+	−	+	
86	BC 35	NH.CO— (CH$_2$)$_{10}$ NH.CO—	C_2H_5	C_2H_5	C_2H_5	I		Cat: 2 mg/kg, 60% paralysis	−	+	−	+	
87	640 H.C.	CH_2.N.CO— CO CH_3 CO CH_3 CH_2.N.CO—	CH_3	CH_3	CH_3			HDD 52 mg/kg					74

TABLE III—(*Concluded*)

No.	Code No.	R	R_1	R_2	R_3	X	M.p. °C	Neuromuscular Action			Ganglionic Action		References
								Intensity	Depolarization	Inhibition of depolarization	Stimulation	Block	
88	642 H.C.	$CH_2.N.CO-$ $CO\ CH_2$ $(CH_2)_2$ $CO\ CH_2$ $CH_2.N.CO-$	CH_3	CH_3	CH_3			HDD 5.4 mg/kg					74
89	644 H.C.	$CH_2.N.CO-$ $CO\ CH_2$ $(CH_2)_4$ $CO\ CH_2$ $CH_2.N.CO-$	CH_3	CH_3	CH_3			HDD 4.98 mg/kg					74
90	648 H.C.	$CH_2.N.CO-$ $CO\ CH_2$ $(CH_2)_8$ $CO\ CH_2$ $CH_2.N.CO$	CH_3	CH_3	CH_3			HDD 4.9 mg/kg					74

* HDD, Head drop dose for rabbits. H.C. = Code numbers used by F. Cheymol *et al.* B.C. = Code numbers of the "Österreichische Stickstoffwerke A.G."

all SDCh present initially was split within two hours. Investigating the hydrolysis at constant temperature (75°C) and varying pH, he found that there is no appreciable hydrolysis at pH 5 within five hours, whereas in the same time about 10% hydrolysis occurs at pH 1 and pH 6, and at pH 8 it is about 60%. Fraser (141) used a biological test (rat diaphragm) and arrived at similar results. At pH 3.4, the hydrolytic loss of a 5% solution of SDCh in glass vials was 5.85% after three months and 22% after one year at room temperature (96). At 37°C the loss under identical conditions was 24.5% in three months and practically total within one year (96).

Few data are available on non-enzymatic hydrolysis of other members of this series of esters. Glick (165) stated that the hydrolysis of maleyldicholine is higher than that of SDCh. Ginzel, Klupp and Werner (158) found it almost equal for SDCh and the ester of adipic acid. Stumpf (338) (unpublished) working with a manometric method, compared the hydrolysis of some homologous dicholinesters of dicarboxylic acids and obtained the following values of μl CO_2 after 30 minutes from 2 ml of 30 mM solution at pH 7.4 and 37°C: carbonyl—95.0, succinyl—7.0, fumaryl—114.0, adipyl—4.4, azelayl—3.7, sebacyl—0.

From these results, the conclusion may be drawn that, within the homologous series of these diesters, the non-enzymatic hydrolysis decreases with increasing chain length and that esters of unsaturated acids show a higher hydrolysis than those of saturated ones. Under the conditions chosen by Stumpf (338) the esters of dicarbamic acids show no hydrolysis at all.

III. METHODS OF ESTIMATION

Owing to the lack of specificity of all chemical methods for the evaluation of ammonium compounds, all estimations of the quaternary compounds under discussion in biological material involve their isolation and separation in pure form. So far only paper chromatography has been used for this isolation and separation from serum or tissue extracts. Whittaker and Wijesundera (367, 368) used n-propanol:formic acid:water (8:1:1) (solution I) or n-propanol:benzyl alcohol:water (solution II) for the chromatographic separation of cholinesters such as SDCh and SMCh on paper. The R_F values for solution I were 0.30 for SDCh and 0.62 for SMCh and for solution II 0.30 for SDCh and 0.17 for SMCh. Solution II can also readily be used for the separation of SDCh, SMCh, choline and succinic acid. Augustinsson and Grahn (14) described a method based on paper chromatography, by which ACh, SDCh, adipyldicholine (AdDCh) and the hexaethyl derivative of this substance as well as sebacyldicholine and related substances can be separated. In this method the authors used a mixture of n-butanol:ethanol:acetic acid:water in a relation of 8:2:1:3 (filter paper, Munktell No. 0B or Whatman No. 4; temperature 20°C; time of ascending migration 16 hours, descending 9 hours; amount of substance used 10–50 μg). Under such conditions the following R_F values were found for the descending migration of the substances mentioned above: ACh: 0.47, SDCh: 0.18, AdDCh: 0.22, M 114 (see Table I, No. 24) 0.25, sebacyldicholine: 0.54. A paper chromatographic separation of SDCh from gallamine, dTC and "myocain" has been described by Wankmüller (361).

The following colour reactions were used to visualize these substances on paper:

1) According to Hestrin's method (194), ACh and the aliphatic dicholinesters of dicarboxylic acids can be spotted by converting them into the corresponding hydroxamic acids with hydroxylamine, which then give a red colour with ferric chloride (366). In distinction from the esters of dicarboxylic acids, those of dicarbamic acids do not give a colour reaction either on paper or in solution. The limit for this estimation is 0.5 to 2 μg (199).

2) The iodine method of Brante (46). SDCh, like many other substances containing nitrogen, gives a brownish-yellow spot on spraying with alcoholic iodine solution. This reaction is reversible since SDCh remains unchanged after evaporation of the iodine and can then be tested in another way.

3) Conversion of the dicholinesters into their phosphomolyldic acid salts and subsequent reduction to molybdic blue with acid stannous chloride according to Chargaff's method (69). The method was used for SDCh but did not prove very specific (367).

4) Treatment of the chromatograms with 0.4% solution of dipicrylamine magnesium salt in aqueous acetone (321). With choline and with a series of mono- and diesters of dicarboxylic acids or of dicarbamic acids, this method yields brownish-yellow spots on a light yellow background (14, 265, 337).

5) Treatment of the paper chromatograms with bromthymol blue in alkaline bicarbonate solution. SMCh and SDCh give yellow spots (47).

6) Treatment of chromatograms with Dragendorf's reagent (280).

Some of the colour reactions mentioned above were worked out for quantitative estimation of dicholine compounds. Samuelsson (321) described a colorimetric method based on the reactions of dipicrylamine and choline derivatives as discovered by Ackermann and Mauer (1). These compounds have well defined melting points (*e.g.*, that of SDCh, m.p. 141–142°C) and form deep yellow solutions in acetone, whose colour follows the Lambert-Beer law. For quantitative estimations of SDCh in solutions meant for injections the precipitation reactions of SDCh with tetraphenyl sodium borate or with ammonium reineckate can be used (318). The first of these reactions is impaired by potassium, ammonium, choline and monocholinester ions. The reineckate of SDCh is soluble in acetone with a red colour and since the intensity of colour follows the Lambert-Beer law over a wide range, it can be used for the colorimetric estimation of SDCh. The measurements should be carried out at 525 mμ and have a sensitivity of 0.1 μg/ml (318). Another colorimetric method is based on the fact that the salts of quaternary ammonium bases with bromthymol blue can be separated from aqueous solution by shaking with chloroform. The coloured salts are soluble in chloroform with a yellow colour whose intensity is proportional to the concentration of the ammonium compounds contained in the original solution. SDCh and hexamethylenedicarbaminoylcholine can be estimated quantitatively in aqueous solutions by this method (Beckman spectrophotometer, wave length 420 mμ; thickness 1 cm, final volume of the chloroform solution 5 ml; sensitivity 10 μg/ml) (337).

Several biological methods have been developed for the estimation of dicholinesters. Compared with the chemical methods, these have the advantage of higher sensitivity and specificity and they are not as easily impaired by other substances occurring in urine or in tissue extracts. Especially the contracture which nearly all of these substances cause in the frogs rectus abdominis muscle can be used to great advantage in their estimation (42, 68, 286, 309). With this method SDCh (129, 281) and hexamethylenedicarbaminoylcholine (52) were quantitatively estimated in the urine of animals or man without previous separation or isolation. The threshold for the estimation of SDCh is 0.1 μg/ml and for hexamethylenedicarbaminoylcholine 0.2 μg/ml. In cats SDCh can be estimated quantitatively with doses of 5–10 μg after treatment of the animals with 1 mg/kg of eserine (281). SMCh and SDCh as well as their mono-, di- and triethyl derivatives can be estimated quantitatively with the rat phrenic nerve-diaphragm preparation (105). With the "drop off test" in mice the lower margin for the estimation of SDCh was found to be 200–300 μg/kg (281).

IV. PHARMACOLOGICAL ACTIONS IN ANIMALS AND MAN

A. Dicholinesters and other di-trialkylammoniumethyl esters of aliphatic dicarboxylic acids

1. Diesters of carbonic, oxalic and malonic acid. a. Carbonyldicholine. Carbonyldicholine was first synthesized by Abderhalden *et al.* (1) and has been investigated recently at the Pharmacological Institute in Vienna (unpublished). This compound differs from all the other members of the dicholine dicarboxylic acid ester (DChDCAE) series, in that it has no actions at all on neuromuscular junctions.

Doses as high as 10 mg/kg cause no paralysis of the striated muscle of cats. 1 mg/ml is without any effect on the isolated diaphragm of the rat or on the isolated frog rectus abdominis preparation.

On the other hand there is rather convincing evidence that carbonyldicholine does possess ganglion-blocking property.

A depressor action on the cat blood pressure which could be neither prevented nor abolished by atropine was observed after doses of 1 to 5 mg/kg. Moreover, carbonyldicholine caused a reduction of the contractions of the cat nictitating membrane on indirect stimulation when given intravenously in doses of 5 mg/kg and did not influence the flow rate of the isolated perfused hind limb of the cat. Comparing the mydriatic actions of carbonyldicholine and hexamethonium on mice a ratio of 0.12 was found, indicating that the latter substance was about eight times more active. The action of carbonyldicholine on loops of isolated ileum of guinea pigs, contracted by pretreatment with ACh, histamine and nicotine was compared with that of hexamethonium. In these experiments neither carbonyldicholine nor hexamethonium showed any actions, in doses up to 50 μg/ml, against the ACh and histamine contractures. The nicotine contractures were abolished by 2.5 μg/ml of carbonyldicholine or by 0.5 up to 1.0 μg/ml of hexamethonium, indicating a relative potency of approximately 0.225. On the isolated frog heart, carbonyldicholine (50–500 μg/ml) has a positive inotropic effect similar to that found by O. Loewi (253) for other ganglion-blocking agents.

All this suggests that the first member of the series of DChDCAE is a ganglion-blocking substance and that its pharmacology is entirely different from the

higher homologues of this series. Carbonyldicholine resembles more those members of the polymethylenebistrimethylammonium series which, like hexamethonium, are inhibitors of ganglionic transmission, but it is about six to eight times less active. Carbonyldicholine is not split by the BChE of horse since its spontaneous hydrolysis could not be augmented by the addition of horse serum (1:10).

b. Oxalyldicholine and malonyldicholine. The dicholinesters of oxalic and malonic acid were synthesized and investigated by Bovet and his group at the "Istituto Superiore di Sanità" in Rome (35, 41, 42, 144, 146, 149, 150). Since oxalyldicholine has a head drop dose of 75 mg/kg in rabbits and a lethal dose of 150 mg/kg it is doubtful whether it really should be included in the class of "artificial curares". No other pharmacological data are available, but at least as regards neuromuscular activity, oxalyldicholine seems to be more related to carbonyldicholine than to the higher members of the DChDCAE series. Malonyldicholine has a more potent neuromuscular activity (head drop dose in rabbits = 2 mg/kg) and is therefore more closely related to the next higher member, SDCh, which proved to be an extremely potent neuromuscular blocking substance. Here too no other pharmacological data are available.

2. Diesters of succinic acid. a. Succinyldicholine. By far the most important representative of the group of substances mentioned in this review is the dicholinester of succinic acid. According to its chemical constitution SDCh has been called "diacetylcholine". However, pharmacological analysis of this substance has been extensive in the last five years and has shown that no animal yet investigated can break down the aliphatic linkage to form ACh.

Neuromuscular actions. The outstanding property of SDCh is that it has a powerful paralysing effect of short duration on mammalian striated muscle. The distance between the two quaternary groups appears to be optimal for this effect (41). This view seems to be corroborated by the fact that in the homologous series of polymethylenebistrimethylammonium compounds the optimal distance is of the same order, *i.e.*, about 14.5 Å. (252). However, Cheymol *et al.* (70, 71, 74, 75) and Kraupp *et al.* (232, 237) have shown, that in the series of dicarbaminoylcholines the optimal distance is entirely different and we shall see that the close correspondence between the DChDCAE and the methonium series may be entirely fortuitous.

A second characteristic property of SDCh and its homologues in the DChDCAE series is the short duration of their action even if paralysis is complete. From the beginning of their investigations, Bovet (42, 44), Phillips (288), and Castillo and de Beer (66) attributed this to the fact that SDCh is hydrolyzed by BChE. Many subsequent findings seem to support this theory, but other factors may contribute to this shortness of action.

It will therefore be best to consider first the results found in various animal species and in isolated muscle preparations, and to keep in mind that even in one species different muscles are apt to vary widely in their reactions, at least quantitatively.

Like decamethonium, SDCh causes a contracture of those muscles of the frog

which are most susceptible to the effect of ACh, especially the rectus abdominis (38, 41, 42, 159, 259, 335). Weaker effects are seen with the sartorius and the smallest effects with the gastrocnemius (42). The onset of the contracture is about twenty times slower than with ACh comparing the time in which the maximal tension is reached (180). The contractures can be abolished by dTC and gallamine and correspond to the effects of substances of the "depolarizing" type of muscle relaxants. The contracture-producing actions of ACh and of SDCh are therefore always synergistic (259).

The contracture of the frog muscle after treatment with SDCh does not lead to any changes in the ATP or ADP content of the muscle, although the creatine phosphate content is decreased (118, 220).

In muscles of the toad (sartorius of *Bufo vulgaris*) SDCh shows twitches which are superimposed on the contracture in the same way as shown by Sommerkamp with ACh (331). However, if large doses of SDCh (5 mg/kg) are injected into the lymph sac of a frog, the animal shows the same flaccid paralysis of its muscles as with dTC, and only with reduced doses one can observe positions of partial contracture as with nicotine (41).

Buttle and Zaimis (61) have shown that, in birds, C-10 causes a contracture of the muscles of the hind limbs, and Bovet demonstrated the same reaction for SDCh (41, 42).

The sensitivity of different muscles of the bird seems to vary, since it was found that small doses cause spastic paralysis only of the legs, the animal being still able to fly, whereas large doses cause contracture of the breast muscles and opisthotonus (41, 42, 159). A convenient preparation for following this reaction quantitatively was developed at this Institute by Ginzel *et al.* (155, 159, 162) who attached the isolated tendon of the gastrocnemius of pigeons to an isotonic lever and recorded the contractions of the muscle both with indirect stimulation from the sciatic nerve and from supramaximal stimulation of the muscle itself. Intravenous injection of a few micrograms of SDCh caused contracture of the muscle which, even if it was maximal, was much shorter than one caused by C-10. If smaller doses were given, so that the tension of the contraction did not reach that of the muscle twitches caused by direct or indirect stimulation, such twitches were superimposed on the contracture in the same way as observed by Brown (49a) with ACh, indicating that indirect stimulation is not interrupted. In the pigeon preparation, as in mammals, tubocurarine caused merely a decrease or a total block for indirect stimuli without affecting direct stimulation at dose levels which caused complete block at the motor endplate. dTC diminished or abolished the contracture caused by SDCh (38, 41, 42, 157).

Mammalian muscles, unlike those of birds and reptiles, usually show no contracture with SDCh, but react with flaccid paralysis due partly to neuromuscular block and partly also to a direct effect on the muscle fibre (26, 38, 39, 41, 42, 66, 159, 183, 288, 349). Stimulating effects on the muscle fibres can always be observed in a varying degree before paralysis (160, 349). There are however at least two circumstances in which a flaccid paralysis does not develop. According

to Veigel (358), the diaphragm of guinea-pigs under barbiturate anaesthesia responds to SDCh with contracture. The second exception among normally innervated muscles is the external muscles of the eye. Hofmann and Lembeck (200) registered the contractions of these muscles on stimulation of the oculomotor nerve in rabbits and found that injections of SDCh caused a contracture (the same was found for C-10 (93)) which could be extinguished by dTC. This again seems to be a typical reaction to depolarizing substances, since the same was found for ACh by Brown and Harvey (50). It is possible that this reaction of the external muscles of the eye is the cause of the increase in intraocular pressure which was observed by Hofmann and Holzer (199b) in man after the injection of SDCh. If we now proceed to review the paralysing effect of SDCh on isolated or partially isolated preparations of mammalian muscle, it seems astonishing how few experiments have been made with the isolated diaphragm of the rat. This is probably due to the rather weak action on this preparation, in which this substance causes only a 50% reduction in tension with concentrations as high as 1 mg per 100 ml (26, 160, 316, 317, 364).

In the intact mammal the intravenous injection of SDCh is followed by marked fasciculations of all striated muscles (349): in man these may be felt as somewhat painful cramps (349). The subsequent paralysis sets in much more rapidly than with curare alkaloids and according to Thesleff (349) is usually maximal about sixty seconds after the beginning of an intravenous injection. The sequence of onset of paralysis is the same as with dTC, the facial and laryngeal muscle usually being affected first and the diaphragm last (84, 347, 349). This corresponds closely to the frequency of nerve impulse which reach the different types of muscles during the course of physiological innervation. Those with short intervals between the single impulse are more readily paralysed. However, the effect is of short duration in all animals even if the initial paralysis was complete.

Thesleff (349) finds that if the dose and the time of paralysis are both plotted on logarithmic scales, a linear relation between dose and effect can be observed. However, it must be remembered that different muscles of one species may show a somewhat different sensitivity to SDCh, *e.g.*, Somers (330) showed that in the cat the tibialis anticus is always more sensitive than the gastrocnemius muscle. The differences are much less pronounced than with C-10 for which Zaimis has given a thorough comparative analysis in different animals (375, 376). The stimulating effect of SDCh, which gives rise to fasciculations after intravenous injections, can be studied best by means of close intraarterial injections according to Brown's technique (49). Like ACh, SDCh also causes an initial twitch; this is then followed by transient depression of excitability to indirect stimulation. The initial twitch can be abolished by pretreatment with small doses of dTC.

Chronically denervated mammalian muscles react to SDCh with a contracture similar to that observed after ACh (41). In the dog Bovet made a remarkable observation (41). The gastrocnemius muscle of one side was chronically denervated and during the subsequent experiment the normal muscle of the other

side was stimulated rhythmically from the sciatic nerve the reaction of the denervated muscle being tested by repeated intra-arterial injections of ACh into the corresponding femoral artery. If 0.1 mg/kg SDCh was then given intravenously, this amount was sufficient to abolish indirect excitability on the normal side. On the denervated side however, the short contractures caused by ACh were either unaffected or even augmented in tension. But if a ten times higher dose of SDCh was given, these effects of ACh on denervated muscles were completely abolished. This seems to show that SDCh, while acting as a "depolarizing" agent in moderate doses, may change its mode of action in excessive concentrations and can act like dTC which, of course, antagonizes the contractures caused by ACh on denervated muscle.

All the details previously mentioned in this paragraph tend to prove that SDCh is, in fact, a depolarizing drug similar to C-10 and probably even more closely related to ACh than the latter. The relevant evidence for such a mode of action will be presented in IV, C, p. 315. An unexpected observation reported for SDCh by Granit *et al.* (169, 170) was that subparalytic doses of SDCh caused a strong transient regular discharge of muscle spindles when the drug was injected intra-arterially. This effect, although facilitated by increase of muscle tension, also occurs in an entirely flaccid muscle with higher doses of SDCh. SDCh still elicited discharges from the muscle spindles when the muscle was completely unresponsive to excitation through alpha motor fibres, though still higher doses were then required. SDCh ultimately also paralyses the gamma endplates. However, there often appears to be a transient stimulating effect before the onset of paralysis.

Ganglionic actions. Although SDCh has a very weak ganglionic activity as compared with the other members of the DChDCAE series, its effects on the vegetative ganglia have been studied in detail and are of practical importance. SDCh causes primarily an excitation and subsequent depression of ganglion cells (159, 349). These effects include all vegetative ganglia which have been studied so far and also the suprarenal medulla. Bovet (41, 42) had already described how large doses of SDCh (2–100 mg/kg in dogs), far in excess of the muscular paralysing threshold, cause a pronounced transient elevation of blood pressure. Thesleff (349) found that the rise in blood pressure with doses between 2 and 50 mg was especially noticeable in cats and was less in rabbits or dogs. He could find no diminution of the effect with repeated doses, but he saw that very large doses (in excess of 100 mg/kg) caused less effect than smaller ones and that they were followed by a slight depression of blood pressure. This was the only lowering of blood pressure noticed by this author, whereas Bovet (41, 42) had described a slight initial lowering effect.

Denervation of both carotids or clamping of these arteries had practically no effect on the pressor action of SDCh (349). However, the action was partly abolished by the application of ganglion-blocking agents such as hexamethonium bromide or tetraethylammonium chloride (349). The pressor effect was also greatly diminished by sympatholytic drugs such as dibenamine (349). After application of this drug there was an initial fall in blood pressure and then a

slight rise when SDCh was injected. Evidently all the actions of SDCh can be explained by its nicotine-like effects on vegetative ganglion cells. It is therefore rather astonishing, that Thesleff (349), recording the action potentials of the inferior mesenteric ganglion, was unable to observe this stimulating effect; with doses up to 10 mg/kg no change of the action potentials could be observed, whereas higher concentrations depressed the ganglion until with 130–140 mg/kg a total block of short duration resulted. This is about six hundred and fifty to seven hundred times the paralysing dose for striated muscle. Such experiments should probably be repeated since at least with the compounds of this series with increased length of the polymethylene chain there can be no doubt of the ganglion-stimulating action. Apparently, however, Granit, Skoglund and Thesleff (in unpublished observations) were also unable to see any increase in the post-ganglionic discharge of action potentials in the inferior mesenteric, the stellate, or the superior cervical ganglion.

Miscellaneous actions. v. Euler *et al.* (107a) found in 1941 that 5–10 μg of ACh injected into the external carotid artery caused a maximal discharge in the afferent fibres from the carotid body in cats without influencing the baroceptor activity. They proposed the theory that this substance might play a role in the transmission of chemical impulses within the chemoceptor cells of this region. Landgreen *et al.* (242, 250) repeated these experiments with SDCh. When they injected 20 μg into the isolated circulation of the carotid body they found a marked increase in the typical action potentials of the chemoceptors. Higher doses (1–2 mg) caused transient complete paralysis.

ACh decreases the threshold for pain caused by injection of histamine and this action can be antagonized by SDCh as well as by decamethonium (C-10) and dTC (327).

The effects of SDCh on the gastrointestinal tract are insignificant. Le Heux (245), in 1921, found that the substance caused no more excitation of the isolated rabbit ileum than choline itself. This was confirmed by Bovet (41, 42) who also failed to see any increase in the effects of ACh or histamine on the tone or peristalsis of such loops of rabbit ileum. Nor could any effect on the peristalsis *in situ* be observed in dogs. In guinea-pig ileum Thesleff (349) found no effect up to a concentration of 50 mg/l and irregular contractions above this concentration. Rapid intravenous injection of as much as 100 mg/kg in cats caused a short inhibition of peristaltic movements and an increase in tone in the duodenum *in situ*. No effect could be seen on the rectal caecum of fowl.

SDCh has no bronchoconstrictor effect, nor does it have any constrictor effect on the uterine muscle of rabbits up to concentrations of 100 mg/l. A few results are available which point to an action of SDCh on the central nervous system. Bovet (38, 43) and Longo and Spaccarelli (256) found that SDCh, even in doses as high as twenty times the normal lethal dose, does not modify the electroencephalogram (EEG), provided sufficient care is taken to maintain adequate artificial respiration. More recently, however, the problem was again taken up by Longo in Bovet's laboratory (255). Injecting SDCh into the carotid artery, this author could demonstrate an effect on the EEG of rabbits of the same type

as that produced by ACh or sensory stimuli; the effect consisted in a transient arousal reaction and was elicited by as small a dose as 3 to 7 μg SDCh as compared to 0.1 to 0.5 μg ACh. Since the carotid body was denervated in these experiments, a stimulation of chemoceptors was eliminated as a possible cause for the arousal reaction. A stimulation of other sensory receptors through SDCh was largely eliminated, since the injection was made into the internal carotid artery by means of a catheter.

Central actions have also been studied from a different point of view, namely with regard to a possible paralysing action of SDCh on the respiratory center (166). Such investigations originated from clinical observations on patients where an abnormally prolonged respiratory arrest after usual doses of C-10 or SDCh was relieved by central stimulating drugs, *e. g.*, lobeline or nikethamide (21, 97, 187). In fact Ellis *et al.* (97, 98) found that, with high doses of SDCh and other muscle-paralysing agents, the return of the diaphragmatic twitch on stimulation of the phrenic nerve occurred appreciably before resumption of spontaneous respiration. During the interval between the restoration of indirect excitability of the diaphragm and occurrence of spontaneous action potentials in the phrenic nerve, these authors were able to restore spontaneous respiration by injecting lobeline. The question of a central depressant effect of muscular paralysing agents on respiration must be discussed with great care, since, as Frey *et al.* (143) rightly point out, with normal doses of such drugs central effects play a very subordinate role, and with high doses both hyperventilation and hypoxia and also the alveolar CO_2-tension must be carefully considered before a central effect can be directly attributed to a drug. With SDCh these authors usually noticed an initial stimulating effect both on the phrenic action currents and on diaphragmatic twitches.

Action on the intracellular and extracellular distribution of potassium. A very interesting effect of depolarizing substances on muscle tissue has been found in experiments by Klupp and Kraupp (230, 231). These authors found that in dogs injections of SDCh, in doses between 100 and 500 μg/kg, cause a considerable rise of the plasma potassium level. 500 μg/kg, for instance, augmented this value maximally (5–6 mg%), and even after forty-five minutes the normal level was not yet reached. When an osmotic diuresis was induced in these animals with mannitol, a dose of 250 μg/kg caused a surplus excretion of potassium in the urine amounting to 0.7–0.8 mequiv.

Two facts make it evident that this surplus potassium derives from the muscle fibre and not from other tissues. 1) *d*-Tubocurarine in doses which by themselves do not liberate detectable amounts of potassium (500 μg/kg in dogs of about 15 kg) completely inhibits liberation of potassium ion by 500 μg/kg SDCh for five hours. 2) The increase in potassium output can also be measured in perfused hindlimbs of cats. In such preparations an intraarterial injection of 100 μg SDCh caused an output of 0.38 mequiv. of potassium. This action began within the first minute, reached its maximum in two minutes and disappeared completely within ten to fifteen minutes. From such experiments it can be computed that one molecule of SDCh liberates at least 1500 potassium ions. Other

depolarizing drugs have an identical influence (*e.g.*, C-10 or octamethylene dicarbaminoylcholine) although the time course is different.

At present these effects can be explained only by supposing that, by lowering the resting potential at the site of the endplates and of the muscle fibre itself, the depolarizing substances cause a disturbance of the Donnan equilibrium existing in these membranes. Such an action would be analogous to the effects of cationic stimulating electrical currents on the membrane of nonmyelinated nerve fibres, in which Hodgkin and Huxley (199) found that, after a short lasting initial inflow of sodium ions, an output of potassium occurs through the membrane, persisting as long as the duration of the electrically induced depolarization.

Enzymatic hydrolysis and cholinesterase inhibition. The first investigation of enzymatic hydrolysis of SDCh was carried out by Glick (165) with horse serum. Using a 1 % solution of SDCh, the rate of hydrolysis was found to be about 4 % of that of ACh. Glick (165) assumed that the close vicinity of two acyl groups in the molecule brings about a steric configuration unfavourable for a close linkage of the esteratic groups with the colloidal particles of the enzymes and their active centre. This slow enzymatic splitting was confirmed by subsequent investigations (13, 14, 30, 42, 44, 66, 89, 109, 122, 123, 128, 130, 133, 140, 141, 159, 160, 181, 233, 248, 251, 259, 288, 342, 365, 366, 368). With different enzyme preparations or substrate concentrations, the hydrolysis was found to range from 1.5 to 5 % of that of ACh. The optimal substrate concentration for butyrylcholinesterase (BChE) was found to be 0.375 % (251) or approximately 5 mM/l (342, 368), and excess of substrate leads to inhibition. The reaction can be stopped by heat inactivation of the enzyme or by anticholinesterases (42, 44, 66, 122, 133, 251, 354); inhibition of the already slow process is difficult to measure. Contradictory statements have been made about the hydrolysis of SDCh by AChE. Some investigators did not find any action of AChE (13, 14, 108, 140, 141, 182, 248) whereas others observed more or less activity. Bovet-Nitti (44), working with erythrocytes of cattle, found minimal activity. Löw and Tammelin (251) and Tammelin (342) however, working with the optimal substrate concentration of 0.375 %, obtained an unquestionable enzymatic hydrolysis of SDCh by horse erythrocytes. Extracts from liver, tibialis muscle, and heart of rabbits, or from the electric organ of the torpedo, all containing mainly AChE (12), hydrolyze SDCh at about the same rate, but much more slowly than ACh (342). According to Lüllmann *et al.* (259), SDCh is split by AChE of human erythrocytes but not by that of rabbit brain or of the leech. For hemolysates of human erythrocytes and for cobra venom the optimal substrate concentrations of SDCh correspond closely to those of ACh (pS: 2–2.5) (342, 343).

No organ as yet investigated seems to have a high specific affinity for SDCh. The enzymatic effects of AChE can also be inhibited by anticholinesterases, although quantitative statements are difficult because the hydrolysis is slow (251).

The SDCh hydrolysis by BChE follows a reaction of zero order (126, 133, 354), but it can be shown with manometric methods that the reaction practically ceases when 50 % of the theoretical amount of CO_2 has been liberated from the

bicarbonate buffer utilized (368). Whittaker *et al.* (365, 366, 368) were able to show that in horse serum the reaction occurs in two steps. First, the monoester of succinic acid and free choline are formed and then the monoester is decomposed to succinic acid and choline, the second reaction starting only when the first is nearly complete and one of the ester linkages of SDCh has been split. Since the monoester is split at a much slower rate, the hydrolysis apparently stops at 60% of the total possible hydrolysis. The same is true for human serum (130, 132, 133, 248, 354).

With paper chromatography Augustinsson and Grahn (14), using SDCh and horse serum, found spots which are not identical with either SDCh or choline and are probably due to the monoester. An exact analysis (368) of this process showed that after 10 to 35% cleavage one finds SDCh, SMCh and choline, after 50% only SMCh and choline, and after 60% SMCh, choline and succinate. Manometric investigations show, moreover, that after 50% cleavage the curve of the enzymatic reaction shows the same course of hydrolysis as an equal mixture of SMCh and choline, whereas the hydrolysis of mixtures of SDCh, choline, and succinic acid follow a different time course. The fact that SMCh is only split when there is no more SDCh available is explained by a much greater affinity of the latter substance to the enzyme. This small affinity of the monoester is also the reason for the fact that horse serum (368) or human serum (122, 128, 132) hydrolyze this substance at a rate which is only approximately $\frac{1}{6}$ to $\frac{1}{10}$ of that of SDCh. This reaction is not inhibited by excess of substrate (368). The monoester is also hydrolyzed by AChE (13).

Fate in the organism. No data are available on the absorption and excretion of SDCh after oral ingestion, but many investigations on the enzymatic breakdown of SDCh in the organism and on its excretion by the kidneys have been carried out after intravenous administration. It is often assumed that there is some relation between the duration of the action of SDCh and the rate of its enzymatic hydrolysis by BChE. This was first stated by Bovet *et al.* (42), by Phillips (288), and by Castillo and de Beer (66). Such relations have actually been found by Bourne *et al.* (34) and Evans *et al.* (108), who were able to demonstrate that in human patients in whom SDCh had an action of excessive duration a low level of serum ChE was usually present. This has been confirmed by several other investigations (32, 33, 62, 110, 134, 179, 182, 188, 246, 300, 301). Moreover, in man (109, 110), dogs (182), and cats (100) the actions of SDCh were considerably diminished by intravenous application of ChE concentrates. (For details see next chapter.) According to Evans *et al.* (108, 110), the duration of apnoea after SDCh injections is inversely proportional to the activity of BChE in serum. A certain correlation between the activity of procaine esterase in plasma and the duration of respiratory paralysis was also found by Foldes *et al.* (128, 129, 133), which however was not as significant as that described by Evans *et al.* (108, 110).

Correspondence between BChE activity of serum and intensity of SDCh effects was also found in a variety of animal species. It appears that man is less sensitive to SDCh than various other mammals (122, 124, 182, 354) and in

agreement with these observations, Foldes *et al.* (124) and Klupp *et al.* (233) were able to demonstrate that human plasma hydrolyzes SDCh quicker than, for instance, cat and dog plasma. Dogs are highly sensitive to SDCh (181, 182) and, in addition, SDCh is hydrolyzed faster by human than by dog serum (124, 233, 354). Similar differences in the actions of SDCh have been found between cats and mice, the latter being less susceptible to SDCh (281). This could be correlated with the observation that in mice the activity of procaine esterase of the serum is found to be higher than in cats (15). However, if butyrylcholine is used as substrate for the plasma enzyme, cats have a higher activity than mice (332). All such experiments are hampered by the uncertainty whether the two enzymes splitting butyrylcholine and procaine are indeed identical (123, 223, 224).

Although many observations tend to prove the connection between the duration of SDCh effects and the activity of BChE, some experiments suggest that other factors beside BChE activity are responsible for the intensity and duration of SDCh action. According to Hall *et al.* (182), the above mentioned higher sensitivity of dogs for SDCh in comparison to man is due not only to the differences of BChE activities of serum but also to the low activity of AChE in this species. The relation of BChE activity to the intensity or duration of the actions of SDCh are explained in the following way: within the organism an equilibrium exists between free SDCh, BChE-SDCh-complex and AChE-SDCh-complex. The complex binding of the latter kind (responsible for the inhibition of AChE at the endplates, the hydrolysis being very slow) would be responsible for the pharmacological action, whereas the complex binding of SDCh with BChE (comparatively quick hydrolysis) would be the cause of the short duration of SDCh action.

No correlation between SDCh action and BChE level in serum was found by Fraser (141), who could not detect any difference in the duration of SDCh paralysis in two groups of rabbits which differed from one another in their plasma ChE activities. The question has also been raised, whether the weak hydrolysis of SDCh can really be of any importance for the elimination of that substance (89). *In vivo* the splitting of SDCh in such minute quantities as are necessary for paralysis is evidently a relatively quick process. It has even been assumed that the hydrolysis of such small amounts of SDCh is not a reaction of zero order but follows a first order reaction, so that an increased concentration of SDCh in the organism would lead to an increased hydrolysis (130). Argent *et al.* (6) have pointed out that BChE activity could only be of importance for the short period in which SDCh circulates in the bloodstream after intravenous injection. But this argument can be criticized, since even when SDCh is attached to its specific receptors at the endplates, there will always be an equilibrium between bound and free SDCh which will be disturbed if the latter is hydrolyzed by BChE. Finally, it must be emphasized that many findings on abnormal prolongation of SDCh action with normal levels of BChE may be quoted where other factors were held responsible (110, 341, 372).

During enzymatic hydrolysis of SDCh, SMCh is formed, and so the question

has been raised whether, after therapeutic application of SDCh, an accumulation of SMCh in the organism can occur and can potentiate the neuromuscular effects of SDCh (122). For this reason Foldes (122) studied the effects of SMCh in man and found that 5–7 mg/kg cause a muscular paralysis after three to five minutes, lasting eight to twelve minutes. It was noticed that an ineffective dose of SMCh markedly potentiated the effects of subsequent doses of the diester. As the monoester has a much smaller effect, it is improbable that its persistence after the therapeutic application of SDCh could be the cause of a prolonged muscular relaxation. On the other hand, Foldes *et al.* (133) argue that with low BChE values in the blood and impaired excretion (which normally covers about 12 to 15%) there might be an influence of the monoester in prolonging the action of infused SDCh.

Compared with the enzymatic destruction, urinary excretion plays only a subordinate role in the elimination of SDCh (133). This was studied after intravenous application of SDCh by Norton and de Beer (281) in cats and mice and by Foldes and Norton (129) in man. After injection of equivalent doses cats excrete somewhat more SDCh than mice. This may be explained by assuming that procaine esterase splits SDCh, as mice have more procaine esterase. Also in cats the excretion of SDCh can be augmented by anticholinesterases. In both species the excretion takes place during the first half hour after injection and amounts to 5 to 15% of the given dose. In man excretion is also nearly complete half an hour after an intravenous injection. With a dosage of 1 mg/kg, man excretes about 2.2% of the injected material. During a slow intravenous infusion of SDCh, sufficient to cause prolonged relaxation of the muscle, about 2.8% is excreted. The concentration in the urine differs widely in different experiments, *e.g.*, between 6.5 and 120 μg/ml, indicating that excretion by the kidney is independent of the rate of urine formation. In man, no correlation was found between SDCh excretion in the urine and procaine esterase activity or duration of apnoea. SMCh is excreted at a much higher rate in man than SDCh. After intravenous application of 0.8 mg/kg of the monoester, an average amount of 9.2% is excreted unchanged in the urine. This fact is attributed to the smaller hydrolysis of SMCh by BChE.

Antagonists and potentiators. On the basis of the fact that SDCh is hydrolyzed by BChE, many investigations have been carried out, in order to demonstrate antagonizing effects of injected BChE preparations or potentiating effects of anticholinesterases on the paralysing action of SDCh.

The antagonizing effect of intravenous BChE administration was first shown in man by Evans *et al.* (108, 110). These authors stated that the duration of apnoea after SDCh and the level of BChE are inversely proportional and approximately follow the formula: duration of apnoea in minutes after 30–50 mg SDCh times units of BChE equals 200. This factor could be diminished by intravenous application of a BChE preparation ("cholase") from 198–252 to 143–208. According to Augustinsson (13) this is the first example of successful therapy with an enzyme preparation of this type. The actions of SDCh are also considerably diminished in animals by intravenous applications of enzyme concentrates (100, 103, 181).

The potentiating effects of anticholinesterases were demonstrated first by Bovet-Nitti *et al.* (41, 44, 254), who showed conclusively that eserine increases and prolongs the effects of SDCh in animals. Similar results with eserine, neostigmine, tetraethyl pyrophosphate (TEPP), and other anticholinesterases were obtained by many other authors (2, 27, 34, 36, 42, 63, 64, 66, 86, 89, 99, 100, 103, 104, 108, 141, 159, 160, 173, 187, 203, 246, 251, 264, 329, 347, 350). According to Ginzel *et al.* (159) the paralysing action of SDCh on the sciatic-gastrocnemius preparation of cats was prolonged, although the degree of maximal paralysis was not increased after pretreatment with eserine (159). On the sciatic-gastrocnemius preparation of pigeons, on the rat phrenic nerve diaphragm preparation, and with close arterial injections in cats, Ginzel *et al.* (159, 160) could demonstrate only very slight potentiating effects of eserine on the actions of SDCh. No effect of eserine was detectable on the contracture caused by SDCh in the frog rectus muscle. On the rat diaphragm preparation potentiating effects of eserine and neostigmine could be obtained for the actions of ACh but not for those of SDCh (259).

The question has not been definitely settled, whether the anticholinesterases exert their potentiating effects on the action of SDCh only by inhibiting the enzymatic hydrolysis of this substance. For instance, Fraser found (141) that the potentiating effects of eserine on the action of SDCh began much later than the maximal inhibition of BChE; and he concluded that BChE activity is not the only determining factor for SDCh paralysis. At least for neostigmine, a direct effect on muscle fibres has been established (311), which might be essential in the potentiation of the paralysing effects of SDCh (89). Moreover, it has often been observed in man that prolonged paralysis of striated muscles by SDCh can even be reversed, in some instances dramatically, by injections of neostigmine (6, 171, 197, 315). Many more substances have been described, which modify the intensity of action of SDCh; some of them are specific inhibitors of BChE, others are specific inhibitors of AChE. Finally, substances will be mentioned, which modify the action of SDCh without interfering with BChE or AChE activity.

Ellis *et al.* (104) studied the modifying action of various anticholinesterases on the effects of SDCh. They used 1,5-bis-(4-allyldimethylammoniumphenyl)-pentane-3-one dibromide (compound 53-67) as a specific inhibitor of AChE and trimethyl-(2-dimethylcarbamyloxy-5-phenyl)benzylammonium bromide (Nu 683) as a specific inhibitor for BChE. On the nerve-muscle preparation of the cat, 53-67 antagonized and Nu 683 potentiated the action of SDCh. On the frog rectus, 53-67 had no effects but Nu 683 enhanced the action of SDCh. In cats the effects with C-10 were equal to those obtained with SDCh. Contrary to Ellis (104), Fraser (141) found no effect on the muscle of cats or fowl with 284C51 (chemically identical with 53-67) on the SDCh action. In experiments by Marotta and Carminati (268), it was found that a derivative of terephthalic acid (302 I.S.), a specific inhibitor of AChE, had no effects on the SDCh action in the rabbit sciatic-gastrocnemius preparation. On the other hand, a derivative of the isomeric phthalic acid (306 I.S.), which had proved to be a specific inhibitor of BChE, undoubtedly prolonged SDCh paralysis. All authors mentioned seem

to agree that inhibitors of BChE prolong SDCh paralysis and that this is probably so because SDCh is hydrolyzed by this enzyme, but it is difficult to prove this connection. With the exception of Ellis in his experiments mentioned above, all authors agree that inhibition of AChE is without significant influence on the actions of SDCh.

According to de Beer *et al.* (89, 100), substances which modify the action of SDCh have the following characteristics. a) Antagonism of SDCh is not related to antagonism of muscarine-like activity of ACh. b) Antagonism of SDCh is related to antagonism of nicotine-like activity of ACh. c) Potentiation of SDCh is related to potentiation of nicotine-like activity of ACh.

The effect of SDCh is therefore not modified by atropine (99). However, Bovet (41) found slight inhibition of the SDCh effects when atropine had been given previously.

Substances influencing the pressor effects of ACh and therefore inhibitors of SDCh include: substituted benzhydrylpiperazines, especially N-benzhydryl-N′-methyl-N′-ethylpiperazine iodide (No. 51-212) (99, 110, 103); ganglion-blocking substances such as pentamethonium, hexamethonium, and tetraethylammonium (41); and sympathicolytic or adrenolytic substances such as diethylaminomethylbenzodioxane (883F), and N-n-propyltetrahydroisoquinoline (No. 660). 51-212 is the most potent of these agents and is about five times stronger than 883F (89, 99, 100, 103).

Synergists, which also potentiate the pressor activity of ACh, were found in a series of dicarboxylic acid di-tertiary-aminoalkylamides and their quaternary ammonium salts (65, 89, 100, 289, 290, 291). Especially potent substances of that kind were different tertiary dipiperidinoethylamines, *e.g.*, those of succinic acid (50-236), glutaric acid (50-254) or adipic acid. The corresponding dipyrrolidinoethylamines were less potent and their quaternary derivatives were even inactive. Di-dimethylaminoethylamides of aliphatic dicarboxylic acids are also active potentiators of SDCh, as well as (in contrast to the substances mentioned above) their quaternary salts. A good example is the corresponding tertiary and the quaternary derivative of succinic acid (49-205 and 49.164). None of these substances have any curare-like activity in doses which show a strong potentiating effect on SDCh.

The substances 49.164 and 50-236 have no anticholinesterase effect worth mentioning. Finally, eserine and also 3-hydroxyphenyldimethylethylammonium chloride (edrophonium) belong to this group of SDCh potentiators (89, 99, 100, 103).

The substances named under b) potentiate the effects of dTC, and the potentiators named under c) antagonize them. All substances as a rule act similarly on C-10 and on SDCh and this again would be in favour of the assumption that their influence on SDCh is not simply due to their effect on cholinesterases.

Ellis *et al.* (103) investigated the modifying influence of some SDCh potentiators and antagonists and also of the substance 49-204 [(1-methyl-2,4′-dimethylaminophenethyl)-piperidine-1,4′-bismethyl iodide] on the activity of SMCh. The substance 50-254 had the same prolonging effect on SMCh as on C-10 and

SDCh activity. In contrast, eserine has a stronger effect on SDCh than on SMCh. In cats a dose of 2 mg/kg acts for about twenty minutes. The substance 51-212, which is one of the most potent antagonists of SDCh, acts even more strongly against SMCh than against SDCh. The substance 49-204, which does not antagonize SDCh or even has a slight potentiating effect on that substance, is a strong antagonist of SMCh and C-10. Injection of purified preparations of BChE decrease the intensity but not the duration of action of SMCh. This is another indication that SMCh belongs to the depolarizing muscle relaxants and that it is more closely related to C-10 than the diester.

Experiments carried out on the rat diaphragm preparation by Rummel and Schulz (316, 317) show that adrenaline antagonizes the paralysing activity of SDCh and other depolarizing drugs. This effect is attributed to a repolarization of the endplate and the nerve endings, but the question remains unsolved whether this is due to adrenaline itself or to some secondary metabolic effect. The same authors also showed that not only dTC and adrenaline are antagonists of SDCh, but also calcium ions (217), quinidine and caffeine which form a group of antagonistic drugs against depolarizing agents. All these substances have a polarizing effect on membranes. Caffeine and calcium ions are also antagonists against dTC (316, 317), the double action of calcium being attributed first to a hyperpolarizing effect on muscle membranes and secondly to an increase of ACh liberation at the motor endplate (67). C-10 can also be antagonized by these substances but not by quinidine (316, 317).

The interactions between SDCh and procaine have been thoroughly investigated by Ellis *et al.* (101, 102, 127, 304). In animals with a high level of BChE and in man these authors found a potentiating effect of procaine most probably due to substrate competition between SDCh and procaine for BChE. In animals with low BChE levels (cats, dogs), either antagonistic or potentiating effects could be observed, depending on the sequence of injections. Since in these animals the substrate competition between SDCh and procaine for BChE plays practically no part, the results are explained by interactions of the following events: interference between ACh and procaine at the endplate receptor, inhibition of ACh liberation by procaine, competition between ACh and procaine for the AChE of the endplate region. All these are determining factors for the interaction between ACh and SDCh at the endplate organ (101, 102, 127, 304).

Experiments by Osterloh (284, 285) show that choline chloride (1–4 mg/kg in cats and dogs) given before or during a permanent infusion of SDCh distinctly enhances the SDCh paralysis. Since this effect can also be seen with C-10, it is not attributed to the inhibition of BChE but rather to increased ACh liberation at the endplate. The action of g-strophanthin on the SDCh effects has been investigated by Westermann (364). This author could demonstrate that on the mouse diaphragm-phrenic preparation g-strophanthin in a concentration of 10^{-5} enhanced the depression of contractures caused by 10^{-5} SDCh. According to Bovet *et al.* (41), veratrine has no influence on the action of SDCh. Suramin sodium (moranyl, germanin) decreases the toxicity of SDCh (41).

The mutual antagonism between depolarizing agents and those which, like

dTC, inhibit depolarization was first described by Paton and Zaimis (286) and is of course applicable also to SDCh: dTC (2, 159, 347), gallamine and the triethyl derivative of SDCh are antagonists of SDCh (41, 163), whereas C-10 and hexamethylenedicarbaminoylcholine both potentiate the actions of SDCh (59). Lüllmann and Förster (259) could, however, show that the antagonism does not hold true for all test objects. While on the frog rectus muscle the synergism and antagonism, mentioned above, is readily observed, in the dorsal muscle of the leech dTC augments the contracture caused by both SDCh and C-10. According to Rummel and Schulz (317), SDCh has no effect on the paralysis of the diaphragm caused by dTC.

Clinical applications. SDCh was first tried clinically by Brücke (57) and independently by Thesleff (83, 84, 85, 346, 348, 350) as a short acting muscular relaxant. SDCh administered by intravenous injection has a rapid action. Soon (twelve to fifteen seconds) after the injection, generalized muscular fasciculations appear (sometimes causing pain) of varying intensity and of fifteen to twenty seconds duration (18, 25, 34, 58, 84, 86, 124, 126, 137, 203, 216, 261, 293, 305, 319, 347, 350, 353, 363, 369). The initial fasciculation depends largely on the speed of the injection (124, 126, 293). Subsequently paralysis sets in, lasting with therapeutic doses from one to twenty minutes (34, 216, 293, 347). A linear relation between log dose and the duration of muscular and respiratory paralysis was found for doses ranging from 0.1 to 0.6 mg/kg of SDCh iodide (347). An increase in the total dose above 100 mg SDCh chloride (corresponding to 150 mg SDCh iodide) causes no further prolongation of muscular relaxation (305, 347). The initial fasciculation, as well as the paralysis, is first seen in the facial muscles and then spreads downwards (18, 261, 269, 347). On recovery from the paralysis respiration and strength of the muscles are restored within not more than three minutes (18, 84, 121, 175, 216, 305). Thesleff and other authors (18, 82, 119, 121, 124, 138, 203, 347) found a relation of 1:2 between the dose which paralyses the peripheral muscles and that which causes arrest of respiration. However, other observers (18, 34, 217, 305, 350) state that it is usually not possible to obtain sufficient relaxation without marked simultaneous impairment of respiration.

In some cases an unexpected prolonged respiratory paralysis occurs after a single dose of SDCh (24, 32, 34, 120, 121, 134, 168, 187, 195, 198, 214, 228, 257, 258, 301, 324, 360, 362). Sometimes this may be due to low levels of serum ChE (32, 34, 86, 94, 108, 121, 130, 134, 139, 246, 301, 340), but in other cases no such connection has been seen (6, 197, 228, 300, 315). In such cases, overdosage of SDCh is presumably the most frequent cause of the prolonged action (6, 119, 174). Great care should be taken with SDCh in cases in which a low serum cholinesterase level and therefore an uncontrollable action of SDCh is to be expected (247, 262).

No appreciable influence of sex and age on the strength and duration of SDCh action has been observed (95, 347).

The method preferable for short surgical procedures is a combination with thiopental sodium (thiopentone sodium) (5, 18, 25, 28, 31, 32, 34, 58, 76, 77, 83, 95, 121, 124, 125, 126, 136, 138, 167, 202, 206, 207, 216, 243, 283, 305, 333, 334, 347), but since the action of SDCh sets in earlier than that of the thiobarbiturate the latter should be administered first (31, 124, 126, 136, 202, 203, 305, 312). Simultaneous administration is also excluded by the instability of SDCh in alkaline solutions, since the pH of a solution of thiopentone sodium is about 8–9 (203, 369). No detectable influence of pretreatment with thiopentone on the duration of SDCh action could be observed (347), apart from some cases in which a prolonging influence has been claimed (21, 95, 276, 305). During anaesthesia with ether, or with cyclopropane no appreciable difference in the duration of muscular paralysis was demonstrated as compared with the values obtained during barbiturate anaesthesia (84, 347, 350).

Prolonged action of SDCh can be maintained by repeated single doses (31, 32, 58, 87, 111, 175, 350), by intravenous infusion (5, 23, 25, 126, 176, 270, 273, 287, 333, 334, 347, 370) and by combination with other relaxant drugs such as dTC (18, 25, 34, 84, 216, 305, 347), gallamine (34, 84, 216, 347), C-10, or Imbretil (34, 59). In such cases the initial thiopentone anaesthesia is frequently replaced by nitrous oxide (32, 34, 58, 84, 121, 124, 138, 175, 177, 283, 334, 357).

Using intravenous infusions, the degree of muscular relaxation can easily be varied by adjusting the rate of infusion, and a desired degree can be maintained without cumulation (303, 347, 350, 369). Occasionally, however, tachyphylaxis has occurred (277). After stopping an infusion the reflexes return as quickly as after a single injection (121, 175, 305).

SDCh is remarkably free from side effects when given in normal clinical doses (25, 34, 55, 58, 83, 84, 126, 172, 202, 216, 261, 270, 350). By intracutaneous injections Thesleff (203, 347, 350) found that it causes no liberation of histamine. Others believe that some histamine may be liberated, but only about $\frac{1}{100}$ of the quantity found after dTC (18). Laryngospasm never occurs with SDCh (126, 216). A slight salivation can be observed in patients who receive SDCh without pretreatment with atropine or scopolamine (18, 34). On the other hand, Foldes and other authors (84, 124, 369) reported mild blocking of parasympathetic effects, such as a slight increase in pulse rate and occasionally dilatation of the pupils.

A few authors have seen a slight rise of blood pressure after single intravenous doses of SDCh, both systolic and diastolic pressure being affected (4, 18, 34, 84, 124, 126, 138, 282, 369). After high doses of SDCh alterations of the electrocardiogram pattern can sometimes be observed (elevation of the T-wave (34)). Finally, injections of SDCh regularly cause a rise in intraocular pressure (199b), probably due to a simultaneous contracture of all external eye muscles and also to ganglionic actions or to asphyxia (325).

The prompt and complete paralysis caused by SDCh, which is followed by an equally rapid recovery, made this relaxant valuable for intratracheal intubation. Details of dosage and techniques are discussed in the following references, 2, 18, 25, 28, 34, 83, 124, 125, 185, 186, 216, 261, 269, 270, 283, 295, 298, 305, 350, 369.

SDCh is also used widely for laryngoscopy (23, 34, 114, 177, 178, 218), oesophagoscopy (34, 107, 207, 227, 260), bronchoscopy (22, 23, 24, 76, 77, 107, 177, 178, 206, 207, 216, 219, 225, 227, 236, 260, 263, 270, 279, 295, 302, 306, 307, 326), cystoscopy (83, 84, 295, 350), gastroscopy (225, 363), bronchography (216, 243, 260), and aortography (84, 350). There is no agreement whether SDCh should be given to inadequately prepared patients in casualty anaesthesia (3, 17, 51, 151, 319, 374). Furthermore, SDCh has been found useful in the treatment of laryngospasm (84, 270, 350) and in various kinds of orthopaedic manipulations (2, 18, 23, 34, 58, 83, 84, 136, 167, 189, 225, 270, 287, 305, 350, 357, 369).

For dosage and details of special techniques of SDCh in thoracic surgery see 78, 83, 84, 273, 369. For the use of SDCh in abdominal surgery see 18, 34, 58, 59, 83, 121, 126, 175, 201, 216, 225, 278, 282, 284, 292, 296, 312, 322, 328, 333, 334, 345, 437.

SDCh with its short action and the absence of toxic side effects is the drug of choice in electroshock therapy (4, 10, 11, 18, 28, 34, 88, 152, 164, 184, 196, 202, 203, 204, 266, 275, 283, 305, 314, 323, 346, 350, 369). For this purpose it was first introduced by Ginzel and Arnold (11, 152) and independently by Thesleff and Holmberg (202, 203, 204, 346, 350). The effects of SDCh make it possible to reduce the convulsions until they are practically negligible, without risking prolonged respiratory arrest. By using electroshocks modified by SDCh the frequency of fatal accidents, of bone fractures, or other major injuries can be greatly diminished (10, 202). For technical details see 10, 196, 202, 203. Other neurological indications for which SDCh has been successfully applied are the intra-arterial therapy of spastic pareses (115, 172), and the treatment of tetanus (58, 135, 172, 373). Experimental investigations on the SDCh action in experimentally produced local tetanus of rabbits were done by Hougs and Andersen (211), who found a complete disappearance of the action potentials of the anterior tibial muscle for a duration of twenty minutes after two successive doses of 0.5 mg/kg SDCh. In myasthenic patients SDCh acts in the same manner as in normal individuals (208, 350).

In obstetrics SDCh is frequently used (58, 106, 126, 249, 305, 336, 349). No untoward reactions of the newborn have been observed, since although it passes the placenta it is destroyed to a great extent during this passage, owing to the considerable concentration of esterase in placental tissue (58, 349). Intrauterine manipulations are greatly facilitated by the use of SDCh because of the relaxation of the perineum (305, 369).

No complete conformity exists as to whether SDCh should be given according to body weight (32, 112, 203). For short periods of relaxation and depending on the duration desired, total doses of 10–100 mg, or 0.3–1.1 mg/kg SDCh are given intravenously (23, 25, 31, 34, 58, 83, 119, 121, 126, 127, 164, 202, 203, 216, 287, 305, 314, 350, 369, 371). According to need these doses are sometimes repeatedly administered (18, 83, 84, 137, 138, 226, 350, 371). For intravenous infusion 1–15 mg per minute are given in a 0.1–0.2% solution (84, 86, 119, 121, 124, 125, 126, 138, 172, 175, 190, 305, 347, 350, 373).

b. Other di-trialkylammoniumethyl esters of succinic acid. Di-monoethyldimethylammoniumethyl succinate, di-diethylmonomethylammoniumethyl succinate and di-triethylammoniumethyl succinate were first investigated pharmacologically by Bovet, who found that their neuromuscular activity decreases with the number of ethyl groups attached to the nitrogen (37, 38, 41, 42, 44, 146, 149, 150, 254). Moreover, Ginzel *et al.* (163), working with these substances were able to show that a progressive change of methyl groups to ethyl groups also alters the character of the neuromuscular paralysis. Working with a nerve-muscle preparation of the pigeon, they showed that the diethyl and triethyl derivatives no longer caused contracture, whereas the monoethyl derivative gave rise to a transient contracture similar to that caused by SDCh, but only occurring with relatively high doses. Additional observations on the rectus muscle of the frog also showed the change from contracture-producing substances to contracture-inhibiting ones with stepwise substitution of methyl by ethyl groups. These observations can be interpreted as a gradual change of the type of paralysis from the depolarizing action of SDCh to a neuromuscular block like that produced by curare with the triethyl derivatives. This conclusion agrees with observations by Bovet *et al.* (41, 42), who were able to demonstrate that the triethyl derivatives of SDCh antagonized the paralysis as well as the contracture-producing activity of SDCh itself. However, the ethyl derivatives do not show all the characteristics of true "curariform" activity because, for instance, they are not antagonized by anticholinesterases (163).

Of the ethyl derivatives of SDCh, only the mono-ethyl compound has been tried in clinical research, first by Valdoni (356) and later by Scurr (323) and Mazzani *et al.* (274). There is no appreciable difference between the clinical effects and mode of action of this substance and of SDCh (28, 131, 182, 248).

Di-monobenzyldimethylammoniumethyl succinate (Table I, 14), di-mononitrobenzyldimethylammoniumethyl succinate (297) and di-monoacetonyldiethylammoniumethyl succinate (Table I, 16) have only a weak blocking action of the "curariform" type. For instance, the monobenzyl derivative showed a paralysing action of only $\frac{1}{30}$ of that found with SDCh on the pigeon nerve-muscle preparation, causing no contracture but only flaccid paralysis. The action of the p-nitrobenzyl derivative in cats is about $\frac{1}{8}$ of that of dTC. The action is reversible by 3-hydroxyphenyldimethylethylammonium just as with dTC (297). The blocking effects on the superior cervical ganglion of the cat are

about $\frac{1}{25}$ of those of dTC. The enzymatic hydrolysis of the diethylacetonyl derivative is of the same order as that of SDCh (338).

3. Diesters of higher homologous aliphatic dicarboxylic acids. a. Glutaryldicholine and adipyldicholine. There is only a small number of published reports on glutaryldicholine. The neuromuscular paralysing activity was first observed by Bovet and co-workers (35, 41, 42, 146, 149, 150) in the progress of an investigation of a large number of different DChDCAE. In agreement with Bovet-Nitti (44), Klupp and Stumpf (233) found that the compound is hydrolyzed by BChE at a rate of approximately 40% of that of ACh in equivalent concentrations. Clinical trials on human patients showed that a satisfactory degree of relaxation cannot be obtained even with doses as high as 200 mg, and this dose already caused a considerable rise of blood pressure (57). Some pharmacological details were worked out by Ginzel *et al.* (156), who found a close quantitative and qualitative similarity between the actions produced by glutaryl- and adipyldicholine (AdDCh). In the cat, AdDCh and glutaryldicholine correspond reasonably well as regards intensity and duration of neuromuscular paralysis (156, 158, 159, 160), and doses as low as 100 μg/kg cause a considerable rise of blood pressure. This undesirable side effect was also noted by Bovet in experiments on dogs (35, 41, 42); further investigations of Ginzel *et al.* (158, 159, 160) revealed a nicotine-like action of AdDCh on sympathetic ganglia and on the chemoceptors of the carotid sinus. The neuromuscular actions of glutaryldicholine and AdDCh were studied on the isolated diaphragm preparation of the rat, where glutaryldicholine was about three times as active as AdDCh. Both the activity of glutaryldicholine and AdDCh can be increased markedly by adding a small dose of eserine to the bath. An explanation for the potentiating effect of eserine is obtained from the fact that glutaryldicholine, as well as AdDCh, is hydrolyzed by cholinesterase preparations: Bovet-Nitti (44) found that BChE and AChE of ox erythrocytes are able to split AdDCh, though the latter enzyme was much less effective. Similar results were obtained by Ginzel *et al.* (160), who were using the nucleus caudatus of dogs as a source for AChE. However, Augustinsson *et al.* (14) did not find any measurable hydrolysis of AdDCh on incubation with AChE of human erythrocytes. Bovet-Nitti (44) and Klupp *et al.* (233) agree that AdDCh and glutaryldicholine are hydrolyzed by BChE of serum of man and horse at about the same rate. The latter authors (233) found in addition that both diesters of choline are hydrolyzed by BChE of dog serum at a much lower rate, taking the speed of hydrolysis of ACh as a basis of reference (2–3% as compared with about 40% in the case of serum of horse or man). Using an electrometric titration procedure, Ginzel *et al.* (160) found that the hydrolysis of AdDCh through BChE concerns only one ester bond, resulting in the formation of the monocholinester of adipic acid. Support for this interpretation was later provided by the chromatographic work of Augustinsson and Grahn (14) who, after enzymatic hydrolysis, demonstrated the appearance of a new spot on the chromatogram, which was due neither to choline nor to AdDCh and therefore could reasonably be ascribed to adipylmonocholine, though final identification was not performed.

The question now arises to what extent the enzymatic hydrolysis of AdDCh and glutaryldicholine is responsible for the short duration of action of these compounds in the organism. The relevant considerations have already been discussed for the case of SDCh; the arguments for and against the theory that hydrolysis is responsible for the short duration of action will be summarized briefly. Highly suggestive for BChE being related to the short action are a number of observations regarding the potentiation of activity of AdDCh after administration of anticholinesterases. For such experiments eserine has been used in cats, dogs, and pigeons; in the isolated diaphragm preparation of the rat neostigmine and TEPP have been used in addition. It is interesting that out of the series of dicholinesters and related compounds investigated by Ginzel *et al.* (159), AdDCh showed the highest degree of potentiation of its paralysing activity after eserine pretreatment of the test objects. Further support for this assumption is obtained from the comparatively high activity of AdDCh in dogs as compared with man (57, 273); this appears to be correlated with differences of speed of enzymatic hydrolysis as described above (233).

However, difficulties are encountered if the degree of potentiation of the neuromuscular blocking activity, caused by pretreatment with eserine, is compared for different members of the series of dicholinesters and related compounds investigated by Ginzel *et al.* (159, 160), as there is no appreciable correlation between the speed of hydrolysis of these diesters by BChE and the potentiation of their paralysing effects by eserine.

In addition to their paralysing action on skeletal muscles, AdDCh and glutaryldicholine under certain circumstances showed excitatory actions, which appeared related to the ganglion-stimulating effects already mentioned: such effects are best observed on the isolated rectus muscle of the frog (180), with close arterial injection in the cat (160), and on avian muscle (157, 163). In the amphibian and avian muscle preparations these compounds produce contracture; with close arterial injection to the anterior tibial muscle of cats a twitch is observed before the onset of the paralysis, and in chronically denervated muscles of cats AdDCh was found to produce a contracture of the same type as that seen with ACh (48, 157). These stimulatory actions of AdDCh and of related compounds can be potentiated by pretreatment with eserine, and it was found by Ginzel *et al.* (160) that there is a satisfactory correlation between the degree of potentiation and the rate of hydrolysis by BChE. It will be noted that the situation is rather different from that which was described for the paralysing actions: following eserine, only the stimulating actions show such increase of activity as would be postulated by the BChE hypothesis outlined before, whereas this relationship does not hold true for the paralysing actions.

The stimulating actions on amphibian, avian, and chronically denervated mammalian muscle, as well as the twitch response of the innervated mammalian muscle after close arterial injection, resemble closely the effects of ACh, decamethonium, or SDCh, and are characteristic of substances which depolarize the neuromuscular junction. In fact, Ginzel *et al.* (163) were able to show that AdDCh lowered the resting potential of frog muscle and of the gracilis muscle of the cat, and they furthermore showed that stimulatory as well as paralysing

actions of AdDCh are antagonized by dTC or related competitive blocking substances, such as the di- and triethylammoniumethyl adipates.

The relative intensity of excitatory and paralysing activities of AdDCh varies for different animal species. Unfortunately, the excitatory actions predominate in man to such an extent that this substance is not suitable for clinical application (57, 263).

b. Other di-trialkylammoniumethyl esters of glutaric and adipic acid. Of the three possible symmetrical ethyl substitution products of glutaryldicholine only di-triethylammoniumethyl glutarate has been tested pharmacologically; Bovet *et al.* (38, 42, 146, 149, 150) found that the head drop dose of this substance was as high as 20 mg/kg. The neuromuscular blocking activity of this compound is therefore about $\frac{1}{40}$ of that of glutaryldicholine (38, 42, 146, 149, 150).

Stepwise replacement of methyl by ethyl groups in the molecule of AdDCh led also to a reduction of the neuromuscular blocking activity, as was first demonstrated by Bovet *et al.* (41, 42). Ginzel *et al.* (157, 163) were able to show that this decrease of activity is associated with a change of the type of action on the neuromuscular junction, in a way similar to that already described for SDCh and its ethyl derivatives. The monoethyl derivative of AdDCh still retains the depolarizing effect of AdDCh, as evidenced by its contracture-producing activity in amphibian and avian muscle and by its ganglion-stimulating effects (42, 320). A rather unexpected phenomenon was observed with di-diethylmonomethylammoniumethyl adipate in the pigeon, with doses ranging from 1–3 mg/100 g body weight. This compound not only produced contracture but at the same time also abolished the contractions elicited by indirect stimulation (157, 163); with higher doses only a transient block of indirect excitability was noted, and if the substance was injected during a prolonged contracture (caused, for instance, by C-10) it immediately abolished the contracture. According to the two last mentioned effects, the substance would have to be classified as a curare-like compound, in contrast to its stimulant and excitatory effect in the lower dosage range. It appears to occupy an intermediate position between a purely depolarizing drug (as AdDCh) and a competitive blocking drug of the dTC type. In fact, the corresponding di-triethyl derivative of ADCh had only the typical properties of a competitive blocking drug and did not produce contractures (157, 163, 353). On isolated frog muscle di-triethylammoniumethyl adipate was found to protect completely against the depolarizing action of ACh, C-10 and AdDCh, whereas in the gracilis muscle of the cat this compound still gave rise to a very transient reduction of the resting potential. The reduction amounted to only two to three millivolts (163), but the depolarizing effect of a subsequently injected dose of AdDCh was markedly reduced (163). These findings indicate that a gradual transition takes place from depolarizing to competitive blocking action, with intermediate stages in which both properties are present in one and the same compound. A theoretical interpretation of this phenomenon, recently developed by Ariens (7, 8, 9), will be discussed in IV C, below.

Nevertheless there is a notable difference between the competitive blocking

activity of di-triethylammoniumethyl adipate and dTC inhibitors (159). As has already been discussed in IV, A, 2b, this may well be due to the enzymatic hydrolysis of the ethyl derivatives, which proceeds at about the same rate as the hydrolysis of AdDCh (160).

c. Pimelyldicholine, suberyldicholine, azelayldicholine, sebacyldicholine and 1,10-decanedicarbonyldicholine. Pimelyldicholine was investigated by Bovet *et al.* (35, 41, 42, 146, 149, 150) who found that the head drop dose in the rabbit amounts to 3 mg/kg. They also noted as a marked side effect a considerable rise of blood pressure, which in dogs was observed in doses as low as 0.02 mg/kg. In contrast to its weak paralysing effect in mammalian muscle, pimelyldicholine caused contractures of the frog rectus muscle in comparatively low concentrations, being in that respect about thirty times more potent than SDCh.

Suberyldicholine and sebacyldicholine show great similarity in their pattern of pharmacological actions, though certain quantitative differences exist. They will be discussed together on the basis of findings reported by Ginzel *et al.* (161) and of unpublished observations made by Brücke *et al.*

The main effect of these two substances, in doses which have no paralysing effect on unanaesthetized dogs, is a powerful stimulation of respiration. This can also be seen in anaesthetized cats and is only insignificantly diminished by section of both vagi. The respiratory stimulation, which is considerably more effective than that of lobeline (suberyldicholine is forty times, sebacyldicholine is ten times more potent) can be completely abolished by denervation of the carotid sinus. Under such circumstances there is even a slight inhibition of respiration, just as with lobeline. When suberyl- or sebacyldicholine are injected into the circulation of the carotid sinus, only very small doses (about 1 μg) are needed to stimulate respiration. Isolated destruction of the chemoceptors of this region by injection of 0.3 ml of a 0.5% solution of acetic acid also abolishes the influence of those substances on respiration, whereas the effect on blood pressure remains unaltered.

It is apparent from these experiments that suberyl- and sebacyldicholine are powerful stimulants of the chemoceptors of the region of the carotid body, with relatively insignificant effects on the aortic chemoceptors. Furthermore, these substances cause a dramatic rise of blood pressure even in subparalytic doses. The pressure effect is not affected by section of both vagi or by denervation of the baroceptor and chemoceptor region of the carotid sinus and it occurs in decapitated cats. No effect on perfusion pressure can be seen in isolated hindlegs of the cat under artificial perfusion, and all pressor effects in intact animals are abolished by ganglion-blocking agents such as hexamethonium.

It is evident, therefore, that the pressor effects of these diesters are mainly caused by stimulation of sympathetic ganglia and probably also by stimulation of the suprarenal medulla and that stimulation of chemoceptors plays a subordinate role in this instance. The strong ganglion-stimulating effect can also be observed in isolated, artificially perfused ganglia. Since all these effects are seen with doses $^1/_{100}$ of those which cause muscular paralysis, the latter effect can hardly be observed in intact animals. On isolated muscle preparations however they act as depolarizing relaxants, causing contracture in avian and frog muscle.

It is noteworthy that apparently only one quaternary N-atom is necessary for the marked nicotinic effect of such substances, for we found in unpublished experiments that replacement of one choline group in sebacyldicholine by methoxyl hardly changes the pharmacological properties. 0.2–0.3 mg/kg of this substance given intravenously in cats causes a rise of blood pressure of about 150 mm Hg, and the effects on respiration are comparable with those of sebacyldicholine. The rather weak paralysing effect on striated muscle is also retained, since such doses cause a decrease of muscle twitches in the gastrocnemius when the nerve is stimulated. Moreover, the second quaternary nitrogen grouping in the DChDCAE series apparently interferes with the ganglionic action of these substances, since the latter activity decreases with shortening of the polymethylene chain. This hypothesis is supported by the fact that in SDCh replacement of one choline group by methoxyl leads to a considerable increase of the ganglion-stimulating activity. For the neuromuscular blocking activity on the other hand, an optimal steric arrangement of the two quaternary nitrogen atoms appears necessary, since increasing the distance between these two atoms, or removal of one of them, reduces the activity.

Preliminary tests with azelaylcholine, for which no published data exist, were carried out in the Vienna Institute. A ganglion-stimulating activity, which was about half as strong as that of suberyldicholine, was noted.

The ganglion-stimulating activity is still further reduced in the case of 1,10-decanedicarbonyldicholine (232). The neuromuscular activity is of the same type and of about the same magnitude as that of sebacyldicholine.

A comparative investigation of the enzymatic hydrolysis of pimelyl-, suberyl-, azelayl-, sebacyl- and 1,10-decanedicarbonyldicholine by sera of man, horse and dog was carried out by Klupp and Stumpf (233). Similar observations have been made by Bovet-Nitti (44) and Ginzel *et al.* (160) with lower members and with some of the substances mentioned here. In the series of the DChDCAE, Klupp and Stumpf (233) found that the rate of hydrolysis increases with the length of the polymethylene chain. For human serum the hydrolysis rate reached its maximum in azelayldicholine with a substrate concentration of 30 μM, whereas for dog serum a maximum of hydrolysis was obtained with sebacyldicholine. In all cases 1,10-decanedicarbonyldicholine was found to be split at a slower rate than sebacyldicholine. The enzymatic breakdown of the latter substance has been investigated in detail by Ginzel *et al.* (160) and Augustinsson and Grahn (14), and it appears probable that the first step of hydrolysis consists in the formation of sebacylmonocholine.

Very few data are available for the enzymatic hydrolysis of these compounds by AChE. It is evidently very slow. A certain dependence of the rate of hydrolysis on the chain length seems to occur, since Ginzel *et al.* (160) showed that sebacyldicholine was hydrolyzed twice as rapidly as AdDCh by esterase from human nucleus caudatus. However, Augustinsson and Grahn (14) found no changes in chromatograms of sebacyldicholine after incubation with human erythrocytes.

d. Other di-trialkylammoniumethyl esters of pimelic, suberic, azelaic, sebacic and 1,10-decanedicarboxylic acid. Few pharmacological observations have been published on the di-triethylammoniumethyl esters of dicarboxylic acids with five,

six, eight, and ten methylene groups. The di-triethylammoniumethyl esters of pimelic and sebacic acid were first described by Bovet *et al.* (42, 146, 149, 267), who found them markedly less potent neuromuscular blocking agents than the analogous trimethyl derivatives. A more detailed pharmacological investigation of di-triethylammoniumethyl suberate and the corresponding sebacyl ester was carried out by Ginzel *et al.* (154, 229). These authors were able to show that these substances have only a very weak curare-like neuromuscular blocking activity but a relatively strong ganglion-blocking action as indicated by a fall in blood pressure and a reduction of the contraction of the nictitating membrane after preganglionic stimulation. The relation between ganglion-blocking (5–15 mg/kg) and neuromuscular paralysing doses in cats was found to be 1:4. In man both these substances caused a short lasting ganglionic blockade and a transient fall of blood pressure (240). Di-triethylammoniumethyl esters of the higher aliphatic dicarboxylic acids investigated so far are hydrolyzed by BChE at about the same rate as the corresponding trimethyl derivatives (338).

In an attempt to find more potent and clinically suitable ganglion-blocking agents, a series of substances was synthesized by the "Österreichische Stickstoffwerke" in which acetonyl, ethyl, acetyl, allyl, n-propyl, and morpholyl groups in combination with methyl or ethyl groups were attached to the two quaternary nitrogens of the suberic and the sebacic derivatives. Some chemical and pharmacological data concerning these substances are listed in the tables, p. 275–279. All these substances exerted a relatively weak curare-like activity together with a more or less pronounced ganglion-blocking action. The most potent ganglion-blocking compound was di-diethylacetonylammoniumethyl sebacate which, in doses of 5 mg/kg, caused a 70% reduction of the contractions in nictitating membrane of the cat stimulated indirectly from the preganglionic fibres. Doses as high as 30 mg/kg were ineffective on neuromuscular transmission. The investigation of the enzymatic hydrolysis of these substances by horse serum resulted in the following general statements. Substitution of one radical by an acetonyl group decreases the velocity of enzymatic hydrolysis. This is more prominent with ethyl derivatives than with dicholinesters. Substitution of one methyl group of the choline radicals by n-propyl increases, substitution of all three methyl groups by n-propyl decreases, the enzymatic hydrolysis.

4. Diesters of unsaturated, branched, or cyclic aliphatic dicarboxylic acids. The dicholinester of fumaric acid was investigated by Bovet *et al.* (41, 42, 146, 149, 150). It was stated that this substance showed a neuromuscular blocking activity which equalled that of AdDCh (head drop dose 0.5 mg/kg). Substitution of one methyl group by ethyl did not alter the paralysing activity (41, 42).

Fumaryldicholine and maleyldicholine both show a higher rate of non-enzymatic as well as of enzymatic hydrolysis than SDCh (165, 338). Using horse serum as the source of enzyme, the hydrolysis of fumaryldicholine amounts to 18% (338) and that of maleyldicholine to 13% (165) of that of ACh in equimolar concentrations. From these results it seems probable that unsaturated DCh-DCAE are hydrolyzed at a faster rate than the corresponding substances with saturated chains.

Attachment of one methyl (41, 42, 146, 149, 150, 313) or one phenyl group (342) to the ethylene moiety in the molecule of SDCh results in a considerable decrease of the neuromuscular paralysing action; for methylsuccinyldicholine the head drop dose is 2 mg/kg and for phenylsuccinyldicholine 8 mg/kg, as compared to a head drop dose for SDCh of 0.2 mg/kg. It has been stated that phenylsuccinyldicholine is able at moderate dose levels to produce severe paralysis of the extremities without at the same time impairing spontaneous respiration in rabbits (342). Phenylsuccinyldicholine was found to produce 50% inhibition of the AChE of erythrocyte hemolysate in a concentration of 10^{-2}; in this respect it is therefore less active than SDCh (342).

Diesters of cycloaliphatic dicarboxylic acids were described by Bovet *et al.* (36, 38, 41, 42, 144, 150), who investigated cyclohexyl-1,2-dicarbonyldicholine and the di-dimethylmonoethylammoniumethyl and di-triethylammoniumethyl ester of 1,2-cyclohexanedicarboxylic acid. Compared with the noncyclic aliphatic compounds, the alicyclic esters showed a considerable decrease of the paralysing action. A remarkable feature of the alicyclic compounds is that progressive ethyl substitution increases the neuromuscular activity, in contrast to SDCh and AdDCh in which ethyl substitution has the opposite effect.

B. Dicholinesters and other di-trialkylammoniumethyl esters of aromatic dicarboxylic acids

Bovet *et al.* (35, 36, 38, 40, 41, 44, 145, 147, 148, 150, 268) have carried out experiments on the pharmacological actions of dicholinesters of phthalic, terephthalic and isophthalic acid and of their trialkylammoniumethyl derivatives, all of which have weak curare-like activity (267). The head drop doses lie between 3 mg/kg for the dicholinester of terephthalic acid (320 I.S.) and 40 mg/kg for that of isophthalic acid (324 I.S.). The neuromuscular activity is maximal for the para-derivatives, less for ortho-derivatives and weakest for the meta-derivatives. Partial or complete replacement of methyl by ethyl groups increases the neuromuscular action in all cases about two to threefold. Hydration of the benzene rings does not alter the activity. This is remarkable since the distance between the nitrogen atoms in the hexahydro derivative of the dicholinester of phthalic acid (320 I.S.) is about the same as in SDCh and yet the substance has only $\frac{1}{25}$ the activity of the latter.

Peripheral muscarinic actions could not be found with these substances. Nicotine-like actions can be demonstrated in the dicholinester of terephthalic acid (320 O.S.) and of isophthalic acid (324 I.S.), whereas the triethylammoniumethyl derivatives lower the blood pressure, block the cardiac vagus, and inhibit the ACh contracture in frog rectus muscle.

The neuromuscular paralysing action of 302 I.S., as tested by measuring the head drop doses and by toxicity tests, is augmented by eserine. This can be explained by an inhibition of enzymatic hydrolysis, to which all diesters of aromatic dicarboxylic acids are susceptible.

According to Bovet-Nitti aromatic dicholinesters are hydrolyzed only by BChE and the following rules can be formulated. 1) The number of choline

radicals (1, 2, or 3) is unimportant for the velocity of hydrolysis. 2) Substitution of one methyl group within the choline radicals by an ethyl group has an influence in so far as the ethyl derivatives are hydrolyzed more slowly than the cholinesters. This is in contrast to the aliphatic cholinesters and their ethyl derivatives respectively. 3) In dicholinesters of aromatic dicarboxylic acids the steric arrangement of the substituents is relevant. The rate of hydrolysis of isophthalyldicholine is 160%, that of terephthalyldicholine 25%, and that of the dicholinester of phthalic acid is 12% of that of benzoylcholine. Bovet-Nitti made an interesting discovery when investigating the hydrolysis of the di-triethylammoniumethyl ester of terephthalic acid (302 I.S.) (45). She showed that whereas eserine in concentrations of 1 in 10,000 completely inhibits the hydrolysis of benzoylcholine it has only a very weak inhibiting action on the hydrolysis of 302 I.S. The di-triethylammoniumethyl esters of the phthalic acids also cause selective inhibition on both types of cholinesterases (35, 45). The di-triethylammoniumethyl phthalate (306 I.S.) inhibits the hydrolysis of benzoylcholine by BChE up to a dilution of 1:10,000 and moreover it inhibits the hydrolysis of the meta-derivative, which normally is hydrolyzed thirteen times more rapidly than 306 I.S. On the other hand, 306 I.S. does not inhibit AChE. In contrast to this substance both the di-triethylammoniumethyl terephthalate and the di-triethylammoniumethyl isophthalate inhibit the activity of AChE, but not that of BChE. 306 I.S. can therefore be characterized as a specific inhibitor of BChE, and 302 I.S. as a specific inhibitor of AChE. The same applies to the corresponding diiodopropylates. Only those substances which inhibit AChE have muscarinic properties *in vivo*.

Experiments by Liljestrand and Zotterman (250) have shown that neither 302 I.S. nor 306 I.S. increases the sensitivity of chemoceptors to oxygen lack, ACh, or other cholinesters.

C. General considerations concerning the mechanism of action and the correlation between chemical structure and pharmacological actions of di-trialkyl-ammoniumethyl esters of dicarboxylic acids

The di-trialkylammoniumethyl esters described in this review can be classified as to their chemical structure according to the following distinctions. a) The nature and the size of substituents attached to the two quaternary nitrogen groups. Variations of these substituents, especially when there is a change from methyl to other radicals change the basic characteristics of the pharmacological actions of such compounds. b) The number and the nature of the bonds and of the distribution of CH or CH_2 groups intercalated between the two esteratic groups. Variation in this respect leads to appreciable changes in pharmacological effect only when aliphatic bonds are changed into aromatic ones. Within the aliphatic series such changes lead only to quantitative changes in activity.

In the aliphatic series, all substances in which the two quaternary nitrogen atoms are substituted only by methyl groups show predominantly depolarizing effects on neuromuscular or ganglionic synapses. Carbonyldicholine, the first member of the homologous series, is an exception. It has no neuromuscular

activity at all, but only a competitive ganglion-blocking action like hexamethonium. The depolarizing action of aliphatic dicholinesters was first demonstrated in a comparison with the action of decamethonium. A direct electrophysiological demonstration of the changes in membrane potentials of muscle fibres was carried out by Ginzel *et al.* (163). Using the method of Burns and Paton (60), these authors measured the changes of the resting potential in the gracilis muscle of the cat under the influence of adipyldicholine (AdDCh). They were able to show that in some cases an intra-arterial injection of 10–30 μg caused a complete depolarization, whereas in other preparations there remained a potential amounting to 40–80 % of the original resting potential. The depolarizing effect lasted eight to ten minutes. Pretreatment with dTC (150 μg) prevented the depolarization for a duration of two hours. In frog muscle the method of Kuffler (241) and Fatt (113) was applied and showed that AdDCh caused only a diminution of the resting potential and this diminution could be enhanced by eserine (10^{-6}). In these experiments no evidence could be found for the assumption that the depolarizing action of AdDCh spreads from the endplate region to the adjoining parts of the muscle fibre. No analogous experiments have been carried out so far to measure the depolarizing action of dicholinesters at the site of the synaptic membranes in ganglia.

Stepwise substitution of methyl groups in the quaternary bases by ethyl groups leads to an alteration of the neuromuscular action. According to the number of ethyl groups present in the molecule, there is a diminution or complete extinction of the depolarizing effects and at the same time a competitive antagonism appears against the neuromuscular actions of ACh (267). Whereas the monoethyldimethyl derivative still retains practically all properties of the trimethyl ester, the diethylmonomethyl derivative shows both depolarizing and competitive antagonistic properties, and the triethyl derivative shows only pharmacological properties which are characteristic of dTC, namely a competitive antagonism against the physiological transmitter substance. Ginzel *et al.* (163) were able to show that the triethyl derivative corresponding to AdDCh causes no depolarization on its own, but only diminishes or, in higher doses, abolishes the depolarization caused by AdDCh. With equivalent doses this effect is much shorter than that of dTC. An analogous action of the triethyl derivative can be shown on frog muscle, where the depolarizing effect of AdDCh is completely abolished over a wide range of dosage.

The progressive substitution of ethyl instead of methyl groups on the quaternary nitrogen in di-trialkylammonium esters also leads to a diminution of the ganglion-stimulating effects of such substances. In some instances, *e.g.*, for the triethyl derivatives of adipic (353), sebacic (154, 158, 229) or suberic acid (154, 158, 229), a ganglion-blocking effect can be seen, which replaces the strong excitatory effect caused by the corresponding dicholinesters. Substantially the same changes can be noticed if an exchange of methyl groups against larger radicals such as propyl, acetonyl, ethylacetyl, or others takes place, and it is of no importance whether this substitution is uniform, or is a mixed substitution with ethyl groups. The diminution of the neuromuscular blocking effects is still more

striking with the triethyl derivatives, and all these substances show more or less ganglioplegic effects which in some of the substances distinctly prevail over the neuromuscular effects.

Partial or complete ethylation of aliphatic cholinesters of dicarboxylic acids does not lead to a diminution of the enzymatic hydrolysis by BChE. Substitution of one radical by an acetonyl group diminishes the hydrolysis more in the ethyl derivatives than in the dicholinesters. Symmetrical replacement of one methyl group by propyl increases, and complete substitution with propyl groups diminishes, the enzymatic hydrolysis.

The relations described here between the pharmacological properties and the nature of the substituents provides another example of the influence which a diminution of the electric charge at the site of the quaternary nitrogen exerts on the synaptic membranes. The diminution of electric charge by the "umbrella-effect" of substituted aliphatic radicals weakens the depolarizing activity, but, on the other hand, it rather enhances the affinity of such substances for the different membranes. The effect is that the greater steric extension of these substances increases the competitive displacement of ACh set free as the physiological transmitter. The relations between the nature of substituents, the density of charges, and the pharmacological actions are considered in more detail in Taylor's review (344).

In the series of aromatic dicholinesters the neuromuscular blocking effect is increased by stepwise substitution with ethyl groups. Precise experiments are lacking, but the very weak neuromuscular paralysing effect of the aromatic series seems to indicate that in this group of cholinesters even the methylated compounds have predominantly curare-like effects. Therefore, ethylation would not change the mode of action.

The ganglionic effects of the di-trialklyammoniumethyl esters of the aromatic series resemble those of the aliphatic group, since here too progressive ethylation of the choline derivatives causes a change from excitatory to paralysing effects. The enzymatic hydrolysis in the aromatic series of cholinesters is distinctly decreased by substituting methyl groups by ethyl groups.

Within the series of aliphatic cholinesters of dicarboxylic acids, the neuromuscular blocking effect increases with the length of the chain from carbonyldicholine to SDCh and then decreases with a further increase in the length of the polymethylene chain. The contracture effect on bird muscle behaves similarly. On the other hand, the dependence of the contracture-producing effect in frog muscle on the chain length of aliphatic dicholinesters shows an inverse behaviour. In the rectus abdominis preparation of frogs, the dicholinester of oxalic acid has an extremely weak effect. With an increase in the chain length the contracture gets more and more pronounced and with sebacyldicholine reaches 74% of that of ACh. It is interesting to note that a similar connexion between chain length and effect as found with the frog rectus muscle holds also true for the ganglion- and endplate-stimulating effects. Whereas carbonyldicholine has a paralysing effect on ganglia, this disappears quickly with an increase in chain length and even SDCh shows a weak ganglionic stimula-

tion which increases steadily with the length of the polymethylene chain to reach its maximum with suberyldicholine. Similarly, the tension of the initial twitch on close arterial injection increases considerably from SDCh to suberyldicholine. Moreover, an exact analysis of the ganglionic effects of SDCh showed that under certain experimental conditions it causes a ganglion-blocking effect both to preganglionic tetanic stimulation and to single shocks, which is apparently not caused by ganglionic depolarization but rather by competitive antagonism to ACh. SDCh therefore offers another example of the fact that a single substance may influence the effector organ by depolarization and, at the same time, by a competitive antagonism against other depolarizing agents. Something similar can be seen with the di-triethyl ammonium adipate on the cat gracilis muscle, where it first decreases the resting potential by 2–3 mV but then inhibits further depolarization by other depolarizing substances (163).

From the existing data, the following general conclusions may be drawn about the influence of chain length on enzymatic hydrolysis for aliphatic cholinesters of dicarboxylic acids, and its relation to pharmacological activity. The rate of hydrolysis increases with the number of interposed CH_2-groups and reaches its maximum with azelayldicholine with human and horse serum and with sebacyldicholine for dog serum. A further increase in chain length diminishes the hydrolysis. On the frog rectus there exists a correlation between the velocity of hydrolysis or the chain length and the enhancement of the contracting effect under the influence of eserine. However, no such correlation can be demonstrated with the muscle of warm-blooded animals, since the increase of effect under the influence of eserine is maximal for AdDCh and decreases both with decreasing and with increasing chain length. The initial twitch does not show the potentiating effect of eserine with certainty. The existing data do not show what influence double bonds within the methylene chain of aliphatic di-trialkylammoniumethyl esters may have on their pharmacological properties. On the neuromuscular activity the influence seems to be slight, if demonstrable at all, as can be seen from the negligible difference between the neuromuscular blocking effect of SDCh and fumaryldicholine (41, 42). However, the rate of non-enzymatic and enzymatic hydrolysis of such substances is greatly increased (338).

If the polymethylene chain is replaced by aromatic groups the neuromuscular paralysing effects of dicholinesters of dicarboxylic acids are considerably decreased. With the three isomeric dicholinesters of phthalic acid, the para-derivative shows the strongest, and the meta-derivative the slightest, activity. No quantative data are available for the ganglionic effects of aromatic dicholinesters.

These substances are hydrolyzed by BChE like their aliphatic congeners, the meta-derivative showing the highest and the ortho-derivative the slowest disruption. Compared with aliphatic di-trialkylammoniumethyl esters, the analogous aromatic substances show a stronger inhibition both of BChE and AChE (45). Finally, it may be remarked that the data presented hitherto indicate that within the series of aliphatic di-trialkylammoniumethyl esters of dicarboxylic acids a branching of the chain or the introduction of alicyclic groups greatly diminishes the neuromuscular and the ganglionic actions.

D. Dicholinesters and di-trialkylammoniumethyl esters of aliphatic dicarbamic acids

1. Dicholinesters of aliphatic dicarbamic acids. Carbachol (Doryl), the cholinester of carbamic acid, introduced by Kreitmair in 1932 (238), has strong muscarinic and nicotinic properties, and according to Bacq and Brown (16) it has an additional very marked paralysing effect on striated muscle. It was of interest, therefore, to see whether the replacement of simple dicarboxylic acids by the corresponding polymethylene dicarbamic acids would modify these properties. This was first tried by Bovet (41), who found that doubling of the carbaminoylcholine molecule led to a substance with decreased muscarinic effect but with hardly any curare-like action. Increasing the size of the molecule, by interposition of two methylene groups between the two carbamic acid groups, does not significantly change these actions (41, 191), although this chemical change leads to substances with about 10–12 atoms intercalated between the two quaternary nitrogen atoms, a configuration which is optimal for the paralysing effect in the "methonium" series and in dicarboxylic esters of choline.

A further increase of the chain length leads to substances with remarkable pharmacological properties. Such substances were first described by Delaby *et al.* (90, 91, 92) and investigated by Cheymol and his co-workers (70, 72, 74, 75). Independently of these experiments this group was thoroughly described by Klupp *et al.* (232, 237) from this institute. There is very good agreement between the results of Cheymol *et al.* and those of Klupp *et al.* All substances with less than 5 methylene groups have less muscarinic action than carbaminoylcholine and less paralysing action than the higher members of the series. With an increase in the chain length the muscarinic effects disappear completely and weak nicotinic effects can be observed. These have their maximum with a chain length of 6–8 methylene groups and decrease gradually with the higher homologues.

As in the series of the "methonium" compounds or the dicholinesters of dicarboxylic acids, there is a marked difference in the pharmacological properties between the monomeric compounds and some of the dimeric substances. The higher homologues of the present series are among the most powerful paralysing agents for striated muscles, but they have lost all muscarinic actions of the original substance, carbaminoylcholine, and retain very little of its nicotinic action. Moreover, these substances with 6–10 methylene groups have a definite anti-acetylcholine effect on the isolated ileum of the guinea-pig. These observations were extended by observations of Herzfeld and Stumpf (193, 339) on the blood pressure of dogs: in these animals octamethylenedicarbaminoylcholine, in doses which cause a long lasting paralysis of striated muscle, diminishes the depressant effects of acetylcholine, methacholine (mecholyl), and benzoylcholine. The same antagonism can be seen in the isolated frog heart (232, 237).

This is surprising, since these substances are powerful inhibitors both of AChE and BChE. Like carbaminoylcholine itself, the dicarbaminoylcholines are not hydrolyzed by these enzymes. Their inhibitory effects are almost equal for both types of esterase, but the higher homologues have a stronger effect on AChE, whereas the lower have a greater effect on BChE. Carbaminoylcholine is rather weak in this respect, but, according to Cheymol (74), heptamethylenedicarbam-

inoylcholine has a 25% inhibitory effect on BChE in concentration of 1.6×10^{-6} and for AChE at 5×10^{-6}. Klupp *et al.* (232), who made a very thorough investigation of this point, found that the octamethylene derivative gave a 50 per cent inhibition of BChE with 10^{-6} and of AChE with 2×10^{-7}. Slight differences between the results of the two groups can be explained by the fact that they used different substrate concentrations and different enzyme preparations for the two kinds of esterases. However, it was established that these substances had an activity which was only $\frac{1}{6}$–$\frac{1}{10}$ that of eserine. In the above-cited experiments Herzfeld and Stumpf (193, 339) were able to show that these inhibitory effects on the cholinesterases are also exerted *in vivo*, and in their experiments both cholinesterases were inhibited 100% after 300 μg/kg of octamethylenedicarbaminoylcholine, when the effects of ACh, benzoylcholine, and mecholyl were already reduced. In spite of this inhibition of cholinesterases these compounds have practically no muscarine-like effects, even when the esterase of the erythrocytes is completely inhibited. As already mentioned, this is probably due to the simultaneous presence of an atropine-like activity.

All these actions, however, are of minor importance compared with the powerful neuromuscular blocking activity of the higher members of this series.

The neuromuscular paralysis exerted by the dicarbaminoylcholines is of the depolarizing type, as indicated by the contracture produced in the frog rectus and in pigeon muscle, as well as by the potassium release from the muscles after intravenous administration of octamethylenedicarbaminoylcholine (230, 231). However, some more recently discovered facts show the limitation of any such classification (cf. V, p. 323). Honetz *et al.* (210) found that in rats a potassium release occurred only after the first dose of hexamethylenedicarbaminoylcholine, whereas a second equal dose half an hour later did not show any effect on the potassium output of the muscles of the hindlimbs. From these findings it may be concluded that the first dose of hexamethylenedicarbaminoylcholine, for a time immediately after the injection, exerted its action by depolarization, yet later on acted more like curare. Recently Herzfeld *et al.* (192) made the observation that in rats and in dogs neuromuscular paralysis caused by hexamethylenedicarbaminoylcholine could be strongly antagonized by neostigmine. From these results it is concluded that the action of the dicarbaminoylcholine on the neuromuscular receptor is of a dual nature. During the onset of action the endplate membrane is depolarized and as a consequence potassium is moving outwards through the membrane. From this point of complete depolarization the membrane potential becomes gradually restored but, as a result of its strong affinity for the endplate receptor, the dicarbaminoyl compound is still fixed to the membrane, now acting as a competitive inhibitor like dTC. At present, this assumption is chiefly based on investigations of the time course of the potassium output and has to be supported by direct electrical measurements of the resting membrane potentials of endplate areas. One of the unexpected results with dicarbaminoylcholines, which are quite effective as anticholinesterases *in vitro*, is that they have no anticurare activity on the isolated rat diaphragm; in fact, there are indications that these substances, in the later stages of their interaction

with muscle fibres (234, 235), act like competitive blocking agents. In the presence of a fully paralysing dose of dTC, however, the paralysis resulting from octamethylenedicarbaminoylcholine plus dTC is largely antagonized by eserine. Evidence has been brought forward in favour of the assumption of a competitive replacement of octamethylenedicarbaminoylcholine by dTC and *vice versa* at the motor endplate, thereby causing an eserine antagonism against that part of the total paralysing effect which is caused by dTC. Evidence for the occurrence of these phenomena at the site of the endplate was obtained from records of endplate potentials in cat gracilis muscle. Although the endplate potential obtained after intravenous injection of hexamethylenedicarbaminoylcholine was increased in height and particularly in duration after administration of eserine, it reached the level required for eliciting a propagated response only after subsequent injections of a small dose of dTC (79).

With respect to the possible clinical application of some of the most potent dicarbaminoylcholines for muscle relaxation, a series of substances has been tested as antagonists against the neuromuscular paralysis caused by the dicarbaminoylcholines. Apart from the antagonistic action of neostigmine mentioned above, Congo red, Germanin, sodium-α-naphthylamine-4-sulphonate and aniline blue showed some antagonism against the paralytic action of hexamethylenedicarbaminoylcholine on the rat gastrocnemius preparation, when given in high doses and after the injection of the relaxant (53). In contrast Cheymol *et al.* (74) found no antagonistic activity of Congo red and Germanin in rabbits when the doses of the antagonist were given 30 minutes before the injection of hexamethylenedicarbaminoylcholine. The same authors found that neostigmine (0.1 mg/kg) and 3-hydroxyphenyldimethylethylammonium chloride (0.4 mg/kg), given 15 minutes before the dicarbaminoylcholine administration, caused an enhancement of the resulting paralysis. Sodium dimethyldithiohydantoinate, which shows some antagonistic action against *d*-tubocurarine, also causes an enhancement of the neuromuscular paralysing action of hexamethylenedicarbaminoylcholine (73).

Hexamethylenedicarbaminoylcholine (Imbretil, Österreichische Stickstoffwerke) proved to be a satisfactory, long acting muscle relaxant for clinical use in surgical operations (59, 209) and in the treatment of tetanus (56). The clinical reports quoted may be consulted for details about the actions of hexamethylenedicarbaminoylcholine in man. Compared by weight, it acts three or four times more strongly than *d*-tubocurarine and besides gives no signs of histamine release. Laryngospasm and bronchospasm do not occur in the course of a paralysis caused by Imbretil. Intubation is facilitated as it is with SDCh, yet as a result of delayed onset of action, intubation with Imbretil should preferably not begin until three minutes after the injection of the relaxant. Untoward side reactions on heart function and circulation could not be observed. The following scheme of doses has proved best for surgical application: 30–50 μg/kg effect a complete relaxation of twenty to thirty minutes duration, 50–70 μg /kg act for thirty to sixty minutes, 70–90 μg/kg for sixty to ninety minutes and 100 μg/kg is necessary for operations which last longer than ninety minutes. 100 μg/kg must not

be exceeded, otherwise the spontaneous respiration is insufficient for a long period of time. The effect of pharmacologically tested antagonists is under clinical investigation. Neostigmine seems to be a good antagonist if given during the later stages of paralysis caused by Imbretil (54, 272). Hexamethylenedicarbaminoylcholine is chiefly eliminated by the kidneys: 67 % of a dose given by intravenous injection is excreted in the urine during the first hour (52).

2. Other di-trialkylammoniumethyl esters of aliphatic dicarbamic acids. A series of ethyl-, benzyl-, morpholyl-, acetonyl-, cyclohexyl- and ethylacetylammoniumethyl esters of some of the polymethylenedicarbamic acids have been prepared by the "Österreichische Stickstoffwerke" and have been tested in the Vienna Pharmacological Institute (unpublished investigations). Generally, it can be said that all these substances show less neuromuscular blocking activity as compared with the analogous choline derivatives. Melting points, paralysing doses and a short characterization of the nature of their neuromuscular and ganglionic action can be found in Table III, p. 282ff. From the values given in Table III it can be seen that the monoethyldimethyl derivatives have retained the neuromuscular potency of the trimethyl derivatives, whereas the triethyl derivatives show a marked decrease in neuromuscular activity. Moreover, in experiments in pigeons the triethylammoniumethyl ester of the hexamethylenedicarbamic acid caused only a flaccid paralysis, indicating that here too substitution of ethyl groups on the quaternary nitrogens changes the mode of action from a depolarizing to a competitive one. In contrast to the cholinedicarbamic esters, the triethyl derivatives have no ganglion-stimulating properties, but cause a ganglionic block paralleling their neuromuscular actions.

Replacement of two methyl groups on the quaternary nitrogen atoms of hexamethylenedicarbaminoylcholine by cyclohexyl leads to a considerable decrease of the neuromuscular blocking activity. The same effect is obtained by the exchange of one methyl group against one benzylmorpholyl or ethylacetyl group. In all these derivatives the original ganglion-stimulating properties have been converted into a relatively weak ganglion-blocking action.

Substitution of ethyl groups for methyl groups on the quaternary nitrogen does not alter the anticholinesterase effect of dicarbaminoylcholines.

V. CONCLUDING REMARKS

It seems unnecessary here to enumerate again the pharmacological properties of muscle-paralysing substances which led to their classification into two types, one causing paralysis by depolarization of the motor endplate and possibly the adjoining parts of the muscle fibre itself and the other acting by competitive displacement of acetylcholine from its receptors. This has lately been admirably done by Paton and Zaimis (286a).

Many of the substances reviewed on these pages seem to fall into one or the other of these categories. The experimental evidence of the last few years has shown, however, that the distinction between those substances which in their actions resemble ACh itself and those which are similar to dTC is no longer fully applicable. For instance, the extensive work of Zaimis (375, 376) with the

muscles of many mammalian species has shown that a substance like C-10 or SDCh, which was thought to be a prototype of the depolarizing series, may have curare-like actions in some species. Even the different muscles of one animal may show differences in their reactions to the same paralysing agent, some showing features which can only be ascribed to competitive displacement.

Even in the muscles of birds whose reaction to depolarizing substances with a contracture seems so typical, Zaimis (375, 376) was able to show that the reaction to decamethonium begins by such a contracture but then changes into a flaccid curare-like paralysis. Work published from this Institute has shown that pretreatment of bird muscles with certain quaternary phosphonium compounds could change the type of reaction obtained with subsequent application of normally depolarizing substances into a curariform paralysis (153). Moreover, examples mentioned in this review show clearly, that comparatively simple changes within the molecule of muscle-paralysing substances may change a depolarizing substance into a curare-like one, although it is noteworthy that in these cases the curare-like properties are usually comparatively weak and can be elicited only by large doses.

The question then arises whether there are two or even more types of muscle fibres or receptors, some reacting to depolarizing substances and others reacting to curare-like agents. Indeed the older morphological literature as reviewed by Riesser (308) or Krüger (239) indicates that there are quite distinct groups of muscle fibres discernible under the microscope. Although such differences within the muscle *fibre* should not be used as an argument for the existence of two kinds of *receptors* for paralysing substances which are concerned only with endplates, Thesleff and Unna (352) think it improbable that "a single drug at given dose levels would have two qualitatively different actions on members of a population of identical muscle fibres". These authors favour the idea of two distinct muscle receptors. This argument cannot be accepted. The difference might be only quantitative, since there is no evidence for the assumption that some muscle fibres remain unaffected while others are paralysed either with a substance like C-10 or dTC. Moreover, the fact that pretreatment with a depolarizing substance like C-10 or SDCh in many instances changes the reaction of a muscle, so that a subsequent injection of the same substance leads to a paralysis with characteristic curare-like features (376), seems to speak against a dual receptor. The most convincing argument against this theory is based on the fact that the release of potassium which always follows the injection of a depolarizing substance, can be entirely abolished by dTC. Here surely the antagonism must take place at the identical receptor. Therefore, while the specific morphology of different muscle fibres may very well influence their mode of contraction (slow or quick muscle fibres) and the functional activity of muscles predominantly composed of one type or of the other, we do not think that it forms the basis of their reactions to depolarizing or curariform muscle-paralysing substances.

It seems more probable that these differences are a property of the different types of molecules themselves and that many of these molecules have indeed a dual action during their application to the muscle fibre. This view is very clearly

supported by experiments of Jenden *et al.* (221, 222) on the isolated lumbrical muscle of the rabbit. The authors used direct and indirect stimulation on this small muscle and showed that C-10 quickly diminished direct and abolished indirect excitability, but that both returned within about forty minutes and then indirect excitability was again blocked for a period of several hours, while direct excitability remained unaffected during this second period of paralysis. Indirect excitability could be restored by neostigmine during the second phase.

Taylor (344) assumes that the primary depolarizing action is in some way connected with the penetration of the paralysing substance to the site of its receptors in the endplate and that the change in concentration gradient is important for this depolarizing activity. The secondary, curare-like effect, on the other hand, occurs only when the substance is firmly attached to the specific receptor.

It seems therefore that even in the presence of an unaltered concentration of a depolarizing substance in the neighbourhood of the endplate, the membrane potential of the endplate can be regenerated. Since such a regeneration of a potential is a process consuming free energy, an active metabolic process must be involved. As a matter of fact, Thesleff (351) has recently been able to show that exactly the process outlined above takes place in the single fibres of frog sartorius when ACh is applied in the presence of neostigmine. There is first a marked depolarization, but the resting potential returns to normal in the presence of ACh. Addition of further ACh fails to depolarize the membrane or to excite the nerve fibre.

It is noteworthy that a very similar theory has been developed quite independently by Ariens (7, 8, 9). This author points to the fact that, in discussing the theory of competitive antagonism, only one of several possible events has hitherto been accurately discussed, namely the case where an active substance is displaced from its receptor by an inactive one. For instance, *p*-aminobenzoic acid as part of an enzyme system has what Ariens calls an intrinsic activity on the metabolism of microorganisms. However, although its affinity to the surface of the microorganism is higher than that of sulphonamides, it can nevertheless be displaced by sulphonamides if these are present in a sufficient concentration. In this case, the intrinsic activity of the displacing substance (sulphonamide) is zero. The affinity to the receptor may even be more pronounced in such an inactive derivative. Now Ariens rightly points out, that a competitive displacement may also take place between two substances both of which have an intrinsic activity of different intensity, and both of which have an affinity of varying strength to the receptor. Extending the mathematical formula of Michaelis and Menten (276) for enzyme substrate reactions, to cover the equilibria which may be formed between a receptor and two substances of different affinity and also of different intrinsic activity, Ariens arrives at a more generally applicable formula. Working on the frog rectus muscle and using C-10, succinyl- and adipyldicholine and their ethyl derivatives, Ariens and de Groot (8) were able to test this theory. The depolarizing (in this case muscle-contracting) property is called the intrinsic activity and its intensity can be obtained from the shape of the various dose-action curves of the different substances. The affinity for the receptor was calcu-

lated from the dose-activity curve and from the "inhibition index", *i.e.*, the ratio: antagonist concentration divided by agonist concentration for 50% of the maximal effect. This affinity would therefore correspond to what we may call "curare-like" action of a given substance. It is evident that Ariens' theory is compatible with the observations of Taylor. He demonstrates that substances with high intrinsic activity cause contraction of the frog rectus muscle, those with zero intrinsic activity are muscle relaxants, whereas the intermediate substances may cause either contraction or relaxation depending on the equilibria which are formed. The frog rectus muscle preparation is ideally suited to establish these relations and Ariens was able to prove that the experimental data corresponded very exactly to those calculated from his formula.

It would be much harder to substantiate the theory on Taylor's preparation or on the muscle of whole animals. In the first case, the depolarizing action would be expressed by the initial depression of both direct and indirect excitability, whereas the affinity would correspond to the long lasting secondary depression of the indirect stimulation. But the competitive displacement would take place between the drug added to the bath and the ACh liberated at the endplate, by indirect stimulation of the muscle from its motor nerve. The concentration of the transmitter would therefore not be equal at different moments of the period of observation. In whole animals the conditions would be still more complicated. Moreover, Ariens' theory can be applied only for periods of observation during which the receptor for ACh remains unaltered. At the present time we are unable to state precisely the characteristics of molecules which would cause a maximum of depolarization of the endplate membrane. However, it can be said, that the strong positive charge on the quaternary nitrogen atom within such molecules and the changes which occur in this charge by various substituents on the nitrogen atom are directly related to the "affinity" of such compounds to the endplate.

Fleckenstein (116, 117) has recently developed a crude, but interesting model for testing the adsorption to negative receptors. He uses collodion tubes filled with M/100 KCl solution to which he adds the substances under investigation in M/50 concentration. In the experiments referred to here, he used C-10, succinyl- and adipyldicholine and their ethyl derivatives as well as dTC and gallamine. Measuring the membrane potential, he found a considerable initial change as the drugs were adsorbed. After forty-five minutes he began to wash the membranes with distilled water and with M/100 KCl and followed the return of the initial potential. From his data it can be seen that nearly all substances quickly lead to a fall of membrane potential, but that those which cause pronounced depolarization of the muscle endplate membrane like ACh or SDCh and its monoethyl derivatives, can be easily washed out, whereas those which are known for their curare-like properties *in vivo* seem to be more strongly adsorbed, therefore causing a longer lasting diminution of the potential. The most persistent substance in Fleckenstein's series was gallamine.

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