A METHOD OF DEMONSTRATING THE HUMORAL TRANSMISSION OF THE EFFECTS OF CARDIAC VAGUS STIMULATION IN THE FROG. By W. A. Bain. From the Department of Physiology, University of Edinburgh. (With three figures in the text.)

(Received for publication 12th September 1932.)

Many who have attempted to repeat Loewi's (1) fundamental work on the frog-heart have found it difficult to get confirmative results, and some have failed to get any results at all. Among these are Asher (2), Heymans and Heymans (3), Hermann and Malméjac (4), and Tournaire, Chabrol, and Malméjac (5). Others again, while obtaining a percentage of positive results, are unable, for various reasons, to accept Loewi's conclusions. Among these are Lambert, Hennequin, and Merklen (6). They oppose Loewi mainly on account of their consistent failure to obtain the humoral transmission of a negative chronotropic effect, which they hold is the indication par excellence of vagal activity and hence of the presence or not of the supposed "vagus substance."

In view of the admitted difficulty of obtaining positive results in such experiments and, in particular, the difficulty of demonstrating chronotropic effects, it may be useful to describe a method whereby the humoral transmission of the effects of vagus stimulation, especially the chronotropic, may be more easily and definitely demonstrated than heretofore.

Loewi, in his experiment, collected the fluid from a vagus-stimulated heart and applied this to the same or to another heart. He considered that it was important in such experiments to employ the smallest possible quantity of fluid in the nerve-stimulated heart, his idea being that the smaller the quantity of fluid in contact with the heart the more concentrated would be the resulting neuromimetic fluid, and thus the more definite the effects on the heart to which this was applied.

Nevertheless it would appear that in Loewi's method a considerable amount of the vagus substance formed during stimulation must be destroyed, before it can be applied to another heart, by the mechanism known to exist in the heart for that purpose. From this consideration it appeared possible that better results might be obtained by a method
in which the irrigating fluid was passed somewhat rapidly through the
heart in the hope that the "vagus substance," almost as soon as it is
formed, would pass into the irrigating fluid and thus away from at
least one of the factors operating for its destruction.

It was desirable that the method should be a "self-recording" one,
the Ringer flowing continually from donor to receiver heart even during
vagus stimulation and consequent arrest of the donor. But in no
technique previously used has it been possible to obtain complete
stoppage of the donor heart without interference with the supply of
Ringer to the recipient heart. For example, in Ten Cate's (7) method,
which most nearly resembles the one about to be described, stoppage of
the donor heart involves a complete cessation of the supply of fluid to
the recipient heart. This difficulty has been got over by the use of a
special cannula which permits a continuous flow of fluid through the
donor heart even when this is at a standstill as a result of vagus
stimulation.

**Method as Applied to the Heart.**

The method employed by me consists in washing Clark's (8)
solution through a donor heart by means of a double Kronecker cannula
so arranged that the fluid, entering by one limb, irrigates part of the
inner surface of the heart—usually the sinus venosus and auricles—
passes out by the second limb of the cannula, and is then immediately
applied to a recipient heart. The cannula is depicted in fig. 1 (E), which
shows the general arrangement of the experiment.

I used at first for the donor heart a simple preparation, consist-
ing only of the isolated heart with its vago-sympathetic cardiac nerve
on each side intact. This proved in some respects unsatisfactory in
view of the intermixture of the vagus and sympathetic in that nerve,
and in later experiments a more complicated preparation was employed.
This consisted of the heart, the medulla oblongata in situ, and the
tissues surrounding the nerves between their cranial exit and the heart.
The nerves are not cleared in this preparation because they are more
easily kept moist and less liable to damage if the surrounding tissue is
left. The vagus is stimulated at its origin by shielded electrodes placed
in contact with the medulla oblongata.

The main necessity in obtaining and fitting up the preparation is
speed. In my experience it is unusual to get a good response from
stimulation either of the medulla oblongata or of the vago-sympathetic
cardiac nerve after thirty minutes have elapsed from the commencement
of the dissection. Whether this is due to an effect on the nerve fibres,
the nerve terminations, or something peripheral to these has not been
determined.

The vae cave having been tied off, the double cannula, as shown in
the diagram (fig. 1), is introduced into the sinus venosus and secured
there by a ligature. Alternatively the cannula may be introduced into the left auricle through an incision in its wall, the inter-auricular septum

being severed, the sinus-auricular valves obliterated, and the venæ cæ
tied off as before.
The inlet tube of the cannula is attached to a perfusion apparatus so arranged that the hydrostatic pressure of the fluid supplied to the donor heart can be adjusted from about 5 cm. to about 20 cm. and maintained constant at any desired level between these limits. This perfusion apparatus is connected with a large reservoir. Clark's solution is supplied to the donor heart and the perfusion pressure adjusted so that fluid drops at a regular rate from the output limb of the cannula, and, even when the heart is completely stopped, continues to fall in amount not appreciably different from before.

A second heart—the receiver—is attached to a short glass tube (H) widely open above but drawn out below to a small cannula which is tied into its sinus venosus. The fluid issuing from the outlet limb of the double cannula in the donor heart is led to this short glass tube, and hence to the recipient heart, by a piece of fine "drainage" tubing, and fills this up to the level of a lateral overflow tube so that the hydrostatic pressure is kept constant. The open end of this piece of tubing dips below the surface of the fluid in the glass tube, H. Care is taken that sufficient fluid is supplied to this tube to ensure a slow drip from the overflow even when the donor heart is quiescent as a result of nerve stimulation. Each heart is attached by a thread to its recording lever, connection between thread and heart being made by a small silver clip.

When both hearts are functioning regularly the medulla oblongata of the donor is stimulated. If a good vagus result is obtained in the donor an effect usually shows itself in the receiver after a short latent period. Fig. 2 is a record obtained by this method. The top line shows the contractions of the receiver heart, the next those of the donor; the third is the signal line, and below this the time-marking is shown in five-
second intervals. It is seen that the effects of vagus stimulation of the donor heart are humorally transmitted to the receiver, complete stoppage of the recipient heart being obtained after a slight preliminary slowing. That the effect on the recipient heart is a specific one can be shown by applying a drop or two of 0.1 per cent. atropine to it and repeating the experiment. Although the donor heart will stop as before, no effect is now shown on the recipient heart.

The most common difficulty which arises is that the stimulated heart does not always give a good vagus response. The simplest way to overcome this difficulty is to raise the hydrogen-ion concentration of the perfusing fluid (with dilute hydrochloric acid) to a pH value of about 6.5. This usually improves the vagus response considerably, and also increases the sensitivity of the recipient heart to the "vagus substance." This sensitivity is also increased if the heart has been perfused continuously for some hours before the experiment proper is begun. It will then be in a "hypodynamic" condition, and will respond readily even to slight concentrations of the "vagus substance" which is passing from the donor heart.

**METHOD AS APPLIED TO OTHER TISSUES.**

The same method of perfusing the donor heart may be used to obtain a transmission of the effects of vagus stimulation to tissues other than the heart, suitable precautions being taken to ensure that the test tissue receives a constant supply of fluid during the experiment.

In fig. 3 is reproduced the record of two experiments (A and B) in which the isolated frog-stomach was used as a test. The top line shows...
the contractions of the donor heart, the next the movements of the stomach, and the third and fourth lines the signal and time-marking as before. The stomach had given a contraction just at the beginning of record A. The contraction was slight, but typical of the previous behaviour of the tissue. The heart-vagus was then stimulated for about fifteen seconds, causing heart stoppage. The stomach almost immediately gave a contraction of relatively large amplitude, attaining its maximum rapidly. The vagus stimulation was discontinued during the relaxation of the stomach. About a minute after the relaxation was complete—and it will be noted the tone of the stomach is now maintained at a slightly higher level than before—a spontaneous contraction again occurred. This had an amplitude slightly greater than the first, but less than half that of the contraction which took place when the "vagus substance" was in contact with the stomach. The rate of contraction and relaxation is also very much less than in the "vagus substance contraction." The second part of the figure (B) shows a tracing taken about six minutes later than the first. Here again both the rate and amplitude of the stomach contraction initiated during vagus stimulation are much greater than normal. The vagus stimulation was in this case continued after the contraction had passed off, and during this time the tone of the stomach musculature was increased above normal.

Summary.

A method is described whereby the humoral transmission of the effects of cardiac vagus stimulation in the frog may be strikingly demonstrated.

REFERENCES.

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