THE PHARMACOLOGICAL ACTION OF SOME ANALOGUES OF PHYSOSTIGMINE

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Received for publication June 1, 1931

Physostigmine (Eserin) is of interest chemically as an example of an alkaloid whose pharmacological action depends on the presence in the molecule of a particular group, in this case the methylcarbamic ester group CH_3NHCOO. It has been shown by Stedman (1, 2), Stedman and Stedman (3), White and Stedman (4) that other compounds containing this group possess a miotic action similar to that of physostigmine. The results of his extensive investigation can be summarized as follows:

All the compounds which possessed miotic activity were basically substituted phenylesters of monoalkylcarbamic acids of the general formula $RNHCOOC_6H_4R'$, where R was a methyl or ethyl group and R' a basic substituent such as $-N(CH_3)_2$ or $-CH_2N(CH_3)_2$, etc.

The activity was greatest when R was a methyl group,—i.e. when the compound was similar to physostigmine in that it contained the group CH_3NHCOO. No activity was observed when R was a phenyl group.

The miotic activity varied according to whether R' was in the *o*, *m* or *p*-position and the activity of the hydrochlorides was in some cases greater and in others smaller than that of the quaternary salts.

There is an evident analogy with the case of cocaine, which is a benzoic ester of a bicyclic alkamine, whereas physostigmine is a methylcarbamic ester of a tricyclic basic phenol. In the same way as many synthetic esters of simpler alkamines possess local anesthetic properties similar to those of cocaine, so do the methylcarbamic esters of the simpler basic phenols synthesized by

Stedman possess pharmacological properties similar to those of physostigmine. It was therefore of interest to investigate the variation of the pharmacological properties in this series on modifying the carbamic acid group on the one hand and the phenolic residue on the other. It also seemed probable that a systematic investigation might result in obtaining a compound suitable for therapeutic use which would be free from the inherent disadvantages of physostigmine, particularly the ease with which it decomposes in solution.

PRELIMINARY WORK

In the first place several of the compounds whose miotic action had been observed by Stedman were investigated for toxicity, miotic action, and action on the rabbit's intestine. It was found that many of the quaternary salts of this series were highly toxic substances, having the characteristic action on the central nervous system and causing increased salivation like physostigmine. The quaternary salts examined were found to have a stronger action on the intestine than the hydrochlorides of the corresponding tertiary bases and to be more readily decomposed. This decomposition takes place in aqueous or alcoholic solution and was also observed by Stedman (1, p. 733). It occurs with elimination of alkyl or aryl isocyanate the odor of which is evident after some hours standing in the cold or immediately on boiling. The carbamic ester group is transformed into a phenolic hydroxyl group according to equation A.

(A) $$RNHCOOC_6H_4R' = RNCO + HOC_6H_4R'$$

As the presence of a solvent, water or alcohol, is necessary for the decomposition to take place below 100°, it is probable that the solvent plays an important part in initiating the decomposition. In aqueous solution the isocyanate then reacts with water according to equation B to form the disubstituted urea.

(B) $$2RNCO + H_2O = (RNH)_2CO + CO_2$$

(C) $$CH_3NCO + H_2NC_6H_5 = CH_3NHCONHC_6H_5$$

The change could be particularly well followed in the case of phenylcarbamic esters, where insoluble diphenyl urea is produced.

In the case of the methylisocyanic esters the dimethylurea formed remains in solution but by adding aniline to the solution insoluble methylphenyl urea is formed according to equation C.

In the presence of excess of alkali the end products of the hydrolysis are the phenol, amine, and alkali carbonate, but even then the first stage is an elimination of isocyanate, the odor of which can be observed if the solution is made only just alkaline. The tentative suggestion had already been made by Stedman (1, p. 733) that the activity of these compounds might be due to the action of one of the products of hydrolysis liberated in the body. An experiment was therefore made with N-bromacetamide $CH_3 \cdot CON\langle^{H}_{Br}$ which readily splits off HBr in vitro producing methylisocyanate. It was found, however, that it produced none of the characteristic symptoms of physostigmine poisoning.

When the solution of the carbamic ester is made slightly acid (pH on the acid side of 5) the decomposition can be greatly suppressed. It was only after this discovery that we were able to obtain concordant results in the evaluation of the pharmacological activity of many of the compounds mentioned below, which were tested in buffered solutions, a method which proved to be satisfactory for experimental purposes.

ESTERS OF DISUBSTITUTED CARBAMIC ACIDS

In order to obtain compounds which might be less readily decomposed than the monoalkylcarbamic esters previously prepared, some dialkyl and arylalkylcarbamic esters were prepared. These contain instead of the group $R \cdot NHCOO$, the group $\begin{matrix} R \\ R_1 \end{matrix}\rangle NCOO-$. It was considered that a decomposition analogous to that of the monosubstituted carbamic esters would be far less likely to take place as it would involve the migration of an alkyl or aryl radical instead of a hydrogen atom as in equation A. If the decomposition took place in an analogous manner a phenolic ether would be produced as in equation D, a course which seemed unlikely.

$$(D) \qquad \begin{matrix} R \\ R_1 \end{matrix}\rangle NCOOC_6H_4R' \longrightarrow RNCO + R_1O \cdot C_6H_4R'$$

In one compound investigated the nitrogen of the carbamic ester group formed part of a heterocyclic ring, R and R_1 being in that case together represented by the pentamethylene group C_5H_{10} so that such a decomposition was completely excluded.

It was of course possible that owing to the improbability of isocyanate liberation, a simple hydrolysis would take place in these cases forming the secondary amine and carbon dioxide. On heating the neutral aqueous solution of such disubstituted carbamic esters no amine could, however be detected or determined quantitatively. The assumption that this class of compounds would be more stable in vitro thus proved to be justified and it was found that many of them had a high pharmacological activity.

PHARMACOLOGICAL INVESTIGATION

The results of the investigation are given in table 1 and concern the following properties: (*a*) Toxicity (intravenously and orally); (*b*) miotic action; (*c*) peristaltic action (on the surviving intestine (Magnus) and in some cases in situ (Trendelenburg); (*d*) action on the frog's heart.

The intensity of these effects could be measured quantitatively with sufficient accuracy for comparison with the corresponding effects of physostigmine. Using the figures obtained as a basis, the compounds could be classified according to their "physostigmine activity." The various properties were not always present in the same degree, a few substances, for instance showing a relatively weak miotic action but having a strong action on intestinal peristalsis. The various pharmacological properties are therefore treated separately in the discussion.

METHODS

1. Toxicity. The toxicity was determined in the usual way, the minimum dose to cause death of over 80 per cent of the animals being recorded. This naturally gives somewhat higher figures for the toxicity than the "50 per cent-deaths" method adopted by White and Stedman (4), but where the same substances have been examined the toxicities are in the same order

as those observed by these authors. In table 2 the values obtained by the "50 per cent-deaths" method are given for comparison in the case of two of the compounds and physostigmine, the values showing agreement with those of White and Stedman for physostigmine. In the compounds marked with an asterisk the toxicity and other pharmacological effects were measured in stabilized solutions of pH about 3.7. These were usually prepared by dissolving 1 gram of substance in 95 cc. of decinormal glycine-sodium-chloride solution + 5 cc. of decinormal hydrochloric acid (Sørensen buffer solution, Clark (5)) and diluting to the required strength with Ringer solution. The buffer solution itself was non-toxic. Mice were usually used for toxicity determinations.

2. Miotic action. This was observed on the cat, 2 drops of the solution to be examined being instilled into one eye and the two eyes subsequently compared at intervals. To obtain a complete comparison of the various compounds it would be necessary to take account of the duration as well as the intensity of the maximum miotic effect produced by a given concentration. The duration of the action was only noted in a few cases in which the action was strongest.

3. Action on the small intestine (rabbit). a. Surviving intestine. The action of the substances on the isolated rabbit intestine suspended in 50 cc. of Dale's solution kept, like the washing solution, at 37° was studied. The apparatus of Guggenheim and Löffler (6), which enables the test to be carried out on two pieces of intestine simultaneously, was used. The test pieces were taken from various parts of the small intestine and were about 2 cm. long. Portions of the ileum were usually found to be more sensitive than those from the duodenum. Each substance was directly compared with physostigmine so as to eliminate as far as possible differences due to varying sensitivity of the test object.

b. Intestine in situ. A few of the substances were also tested by the method of P. Trendelenburg (7) on the intestine in situ of a rabbit kept in deep narcosis by 0.5 cc. per kilogram of "Roche-Numal," a solution containing 10 per cent of allylisopro-

pyl barbituric acid. The injections were made into the vena jugularis, into which a cannula which could be closed by a cock was fixed.

4. Action on the frog-heart. The heart action was studied on the isolated esculenta heart by the method of Straub.

5. Action on blood pressure and respiration. The blood pressure tests were carried out on the rabbit narcotized as above. The method of P. Trendelenburg (8), which enables the experiment to be carried out for many hours on one animal without fear of coagulation, was used.

In investigating the action on respiration both the effect on the volume and on the frequency of respiration was observed. A gasometer of 1 liter capacity with two one-way valves regulating inspiration and expiration was connected by a cannula to the trachea of the animal. A spindle on the gasometer makes a complete revolution when 1 liter of gas passes through the meter and carries two radial wires in the form of a cross. Each time 250 cc. of gas have passed through the meter one of the wires closes a circuit actuating an electromagnet and a stroke is registered. In parallel with the meter a Marey's tambour was arranged to register the respiratory frequency on the same graph as blood-pressure and volume.

DISCUSSION OF RESULTS (TABLE 1)

Toxicity. In discussing the toxicity it is convenient to divide the compounds into three classes, the members of which show certain similarities: (*a*) Salts of weak tertiary aromatic bases from which the base is liberated in neutral or slightly alkaline solution (i.e., in the intestine); (*b*) salts of strong tertiary bases having the nitrogen in the side chain, from which the base is liberated only in alkaline solution; (*c*) quaternary salts which remain in solution in alkali.

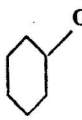
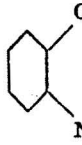
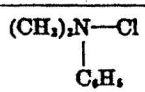
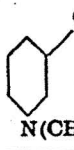
The members of class (*a*) are in general less toxic than those of classes (*b*) and (*c*). When given orally salts of class (*a*) are considerably less toxic than when injected intravenously, possibly because the free base is only slowly absorbed through the intestinal walls. Salts of class (*b*) show chemically the closest rela-

tionship to physostigmine and their toxicities are of the same order as that of the natural alkaloid. The difference between the lethal doses by the oral and intravenous route is here less pronounced, although several of the compounds are, like physostigmine, unstable and might be expected to decompose in the alimentary tract. The relatively high toxicity of physostigmine by the mouth might possibly be due to the inhibiting action it exerts on hydrolysis by esterases (Stedman (9)) as it might conceivably inhibit the action of the enzymes of the alimentary tract sufficiently to prevent to some extent its own hydrolysis.

Among the compounds of class (*c*) are some highly toxic substances, but throughout this class there is a large decrease of toxicity when the compounds are given orally. Irrespective of whether we are dealing with compounds which are stable in neutral aqueous solution (substances 3, 4, 32, 33, 34, 35, 36, 38, 40, 42) or substances which readily decompose on boiling their solutions (10, 11, 13, 14, 16, 17, 18, 20, 21, 23, 25), the ratio of the oral to the intravenous lethal dose is more than 10 and occasionally over 100 in the different compounds. We do not consider that this similarity of behavior between the stable and unstable substances is due to the fact that the latter were tested in buffered solutions which were stable to boiling for a short time, because the effect of the buffer salts would be overcome in the alimentary tract. The solutions were only buffered to prevent decomposition during testing.

A large difference between the values obtained for the lethal doses of a compound when administered intravenously or orally indicates either that it is only slowly absorbed in the alimentary tract so that a lethal concentration is only attained with high doses, or that it is rapidly eliminated either unchanged or after decomposition or combination with another substance in the body. Various considerations have led us to abandon the view that the cause of the reduced toxicity when given orally is that the substances are all unstable in vivo. In the first place we became doubtful of this hypothesis when it was found that in vitro stability, as mentioned above, was no criterion of high oral toxicity. Further the phenol, substance 4 which cannot undergo

TABLE 1

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
1	Methylcarbamic ester of phenol		84°	>50	>1,000	0.2 per cent, no action	1×10^{-5}, no action	
2	Methylcarbamic ester of 2-nitrophenol		56°	33	>50			
3	Trimethylphenyl ammoniumchloride		234°	15	200–300	2 per cent, inactive	2×10^{-6}, definite; 1×10^{-5}, strong action	
4	3-Oxyphenyl-trimethylammonium-iodide		182°	25–30	200–250	2 per cent, inactive	1×10^{-5}, strong action; 1×10^{-6}, definite action	
5	3-Acetoxyphenyl-trimethylammonium-methylsulfate		121°	7.5–10	1,000	1 per cent, doubtful	5×10^{-6}, weak action	

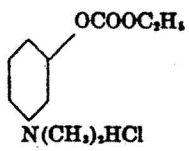
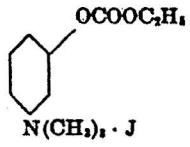
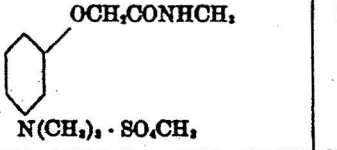
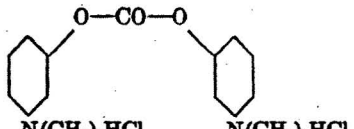
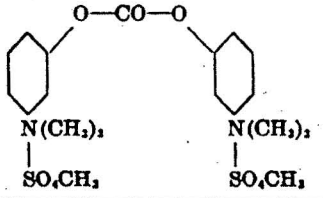
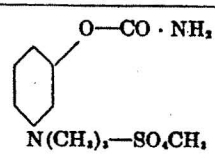
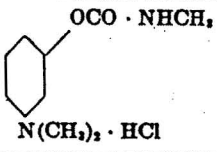
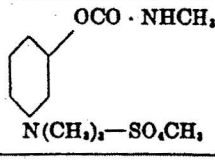
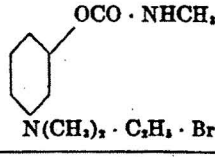
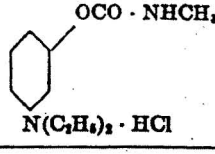
6	3-Ethylcarboxydimethylaminophenol-hydrochloride		131°		About 500	1 per cent, inactive	2 × 10^{-5}, doubtful action	
7	3-Ethylcarboxyphenyltrimethylammoniumiodide		153°	25	>500	5 per cent inactive	2 × 10^{-6}, doubtful; 1 × 10^{-5}, slight contraction	
8	m-Dimethylaminophenoxyacetmethylamide-dimethylsulfate		121°	7.5	1,000	2 per cent inactive	1 × 10^{-5}, no action	
9	Bis-(3-dimethylaminophenyl)-carbonate hydrochloride		210°		2,000–2,500	Only soluble in excess HCl. Too irritant for testing		
10	Bis-(3-Trimethylphenylammonium)-carbonate-di-methylsulfate		195°	12.5	1,000	1 per cent, no action	1 × 10^{-5}, definite action	1 per cent, no definite action

TABLE 1—*Continued*

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
11*	Carbamic ester of 3-Oxyphenyltrimethylammoniummethylsulfate		137°	0.7	500	1 per cent, inactive	5×10^{-6}, strong contraction	1 per cent, no definite action
12	Methylcarbamic ester of 3-oxyphenyldimethylaminehydrochloride		170°	15	55	5 per cent, temporary	1×10^{-4}, excitation	0.1 per cent, cessation of beats in diastole
13*	Methylcarbamic ester of 3-oxyphenyltrimethylammoniummethylsulfate		157–160°	0.1	2.5	1 per cent, temporary	0.5×10^{-4}, definite contraction	1 per cent, cessation of beats in diastole
14*	Methylcarbamic ester of 3-oxyphenyldimethylethylammoniumbromide		164°	0.15	5–8 (rat)			
15	Methylcarbamic ester of m-oxyphenyldiethylamine hydrochloride		144°	5	20		1×10^{-6}, active; 1×10^{-5}, strongly active	

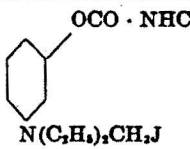
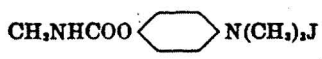
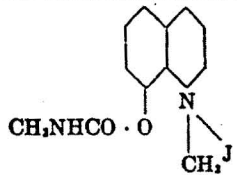
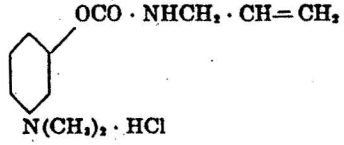
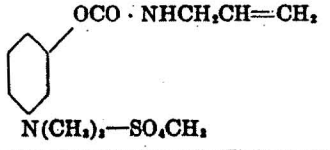
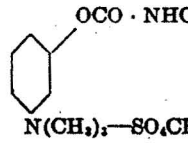
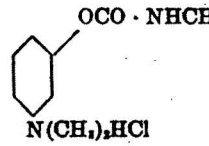
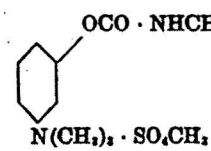
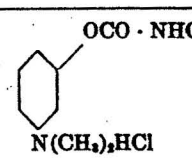
16*	Methylcarbamic ester of oxyphenylmethyldiethylammonium-iodide		136°	0.1	20	0.5 per cent, strongly active	0.5–1.0 × 10^{-7}, definite action	
17*	Methylcarbamic ester of p-oxyphenyltrimethylammonium-iodide		165°	2	50		1 × 10^{-5}, excitation	1 per cent, cessation of beats in diastole
18*	Methylcarbamic ester of 8-oxyquinoline methiodide		154°	0.1	200	1 per cent, no definite action	2 × 10^{-5}, definite contraction	0.01 per cent, increase of amplitude; 0.1 per cent, cessation of beats in diastole
19	Allylcarbamic ester of m-oxyphenyldimethylamine hydrochloride		155°	150	500	2 per cent, inactive (too irritant)	2 × 10^{-5}, strong paralysis; 0.5 × 10^{-6}, definite excitation	
20*	Allylcarbamic ester of m-oxyphenyltrimethylammonium-methylsulfate		112°	0.75	25	1 per cent, definite	0.5–0.25 × 10^{-6}, definite contraction	1 per cent, increase of amplitude

TABLE 1—*Continued*

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
21*	Ethylcarbamic ester of m-oxyphenyltrimethylammonium-methylsulfate		131°	1	100	1 per cent, weak		
22	Benzylcarbamic ester of 3-oxyphenyldimethylaminehydrochloride		180°	50.0	500	Only soluble in excess acid		
23*	Benzylcarbamic ester of 3-oxyphenyltrimethylammonium-methylsufate		159°	0.1	33	1 per cent, no definite action	0.5–0.25 × 10^{-6}, definite action, similar to physostigmine	0.1 per cent, slight increase of amplitude; 0.01 per cent, no action
24	Phenylcarbamic ester of 3-oxyphenyldimethylaminehydrochloride		158°	20–30	About 500			

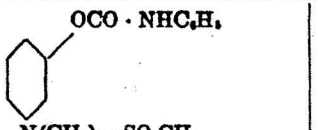
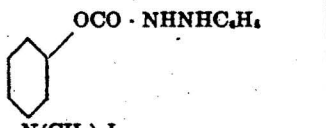
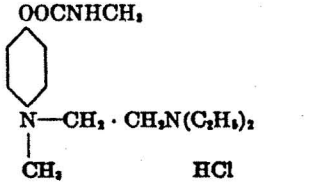
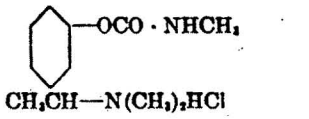
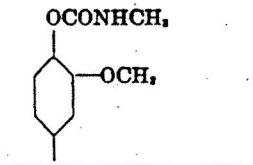
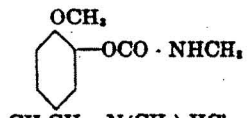
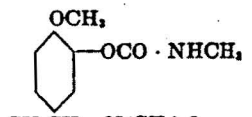
25*	Phenylcarbamic ester of 3-oxyphenyltrimethylammonium-methylsulfate		156°	2 (buffered); 6–7 (unbuffered)	125–166	1 per cent, inactive	1×10^{-4}, doubtful; 2×10^{-4}, definite action	
26	Phenylhydrazinoformic ester of 3-oxyphenyltrimethylammoniumiodide		158°	0.25	200	1 per cent, no definite action	0.3×10^{-4}, definite action; 0.5×10^{-4}, strong action; 1×10^{-4}, paralytic action	0.1 per cent, slight decrease of amplitude
27	Methylcarbamic ester of 4-oxyphenyldiethylaminoethylmethylamine hydrochloride		159°	0.1	25	2 per cent, no definite action	2×10^{-4}, no definite action	
28†	Methylcarbamic ester of α,3-hydroxyphenylethyldimethylamine hydrochloride (Miotine)		169°	1.0	2.0	0.1–0.5 per cent, several hours; 1–2 per cent, 24 hours	$0.2–0.25 \times 10^{-4}$, definite action	0.1 per cent, slight decrease of tone; 0.5 per cent, large decrease of tone

TABLE 1—*Continued*

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
28a†	Methylcarbamic ester of α-(4-hydroxy-3-methoxyphenyl)-ethyldimethylamine hydrochloride		145° (decomposed)	1–1.5	25	0.5 per cent, strong after 30 minutes	1–2 × 10^{-5}, definite increase of tone	0.1 per cent, slight decrease of tone
29†	Methylcarbamic ester of α-(3-hydroxy-4-methoxyphenyl)-ethyldimethylamine hydrochloride			6		1 per cent, maximum after 2 hours; next day indefinite; 0.5 per cent, definite after 2 hours	1 × 10^{-6}, slight action; 1 × 10^{-5}, large increase of tone	0.1 per cent, slight decrease of tone
30†	Methylcarbamic ester of α-(3-hydroxy-4-methoxyphenyl)-ethyltrimethylammoniumiodide		177°	5		0.5 per cent, strong after 2 hours; next day indefinite	2 × 10^{-6}, slight increase of tone; 5 × 10^{-6}, moderate increase of tone	0.1 per cent, no certain action

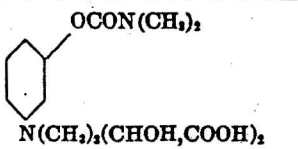
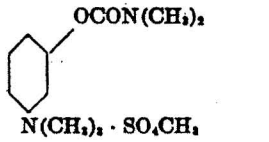
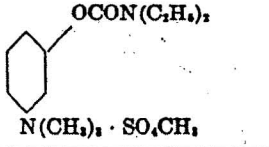
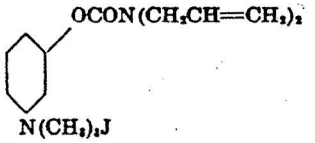
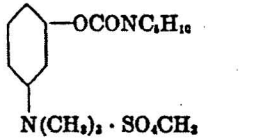
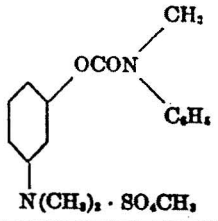
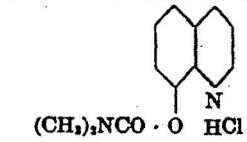
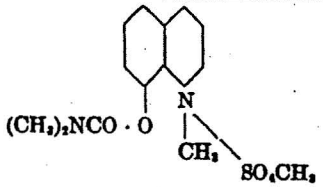
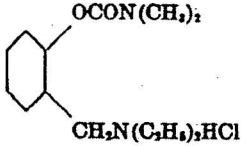
31	Dimethylcarbamic ester of 3-oxyphenyldimethylaminetartrate		Not crystallized	60		1 per cent, slight miosis	0.4 × 10^{-6}–1 × 10^{-5}, no definite action	
32	Dimethylcarbamic ester of 3-oxyphenyltrimethylammoniummethylsulfate		143°	0.5	12–16	0.5–1 per cent, miosis several hours	0.4–0.2 × 10^{-6}, contraction	1 per cent, slight decrease of amplitude; 0.1–0.01 per cent, doubtful action
33	Diethylcarbamic ester of 3-oxyphenyltrimethylammoniummethylsulfate		137°	8	71	1–4 per cent, no definite action	1–2 × 10^{-5}, no definite action	
34	Diallylcarbamic ester of 3-oxyphenyltrimethylammoniumiodide		110°	10	>250	4 per cent, indefinite	2 × 10^{-5}, indefinite	
35	Pentamethylenecarbamic ester of 3-oxyphenyltrimethylammoniummethylsulfate		159°	6	500	2 per cent, inactive	2 × 10^{-6}, slight; 1 × 10^{-5}, strong action	

TABLE I—*Continued*

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
36	Methylphenylcarbamic ester of 3-oxyphenyltrimethylammoniummethylsulfate		163°	3.5	75	1–2 per cent, slight miosis	0.4×10^{-6}, strong action	0.1 per cent, no definite action
37	Dimethylcarbamic ester of 8-oxyquinolinehydrochloride		193°	150	400	0.5–1 per cent, definite	1×10^{-5}, no definite action	
38	Dimethylcarbamic ester of 8-oxymethylquinoliniummethylsulfate		139°	0.5	200	0.25–0.5 per cent, definite for several hours	0.2×10^{-6}, definite contraction	
39	Dimethylcarbamic ester of 2-oxybenzyldiethylamine		Not crystallized	1.5	5	1 per cent, slight	1×10^{-6}, no action; 2×10^{-6}, slight contraction; 2×10^{-5}, strong contraction	

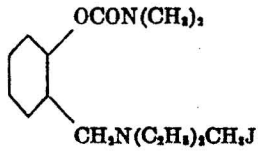
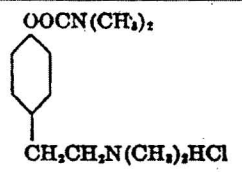
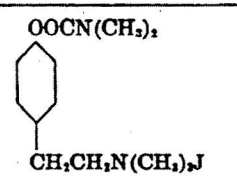
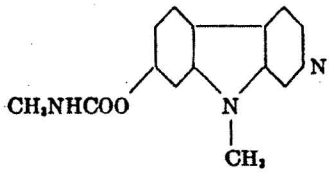
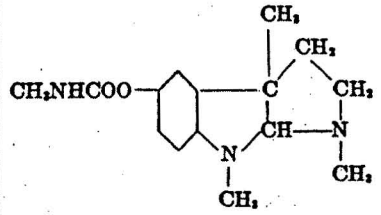
40	Dimethylcarbamic ester of 2-oxybenzyl-methyldiethylam-moniumiodide		156°	0.5	75–100	1 per cent, no definite action	1 × 10⁻⁶, no action; 2 × 10⁻⁶, slight contraction; 1 × 10⁻⁵, strong contraction	
41	Dimethylcarbamic ester of Hordenine hydrochloride		206°	15	75	1 per cent, trace; 2 per cent, weak miosis	2 × 10⁻⁵, no action	
42	Dimethylcarbamic ester of Hordenine methiodide		234°	55	>100	1 per cent, indefinite; 2 per cent, trace	2 × 10⁻⁵, no action	
43†	Methylcarbamic ester of Harmol hydro-chloride			66		1 per cent, weak; 0.5 per cent, indefinite miosis	1 × 10⁻⁵, no definite action; 2 × 10⁻⁵, weak action; 4 × 10⁻⁵, weak action	0.1 per cent, slackening or cessation of pulsations

TABLE 1—*Concluded*

NUMBER	NAME	STRUCTURAL FORMULA	MELTING POINT	LETHAL DOSE (MOUSE, MGM. PER KGM.)		MIOTIC ACTION (CAT)	ISOLATED RABBIT INTESTINE	ISOLATED FROG HEART
				Intravenously	Per os			
44	Physostigmine			0.5	3	0.1–0.5 per cent, definite several hours; 1–2 per cent, 24 hours	0.25–0.5 × 10^{-6}, contraction	0.1 per cent, strong decrease of tone or cessation of beats
45	Physostigmine methiodide	$C_{15}H_{21}O_2N_3, CH_3I$	188°	0.75–1	250–300	0.1 per cent, active	0.2 × 10^{-6}, contraction	

* Tested in buffered solution.

† We are indebted to Dr. and Mrs. E. Stedman (16) for kindly supplying the specimens of these five compounds.

such a hydrolysis, is also 10 times less toxic orally than intravenously and its carbonic ester (substance 10) is much less toxic orally than the phenol which it would form to the extent of over 90 per cent if hydrolyzed. It would be necessary to assume a detoxication of the phenol in the organism by combination to explain these observations.

It will also be seen from table 1 that physostigmine behaves similarly after conversion into its quaternary salt. Although the ratio of the oral to the intravenous lethal dose of physostigmine sulfate is only 6, the corresponding ratio in the case of the quaternary salt physostigmine methiodide (substance 45) is over 100, although there is no great difference in the stability of the two salts.

On careful consideration it was found that there are many indications which point to the fact that the large difference in toxicity by the two routes is a general characteristic of quaternary ammonium compounds. Thus curare, which contains as active principle the quaternary base curarine, is 70 times less toxic to rabbits orally than subcutaneously (K. Sauer (10)) and a similar relation holds with other animals. A discussion of the reasons for this is given by R. Boehm (11) who points out that it seems to be due to their rapid elimination, various experimenters having shown that curarine is found almost quantitatively in the urine (S. Jakabházy (12)). A similar observation was made by M. Führer (13), in the case of methyl green, the diquaternary salt of methyl violet. He found that rabbits showed no reaction to 15 times the intravenous lethal dose if it was given orally. In order to have another example of a simple quaternary salt, free from ester or hydroxy groups, the toxicity of trimethylphenylammonium chloride $C_6H_5N(CH_3)_3Cl$, substance 3, was determined on mice and found to be 15 mgm. per kilogram intravenously and 250 mgm. per kilogram orally.

It therefore seems probable that a much reduced toxicity orally compared with intravenously, is a general characteristic of quaternary ammonium compounds, probably because they are rapidly eliminated from the blood stream. In consequence it appears justifiable to conclude that the high value for the

ratio of the oral to the intravenous lethal dose of the quaternary salts of substituted carbamic esters is not solely due to the fact that they are hydrolyzed in vivo although hydrolysis probably takes place to some extent, particularly in the case of those compounds which are unstable in vitro.

Miotic action. Only those substances which were carbamic esters of phenols containing a basic substituent exhibited any miotic action. Relatively slight modifications of the carbamic ester group caused the miotic activity to be weakened as in substance 36, or to disappear, as in substances 25, 33 and 34. The quaternary salts of the aromatic bases examined were usually definitely stronger in their action than the hydrochlorides of the corresponding tertiary bases, but this may be due to the position of the basic substituent as observed by Stedman (1, p. 732). The mono-quaternary salt of physostigmine is almost as active as physostigmine, thus forming a contrast to cocaine which loses its characteristic properties on conversion to a quaternary salt (Ehrlich (14)).

The dimethylcarbamic esters 31, 32 and 38 were definitely more active than the monomethylcarbamic esters of the corresponding phenol 12, 13 and 18. The dimethylcarbamic esters 39, 40, 41 and 42 which contain the basic group in a side chain also showed definite activity, that of the hydrochlorides of the tertiary bases being in these cases higher than that of the quaternary salts. The corresponding monomethylcarbamic esters were not prepared. The methylphenylcarbamic ester 36 produced relatively weak miosis and the phenylcarbamic ester 25 was still less active. The ethylcarbamic ester 21 was definitely active in a concentration at which the diethylcarbamic ester 33 was inactive. The monoallylcarbamic ester derivatives 19 and 20 seem to be at least as active as the methylcarbamic esters of the same phenol 12 and 13 but the diallylcarbamic ester 34 showed no miotic activity.

The conclusion of Stedman that the monomethylcarbamic esters are the most active in producing miosis can therefore be extended by the statement that the dimethylcarbamic esters are in some cases more active than the monomethylcarbamic esters

and the methylphenylcarbamic esters more active than the phenylcarbamic esters. The ethylcarbamic esters are weakly active as found by Stedman, the allylcarbamic esters are more active, but the diethyl- and diallylcarbamic esters are inactive.

Action on the intestine. The case of miotine (substance 28), the only synthetic salt of a tertiary base approaching physostigmine in activity, has been fully investigated by its discoverer Stedman. The discussion below is therefore confined to the quaternary ammonium salts which appear in most cases to be more active than the hydrochlorides of the corresponding tertiary bases in stimulating intestinal peristalsis.

Several of the simpler quaternary ammonium compounds which do not contain a carbamic ester group cause a strong contraction of the surviving intestine. Thus substances 3, 4 and 7 exert an influence at concentrations of one or two parts per million and are about equal in activity to the carbamic esters 11, 25, 30, 35 and 40, and more active than the carbamic esters 17, 18, 33, 34 and 42, so that the action on peristalsis is less specific than the miotic action. Among the carbamic esters themselves those substances producing the strongest miosis usually but not invariably had the strongest action on intestinal peristalsis. The most notable exceptions are substances 23 and 36 which have a weaker miotic action than many of the carbamic esters examined but exert about as strong an action on the intestine as physostigmine. Further, substance 26 which contains the group $-OCO-NHNHC_6H_5$ instead of the true carbamic ester group has no miotic action in 1 per cent solution but stimulates peristalsis in a similar degree to physostigmine. The pharmacological data for substance 26 are in fact almost quantitatively identical with those obtained with substance 23 the corresponding benzylcarbamic ester, from which it only differs by having the group $-NHC_6H_5$ instead of $-CH_2C_6H_5$ attached to the nitrogen of the carbamic ester group.

It appeared that the esters of unsubstituted carbamic acids $H_2N \cdot COO-$ are not highly active in stimulating intestinal movement if we can judge from the single case of substance 11 which stimulates the isolated intestine only in a concentration

of 5×10^{-6}; the corresponding methylcarbamic ester, substance 13, acts strongly at a concentration of 1×10^{-6}, and the ester of dimethylcarbamic acid, substance 32, has a strong action in a concentration of 0.2×10^{-6}. Similarly the methylcarbamic ester, substance 18, acts only at a concentration of 2×10^{-6} while its dimethyl analogue substance 38 acts in concentrations of 0.2×10^{-6}. It is evident therefore that the presence of a methyl radicle on the carbamic ester group increases the activity and that the effect is potentiated in these cases by the presence of a second methyl group. The phenylcarbamic ester 25 is almost inactive at a concentration of one part per million while the methylphenylcarbamic ester 36 is active at a fifth of this concentration and is hence rather more active than the monomethylcarbamic ester 13 and at the same time the disubstituted ester is less toxic than either of the monosubstituted carbamic esters which can be regarded as its parent substances.

The diethyl and diallyl compounds 33 and 34 are relatively inactive, although the monoallylcarbamic ester, substance 20, is at least as active as, and less toxic than physostigmine. Substance 16 probably exerted the strongest action on the intestine of all the compounds examined but is unsuitable for therapeutic use on account of its high toxicity and instability. For this reason the more stable and less toxic substances 32 and 36 which have an activity as great as that of physostigmine and are much more stable than the latter, were chosen as most suitable for further investigation.

A characteristic property which distinguishes the carbamic esters from the other quaternary salts which have an effect on intestinal peristalsis is that the former are usually much more difficult to wash out of the test portion of intestine than physostigmine, so that it was often necessary to change the test object after only a single one of these compounds had been tested. The action of these derivatives could, like that of physostigmine, be counteracted by atropine, but the use of atropine also rendered the intestine unsuitable for further use. Owing to variations in the sensitivity of different test pieces of intestine the figures obtained must therefore be regarded as purely relative, but by the

performance of a large number of experiments using the same technique it was possible to be reasonably certain that the activities given in table 1 satisfactorily represent the true relative values.

INVESTIGATION OF THE DIMETHYL- AND METHYLPHENYL-CARBAMIC ESTERS OF 3-OXYPHENYLTRIMETHYLAMMONIUM METHYLSULFATE (SUBSTANCES 32 AND 36)

Most of the substances in table 1 were tested only in order to obtain an idea of their activity relative to physostigmine but after this preliminary survey two of the substances, 32 and 36, were subjected to a more thorough pharmacological investigation in order to compare them fully with physostigmine. They have the advantage of being less readily hydrolyzed than the natural alkaloid and the tests described below were carried out with unbuffered solutions in 0.9 per cent sodium chloride which had been sterilized at 100° in ampoules and stored for some months without diminution in activity. The physostigmine sulfate solutions were of course freshly prepared. Substance 38, which is equally active but was only recently synthesized, would probably show values very similar to those for substance 32 on more thorough investigation.

These substances as well as most of the quaternary salts described in table 1 are methylsulfates of quaternary bases. These salts were selected because they are stable in air and being free from iodine their solutions cannot become yellow on keeping, owing to liberation of free iodine by oxidation of traces of hydriodic acid, as often occurs with quaternary iodides particularly after sterilizing at 100°.

The results of this further investigation are summarized in table 2.

1. Toxicity. One of the first symptoms on the rabbit is a masticatory motion of the jaws of the animal, indicating the beginning of increased salivation. A short initial period of excitement can often be observed, the animals jump about the cage and become restless. Soon characteristic twitchings of the skin of the whole animal begin and the toes are spread out. Copious sali-

TABLE 2

	PHYSOSTIGMINE	SUBSTANCE 32	SUBSTANCE 36
Lethal dose (mgm. per kgm. mouse) Intravenously	0.4 (2/20 died) 0.5 (12/15 died)	0.3 (7/15) 0.4 (15/20)	2.0 (0/11) 2.5 (13/30) 3.0 (14/15)
Lethal dose (mgm. per kgm. mouse) Subcutaneously	0.75	1.0	3.0
Lethal dose (mgm. per kgm. mouse) Oral	3.0	12–16	75
Lethal dose (mgm. per kgm. rabbit) Intravenously	0.5–0.8	0.25	0.5
Lethal dose (mgm. per kgm. rabbit) Subcutaneously	3.0	0.5–0.75	1.0
Rabbit intestine: a. Isolated	One part in 5 to 7½ millions	One part in 5 to 7½ millions	One part in 5 to 7½ millions
b. In situ	0.02 mgm. per kgm.	0.02 mgm. per kgm.	0.02 mgm. per kgm.
Isolated frog heart	0.1 per cent usually stops the heart in diastole	0.1 per cent, doubtful action. Occasional slight loss of tone	0.1 per cent, no definite action
Curare antagonism (cat)	Definite	Definite	Absent
Blood pressure (rabbit intravenously)	0.1 mgm. per kgm., no action	0.1 mgm. per kgm., no action	0.1 mgm. per kgm., no action

vation and some defecation and lachrymation occurs. The respiratory frequency rises, the pulse rate diminishes. Later the animal lies on its side, respiration becomes gradually labored and after convulsive seizures the animal dies from respiratory failure at a time when the heart continues to beat and the muscles to twitch. Rats and mice behave similarly and the effect on the cat differed only in that an emetic action was observed even with sublethal doses. These symptoms are characteristic of this group of compounds (cf. White and Stedman, p. 264, for miotine) and were very marked with substance 13 whose toxicity has also been carefully investigated. Post-mortem examination often showed edema of the lungs in animals which had died slowly from the effects of the drug.

The dose causing 50 per cent mortality is 0.45 mgm. per kilogram mouse intravenously for substance 32 and physostigmine. Substance 36 has about a fifth of this toxicity (2.5 mgm. per kilogram. On subcutaneous injection physostigmine and substance 32 are less toxic than intravenously, whereas the lethal dose of substance 36 for mice is the same intravenously and subcutaneously. Physostigmine is more toxic than the other two substances when given by the mouth.

On the rabbit substance 36 and physostigmine are equally toxic intravenously while substance 32 is twice as toxic.

2. The miotic action of substance 32 on the cat is almost equal to that of physostigmine, the concentrations at which an action is just visible with certainty being 0.01 per cent for physostigmine and 0.05 per cent for substance 32. Substance 36 has only a fraction of this activity, a very weak but definite action is obtained on using a 1.0 per cent solution. This may be expressed by stating that physostigmine in 0.01 per cent solution has the same action both as regards intensity and duration as a 0.05 per cent solution of substance 32 or a 1 per cent solution of substance 36.

3. Rabbit intestine. On the surviving rabbit intestine physostigmine was always definitely active at a concentration of one in a million and usually showed a definite increase of tone at one part in five millions and in a few cases at one part in seven and

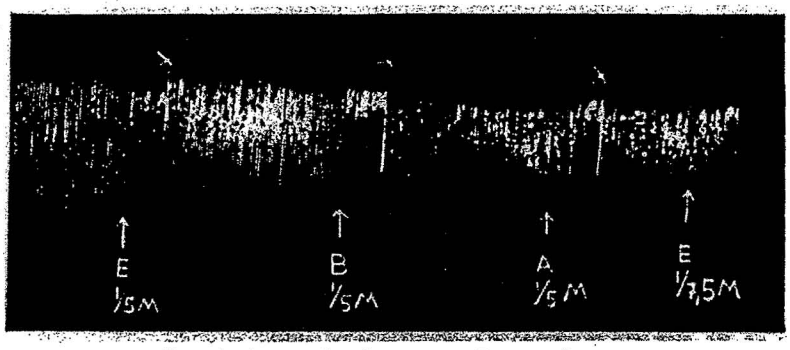


FIG. 1. ACTION ON SURVIVING RABBIT ILEUM

E, physostigmine; *A*, substance 32; *B*, substance 36. (Concentrations one in five to seven and a half millions.) At X washed out with Dale's solution.

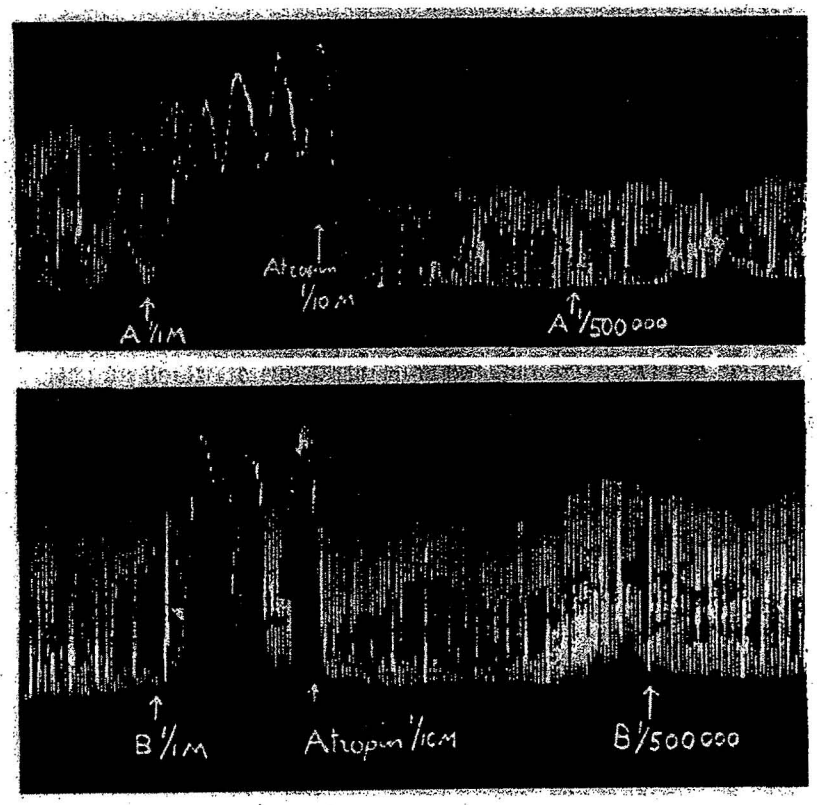


FIG. 2. ATROPINE ANTAGONISM

A, substance 32; *B*, substance 36

a half millions. Substances 32 and 36 are about as active as physostigmine as will be seen from figure 1. The action of all three substances on the surviving intestine could be completely antagonized by atropin as shown in figure 2.

The intestine in situ is definitely stimulated by intravenous

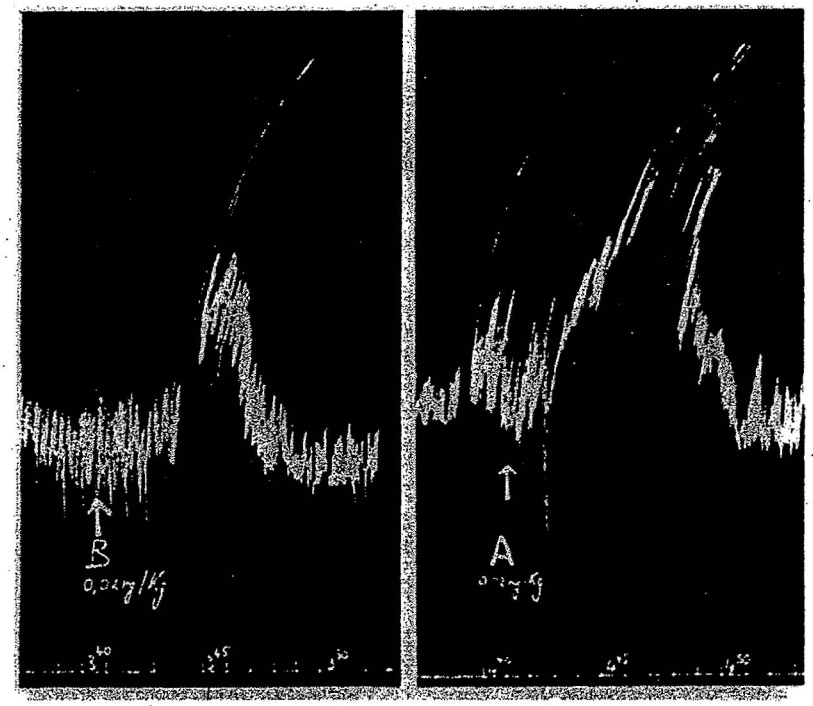


FIG. 3. ACTION ON SMALL INTESTINE IN SITU

Rabbit, 3.1 kgm., ♀. Narcosis 0.5 cc. per kilogram "Roche-Numal" intravenously. *B*, 0.02 mgm. per kilogram substance 36, definite stimulation followed by defecation; *A*, one hour later 0.02 mgm. per kilogram substance 32, the stimulation of the intestine was again followed by defecation. Time in minutes.

injection of 0.02 mgm. and defecation follows (fig. 3). No certain difference can be observed in the action of the three substances.

4. On the isolated frog-heart all three have a negative inotropic action. Substances 32 and 36 have a definitely weaker action

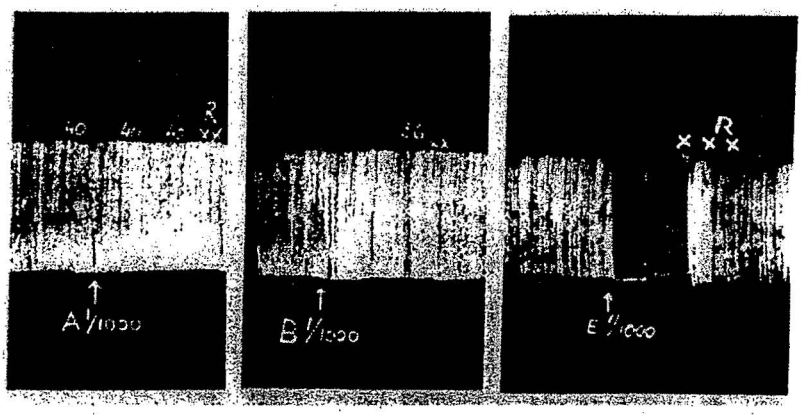


FIG. 4. ACTION ON ISOLATED ESCULENTA HEART

The figures give frequency per minute. *A*, substance 32; *B*, substance 36. At X washed out with Ringer's solution. *E*, physostigmine.

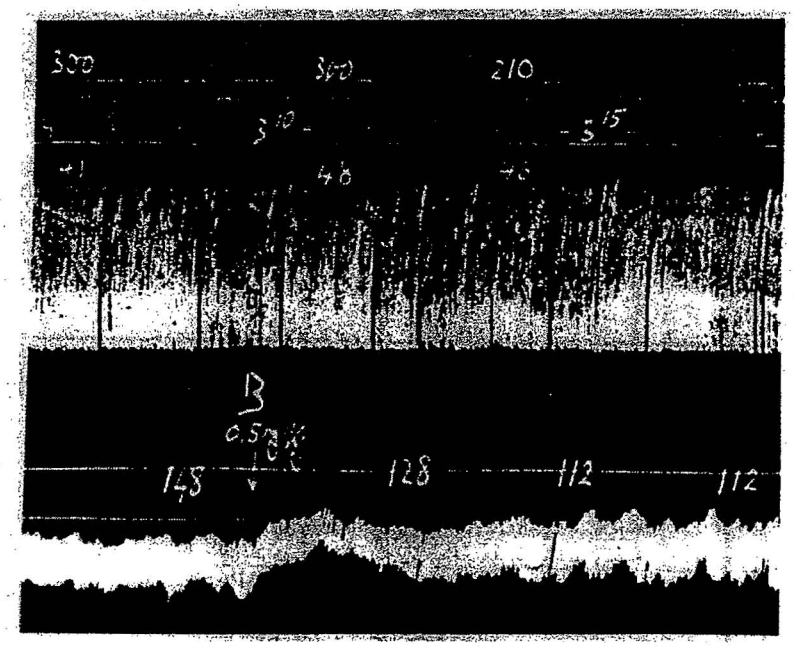


FIG. 5. ACTION ON BLOOD PRESSURE

Rabbit 180, 3.2 kgm. Top curve, volume of respired air; each stroke corresponds to 250 cc. The figures denote volume respired in one minute. Immediately below is given the time in minutes. The figures above the curve of respiratory frequency denote the number of respirations per minute. The bottom curve registers the carotid blood pressure, the figures giving pulse frequency. At *B*, 0.50 mgm. per kilogram substance 36.

(fig. 4), physostigmine often stopping the heart in diastole at a concentration of 0.1 per cent whereas substances 32 and 36 at most cause a slight decrease of the amplitude. A 1 per cent

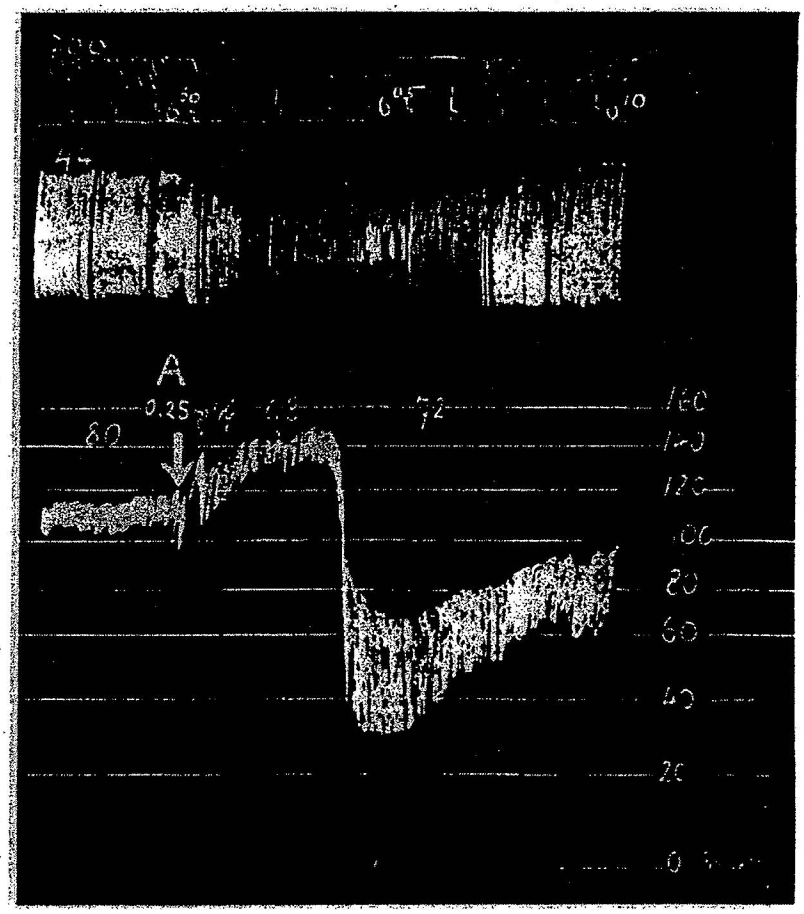


FIG. 6. ACTION ON BLOOD PRESSURE
Rabbit 105, 3.1 kgm. Remaining description as in figure 5. At *A*, 0.25 mgm. per kilogram substance 32. Time in minutes.

solution of physostigmine almost invariably stops the heart in diastole, whereas solutions of this concentration of substances 32 and 36 usually only cause a decrease of amplitude.

5. Action on blood pressure and respiration. The action on blood pressure is slight in doses up to 0.1 mgm. per kilogram which is considerably above the dosage required to stimulate intestinal activity (fig. 5). The reduction of blood pressure and increase of respiratory activity becomes more marked the nearer toxic doses are approached, the pulse rate being slackened as with

TABLE 3

Cat, 2.7 kgm. Deep narcosis with 0.5 cc. per kilogram "Roche Numal" intraperitoneally. Excitation of N. ischiadicus by induction coil. Substances injected into the jugular vein.

TIME	INJECTED	LIMITING DISTANCE OF COIL AT WHICH EXCITATION WAS OBSERVED
		mm.
11:10		100
11:12	5 mgm. curare	
11:17		50
11:18	5 mgm. curare	
11:25		40
11:26	10 mgm. curare	
11:30		10 (no action)
11:32	0.5 mgm. substance 32	
11:34		40
14:15	10 mgm. curare	50
14:17		
14:25		10 (no action)
14:26	3 mgm. substance 36	
14:29		10
		10
		10
14:52	0.5 mgm. substance 32	
14:54		80
14:58		40

physostigmine. With toxic or almost toxic doses the blood pressure is reduced almost to zero, as will be seen from figure 4. The respiration continues but the intake of air apparently ceases entirely, the volume registered being reduced almost to zero. If the animal recovers and breathing recommences, the blood pressure also increases. If the animal dies beating of the heart continues several minutes after respiration has ceased (figs. 5 and 6).

Of the three substances, physostigmine, substance 32 and 36, the last has the smallest effect on blood pressure or respiration, in agreement with its lower toxicity.

6. Antagonism to curare. It has been shown by White and Stedman (4), that miotine shows the strong antagonism characteristic of physostigmine to the paralytic action of curare on the nerve endings. Many other quaternary ammonium compounds besides curarine possess a similar action to curare, while choline, which is also a quaternary ammonium compound, on the contrary inhibits the action of curare (Abderhalden and Müller (15)).

It was consequently difficult to foretell how substances 32 and 36, which have many of the properties of physostigmine, but are on the other hand quaternary ammonium compounds, would behave as regards their effect on the curare action. Experiment showed that substance 36, as will be seen from table 3, had no antagonistic action to curare, while substance 32 showed similarity to physostigmine in its antagonism to the action of curare in causing a paralysis of the motor nerve endings.

SUMMARY

1. A series of alkyl, aryl, dialkyl and aryl-alkyl carbamic esters of phenols containing a basic substituent directly or indirectly attached to the phenyl radicle have been examined for physostigmine-like action, the toxicities, miotic action and effect on intestinal peristalsis being tabulated.

2. Several related compounds which do not contain both a carbamic ester group and a basic substituent show no activity.

3. The physostigmine-action is strong in methyl-, dimethyl-, allyl-, benzyl- and methylphenyl-carbamic esters of phenol-bases, weak in ethyl- and phenyl- and absent in diethyl and diallylcarbamic esters of this series. The esters of disubstituted carbamic acids are stable.

4. The quaternary salts of the aromatic bases were more active than the hydrochlorides of the corresponding tertiary bases. When the basic radicle was in the side chain the difference was less marked or reversed.

5. The dimethyl- and methylphenyl-carbamic esters of 3-oxyphenyl-trimethylammonium methylsulfate have been fully investigated. They are at least as active as physostigmine in stimulating intestinal peristalsis. The miotic activity of the dimethylcarbamic ester is similar to that of physostigmine, that of the methyl-phenyl-carbamic ester being weak. The latter does not show an antagonistic action to curare. The symptoms produced by toxic doses are similar to those produced by physostigmine.

In conclusion we have pleasure in expressing our thanks to the heads of the scientific department for their interest and guidance during this work.

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