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THE CHEMICAL TRANSMITTER OF VAGUS EFFECTS TO THE STOMACH.

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Since Loewi [1921] first demonstrated the chemical transmission of the inhibitor effect of vagus impulses on the frog's heart, a similar mechanism has been recognized in the transmission of other peripheral parasympathetic effects. The reluctance of many investigators in this field to identify the transmitter as acetylcholine itself, and still to speak of it as "acetylcholine-like," can be justified by the absence, hitherto, of direct chemical evidence of identification. The resistance of certain parasympathetic effects to atropine, which easily annuls the corresponding effects of acetylcholine, has seemed to others to impose an even stronger objection to such identification; and it must be admitted that an assumption that acetylcholine acts as the general parasympathetic transmitter requires additional suppositions to explain this anomalous difference [cf. Dale, 1929; Dale and Gaddum, 1930]. It should, however, be noted that the action of atropine, if regarded as decisive against acetylcholine itself, is equally so against any choline derivative; so that, if the transmitter on this account is held not to be acetylcholine, it cannot be "acetylcholine-like." Among the parasympathetic effects often cited in connection with this difficulty caused by resistance to atropine, are those of the vagus and the pelvic nerves on the muscular walls of the alimentary canal and the bladder. We decided to attempt to obtain the chemical transmitter effective in these actions, and to test its properties. Hitherto our observations have only been completed for the action of the vagus on the stomach. This action has, for the purpose in view, two disadvantages: the action of the vagus on the stomach muscle is not very highly resistant to atropine, and we have not found it possible to separate the circulation of the muscular wall from that of the mucous membrane, on the glands of which the vagus has a secreto-motor action. On the other hand, the augmentor action of the vagus on the activity of the small intestine, which resists atropine in larger doses, presents additional difficulties for our purpose, in that it has not the same definite relation

to the period of stimulation as has that on the stomach. It seems desirable, therefore, to publish the completed experiments on the gastric vagus, and to leave open to further investigation the less clear-cut effect of this nerve on the small intestine, and the action of the pelvic nerve on the colon.

Methods.

(1) *Natural circulation.* Dogs have been used as subjects of all the main experiments. After preliminary anæsthesia with ether, under which the required cannulæ were inserted, a 1 p.c. solution of chloralose in warm saline was given by slow intravenous infusion to a total of 15–20 c.c. per kg., according to the amount needed to produce a deep anæsthesia, which was maintained by a further infusion, when necessary, in the later stages of a long experiment. The abdominal cavity was opened by a median incision, and with successive ligature of the arteries and veins the whole of the large and small intestines was removed, nearly up to the pylorus. Care was taken in removing the duodenum, which was ligatured a few centimetres below the pylorus, to preserve any branch from the pancreatico-duodenal artery to the pyloric end of the stomach. The main gastric artery to the fundus, and the gastric veins draining into the splenic vein, were carefully preserved in the subsequent removal of the spleen and the remainder of the pancreas. A cannula, filled with saline solution and provided with a clamped rubber tube, was now inserted into the lower end of the portal vein, just above its formation by union of the mesenteric veins. Opening of the clamp on the tube leading from this cannula, accompanied if necessary by compression of the portal vein just below the liver, led to the outflow for collection of venous blood coming entirely from the stomach.

The abdominal wall was now closed, leaving egress for the tube leading from the portal vein. The dog being turned on its right side, a window was made in the left side of the lower chest wall by removing the middle portions of two or three ribs, with the attached intercostal muscles, care being taken to secure complete hæmostasis. The vagi were then dissected off the wall of the œsophagus from just below the roots of the lungs to about 1 cm. above the diaphragm. They were tied and cut above, and their peripheral ends prepared so that they could be laid together across a pair of chlorided silver-wire electrodes, when this was inserted through the window in the chest by a long insulating holder.

The blood of the animal was now rendered incoagulable by an intravenous injection of heparin, or of the dye "chlorazol-fast pink" [see Huggett and Rowe, 1933], which we have found very effective and

suitable for experiments of this kind. Eserine was injected, in a quantity judged to be sufficient to protect acetylcholine or a similarly sensitive choline ester from destruction by the blood esterase, during passage from the site of liberation to the collecting cannula. As will be seen, it is doubtful whether more than partial protection was ever achieved. With the eserine, in a dose of 1–2·5 mg. per kg. of body weight, we gave a small dose—usually a total of 0·5 mg.—of atropine, to prevent a retardation of the heart beat by the eserine sufficient to impair the circulation. The first control sample of portal (gastric) blood was taken about 10 min. after administration of these alkaloids, to allow the inhibitory effect of eserine on cholinesterase to become fully established. Each sample, consisting of 10 c.c. of blood, was taken in measured time into a graduated cylinder, which already contained 0·2 c.c. of 1:1000 eserine, so that the completed sample contained 1 : 50,000 of additional eserine. The activity of the samples was, so far as possible, tested without delay, but, when delay was necessary, the cylinders containing them were immersed in pounded ice. An actual trial showed that a sample containing 1 : 50,000 eserine, and thus cooled to about 0°, could be kept for even an hour without significant loss of activity.

(2) *Artificial perfusion.* As for experiments with natural circulation, the dog was anæsthetized with ether followed by chloralose, and the dissection for removal of the intestines, spleen and pancreas followed a similar course. Great care was taken to tie even the smallest vessels, and the omentum was removed, except for the strip involving the veins draining the stomach into the splenic vein. The hepatic artery was now doubly ligatured near to the liver and cut across, and a ligature laid ready for tying round the portal vein at the same level. The cœlic axis, with the gastric and hepatic arteries, now its only functional branches and supplying only the stomach, was dissected clear from its bed and from the surrounding solar plexus, and made ready for rapid insertion of a perfusion cannula, and for removal from the body immediately thereafter. The chest was then opened widely, under artificial respiration, by splitting the sternum, and the vagi were tied and cut just below the roots of the lungs, and dissected clear from the œsophagus to just above the diaphragm. The œsophagus was then opened, and a wide glass cannula was inserted so as to project into the lumen of the stomach through the cardiac orifice, the œsophagus being tied firmly round the neck of the cannula. The glass cannula was made long enough at the other end to project through the opening of the thermostat chamber, to which the isolated stomach was later removed.

The arterial perfusion cannula was now inserted into the cœliac axis, the portal vein tied near the liver, the cannula in the lower end of the portal vein opened, and perfusion of the stomach with Locke-Ringer solution was begun. The perfusion was carried out by a single-chamber Dale-Schuster pump, immersed in a water bath at about 39° C. A long rubber tube led the warm Locke's solution to the arterial cannula, while the stomach with its attachments was being removed from the body and transferred to the thermostat chamber. This was essentially the chamber described and figured by Bauer, Dale, Poulsson and Richards [1932] for perfusion of the isolated liver. In the present case the wide glass tube, tied into the lower end of the œsophagus, passed out through the gap on one side of the ebonite rim of the chamber, to be fixed by a clamp. Through the same gap passed the insulating holder of the stout silver electrodes, across which the vagi were laid inside the chamber. Through the gap in the opposite side of the rim passed the long beaks of the arterial and venous cannulæ, and a string by which the pylorus was anchored to an external support. The stomach lay in the chamber on a sling of netting, fixed at its cardiac and pyloric ends. The gaps in the rim being filled with putty, the chamber was now closed by the glass lid, previously warmed, and the temperature inside it rapidly rose to about 38° C., which was also that of the solution in the arterial cannula. The air in the chamber was enriched by a stream of oxygen, which bubbled through warm Ringer's solution before entering the air space. The arterial pump was adjusted to give a moderate rate of perfusion, 1 c.c. flowing from the venous cannula in about 2·5–3 sec., or 100 c.c. in 4–5 min.

Only a few minutes elapsed between the removal of the stomach from the body and the closure of the thermostat chamber, and not more than a momentary interruption of the arterial perfusion occurred during the transfer. A change was now made from the plain oxygenated Locke's solution used for this preliminary perfusion to similar Locke's solution containing 1 part of eserine in 300,000–500,000. This caused well-marked spontaneous contractions of the whole stomach, which subsided with continued perfusion. Samples of the control venous fluid could then be taken, and other samples during stimulation of the vagi.

Results.

(1) *Natural circulation.* Section of the vagi cannot be regarded as completely stopping parasympathetic stimuli to the muscle coats of the stomach wall. The ganglionic plexus between the muscle coats, with the

cells of which the preganglionic fibres of the vagus are generally supposed to make connections, keeps the stomach wall in spontaneous activity, apart from impulses from the vagi. That this spontaneous activity is maintained by peripheral liberation of a choline ester is suggested by its intensification by eserine. It is not surprising, therefore, to find that the control samples of portal blood, collected after eserine has been administered, already contain recognizable amounts of a substance resembling acetylcholine in action, when tested on the eserinized leech muscle or on the blood-pressure of the anæsthetized cat. It had already been shown by Feldberg and Rosenfeld [1933] that the portal blood in an animal with intact intestines may show clear evidence of the presence of a substance acting like acetylcholine, after a large dose of eserine has been injected, and without any nerve stimulation. We could not, therefore, in the case of the stomach, obtain a clear-cut difference between "resting" venous blood and "active" venous blood; for there is no resting state of the organ. What we could expect to find was a quantitative difference, in the direction of an increase of the activity due to the chemical transmitter, in the blood taken during effective vagus stimulation, as compared with that of the blood taken before vagus stimulation, or again at a later stage, when the activity due to the stimulation had subsided. Such differences we regularly observed; but we found, in the course of the earlier experiments, that in order to obtain a large difference, so that the activity of the "vagus" blood was $2\frac{1}{2}$ to 4 times that of the "control" blood, it was necessary to stimulate the vagus strongly, so that the stomach wall was thrown into vigorous contractions.

An extract from a protocol will conveniently indicate the procedure and the nature of the results of an experiment.

Exp. 1. Dog. 13 kg.

Experiment begun 10 a.m. Anæsthesia and dissection as under "Methods."

1 p.m. A cat was anæsthetized with ether followed by chloralose and prepared for testing samples on blood-pressure. Two symmetrical strips of the body wall of a leech suspended in aerated Ringer solution and sensitized by addition of 1 : 200,000 eserine. Eserine in a concentration of 1 : 50,000 was added to all blood samples as collected, except when otherwise stated.

1.50 p.m. Blood of dog made incoagulable with "chlorazol-fast pink." Samples of portal blood, tested in 1 in 4 dilution had a slight, non-specific action on the leech preparations with a first, but not with a renewed application.

2.8 p.m. 0·5 mg. atropine and 30 mg. eserine intravenously.

2.20 p.m. Control sample I of portal blood. Tested on leech. In 1 : 4 dilution it produces a rapid contraction (Fig. 1, *A*), corresponding to that earlier produced by A.C.[1] $1 : 200 \times 10^6$. The concentration in the undiluted blood is, therefore, about $1 : 50 \times 10^6$.

[1] The symbol A.C. is here used for brevity to represent acetylcholine.

2 c.c. undiluted blood, injected intravenously into cat, produce just perceptible fall of arterial pressure. Effect too small for accurate measurement.

2.43 p.m. Control sample II. Tested on leech in 1 : 10 dilution (probably about 1 : 500 × 10^6 A.C.) causes very small slow contraction (Fig. 1, *B*).

2.50–2.52 p.m. Stimulate vagi in thorax, with secondary coil at 6 cm. Powerful and continuous contractions of stomach wall. Blood samples III and IV collected during, and sample V after end of stimulation. Samples III and V were collected with the normal addition of 1 : 50,000 eserine, and placed in ice. Sample IV was collected without additional eserine and kept at room temperature.

2.52 p.m. Sample III tested for action on cat's blood-pressure in comparison with a standard solution of A.C., 1 : 10^7. Repeated doses of 2 c.c. of the undiluted blood produced falls of arterial pressure of the same depth as those produced by 0·1 γ of A.C. After 0·1 mg.

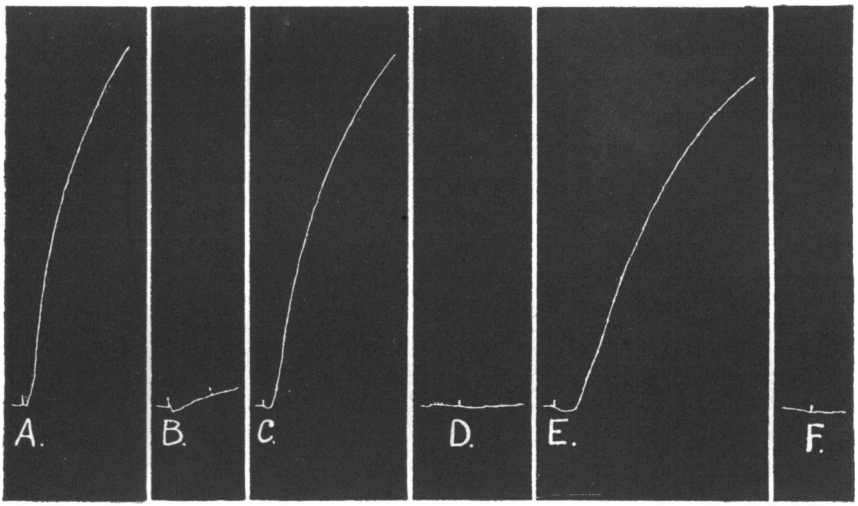

Fig. 1. Leech muscle preparation in eserine 1 : 200,000. See protocol of Exp. 1.

of atropine a further 2 c.c. of blood caused only a slight rise of blood-pressure. The action of the blood would be accounted for by the presence in it of A.C. to the extent of 1 : 20 × 10^6.

2.53 p.m. Sample III tested on the leech in 1 : 10 dilution, causing a contraction (Fig. 1, *C*) similar to that (Fig. 1, *A*) caused by sample I in 1 : 4 dilution. Again the activity of sample III is estimated at 1 : 20 × 10^6 A.C., or 2½ times that of sample I.

3.35 p.m. Sample IV, kept at room temperature without additional eserine for 43 min., tested on leech in 1 : 10 dilution. No effect (Fig. 1, *D*).

3.51 p.m. Sample V, kept at 0° C. with added eserine for nearly 1 hour, tested similarly in 1 : 10 dilution, producing a contraction (Fig. 1, *E*), only slightly less rapid than that in Fig. 1, *C*. Sample V, collected just after stimulation, may have been initially a little less active than sample III; or the leech preparation may have been declining in sensitiveness. Fig. 1, *D* and *E* show, in any case, that the substance appearing in the gastric venous blood on vagus stimulation is relatively stable at 0° C. in the presence of additional eserine, and rapidly disappears from the blood at room temperature and without added eserine.

3.53 p.m. Another control sample, VI, tested on the leech in dilution 1 : 10, had no

effect (Fig. 1, *F*). In 1 : 4 dilution it produced a contraction comparable to that of Fig. 1, *B*. Probably its activity, therefore, was not much higher than that of A.C. $1 : 100 \times 10^6$.

4.2–4.3½ p.m. Renewed vagus stimulation, during which sample VII of portal blood was collected. Tested on the leech, in comparison with dilutions of acetylcholine, it produced a contraction corresponding to an original concentration of A.C. $1 : 25 \times 10^6$.

Experiment terminated.

It will be seen that such an experiment demonstrates that:

(1) There appears in the portal (gastric) venous blood, after injection of a large dose of eserine, a substance not previously present, which has on the eserinized leech muscle a stimulating action comparable to that of acetylcholine in high dilution.

(2) On stimulating the vagi this activity of the portal blood shows a 2½ fold increase, and the blood now shows a depressor action on the cat's blood-pressure, matched by acetylcholine in a concentration identical with that matching the stimulant action on the leech. This action on the blood-pressure is completely abolished by a small dose of atropine.

(3) An hour after the stimulation, the specific activity of the portal blood is lower than before stimulation. Renewed stimulation of the vagi raises it again to a level close to that attained in the first stimulation.

(4) The substance appearing in the blood on vagus stimulation disappears rapidly from the shed blood at room temperature, but it persists for one hour, with little or no loss, if the blood is treated with eserine and kept at 0° C.

In another experiment a sample of the gastric venous blood, collected during vagus stimulation under conditions similar to the above, was tested in the same (1 : 10) dilution on two symmetrical preparations from the same leech, of which one had been given preliminary treatment for half an hour with 1 : 100,000 eserine, while the other was suspended in plain Ringer's solution. The results are shown in Fig. 2, *A* and *B*. It will be seen that the characteristic stimulant action shown in record *A* is entirely absent in *B*. Although the blood contained eserine in sufficient quantity, even after dilution, eventually to sensitize the leech, the process is a slow one.

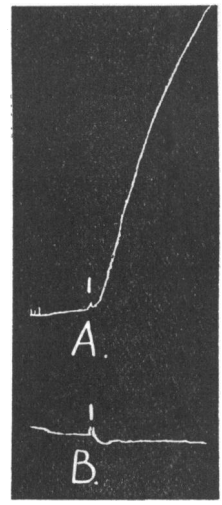

Fig. 2. Symmetrical strips of leech, *A* in eserine 1 : 100,000, *B* in plain Ringer. Application to both of "vagus" blood samples diluted 1 : 10.

The substance appearing in the blood from the stomach during vagus stimulation is, accordingly, one which is destroyed

by the blood, but protected by addition of eserine, and one of which the stimulating action on leech muscle is very much enhanced by preliminary treatment of the muscle with eserine.

In another experiment, otherwise similar to that described above in detail, a smaller dose of eserine (1 mg. per kg.) was injected into the dog, and the control sample of gastric venous blood showed a lower activity, corresponding, in the test on the eserine-treated leech approximately to that of A.C. $1 : 200 \times 10^6$. The activity of the blood collected during vagus stimulation showed in this case a fourfold increase, corresponding to A.C. $1 : 50 \times 10^6$. The same samples, and another collected during a subsequent period of vagus stimulation, were tested more thoroughly on

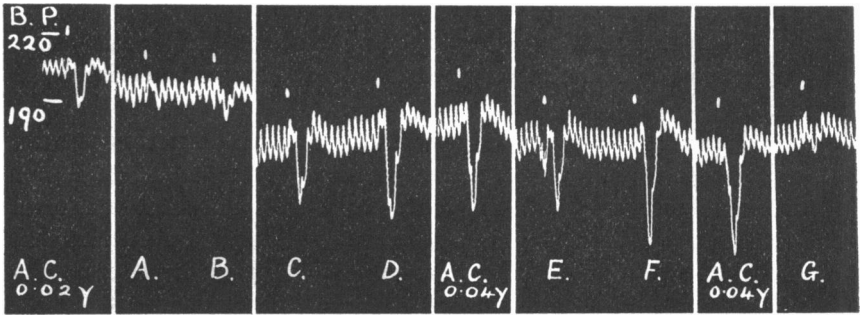

Fig. 3. Cat under chloralose. Carotid blood-pressure. At *A* and *B* 1 and 2 c.c. of control blood, at *C* and *E* 1 c.c., at *D*, *F* and *G* 2 c.c. of two "vagus" samples. Before *G* 0·1 mg. atropine. For details see text.

the blood-pressure of a cat under chloralose, an attempt being made to balance their activities against those of known doses of acetylcholine. Fig. 3 shows the result. Injections at *A* and *B*, of 1 and 2 c.c. respectively of the control portal blood, produce effects too small to be balanced accurately against acetylcholine. It can safely be estimated, however, that the effect of 2 c.c. at *B* is less than that of the preliminary dose of $0{\cdot}02\gamma$ A.C.; so that the blood is definitely weaker than A.C. $1 : 100 \times 10^6$, and probably not stronger than A.C. $1 : 200 \times 10^6$, which matched it in the test on the leech. The effect, at *C*, of 1 c.c. of blood collected during the first vagus stimulation, and at *D* of 2 c.c. of the same blood, can be compared with that of $0{\cdot}04\gamma$ A.C. which follows them immediately; and it can be seen that 1 c.c. causes a smaller, and 2 c.c. a somewhat larger effect than the control dose of A.C. This sample, accordingly, is somewhat stronger than A.C. $1 : 50 \times 10^6$. At *E* and *F* injections of 1 and 2 c.c.

were made of a sample collected during a second period of vagus stimulation. In this case the effect of 2 c.c. is equal to or very slightly less than that of 0·04γ A.C. We may tabulate the results of these estimates as follows.

Blood sample	Equivalent activity in concentration of A.C.
Control	$< 1 : 100 \times 10^6$; not $> 1 : 200 \times 10^6$
1st vagus sample	$\leqq 1 : 50 \times 10^6$
2nd ,,	$\leqq 1 : 50 \times 10^6$

In both vagus samples, therefore, the activity on the cat's blood-pressure, as on the leech muscle, was approximately 4 times that of the control sample, and matched the same concentration of A.C. as in the leech experiment.

Finally the cat received an intravenous injection of 0·1 mg. of atropine, and, at *G* in Fig. 3, a further injection of 2 c.c. of the second sample of vagus blood, similar to that given at *F*, was now without significant action.

(2) *Artificial perfusion.* The general results of these experiments were very similar to those with natural circulation. The method had certain advantages, however, which enabled us to make some additional tests. The rate of the perfusion could be very closely controlled by adjustment of the stroke of the pump. With natural circulation we were, of course, obliged to take the venous blood at the rate at which it entered the portal vein. We did not, in fact, observe any important change in the rate during vagus stimulation; but the rate of outflow, both in control and vagus periods, was only controlled, and then not very exactly, during the time of collection of a 10 c.c. sample in each case. With artificial perfusion the whole effluent could be collected for any required period. We regularly, indeed, took a sample of 100 c.c. of control fluid, recording accurately its time of collection, and again a sample of 100 c.c. during vagus stimulation. The outflow under the latter conditions showed an obvious retardation during the initial strong contractions caused by the onset of vagus stimulation. This might be compensated naturally by accelerated flow later in the period; if not, the stroke of the pump was slightly increased, so as to make the time for collection of the full sample of 100 c.c. practically equal to that for the control sample.

As to the direct comparisons of the activities of the control and stimulation fluids, as collected, it need only be said that they showed similar ratios to those obtained for the corresponding samples of blood with natural circulation, varying from about 1 : 2·5 to 1 : 4 in different experiments. The estimates in terms of acetylcholine, also, corresponded

again for the "muscarine" action on the cat's blood-pressure, and the "nicotine" action on the leech. The fluid being Locke's solution instead of blood, it was further possible to use the isolated frog's heart for direct comparisons, the fluids being diluted with water to 1·4 times their volume, and tested on a heart previously beating on the unperfused eserinized Locke's solution diluted to the same extent. Fig. 4 shows the effects on such a heart, at *A* of control fluid collected before stimulation, at *B* of fluid collected during it, at *C* of fluid collected, 8 min. after it ended, at *D* of fluid collected during a second period of vagus stimulation,

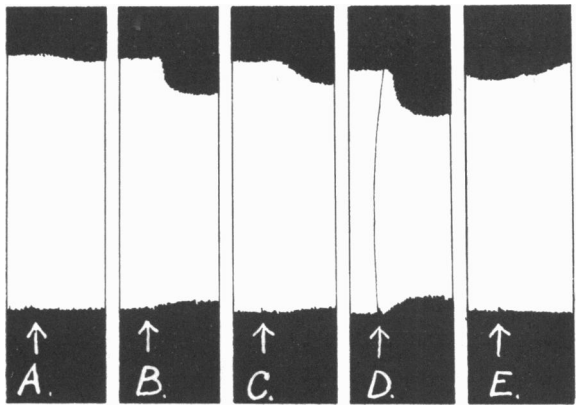

Fig. 4. Isolated frog's heart (Straub). Ringer contains 1 : 700,000 eserine. Tested with perfusion effluents collected: *A*, before stimulation; *B*, during first vagus period; *C*, 8 min. later; *D*, during second vagus period; *E*, same as *D*, after atropine.

and at *E* of the same fluid as at *D*, after treating the heart with a trace of atropine.

The main advantage of the experiments with artificial perfusion, however, was the possibility of collecting a comparatively large volume of fluid, containing the transmitter of the vagus effect in a relatively clean solution. The saline effluent containing it could be taken to dryness by evaporation under diminished pressure, the inorganic salts eliminated, except for traces, by extracting the carefully dried residue with absolute alcohol, and, after removal of the alcohol by renewed evaporation, the transmitter could be obtained in solution at any desired concentration, and in a quantity sufficient to enable its activity to be determined within fairly narrow limits, in terms of acetylcholine, by a series of different physiological reactions. For this purpose the venous effluents were

collected in graduated vessels already containing the small volume of $N/10$ HCl required to bring the reaction of the total quantity collected to about pH 4, at which acetylcholine is very stable in watery solution. Sufficient eserine being present to suppress the action of any cholinesterase, such fluids could be evaporated under diminished pressure, the process being at intervals interrupted to adjust the reaction, so that the acidity did not seriously exceed pH 4.

In this way samples of 100–200 c.c. of control and stimulation fluids were treated, so that the activity was contained in any desired fraction of the original volume of Locke's solution, without excess of salt. The eserine present in the original fluid was not eliminated; but even assuming that it was concentrated without destruction, an original concentration of $1 : 5 \times 10^5$, which was adequate to protect the transmitter under the conditions described, even if concentrated tenfold to $1 : 5 \times 10^4$, would still give a practicable concentration for presensitizing preparations of leech muscle, or of the skeletal muscle or heart of the frog, and would have no disturbing effect on tests made with intravenous injections into a cat under chloralose. Tests on the control and stimulation fluids, concentrated in this way, showed that they had preserved the original ratio of their activities, with but little absolute loss. Attention was accordingly directed, in two experiments, to a more careful determination of the activity of the fluid collected during stimulation, in a series of different physiological reactions, in terms of acetylcholine. The two experiments gave quite similar results, and it will suffice to record them in greater detail and illustrate them in the case of one.

In the particular experiment from the records of which Fig. 5 was constructed, the unconcentrated control and stimulation fluids gave the effects on the frog's heart shown in Fig. 4. A relatively low concentration (1 : 500,000) of eserine had been used for the perfusion, and even the stimulation fluid had an activity of only about A.C. $1 : 80 \times 10^6$. After evaporation to dryness, however, and removal of salts, a solution having one-third of the original volume, and therefore about three times the original activity, was found suitable for the test on a cat's arterial pressure. For the tests on the eserinized frog's heart and skeletal (rectus abd.) muscle the concentrate was further diluted with 40 p.c. of its volume of distilled water. For the test on the sensitive leech preparation it required further dilution with 5 times its volume of the appropriate Ringer's solution—*i.e.* to less than half the original strength. The strength of the threefold concentrate was first determined on the cat's blood-pressure. In Fig. 5 *a*, *A* and *C* show the effects of injections of 1 c.c. each of

A.C. $1 : 40 \times 10^6$ and $1 : 20 \times 10^6$ respectively, and *B* that of 1 c.c. of the concentrate. It will be seen that the action of the latter is "bracketed" by those of the two doses of acetylcholine, and, so far as can be estimated, lies about midway between them. For the other tests these two stock dilutions of acetylcholine were diluted in exact parallel with the concentrate, so that the ratios between the doses giving the different effects at *A*, *B* and *C* remained constant, at the different dilutions above indicated. It will be seen that the effect at *B* is similarly bracketed by those at *A* and *C* in every test, and that the activity of the concentrate is approximately midway between those of the two dilutions of acetylcholine. We may conclude, accordingly, that the activity of the transmitter, released by stimulation, is constant in relation to that of acetylcholine in all four tests, and that the activity of the concentrated effluent, before further dilution, was in each case approximately equivalent to that of A.C. $1 : 30 \times 10^6$.

In two experiments the stability of the transmitter was tested by making the solution strongly alkaline and acid respectively; with the result that the activity disappeared rapidly from the alkaline solution at room temperature, but remained practically unchanged in the acid solution under the same conditions.

One further experiment may be mentioned, in which the concentrated stimulation fluid, when injected into the aorta through the stump of the cœliac artery in an eviscerated cat, produced an output of

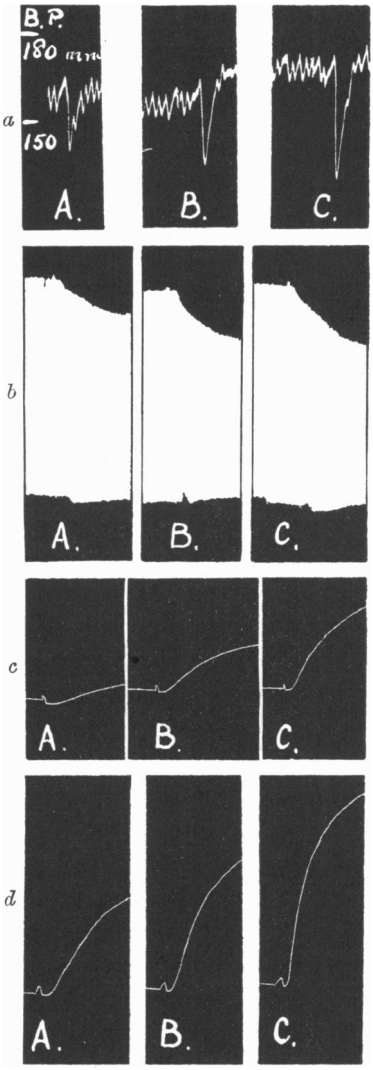

Fig. 5. Tests of concentrated vagus effluent on: *a*, blood-pressure of cat under chloralose; *b*, frog's heart (Straub); *c*, frog's rectus abdominis; *d*, leech muscle. In each case *B* shows the effect of the perfusion concentrate, given in constant ratio to two strengths of A.C., at *A* and *C*, that at *C* being double that at *A*. See text.

adrenaline from the suprarenal gland, exactly comparable to that produced by the corresponding dose of acetylcholine [cf. Feldberg and Minz, 1931].

Discussion.

The active substance which is found already in the venous effluent from the stomach under eserine, in the absence of vagus stimulation, shows such a pronounced increase—as much as fourfold—during vagus stimulation, that there is no reason for doubting its function as the transmitter of the effects of vagus impulses to the active structures of the stomach wall. In all the properties which a variety of tests revealed, this substance was found to be indistinguishable from acetylcholine. Such properties are its rapid destruction by blood in the absence of eserine; the great increase of its activity on different tissues caused by treating them with eserine; the constant quantitative relation of its activity to that of acetylcholine in different tests, some depending on the "muscarine action" others on the "nicotine action" of that substance; the annulment of its "muscarine" actions by small doses of atropine; and its instability to alkali, even in the presence of a protective concentration of eserine. There is no reason to doubt that it is acetylcholine, though our hope that, by artificial perfusion, we might be able to obtain the transmitter in sufficient concentration to justify an attempt at chemical isolation, has not been realized.

The point of liberation of this acetylcholine by impulses in the vagus is less certain. We have already mentioned the possibility that it might come from the mucous membrane, rather than the muscle coats. We regard this possibility as discounted by the fact that we only observed a large increase in the output when stimulation of the vagus threw the muscular wall into vigorous contraction. The fact that, as McSwiney [1933] has shown, the augmentor effect of vagus impulses on the stomach musculature is enhanced by eserine, was already in favour of the transmission of such effects by a choline ester. Even if we assume, however, that vagus impulses concerned with increase of muscular activity are responsible for liberation of at least an important part of the acetylcholine detected, we must still, in the light of the latest evidence [cf. Feldberg and Gaddum, 1934], consider whether the release occurs in transmitting preganglionic impulses to the ganglionic plexus, or in transmitting the effect of postganglionic impulses to the plain muscle fibres. It is possible that both may be concerned. The considerations suggested by Feldberg and Gaddum (*loc. cit.*) as to the origin of the acetylcholine released in the heart by vagus impulses, apply probably with greater

force to the case of the stomach wall, with its rich ganglionic plexus. It must be admitted, however, that the probabilities with regard to several of the points involved are derived from what may be misleading analogies. Thus, it is probable that parasympathetic preganglionic fibres are cholinergic, as those of the true sympathetic system are now known to be on direct evidence; and it is probable that the action of acetylcholine on parasympathetic ganglion cells is of the "nicotine" type, resistant to atropine, as it was long ago shown to be on true sympathetic ganglion cells [Dale, 1914]. Neither of these probabilities, however, has been brought to the test of direct experiment, and the difficulties of doing so will not be readily surmounted. We must be satisfied meanwhile to regard it as probable that the transmission of preganglionic vagus impulses to the cells of the ganglionic plexus in the stomach, and that of post-ganglionic impulses to the plain muscle cells, are both dependent on release of acetylcholine, and that the sensitiveness of this vagus effect to atropine is due to blockage of the "muscarine" effect on the plain muscle, rather than of the transmission at the ganglionic synapses.

It should be emphasized that we have not observed any kind of physiological activity in the venous effluent from the stomach, increasing with vagus stimulation, except that due to a substance indistinguishable from acetylcholine; and, further, that this increase of acetylcholine in the venous fluid must correspond to such an increase at the actual site of liberation as would account for all the augmentor effects of the vagus on the activity of the stomach, with which we have been concerned. There is no reason for differentiating these effects from those of the vagus on the heart, with regard to the chemical mechanism of their transmission. If acetylcholine is accepted as the transmitter in both these cases, it may further be regarded as very improbable that the mechanism should suddenly change at the pylorus, and that the augmentor effects of the vagus on the activity of the small intestine should be transmitted by a radically different method. The evidence now obtained as to the method of transmission in the stomach, seems to us, therefore, to weaken further the significance which some have accorded to the relatively high resistance to atropine of the effects of the vagus on the small, and of the pelvic nerve on the large intestine.

Summary.

The venous blood, or perfusion fluid, from the stomach wall contains acetylcholine in recognizable concentration, when eserine has been injected or added to the perfusion.

Stimulation of the thoracic vagi, adequate to cause contractions of the stomach wall, causes a manifold increase in this acetylcholine.

Acetylcholine acts as the chemical transmitter of vagus effects to the stomach.

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