TABLE II.
Chart Showing the Difference Between Percentage of Fat in Stools and Percentage of Fat Excreted.

Dog Date	VI 12/20/46	VI 1/8/47	I 12/20/46	I 1/8/47	VII 12/23/46	VII 1/8/47
Wt dry stool (g)	107	65.9	113	110	55	28
Total (g) fat excreted	18.3	19.9	14.9	21.6	4.6	3.5
% dry weight	17.1	30.2	13.2	19.7	8.4	12.6
Fat fed (g)	38.1	38.1	38.1	38.1	38.1	38.1
% fat excreted	48.1	52.3	39.2	56.8	12.2	9.2
	Dogs deprived of external pancreatic secretion				Normal dog	

Weights indicate average daily weight. All experiments were carried out over 3-day periods.

The percentage of fat in the dried stool in normal dogs on a standard diet varies widely. In healthy human beings on the Schmidt diet, the average in our clinic has been 25%. In steatorrhea of pancreatic origin the percentage of fat in the dried stool is frequently less than the maximum occurring in health; hence no definite conclusion regarding the absorption of fat can be drawn from the percentage of fat in the stool unless it is greatly elevated. In Table II are given the results of absorption studies on 2 dogs deprived of pancreatic secretion, and for comparison those on a normal dog. All were fed the same amount of fat daily, namely 38 g, and all had a percentage of fat in the dried stools that was within normal limits, yet the dogs without pancreatic digestion lost in their feces from 39.2% to 56.8% of the fat fed. It is evident from this that the amount of fat in the stools is all important. In obstruction of the pancreatic ducts the weight of the dried stools is definitely increased as is shown in Table II.

Summary. Data on the absorption of fat and nitrogen following folic acid therapy in dogs deprived of external pancreatic secretion have been presented. Folic acid was shown to be of no value as far as absorption of these substances was concerned.

The importance of employing a standard diet of known composition and of determining the amount of fat lost in the stools and not relying on the percentage of fat present in the dried stool is emphasized.

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Blocking and Protecting Actions of Amines and Ammonium Compounds on a Crustacean Synapse.

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The central nervous system of the crayfish provides a highly satisfactory preparation for the study of synaptic transmission. It contains 4 giant fibers, up to 250μ in diameter, which extend the entire length of the ventral nerve cord. These fibers synapse with motor fibers in each of the abdominal ganglia. A single impulse in a giant fiber normally causes a single response in the motor fibers (Wiersma[1]).

Nicotine is a particularly active drug on this synapse, causing an initial facilitation followed by block. However, approximately 40 minutes after block by nicotine, transmis-

[1] Wiersma, C. A. G., *J. Neurophysiol.*, 1947, **10**, 23.

sion re-appears, regardless of whether the preparation has been rinsed with fresh perfusion fluid or allowed to remain in the nicotine solution. In either case, preparations which have recovered from nicotine block are protected against further action of nicotine, since renewed application of this drug, in the same or even in higher concentration, is without effect (Wiersma and Schallek[2]; Schallek and Wiersma[3]).

These authors also found that high concentrations of nornicotine and anabasine, 2 compounds closely related in structure to nicotine, block transmission across this synapse. Lower concentrations have no blocking action, but they are able to protect the synapse against the effects of a subsequent application of nicotine.

In amphibia and mammals nicotine shows both stimulatory and inhibitory effects on ganglionic and central synapses of the autonomic nervous system as well as at the peripheral neuro-muscular junctions of the somatic nervous system. The actions of the aliphatic primary amines and trimethylammonium salts have been found to be similar to those of nicotine upon various mammalian test preparations (Alles[4]). The present study describes the actions of amines and trimethylammonium salts on synaptic transmission in the crayfish. Observations were made on both the direct action of these compounds and on their ability to protect the preparation against the action of nicotine.

Methods. The procedure used was based on that described by Wiersma and Schallek[2] and Schallek and Wiersma.[3] The abdominal nerve cord of the crayfish *Cambarus clarkii* was freed from surrounding tissue; the preparation was then placed in a bath containing 100 cc of crayfish perfusion fluid (van Harreveld[5]). Stimulating electrodes were placed on the nerve cord between the 5th and 6th abdominal ganglia, while the response, which was led off a motor root of the 2nd or 3rd ganglion, was observed on the screen of a cathode-ray oscilloscope. Stimuli were delivered at a rate of 30 per minute. Control tests showed that in the absence of drugs the post-ganglionic response to this rate of stimulation remained unchanged for several hours. Drug concentrations mentioned in this paper are final values after dilution in the bath.

In testing amines and ammonium compounds, their direct action was noted by finding the minimum concentration causing block within 10 minutes. To avoid possible osmotic effects, concentrations higher than 5×10^{-3} g/cc were not used. After finding the minimum blocking concentration, lower concentrations of the drug were tested for their protection against nicotine. Control tests on other preparations had shown that in the absence of other drugs the nicotine dose used (1 or 2×10^{-5}) blocked transmission in 2 to 5 minutes. The nicotine was always added to the bath 10 minutes after application of the compound being studied. If synaptic transmission still occurred 10 minutes later, the synapse was considered to be protected against the blocking action of nicotine. If nicotine block occurred in 5-10 minutes, the concentration of the drug being tested was considered to show a trace of protection against nicotine.

This procedure is illustrated by the following experiments on heptyl amine sulfate:

A. 1×10^{-3} g/cc blocks in 1 min. Hence this dose is above threshold for block.

B. 5×10^{-4} blocks transmission in 10 min. This concentration is the threshold for block as defined in this study.

C. 1×10^{-4} has no effect in 10 min. Nicotine 1×10^{-5}, given at this time, produced only a transient depression. Hence this concentration of heptyl amine is below threshold for block, but above threshold for protection against nicotine.

D. 5×10^{-5} has no effect in 10 min. Nicotine 1×10^{-5} now blocks in 8 min. Hence this is approximately the threshold dose for protection against nicotine.

2 Wiersma, C. A. G., and Schallek, W., *J. Neurophysiol.*, 1947, **10**, 323; *Science*, 1947, **106**, 421.

3 Schallek, W., and Wiersma, C. A. G., *J. Cell. Comp. Physiol.*, 1948, **31**, 35.

4 Alles, G. A., *Univ. Calif. Publ. Pharmacol.*, 1941, **2**, 1.

5 Harreveld, A. van, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 428.

TABLE I.
Action of Amine Compounds.
Minimum bath concentrations in g/cc for action indicated.
Concentrations higher than 5×10^{-3} were not used.

Compound	Block	Protection against 1×10^{-5} nicotine
Butyl amine sulfate	None	None
Amyl " "	"	5×10^{-4}
Hexyl " "	5×10^{-3}	5×10^{-4}
Heptyl " "	5×10^{-4}	5×10^{-5}
Octyl " "	5×10^{-5}	1×10^{-5}
Dodecyl " HCl	1×10^{-5}	2×10^{-6} (trace)
Butyl methylamine HCl	None	None
Amyl " "	1×10^{-3}	1×10^{-4}
Hexyl " "	1×10^{-3}	1×10^{-5}
Heptyl " "	1×10^{-4}	1×10^{-5}
Dodecyl " HBr	5×10^{-5}	5×10^{-6} (trace)
Butyl dimethylamine HCl	1×10^{-3}	1×10^{-4}
Amyl " "	1×10^{-3}	1×10^{-4}
Hexyl " "	5×10^{-4}	1×10^{-4}
Heptyl " "	2×10^{-4}	1×10^{-4}
Octyl " "	1×10^{-4}	5×10^{-6}
Dodecyl " "	5×10^{-5}	5×10^{-7} (trace)

E. 1×10^{-5} has no effect in 10 min. Nicotine 1×10^{-5} now blocks in 4 min. Hence this concentration shows no protection against nicotine.

It is obvious that a great many experiments would be needed for exact quantitative determinations of the actions of each compound. The threshold values presented in this study should be considered only as good approximations.

Observations. Amine Compounds of Table I. Each of 3 series of alkyl amines shows a variable but progressive increase in blocking activity as the alkyl chain is lengthened. With the lower alkyl compounds the dimethylamines are generally more active in this respect than the corresponding methylamines and they in turn more active than the amines. Dodecyl amine, however, appears to be more active than either its methyl or dimethyl derivatives.

The ability of these alkyl compounds to protect the synapse against blocking by nicotine also shows a general increase with the length of the alkyl chain through the octyl compounds. The dodecyl amines, however, show only partial protection against subsequent nicotine blocking, although it is notable that this partial protection is shown by high dilutions. It is possible that the protection which might be expected to be produced by the dodecyl compounds is largely obscured by their powerful direct blocking action. Evidence for this view is presented below.

Trimethylammonium Compounds of Table II. The methyl to octyl trimethylammonium salts show no blocking activity in the highest concentrations tested (5×10^{-3} g/cc). The dodecyl compound was quite active in this respect, but less so than the other dodecyl compounds.

The ability of these alkyl trimethylammoniums to protect the synapse against blocking by nicotine shows a general increase with the length of the alkyl chain. While the lower members of the series show less protecting action against the effect of nicotine than the corresponding amine compounds, the dodecyl compound is quite comparable in effect.

The amyl trimethylammonium salt was notable in being the only substance found in this study, aside from nicotine itself, that caused a consistent facilitation of synaptic transmission. A weak facilitation was occasionally noted with the closely related butyl and hexyl trimethylammonium salts. It should be noted that observations of facilitation are far more variable and dependent upon the state of the particular preparation than

TABLE II.
Action of Trimethyl Ammonium Compounds.
Minimum bath concentrations in g/cc for action indicated.
Concentrations higher than 5×10^{-3} were not used.

Compound		Block	Protection against 1×10^{-5} nicotine
Methyl trimethylammonium	I	None	—
Ethyl "	"	"	—
Propyl "	"	"	—
Butyl "	"	"	None
Amyl "	"	" *	5×10^{-3}
Hexyl "	"	"	2×10^{-4}
Heptyl "	"	"	5×10^{-4}
Octyl "	"	"	5×10^{-5}
Dodecyl "	Br	5×10^{-4}	5×10^{-7} (trace)

* Shows facilitation at 1×10^{-3}.

TABLE III.
Protection Against Nicotine.
Blocking time in minutes after 2×10^{-5} g/cc of nicotine given 10 minutes after concentrations of trimethylammonium iodides.

Conc. of trimethylammonium compound	Hexyl	Heptyl	Octyl	Dodecyl
5×10^{-3} g/cc	>60	7	6	—
1×10^{-3} "	26	20	16	—
5×10^{-4} "	12	5	>60	—
1×10^{-4} "	8	—	10	2
1×10^{-5} "	—	—	6	5
1×10^{-6} "	—	—	—	7
1×10^{-7} "	—	—	—	3

Nicotine controls, without protecting compounds, block in 2-5 minutes.

are observations of inhibition.

Dodecyl Compounds. It was suggested above that the failure of these compounds to show complete protection against the action of nicotine might be correlated with their powerful direct blocking activities. Evidence in support of this is given in Table III where the blocking time required by a test dose of nicotine is shown in relation to the concentration of the protecting compound used.

The hexyl compound, as well as all compounds below it in this series, shows maximum protection against the effect of nicotine at the highest concentration tested (5×10^{-3} g/cc). A different situation appears with the heptyl, octyl and dodecyl compounds. Here the highest concentration which does not block by itself in 10 minutes shows little or no protection against the test dose of nicotine. Maximum protection is afforded by pretreatment with a lower concentration of ammonium compound. It seems reasonable to assume that the decreased protection apparent with the higher concentration of these compounds is caused by a synergism between their own not fully developed blocking action and that of the added test dose of nicotine. This synergism may likewise account for the fact that the dodecyl compounds, while exerting the most powerful blocking actions of the series, show no more than a trace of protection.

Discussion. The present study is the first to compare the actions of primary, secondary and tertiary amines on the same preparation. Alles[4] studied the alkyl primary amines extensively on mammalian test preparations. He found that their inhibitory actions on gut of the guinea pig and rabbit, and their antagonism to the actions of adrenalin, acetylcholine and histamine, increased regularly with the length of the alkyl chain. These relationships appear to be the same as found in the present paper for the direct and indirect actions of each of the series of primary, secondary and tertiary amines.

With regard to the alkyl trimethylammoniums, two groupings of depressant effects

seem necessary. These are the curariform actions, usually studied with frog nerve-muscle preparations, and the muscarinic actions, usually studied with the frog heart or the mammalian circulatory system. Studies on the curariform action of a series of these salts showed that the amyl through octyl compounds were about equal in activity, while the dodecyl compound was much less active (Ing and Wright[6]). Studies on the muscarinic activities of the methyl to nonyl trimethylammonium salts clearly showed the butyl and amyl members of the series to be the most active (Kulz,[7] Raventos,[8] Alles and Knoefel[9]). In marked contrast to both these results, the dodecyl compound is the only one which shows any blocking activity on the crayfish synapse.

The stimulant or nicotinic actions of the alkyl trimethylammoniums have been studied on the leech muscle, frog rectus abdominis, gut of rat or rabbit, and circulatory system of the dog. The butyl, amyl or hexyl compounds were found to be the most active of the series (Kulz and Achenbach,[10] Raventos,[8] Alles and Knoefel[9]). Fitting in with these observations, we find in the present work that the amyl compound is noteworthy in showing a marked facilitation of the synapse studied, while the butyl and hexyl compounds occasionally show a weak facilitating action.

The antagonism of nicotine by quaternary ammonium compounds has not been studied previously. However, Raventos[8] and Clark and Raventos[11] investigated the antagonism of the nicotinic actions of acetylcholine by a series of alkyl trimethylammonium compounds. They found that the blocking activity of this series increased with the length of the alkyl side chain. In the present paper a similar relationship has been found for the blocking activity against the action of nicotine. It therefore appears that the blocking action of these compounds to nicotine on the crayfish synapse corresponds to their blocking action to acetylcholine on other preparations. This is remarkable, for no direct action of acetylcholine has been observed on synaptic transmission in the crayfish; neither facilitation, nor block, nor protection against nicotine (Prosser,[12] Schallek and Wiersma[3]).

Summary. This paper describes the effects of various amines and ammonium compounds on synaptic transmission in the central nervous system of the crayfish.

In the series from butyl to octyl amine, the activity of the drug in blocking synaptic transmission increases with the length of the alkyl side chain. Dodecyl amine shows greater activity than octyl amine. The corresponding alkyl methylamines and dimethylamines show a similar increase. The trimethylammonium series shows no blocking activity until the dodecyl compound is reached. The amyl trimethylammonium compound is unique in showing a consistent facilitation of synaptic transmission.

The ability of sub-threshold doses of these drugs to protect the preparation against the blocking action of nicotine reaches a maximum with the octyl compounds. The protecting activity of the trimethylammonium series is generally weaker than that of the other compounds. Although the dodecyl compounds show only a trace of protection against nicotine, this weak protection is shown at very high dilution.

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