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THE ACTION OF CHEMICAL VESICANTS ON CHOLINESTERASE

By R. H. S. THOMPSON

From the Department of Biochemistry, Oxford

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Towards the end of 1941 information began to accumulate suggesting that some of the systemic effects produced by certain chemical warfare vesicants had a resemblance to the muscarine-like actions of acetylcholine. In view of this, and on account of the physiological importance of acetylcholine, both in synaptic and neuro-muscular transmission as well as in its parasympathomimetic effects, it was decided to investigate the action of certain vesicant substances on cholinesterase.

The work described here has already been submitted to the Ministry of Supply in three reports (Thompson, 1942*a*, *b*, 1943).

At the outset of the work it was decided to investigate a 'tissue' cholinesterase rather than the esterase present in the serum, as it was thought that considerable differences in properties might exist between the enzyme present locally in the neighbourhood of nerve endings, where rapid inactivation of acetylcholine is essential, and the enzyme of the serum. Alles & Hawes (1940) and Glick (1941) had already reported on differences in the specificity and other properties of cholinesterase preparations from different sources, and the view that cholinesterase activity is exhibited by at least two types of enzyme (a specific cholinesterase and a non-specific pseudo-cholinesterase) has since been amply confirmed (Richter & Croft, 1942; Mendel & Mundell, 1943; Mendel, Mundell & Rudney, 1943; Mendel & Rudney, 1943).

For this reason the pigeon brain was used as the source of the enzyme in the initial experiments, rat skin and guinea-pig serum being used later. In this connexion it is of interest to note that Nachmansohn & Rothenberg (1944) have claimed that nervous tissue, unlike other tissues, contains only the specific cholinesterase.

METHODS

Vesicants

The following vesicants, kindly supplied by the Chief Superintendent, Experimental Station, Porton, have been studied:

- ββ′-Dichlorodiethyl-*N*-methylamine.
- Mustard gas (ββ′-dichlorodiethylsulphide).
- Trichlorotriethylamine.
- Carbomethoxy-β-chloroethylnitrosamine.
- Lewisite (β-chlorovinyldichlorarsine).
- Phenyldichlorarsine.
- Ethyldichlorarsine.

Enzyme preparations

(1) *Brain.* The enzyme was prepared from pigeon brain by a modification of the method of Nachmansohn (1939) by grinding with bicarbonate-Ringer's solution (containing calcium), one brain being finally dispersed in 30 ml. Ringer's solution. The dispersion was then filtered through muslin at room temperature, and 0·3 ml. of the filtrate used for each test.

(2) *Skin.* Rat skin, clipped free from fur, was removed from the animal immediately after killing by decapitation. The underlying fat and connective tissue were dissected away with a scalpel, and the skin sliced with a razor. 100–150 mg. of slices were used for each test.

(3) *Serum.* Blood was drawn by cardiac puncture from guinea-pigs, and the serum, separated by centrifuging, was diluted with bicarbonate buffer (pH 7·4) so that 1·0 ml. of the dilution contained 0·15 ml. serum. Activity was determined on 1·0 ml. aliquots of the dilutions.

Estimation of esterase activity

Esterase activity was determined by Ammon's (1933) adaptation of the Warburg technique, in which the acid produced by enzymic hydrolysis is measured by CO_2 evolution from bicarbonate buffer.

All measurements were carried out at pH 7·4 and 38° C., the gas space in the flasks being filled with 95% N_2 + 5% CO_2 immediately before placing the flasks in the bath.

In all cases, except where stated, 8 mg. of acetylcholine chloride (British Drug Houses) were tipped in from the side-bulbs after temperature equilibration to give a final concentration of 0·015 M. In most cases the vesicant, dissolved in bicarbonate buffer, was added to the main flask immediately after the addition of the enzyme, i.e. about 15–20 min. before the acetylcholine was tipped in.

In each experiment flasks were set up to control the rate of non-enzymic hydrolysis of acetylcholine, and in the earlier experiments to control also the rate of CO_2 evolution by the preparation in the absence of added acetylcholine. Both non-enzymic hydrolysis and acid production by the enzyme preparation alone were in all cases small.

The manometers were in most cases read at 10 min. intervals, measurements in all experiments being carried out in duplicate.

RESULTS

Effects on brain cholinesterase

The initial experiments of this series were carried out with $\beta\beta'$-dichlorodiethyl-*N*-methylamine. Both the free base and the hydrochloride were studied, the compound in each case being dissolved in bicarbonate buffer immediately before addition to the enzyme. It will be seen (Fig. 1) that a concentration of approximately 0·15 mM. of the hydrochloride (or slightly more of the free base) produces approximately 50% inhibition of enzymic activity.

In the experiments summarized in Fig. 1 the enzyme was in contact with the poison for 5 min. at room temperature (during which time the flasks were filled with gas), followed by 10 min. in the bath (for temperature equilibration) before the acetylcholine was tipped in. If, however, acetylcholine was also present from the time of commencement of poisoning the degree of inhibition was markedly reduced; in the experiments summarized in Table 1 acetylcholine and the inhibitor were in the main compartment of the flasks, the enzyme being tipped in from the side-bulbs after temperature equilibration.

By contrast with the results shown in Fig. 1, in which 0·17 mM. $\beta\beta'$-dichlorodiethyl-*N*-methylamine produced over 50% inhibition, it will be seen that this concentration produced only 10% inhibition if 3 mg. acetylcholine were present

when the poison was tipped in, while 30 mg. acetylcholine protected against concentrations as high as 1·34 mM. $\beta\beta'$-dichlorodiethyl-*N*-methylamine.

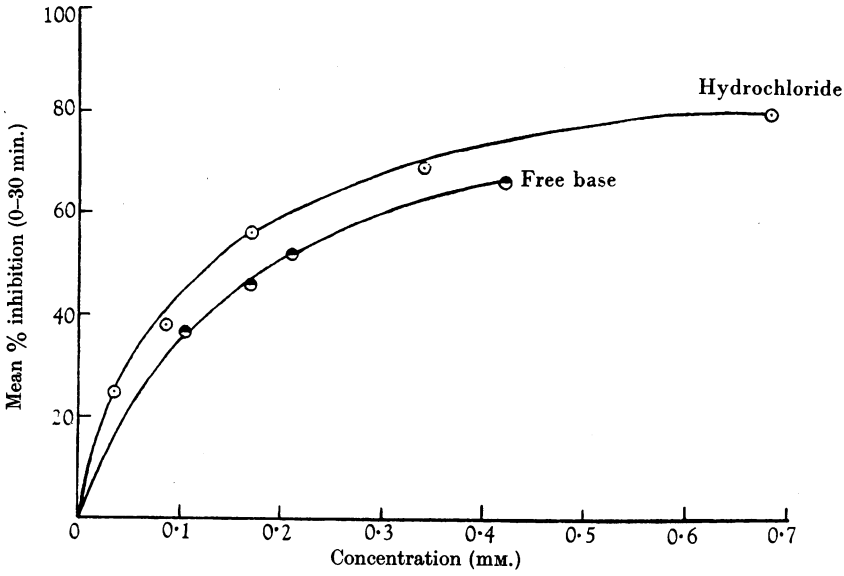

Fig. 1. Inhibition of brain cholinesterase by $\beta\beta'$-dichlorodiethyl-*N*-methylamine.

It has been shown by Matthes (1930) that the inhibitory effect of eserine on cholinesterase can be completely reversed by dialysis. Since, with the preparations used here, the enzyme is largely present in the insoluble, centrifugable fraction of the brain dispersion, it was decided to determine whether the enzyme,

TABLE 1. Effect of concentration of acetylcholine on the toxicity of $\beta\beta'$-dichlorodiethyl-*N*-methylamine to brain cholinesterase, the substrate being present from the commencement of poisoning

Concentration of inhibitor (mM.)	Mean % inhibition (0–30 min.) at the initial concentrations of substrate stated (mg. acetylcholine chloride/3 ml.)		
	3	15	30
0·17	10	—	—
0·68	24	—	—
1·34	34	4	1

after poisoning by the vesicant, could be reactivated by centrifugation and resuspension of the precipitate in fresh bicarbonate buffer. A brain preparation was therefore divided into two parts, one of which was centrifuged for 5 min., and the precipitate resuspended in the same volume of bicarbonate buffer. The percentage inhibitions produced by 0·17 mM. $\beta\beta'$-dichlorodiethyl-*N*-methylamine on the fresh and centrifuged fractions were determined and compared with the inhibition remaining in a fresh preparation poisoned with the same amount of the vesicant, but centrifuged after 15 min. and resuspended

in fresh bicarbonate buffer. Table 2 shows the result of this experiment, which suggests that under these conditions some degree of reversal of poisoning can be brought about by resuspending the poisoned enzyme in a fresh medium.

TABLE 2. Percentage inhibition produced by 0·17 mM. $\beta\beta'$-dichlorodiethyl-*N*-methylamine, (*a*) on a fresh preparation of brain cholinesterase; (*b*) on preparation (*a*) after centrifuging and resuspending in its original volume of $NaHCO_3$ buffer; (*c*) on preparation (*a*), centrifuged and resuspended in the same volume of fresh buffer after 15 min. poisoning

	Percentage inhibition			
	0–10 min.	10–20 min.	20–30 min.	Mean
(*a*) Fresh enzyme	59	53	59	57
(*b*) Centrifuged enzyme	66	66	71	68
(*c*) Fresh enzyme, poisoned and then centrifuged and resuspended	19	24	26	23

It has also been shown that the non-vesicant $\beta\beta'$-dihydroxydiethyl-*N*-methylamine is non-toxic to brain cholinesterase, a concentration of 0·56 mM. producing only 3% inhibition.

Goodman & Gilman (1942), who remarked on the close similarity between the effects of $\beta\beta'$-dichlorodiethyl-*N*-methylamine and the muscarine-like actions of acetylcholine, have also stated that trichlorotriethylamine frequently produces salivation, vomiting and defaecation in poisoned animals. Trichlorotriethylamine and two other non-arsenical vesicants, mustard gas and carbomethoxy-β-chloroethylnitrosamine were therefore also tested with respect to their action on brain cholinesterase.

Table 3 shows the percentage inhibitions produced by varying amounts of these compounds, together with results obtained with $\beta\beta'$-dichlorodiethyl-*N*-methylamine for comparison.

TABLE 3. Inhibition of brain cholinesterase produced by four non-arsenical vesicants. (Numbers in brackets denote number of experiments)

Vesicant	Mean % inhibition produced by the concentration (in μg./3 ml.) stated			
	50	100	200	500
$\beta\beta'$-Dichlorodiethyl-*N*-methylamine HCl	43 (3)	56 (6)	69	—
Trichlorotriethylamine HCl	22	55 (4)	75	—
Carbomethoxy-β-chloroethylnitrosamine	—	12	20	36 (4)
Mustard gas	—	—	—	28 (7)

From Table 3 it will be seen that trichlorotriethylamine is also an active inhibitor of this enzyme, a concentration of 0·15 mM. producing rather more than 50% inhibition. Carbomethoxy-β-chloroethylnitrosamine and mustard gas are less potent inhibitors, concentrations of approximately 1 mM. being required to produce inhibitions of 36 and 28% respectively.

It was of interest to determine next whether the arsenical vesicants also inhibited cholinesterase. Before commencing the work described above on $\beta\beta'$-dichlorodiethyl-*N*-methylamine, it had been shown in this laboratory that

sodium arsenite is highly toxic to brain cholinesterase. As early as 1939 Massart & Dufait had stated that horse-serum cholinesterase is inhibited by arsenite, and with a tissue cholinesterase (brain) I have found that concentrations of arsenite as low as 0·17 mM. produce approximately 50% inhibition.

The results obtained with sodium arsenite and the arsenoxides derived from phenyldichlorarsine, ethyldichlorarsine and lewisite are given in Table 4.

TABLE 4. Inhibition of brain cholinesterase by arsenicals.
(Numbers in brackets denote number of experiments)

Arsenical	Mean % inhibition produced by the concentration (in μg./3 ml.) stated			
	50	100	200	500
Sodium arsenite	52	69 (4)	—	—
Phenyldichlorarsine	19	30	40 (2)	57 (3)
Ethyldichlorarsine	—	—	—	35 (2)
Lewisite	—	2	2	19 (5)

The three organic arsenicals are each less toxic to this enzyme than sodium arsenite, lewisite in particular being a poor inhibitor at the concentrations studied; in each case, however, a significant inhibition is produced by concentrations of approximately 1 mM. (500 μg./3 ml.). Using still higher concentrations of lewisite (M./250) Mackworth (1942) obtained 30% inhibition of the serum enzyme.

Cholinesterase activity of normal and contaminated skin

The presence of a specific cholinesterase in skin has already been described (Thompson & Whittaker, 1944), and the effect on this enzyme of contamination of the skin with vesicants was investigated as follows:

Young, white rats, weighing from 30 to 70 g., were used. Two areas of skin, each approximately 10–15 sq. cm. on each side of the animal, were closely clipped free from fur with scissors, the animal being kept lightly anaesthetized with ether. After the effects of the anaesthetic had passed off, one of the clipped areas was contaminated with the vesicant, which was dropped on to the skin from a pipette and spread over the area with a glass rod. The rat was then returned to its cage and kept under observation for 1 hr., at the end of which time it was killed by decapitation. Both areas of clipped skin, contaminated and uncontaminated, were then removed, and their cholinesterase activities compared. By this means the enzymic activity of the contaminated skin was always compared with that of normal skin from the same animal, thus obviating any differences in cholinesterase content that might exist between the skins of different animals.

Three non-arsenical vesicants, mustard gas, ββ′-dichlorodiethyl-*N*-methylamine and carbomethoxy-β-chloroethylnitrosamine, were investigated along these lines. In each case, except the first two experiments with mustard gas, contamination was carried out with the undiluted vesicant. A series of

determinations of the percentage inhibition of cholinesterase activity in the skin of different animals contaminated with these vesicants is given in Table 5. It will be seen that contamination of the skin with carbomethoxy-β-chloroethylnitrosamine produces an inhibition of enzymic activity in the affected skin amounting to 79–92%. Contamination with mustard gas or ββ′-dichlorodiethyl-*N*-methylamine yields more variable results, in the case of mustard gas inhibitions up to 50% (mean value = 33%) being obtained, and in the case of ββ′-dichlorodiethyl-*N*-methylamine up to 40% (mean value = 29%).

TABLE 5. Percentage inhibition of cholinesterase activity in rat skin contaminated with non-arsenical vesicants

(Contamination = 1 hr. with undiluted vesicant except where otherwise stated.)

Carbomethoxy-β-chloroethylnitrosamine	Mustard gas	ββ′-Dichlorodiethyl-*N*-methylamine
80	22*	39
84	37*	12
79	50	39
92	21	27
	27	28
	47	
	30	
Mean = 84	33	29

* 2 hr. contamination with 50% mustard gas in ethanol; control skin painted with ethanol only.

In all cases marked erythema of the contaminated skin was observed at the time of killing, and on removing the skin considerable oedema, often partly gelatinous in nature, was present in the subcutaneous tissues. The rats contaminated with mustard gas or ββ′-dichlorodiethyl-*N*-methylamine invariably showed profuse salivation and lachrymation for some time before killing. In three of the experiments the tears were seen to be stained red; a small quantity of the tears was therefore collected and examined in ultra-violet light, when they were seen to show a pronounced red fluorescence; the possible significance of this finding is discussed later. In addition to these signs the rats contaminated with ββ′-dichlorodiethyl-*N*-methylamine were considerably collapsed at the time of killing.

In the experiments with carbomethoxy-β-chloroethylnitrosamine, on the other hand, excessive salivation and lachrymation were not present, and the rats were in good condition at the end of the hour; locally, however, erythema and considerable oedema were invariably present.

Owing to the rapid onset of collapse and death after contamination of a large area of skin with one of the arsenical vesicants, it was not possible by this method to investigate the effects of similar contaminations with any of these compounds. One experiment, however, was carried out with sodium arsenite in order to determine whether the cholinesterase in skin is poisoned in vitro by a trivalent arsenical; this was found to be the case, the addition of 0·17 mM. sodium arsenite to normal rat skin slices resulting in 36% inhibition of activity.

In vivo effects on level of serum cholinesterase

In view of the rapid onset of signs of systemic intoxication in animals heavily contaminated with mustard gas, a comparison was made of the serum cholinesterase level in normal animals and in animals after heavy contamination of the skin with this vesicant.

Owing to the relatively low cholinesterase content of rat blood (Stedman, Stedman & White, 1933), guinea-pigs have been used throughout.

Blood was drawn by cardiac puncture from the living animal, and esterase activity determined on a dilution of the serum obtained as already described.

In each experiment the activity of normal serum, freshly obtained, was compared with that of serum from the contaminated animal, a total of twelve normal and twelve experimental animals being examined. The experimental animals were contaminated on the clipped skin of the back with 100 mg. mustard gas/kg. body wt., the serum being drawn 18–50 hr. after contamination, i.e. at a time when all the animals were showing signs of systemic poisoning (excessive salivation).

The results, summarized in Table 6, show that there is a significant fall in the cholinesterase level of the serum of guinea-pigs following contamination with mustard gas. No evidence was obtained suggesting that the fall was any greater after 50 hr. than after 18 hr.

TABLE 6. Serum cholinesterase level in normal guinea-pigs and in guinea-pigs contaminated with mustard gas

(Contamination = 100 mg. mustard gas/kg. body wt. Serum tested 18–50 hr. later.)

Enzymic activity (μl. CO_2/ml. serum/min.)	
Normal	Contaminated
28·2	16·5
31·2	20·2
26·5	10·3
39·6	17·8
21·6	19·7
23·1	17·7
16·1	14·8
23·2	15·5
24·1	15·8
29·7	13·9
31·0	13·4
31·0	13·7
Mean = 27·1 ± 1·75	15·8 ± 0·81

Difference of means = 11·3 ± 1·93

$t = 5{\cdot}85$ ($n = 22$); $P = 0{\cdot}001$.

Evidence of muscarine-like effects produced by vesicants

Chromodacryorrhoea

Before considering the wider experimental evidence pointing to the development of muscarine-like effects in animals poisoned with various vesicants, the

significance of the red tears secreted by contaminated rats, mentioned earlier, will be discussed.

It has been repeatedly noticed in this laboratory that white rats poisoned with mustard gas, given either by injection or by application to the skin, frequently develop in the course of the next 4 or 5 days a characteristic condition of the eyes, consisting of an accumulation of dried reddish brown matter around the palpebral margins. In the course of the experiments already described in this paper, it was noticed that three rats that had received heavy contamination of the skin, one with mustard gas and two with $\beta\beta'$-dichlorodiethyl-*N*-methylamine, were freely secreting deep red tears 1 hr. after contamination; as already mentioned these tears showed a pronounced red fluorescence in ultra-violet light.

It seemed therefore as if these contaminated animals were showing the condition of chromodacryorrhoea recently described by Tashiro, Smith, Badger & Kezur (1940). Freud (1933), quoted by Selye (1937), observed that injected acetylcholine causes white rats to shed tears which he described as being tinged with blood. Tashiro, Smith, Badger & Kezur (1940) have extended these observations, and have described a flow of red tears commencing 2–5 min. after intraperitoneal injection of acetylcholine, usually following salivation and the secretion of clear tears.

In a more recent paper Tashiro, Badger & Younker (1940) have shown that Freud's assumption that the red colour of the tears is due to haemoglobin is incorrect. The pigment possesses absorption bands close to but not identical with those of oxyhaemoglobin; moreover, the lachrymal pigment shows a strong red fluorescence. They have suggested that the pigment may be identical with, or related to, the porphyrin in the Harderian glands of rats described by Derrien & Turchini (1924).

It was decided therefore to compare the ocular condition developing after contamination of rats with mustard gas or $\beta\beta'$-dichlorodiethyl-*N*-methylamine with the chromodacryorrhoea produced by acetylcholine.

In confirmation of the earlier work it was found that red tears may be regularly and rapidly produced in white rats by the injection of acetylcholine. Acetylcholine chloride, 5–6 mg./kg. body wt. injected subcutaneously, produced a copious flow of dark red, opaque tears commencing 3–4 min. after the injection; the flow was maintained for a period of 5–15 min.

Two rats were next given 5 mg. eserine sulphate/kg. body wt. subcutaneously; profuse lachrymation and salivation, accompanied by twitching movements, developed and were followed, half an hour after the injection, by a copious excretion of the typical red opaque tears.

Two rats received, by subcutaneous injection, 47 and 55 mg. mustard gas (dissolved in cellosolve)/kg. body wt. respectively. On the following day the rat that received the larger dose began to secrete red tears, and died late that

afternoon. On the second morning after the injection the other rat was found to be secreting red tears profusely; a large amount of dried dark red matter was attached to the lid margins, while the inner canthus of each eye was filled with red fluid. The secretion of red tears was continued throughout the 2nd and 3rd days, and on the morning of the 4th day the animal was found dead.

50 mg. $\beta\beta'$-dichlorodiethyl-*N*-methylamine were next injected into two rats. A flow of red tears occurred in both animals, commencing about 50 min. after the injection and about 10–15 min. before death.

Both mustard gas and $\beta\beta'$-dichlorodiethyl-*N*-methylamine can therefore give rise to a secretion of red tears in white rats, comparable to that produced by injected acetylcholine or eserine.

Other evidence

As pointed out earlier, considerable experimental evidence exists in both English and American literature pointing to the slow development of muscarine-like effects in animals after contamination with various vesicants.

The onset of lachrymation, salivation and diarrhoea after application of $\beta\beta'$-dichlorodiethyl-*N*-methylamine has been described by Cameron & Short (1942*a*), while in America Goodman & Gilman (1942) have described the fall in blood pressure, salivation, meiosis and increased gastro-intestinal activity that occurs after poisoning with this compound.

The case for the development of muscarine-like effects after administration of mustard gas does not seem to have been stressed. Apart from the haemo-concentration, however (Cameron, 1941), Cameron & Short (1942*b*), in confirmation and extension of work by Florey (1940), have described the acute and often haemorrhagic gastritis, associated with haemorrhages in the small and large intestine after application of mustard gas to the skin. Courtice & Cameron (1942) investigated the blood changes, and concluded that 'a picture of so-called shock is produced as a result of the loss of plasma, probably mainly local at the site of application of the mustard gas, and secondly another train of functional changes develops and continues to progress long after the initial shock has subsided. The fundamental lesion in this latter disturbance appears to be a gastro-enteritis which causes diarrhoea and an increased salivation.'

DISCUSSION

The ability to inhibit brain cholinesterase has been shown to be, in varying degrees, a common property of all the vesicants studied here. Three of them, when applied to the skin of living animals, have also been found to reduce the cholinesterase activity of this tissue.

In this respect the enzyme in horse serum is in striking contrast to that in brain; using the serum enzyme Mackworth (1942) obtained only 5% inhibition of activity with 0·33 mM. $\beta\beta'$-dichlorodiethyl-*N*-methylamine. In vivo, however, in animals contaminated with lethal amounts of mustard gas, a

lowering of the level of serum cholinesterase has been found, although no attempt has been made to discover if this is a direct effect of the absorbed mustard gas or products of mustard gas, or whether it is secondary to other changes brought about by the vesicant.

The muscarine-like actions of certain of the nitrogenous vesicants have already been commented on, and evidence has also been brought forward showing that, like acetylcholine or eserine, mustard gas and $\beta\beta'$-dichlorodiethyl-*N*-methylamine can bring about the secretion of red tears when injected into white rats.

In view of these findings it is suggested that some of the systemic effects produced by certain of the vesicants may be due, in part at any rate, to an inhibition of the cholinesterase at cholinergic nerve-endings. A failure to hydrolyse liberated acetylcholine could well account for certain of the changes observed. In this connexion it is also of interest to compare the leucopenia observed by Cameron (1941) in animals poisoned with mustard gas with that observed by Dale & Laidlaw (1919) after the slow infusion of acetylcholine; in both cases the polymorphonuclear cells were more affected than the lymphocytes.

It is clear from what is now known about the effects of these vesicants that this is only one of several biochemical actions exerted by them at these concentrations. Moreover, by comparison with eserine they are relatively weak inhibitors of cholinesterase in vitro. It is not suggested therefore that any effect that they may exert on the cholinesterase in skin is playing a central role in the pathogenesis of vesication, but since all the vesicants studied produce systemic effects which could in part be explained by cholinergic activation it is of interest, despite the wide range of effects produced by them on cholinesterase in vitro, to review the known facts concerning the local effects of an accumulation of acetylcholine in the skin, such as might result from inhibition of the cholinesterase.

In a study of urticaria of nervous origin Grant, Bruce Pearson & Comeau (1936) concluded that the condition was brought about by the release of acetylcholine in the skin as a result of stimulation of cholinergic nerve fibres. This conclusion was based partly on the results observed after the electrophoretic introduction into the skin of various choline derivatives and pharmacologically related substances. It was found that the introduction of carbaminoylcholine into the skin of these patients regularly produced an urticarial attack. Acetylcholine was less certain in its action unless eserine was also present; whealing, which could be abolished by atropine, also occurred in response to warming the legs.

According to these authors acetylcholine with eserine, or carbaminoylcholine, introduced electrophoretically into the skin of normal subjects produces only a local reddening, lasting 10–15 min., without flare or wheal.

Alexander, Elliott & Kirchner (1940), on the other hand, claim that eserine is a powerful urticariogenic substance, although few experimental details are given. In view of these divergent results a few rough experiments were carried out in this department with acetylcholine, carbaminoylcholine and eserine. Using a 2 mA. current from a 45 V. battery for either 2 or 3 min. it was found that 1:100 carbaminoylcholine or eserine sulphate produces whealing of the skin; the response was more marked with eserine, wheals commencing to appear 2 min. after removing the electrode, rapidly becoming confluent over the entire area covered by the electrode, and persisting for $1\frac{1}{2}$–2 hr. Eserine (1:1000) was also found to produce wheals; no higher dilution was tested. Acetylcholine (1:100) caused intense erythema, lasting for about 1 hr., and pilo-erection, but no wheals. Although these lesions are obviously fundamentally different from the necrosing blisters produced by chemical vesicants, it is regarded as significant that a recognized inhibitor of cholinesterase (eserine) can cause erythema and whealing of skin when introduced into it.

SUMMARY

1. It has been shown that a number of chemical vesicants, both arsenical and non-arsenical, are inhibitors of brain cholinesterase.

2. When applied to the skin of rats, mustard gas and two other nitrogen-containing vesicants have also been shown to produce an inhibition of the cholinesterase activity of the contaminated skin.

3. There is a significant reduction in the cholinesterase content of the serum of guinea-pigs after heavy contamination with mustard gas.

4. Both mustard gas and $\beta\beta'$-dichlorodiethyl-*N*-methylamine, when injected into white rats, can give rise to the secretion of red tears, comparable to those produced by injected acetylcholine or eserine.

5. The possible significance of these findings in connexion with the systemic and local effects produced by contamination of the skin with chemical vesicants is discussed.

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