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SOME ASPECTS OF THE PHARMACOLOGY OF AN HOMOLOGOUS SERIES OF CHOLINE ESTERS OF FATTY ACIDS

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The pharmacological effects of a series of fatty acid-choline esters have been studied on the isolated rabbit heart, the isolated guinea-pig ileum and the rat stomach. The effect changed with increasing chain length, and three different types of response were observed. The short-chain compounds produced depression of the isolated rabbit heart and spasm of the isolated guinea-pig ileum. Only one, butyrylcholine, had an erratic stimulating effect on hydrochloric acid secretion by the partially vagotomized rat stomach. Medium-chain compounds had a stimulating effect on the isolated rabbit heart, a mixed spasmogenic and relaxant effect on the isolated guinea-pig ileum, and no effect on the rat stomach. Long-chain compounds blocked the effect of acetylcholine on the isolated rabbit heart and the isolated guinea-pig ileum; they also depressed spontaneous hydrochloric acid secretion by the rat stomach. The nature of these three types of response is discussed.

The present investigation is concerned with the pharmacology of the choline esters of an homologous series of saturated straight-chain monobasic fatty acids.

Previous work was mainly concerned with the effect of these compounds on blood pressure. Hunt and Taveau (1911), in their extensive investigation of the pharmacology of a large number of choline derivatives, included the esters from acetylcholine (ACh) to pentanoylcholine and in addition palmitoylcholine, all of which occur in the series used in this work. They found that with increasing chain length the depressor effect declined, and a pressor effect was observed which in some cases became manifest after atropine but in others without atropine. These findings were confirmed and elaborated by le Heux (1921), Simonart (1932), Chang and Gaddum (1933), and Bovet and Bovet-Nitti (1948).

Fourneau and Page (1914) synthesized a series of these esters with even numbers of carbon atoms in the chain. Haemolytic action was most pronounced with the palmitic and stearic acid esters; it diminished with decreasing chain length, and was no longer found with members below dodecanoylcholine. In the present investigation an almost complete series of these esters was available. The effect of the chain length upon the pharmacological action was examined on the isolated mammalian heart, the motility of the small intestine, and the secretory response of the stomach.

METHODS

Isolated Rabbit Heart.—The usual Langendorff type of preparation with retrograde aortic perfusion was used. The composition of the perfusion solution used was: NaCl 9 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaHCO_3$ 0.5 g., dextrose 1.0 g., distilled water 1 litre. The compounds tested were injected through the rubber-capped side-arm of the perfusion apparatus.

Isolated Guinea-pig Ileum.—The ability of some of the compounds to induce spasm was expressed in terms of the concentration which caused an effect equal to that produced by $10^{-6.5}$ M-ACh. This was determined upon the isolated guinea-pig ileum suspended in the 2.0 ml. bath of an automatic assay apparatus, similar to that described by Boura, Mongar, and Schild (1954). A 2 min. cycle was employed. The lever used was constructed according to the dimensions given by Schild (1947), and was not allowed to excurse more than 30° from the horizontal. This ensured an approximately linear relationship between the recorded height of contraction and the actual shortening of the muscle. Three preselected concentrations of the test spasmogen and the fixed concentration of ACh were set up in the apparatus

together. ACh was added repeatedly until a steady response had been obtained, when the remaining bottles of test spasmogen were switched in. Six contractions to each concentration of the test spasmogen and six to the standard spasmogen were obtained, the doses being added in random fashion. At the end of the cycle, a second test spasmogen was assayed in a similar manner. The heights of all contractions were measured in mm. Mean responses were computed, and those for the three concentrations of test spasmogen were plotted as ordinates against the negative logarithms of the corresponding molar concentrations as abscissae, and a curve drawn through these three points. The activity ratio for the test spasmogen was then determined graphically by dropping a perpendicular from the point on the curve corresponding to the mean response to ACh. The intersection of this line with the abscissa represented the ACh equivalent for the test spasmogen. Two determinations were carried out for each spasmogen using ileum from two guinea-pigs, and the means calculated. The results from the two experiments were in good agreement.

Hexamethonium iodide (HMI) was used to determine whether the lower members of the series produced spasm of the intestinal muscle by a ganglionic ("nicotinic") or a direct ("muscarinic") type of action. Equilibrium was attained with the standard spasmogen (ACh 0.05 μg./ml.) and then a single response to the test spasmogen was obtained. The tissue was then bathed in Tyrode solution containing 3.0 μg. HMI/ml. for exactly 2 min. The spasmogens were again added, and the effect of HMI on the response determined.

pA_2 values (Schild, 1947) for the esters against ACh were determined after 10 min. contact between the tissue and the antagonist. Percentage effects were calculated with respect to the difference between the response to the single and to the double dose of ACh, and not as % of the maximum. The tissue was always allowed to recover from the effect of the antagonist before proceeding with the experiment. Where this did not occur, as for example with the higher esters, fresh ileum from the same animal was used. Four determinations of pA_2 were made for each ester, using ileum from different guinea-pigs.

Errors in the quantitative assessments of activity due to adsorption of the esters on to the glassware (Marshall, 1955) were avoided by immersion of all glass apparatus in 33% HNO_3 overnight, followed by thorough washing in hot tap water and distilled water before subsequent use. No detergent was used.

In Vivo Experiments on the Secretory Response of the Rat Stomach.—White male rats weighing 200 to 250 g. were used. The abdomen was opened under ether anaesthesia and the pylorus exposed and ligated. A glass cannula carrying a short length of rubber drainage tubing was introduced through a small incision just orally to the ligature. Through this cannula the stomach was rinsed, after which the cannula was removed and a second ligature applied. The rats were then allowed to recover from the anaesthetic and left for 6 hr. At the end of this period the rats were anaesthetized once more, the total stomach contents measured, the *p*H determined with indicator papers, free HCl titrated with N/100 NaOH using methyl orange as indicator and total chlorides determined using Patterson's micro-technique (Harrison, 1943).

The esters were injected into the adductors of the thigh in aqueous solution. The control rats received a corresponding injection of tap water. In order to demonstrate stimulating effects, spontaneous secretion was reduced by partial vagotomy. The oesophagus was cut about 0.5 cm. above the cardiac sphincter between ligatures. A glass cannula was introduced through the opening pointing caudally into the cardiac end of the stomach. The stomach was rinsed through this cannula until the returning fluid was clear. Following this, 5 ml. of warm tap water was left in the stomach. By this means, the anterior vagus was cut, the posterior vagus damaged, and the rat stomach secreted small amounts of free HCl over a period of 2 hr. It responded to such secretory stimuli as histamine, neostigmine, and carbachol.

In all instances the iodides of the esters were administered, except for palmitoylcholine, which was used as the more soluble bromide.

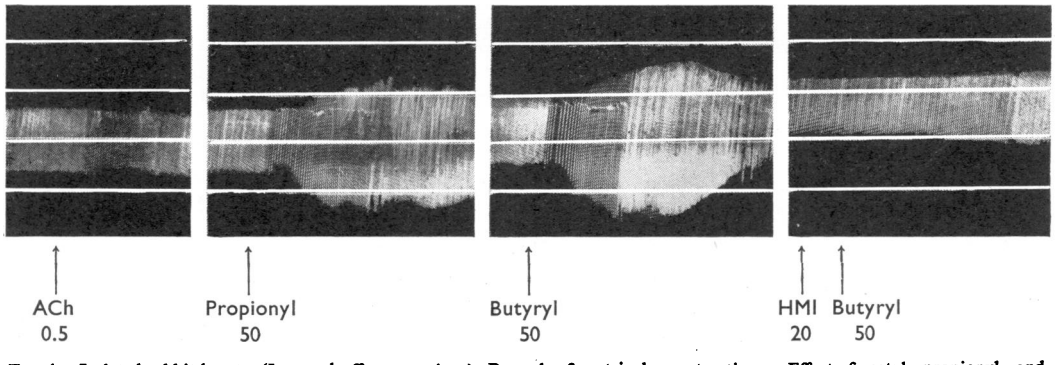

FIG. 1.—Isolated rabbit heart. (Langendorff preparation.) Records of ventricular contractions. Effect of acetyl-, propionyl-, and butyrylcholine. The action of butyrylcholine is greatly reduced by hexamethonium (HMI). Doses in μg.

Results

Effect on the Isolated Rabbit Heart

Three different types of effect were observed. The cardiac slowing and reduction of systolic contraction which characteristically occur with ACh were produced by the esters up to heptanoylcholine, although very much larger doses (50 μg.) had to be employed (Fig. 1).

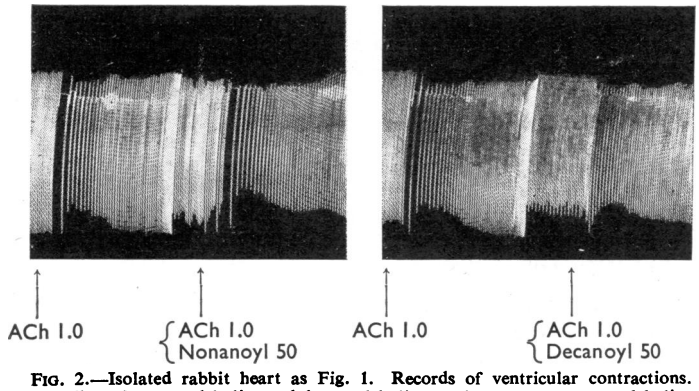

FIG. 2.—Isolated rabbit heart as Fig. 1. Records of ventricular contractions. Effect of nonanoylcholine and decanoylcholine on the response to acetylcholine (ACh). Amo unts expressed in μg.

With propionylcholine a stimulating effect made its appearance immediately following the depressant effect. This stimulation, which affected both the rate and height of contraction, was maximal with butyrylcholine, but was weaker with the pentanoyl and hexanoyl compounds and was not produced by the heptanoyl compound. When a dose of 20 μg. of hexamethonium was given immediately before the dose of ester, the stimulating effect of the propionyl compound was abolished, and that of the butyryl

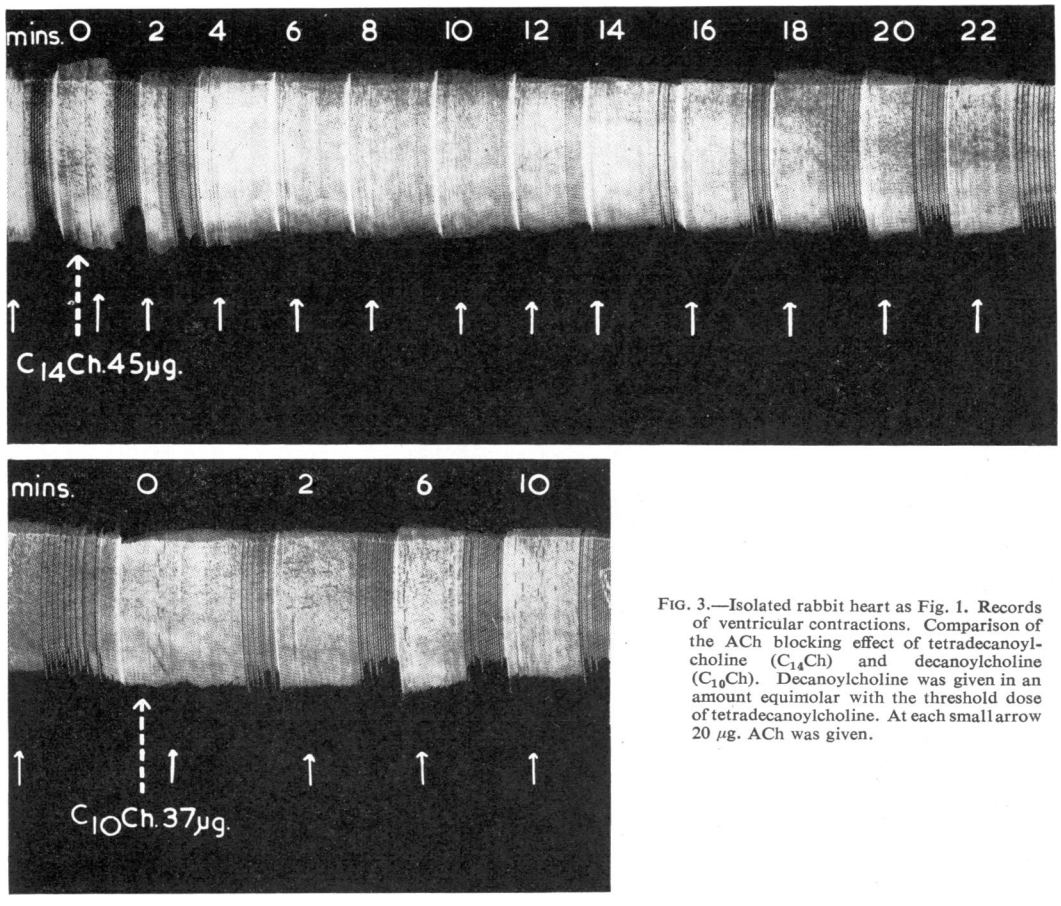

FIG. 3.—Isolated rabbit heart as Fig. 1. Records of ventricular contractions. Comparison of the ACh blocking effect of tetradecanoylcholine (C_{14}Ch) and decanoylcholine (C_{10}Ch). Decanoylcholine was given in an amount equimolar with the threshold dose of tetradecanoylcholine. At each small arrow 20 μg. ACh was given.

and pentanoyl esters was reduced by 70% and 85% respectively (Fig. 1).

The octanoyl and nonanoyl compounds seemed to be without effect on the isolated rabbit heart. When the chain length was increased further, however, a new phenomenon occurred which was first observed with the decanoyl compound (Fig. 2). This ester had a transitory blocking effect on the response to ACh when administered simultaneously. With a further increase in chain length the effect became more pronounced. Frequently it appeared after a short latent period and usually reached its maximum after 4 to 20 min. An assessment of the relative potency of these long-chain esters was made difficult by the persistence of their ACh blocking effect, which, in some instances, lasted for several hours. Occasionally the original response to ACh could no longer be obtained at all.

The effect of the esters was therefore compared by determining the threshold value for stearoylcholine and tetradecanoylcholine and comparing the effect of these compounds with that of the adjacent members used in corresponding amounts. Under such conditions stearoylcholine, palmitoylcholine, tetradecanoylcholine, and dodecanoylcholine produced the same effect. Decanoylcholine, however, was distinctly less potent in depressing the ACh response than any of the longer-chain esters (Fig. 3).

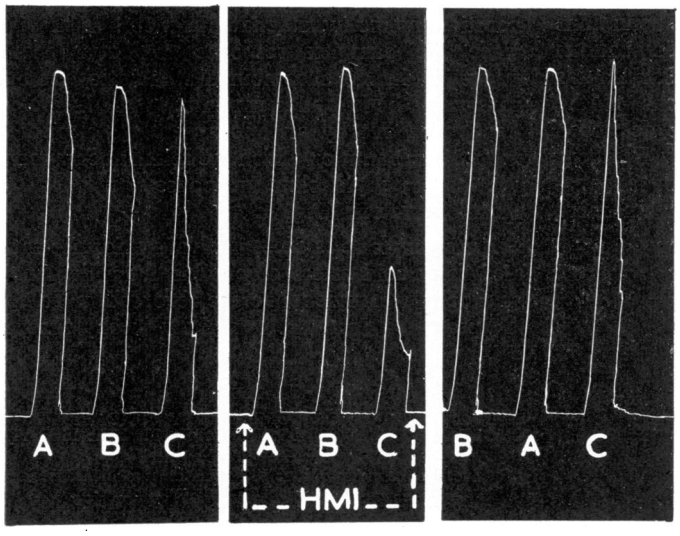

FIG. 4.—Isolated guinea-pig ileum. Hand-operated organ bath, 10 ml. capacity. Effect of hexamethonium (HMI) 3·0 μg./ml. on the response to 0·07 μg./ml. of ACh (A), 2·5 μg./ml. of propionylcholine (B) and 125 μg./ml. of butyrylcholine (C).

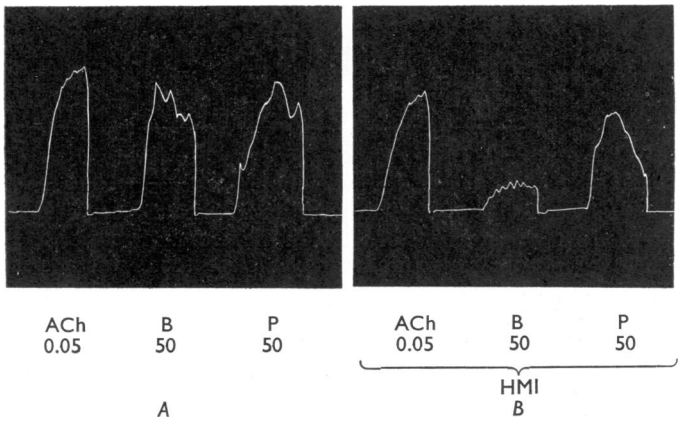

FIG. 5.—Isolated guinea-pig ileum. Automatic organ bath, 2 ml. capacity. Fast moving drum. Effect of hexamethonium iodide (3 μg./ml.) on the response to ACh, butyrylcholine (B) and pentanoylcholine (P). *A*, In the absence of hexamethonium iodide. *B*, In the presence of 3 μg. hexamethonium iodide per ml. Amounts expressed in μg./ml.

Effect on the Isolated Guinea-pig Ileum

Spasmogenic Activity.—The following molar concentrations determined in duplicate were equiactive with ACh $10^{-6\cdot5}$ M (0.05 μg./ml.): propionylcholine $10^{-4\cdot7}$, $10^{-4\cdot6}$; butyrylcholine $10^{-3\cdot6}$, $10^{-3\cdot6}$. A rapid fall in spasmogenic activity occurred with increasing chain length. The mode of action changed in the transition from propionyl to pentanoylcholine. After a 2 min. exposure to 3.0 μg./ml. of HMI, the responses to ACh and propionylcholine were unaffected (Fig. 4). The responses to both butyryl and pentanoylcholine, however, were considerably affected by the same concentration of HMI (Fig. 5).

The responses to pentanoylcholine were less affected by HMI than those to butyrylcholine (Fig. 5); in the same experiment the ACh responses were also partially inhibited by HMI. However, this did not always occur, and there was considerable variation in the effect of HMI on the responses to ACh with different samples of tissue. In some experiments, using a hand-

operated organ bath, the effect of HMI 3.0 μg./ml. on the response of the isolated guinea-pig ileum to ACh varied from 5 to 10% inhibition to 3 or 4% potentiation. Of the spasmogens tested with HMI in the present work, butyrylcholine appeared to be most sensitive (Figs. 4 and 5). Both this compound and pentanoylcholine, in distinction to ACh and propionylcholine, produced contractions of the guinea-pig ileum which were characterized by a rapid increase of tone, followed by a phase during which the tonus fluctuated and gradually fell (Fig. 5). HMI, besides diminishing the height of these contractions, also inhibited the fluctuations of tone; with pentanoylcholine the fluctuations were converted to a pure relaxation. Barium has been observed to produced contractions of the guinea-pig ileum which resemble those described above, and this substance was stated by Feldberg (1951) to promote contraction partly by a ganglionic type of action. In order to obtain more

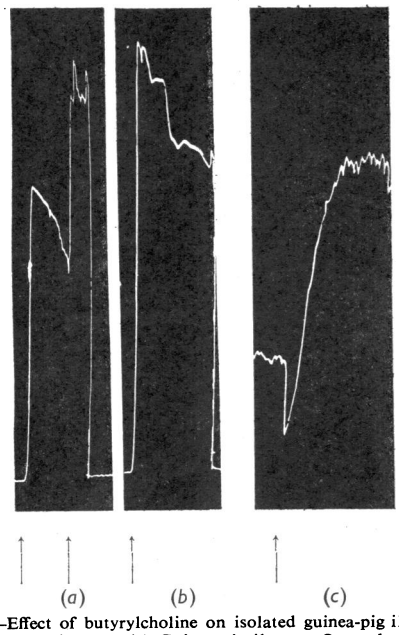

FIG. 6.—Effect of butyrylcholine on isolated guinea-pig ileum and rabbit duodenum. (*a*) Guinea-pig ileum. Organ bath 20 ml. capacity. At arrows, 500 μg. of butyrylcholine. (*b*) Guinea-pig ileum. Organ bath 20 ml. capacity. Effect of 10 μg. of neostigmine methylsulphate administered immediately before 250 μg. of butyrylcholine at arrow. (*c*) Rabbit duodenum. Organ bath 50 ml. capacity. At arrow, 250 μg. of butyrylcholine.

information regarding this complex response to butyrylcholine, further experiments were carried out with this substance.

Three possible explanations for the secondary relaxation came to mind. First, such relaxation might have been due to a non-specific depression of the muscle caused by the substance itself. Such a possibility, however, could be excluded by the finding that a subsequent administration of the same amount of butyrylcholine during the stage of relaxation still produced a spasmogenic effect (Fig. 6a). Secondly, relaxation might have been due to rapid hydrolysis of butyrylcholine by pseudocholinesterase, to the action of which it has been shown to be sensitive (Bayliss and Todrick, 1955). Such a possibility is excluded by the finding that secondary relaxation is not abolished by neostigmine methyl sulphate (Fig. 6b). In the third place the complex response to butyrylcholine might be due to a combined stimulation of both parasympathetic and sympathetic ganglia. This was borne out by an experiment on a piece of rabbit duodenum on which the relaxant component of the butyrylcholine response preceded the spasmogenic one (Fig. 6c).

Antagonism to ACh.—pA_2 values for the saturated fatty acid esters of choline from heptanoylcholine to stearoylcholine are recorded in Table I. A peak of anti-ACh activity was observed with tetradecanoylcholine. Heptanoylcholine and octanoylcholine were approximately equiactive as antagonists of ACh on the ileum. From octanoylcholine to dodecanoylcholine there was a steady rise in activity; the mean increase for each $-CH_2-$ increment in the carbon chain over this range of the series was 0.44 *p*A units. From tetradecanoylcholine to stearoylcholine the mean decrease in pA_2 for each $(-CH_2-)_2$ increment in the carbon chain was 0.32 *p*A units. Thus, the increase in activity up to tetradecanoylcholine took place at a greater "rate" than the decrease in activity above tetradecanoylcholine.

TABLE I

THE pA_2 VALUES OF SOME FATTY ACID ESTERS OF CHOLINE, MEASURED AGAINST ACh ON THE ISOLATED GUINEA-PIG ILEUM

All values are the means of four determinations: standard deviations in parentheses. All of the compounds were iodides, except the palmitoyl ester which was used as the bromide.

$$R—CO.O.CH_2.CH_2.\overset{+}{N}(CH_3)_3\ X^-$$

Compound	R	pA_2
Heptanoylcholine	$-C_6H_{13}$	4·74 (0·17)
Octanoylcholine	$-C_7H_{15}$	4·69 (0·01)
Nonanoylcholine	$-C_8H_{17}$	4·97 (0·11)
Decanoylcholine	$-C_9H_{19}$	5·52 (0·08)
Undecanoylcholine	$-C_{10}H_{21}$	5·99 (0·15)
Dodecanoylcholine	$-C_{11}H_{23}$	6·46 (0·06)
Tetradecanoylcholine	$-C_{13}H_{27}$	6·79 (0·20)
Palmitoylcholine	$-C_{15}H_{31}$	6·37 (0·13)
Stearoylcholine	$-C_{17}H_{35}$	6·16 (0·21)

Effect on Gastric HCl Secretion of Rats

Fig. 7 shows the effect of the choline esters of fatty acids with even numbers of carbon atoms on the spontaneous gastric secretion of the rat. The lower members of this series are readily soluble, and large doses (165 mg./kg.) were used. The first three members, butyryl-, hexanoyl-, and octanoylcholine, did not produce any demonstrable effect. The results were all within the range of the controls. Although the mean of the results obtained with butyrylcholine (0.53 m.equiv) was somewhat higher than that of the controls (0.456 m.equiv), this difference was not significant. The solubility of the choline esters decreases with increasing chain length, and the maximum concentration obtainable with palmitoylcholine was only 3.3 g./l. The amount administered was therefore reduced to 33 mg./kg., and was used even for the more soluble compounds for the sake of uniformity. All the higher members of this series, starting with dodecanoylcholine, produced a marked inhibition of HCl secretion, although with decanoylcholine only the larger dose was effective. The *P* values were calculated according to White's modification of Wilcoxon's ranking method (White, 1952). The *P* value for all esters up to palmitoylcholine was below 0.001 (Table II). Significant inhibition was still obtained with the stearoyl ester ($P=0.05$).

TABLE II

INHIBITORY EFFECT OF THE LONGER-CHAIN FATTY ACID-CHOLINE ESTERS ON THE 6 HR. SPONTANEOUS SECRETION OF FREE HCl BY THE RAT STOMACH

Ester	Dose (mg./kg.)	No. of Animals Used	Mean Values in m.equiv.	*P*
Control	—	12	0·465	
Decanoyl	165	6	0·0115	0·001
Dodecanoyl ..	33	5	0·034	0·001
Tetradecanoyl ..	33	6	0·132	0·001
Palmitoyl	33	8	0·085	0·001
Stearoyl	33	4	0·208	0·05

As the experiments on the heart and ileum had shown that the lower esters have a parasympathomimetic effect, it seemed possible that they might stimulate gastric secretion. When rats in which the spontaneous secretion had been reduced by partial vagotomy were injected with the shorter-chain esters, the results shown in Fig. 8 were obtained. Secretion was significantly raised with butyrylcholine, but remained unchanged when hexanoylcholine or octanoylcholine were used.

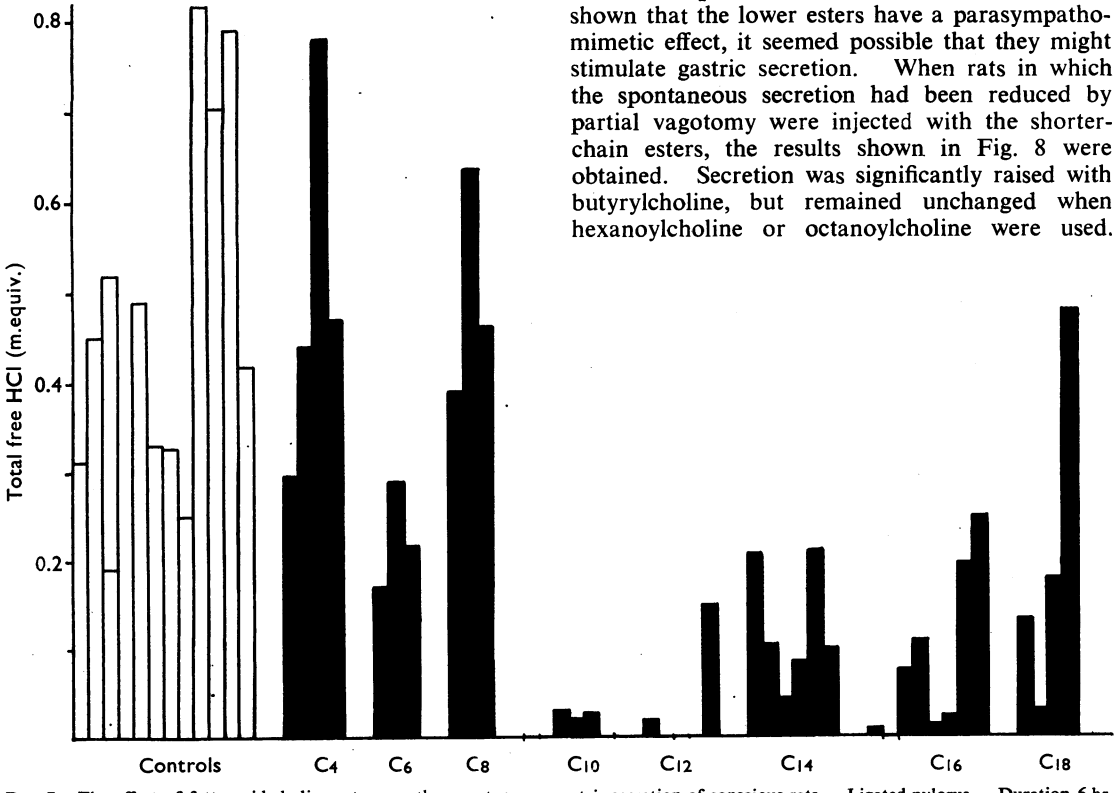

FIG. 7.—The effect of fatty acid-choline esters on the spontaneous gastric secretion of conscious rats. Ligated pylorus. Duration 6 hr. Each column represents the total free HCl secreted by one rat. Black columns represent the response after the intramuscular injection of the fatty acid-choline esters from butyrylcholine (C_4) to stearoylcholine (C_{18}). Blank columns represent the response after a corresponding injection of tap water. Compounds C_4 to C_{10} were given in doses of 165 mg./kg. and compounds C_{12} to C_{18} in doses of 33 mg./kg.

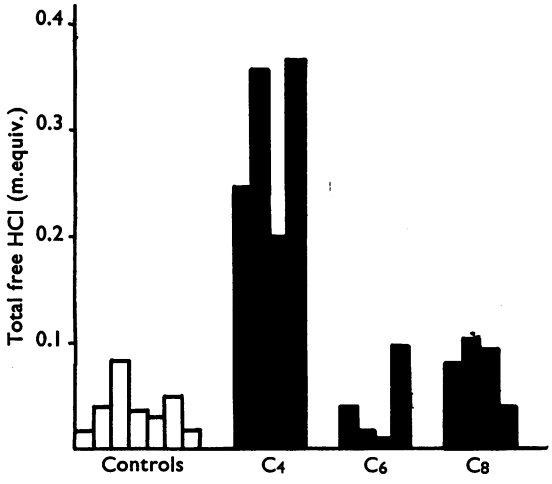

FIG. 8.—The effect of fatty acid-choline esters on the spontaneous gastric secretion of conscious rats. Ligated pylorus. Partial vagotomy. Distension with water. Duration 2 hr. Each column represents the total free HCl secreted by one rat. Black columns represent the response after the intramuscular injection of 165 mg./kg. of the fatty acid-choline esters from butyrylcholine (C_4) to octanoylcholine (C_8). Blank columns represent the response after a corresponding injection of tap water.

DISCUSSION

Three different types of effect of the fatty acid-choline esters could be demonstrated in the present investigation. With increasing chain length the muscarinic effect of ACh was replaced by a ganglionic effect and eventually by an ACh blocking action.

Muscarinic Effect.—The rapid decline in muscarinic action beyond ACh is well known and has been demonstrated as a waning depressor effect on blood pressure by Hunt and Taveau (1911), Chang and Gaddum (1933), and Bovet and Bovet-Nitti (1948), and as a decrease of the spasmogenic effect on isolated intestine by le Heux (1921) and by Chang and Gaddum (1933). In the present work on the isolated intestine and on the isolated heart, there was a steep decline in the depressant effect on rate and amplitude of contraction in the series of esters studied.

It has generally been assumed that the spasmogenic effect of ACh on the intestine, as well as the depressant effect on blood pressure and on the heart, is due to a direct action on parasympathetically innervated end-organs. In the case of the isolated heart, this view has recently been challenged by Perry and Talesnik (1953), who found that large doses (6 mg.) of hexamethonium abolished the depressant effect of ACh; they therefore concluded that ACh acted on intracardiac parasympathetic ganglia. Recent work by Douglas and Gray (1953) and Evans and Schild (1953), however, has shown that HMI has an effect on ganglion-free preparations, so that such a conclusion does not necessarily follow.

With further increase in chain length the muscarinic effect decreases still further. Hunt and Taveau (1911), Simonart (1932) and Bovet and Bovet-Nitti (1948) reported a slight depressor effect with butyrylcholine; Chang and Gaddum (1933) found "no real depressor effect" with this ester, but observed a spasmogenic effect on the isolated rabbit intestine. In the present work a slight depressant effect on the heart could be found occasionally with the esters from butyrylcholine to heptanoylcholine and a spasmogenic effect with butyrylcholine on the isolated guinea-pig ileum, though it is unlikely that these effects are due to a muscarinic mechanism.

Ganglionic Effect.—The pressor effect which is produced by ACh after atropine has been attributed to a stimulating effect on sympathetic ganglia leading to a release of sympathomimetic amines from adrenergic nerve endings and from the adrenal medulla. A similar response with some of the higher esters after atropine was found by Hunt and Taveau (1911), Chang and Gaddum (1933), and Simonart (1932). The last author claimed that this effect increased with increasing chain length from ACh to butyrylcholine. Bovet and Bovet-Nitti (1948) found a pressor effect without previous atropinization with the esters pentanoylcholine, hexanoylcholine and octanoylcholine.

The present experiments were carried out in the absence of atropine. On the isolated heart, the first appearance of an effect which was the reverse of that produced by ACh occurred with propionylcholine and consisted in an increase in rate and extent of contraction. With increasing chain length, this effect increased further, reaching its maximum with butyrylcholine, waned again with pentanoylcholine and still further with hexanoylcholine, and could no longer be detected with heptanoylcholine. As this response could be effectively blocked with HMI, stimulation of intracardiac sympathetic ganglia seemed the most likely explanation.

A stimulating action on the isolated rabbit or cat heart by ganglionic stimulants such as nicotine and ACh after atropine was described by Hoffmann, Hoffmann, Middleton and Talesnik (1945) and more recently on the isolated rabbit auricles by Kottegoda (1953) and Ginzel and Kottegoda (1953). The latter authors found that this effect could be blocked by HMI, and assumed

the presence of peripheral sympathetic ganglia within the auricular tissue.

On the isolated guinea-pig ileum, the first evidence suggestive of a ganglionic component of the response occurred with butyrylcholine. Although this compound, as well as pentanoylcholine, still produced a spasmogenic effect, the response differed in several aspects from that produced by ACh and propionylcholine. Whereas the response to the two latter esters was little influenced by HMI, this ganglionic blocking agent almost completely abolished the response to butyrylcholine, which suggested that the spasmogenic effect in this case was exerted on the intramural parasympathetic ganglia. The experimental evidence illustrated in Fig. 6 suggested that the stimulation of sympathetic intramural ganglia was responsible for the spasmolytic component of the response to butyrylcholine. The existence of such ganglia has been suggested by Ambache (1951), who demonstrated the reversed action of nicotine on the isolated mammalian intestine after cholinergic paralysis with *Clostridium botulinum* toxin. HMI, given in an amount that almost abolished the spasmogenic effect of butyrylcholine (3.0 μg./ml.), did not appear to affect this rapid relaxation, so that the sympathetic ganglia involved would appear more resistant to HMI than the parasympathetic ones. Only a small effect of this sort was produced on the response to pentanoylcholine, which seemed to point to a mainly non-ganglionic mechanism. The response to this ester, however, was erratic and in many instances no spasmogenic effect could be found.

ACh Blocking Effect.—The ACh blocking effect of these fatty acid-choline esters has not previously been described, though such an effect has been reported with other choline esters such as the benzilic acid ester by Ing, Dawes and Wajda (1945), dibutoline by Swan and White (1944), and benzoyl choline by Akcasu, Sinha and West (1952).

The blocking effect appeared in all three test preparations used. On the isolated heart and on the rat stomach the first member of the series to show the effect was decanoylcholine; heptanoylcholine had considerable activity on the isolated guinea-pig ileum. The only preparation on which accurate quantitative assessment could be carried out was the isolated guinea-pig ileum. There was a well-marked and progressive increase of anti-ACh activity from octanoylcholine to tetradecanoylcholine and a decrease from tetradecanoylcholine to stearoylcholine.

It seems possible that this phenomenon is associated with a gradation in physical properties, as the series is ascended. It will be seen that from nonanoylcholine to tetradecanoylcholine the differences in pA_2 values between adjacent homologues gradually fall (0.55 between nonanoylcholine and decanoylcholine, 0.45 between decanoylcholine and undecanoylcholine and between undecanoylcholine and dodecanoylcholine, 0.33 between dodecanoylcholine and tetradecanoylcholine). A similar phenomenon is also seen when the physical or physico-chemical properties of other homologous series are considered, such as the boiling points of the paraffins and the melting and boiling points of the monobasic carboxylic acids. No supplies of the tridecanoyl ester were available, hence the exact position of peak atropine-like activity could not be ascertained.

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APPENDIX

Preparation of the Choline Esters of Fatty Acids

By Zu-Yoong Kyi and W. Wilson

Several of the choline ester salts made during the present investigation were described by Fourneau and Page (1914), who failed to state the melting points of the iodides. Loury (1939) outlined a more convenient method of preparation for choline ester salts, but gave no details. In the present work, Loury's method was used, as follows:

$$R.CO.Cl + HO.CH_2.CH_2.N(CH_3)_2 \longrightarrow R.CO.O.CH_2.CH_2.N(CH_3)_2 \xrightarrow{CH_3I} R.CO.O.CH_2.CH_2.\overset{+}{N}(CH_3)_3\ I^-$$

The products so obtained are listed in Table III. The iodides of the higher members were only slightly soluble in water, and it was necessary to make the corresponding bromides. This was easily accomplished by using methyl bromide instead of methyl iodide in the above reaction scheme. Abderhalden, Paffrath, and Sickel (1925) quote m.p. 72° C. (for palmitoylcholine bromide, which is substantially lower than the m.p. 185° C. observed by us. However, previous workers have observed polymorphism in choline ester salts (Fourneau and Page, 1914; Loury, 1939). These salts decompose slightly above their melting points, and trimethylamine salts are formed:

$$R.CO.O.CH_2.CH_2.\overset{+}{N}(CH_3)_3\ I^- \longrightarrow R.CO.O.CH{:}\ CH_2 + N(CH_3)_3, HI$$

Experimental. — Iodides: 2-Dimethylaminoethanol (1 mole) was added slowly to the appropriate acid chloride (1.1 moles) (prepared by the method of Bauer, 1946), dissolved in dry benzene (5 to 10 moles), with cooling to 0° C. After 24 hr. water was added and the organic solvent layer removed. With the higher members, it was necessary to add alcohol as well as water to dissolve the solids. Aqueous NaOH was added to the aqueous layer, and the basic ester isolated by chloroform or ether extraction. The ester was dissolved in ethanol or *iso*propanol and refluxed with methyl iodide. For the first three members of the series, methyl iodide was added to the crude chloroform extract. The products are listed in Table III.

Bromides: The basic esters, prepared as already described, were boiled with a solution of methyl bromide (approximately 15%) in chloroform; the solvent was distilled off, and the residue recrystallized.

Thermal Decomposition of Choline Ester Salts. —Dodecanoylcholine iodide (5 g.) was heated at 200 to 230° C. (bath temperature) for 30 min. Extraction of the black mass with hot ethanol gave trimethylammonium iodide (1 g., 44%). The same product was obtained from propionyl and palmitoylcholine iodides.

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Table III

Choline Ester Iodides. $R.O.CH_2.CH_2.\overset{+}{N}(CH_3)_3\ I^-$

Name	Recrystallized from	Yield %	m.p. °C.	Formula	% Found				% Calculated			
					C	H	N	I	C	H	N	I
Propionyl ..	Ethanol	32	130·5–132	$C_8H_{18}O_2NI$	33·7	6·3		43·9	33·45	6·3		44·25
Butyryl ..	*iso*Propanol	77	93–94	$C_9H_{20}O_2NI$	36·1	6·6		42·3	35·9	6·6		42·2
Pentanoyl ..	Ethanol	69	107–108	$C_{10}H_{22}O_2NI$	38·5	6·9		40·45	38·1	7·0		40·3
Hexanoyl ..	,,	76	125–126	$C_{11}H_{24}O_2NI$	40·3	7·2		38·3	40·1	7·3		38·6
Heptanoyl ..	,,	40	137–138	$C_{12}H_{26}O_2NI$	42·3	7·4		36·9	42·0	7·6		37·0
Octanoyl ..	,,	59	146–147	$C_{13}H_{28}O_2NI$				35·6				35·6
Nonanoyl ..	,,	41	154·5–156	$C_{14}H_{30}O_2NI$	44·5	8·0		34·8	45·3	8·1		34·2
Decanoyl ..	Methanol	52·5	158–159	$C_{15}H_{32}O_2NI$	45·9	8·4		32·8	46·75	8·3		33·0
Undecanoyl ..	,,	66	164–165	$C_{16}H_{34}O_2NI$	48·2	8·4		31·9	48·1	8·5		31·8
Dodecanoyl ..	,,	77	164–166	$C_{17}H_{36}O_2NI$				30·6				30·75
Tetradecanoyl ..	,,	75	162–163	$C_{19}H_{40}O_2NI$	51·8	9·0			51·7	9·1		
Palmitoyl ..	,,	80	160–161	$C_{21}H_{44}O_2NI$			2·7	26·6			3·0	27·1
Palmitoyl *bromide*	Acetone-ethanol	86	185	$C_{21}H_{44}O_2NBr$								
Stearoyl ..	Methanol	77	161–162·5	$C_{23}H_{48}O_2NI$	55·3	9·4		25·0	55·5	9·7		25·55