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The Inhibition of Acetylcholine Synthesis in Brain by a Hemicholinium

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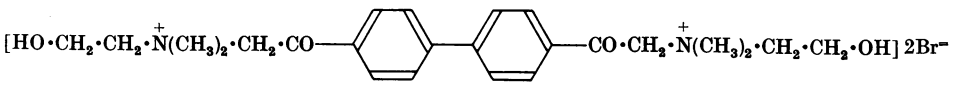
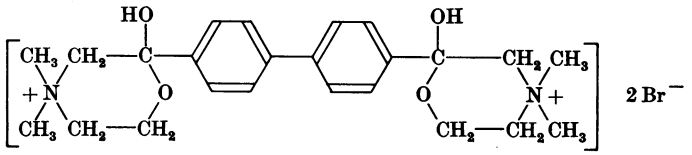
Long & Schueler (1954) described a series of bisquaternary ammonium compounds based on αα′-dibromo-4:4-bisacetophenone including one, obtained by treating the dibromo compound with dimethylaminoethanol, which was formulated as in Fig. 1*a*. As it was extremely toxic when injected into animals but possessed only weak anticholinesterase activity, and as the infrared spectrum showed strong bands due to ether groups, Schueler (1955) suggested the hemiacetal formulation (Fig. 1*b*) and described the compound as a 'hemicholinium'. The compound of Fig. 1 is usually referred to as HC3.

Long & Schueler found that, when injected into animals, HC3 paralysed the respiration; the paralysis developed after some delay and was reversed by injecting choline or anticholinesterases. MacIntosh, Birks & Sastry (1956) found that low concentrations of HC3 (0·01–0·1 mM) inhibited the synthesis of acetylcholine in the perfused superior cervical ganglion of the cat and in minced brain of the mouse; the inhibition was reversed by adding choline in similar concentrations. MacIntosh *et al.* (1956) therefore suggested that HC3 somehow inhibited the formation of acetylcholine. However, choline acetylase extracted from acetone-dried powder of brain was hardly inhibited at all by HC3, even when the concentration of HC3 was one thousand times that of choline. This made it unlikely that the inhibition of acetylcholine formation thought to occur *in vivo* was due to a direct effect of HC3 on choline acetylase.

The possibility remained, however, that the absence of inhibition *in vitro* was either because the enzyme was changed during its extraction or because HC3 competed with choline for access to the enzyme in the cell. These alternatives had to be examined because in the brain 75 % or more of the choline acetylase is associated with intracellular particulate fractions that are fully active only after



(*a*)



(*b*)

Fig. 1

they have been treated with an organic solvent such as ether. This suggests that a structural barrier has to be disrupted before the enzyme is fully active. HC3 may therefore act by competing with choline in its movement through such a barrier. This paper shows the effect that HC3 has upon the choline-acetylase activity of particles obtained from guinea-pig brain. When the choline concentration is low HC3 inhibits the enzyme as long as the particles are intact but not after they have been treated with ether. A preliminary account of this work has been given (Gardiner, 1957).

MATERIALS AND METHODS

Abbreviation. TEPP, Tetraethyl pyrophosphate.

HC3. This was synthesized as described by Long & Schueler (1954) (Found: Br, 27·0. Calc.: Br, 27·6%). The u.v.-absorption spectrum and the chemical properties were as described by Schueler (1955).

Pigeon-liver extract. This was prepared according to Kaplan & Lipmann (1948).

Acetyl-coenzyme A. This was prepared from coenzyme A (Pabst Laboratories) by acetylation with acetic anhydride (Stadtman, 1957).

Tetraethyl pyrophosphate. A commercial sample (L. Light and Co. Ltd.) was redistilled under reduced pressure with appropriate precautions (Toy, 1948). The fraction boiling at 96–98° under 0·05–0·1 mm. Hg was collected and kept in 0·5 ml. portions in sealed ampoules. After the ampoules were opened they were kept in a stoppered tube and discarded after a fortnight. Solutions of TEPP were made up in 0·01 M-potassium phosphate buffer, pH 7·4, immediately before use.

Acetylcholine. This was estimated pharmacologically on the isolated frog rectus abdominis with acetylcholine perchlorate (British Drug Houses Ltd.) as standard. The muscle was sensitized towards acetylcholine by treatment with TEPP (Hobbiger, 1950). The semi-automatic device described by Boura, Mongar & Schild (1954) was used; the samples were added manually at the appropriate times.

The solutions to be assayed contained substances other than acetylcholine which affected the frog muscle. Their effect was compensated for by adopting the method of Feldberg (1950). The comparison solution was freed from acetylcholine as follows: one drop of Universal Indicator (British Drug Houses Ltd.) was added to 2 ml. of the solution to be assayed, NaOH (0·33 N) was added dropwise until the colour changed to purple, and one more drop was added. The alkaline solution was heated in a stoppered test tube for 2 min. in boiling water, chilled in ice–water and neutralized with HCl (0·33 N). The volume was made up to 4 ml. with frog Ringer solution. This treatment was sufficient to destroy the acetylcholine; greater alkalinity changed the sucrose or HC3, or both, in the solution so that they affected the frog rectus.

With small quantities of acetylcholine, or for insensitive preparations of the muscle, an approximately fivefold increase in sensitivity could be obtained by adding a potentiating mixture. This contained M-potassium phosphate buffer, pH 7·4 (1 ml.), 0·2 M-choline chloride (0·5 ml.) and frog Ringer solution to 25 ml. Since this mixture by itself made the rectus contract the maximum amount that could be added was four-fifths of the dose that produced a just perceptible contraction. The same amount was added to all test solutions by means of a syringe arranged to deliver a pre-set volume.

Mouse- and guinea-pig-brain minces. The animals were killed by decapitation and the brains were removed and chilled. The cold tissue was placed in a glass tube closed at one end by a close-fitting plunger and at the other by two layers of fine nylon stocking. The mince was prepared by extruding the tissue slowly through the nylon mesh; as it emerged it was scraped off and transferred to a chilled weighed flask. The mince was suspended in a modified Locke's solution containing bicarbonate (Mann, Tennenbaum & Quastel, 1939), and samples, with the appropriate additions of HC3, were incubated in Warburg manometers. The incubated mixtures were prepared for the assay of acetylcholine as described by Mann *et al.*

Guinea-pig-brain suspension. The brains of two or more animals were removed as described and ground in an all-glass homogenizer in 9 vol. of ice-cold 9% (w/v) sucrose solution. The sucrose solution contained ethylenediaminetetra-acetic acid (1·0 mM) and was adjusted to pH 7·4 with NaOH. The tissue suspension was centrifuged at 1000*g* for 10 min. at 0–1° to remove intact cells and nuclei. The supernatant was a cell-free suspension which contained the choline acetylase. The suspension was used as such, or it was centrifuged at 15 000*g* for 10 min. to sediment the mitochondrial fraction (Gardiner, 1959). This was resuspended in sucrose solution. It contained less soluble choline acetylase, and also less choline.

Each enzyme preparation was divided into two parts. One part was stored in ice–water for no longer than 1 hr. before use. The other was treated with ½ vol. of peroxide-free ether and kept for 20–30 min. in ice–water. The ether was removed by blowing N_2 through the suspension just before use; frothing was prevented with silicone anti-foam (Midland Silicones; type A).

Choline-acetylase assays. The reaction was carried out in stoppered graduated test tubes, which had been selected to withstand centrifuging. The reaction mixtures were kept ice-cold until all components had been added; they were then incubated at 37° with gentle shaking. To stop the reaction, enough acid was added to make the pH 3–4, and the tubes were heated for 5 min. in boiling water. After cooling thoroughly in ice–water the mixture was neutralized with NaOH, made up to 5 ml. with frog Ringer solution and centrifuged. The supernatant, which contained the acetylcholine, was frozen and stored at −20° until assay.

RESULTS

Minced mouse and guinea-pig brain. MacIntosh *et al.* (1956) found that when HC3 was added to minced mouse brain it inhibited the formation of acetylcholine. This was confirmed; the inhibition was less than they had found, but so also was the synthetic activity. HC3 also inhibited the synthesis of acetylcholine in minced guinea-pig brain; Table 1 shows that HC3 (0·1 mM) reduced the synthesis by up to four-fifths in guinea-pig brain and by only two-thirds in mouse brain.

Cell-free homogenates of brain. The effect of HC3 was tested on the synthesis of acetylcholine

by cell-free homogenates of brain prepared as described in the Materials and Methods section. The work of MacIntosh *et al.* (1956) had suggested that HC3 would inhibit the synthesis of acetylcholine only if the concentration of choline was much below that usually used for determining the activity of the synthesizing enzymes. This was confirmed. Table 2 shows that HC3 inhibited the formation of acetylcholine only when the concentration of added choline was less than 25 μM.

Under these conditions the amount of acetylcholine synthesized was small and variable. Hebb (1955) suggested that this might be due to an inadequate supply of acetyl-coenzyme A. The brain suspension was therefore fortified with pigeon-liver extract and citrate. Table 3 shows that when this was done more acetylcholine was formed even in the absence of added choline, and in the presence of 0·1 mM-HC3 this synthesis was reduced by 40 %. When 25 μM-choline was added the synthesis was reduced by only 14 %.

Particulate choline acetylase of guinea-pig brain. To obtain information about the site at which HC3 inhibited acetylcholine formation it was necessary to simplify the system, for the following reasons: Crude homogenates of brain contain choline acetylase in at least two forms (Hebb & Smallman, 1956). About 25 % of the total activity is soluble, and there is evidence that soluble choline acetylase is not inhibited by HC3 (MacIntosh *et al.* 1956). As long therefore as soluble choline acetylase was present, its activity might have masked any inhibition by HC3 of insoluble choline acetylase. Further, it was desirable to reduce the concentration of endogenous choline.

For these reasons, the crude homogenates were used to prepare mitochondrial fractions, which Hebb & Smallman (1956) had shown to contain most of the choline-acetylating activity.

Hebb (1955) and Reisberg (1957) reported that choline acetylase was inhibited by excess of acetyl-coenzyme A and that the rate of acetylcholine synthesis was greater if the precursors of acetyl-coenzyme A were present rather than acetyl-coenzyme A itself. However, the mitochondrial fraction of brain synthesized moderate amounts of acetylcholine when incubated with choline and acetyl-coenzyme A itself. Fig. 2 shows that, in the presence of 10 mM-choline, as the concentration of acetyl-coenzyme A was increased so was the

Table 1. *Inhibition of acetylcholine synthesis in minced brain by HC3* (0·1 *mM*)

The flasks contained 150 mg. wet wt. of brain mince, 0·5 mg. of eserine sulphate, where indicated 0·32 μmole of HC3, and bicarbonate–Locke's solution to 3·2 ml. They were incubated, with gentle shaking, in an atmosphere of $O_2 + CO_2$ (95:5) for the time stated below.

Animal	Incubation (hr.)	Acetylcholine formed (μm-moles/g. wet wt.) Without HC3	With HC3	Inhibition by HC3 (%)
Mouse	3½	39	12	69
Guinea pig	4½	54	9	83
Guinea pig	1½	39	10	74
Guinea pig	1¼	41	8·7	79

Table 2. *Inhibition of synthesis of acetylcholine by HC3 in cell-free homogenates of guinea-pig brain*

Reaction mixture contained in 2 ml.: 1 ml. of brain suspension, 0·8 ml. of substrate mixture, choline chloride solution, HC3 solution, or water as appropriate. Substrate mixture contained in 8·5 ml.: 1 ml. of M-potassium phosphate buffer, pH 7·4, 1 μmole of coenzyme A, 0·1 m-mole of adenosine triphosphate, 0·2 m-mole of sodium acetate, 0·1 m-mole of L-cysteine hydrochloride, 0·4 m-mole of $MgCl_2$, 20 μmoles of TEPP. Incubation was for 1 hr. at 37°. Results are corrected for acetylcholine content of homogenate and synthesis from endogenous substrates.

No. of experiments	Concn. of HC3 (μM)	Concn. of added choline (μM)	Net acetylcholine formed (μm-moles/g. wet wt.)	Inhibition (%)
1	0	10 000	330	—
	50	10 000	330	0
2	0	50	7	—
	50	50	7	0
1	0	25	14	—
	50	25	12	14
1	0	None	12	—
	50	None	3	75

Table 3. *Inhibition of acetylcholine synthesis by HC3 in cell-free homogenate of guinea-pig brain with augmented acetyl-coenzyme A synthesis*

The reaction mixtures contained in 2 ml.: 1 ml. of brain suspension, 2 μmoles of TEPP, 0·7 ml. of substrate mixture, choline chloride solution, HC3 solution, or water as appropriate. The substrate mixture contained in 7·4 ml.: 2 ml. of 'aged' pigeon-liver extract, 0·5 ml. of M-potassium phosphate buffer, pH 7·4, 0·3 m-mole of citric acid, 0·4 m-mole of sodium acetate, 0·15 m-mole of L-cysteine hydrochloride, 0·1 m-mole of adenosine triphosphate, 1 μmole of coenzyme A, 5N-KOH to adjust pH to 7·4, water to volume. Incubation was for 1 hr. at 37°. Results are corrected for acetylcholine content of homogenate and synthesis from endogenous reactants.

No. of experiments	Concn. of HC3 (μM)	Concn. of added choline (μM)	Average synthesis of acetylcholine (μm-moles/g. wet wt.)	Activation (+) or inhibition (−) (%)
		Untreated homogenate		
5	0	25	34	—
	100	25	26	−14
5	0	None	25	—
	100	None	15	−40
		Ether-treated homogenate		
5	0	25	60	—
	100	25	73	+22
5	0	None	43	—
	100	None	59	+37

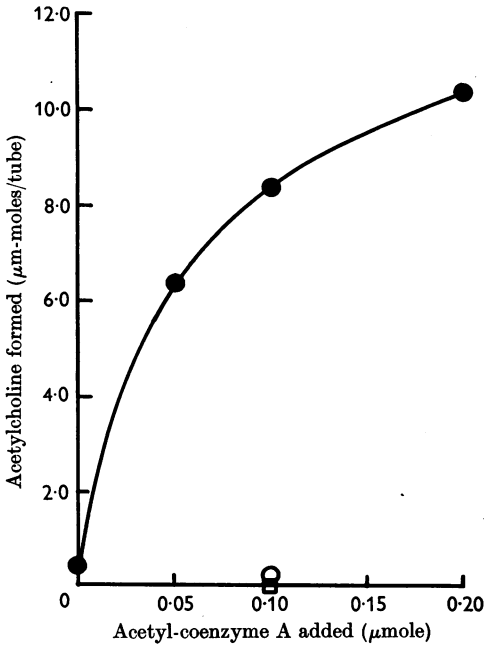


Fig. 2. Synthesis of acetylcholine from added acetyl-coenzyme A by mitochondrial fraction from guinea-pig brain. Reaction mixtures contained in 2 ml.: 1 ml. of mitochondrial suspension, 50 μmoles of NaH_2PO_4, 50 μmoles of $Na_4P_2O_7,10H_2O$, acetyl-coenzyme A as indicated, 0·02 m-mole (●) or 20 μm-moles (○) of choline chloride or none (□). Incubation was for 1¼ hr. at 37°.

amount of acetylcholine formed. Acetyl-coenzyme A (0·1 μmole) was used in subsequent experiments. When no choline was added no acetylcholine was formed. Thus the enzyme activity was limited by lack of choline rather than by excess of added acetyl-coenzyme A. When 20 μm-moles of choline was added some acetylcholine was formed, but even with so little choline the proportion of it that was acetylated was small.

The conditions so established were used for determining the effect of HC3 on the synthesis of acetylcholine by mitochondrial fractions of brain. Table 4 shows that when the concentration of added choline was 25 μM, HC3 at a concentration of 0·1 mM inhibited acetylcholine synthesis by nearly 50%.

Effect of varying the concentrations of choline and HC3 on the synthesis of acetylcholine by brain particles. In two experiments higher concentrations of both choline and HC3 were used; the results are in Table 5. When the concentration of HC3 only was increased the inhibition of acetylcholine synthesis was increased. However, if the choline concentration was also increased inhibition by HC3 was diminished. The effect of increasing the concentration of choline was not offset by increasing that of HC3.

Acetylcholine synthesis from endogenous reactants. When a homogenate of guinea-pig brain was incubated with an anticholinesterase but without added substrates a little acetylcholine was syn-

thesized. Table 6 shows that this synthesis was not affected by HC3. When the mitochondrial fraction was incubated in this way traces of acetylcholine were usually formed but again HC3 had no effect.

Cell-free suspension of guinea-pig brain after treatment with ether. Samples of the brain homogenates were treated with ether (see Methods) and the effect of HC3 on acetylcholine synthesis was determined. The results in Table 3 show that more acetylcholine was produced when homogenates had been treated with ether than when not, and the synthesis by ether-treated homogenates was not inhibited by HC3. On the contrary, in the presence of HC3 the formation of acetylcholine was usually increased. The average increase produced by 0·1 mM-HC3 was 37 % when no choline was added and 22 % when choline was added at a concentration of 25 μM.

Mitochondrial fraction after ether treatment. The effect of HC3 on mitochondrial fractions that had been treated with ether was similar to that on the homogenates. Table 4 shows that the choline acetylase was between two and four times as active and, again, HC3 (0·1 mM) produced not inhibition but activation. The effect was less than with the homogenates for the average activation was only 10 %.

Table 4. *Inhibition by HC3 of acetylcholine synthesis by mitochondrial fraction of guinea-pig brain with added acetyl-coenzyme A*

The reaction mixtures contained in 2 ml.: 0·1 μmole of acetyl-coenzyme A, 2 μmoles of TEPP, 50 μmoles of NaH_2PO_4, 50 μmoles of $Na_4P_2O_7,10H_2O$, 0·05 μmole of choline chloride, 1 ml. of suspension of mitochondrial fraction of brain and 0·2 μmole of HC3 if present. Incubation was for 1½ hr. at 37°. The results are corrected for acetylcholine content of particles and for any synthesized from endogenous choline.

Prep. no.	Acetylcholine formed (μm-moles/ml. of suspension) Without HC3	With HC3	Activation (+) or inhibition (−) (%)
	Untreated mitochondrial fraction		
1	3·3	2·5	−25
2	5·2	2·6	−50
3	3·3	1·9	−43
4	2·5	1·0	−60
5	5·3	2·5	−53
	Ether-treated mitochondrial fraction		
1	7·0	9·0	+28
2	16·0	16·0	Nil
3	7·0	7·1	+ 1
4	9·4	10·4	+11
5	16·3	18·2	+12

DISCUSSION

The results have shown that the synthesis of acetylcholine by intracellular particles obtained from brain was inhibited by HC3, but not if the particles were first treated with ether. Treatment with ether is thought to disrupt the membrane which encloses intracellular particles. The observations suggest, therefore, that HC3 inhibits not by a direct effect on choline acetylase but by an indirect effect which involves the integrity of the particles.

It was found, further, that the degree of inhibition depended, amongst other things, upon the concentration of choline in the medium: as the concentration of choline was reduced below about

Table 5. *Inhibition of acetylcholine synthesis by HC3: effect of various concentrations of choline and HC3*

The reaction mixtures contained in 2 ml.: 0·1 μmole of acetyl-coenzyme A, 2 μmoles of TEPP, 50 μmoles of NaH_2PO_4, 50 μmoles of $Na_4P_2O_7,10H_2O$, 1 ml. of suspension of mitochondrial fraction of brain. Other additions were as listed below. Incubation was for 1½ hr. at 37°.

Choline added (μmole)	0	0·05	0·1	0·2	0	0·05	0·1	0·2	0	0·05	0·1	0·2
HC3 added (μmole)	0				0·2				0·4			
Assay results (μm-moles of acetylcholine/ml. of suspension)	1·5	3·9	6·0	8·0	1·5	2·8	5·8	8·0	1·5	2·3	5·3	7·0
Results corrected for acetylcholine content of enzyme suspension	0·5	2·9	5·0	7·0	0·5	1·8	4·8	7·0	0·5	1·3	4·3	6·0
Results corrected for synthesis from endogenous choline	—	2·4	4·5	6·5	—	1·3	4·3	6·5	—	0·8	3·8	5·5
Inhibition (%)	—	—	—	—	—	48	5	0	—	68	12	15
Assay results (μm-moles of acetylcholine/ml. of suspension)	2·2	4·2	8·8	9·7	2·2	3·0	8·5	9·3	2·2	2·6	7·9	9·3
Results corrected for acetylcholine content of enzyme suspension	0	2·0	6·6	7·5	0	0·8	6·3	7·1	0	0·4	5·7	7·1
Results corrected for synthesis from endogenous choline	—	2·0	6·6	7·5	—	0·8	6·3	7·1	—	0·4	5·7	7·1
Inhibition (%)	—	—	—	—	—	60	5	5	—	80	14	5

Table 6. *Synthesis of acetylcholine from endogenous reactants in the presence and the absence of HC3 (0·1 mM)*

Mixtures contained in 2·2 ml.: 2 ml. of homogenate or suspension of mitochondrial fraction of guinea-pig brain, 2 μmoles of TEPP, and, if present, 0·2 μmole of HC3. Incubation was as in previous experiments.

Enzyme prep.	Acetylcholine formed (μm-mole/ml. of suspension)	
	Without HC3	With HC3
Cell-free homogenate	1·1	1·1
Mitochondrial fraction	0·35	0·35

50 μM the inhibition produced by a given concentration of HC3 increased. The results suggest the possibility that HC3 inhibits acetylcholine synthesis by competing with choline for access to the enzyme. Putting the observations together, it is a reasonable conclusion that HC3 is able to block the passage of choline across the membrane enclosing the particles.

Hebb & Smallman in their studies on the intracellular distribution of choline acetylase considered the possibility that the enzyme was enclosed within a barrier or membrane. More recent work (Hebb & Whittaker, 1958; Bellamy, 1959; Whittaker, 1959) supports this idea by showing that both choline acetylase and acetylcholine are associated with particles that have sedimentation characteristics similar to those of mitochondria but which may be distinguished from them. Whittaker (1959) has shown that organic solvents will damage these particles to such an extent that they no longer retain acetylcholine. By milder treatment only some of the acetylcholine is released but a barrier remains which prevents its return. It seems likely that this is the membrane through which the passage of choline is restricted and at which HC3 competes with it.

Such a barrier to the entry of choline would explain why acetylcholine is synthesized so slowly in intact particles. The existence of the barrier might also explain why HC3 has no effect on the very slight endogenous synthesis of acetylcholine, because this is presumably made from choline that has already penetrated into the particles and is free inside them. MacIntosh, Birks & Sastry (1958) have provided other evidence that HC3 affects a transport mechanism for choline. They have shown that in the chicken HC3 inhibits the excretion of choline by the kidney, a function that does not involve choline acetylase.

The present results do not support the possibility that HC3 acts by inhibiting choline acetylase directly, nor that there is no inhibition after treatment with ether because the enzyme itself is changed in some way. If this were so one would expect that the inhibition would increase as the structure of the tissue was broken down, since both choline and HC3 should have easier access to the enzyme; in fact, the inhibition decreased.

The results obtained with other preparations support the conclusion that the effectiveness of HC3 depends upon the structural integrity of tissues. Thus in the whole animal HC3 is extremely toxic. In intact nervous tissue, i.e. in the superior cervical ganglion of the cat, perfusing with low concentrations of HC3 (0·01–0·1 mM) inhibits the production of acetylcholine. In minced brain, in which the nerve cells are still largely intact, HC3 inhibits but less strongly. When the cells in turn are disrupted HC3 still inhibits the synthesis of acetylcholine that proceeds in intracellular particles. When finally these particles are broken down by ether, HC3 inhibits no longer; nor does it inhibit choline acetylase extracted from acetone-dried powders of brain, in which all structure has presumably been destroyed. It seems, then, that HC3 inhibits the activity of choline-acetylating systems by competing with choline for passage through membranes.

An unexpected observation was that the amount of acetylcholine formed by ether-treated preparations acting upon low concentration of choline increased when HC3 was present. This increase was not reported by MacIntosh *et al.* (1956), who used soluble choline acetylase. It seems, therefore, that activation by HC3, like inhibition, was related to the complexity of the enzyme preparation, in that activation was greatest with the homogenates and less with the particles; it is apparently absent with extracts of acetone-dried powder. Even the most active preparations acetylated only 30–40 % of the added choline. Therefore, one reason for the activation by HC3 may be that it inhibits other enzymes utilizing choline and that, because of this, more of the limited amount of choline present is available for acetylation.

In the whole animal HC3 is very toxic, and the problem remains whether the toxicity may be explained by the inhibition of acetylcholine synthesis. The experiments described in this paper offer little support for this idea in that the concentrations of HC3 needed to inhibit acetylcholine synthesis were much higher than the concentrations which are toxic *in vivo*. Thus if the LD_{50} for a guinea pig were uniformly distributed throughout the animal the concentration would be 0·1 μM, and, if throughout the extracellular fluid, 0·5 μM. Against that the concentration required *in vitro* to inhibit brain choline acetylase by 50 % was 0·1 mM. The discrepancy between the concentrations of HC3 required to inhibit choline acetylation *in vitro* and those toxic to the whole animal may be

explained by a readier access of choline to the enzyme *in vitro*.

It has always been difficult to accept that the toxicity of HC3 is due to effects on the central nervous system (Schueler, 1955; Kasé & Borison, 1958) because it is unlikely that a bisquaternary ammonium compound of this type would penetrate sufficiently. Recently, evidence has appeared suggesting that HC3 interrupts neuromuscular transmission in the respiratory musculature (Longo, 1959; Holmes & Wilson, 1960). This effect appears only when the rate of stimulation is high and sustained; this would deplete the acetylcholine in the nerve endings and would aggravate any limitation on its resynthesis. Moreover, the effect was rapidly and readily reversed by choline, which is in keeping with the nature of the inhibition found in the present work.

SUMMARY

1. The inhibition by HC3 of acetylcholine synthesis in minced mouse brain has been confirmed. The inhibition was observed also in minced guinea-pig brain.

2. The synthesis of acetylcholine by homogenates of guinea-pig brain or by particles obtained from them was inhibited by HC3 only if the concentration of choline was low.

3. When these preparations were treated with ether the increased synthesis of acetylcholine that occurred was activated by HC3. This effect was more marked with the cruder preparations.

4. It was concluded that HC3 does not inhibit choline acetylase directly but that it competes with choline for access to the enzyme through structural barriers in the cell.

5. The relation of these findings *in vitro* to the high toxicity of HC3 is discussed.

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Incorporation of [2-^{14}C]Mevalonic Acid and [2-^{14}C]Acetic Acid into Lipids of Mycobacteria

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The present investigation was started with a view to studying the biosynthesis of vitamin K_2, which is known to occur in mycobacteria (Snow, 1952; Brodie, Davies & Fieser, 1958). Vitamin K_2 isolated from *Mycobacterium tuberculosis* (Brevannes) has nine isoprene units in its side chain (Noll, 1958; Noll, Ruegg, Gloor, Ryser & Isler, 1960). Evidence has been presented by Brodie & Ballantine (1960) that vitamin K has an essential role in electron transport and oxidative phosphorylation in preparations from mycobacteria. It is known that animal tissues do not synthesize

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