washed acidic, aqueous solution was made strongly basic with cold aqueous NaOH and extracted $CHCl_3$. The washed (water) and dried ($MgSO_4$) chloroform solution was evaporated *in vacuo*, and the residue was purified either by distillation or crystallization.

Method B is identical with method A except that chloroform is used as the reaction solvent instead of absolute ethanol.

Method C.—A mixture of 0.10 mole of orthoester, 0.10 mole of β-aminoethylhydrazine, and 50 ml of ethyl acetate was refluxed for 20 hr and then distilled *in vacuo*.

Method D is identical with method C except that $CHCl_3$ is used as the reaction solvent instead of ethyl acetate.

Method E is the same as method C except that absolute ethanol is used as the reaction solvent instead of ethyl acetate.

Method F is the same as method B except that the methyl iminoester hydrochloride is used instead of the ethyl iminoester hydrochloride.

Method G is the same as method F except that methanol is used as the reaction solvent instead of $CHCl_3$.

1,4,5,6-Tetrahydro-*as*-triazine.—A mixture of 30 g (0.40 mole) of β-aminoethylhydrazine and 80 g (0.54 mole) of ethyl orthoformate was heated at reflux temperature for 24 hr and then distilled, bp 98–100° (0.3 mm), yield 20%. *Anal.* Calcd for $C_3H_7N_3$: C, 42.34; H, 8.29; N, 47.37. Found: C, 41.82; H, 8.57; N, 47.27.

1,4,5,6-Tetrahydro-*as*-triazine monopicrate was prepared in ethanol and after recrystallization from water; mp 136–138°. *Anal.* Calcd for $C_9H_{10}N_6O_7$: C, 34.40; H, 3.21; N, 26.75. Found: C, 34.61; H, 3.47; N, 26.95.

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The Synthesis and Pharmacology of ^{131}I-Labeled 1,10-Bis(trimethylammonium)-5-chloro-6-iodo-5-decene Dihalide and Related Neuromuscular Blocking Agents[1]

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Synthesis of ^{131}I-labeled 1,10-bis(trimethylammonium)-5-chloro-6-iodo-5-decene dihalide has been accomplished by several different methods. It is a stable depolarizing neuromuscular blocking agent. Some related unsaturated diquaternary amines of interest in structure–activity relationships were also prepared.

Stable depolarizing neuromuscular blocking agents such as 1,10-bis(trimethylammonium)decane dihalide [decamethonium (I)] characteristically produce a dual block on isolated nerve–muscle preparations.[3] It has been suggested[4] that the second phase of this biphasic block is related to the penetration of the drug into the skeletal muscle fiber. This hypothesis can be studied most conveniently by using labeled depolarizing drugs.

Decamethonium (I) labeled with N-methyl-^{14}C has been prepared.[5] However, labeling with ^{131}I

$$(^{14}CH_3)_3N^+(CH_2)_{10}N^+(^{14}CH_3)_3$$

I

would be advantageous, since γ-emitting isotopes permit easier and more accurate counting than is possible with β emitters. Furthermore, the counting is simplified since no disintegration of the tissue or extraction of the drug is necessary. Hence ^{131}I-labeled 1,10-bis(2-iodoethyldimethylammonium)decane dichloride [iodocholinium (II)] was synthesized[4a,b,6] However,

$$^{131}ICH_2CH_2 \quad CH_2CH_2{}^{131}I$$
$$(CH_3)_2N^+(CH_2)_{10}N^+(CH_3)_2$$

II

it is difficult to obtain pure iodocholinium by the method employed; the product appears to be unstable and a biphasic block is not observed consistently. These difficulties have been overcome largely by the synthesis of 1,10-bis(trimethylammonium)-5-chloro-6-iodo-5-decene dihalide [TID-5[1d] (III)].

$$(CH_3)_3N^+(CH_2)_4CI{=}CCl(CH_2)_4N^+(CH_3)_3$$

III

Some unsaturated diquaternaries related to I, which are of interest in structure–activity studies, have been prepared also. These compounds and some of the intermediaries can serve as a convenient starting material for specific tritium labeling of neuromuscular blocking agents.

Partial hydrogenation of 1,10-dichloro-5-decyne (IV)[7] gave 1,10-dichloro-5-decene (V), which, when heated with anhydrous trimethylamine, gave 1,10-bis(trimethylammonium)-5-decene dichloride (VI). Addition of iodine monochloride to VI in glacial acetic acid or methanol–dimethylformamide failed to give the expected 1,10-bis(trimethylammonium)-5-chloro-6-io-

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$$RCH{=}CHR \xrightarrow{ICl} RCHCHIR$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad\quad OR^1$$
$$R = (CH_2)_4N^+(CH_3)_2X$$
$$R^1 = CH_3 \text{ or } OCCH_3$$

dodecane dichloride (VII), presumably because side reactions of the following type took place.

The synthesis of VII using inert solvents was not pursued. It was recognized that III would be a more stable compound than VII, because the presence of a double bond at the 5,6 positions in the decamethylene chain would confer added stability to the molecule.

1,10-Bis(trimethylammonium)-5-chloro-6-iodo-5-decene dichloride (III) was prepared by two different procedures. First, iodine monochloride was added to either IV or 1,10-diiodo-5-decyne (VIII) to give 6-iodo-1,5,10-trichloro-5-decene (IX) and 6-chloro-1,5,10-triiodo-5-decene (X), respectively. Compounds IX in DMF and X in methanol were then converted to III by treatment with trimethylamine. Secondly, 1,10-bis(trimethylammonium)-5-decyne dihalide (XI) was obtained by treating either IV or VIII with trimethylamine. Iodine monochloride was added to the chloride salt of XI to give III. The suspension of XI in acetonitrile momentarily became clear when iodine monochloride was added, which presumably could be related to the initial formation of a polyhalogen complex[8] which would be very soluble in acetonitrile.

Compound III labeled with ^{131}I was prepared by both procedures. Using the second method, excess iodine monochloride was removed with divinyl ether, since use of aqueous sodium thiosulfate solution would contaminate III.

The stability of III in physiological salt solution[9] was examined. The results obtained indicate that III is stable under the conditions employed and hence the detected radioactivity should represent solely III. However, the possibility that III may be metabolized in the body,[5b,10] *e.g.*, by transmethylation, cannot be overlooked.

For comparative studies 1,10-bis(trimethylammonium)-5-decyne diiodide (XII) and 1,10-bis(tri-*n*-propylammonium)-5-decyne diiodide (XIII) were prepared by treating VIII with the appropriate tertiary amines.

Experimental Section

General.—Melting points were determined with a Koller micro hot stage. The melting points and boiling points are uncorrected. Microanalyses were done by Berkeley Analytical Laboratory, Berkeley, Calif., and Clark Microanalytical Laboratory, Urbana, Ill. Dowex 1-X10 anion-exchange resin (total capacity: 1.8 meqiv/wet g) in the chloride form was used.

[^{131}I]Iodine Monochloride. A.—Carrier-free (less than 0.1 mg/ml) elemental $^{131}I_2$ in nitromethane (5 ml, total activity 10 mcuries) was obtained from Tracerlab, Inc. To this solution was added ICl[11] (48 mg, 0.30 mmole) in nitromethane (1 ml).

B.—A solution of elemental $^{131}I_2$ (64 mg, 0.25 mmole) in CCl_4 (10 ml, total activity 15 mcuries) was prepared. Chlorine was bubbled through CCl_4 (100 ml) for 1 hr, diluted 1:10 with CCl_4, and titrated. Chlorine (18 mg, 0.25 mmole) in CCl_4 (3 ml) was added to the ^{131}I solution to form ^{131}ICl (*cf.* ref 12).

1,10-Dichloro-5-decyne (IV), prepared by the method of Cram and Allinger,[7] had bp 115–119° (4 mm) (lit.[7] bp 122–125° (5 mm)).

Anal. Calcd for $C_{10}H_{16}Cl_2$: C, 57.98; H, 7.79. Found: C, 57.42; H, 7.74.

1,10-Diiodo-5-decyne (VIII).—Compound IV (5.00 g, 24.1 mmoles) and NaI (10.86 g, 72.3 mmoles) in dry acetone (100 ml) was heated with stirring under reflux for 24 hr (*cf.* ref 13). After filtration the solvent was evaporated *in vacuo*. The residue was extracted with ether, and the combined ethereal extracts were washed with aqueous 0.1 *N* sodium thiosulfate solution (5 ml) and water (10 ml) and dried ($MgSO_4$). Evaporation of the ether gave a slightly yellow oil of crude VIII (7.42 g, 79%).

1,10-Dichloro-5-decene (V).—A solution of IV (10.00 g, 47.4 mmoles) in ethanol (200 ml, 95%) was partially hydrogenated in the presence of synthetic quinoline (1.75 g, 13.6 mmoles) and 10% Pd–C powder (1000 mg). The uptake of 1 equiv of hydrogen ceased abruptly after 3 hr. The suspension was filtered and the filtrate was poured into water (500 ml). The olefinic dichloride was extracted with ether; the ether extract was washed with 1 *N* HCl (20 ml), aqueous 1 *N* Na_2CO_3 solution (10 ml), and water (15 ml). The ethereal solution was dried ($MgSO_4$) and the ether was evaporated. The residue distilled through a 64-cm Podbielniak column under nitrogen *in vacuo* gave 7.72 g (77%), bp 108–110° (5 mm), $n^{25}D$ 1.4744.

Anal. Calcd for $C_{10}H_{18}Cl_2$: C, 57.42; H, 8.68. Found: C, 57.19; H, 8.33.

6-Iodo-1,5,10-trichloro-5-decene (IX).—A solution of ICl (3.28 g, 20.2 mmoles) in CCl_4 (40 ml) was added to IV (4.14 g, 20.0 mmoles) in the same solvent (10 ml) and placed in complete darkness for 30 min. The solution was washed with aqueous 1 *N* sodium thiosulfate solution (10 ml) and water (30 ml) and dried ($MgSO_4$) in darkness. The solvent was distilled *in vacuo* under subdued light leaving a slightly yellow oil of crude IX (6.50 g, 89%).

1,10-Bis(trimethylammonium)-5-decene Dichloride (VI).—Anhydrous Me_3N (3.35 g, 56.8 mmoles) and V (2.22 g, 10.6 mmoles) were heated in a sealed tube at 50–55° for 1 week. Evaporation of excess Me_3N gave a residue which was washed with ether (30 ml) leaving some crystalline material. The latter was dissolved in a mixture of DMF (8 ml) and methanol (6 ml) and poured into excess benzene giving a yellow oil which rapidly crystallized. Dried *in vacuo* (P_2O_5), VI weighed 1.25 g (36%), mp 198–204°.

Anal. Calcd for $C_{16}H_{34}Cl_2N_2$: C, 58.70; H, 11.38. Found: C, 58.37; H, 11.26.

1,10-Bis(trimethylammonium)-5-decyne Dichloride (XI). A.—Anhydrous Me_3N (13.0 g, 0.22 mole) and IV (3.67 g, 17.7 mmoles) were heated in a sealed tube at 50–55° for 1 week. Evaporation of excess Me_3N gave a residue which was precipitated from methanol (5 ml) with ether (50 ml) giving an oil which rapidly crystallized. Dried at 105° *in vacuo*, XI weighed 1.824 g (32%), mp 230–250° (gradual browning).

Anal. Calcd for $C_{16}H_{34}Cl_2N_2$: C, 59.06; H, 10.53; N, 8.61. Found: C, 58.58; H, 9.55; N, 7.78.

B.—Anhydrous Me_3N (15.0 g, 0.25 mole) and crude VIII (14.7 g, 37.6 mmoles) in dry methanol (100 ml) were treated under reflux for 4 hr. The methanol was partially evaporated until crystallization began. The crystals were collected and dissolved in water, and the solution was passed through a column of exchange resin (168 g). The effluent was distilled *in vacuo* and the residue precipitated from methanol (10 ml) with excess ether. Reprecipitated from methanol–ether XI dried *in vacuo* (P_2O_5, 105°) weighed 5.93 g (48%), mp 235–248° (gradual browning).

Anal. Calcd for $C_{16}H_{34}Cl_2N_2$: C, 59.06; H, 10.53; N, 8.61. Found: C, 58.99; H, 10.55; N, 8.46.

1,10-Bis(trimethylammonium)-5-decyne Diiodide (XI).—Me_3N (1.0 g, 17 mmoles) and crude VIII (174 mg, 0.45 mmole) in methanol (4 ml) were heated under reflux for 11 hr. The cooled solution was poured into excess ether with continuous stirring to precipitate white crystals. Reprecipitated twice from methanol–ether, XI weighed 133 mg (59%).

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(9) The concentration of ions in water expressed in mmoles/l. is: Na^+, 144.2; K^+, 5.9; Ca^{2+}, 1.9; Mg^{2+}, 0.61; Cl^-, 127.0; HCO_3^-, 25.0; SO_4^{2-}, 0.61; and glucose, 11.1.

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Anal. Calcd for $C_{16}H_{34}I_2N_2$: C, 37.81; H, 6.74; I, 49.94. Found: C, 37.85; H, 6.56; I, 51.84.

The following compounds were prepared similarly.

1,10-Bis(triethylammonium)-5-decyne Diiodide (XII).—Recrystallization from 2-propanol–methanol gave white nonhygroscopic crystals (82%), mp 212–214°.

Anal. Calcd for $C_{22}H_{38}I_2N_2$: C, 45.22; H, 6.55; N, 4.79. Found: C, 45.06; H, 7.85; N, 4.95.

1,10-Bis(tri-*n*-propylammonium)-5-decyne Diiodide (XIII).—Recrystallization from 2-propanol gave white nonhygroscopic crystals (75%), mp 330°.

Anal. Calcd for $C_{28}H_{58}I_2N_2$: C, 49.70; H, 8.64; N, 4.14, Found: C, 49.87; H, 8.62; N, 4.10.

Attempted Synthesis of 1,10-Bis(trimethylammonium)-5-chloro-6-iododecane Dichloride (VII).—The addition of excess ICl (varying from 110% to 200% of the stochiometrically required amount) to VI was tried using either glacial acetic acid or a mixture of 5% methanol and 95% DMF as a solvent. The reaction was allowed to take place in darkness at room temperature varying the reaction time from 30 min to 30 hr. The addition product was precipitated as an oil with excess ether or benzene. The oil was dissolved in water and passed through a column of the exchange resin. The eluate was distilled *in vacuo* to leave a pink oil which turned red within 24 hr. The oil failed to crystallize by either freeze drying or from several of the common alcohols.

1,10-Bis(trimethylammonium)-5-chloro-6-iodo-5-decene Dihalide (III). A.—Anhydrous Me_3N (4.72 g, 80 mmoles) and crude IX (6.60 g, 17.9 mmoles) in DMF (5 ml) were heated in a sealed tube at 55° for 16 hr. Evaporation of excess Me_3N and addition of ether (10 ml) precipitated light brown crystals. They were washed with ether (10 ml) and recrystallized from methanol–2-propanol. After drying *in vacuo* (P_2O_5, 105°), III weighed 4.65 g (48%), mp 210–215° (charring).

Anal. Calcd for $C_{16}H_{34}Cl_3IN_2$: C, 39.40; H, 7.03; I, 26.02. Found: C, 39.45; H, 6.91; I, 26.13.

B.—The addition of ICl (98 mg, 0.60 mmole) in CCl_4 (2 ml) to VIII (224 mg, 0.57 mmole) in CCl_4 (3 ml) in the same manner as described for IX afforded crude 6-chloro-1,5,10-triiodo-5-decene (X). Compound X and Me_3N (0.80 g, 14 mmoles) in methanol (4 ml) were heated under reflux for 17 hr. The cooled solution was poured into excess ether to precipitate III as the diiodide salt. Reprecipitation from methanol–ether gave 86 mg (22%).

Anal. Calcd for $C_{16}H_{34}Cl_3N_2$: C, 28.65; H, 5.11; Cl, 5.29; I, 56.77. Found: C, 28.84; H, 5.48; Cl, 4.25; I, 54.78, 57.60, 54.51.

The **picrate** had mp 190–192°.

Anal. Calcd for $C_{28}H_{38}ClIN_8O_{14}$: C, 38.52; H, 4.39; I, 14.54. Found: C, 39.04; H, 4.12; I, 11.01.

C.—A freshly prepared solution of ICl (109 mg, 0.67 mmole) in acetonitrile (2 ml) was added to a suspension of XI (198 mg, 0.61 mmole) in the same solvent (5 ml). The suspension, which became almost clear temporarily, was stirred for 1 hr in darkness. The solvent was evaporated leaving brown solid material, which was only partially soluble in water. The aqueous solution was passed through a column of the exchange resin. Distillation of the effluent *in vacuo* left a yellow oil, which was freeze dried to give white crystals. After drying *in vacuo* (P_2O_5), III weighed 233 mg (75%), mp 212–216°.

Anal. Calcd for $C_{16}H_{34}Cl_3IN_2$: C, 39.40; H, 7.03; I, 26.02; N, 5.74. Found: C, 39.18; H, 6.82; I, 23.12; N, 4.24.

Crude III was prepared also in a similar manner using nitromethane as the solvent with a 78% yield. A sample was precipitated from 2-propanol–methanol–ether.

Anal. Calcd for $C_{16}H_{34}Cl_3IN_2$: C, 39.40; H, 7.03; Cl, 21.81; I, 26.02. Found: C, 39.37; H, 6.88; Cl, 21.53; I, 25.6.

1,10-Bis(trimethylammonium)-5-chloro-6-iodo-5-decene Dichloride Labeled with ^{131}I (III). A.—A solution of ^{131}ICl (49 mg, 0.30 mmole) in nitromethane (6 ml) was added to XI (92 mg, 0.28 mmole) in the same solvent (5 ml) and placed in darkness for 1 hr. Freshly distilled divinyl ether (100 mg) was added to remove excess ^{131}ICl. The addition of excess diethyl ether precipitated white crystals and a brown oil. After the solvents were decanted, the residue was washed with diethyl ether and dissolved in water. The aqueous solution was passed through a column of the exchange resin (3 g).

The **picrate** was precipitated as yellow crystals from an aliquot of the effluent, mp 190–191°.

Anal. Calcd for $C_{28}H_{38}Cl_3IN_8O_{14}$: I, 14.54. Found: I, 13.89.

B.—A solution of ^{131}ICl (82 mg, 0.51 mmole) in CCl_4 (10 ml) was added to IV (100 mg, 0.48 mmole) and placed in darkness for 30 min. The solvent was distilled *in vacuo* leaving a yellow oil of crude IX. Anhydrous Me_3N (1.12 g, 19 mmoles) in DMF and IX was heated at 55° in a pressure tube for 18 hr. Excess Me_3N was evaporated and DMF was removed from III, which had precipitated. The crystals were washed with ether and recrystallized from methanol–2-propanol.

Anal. Calcd for $C_{16}H_{34}Cl_3IN_2$: I, 26.02. Found: I, 22.02.

The purity of ^{131}I-labeled III was examined by ascending chromatography on Whatman No. 4 filter paper using 1-butanol–ethanol–water–acetic acid (8:2:3:1, v/v). The chromatograms were sprayed with Dragendorff's reagent[14] and showed only one clearly marked spot. Strips of the chromatograms were scanned for radioactivity. Only one peak was recorded which corresponded to the position of the labeled III.

Stability of III. A.—A 2.7×10^{-4} *M* solution of ^{131}I-labeled III in physiological salt solution[9] was maintained at 37° and aerated with a mixture of 95% O_2 and 5% CO_2. One aliquot was removed at the beginning of the experiment and another 19 hr later and both were chromatographed as described above. Each of the chromatograms showed only a single peak which corresponded to the position of the spot seen after spraying with Dragendorff's reagent.

B.—A 2.4×10^{-3} *M* solution of III was treated similarly for 8 hr. The picrate was precipitated from an aliquot of this solution at the beginning (A) and at the end (B) of the experiment.

Anal. Calcd for $C_{28}H_{38}Cl_3IN_8O_{14}$: I, 14.54. Found: I, 14.61 (A), 14.87 (B).

Estimation of Neuromuscular Paralysis and Acute Toxicity.—Mice of both sexes weighing 27–36 g were used, 20 for each study. Decamethonium, III, and (+)-tubocurarine were injected into a tail vein. Doses are expressed as milligrams per kilogram. Neuromuscular paralysis was determined by the ability of the mice to remain on a wire grid inclined at 60°. The observation time for this procedure and lethality was 5 min. The ED_{50} (paralysis) and the LD_{50} and their standard deviations were established by the "up and down" method of Dixon and Massey.[15] The therapeutic index (LD_{50}/ED_{50}) was calculated after conversion of the doses into micromoles per kilogram.

Chicks were used for III using the method of Buttle and Zaimis.[16]

Effect of III, Decamethonium, and Succinylcholine on Neuromuscular Transmission and Blood Pressure of Cats.—Adult cats of either sex were anesthetized with α-chloralose (70 mg/kg). Six sciatic tendoachilles preparations were used. Isometric contractions elicited by stimulation of the sciatic nerve were recorded by means of a Grass force displacement transducer and an Offner recorder. The arterial pressure was recorded from the carotid artery. The drugs were injected into the femoral artery.

Effect of III on the Isolated Nerve–Diaphragm Preparation of Guinea Pigs.[3c]—Twenty-two preparations were used. The muscles were stimulated, alternately indirectly and directly as described previously,[3a] at a total rate of 6 stimuli/min and the isometric contractions were recorded as above. The physiological salt solution[9] was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°. Doses are expressed in moles per liter.

Effect of III on Other Isolated Muscle Preparations Stimulated Transmurally.—Seven human intercostal muscles,[17] 22 rabbit lumbrical muscles,[3b] and 8 cat lumbrical muscles from the hindpaw of cats weighing approximately 2 kg were used. The muscles were stimulated by the "short shock" technique.[17,18] The Mg^{2+} concentration of the salt solution was lowered to 0.23 m*M* for the lumbrical muscles. Other experimental details are similar to those described above.

Results

ED_{50} and LD_{50}.—These values for III, decamethonium, and (+)-tubocurarine are shown in Table I.

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(15) W. T. Dixon and F. T. Massey, "Introduction to Statistical Analysis," McGraw-Hill Book Co., Inc., New York, N. Y., 1957, p 319.

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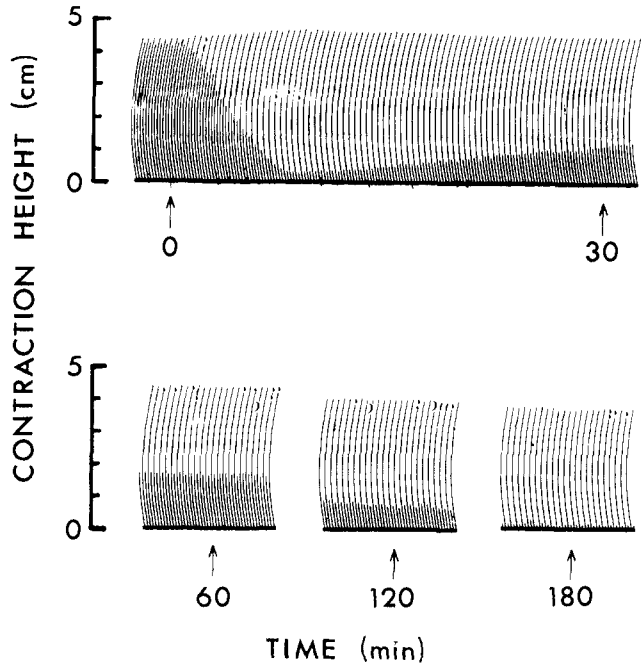

Figure 1.—The blocking effect of 10^{-5} *M* III on the isolated rabbit lumbrical muscle. Ordinates: contraction height. Abscissas: time after the addition of III. The muscle was stimulated alternately transmurally and directly.

TABLE I
NEUROMUSCULAR BLOCKING POTENCY AND TOXICITY

Compd	ED_{50}, mg/kg[a]	LD_{50}, mg/kg[a]	Therapeutic index
III	0.43 (0.12)	1.11 (0.30)	2.58
Decamethonium	0.37 (0.14)	0.68 (0.12)	1.84
(+)-Tubocurarine	0.06 (0.015)	0.17 (0.047)	2.83

[a] The numbers in parentheses are the standard deviations.

Effect on Chicks.—The effect of III was very similar to that produced by decamethonium.[16a] Intravenous injection of 4×10^{-7} moles/kg of III produced an immediate tonic extension of the legs and contracture of the dorsal neck muscles which lasted for about 3 min. Higher doses increased both the duration and intensity of this effect until the birds died of muscular paralysis.

Effect on Neuromuscular Transmission and Blood Pressure to Cats.—Compound III produced a temporary small increase in muscle tension together with fasciculations and contracture. Approximately the same degree and duration of neuromuscular blockade was seen with 0.15 μmole/kg of III, 0.10 μmole/kg of succinylcholine, and 0.05 μmole/kg of decamethonium. None of these compounds altered the blood pressure. Marked tachyphylaxis was seen with III and decamethonium.

Effect on the Isolated Rabbit Lumbrical Muscle.—Figure 1 shows the characteristic biphasic block of 10^{-5} *M* III. The initial rapidly developing block, phase I, is followed by partial recovery of neuromuscular transmission in the continued presence of the drug. A second block, phase II, then begins and increases slowly. The response to III differs from that seen with decamethonium by: (a) less marked recovery of neuromuscular transmission between phase I and II; (b) absence of any depression of the direct muscle response during phase I; and (c) it takes 1–2 hr longer for phase II block to reach a steady state. Repeated additions of III followed by washing of the preparation caused a progressively less marked phase I until the latter eventually was absent. This tachyphylaxis is characteristic of the depolarizing neuromuscular blocking agents. The phase I block also became less pronounced when the Mg^{2+} concentration of the same solution was raised to 0.61 m*M*. Neostigmine, K^+, and a 10° fall in temperature antagonized the phase II block seen with III and decamethonium, indicating that this phase is an antidepolarization block.

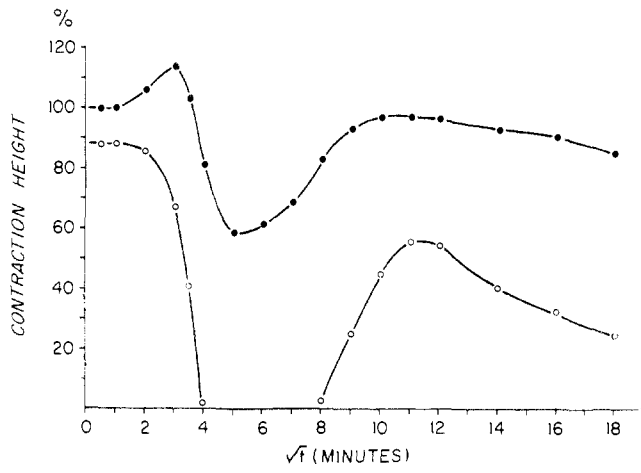

Figure 2.—The dual block of 4.1×10^{-6} *M* III on the isolated cat lumbrical muscle. Ordinate: per cent contraction height; ●—●, the response to direct stimulation expressed as a percentage of initial response to direct stimulation; ○--○, the response to nerve stimulation expressed as a percentage of the response to direct stimulation. Abscissa: the square root of time.

Effect on the Isolated Cat Lumbrical Muscle.—Compound III produced a typical dual block (Figure 2). The phase I block was more marked and distinct in the cat preparation than in the rabbit and was associated with depression of the direct muscle response. Tachyphylaxis to phase I block, and an increase in phase II block was observed with (+)-tubocurarine.

Effect on the Isolated Human Intercostal Muscle.—A dual block similar to that described for decamethonium[17] was seen with III ($1.6 \times 10^{-5} M$).

Effects on the Isolated Guinea Pig Nerve–Diaphragm Preparation.—Figure 3 shows the characteristic response of this preparation to different concentrations of III. The very small phase I block and complete absence of any initial potentiation of the direct response seen with III is in marked contrast to that observed with decamethonium.[3c] The action of Mg^{2+} is curare-like[19] and in high concentrations Mg^{2+} can produce neuromuscular block itself.[20] Hence, lowering of the Mg^{2+} concentration should facilitate the development of phase I block. However, complete elimination of this ion from the salt solution did not enhance the phase I block seen with III. Six hours after addition of four different concentrations of III, apparent steady neuromuscular block occurred. The ED_{50} (nine experiments) was estimated by graphical interpolation to be $1.1 \times 10^{-5} M$, as compared to $2.7 \times 10^{-6} M$ for decamethonium as previously determined.[3c]

Compounds XI–XIII were tested also on this preparation. Compound XI produced a typical dual block

[19] L. Englbach, *Pharmacol. Rev.*, **4**, 396 (1952).
[20] P. E. B. Holmes, D. J. Jenden, and D. B. Taylor, *J. Pharmacol. Exptl. Therap.*, **103**, 382 (1951).

as seen with decamethonium and hence belongs to the group of depolarizing neuromuscular blocking agents. The block seen with XII and XIII was similar to that observed with the antidepolarizer, (+)-tubocurarine.

Discussion.—Even the slightest modification of the decamethonium molecule usually either decreases the potency or changes it from a depolarizing drug to an antidepolarizer, or both.[21] Thus the introduction of the halogen atoms and the double bond in the 5,6 positions of the decamethylene chain, as in III, caused a change in potency and therapeutic index. Furthermore, on the isolated preparations, the ability of III to produce the characteristic biphasic block and initial contracture, together with fasciculations normally seen with depolarizers, was less marked as compared with decamethonium. In particular this was the case for the isolated guinea pig diaphragm, as III produced only a slight phase I block. The slowly developing block seen with III on this preparation is not typical of either the depolarizers or antidepolarizers. Presumably, this block is characteristic of compounds which have a very weak ability to depolarize the motor end plate.

Recently, tritiated decamethonium has become available and has proved to be useful for *in vitro* studies,[4e,22]

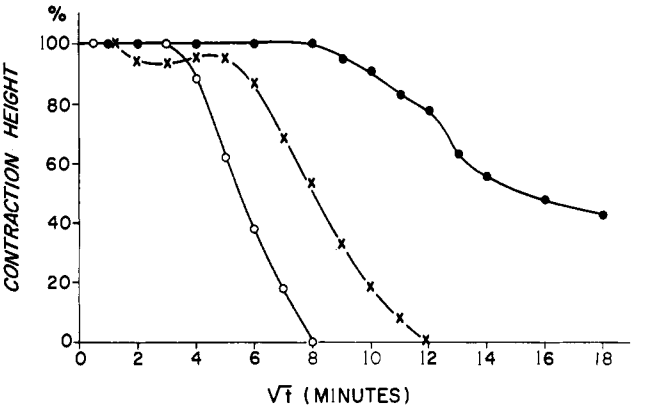

Figure 3.—The blocking effect on three isolated guinea pig diaphragm preparations of different concentrations of III: ●—●, 10^{-5} *M*; ×—×, 2.5×10^{-5} *M*; ○—○, 5.0×10^{-5} *M*. Ordinate: the response to nerve stimulation expressed as a percentage of the response to direct stimulation. Abscissa: the square root of time.

However, III labeled with ^{131}I would still be particularly suited for *in situ* experiments where continuous external monitoring of its radioactivity is desirable, *e.g.*, in a muscle.

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(22) (a) O. A. Nedergaard, Ph.D. Dissertation, University of California, Los Angeles, 1964; *Dissertation Abstr.*, **25**, 1254 (1964); (b) D. B. Taylor, R. Creese, O. A. Nedergaard, and R. Case, *Nature*, **208**, 901 (1965); (c) O. A. Nedergaard and D. B. Taylor, *Experientia*, **22**, 521 (1966).

The Synthesis and Evaluation of the Local Anesthetic Activity of a Series of 4-(ω-Alkylaminoacylamino)salicylate Esters[1,2]

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A series of ω-alkylaminoacyl derivatives of 4-aminosalicylic acid esters (methyl through hexyl, plus 2-diethylaminoethyl) were synthesized and their hydrochlorides were tested for local anesthetic activity. The synthesis of the diethylaminoethyl ester series was examined in some detail since these compounds easily undergo alcoholysis and aminolysis. These reactions were ascribed to an intramolecular *o*-hydroxy catalysis. Only derivatives of the methyl, ethyl, and diethylaminoethyl esters exhibited significant local anesthetic activity. Compared to lidocaine, these compounds were generally more irritating, less toxic, and less active. When the compounds exhibiting local anesthetic activity were quaternized with methyl iodide, local anesthetic activity was lost while the toxicity increased.

Although Drill[3] states that, as a general rule, effective local anesthetics rarely contain either free carboxy or hydroxy groups, Clinton and co-workers[4] and Ludueña and Hoppe[5] have reported that a series of dialkylaminoalkyl 4-alkylaminosalicylates showed a high degree of infiltration and topical anesthetic activity. Keil and Rademacher[6] also reported that some alkylaminoethyl 4-aminosalicylates possessed local anesthetic activity similar to the corresponding esters of 4-aminobenzoic acid. Oxycaine, the 2-hydroxy analog of procaine synthesized by Grimme and Schmitz,[7] has been shown by

(1) The investigation at the University of Athens was supported by a research grant from the Royal Hellenic Research Foundation.

(2) A preliminary report of part of this work has been presented at the 21st International Congress of Pharmaceutical Sciences, Pisa, Italy, Sept. 4–8, 1961, by G. T. and C. S. Preliminary announcements have appeared: G. Tsatsas and C. Sandris, *Proc. Acad. Athens*, **35**, 372 (1960); G. Tsatsas, C. Sandris, and D. Kontonassios, *ibid.*, **37**, 54 (1962). This paper comprises a portion of a thesis presented by D. K. at the University of Athens.

(3) A. V. Drill, "Pharmacology in Medicine," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1958, p 98.

(4) R. O. Clinton, S. C. Laskowski, U. J. Salvador, and M. Wilson, *J. Am. Chem. Soc.*, **73**, 3674 (1951).

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(6) W. Keil and E. Rademacher, *Arzneimittel-Forsch.*, **1**, 154 (1951).

(7) W. Grimme and H. Schmitz, *Ber.*, **84**, 734 (1951).