612.89:547.435–292

THE CHEMICAL TRANSMITTER AT SYNAPSES IN A SYMPATHETIC GANGLION.

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(*Received March* 12, 1934.)

IT was shown by Dale [1914] that acetylcholine and other choline esters have two types of action:

(1) "Muscarine" actions, paralysed by atropine, and reproducing the peripheral effects of stimulating parasympathetic nerves.

(2) "Nicotine" actions, of which the most conspicuous is the stimulant action on the cells of sympathetic ganglia, and probably of all autonomic ganglia. These "nicotine" actions are unaffected by atropine, but are paralysed by large doses of nicotine.

The correspondence between the "muscarine" actions of choline esters and peripheral parasympathetic effects has found its explanation in the evidence, rapidly accumulating since Loewi's first demonstration on the frog's heart vagus [1921], that parasympathetic nerves produce their effects by peripheral release of a choline ester, having properties similar to those of acetylcholine; that, in the terminology suggested by Dale [1933], postganglionic parasympathetic fibres are "cholinergic." Since acetylcholine itself occurs in the animal body, and is the only ester of choline known to do so, it is generally accepted as the probable chemical transmitter concerned. Till recently there was no evidence that the "nicotine" action of acetylcholine, or of any choline derivative, might also be concerned in the chemical transmission of nervous effects. In the course of a study of the distribution of choline esters, and of acetylcholine in particular, in the animal body, Chang and Gaddum [1933] found that extracts of the sympathetic chain of a horse contained comparatively large amounts of a substance having the physiological properties of acetylcholine, and were led to suggest that this substance might play a part in the normal transmission of impulses through the ganglia, having

in view its stimulant action on ganglion cells. The first direct evidence, however, for such a cholinergic function of preganglionic sympathetic fibres was obtained in experiments by Feldberg and Minz [1933] and Feldberg, Minz and Tsudzimura [1933] on the secretion of adrenaline from the suprarenal medulla in response to splanchnic stimulation.

The experiments here described have furnished evidence that the preganglionic fibres ending in relation to the nerve cells of a sympathetic ganglion are also cholinergic. The method used has been essentially one described by Kibjakow [1933], who perfused the superior cervical ganglion in an anæsthetized cat with oxygenated Locke's solution, and recorded the contractions of the nictitating membrane, which, under these conditions, were still evoked by stimulation of the cervical sympathetic nerve. Kibjakow collected the Locke's solution flowing from the vein of the perfused ganglion, and reinjected it into the fluid flowing to the artery of another, or in some cases of the same perfused ganglion. He found that such reinjection of the venous fluid collected during strong stimulation of the cervical sympathetic nerve caused contraction of the nictitating membrane, comparable to that caused directly by nerve stimulation, but that fluid flowing from an unstimulated ganglion had no such action. He regarded this as evidence of chemical transmission in the ganglion, and Chang and Gaddum suggested that the substance concerned might be acetylcholine. It will be seen that our experiments give definite evidence of the liberation of acetylcholine under such conditions, though we have not yet been able to reproduce the reinjection effect, described by Kibjakow, with any regularity.

Methods.

Cats were anæsthetized with chloralose and the ganglion prepared for perfusion by Kibjakow's method. The upper part of the carotid artery was exposed. A transverse vein was divided between ligatures and a lymphatic gland removed. The carotid artery was doubly ligatured and divided beyond the point where the artery to the ganglion arises. All the arterial branches except those running to the tissue round the ganglion were tied and divided. The small internal carotid artery was separated from the postganglionic sympathetic branches and divided. Numerous small veins were also divided, leaving only the vein from which the perfusion fluid was to be collected, with the small vein from the ganglion opening into it. The most convenient vein for this purpose is the internal

jugular. When this vein was too small, as it often was, the fluid was collected from the vein which emerges from the vertebral column through the atlantal foramen, and joins the jugular vein near the ganglion; the jugular vein being, in that case, tied above and below the entry of the vein from the ganglion.

Suitable lengths of the cervical sympathetic nerve and the common carotid artery were freed and the cat's head was then fixed in position, by a clamp holding the upper jaw, so that the contraction of the nictitating membrane could be recorded, by attaching its free edge, by means of a cotton thread passing round a pulley, to a lever. "Chlorazol fast pink" [Huggett and Rowe, 1933], 0·5 c.c. of an 8 p.c. solution per kg., was injected intravenously to prevent coagulation of the blood during manipulation. The common carotid artery being now ligatured and cut, a cannula was tied into its peripheral end and the perfusion started. A small cannula was then tied into the vein to collect the perfusion fluid.

In some experiments the perfusion fluid was Locke's solution of the original composition. In other experiments it was slightly modified to the following composition: NaCl 0·9 p.c., KCl 0·02 p.c., $CaCl_2$ 0·02 c.c., $NaHCO_3$ 0·03 p.c., glucose 0·1 p.c. The significant modification was the reduction of the KCl from 0·042 to 0·02 p.c., since Locke's solution contains sufficient potassium to interfere with some of the pharmacological tests. The above solution, when diluted in the ratio of 1 : 1·4, is almost identical with the original Ringer's solution for the frog. The isolated tissues from frogs and leeches, which were used in some of the tests, were kept in this diluted fluid until the active solutions were actually applied to them, and the perfusion fluid was diluted with water in the ratio 1 : 1·4 for testing. Except in one or two special experiments the perfusion fluid also contained eserine in a concentration of 1/500,000.

Some of the earlier experiments were unsuccessful, owing to failure to realize the necessity for very careful filtration of the perfusion fluid. Filtration through paper is unsuitable for this purpose, and in most of the experiments the perfusion fluid was passed before use through filters made of sintered Jena glass.

The perfusion fluid was contained in a reservoir suspended about 100 cm. above the cat, and connected to the arterial cannula by rubber tubing. In the course of this was interposed a wide glass tube, containing in its upper part an air space through which the fluid passed in drops from a nozzle, the frequency of these giving a rough indication of the rate of perfusion. The fluid then passed through a filter composed of fine linen or glass-wool and thus to the arterial cannula. The arterial cannula

was warmed electrically to about 40° C. by means of resistance wire wound round it. The temperature, controlled by a rheostat, was measured either by a thermometer in a side tube or by a thermocouple. The rate of flow was 0·1–0·3 c.c. per min.

An induction coil was used for stimulating the nerve through platinum electrodes, the primary circuit being interrupted by the automatic hammer. The nerve was moistened continually during the experiment. Stimulation was continued over periods up to ½–1 hour, but it was interrupted during 1–2 min. out of every 3 or 4 min. The distance of the secondary coil was adjusted so that the effect was supramaximal.

The methods used for testing the solutions can be briefly described. For experiments on the cat's blood-pressure chloralose was used as an anæsthetic, the viscera being sometimes removed and eserine injected to sensitize the preparation. The test on eserinized leech muscle was carried out by the method described by Minz [1932] and by Feldberg and Krayer [1933], with minor modifications. In order to economize material the volume of the bath was only 2 c.c. Thin strips of muscle were used, and it was found that the behaviour of symmetrical strips from the same leech was almost identical. The actions of two different solutions can thus be roughly compared by applying one to each strip of muscle. It was found that Locke's solution diluted in the ratio 1 : 1·4 was particularly suitable for use in the bath in these tests. The muscle relaxed rapidly when the acetylcholine was washed out, probably because the solution contained a relatively large amount of potassium, and it was usually possible to detect 0·002γ of acetylcholine, which corresponds to a concentration of 2γ per litre (10^{-9}). Solutions were also tested on the frog's heart isolated by Straub's method, on the frog's rectus abdominis [Chang and Gaddum, 1933] and on the rabbit's auricle [Clark, 1921]. All three preparations were treated with eserine. The last two were suspended in baths of 2 and 3 c.c. respectively.

Results with different tests.

Five different pharmacological methods have been used to test the perfusion fluids for activity. In every case fluid collected during stimulation has been found to have an action indistinguishable from that of acetylcholine, while fluid collected either before or after stimulation was quite inactive. The results obtained in these different tests will be considered separately. The choice of methods is limited by the fact that the activity of the solutions is too small to be detected by any but the most

sensitive tests. The rabbit's intestine is unsuitable as a test object, because of the presence in the perfusion fluid of sufficient eserine to interfere seriously with the behaviour of the intestinal muscle.

Arterial blood-pressure of a cat.

Fig. 1 shows a record of the arterial pressure of a cat (cf. also Fig. 7). 1 c.c. of perfusion fluid collected before stimulation of the nerve was

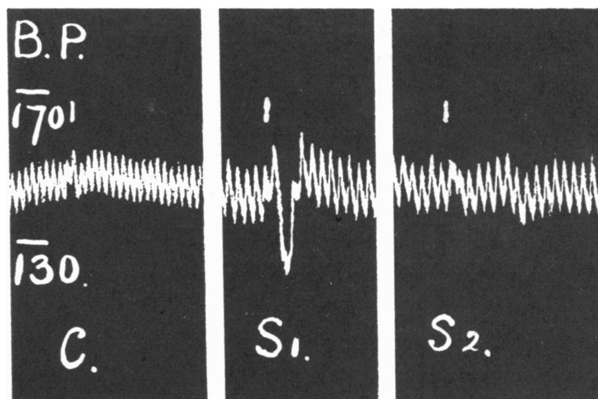

Fig. 1. Cat B.P. S_1 and S_2, fluid collected from the ganglion during stimulation; S_1, before and S_2 after atropine. *C*, control fluid; no stimulation.

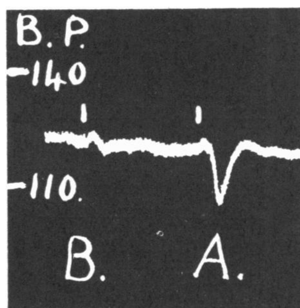

Fig. 2. Cat B.P. Fluid collected from the ganglion during stimulation. Treated *A* with dilute acid; *B* with dilute alkali.

injected at *C* and had no action. On the other hand, 1 c.c. of fluid collected during stimulation caused a fall of blood-pressure (S_1). This effect did not occur when the same injection was repeated after the intravenous injection of 0·1 mg. of atropine (S_2).

Fig. 2 shows that the active substance, producing this fall of blood-pressure in cats, was unstable in alkali. In this experiment the special

saline solution, of which the formula is given above, was used. Fluid collected during stimulation was divided into two portions of 0·9 c.c. each. To one 0·1 c.c. of $N/1$ NaOH was added and to the other 0·1 c.c. of $N/10$ HCl. Both solutions were kept for nearly 40 min. and then neutralized, by the addition of 0·1 c.c. of $N/1$ HCl or $N/10$ NaOH, and injected intravenously into a cat. The portion kept in acid solution was still fully active (*A*) and the portion kept alkaline was inactive (*B*). The effect on the cat's blood-pressure is a particularly suitable reaction for experiments of this kind, because it is unaffected by small changes in the acidity and tonicity of the solution.

Leech muscle.

Eserinized leech muscle provides the most sensitive of the tests we have used for acetylcholine. The effect of fluid collected during stimulation is shown in Fig. 3 *A*, and Fig. 6 *A*. In these figures the small abrupt rise of the record which precedes the contraction of the muscle is not a specific effect, but is due to the changing of the fluid in the bath. The tracing labelled *D* in Fig. 6 shows that fluid collected several minutes after stimulation was inactive. The small rise in the record which this fluid produced is indistinguishable from that produced at *R* by a simple change of the solution in the bath.

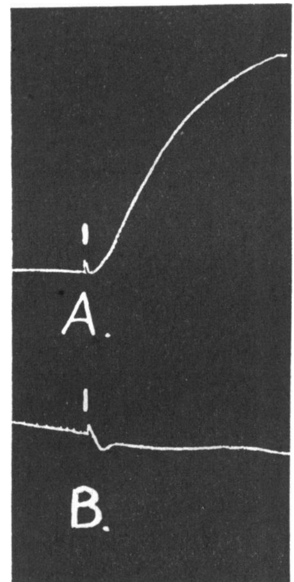

Fig. 3. Above: leech muscle treated with eserine. Below: untreated leech muscle. Effect of fluid collected from the ganglion during stimulation; diluted 1 : 14.

Fig. 3 shows that leech muscle which had not been sensitized to acetylcholine by means of eserine was also insensitive to the active solutions obtained from the ganglion. This experiment was complicated by the fact that the active solutions themselves contained sufficient eserine ultimately to sensitize the muscle, and success depended on the fact that the sensitization was a slow process. This made it possible to show the contrast between the reaction of a piece of muscle which had been previously sensitized and that of one which had not. In the latter case solutions of acetylcholine and active solutions obtained from the ganglion both produced a contraction which was feeble, and which did

not begin till several minutes after the solution had been applied to the muscle. In the experiment shown in Fig. 3 the right and left halves of the anterior part of the muscle from the back of a leech were used for comparison. The left half had been suspended for half an hour in a solution containing eserine (1/100,000); the right half had been kept in a solution free from eserine. The same sample of perfusion fluid, collected during stimulation of the nerve, was tested on both pieces of muscle in a final dilution of 1/14. This degree of dilution was sufficient to make the solution inactive on the normal muscle, though it produced a definite effect on the eserinized muscle. Later, the right half of the muscle was also eserinized, and was then found to be even more active than the left half, both to acetylcholine and to fluid collected during stimulation. The contrast shown in Fig. 3 was therefore due to eserine, and not to asymmetry of the leech. In another experiment it was shown, by a method similar to that described above, that the substance acting on the leech was unstable in alkali.

Frog's rectus abdominis.

The upper part of Fig. 7 shows at *S* the effect on the eserinized frog's rectus abdominis of fluid collected from the perfused ganglion. Fluid collected before stimulation was absolutely ineffective (*C*), while fluid collected during stimulation produced a contraction similar to that due to acetylcholine.

Another portion of the same sample was also tested on the corresponding piece of rectus muscle, taken from the other side of the same frog, but not treated with eserine. This muscle was less sensitive than the eserinized one, both to acetylcholine and to the active perfusion fluid. The latter, however, produced a definite effect, and the estimate of its activity in terms of acetylcholine agreed roughly with the estimate obtained in the experiments with the eserinized rectus, the result of which is shown in Fig. 7.

Frog's heart. (Straub's method.)

Fig. 4 and the upper part of Fig. 6 show the effect on the frog's heart of fluid collected from the perfused ganglion. The effect is indistinguishable from that of acetylcholine and is abolished by atropine (Fig. 4). Fluid collected during stimulation was active and fluid collected in the absence of stimulation was not (Fig. 6 *D*).

Rabbit's auricle.

Fig. 5 shows the effect on the rabbit's auricle. At *S* fluid (0·5 c.c.) collected during stimulation was added to the bath and at *C* fluid (0·5 c.c.) collected without stimulation. The latter had no action. The effect of the active solution was abolished by atropine.

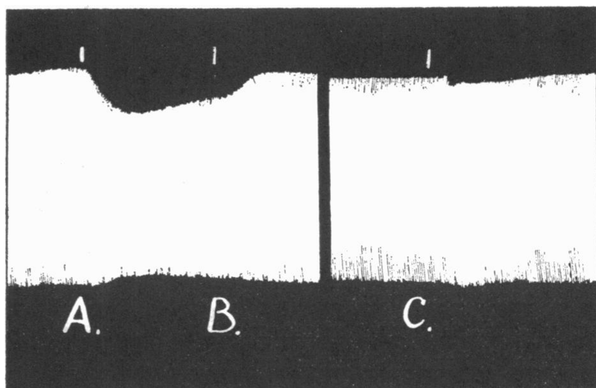

Fig. 4.

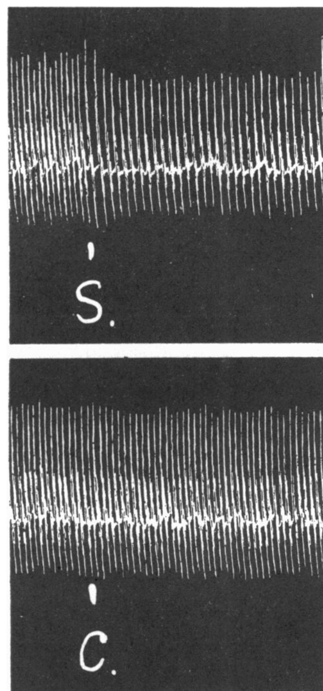

Fig. 5.

Fig. 4. Frog's heart (Straub's method). *A*, fluid collected from the ganglion during stimulation: *B*, 1γ atropine: *C*, same as *A* after atropine.

Fig. 5. Rabbit's auricle. Eserine concentration of the bath 2×10^{-6}. *S*, fluid collected from the ganglion during stimulation: *C*, control fluid; no stimulation.

The identification of the active substance as acetylcholine.

The evidence which has been presented shows that the perfused solution acquired during stimulation various different pharmacological properties similar to those of choline, or a choline ester. The resemblance extends to the fact that the effects on the cat's blood-pressure, the frog's heart and the rabbit's auricle were abolished by atropine. In the case of

several tests there is evidence that the effect was not due to choline itself. Thus the substances acting on the cat's blood-pressure and the leech were unstable in alkali, and the effects of the substances acting on the leech and the frog's rectus were increased by eserine. These facts suggest that one or more choline esters were present in the solution. Since acetylcholine is the only choline ester which has hitherto been isolated from animal tissues, we have made some experiments to test the theory that all these effects were due to the presence of acetylcholine. If an unknown substance, in quantity too small for chemical recognition, shows the pharmacological properties of a choline ester, it can be more closely identified by making simultaneous quantitative comparisons of its activity with those of different pure esters, by different pharmacological methods [Chang and Gaddum, 1933]. If in these different quantitative comparisons the activity of the unknown shows a constant relation to that of a pure ester, the latter is very probably identical with the unknown active principle responsible for all the effects.

Comparisons of this kind are shown in Figs. 6 and 7. In these figures the concentrations given are not the actual final concentrations in contact with the tissue, but the concentrations of standard solutions which, in the process of testing, were diluted to the same extent as the perfused fluid. Thus Fig. 6 shows that the effect of the active fluid, both on the frog's heart and the leech muscle, was greater than that of a solution containing 15γ of acetylcholine per litre and less than that of a solution containing 30γ per litre. In the test on the leech all these solutions were diluted three times so that the actual concentrations of acetylcholine were 5 and 10γ per litre. In the test on the frog's heart they were diluted to an unknown extent by adding 0·4 c.c. in each case to the same, but not accurately known, volume of solution in the cannula.

Fig. 7, which is taken from another experiment, shows that in this case the effect of the active solution, both on the eserinized frog's rectus and on the cat's blood-pressure, was more than that of 30γ of acetylcholine per litre and almost equal to, but in both cases slightly less than that of 50γ per litre. In the experiment on the rectus the solutions were diluted 1 : 1·4 times. The effects on the blood-pressure were obtained by injecting 1 c.c. of each solution undiluted.

In two other experiments similar comparisons were made between the effects on the cat's blood-pressure and those on the frog's heart and the rabbit's auricle respectively. In both cases the results agreed quantitatively with one another, in much the same way as they did in the experiments shown in Figs. 6 and 7. It would not, of course, be possible

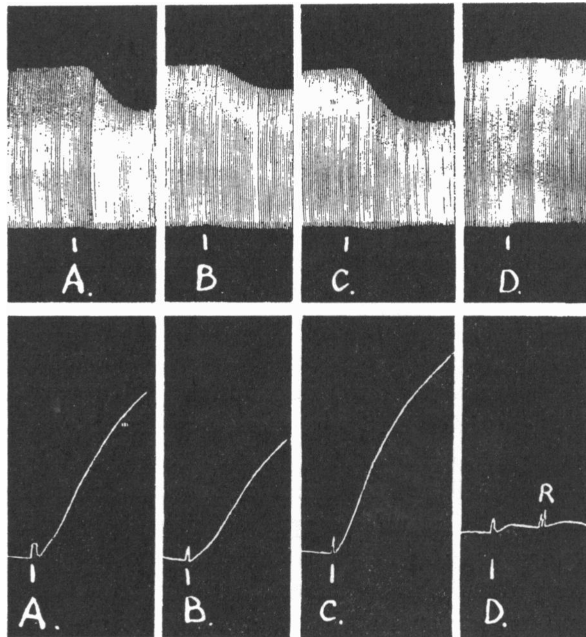

Fig. 6. Above: frog's heart (Straub's method). Below: leech muscle treated with eserine. *A*, fluid collected from the ganglion during stimulation: *D*, control fluid: *B*, *C*, acetylcholine (15 and 30γ per litre respectively).

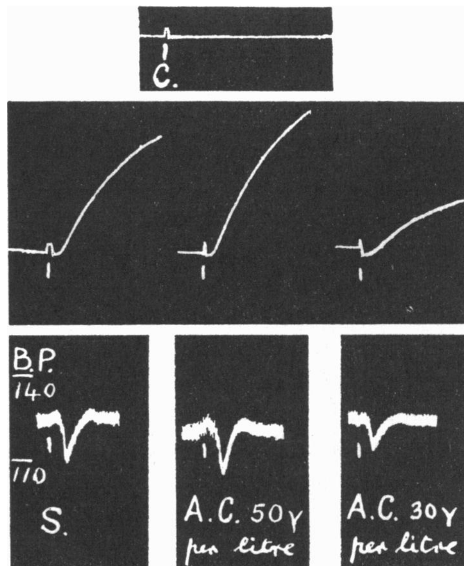

Fig. 7. Above: Frog rectus abdominis treated with eserine. Below: Cat B.P. *S*, fluid collected from the ganglion during stimulation: *C*, control fluid; no stimulation. A.C. acetylcholine.

in experiments of this kind to detect very small quantitative differences between the results of different tests. If larger quantities of active solution had been easily available more accurate comparisons could have been made, but the results were probably accurate enough for the purpose in view. If the tests on the normal and on the eserinized rectus are considered as two tests, these experiments show that six different pharmacological tests agreed in the quantitative estimate which they gave of the potency of the active solution in terms of that of acetylcholine. Three of these tests are concerned with the muscarine-like actions of acetylcholine.

Since, it is well known, the relative potencies of different choline esters may vary widely when different methods of testing are used, such results provide strong presumptive evidence for the view that the effects seen in all six tests were, at least, mainly due to acetylcholine, and not to other choline esters.

THE RATE OF LIBERATION OF ACETYLCHOLINE UNDER DIFFERENT CONDITIONS.

When the perfusion fluid contained no eserine, no activity was detected in any of the tests even when the nerve was stimulated. The addition of eserine to the perfusion fluid did not by itself cause the appearance of activity, as it does in the case of the intestine [Feldberg and Kwiatkowski, 1933] or stomach [Dale and Feldberg, 1933], and no activity was detected in fluid collected without stimulation of the cervical sympathetic, except occasionally in the first sample collected after the perfusion was started. In some cases the sensitivity of the tests on leeches was such that $1\cdot5\gamma$ of acetylcholine per litre would have been detected. Such quantities were not present in the absence of stimulation.

The concentration present in the fluid collected during stimulation varied between 20 and 85γ per litre. Since the rate of flow was 0·1–0·3 c.c. per min., a normal rate for the passage into the perfusion fluid of acetylcholine liberated during stimulation would be of the order of $0\cdot01\gamma$ per min.

THE EFFECTS OF INJECTIONS INTO THE PERFUSION FLUID.

The perfused ganglion forms a convenient preparation for the study of the effects of drugs on a ganglion. Under the conditions of our experiments the injection of acetylcholine into the fluid flowing to the ganglion caused a contraction of the nictitating membrane, but the concentration

necessary to produce this effect was usually slightly higher than the concentration present in the fluid collected during stimulation. In some experiments a solution containing 100γ of acetylcholine per litre produced a definite response, but in others it was necessary to use concentrations 100 times as large as this. We have repeatedly tried to confirm Kibjakow's observation that the reinjection of fluid collected during stimulation caused a contraction of the nictitating membrane. If the ganglion was perfused with plain Locke's solution, as in Kibjakow's experiments, we always obtained negative results. In two of the experiments in which the ganglion was perfused with Locke's solution containing eserine, reinjection of the stimulation effluent caused well-marked contractions of the membrane, but in numerous others no perceptible effect was obtained. This was to be expected, since the concentration of acetylcholine, in the venous fluid detected by the other tests, was usually insufficient to have a detectable action on the ganglion, even when eserine was present.

Discussion.

Evidence has been presented that stimulation of the cervical sympathetic nerve causes the liberation of acetylcholine under the conditions of the experiment. The superior cervical ganglion was not the only tissue perfused, but it is the only tissue from which the acetylcholine is likely to have come. The adjacent vagus ganglion is purely a sensory ganglion, and there is no reason to suppose that it would be affected by stimulation of the cervical sympathetic nerve. The other tissue in the neighbourhood, which was also perfused, was insignificant in bulk compared with the two ganglia, and contained no structure having any probable concern with the phenomenon.

Accepting the fact that acetylcholine is liberated in the superior cervical ganglion by impulses reaching it in the preganglionic fibres, we must consider the probability of its function in the transmission of the effect of a nervous impulse at a synapse. Since acetylcholine has a very powerful stimulating action on ganglion cells, the suggestion is obvious that a small quantity liberated at a synapse by the arrival of a preganglionic impulse provides the effective stimulus for the ganglion cell. The observations of Bishop and Heinbecker [1932], Brown [1934] and Eccles [1934] have shown that each preganglionic impulse gives rise to a single impulse in postganglionic fibres, that the delay at the synapse is not more than 2σ, and that the refractory period is very short. If, therefore, the synaptic transmission of each impulse is effected by release of a small charge of acetylcholine, this must occur in close relation to the

ganglion cell; and, since the stimulating effect of excess of acetylcholine on ganglion cells is followed by a depressant action, it must be very rapidly removed after it has transmitted a stimulus. The concentrations found in our experiments in the venous effluent, representing only the acetylcholine which has diffused from the site of its release into the fluid perfusing the blood vessels, cannot be expected, when a small volume is reinjected into the slow arterial stream, to reproduce effects comparable to those of preganglionic stimulation. It would create no difficulty for the theory of impulse transmission by acetylcholine, if the concentration in the venous fluid were too low to have any obvious stimulating action when thus applied. In most of our experiments it had none. There are other cases, in which a humoral transmission is well established, where the dilution found in a venous effluent is too low to reproduce the nervous effect with direct application. On a few occasions, however, arterial reinjection of a small volume of the actual venous fluid has produced definite stimulation of the ganglion cells, as shown by contraction of the nictitating membrane; and we have regularly obtained powerful stimulation by similarly injecting, in 0·1 or 0·2 c.c., a dose of acetylcholine comparable to the quantity appearing in 1 or 2 c.c. of the fluid during a few minutes of stimulation. It is safe, therefore, to conclude that the concentration of acetylcholine produced, at the site of its release in the ganglion, by a preganglionic impulse, is such that it must act as a powerful stimulus to the ganglion cells. If its appearance were held to have no functional relation to the discharge from the ganglion cells of the postganglionic impulses, we should have to postulate another form of excitation of the cells, also produced by preganglionic impulses, and also effective in stimulating plain muscle through the postganglionic fibres, but not electrically recognizable as impulses in those fibres. It seems simpler, on the facts yet available, to suppose that the release of acetylcholine is effective in transmitting the known form of nervous activity, in separate impulses, across the synapse.

Certain difficulties must be briefly considered. Kibjakow, whose technique of perfusion we have in essentials adopted, used plain Locke's solution, without eserine. Under such conditions, he obtained, during preganglionic stimulation, a venous effluent which, on arterial reinjection, caused good contractions of the nictitating membrane. Without eserine in the perfusion we never obtained a venous fluid having any stimulating action on ganglion cells, or on any other reactive organ on which we tested it. Under Kibjakow's conditions acetylcholine does not, in our experience, appear in the venous fluid, and, if it did, it would not be

effective on reinjection. Without eserine in the perfusion, arterial injection of acetylcholine is only effective in concentrations far greater than the highest we obtained, with eserine, in the venous fluid. Although we used his method, Kibjakow's results seem to have little relation to ours, and we are frankly unable as yet to explain them.

The demonstrated action of eserine, in protecting acetylcholine on its way from or to the ganglion cells through the blood vessels of the ganglion, raises the question of its influence on the effect of preganglionic stimulation. We found it difficult to predict what its action would be. Its potentiation of effects like those of the heart vagus, where the effect of one impulse on the rhythm is prolonged, and that of a series rendered more intense by summation, owing to the delayed enzymatic destruction of acetylcholine after release, seemed to provide no obvious analogy for its probable effect on transmission through the ganglion. It seemed that, in this case, eserine could only potentiate the effect of a preganglionic impulse, or of a series of such impulses, if it enabled a larger number of ganglion cells to be effectively stimulated. Some experiments, still in progress, by one of us (W. F.) with A. Vartiainen, appear to show a pronounced potentiation by perfusion of a very dilute (1 in 10^6) solution of eserine through the ganglion, the responses to successive short groups of submaximal shocks to the preganglionic nerve being recorded. Earlier experiments, with continued perfusion of stronger solutions of eserine had shown depression of the response as the principal effect. This secondary depressant effect of eserine is the only one detected by Eccles [1934], in his experiments recording the action potentials set up in the ganglion and its postganglionic branches by single and repeated volleys in the preganglionic nerve. His conclusion, from this evidence, that "the liberation of acetylcholine is therefore not responsible for the transmission of impulses through the ganglion," seems to us premature at the present stage. We still believe such transmission to be the most reasonable interpretation of our own results, including the few which we have completed on the action of eserine.

It is tempting to generalize from these conclusions and to suggest that all preganglionic autonomic nerve fibres may be cholinergic [Dale, 1933]. If this is true of parasympathetic as well as sympathetic nerves, the question arises whether the "Vagusstoff" of Loewi may not have been liberated at ganglionic synapses. It is very improbable, however, that it was entirely, or even largely derived from this source. The effects of eserine and atropine on the action of the heart vagus, as well as the analogy from Engelhart's [1931] experiment on the pupil, strongly

support the view that the postganglionic vagus fibres to heart muscle are cholinergic. But it is still possible, and even probable, that the preganglionic vagus fibres are also cholinergic, and, since their ganglionic synapses lie in the heart tissue, their mechanism may also make some contribution to the "Vagusstoff."

Summary.

1. The superior cervical ganglion of a cat was perfused with Locke's solution containing eserine, and pharmacological tests were applied to the fluid emerging from the vein.

2. Stimulation of the cervical sympathetic caused the liberation from the ganglion of a substance which was pharmacologically identified as acetylcholine. The identification depended on quantitative agreement between estimates, obtained by six different methods, of the potency of the active solutions relative to that of acetylcholine.

3. These observations support the theory that the mechanism by which each nerve impulse normally passes the synapse consists in the liberation of a small quantity of acetylcholine.

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