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SYNTHESIS OF ACETYLCHOLINE IN SYMPATHETIC GANGLIA AND CHOLINERGIC NERVES

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The evidence for the theory of chemical transmission by acetylcholine across ganglionic synapses points to the preganglionic nerve endings as the seat where the acetylcholine is liberated. When released it is quickly replaced by synthesis so that the normal store of acetylcholine does not diminish even after prolonged preganglionic nerve stimulation [Brown & Feldberg, 1936*b*; Kahlson & MacIntosh, 1939]. Thus it appeared likely, as suggested by Brown & Feldberg, that synthesis of acetylcholine is not a property of the cells in the sympathetic ganglia but of the preganglionic endings and, in that case, it may not be confined to the endings but may occur along the whole length of a cholinergic nerve. The experiments described in this paper were carried out to test this conception.

Synthesis of acetylcholine has been described in the central nervous system by Quastel, Tennenbaum & Wheatley [1936] and Mann, Tennenbaum & Quastel [1938, 1939]. Although some of their interpretation is no longer valid [Trethewie 1938; Stedman & Stedman, 1939], they were the first to show that tissue slices, or suspensions of minced brain, synthesize acetylcholine when respiring. Their results have been confirmed by Trethewie [1938]. About the same time Stedman & Stedman [1937, 1939] found that minced or ground brain, incubated in a medium of chloroform or ether, synthesizes acetylcholine. Synthesis in sympathetic ganglia has been observed in the perfused superior cervical ganglion of the cat during prolonged stimulation of the cervical sympathetic [Brown & Feldberg, 1936*b*; Kahlson & MacIntosh, 1939]. No direct experiments have been carried out to demonstrate synthesis in cholinergic nerves, but there are some observations pointing in this direction. Von Muralt [1937] found that a nerve 'shot into liquid air' during stimulation gave an increased yield of acetylcholine on extraction, and Rosenblueth, Lissák & Lanari [1939] observed a similar increase in nerves extracted a few minutes after prolonged stimulation.

Methods

We used a modification of the method described by Mann *et al.* [1938] for brain tissue, and studied synthesis of acetylcholine in finely divided tissue from normal sympathetic ganglia, from sympathetic ganglia at different stages of preganglionic nerve degeneration, and from the normal and degenerating cholinergic nerves of sheep and cats.

The sheep were bled under spinal duracain or intravenous nembutal anaesthesia and the ganglia and nerves removed immediately after death. The cats were anaesthetized with ether, followed by intravenous injection of chloralose, the ganglia and nerves being removed from the living animal or immediately after death. In some cats they were removed aseptically under ether anaesthesia. The ganglia of one side were used as controls. In order to obtain comparable samples of nerve tissue, each nerve was cut into pieces about 1 cm. in length, which were used alternatively along the nerve for the two samples. The ganglia and nerves were dried between filter paper, weighed and extracted or incubated.

For the determination of the normal acetylcholine content of the tissue the following procedure of acid extraction with boiling was adopted. Ganglia and nerve samples weighing less than 50 mg. were brought into a solution made up of 0·5 c.c. *N*/3 HCl, 1 c.c. bicarbonate-free Locke solution, 0·3 c.c. phosphate buffer solution and eserine, to give a concentration between 1 in 2000 and 1 in 10,000. With greater amounts of tissue double the volume of the solution was used. The tissue was either divided with scissors or ground with silica in this acid solution, and then 0·3 c.c. of distilled water were added for each c.c. to make it isotonic for frog's tissue. The mixture was boiled for 1–2 min. and kept in the refrigerator until assayed for acetylcholine on the rectus abdominis muscle of the frog. Before the assay the solution was neutralized in the cold with 0·5 or 1 c.c. respectively of *N*/3 NaOH and made up with the solution used for the frog's rectus test, so that each c.c. corresponded to a given amount (2–10 mg.) of tissue. The use of *N*/3 HCl has the advantage of giving a physiological NaCl solution when neutralized with *N*/3 NaOH. When, in control experiments, acetylcholine was added to Locke solution and treated in the same manner no measurable loss occurred.

For the incubation, the tissue was divided with scissors or ground in a solution similar to that used for extraction, but containing no HCl or distilled water. The *p*H of this buffered solution was 7·3. It was incubated in a water bath at 37–39° C. with oxygen bubbling through it. After the incubation 0·3 c.c. of distilled water per c.c. solution and 0·5 or 1 c.c. *N*/3 HCl, respectively, were added to the mixture, which was boiled for 1 or 2 min. and then treated in the same way as the control sample. In some experiments both samples were cut or ground in a solution containing no eserine and no HCl; one sample

was then extracted in the usual manner by adding HCl, H_2O and boiling, and the other sample was incubated after the addition of eserine. The acetylcholine yield, from most of the tissues examined, was so high that a solution equivalent to only 0·5–5 mg. tissue per c.c., was necessary for the test on the rectus muscle of the frog. In this case, the amounts of potassium or choline present were too small to interfere with the assay. When the acetylcholine yield was less than 2μg./g. the estimation was usually carried out on the arterial blood pressure of the cat in chloralose anaesthesia.

The addition of tissue extract to the frog's rectus greatly sensitizes the muscle to a subsequent dose of acetylcholine. The sensitization wears off after several minutes and with repeated doses of acetylcholine. The following precautions were taken in order to avoid errors from this source. The first addition of extract was used as a sensitizing dose for the muscle, and for obtaining a crude estimation of the concentration of acetylcholine present in it. The extracts were then tested in alternating rotation with an acetylcholine solution, the strength of which (1 in 20 millions to 1 in 50 millions) was kept constant. The intervals between two contractions were kept between 4 and 7 min. A concentration of extract was used, the effect of which was definitely smaller (or greater) than that of the acetylcholine solution tested before or after it. The concentration of the extract was then increased (or decreased) with each test by 10–20%, until the effect was definitely greater (or smaller) than that of the acetylcholine solution. By keeping this solution constant throughout an assay any change in sensitization of the muscle is noticed, and does not interfere with the quantitative estimation. The acetylcholine yield of the tissue is expressed in μg. acetylcholine chloride/g. fresh tissue.

Results

Synthesis of acetylcholine in sympathetic ganglia

Brown & Feldberg [1936*a*] have shown that, in cats, the acetylcholine content per g. tissue of the right and left superior cervical ganglion does not differ by more than 15%. The difference becomes smaller when ganglia from several cats are extracted together (Table 1). There appears also to be no great difference in the acetylcholine content of the two superior cervical ganglia in sheep, the differences in the two experiments of Table 1 being 0 and 2% re-

Table 1. Acetylcholine content of superior cervical ganglia

Animal	Mg. of ganglionic tissue extracted		Acetylcholine in μg./g.		Difference in %
	Right gl.	Left gl.	Right gl.	Left gl.	
1 sheep	65·0	58·4	18·2	17·9	2
1 sheep	175·6	178·8	11·4	11·4	0
2 cats	24·8	27·6	26·7	25·9	3
3 cats	37·8	33·8	26·2	27·6	5
4 cats	46·4	46·4	28·7	29·4	3

spectively. The variations from animal to animal are greater. In sheep, in which the acetylcholine concentration of the ganglia was found to be lower than in cats, the acetylcholine equivalent/g. fresh tissue varied in seven sheep from 8·3 to 18·2μg./g. (average 13·0μg./g.). The corresponding figures from thirty-five cats were 23·5 and 36·8μg./g. (average 28·0μg./g.).

Incubation of ground or chopped ganglia. Incubation of an excised ganglion brings about an increase in the amount of acetylcholine extractable from it. The amount yielded, however, depended upon whether the ganglia, before incubation, were divided with scissors for 1 or 2 min. or finely ground in a mortar with silica. In the latter condition synthesis of acetylcholine was small and did not occur regularly (Table 2); in the former it was pronounced, sometimes doubling, or even trebling, the acetylcholine yield during a period of incubation of about 2 hr (Table 3). The increase was obtained when incubation was started from a low level of acetylcholine in the ganglionic tissue or from its normal high level. To obtain a low level the ganglia were cut into small pieces or ground in saline solution containing no eserine, the acetylcholine released during the cutting up or grinding being destroyed by the cholin-

TABLE 2. Effect of 2 hr. incubation at 38–39° C. on the acetylcholine yield of ground up sympathetic ganglia

Animal	Acetylcholine in μg./g.		Difference in %	Treatment of ganglia
	Control gl.	Incubated gl.		
1 cat	4·0	3·6	−10	Eserine added after grinding
2 cats	32·9	27·9	−15	Ground in eserine
1 sheep	13·3	16·0	+20	Ground in eserine
1 sheep	2·9	3·5	+21	Eserine added after chopping the tissue with scissors for 5 min.

TABLE 3. Effect of incubation at 38–39° C. on acetylcholine yield of sympathetic ganglia chopped with scissors

Animal	Acetylcholine in μg./g.		Increase in %	Period in min. of		Plasma present	Notes
	Control gl.	Incubated gl.		Chopping	Incubation		
1 sheep	8·3	20·0	141	2	130	9% horse pl.	Both sides chopped in saline without eserine
1 sheep	7·8	17·8	128	2	110	—	
1 sheep	11·1	30·3	173	2	120	—	
1 cat	4·3	11·4	165	2	150	15% cat's pl.	
1 cat	13·5	26·0	93	1	130	12% cat's pl.	
1 cat	6·7	14·3	113	2	120	7% horse pl.	
1 sheep	18·9	43·5	130	2	120	—	Control gl. chopped in HCl; incubated gl. chopped in eserinized saline solution
1 sheep	13·9	27·1	95	2	120	8% horse pl.	
1 sheep	8·3	27·8	219	2	120	10% horse pl.	
1 cat	27·0	52·6	94	1	130	—	
1 cat	26·3	53·8	105	1	90	—	
3 cats	28·6	47·1	65	2	110	—	
3 cats	27·1	58·3	115	2	120	6% cat's pl.	
2 cats	36·8	65·8	79	2	120	—	
4 cats	23·5	40·0	70	2	120	—	

esterase. The control ganglia were then extracted with HCl and eserine was added to the flask containing the ganglia for incubation. This procedure accounts for the relatively low acetylcholine values of the controls in the first and last experiment of Table 2 and in the six first experiments of Table 3. In the other experiments the control ganglia were divided with scissors or ground in eserinized HCl, and the ganglia used for incubation in buffered eserinized saline solution.

The addition of plasma in a concentration of 7–15% to the medium did not appreciably influence the result, although the average increase without plasma was 106, with plasma 134%.

The difference in the results with finely ground ganglia and ganglia cut into small pieces suggests that the property of synthesizing acetylcholine is dependent upon the integrity of some structural part of the tissue, and that mechanical destruction, if carried out too extensively, leads to a loss of this property. That this occurs when a ganglion is divided too finely with scissors is shown by the last experiment of Table 2 in which the ganglion was cut up for a period of 5 min.

Synthesis of acetylcholine apparently occurs only when the acetylcholine store of the tissue is diminished, i.e. after acetylcholine has been released, the tissue being unable to build up an acetylcholine concentration higher or much higher than the normal value. This conclusion is based upon experiments in which, after incubation, the content of the flask was centrifuged, and the assay of acetylcholine carried out separately with the particulate matter and the supernatant fluid, a procedure adopted in four of the experiments recorded in Table 3. The results are tabulated separately in Table 4. Only once was there a yield of acetylcholine from the particulate matter higher (23%) than that of the control ganglion; in the other three experiments the ratio was reversed.

TABLE 4. Distribution of acetylcholine between particulate matter and fluid of incubated ganglion

Acetylcholine content in μg./g. in			
	Incubated gl.		
Control gl. (a)	Particles (b)	Fluid (c)	(b) as percentage of (a)
28·6	15·7	31·4	55
23·5	22·2	17·8	94
27·1	33·3	25·0	123
13·9	12·9	14·2	93

Cutting ganglia into small pieces. If we assume that the synthesis occurs to replace acetylcholine, which has been released or diffused out of the particulate matter, the increase found after incubation must have been greatly dependent upon the acetylcholine loss from the tissue, and cannot give a true picture of the rate of synthesis of acetylcholine in ganglionic tissue. According to this

view, a stimulus leading to release of acetylcholine should be followed by synthesis or replacement. Chopping the ganglia with scissors ought to be a powerful stimulus of this kind and, in fact, leads, without subsequent incubation, to an increase in the acetylcholine yield of the ganglion. The results are shown in Table 5. The ganglia of one side had been divided with scissors in

TABLE 5. Acetylcholine yield of superior cervical ganglia chopped with scissors in HCl (*a*) and in eserinized saline solution (*b*)

	Acetylcholine in μg./g. of				
		(*b*)			
Animal	(*a*)	Total	Particle	Fluid	Increase of (*b*) in %
4 cats	25·0	40·2	13·3	26·9	61
4 cats	31·3	33·5	14·3	19·2	7
1 sheep	10·9	14·1	4·6	9·5	29

buffered eserinized saline solution for 2 min., the mixture was at once centrifuged, and the particulate matter and the supernatant fluid extracted separately with HCl. The control ganglia had been chopped in HCl. In all three experiments the ganglia chopped in eserinized saline solution had yielded a greater amount of acetylcholine than the ganglia chopped in HCl, the increase varying between 7 and 61%. In two of these experiments the amounts of acetylcholine brought into the solution by chopping the ganglia amounted to practically the total acetylcholine content originally present in them, indicating that the amount found in the particulate matter corresponded to the amount synthesized during the procedure of chopping. In the other experiment, in which there was a total increase of only 7%, less than two-thirds of the acetylcholine originally present in the ganglion had been released. The acetylcholine found in the particulate matter, in all three experiments, corresponded to about half the original amount. This value is lower than that obtained in the experiments of Table 4 after incubation. It appears therefore that the greater total yield on incubation results mainly from the fact that the acetylcholine is built up in the particulate matter during this period.

Synthesis in ganglia during degeneration of preganglionic fibres. We can confirm previous observations [Brown & Feldberg, 1936*a*; MacIntosh, 1941] on the disappearance of acetylcholine from the superior cervical ganglion of the cat following section of the cervical sympathetic trunk. Table 6 gives the actual values obtained, which, in Fig. 1, are expressed as percentages of the normal average acetylcholine content of the ganglion (28μg./g.). The crosses in the figure are the results obtained by MacIntosh. The acetylcholine content of the ganglia 4–7 days after section of the sympathetic trunk was too low to be assayed on the frog's rectus muscle with the small amounts of tissue at our disposal. It was determined on the arterial blood pressure of the cat and some of the depressor action, which was atropine sensitive, may have been due to

TABLE 6. Content and synthesis of acetylcholine in superior cervical ganglia during degeneration of preganglionic nerve

No. of cats	Days after cutting cerv. symp.	Acetylcholine in μg./g. of Control gl.	Acetylcholine in μg./g. of Incubated gl.	Increase in %
1	1	16·7	47·2	+185
2	1	25·6	49·8	+ 95
2	2	8·8	7·1	− 19
3	2	5·3	8·3	+ 57
3	3	3·2	3·2	0
3	4	About 1·4	About 1·4	0
2	6	About 1	Little less than 1	0
2	7	About 1·5	About 1·3	− 13

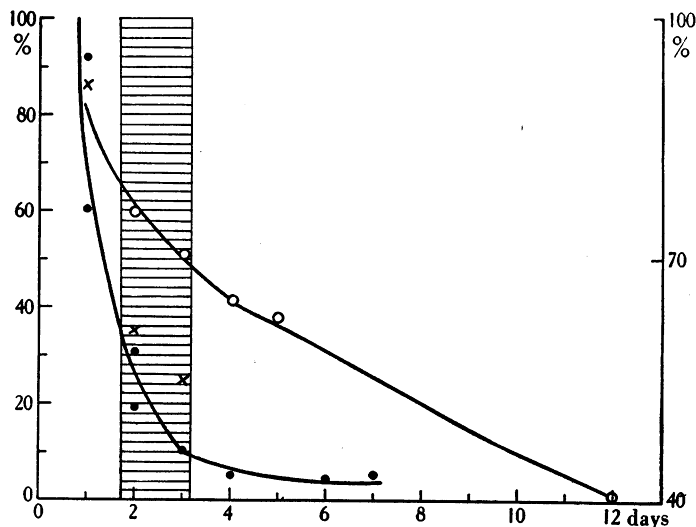

Fig. 1. Effect of preganglionic denervation on acetylcholine (•) and cholinesterase (o) content of the superior cervical ganglion of cats. Ordinates: percentages of normal acetylcholine content (on the left) and of normal cholinesterase content (on the right) of the ganglion. Abscissae: time in days after cutting the cervical sympathetic nerve. Shaded area: approximate period of impairment and loss of synaptic transmission with intact nervous conduction. The cholinesterase values are from Couteaux & Nachmansohn [1940]. (For details see text.)

the presence in the extracts of choline, which does not diminish in the ganglion after preganglionic nerve degeneration [Brown & Feldberg, 1936*b*]. For comparison, the decrease in cholinesterase content in the ganglion after preganglionic denervation is given in Fig. 1. Brücke [1937] had first observed this effect, but the values given are from Couteaux & Nachmansohn [1940], according to whom the cholinesterase decreases to 40% of its original value, and then remains constant. Even if the fact is taken into account that the cholinesterase does not disappear completely, its concentration decreases more gradually than that of acetylcholine, as can be seen from a comparison of the two curves.

After degeneration of the preganglionic fibres, the superior cervical ganglion loses its property of synthesizing acetylcholine which, therefore, is associated, not with the ganglion cells, but with the preganglionic nerve endings in the ganglion. The time when this property is lost coincides with the time when transmission of nervous impulses across the ganglionic synapses becomes impaired or fails. According to Coppée & Bacq [1938] synaptic transmission is impaired 40–50 hr. and abolished 45–72 hr. after cutting the preganglionic nerves. According to MacIntosh [1938] 'difficulties in transmission start after 48 hr. and it is completely or nearly completely abolished after 72 hr.' although at this time the nerve fibres still conduct impulses. In Fig. 1 the shaded area approximately represents this period of impairment and loss of synaptic transmission with intact nervous conduction. During this period, the ability of the ganglion to synthesize acetylcholine was found to become wanting. Synthesis was well pronounced 24 hr. after nerve section; after 48 hr. it was absent in one experiment (upper point on the curve), and diminished in the other (lower point). The latter experiment was carried out with the ganglia of three cats, and the diminution in synthesis may have taken place in all three ganglia, or synthesis may have been absent in one or two ganglia and normal or diminished in the remainder. The ganglia examined 3 days or later after section of the cervical sympathetic nerve no longer synthesized acetylcholine.

Synthesis of acetylcholine in cholinergic nerves

Cervical sympathetic nerve. We can confirm MacIntosh's observation [1941] of a high acetylcholine concentration in the cervical sympathetic trunk of cats, and the same is true for the cervical sympathetic of sheep. It varied in seventeen cats between 21·5 and 40·0 μg./g. (average 31·2 μg./g.) and in eight sheep between 11·9 and 22·7 μg./g. (average 18·0 μg./g.). There is no strict parallelism between the values obtained for the ganglia and their preganglionic nerves, although in more than a third of the experiments the differences were less than 20 %. Table 7 shows that the yield from the nerves was higher in more than half of the experiments; in a few instances the ratio was reversed.

TABLE 7. Acetylcholine content of superior cervical ganglion and its preganglionic nerve

Animal	Acetylcholine content in μg./g. of: Ganglion	Nerve	Animal	Acetylcholine content in μg./g. of: Ganglion	Nerve
Sheep	17·9	16·0	4 cats	25·0	33,3
Sheep	18·9	22·2	4 cats	31·3	31·3
Sheep	13·5	13·3	3 cats	28·6	29·4
Sheep	10·0	21·7	3 cats	27·1	40·0
Sheep	8·3	11·9	3 cats	27·6	21·5
Sheep	10·9	15·4			

As with ganglia, synthesis was small or absent when the nerves were ground with silica and incubated. The results obtained with nerves divided with

TABLE 8. Effect of incubation at 38–39° C. on acetylcholine yield of cervical sympathetic chopped with scissors

Animal	Acetylcholine in μg./g. of: Control nerve	Incubated nerve	Increase in %	Period in min. of: Chopping	Incubation	Plasma present	Notes
Sheep	3·3	5·7	73	1½	140	—	
Sheep	3·3	7·1	115	1½	140	9% horse pl.	Both samples chopped in saline without eserine
Sheep	8·3	11·4	37	1	90	13% sheep ser.	
Sheep	5·4	9·5	76	1	120	25% sheep pl.	
4 cats	33·3	75·8	128	1	140	—	
3 cats	29·4	50·0	71	1	105	12% cat pl.	

scissors and incubated for 90–140 min. in buffered eserinized saline solution are given in Table 8. The sheep's nerves were divided in saline solution containing no eserine; the control sample was then extracted with HCl and the other sample incubated after the addition of eserine. This procedure accounts for the low initial values of acetylcholine. In the experiments on cats the nerves used as controls were divided in HCl, those to be incubated were divided in eserinized buffered saline solution. The increase of acetylcholine after incubation amounted to between 37 and 128% (average 100%), a value slightly lower than that obtained for the ganglia. The addition of small amounts of eserinized plasma or serum to the medium used for incubation did not appear to have a definite effect on the acetylcholine yield. As in the experiments on ganglia, the increase of acetylcholine observed does not represent a true picture of the rate of its synthesis in nervous tissue. Table 9 shows that chopping the nerves

TABLE 9. Acetylcholine yield of cervical sympathetic chopped with scissors in HCl (*a*) and in eserinized saline solution (*b*)

Animal	Acetylcholine in μg./g. of: (*a*)	(*b*)	Increase of (*b*) in %	Time of chopping nerve in min.
1 sheep	15·4	22·2	44	1½
4 cats	31·3	42·9	37	1
3 cats	21·5	36·4	69	1

with scissors for 60–90 sec. in eserinized saline solution, and then extracting them at once with HCl leads to an increase of 37–69% (average 50%), i.e. about half the increase in the two incubation experiments, in which the nerves were chopped in the presence of eserine, may have been brought about during the process of chopping.

Degenerating cervical sympathetic nerve. The acetylcholine concentration in the cervical sympathetic decreases progressively in the first few days after section of the nerve, the greatest decrease occurring, as in ganglia, between the first and third day. The results are given in Fig. 2, the acetylcholine content being expressed as percentage of the normal acetylcholine content of 31·2 μg./g.

fresh nerve. The sympathetic nerves were from the cats from which the ganglia had been taken, the rate of disappearance of acetylcholine being the same in both tissues. A similar decrease during nervous degeneration has been observed in motor nerves [Lissák, Dempsey & Rosenblueth, 1939] and in the vagus [MacIntosh, 1941].

The cervical sympathetic trunk loses its property of synthesizing acetylcholine at about the same early stage of degeneration (or perhaps a little earlier) as the ganglion. Forty-eight hours after section, the nerves no longer synthesized acetylcholine. They were from cats in which the ganglia had also lost their ability to synthesize acetylcholine. The nerves from the other experiment, in which synthesis by the ganglia had been observed at this stage of nerve degeneration, were not examined. All the sympathetic nerves examined later than 48 hr. after section no longer synthesized acetylcholine. The acetylcholine of these nerves was assayed on the arterial blood pressure of the cat. One experiment was carried out 24 hr. after nerve section. There was an increase of acetylcholine with incubation of only 12%. Further experiments are necessary to decide if synthesis disappears earlier in nerve fibres than at their endings.

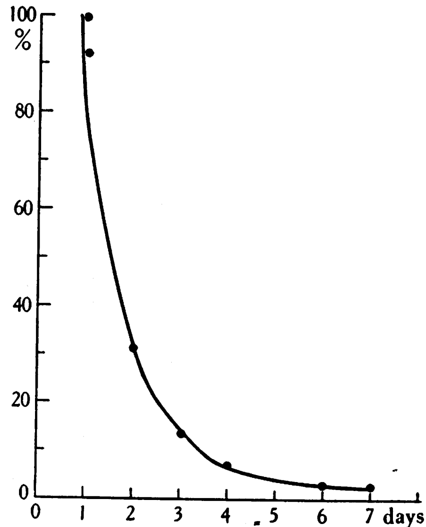

Fig. 2. Acetylcholine content in degenerating cervical sympathetic trunk of cats. Ordinates: percentages of normal acetylcholine content of the nerve. Abscissae: time in days after cutting the nerve. (For details see text.)

Vagus nerve. Table 10 shows the synthesis of acetylcholine occurring in samples of the vagus nerve chopped with scissors and incubated in eserinized saline solution. The weight of the tissue used for each sample was about 370 mg. in the sheep experiment and between 25 and 70 mg. in the cat experiments. The period of chopping was 2 min. for the sheep's nerves and 1 min. for the cat's nerves. The nerves in the first four experiments were chopped before the eserine or the HCl respectively was added to the buffered saline solution, which accounts for the low control values of acetylcholine. In the last three experiments the controls were chopped in eserinized HCl and those used for synthesis in buffered, eserinized saline solution. A proportion of the increased yield of acetylcholine in the last two incubation experiments must have occurred during the procedure of chopping. This is evident from the last experiment of the table. The increase of 40% was obtained by chopping the nerves in eserinized saline solution without subsequent incubation.

TABLE 10. Effect of incubation at 38–39° C. on acetylcholine yield of vagus nerve chopped with scissors

Animal	Acetylcholine in μg./g. of Control nerve	Acetylcholine in μg./g. of Incubated nerve	Increase in %	Incubation period in min.	Plasma present	Notes
Sheep	0·9	2·0	122	120	—	Both nerves chopped in saline without eserine
Cat	4·0	9·4	135	120	16% cat pl.	
Cat	3·8	7·3	90	150	12% horse pl.	
Cat	4·2	5·6	33	130	13% cat pl.	
Cat	9·4	13·0	39	120	—	
Cat	8·8	15·3	74	120	12% cat pl.	
Cat	7·8	10·9	40	0	—	

Spinal nerves and nerve roots. In one experiment two samples of cat's phrenic nerves were chopped with scissors for 1 min. in buffered saline solution without eserine. One sample was then extracted with HCl, the other incubated for 70 min. after adding eserine to it. The acetylcholine equivalent of the control was 6·2, of the incubated sample 8·2μg./g. tissue, an increase of 34%. In two experiments the lumbar motor roots were removed from a cat. Control samples were extracted with HCl, the others incubated in the usual way for 130 min. The acetylcholine yield of the controls was 22·2 and 20·8μg./g., that of the incubated samples 42·5 and 42·7μg./g., increases of 92 and 105% respectively. The corresponding sensory roots from the same cats were treated similarly, and the extracts assayed on the arterial blood pressure of the cat. The intravenous injection of extract equivalent to 20 mg. tissue scarcely gave any fall of blood pressure, if there was a difference the greater depressor action was with the controls. The depressor effect was less than that of 0·005μg. of acetylcholine, i.e. there was certainly not more than 0·25μg./g. acetylcholine in these roots, and there was no indication of it having been synthesized during the process of chopping and the subsequent incubation.

DISCUSSION

Our initial conception that synthesis of acetylcholine in a sympathetic ganglion is a property not of the ganglion cells but of the preganglionic nerve endings in the ganglion, is borne out by the fact that the superior cervical ganglion of the cat loses this property after the cervical sympathetic has been cut. It is possible that the cells in parasympathetic ganglia, which, unlike most of the sympathetic ganglion cells, belong to a cholinergic neuron, would be found to exhibit this property and to do so independently of the integrity of their preganglionic nerve endings, and the same might be true for those sympathetic ganglion cells which give rise to postganglionic cholinergic axons. So far no experiments in this direction have been carried out. In the cat the superior cervical ganglion appears to have at the most only a few of such cells [Bacq, 1935]. The fact that loss of synthesis occurs simultaneously with impairment

and loss of synaptic transmission, but at a time when nervous conduction is intact, suggests the ability to synthesize acetylcholine as a necessary preliminary for normal, and particularly for sustained, transmission across ganglionic synapses. The disappearance of acetylcholine in sympathetic ganglia following section of their preganglionic nerves probably results also from the fact that the preganglionic nerve endings have lost their power to replace any acetylcholine when it has been released and destroyed. These conclusions are reinforced by the findings that cholinergic nerves synthesize acetylcholine in apparently the same manner as sympathetic ganglia, that both lose this property at the same early stage of nervous degeneration and that, in both, this stage is associated with a great drop in the acetylcholine content of the tissue. The loss of ability to synthesize acetylcholine, preceding the loss of nervous conduction, is among the first functional changes occurring in degenerating cholinergic nerves.

If we assume synthesis of acetylcholine to occur throughout the whole course of the fibres of cholinergic nerves up to their final endings, the structural element most likely to be concerned with it would be the tissue of which the axon and its endings are built up. MacIntosh [1941] has pointed out that the presence of acetylcholine in the fibres in a concentration of about the same order as that found in the ganglion suggests a manifold higher concentration at the preganglionic endings than along the course of the nerve fibres. We do not know if this increase in acetylcholine is brought about by a greater ability of the tissue at the endings to synthesize acetylcholine or to an accumulation, at the endings, of the tissue of which the axons are composed. Whenever the acetylcholine is released from this tissue in such a way as to be destroyed or washed away, if eserine is present, it is at once restored by synthesis. Similarly, synthesis of sympathin or adrenaline, the transmitter of adrenergic nerves, might perhaps occur after its release, along the whole course of the nerve up to its final endings. No function can yet be postulated for the release of the transmitter substances along the course of a nerve during the passage of an impulse. It has been demonstrated, however, for different cholinergic and adrenergic nerves [for literature see Lissák, 1939]. There is, as yet, no evidence that the release of acetylcholine, with subsequent synthesis, is necessary for the passage of an impulse in cholinergic nerves, a possibility envisaged by von Muralt [1937]. Otherwise the loss of synthesis should not precede that of conduction in a degenerating nerve. At the nerve endings the released acetylcholine finds a sensitive effector structure upon which to act, and, since it is restored immediately, synaptic transmission is permitted at the high frequency at which nerve impulses may follow each other for a long period of stimulation.

It would be misleading to deduce, from the amount of acetylcholine synthesized in our experiments during a given period, the rate of synthesis as it occurs in the body after a nerve impulse arrives at the nerve ending. The experi-

ments in which ganglia or nerves were cut into small pieces for a minute or two without subsequent incubation give some idea of the speed of synthesis, in response to the release caused by the stimulus of injury, under the conditions of the experiment. An even more striking instance of the rate of synthesis is given by the observation of von Muralt on the increased acetylcholine yield of nerves 'shot into liquid air' during stimulation. Synthesis or replacement of acetylcholine when released by nervous impulses may well occur within the refractory period of the nerve. Brown & Feldberg [1936*b*] have discussed such a possibility for synthesis of acetylcholine at preganglionic endings in sympathetic ganglia, when the acetylcholine has been released by preganglionic nerve stimulation.

Our experiments suggest that synthesis of acetylcholine is dependent upon some structural part of the tissue, since grinding the ganglia or nerves with silica greatly reduced or destroyed this property. The conditions for synthesis to take place, however, may vary in different tissues. For instance, brain tissue does not lose this property on grinding with silica. Nevertheless, in this tissue also, synthesis may be associated with the presence of particles such as cell debris or granules. According to Mann *et al.* [1939] spinning down and removing the particles of a suspension of minced brain leaves a supernatant fluid devoid of the property of synthesizing acetylcholine. In addition, they state that synthesis only occurs in a minced brain suspension when it is using oxygen, and that it is no longer obtained after the suspension has been frozen. Stedman & Stedman, on the other hand, observed synthesis in chloroform and ether suspensions of brain tissue under conditions which are incompatible with cellular activity.

SUMMARY

1. Sympathetic ganglia (superior cervical ganglion) and cholinergic nerves (cervical sympathetic, vagus, phrenic nerve and motor roots) divided with scissors into small pieces and incubated for 1–2 hr. in buffered saline solution, containing eserine, synthesize acetylcholine. This property appears to be dependent upon the intactness of some structural part of the tissue, since it becomes lost or greatly reduced when the mechanical destruction is carried too far, as by grinding the tissue with silica. No synthesis of acetylcholine has been observed with sensory roots.

2. Chopping sympathetic ganglia or cholinergic nerves with scissors for 1 or 2 min. leads, without subsequent incubation, to some synthesis of acetylcholine. Synthesis occurs apparently only in order to replace released acetylcholine and to restore its original concentration in the tissues. The procedure of chopping the tissue may release, and bring into solution, amounts of acetylcholine corresponding to those originally present in the tissue, but about half of this amount is resynthesized in the particulate matter of the suspension during the 1 or 2 min. of chopping.

3. When the cervical sympathetic trunk is cut in a preliminary operation, the distal part of the nerve and the superior cervical ganglion lose their property of synthesizing acetylcholine at an early stage of nervous degeneration (after about 48 hr.). The loss of synthesis occurs with the great drop in the acetylcholine content of the tissue and must be considered responsible for it. The loss precedes the loss of nervous conduction. In the ganglion it coincides with the time when synaptic transmission becomes impaired and lost. It is concluded that synthesis of acetylcholine in sympathetic ganglia is a property of the preganglionic endings and a necessary preliminary for normal and particularly sustained synaptic transmission.

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