J. Physiol. (1957) 136, 174–197

THE LOCATION OF ATRIAL RECEPTORS IN THE DOG: A PHYSIOLOGICAL AND HISTOLOGICAL STUDY

By J. C. G. COLERIDGE, A. HEMINGWAY, R. L. HOLMES and R. J. LINDEN

From the Departments of Physiology and Anatomy, School of Medicine, University of Leeds

(*Received* 29 *October* 1956)

The patterns of discharge from atrial receptors in the cat have been well described (Amann & Schaefer, 1943; Jarisch & Zotterman, 1948; Whitteridge, 1948; Dickinson, 1950*a*; Neil & Zotterman, 1950; Paintal, 1953*a*), but only some of these workers have given attention to the precise location of the receptors initiating these discharges (Jarisch & Zotterman, 1948; Paintal, 1953*a*). Much less is known about similar receptors in the dog (Dawes & Widdicombe, 1953; Henry & Pearce, 1956) although technically, because of size, it should be easier to perform experiments to locate them and to make clear their physiological significance.

Various types of subendocardial and perimuscular formations have been described in the heart and great veins of the cat and the dog (as summarized by Mitchell, 1956). However, despite this information, our knowledge of the atrial receptors is incomplete because previously physiological and anatomical studies have been pursued independently, and, as far as it is known, no experiments have been performed to identify histologically the receptor whose impulses were being recorded.

Recently, experiments have been carried out in this laboratory to investigate the physiological mode of stimulation of receptors situated in the heart and great vessels of the dog and the reflex effects produced by their stimulation, particularly with reference to the regulation of the heart rate (Coleridge & Linden, 1955*a*, *b*). The work now to be described has been concerned with the possibility of accurate localization of receptors in atrio-venous tissues and with their histological identification. Action potentials have been recorded from slips of the vagus nerves and, after localization of the site of origin of these impulses, histological studies have been carried out.

A preliminary account of these findings has already been given (Coleridge, Hemingway, Holmes & Linden, 1956; Holmes, 1956*a*, *b*).

METHODS

Successful experiments were carried out on seventeen dogs varying in weight from 11 to 27 kg. After an initial subcutaneous injection of morphine sulphate (3 mg/kg), the dogs were anaesthetized 1 hr later by an intravenous injection of 0·25 ml./kg of a 1:1 mixture of Dial-urethane (allobarbitone-urethane, Ciba) and sodium pentobarbital solutions. After another hour this was followed by a second injection of morphine sulphate (1·5 mg/kg subcutaneously).

A cannula was inserted low down in the trachea, and the lungs were ventilated by a Starling 'Ideal' pump. The sternum was split in the mid line, and the internal mammary vessels were ligated and cut. The cut edges of the sternum were retracted widely to give a good exposure of the heart. To facilitate the subsequent localization of receptors, the pericardium was opened in the mid line and the pericardial edges were sewn to the chest wall to form a cradle supporting the heart. Soft string ligatures were passed round the superior and the inferior venae cavae about 1 cm outside the pericardium and also round the main pulmonary arterial trunk; care was taken not to include any nerves in the ligatures. Snares were formed round the vessels by passing the ends of the ligatures through short lengths of polythene tubing.

Optical registration of cardiovascular pressures and of respiration was carried out with manometers previously described (Coleridge & Linden, 1954). Right atrial pressure was recorded with a cannula inserted through the right external jugular vein. In some experiments arterial blood pressure was also recorded from the ascending aorta with a cannula inserted through the left common carotid artery. Respiration was recorded with an air-filled system attached to a side arm on the tracheal cannula. Although cannulae should be clamped rigidly for perfect optical registration of pressures, this condition had to be sacrificed to some extent to leave room for the microscope and for the dissection of vagal fibres; it was also essential to have good access to the heart to carry out the various localization procedures. Hence some optical records showed artifacts. This did not decrease the value of the pressure records in so far as they were used predominantly for the timing of the events in the cardiac cycle.

In general, the technique of dissection of the cervical vago-sympathetic trunk followed that previously described (Whitteridge, 1948; Paintal, 1953*b*). The skin edges of a mid-line incision were retracted to form a trough, and the carotid sheath was exposed. The nerve trunk was separated from the carotid artery and laid on a black Perspex platform. In early experiments the nerve was approached by cutting a window in the front of the sheath but this method had to be abandoned because of profuse bleeding. In the later experiments complete haemostasis was achieved as follows: any vessels running over the surface of the sheath were tied off on each side of the platform; the sheath was removed completely from about 1·5 cm of the nerve; and the cut ends of the sheath were packed off with small pledgets of cotton-wool. Warm liquid paraffin was poured into the trough when bleeding had been checked. A nerve bundle was cut from the nerve trunk with a knife made from a fragment of safety-razor blade and, after being separated downwards, was laid on the silver electrodes. Functional single-fibre preparations were obtained by repeated subdivision of the original nerve bundle. When making single-fibre preparations in the cat, it seems to be the usual practice to subdivide the original nerve bundle with fine needles or razor-blade knives (Whitteridge, 1948; Paintal, 1953*b*). However, in the dog it was found to be considerably more difficult to obtain a clean separation of nerve fibres either with needles or knives—possibly because of the relatively greater amount of connective tissue within the substance of the nerve. More single-fibre preparations were obtained when the bundles were split as follows. The original fascicle was taken off the electrodes and laid without bending on the black platform; the end was gently teased apart and grasped with two pairs of fine forceps; the bundle was then split by gently separating the forceps. This subdivision was repeated until a functional single-fibre preparation was obtained. Most of the dissection was undertaken with a binocular microscope (magnification $\times$ 10–30). It was found necessary to carry out this dissection after opening the chest. The technique of isolating the atrial fibres first and then opening the thorax to identify the site of the receptor, employed successfully by Paintal (1953*a*) in

the cat, was attempted in some of our early experiments. However, this method was abandoned because either the fragile single-fibre preparation was pulled off the electrodes by the movements unavoidably associated with the opening of the chest, or the fibre was no longer active by the time the chest and pericardium had been opened and snares placed round the vessels.

The action potentials were amplified by a conventional R-C coupled amplifier and were displayed with an e.c.g. on a double-beam cathode-ray tube. By suitable optical systems a combined photographic recording was effected of the two oscilloscope traces, the beams from the optical manometers, a 50 c/s time trace and a signal marker. A loudspeaker and a second oscilloscope with the sweep triggered by the R wave of the e.c.g. aided the preliminary analysis of the discharge.

Receptors were located approximately by tightening, in turn, the ligatures around the superior and inferior venae cavae and the pulmonary artery. Right and left atrial receptors which had been distinguished by occlusion of the pulmonary artery were further localized by finger pressure on the outside of the veins and atria. A more accurate localization was then carried out. A fine glass rod was inserted through a small incision in the atrial appendage; leakage of blood was prevented by a purse-string suture. The interior of the atrium and the venous orifices were then explored with the tip of the glass rod to find the point which, when pressed, gave a maximal discharge in the single-fibre preparation.

With the method described so far, it was usually possible to localize the receptor to a small area of atrio-venous wall, not more than 1–2 cm in diameter; but in order to limit an area for subsequent histological examination it was necessary to obtain a punctate localization. Because of the anatomical disposition of the great veins and atria, this was only possible if the heart was opened and the atrio-venous cavities exposed widely. This final stage in the functional identification of the site of the receptor was attended with several difficulties. Although a single-fibre preparation sometimes continued to discharge in response to an appropriate stimulus for several minutes after the animal had been killed, on other occasions the fibre soon became inactive. Any movement was likely to displace the fibre from the electrodes. Also the dissection of the heart had not to be obscured by blood. The technique evolved was as follows. The point of approximate localization was marked by fine Spencer Wells forceps. The dog was deeply anaesthetized by the intravenous injection of several ml. of sodium pentobarbital solution. The abdominal wall was opened in the mid line, and the abdominal aorta and inferior vena cava were cut across; the dog bled to death into the abdominal cavity which was packed with cotton wool. Depending on the previous determination of the site of the receptor, the right or left ventricle was opened from the apex towards the atrio-ventricular ring, and the incision was carried through the atrio-ventricular orifice into the anterior wall of the atrium. The atrium was opened widely and held by several forceps. After blood had been mopped up, the interior of the heart was explored with the tip of the glass rod to find the point from which a continuous high-frequency discharge could most easily be elicited. If the preliminary exploration of the venous orifices indicated that the receptor lay in a vein rather than in the body of the atrium, the vein was opened up and explored. In addition to pressure with the end of the glass rod, receptors were also stimulated by localized stretching of the wall between the fine forceps.

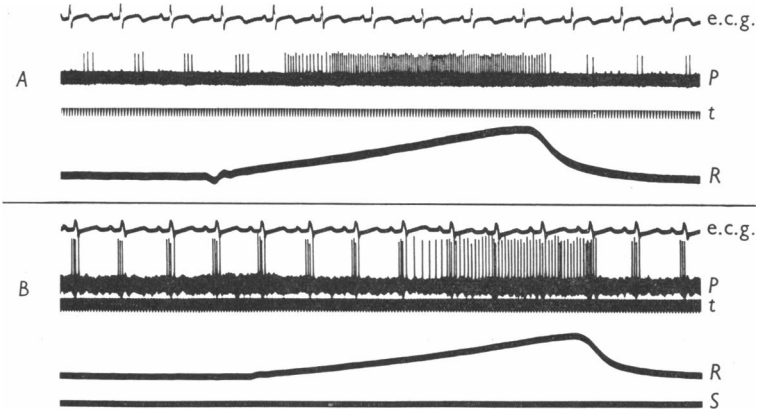
Finally, the point stimulated was marked accurately with fine threads and the whole region was removed and fixed in 10% formalin for 3–5 days. Smaller pieces which included the localized receptor areas were then embedded in paraffin, sectioned serially at 15 μ, and impregnated with silver according to the technique of Holmes (1947). Sections were usually cut in a plane at right angles to the wall, but the curvature which persisted in some specimens caused these to be cut tangentially.

In addition to the examination of the localized areas, a wider survey was made, in other dogs, of the atria and veins after they had been stained by the methylene blue method described by Mitchell (1953).

RESULTS

Initial selection of atrial fibres

It was necessary to differentiate atrial fibres from other fibres whose discharge showed a cardiac rhythm. The two commonest types of fibre with a cardiac rhythm encountered in these experiments were arterial baroceptor fibres and fibres arising from pulmonary stretch receptors which, in addition to the characteristic continuous discharge during inflation, showed a discontinuous cardiac rhythm with intervals of silence. These were identified and discarded.



Text-fig. 1. Fibres from two pulmonary stretch receptors showing an adventitious cardiac discharge. *A* shows a volley during late ventricular systole; *B* shows a discharge during atrial systole. In this and in subsequent figures the following abbreviations are used: e.c.g., electrocardiogram; *P*, action potentials in vagal slip; *t*, time trace ($\frac{1}{50}$ sec); *R*, tracheal pressure (upstroke representing inflation); *Z*, zero reference line; *RAP*, right atrial pressure (cm H_2O); *S*, signal marker. In *B* the spikes have been retouched to aid reproduction.

Arterial baroceptors. In the majority of cases these were identified easily by their characteristic pattern of discharge and by their relationship to the e.c.g. Typically they showed an initial crescendo of impulses in early systole followed by a diminuendo, whereas the late systolic discharge in atrial fibres usually showed a gradual increase in frequency which reached its peak about the summit of the '*v*' wave of the atrial pressure curve.

Pulmonary stretch receptors. Pulmonary stretch receptors which showed a discharge with both a respiratory and a cardiac rhythm have been described previously in the cat (Adrian, 1933; Whitteridge, 1948). That lung receptors might show an adventitious cardiac rhythm is to be expected since structures near the lung roots will be deformed by pulsations of the heart and great vessels; also, receptors within the substance of the lung may well be stimulated more directly by pulsations transmitted from the small branches of the pulmonary artery.

These adventitious cardiac rhythms were found at different phases of the cardiac cycle (Text-fig. 1 *A*, *B*). Though most of them occurred in late ventricular systole (*A*), some fibres showed a burst of impulses during atrial systole (*B*). Hence these 'bogus' cardiovascular rhythms were often a nuisance when looking for fibres with a true atrial rhythm. However, as others have reported, the true nature of the discharge in these fibres was at once apparent in the majority of cases when the lungs were inflated: the adventitious cardiac rhythm became less obvious and the respiratory rhythm became dominant (Text-fig. 1).

After the elimination of obvious respiratory and arterial baroceptor fibres, twenty-five functional single-fibre preparations, showing what were thought to be patterns of discharge characteristic of atrial receptors, were isolated from the cervical vagus. Seventeen of these fibres were localized subsequently to the atrio-venous junctional regions.

Patterns of discharge in atrial fibres

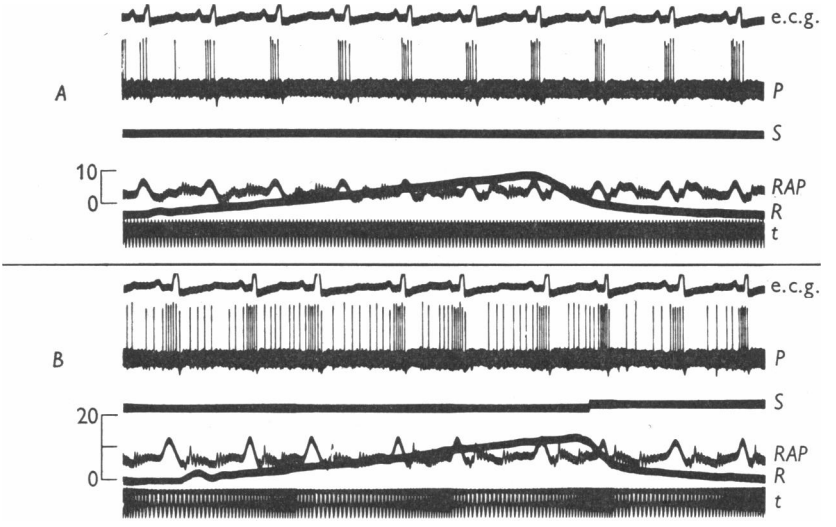
As a result of his observations on the cat, Paintal (1953*a*) divided atrial receptors into two types. Type A, which were said to respond to changes in atrial pressure, characteristically showed a discharge of impulses in time with the pressure wave developed in atrial systole; they also showed a more variable burst of activity corresponding with the '*v*' wave of the atrial filling phase. On the other hand, type B, which Whitteridge (1948) and Pearce & Whitteridge (1951) had thought previously to be pulmonary vascular receptors, were said by Paintal to be atrial receptors which discharged in response to atrial distension. The characteristic pattern of discharge in this second group of fibres was a late systolic burst of impulses corresponding approximately with the atrial '*v*' wave.

In the present series of experiments on dogs, as far as the time relationships of the discharges were concerned, the patterns of impulse activity appeared to fall into the two groups described by Paintal.

In three fibres (all subsequently located to the right side of the heart) there was a discharge of impulses in time with the '*a*' wave in the atrial pressure record, i.e. corresponding with atrial systole and a decrease in atrial volume. These fibres also showed a discharge of impulses during late systole, which varied in number and in frequency. Though not always evident, this late systolic activity could usually be brought out by any procedure which caused an increase in atrial filling (Text-fig. 2). In this experiment, an intravenous infusion of 200 ml. saline brought out a pronounced discharge during late systole which became continuous with the discharge at the '*a*' wave.

Fourteen fibres showed a pattern of discharge whose time relationships appeared characteristic of Paintal's type B atrial receptors (Text-fig. 3);

four were localized to the right atrio-venous system and ten to the left. The main activity in these fibres occurred as a grouped discharge of impulses in time with the atrial '*v*' wave; in addition, there was occasionally a brief burst of impulses coincident with the '*c*' wave in the atrial pressure record. In none of these fibres were there any impulses synchronous with the maximum pressure developed during atrial systole, although in five fibres a brief burst of impulses of high frequency was seen at the beginning of the atrial '*a*' wave. At the usual camera speed, the action potentials in this small



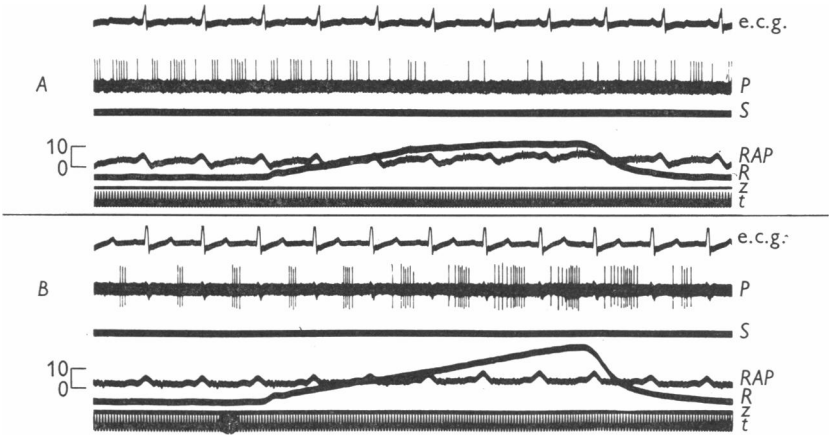
Text-fig. 2. Fibre from right atrio-venous system with a pattern of discharge whose time relationships appeared characteristic of Paintal's type A atrial fibres. *A* shows a volley of impulses synchronous with the '*a*' wave in the atrial pressure record; *B* shows the same preparation after a late systolic volley had been brought out by an infusion of 200 ml. 0·9% (w/v) NaCl solution into the femoral vein (the signal marker indicates the end of the infusion). The camera was running irregularly in *B*.

group were superimposed and appeared as a rather thickened single line (Text-figs. 3*A*, 4); however, it was possible to demonstrate the multiple nature of the discharge when the oscilloscope was switched over to a faster sweep.

All these discharges showed a pronounced respiratory rhythm whose pattern was not always related to the position of the receptor. Though right atrial pressure always increased during inflation of the lungs, some right atrio-venous receptors showed an increase of activity during inflation (Text-fig. 3*B*), while others showed a decrease (Text-fig. 8*A*). The same was true of receptors on the left side of the heart.

Because there was not always the expected change in the impulse pattern

with variations in atrial pressure, it seemed that Paintal's classification of atrial receptors into two distinct functional groups (one responding to pressure, the other to alterations in length) could not be applied to the present results obtained in the dog with the chest opened, but any further analysis of this problem of the stimulus will not be undertaken in this paper.



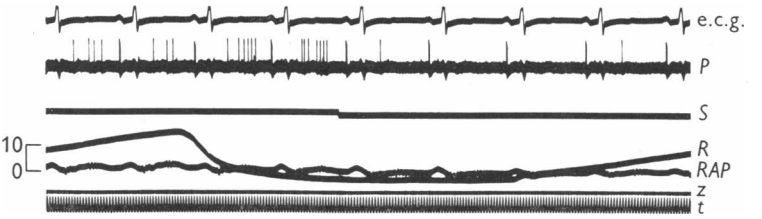
Text-fig. 3. Atrio-venous fibres with patterns of discharge whose time relationships appeared characteristic of Paintal's type B atrial fibres. *A*, fibre from left atrio-venous system; *B*, fibre from right atrio-venous system. The brief discharge of high frequency which occurs at the beginning of atrial systole in *A* can be seen as a thickened single line at the height of inflation.

Localization of atrial receptors

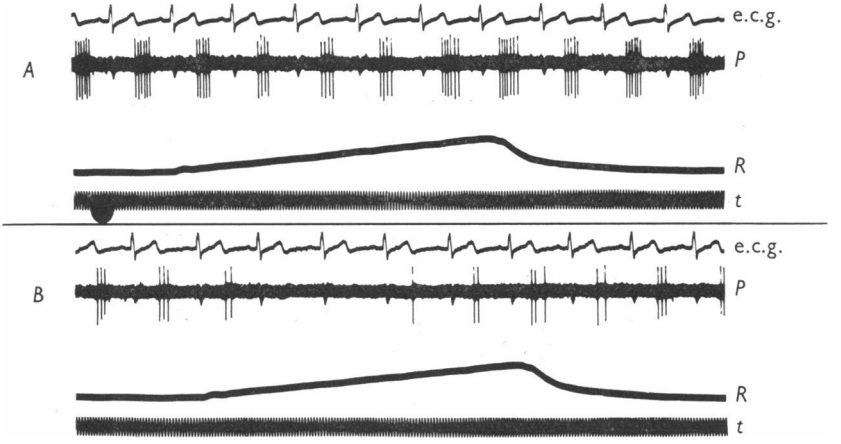
Occlusion of superior and inferior venae cavae. A reduction in atrial fibre discharge always occurred when the ligatures around the venae cavae outside the pericardium were tightened; that is, all receptors appeared to be central to the occluding ligatures, and there was no evidence of any receptors in the extrapericardial part of the venae cavae peripheral to the ligatures. The effect of this venous occlusion on both right and left atrial receptors is illustrated by the records shown in Text-figs. 4–6. It can be seen that venous occlusion had a marked effect on the impulses at the '*v*' wave in the atrial pressure record, but it had little effect on the brief burst of impulses in time with the beginning of the atrial '*a*' wave (Text-fig. 4).

As might be expected from what is known about the relative blood flows in the great veins (Levy & Blalock, 1937), occlusion of the IVC produced a greater effect than occlusion of the SVC. In one experiment the fibre discharge was reduced when the snare around the SVC was tightened (Text-fig. 5), but it was completely abolished when the SVC ligature was released and that around the IVC was tightened (Text-fig. 6).

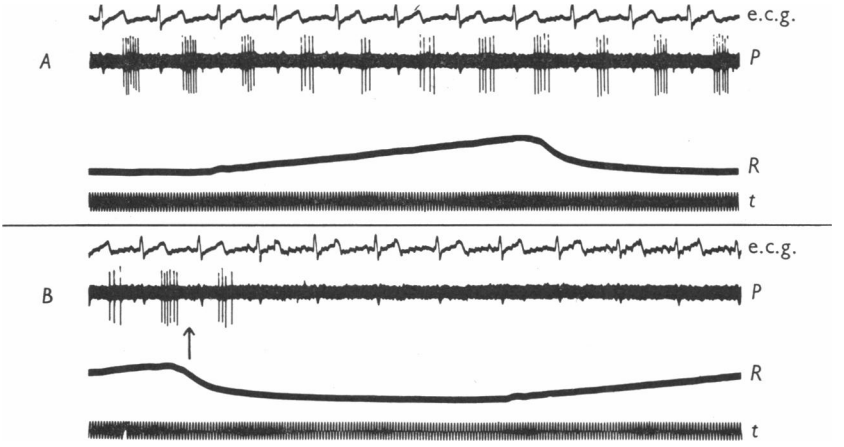
Occlusion of pulmonary artery. Occlusion of the pulmonary artery differentiated clearly between receptors on the right and left sides of the heart, and



Text-fig. 4. Effect of occlusion of inferior vena cava on discharge in fibre from right atrio-venous system. The onset of occlusion of IVC is indicated by signal marker. Note that the brief burst of impulses of high frequency at the beginning of the atrial '*a*' wave is unaffected by occlusion.



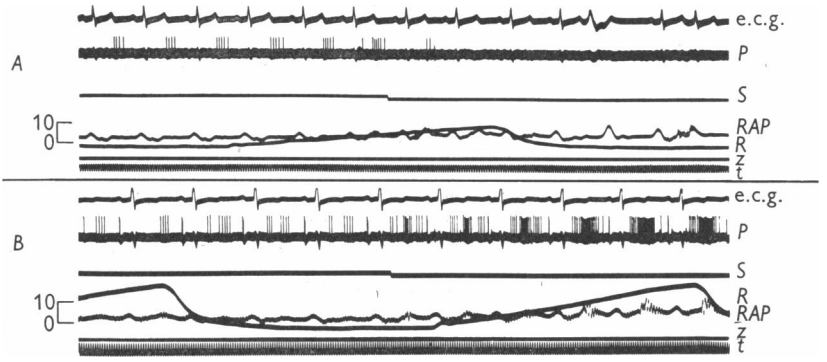
Text-fig. 5. Effect of occluding superior vena cava on discharge in fibre from left atrio-venous system. *A*, normal; *B*, after occlusion of SVC. The spikes below the base line have been retouched to aid reproduction.



Text-fig. 6. Same fibre as in Text-fig. 5. Effect of occluding inferior vena cava. *A*, normal; *B*, IVC occluded at arrow. The spikes below the base line have been retouched to aid reproduction.

proved to be the most useful single procedure in the initial stages of localization. In every experiment in which pulmonary arterial occlusion reduced or abolished the discharge the fibre was found subsequently to originate on the left side (Text-fig. 7 *A*), whereas occlusion always resulted in an increased discharge in right atrial fibres (Text-fig. 7 *B*). Opposite effects were observed when the pulmonary arterial snare was released.

Punctate localization. When the receptor had been located to the right or left side of the heart, a more detailed search was carried out. The method employed can be illustrated best by following a typical functional single fibre through the stages of localization.

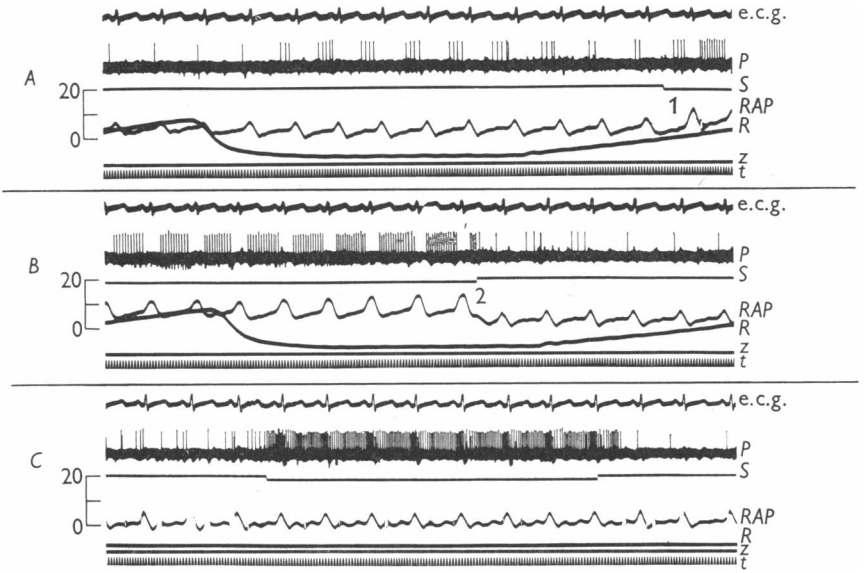


Text-fig. 7. Effect of pulmonary arterial occlusion on the discharge in atrio-venous fibres. *A*, fibre from left atrio-venous system; *B*, fibre from right atrio-venous system. Signal marker indicates onset of pulmonary arterial occlusion.

Pulmonary arterial occlusion had indicated that the receptor was in the right side of the heart. Gentle pressure with the finger on the right ventricle and pulmonary trunk elicited no increase in discharge, but there was a pronounced effect when the right atrium was pressed (Text-fig. 8 *A*, *B*). A fine glass rod was inserted through an incision in the atrial appendage and a purse-string suture was tightened to prevent loss of blood. The interior of the atrium and the openings of the venae cavae were explored with the tip of the rod to find the point from which a discharge could most easily be elicited. The result of this exploration can be seen in Text-fig. 8 *C*, which shows the alteration in discharge produced by punctate stimulation of the posterior wall of the superior vena cava about 1 cm above the atrio-venous junction.

The animal was killed and the right ventricle and atrium were opened; the incision was extended for about 2 cm into the anterior wall of the superior vena cava. The cut edges of the atrium and vein were retracted with fine forceps, and exploration with the glass rod was continued. Careful stimulation confirmed that the receptor lay in the posterior wall of the superior vena cava (inside the pericardium) about 1 cm above the atrio-venous junction slightly

to the left of the mid line (Text-fig. 9*A*). A marked discharge could also be elicited by stretching that part of the caval wall gently between two pairs of fine forceps (Text-fig. 9*B*). Finally the point was pressed again, this time with the tip of a needle, and a brief discharge was obtained with a maximum frequency of 156 impulses/sec (Text-fig. 9*C*). This last stimulation may have damaged the receptor because, in spite of continued pressure, no further discharge was obtained. The point was marked with fine threads and the tissue taken for histological examination.

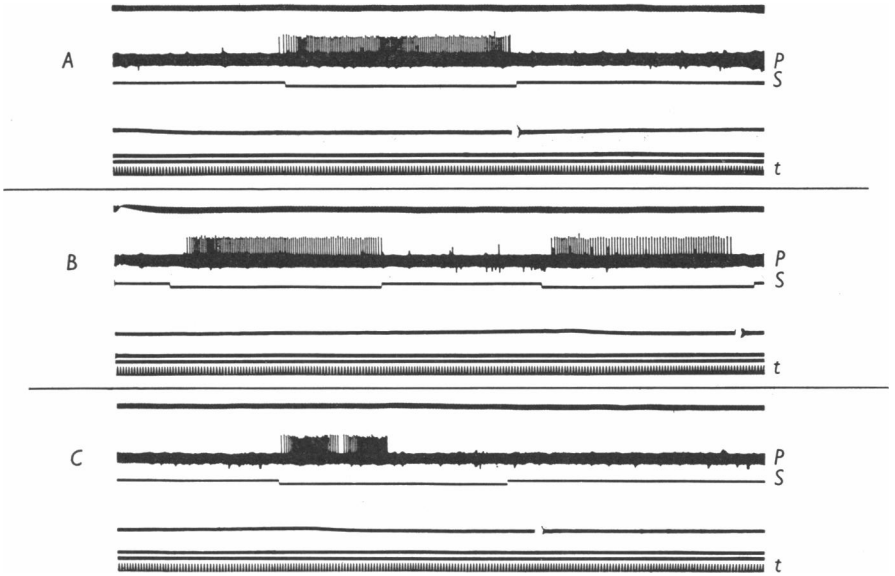


Text-fig. 8. Stages in the localization of an atrio-venous receptor (finally located in posterior wall of superior vena cava). The records in *A* and *B* are continuous; the right atrium was pressed between 1 and 2. *C* shows the alteration in discharge produced by pressure with the tip of a glass rod (inserted through the right atrial appendage) on the posterior wall of the SVC about 1 cm above the atrio-venous junction. The respiratory manometer was disconnected in *C* to give better access to the heart.

Exact determination of the site of a receptor in the left atrio-venous system was found to be rather more difficult. The left atrium and pulmonary venous junctions lie behind the heart. Consequently, it was more difficult to determine the approximate site of the receptor by external pressure before the heart was opened. Furthermore, a much wider exposure was necessary to see the interior of the left atrium and veins, and this carried a greater risk of destroying the nervous pathways. In addition, there were at least six veins opening into the left atrium to be explored.

Nevertheless, the final localization of three left atrio-venous receptors was achieved. In one experiment preliminary exploration had shown that the

receptor was situated approximately at the junction of the right pulmonary veins and left atrium. The heart was opened, and the tip of the glass probe was inserted just inside the orifice of the right lower pulmonary vein (Text-fig. 10*A*): this produced a continuous discharge with a maximum frequency of 21 impulses/sec. The rod was then pushed a further 1–2 mm into the vein and the maximum frequency of discharge increased to 85 impulses/sec. The vein

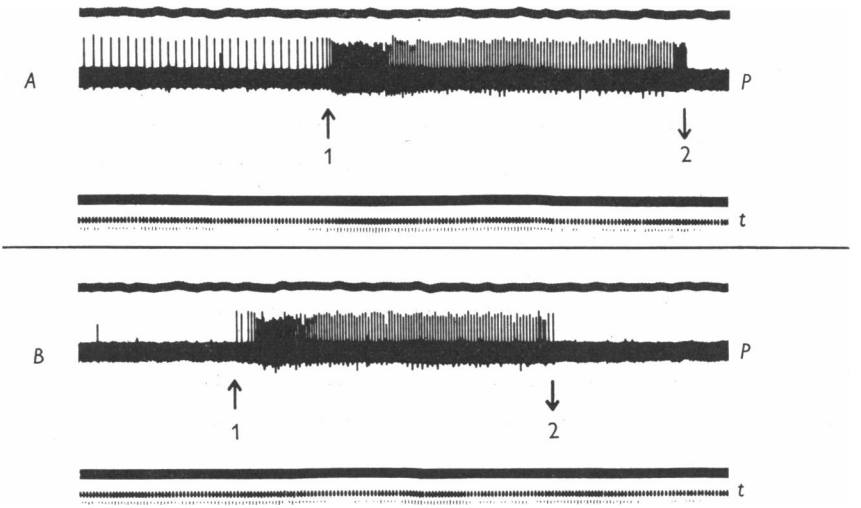


Text-fig. 9. (Continued from Text-fig. 8.) Stages in the localization of an atrio-venous receptor after killing the animal and opening the right ventricle, atrium and superior vena cava. *A*, pressure with the point of a glass rod on the posterior wall of the SVC about 1 cm above the atrio-venous junction. *B*, wall of SVC stretched twice between two pairs of fine forceps. *C*, pressure with tip of needle on same point as in *A*; receptor probably damaged and no further discharge could be elicited.

was opened, the cut edges were held with fine forceps, and the endothelial surface of the vein was explored carefully with the rod. Gentle pressure on the posterior wall, 2 mm proximal to the atrio-venous junction, elicited a discharge with a maximum frequency of 127 impulses/sec, whereas gentle pressure all round this point had no effect (Text-fig. 10*B*).

Certain details of the technique of localization must be emphasized. It was essential both to apply only a localized stimulus and to be able to see clearly the region stimulated. These points, though self-evident, appear to have been overlooked by some previous investigators who attempted to determine the site of atrial receptors (in the much smaller heart of the cat) by external pressure with the finger on the intact beating heart. Early in the present experiments it was found that pressure with the finger accomplished

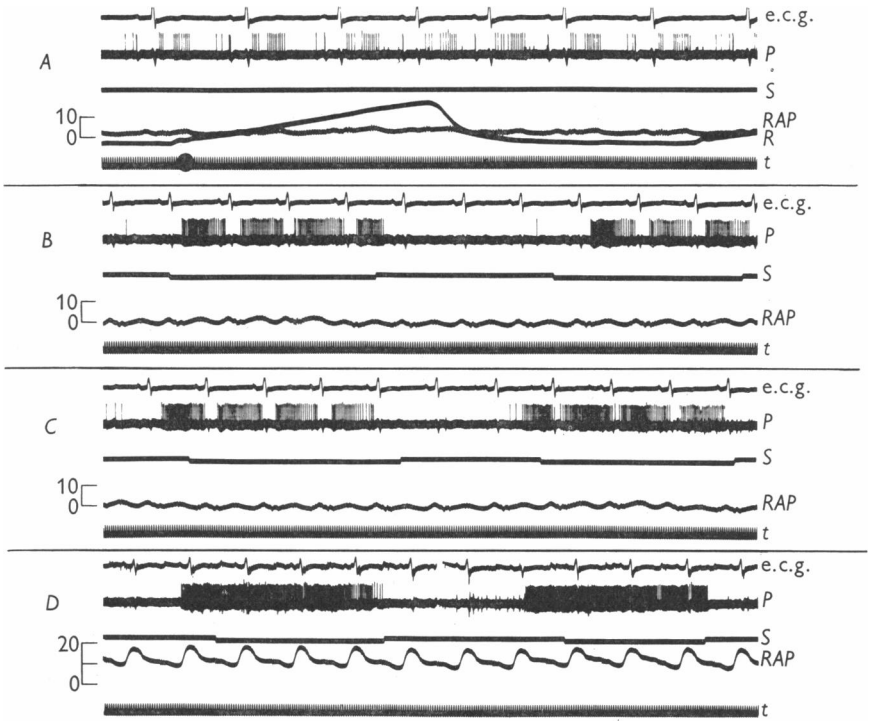
two things: it most effectively obscured the field of vision and it applied the stimulus to a wide area. As a result, misleading observations were sometimes made. Furthermore, light pressure with the finger on the left atrium often resulted in an appreciable increase in right atrial pressure, and on more than one occasion pressure with the finger on the outside of the intact left atrium brought about a marked discharge from a receptor which subsequently was localized to the right side.



Text-fig. 10. Final stages in the localization of a receptor in the left atrio-venous system after the dog had been killed and the left ventricle and atrium had been opened. The tip of a fine glass probe was inserted just inside the orifice of the right lower pulmonary vein: this produced a continuous discharge (*A*); at 1, the probe was pushed a further 1–2 mm into the vein; the probe was completely withdrawn at 2. The vein was opened. *B* shows the discharge produced by punctate pressure (between 1 and 2) on the posterior wall of the vein 2 mm from the atrio-venous junction.

Though external pressure with the glass rod was more satisfactory than finger pressure, it also could elicit a discharge from a receptor some distance away, if the wall between the point of stimulation and the receptor were stretched. This is well illustrated by the following experiment. The discharge in one right atrio-venous fibre is shown in Text-fig. 11 *A*. The lateral wall of the right atrium was pressed gently with the tip of the rod: this produced an appreciable discharge (*B*). A similar effect was produced when the atrium was held by forceps and the SVC was drawn gently up by another pair of forceps, thus stretching the segment of vein and atrium between them (*C*). But it can be seen that although the receptor discharge was augmented in response to pressure at a point or to the stretching of a segment of the atrio-venous wall, the discharge was discontinuous and had a cardiac rhythm with

a silent period roughly at the end of atrial systole. On the other hand, a continuous discharge was finally produced by direct stimulation at a point on the medial wall of the SVC about 1 cm above the atrio-venous junction (*D*), and it was inferred that the receptor was within the wall beneath this point. The position of this receptor was confirmed after the animal had been killed and the atrium and cava opened.



Text-fig. 11. Impulses in fibre from receptor in wall of superior vena cava. *A*, normal record. *B*, two punctate stimulations of lateral wall of right atrium. *C*, junction of SVC and right atrium stretched twice between two pairs of fine forceps. *D*, two punctate stimulations of a point lying between the two forceps in the SVC close to the atrio-venous junction. In *D*, the atrial pressure record is distorted because the heart was displaced to give access to the SVC; the respiratory manometer was disconnected after *A*.

Because of the anatomical disposition of the great veins and atria, receptors could only be localized with certainty when these vascular structures had been opened up, and this was particularly so in the case of receptors on the left side of the heart. A wide dissection was also essential to ensure that pressure was not transmitted to structures lying behind the heart and veins. This was strikingly demonstrated by the following observations. In one experiment localized pressure on the posterior wall of the SVC caused an increase in the discharge in a fibre with a pronounced cardiovascular rhythm;

after careful exploration the receptor was found to be in a main bronchus behind the SVC. In another experiment localized pressure on the posterior wall of the left atrium, after the animal had been killed and the heart opened, elicited a high frequency discharge from a receptor which was eventually found to be located in the anterior wall of the oesophagus. In both cases, the adventitious cardiac discharge showed a marked response to such procedures as occlusion and release of the pulmonary artery. This was to be expected, since these structures were immediately related to the right and left atria.

Position of atrial receptors

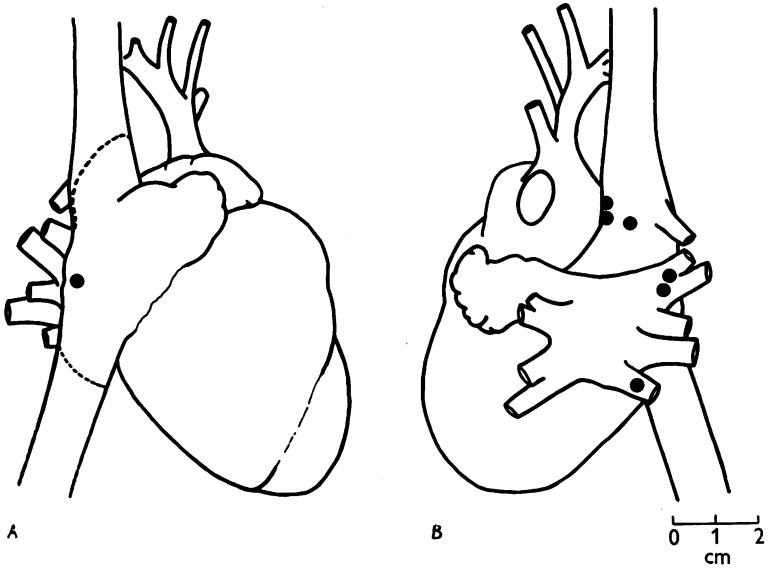
For accurate definition of the site of a receptor, we attempted to differentiate between 'vein' and 'atrium'. With the heart and vessels *in situ* the atrio-venous junction could be identified with some certainty, and it was possible to describe the site of a receptor anatomically according to its position in a vein or in the atrial wall. When the heart was opened, the venous orifices presented a discrete junction between the diverging walls of the atrial cavity and the tubular lumen of the veins into which a rod could be slipped. Nevertheless, it became difficult subsequently to justify this differentiation on histological grounds because of the sheath of myocardium extending from the atria into the proximal parts of the venae cavae and pulmonary veins.

Out of the seventeen fibres whose endings were localized by the procedures described, seven were found to arise from receptors on the right side, and ten from receptors on the left side of the heart. In the case of ten of the seventeen fibres localization of the receptor was carried no further than to the neighbourhood of a particular atrio-venous junction because the action potentials disappeared before exact localization had been completed. Therefore it was not possible to attempt to identify the receptors histologically. In a further seven cases punctate localization was completed after the heart and veins had been fully opened and the point of stimulation could be clearly seen. The positions of these seven receptors are depicted in Text-fig. 12. Three receptors were found in the intrapericardial part of the superior vena cava on the posterior or medial wall; and three were located to the right pulmonary veins immediately proximal to the atrio-venous junction. Only one receptor was localized to the wall of the atrium proper: this was found in the lateral wall of the right atrium half way between the superior and inferior caval openings. All receptors were found inside the pericardium.

Because of the disposition of the apparatus, dissection of the right vagus was easier than of the left and the latter was examined on only two occasions. It is clear that fibres from both sides of the heart pass into the right vagus; the receptors in connexion with the two left vagal fibres which were dissected were not localized precisely but were thought to be in the left heart.

Histological findings

Silver impregnation. In the seven dogs in which it had been possible to determine accurately the site of a receptor, the point which had responded to punctate stimulation was carefully marked and was examined histologically after silver impregnation. All these specimens, with the exception of one from the lateral wall of the right atrium, were from the superior vena cava and the pulmonary veins. In these junctional regions the venous and atrial walls were histologically indistinguishable: the venous adventitia of connective



Text-fig. 12. The position of seven atrio-venous receptors whose site had been determined accurately by punctate stimulation. *A*, right lateral view of heart and great vessels; the dotted line indicates the pericardial reflexion on the superior and inferior venae cavae. *B*, posterior view; for clarity, the pulmonary artery has been omitted.

tissue was continuous with the outer epicardial layer of the heart; a layer of cardiac muscle extended for some distance along the veins; and the intima, which was thicker than in the extrapericardial parts of the veins, merged imperceptibly with the atrial endocardium. Hence the receptor areas examined resembled the heart more closely than the veins, and in the following account the terms 'epicardium', 'myocardium' and 'endocardium' will be used to describe the three layers of both atrial and venous wall.

The outer epicardial layer, of connective tissue with a variable amount of fat, contained numerous blood vessels and bundles of nerve fibres, often associated with small ganglia. Nerves and vessels could be followed into the

myocardium, where ganglia were also observed, lying between bundles of muscle fibres and often adjacent to a small artery. Nerves ending on muscle fibres were occasionally seen.

A more extensive study was made of the endocardium, as it was here that nervous structures were found which seemed to be the most likely mediators of the recorded impulses. The endocardial connective tissue in these sites contained a variable amount of smooth muscle. Thick myelinated nerve fibres were seen at the junction between the endocardium and the myocardium, or within the deeper endocardial layers, where they ran parallel to the endocardial surface. Occasional fibres could be seen passing from the muscle into the endocardium.

Some of these fibres could be traced to end formations. These occupied most of the thickness of the endocardium (Pl. 1, fig. 3), and were formed by the branching, within a circumscribed area, of the thick fibres into finer fibres and terminal filaments (Pl. 1, figs. 1, 2); the myelin sheath was lost beyond the point at which branching occurred. The thick fibres appeared somewhat varicose, while the finer ones were closely associated with deeply impregnated cellular elements. The background tissue in these circumscribed zones showed a lighter impregnation and greater numbers of nuclei than that of the surrounding connective tissue. The shape of a typical end formation, inferred from observations made on sections cut at various angles, was that of a flattened plate with its greatest area parallel to the endocardial surface. Sometimes a number of these endings, from one or more fibres, lay within a very small area of endocardium.

Every specimen, in which 'functional' localization of a receptor area had been achieved, showed thick endocardial nerve fibres running to one or more branching nerve endings of this type. Furthermore, these circumscribed endocardial end formations were the only discrete nervous structures, common to all the silver impregnated specimens, which on the basis of their structure were likely to be receptors.

In addition to the end formations just described, one piece of tissue (taken from the junction of the right lower pulmonary vein with the left atrium) showed a fully impregnated subendothelial nerve network, similar to that figured by Meyling (1953) from methylene blue preparations. In this case curvature of the specimen had resulted in tangential sections through the endocardium passing at one point exactly in the plane of the network, and the typical pattern of branching fibres and interstitial cells was demonstrated over an area of $1{\cdot}0 \times 0{\cdot}5$ mm. Direct comparison showed that this network differed markedly in structure from the end formations just described. In other and more oblique sections, elements of the nerve net sometimes appeared as branched fibres running towards the endocardial surface and ending freely, but study of serial sections revealed that they were part of the network.

Such study also distinguished between small nerve bundles and true terminal structures.

Methylene blue preparations. Confirmation of what had been seen in silver impregnated material was sought by the study of whole thickness preparations stained by methylene blue, after the technique of Mitchell (1953); and a histological survey was also made of different parts of the atria and the adjacent portions of the venae cavae and pulmonary veins. Two definite types of nervous structure were seen in the atrial and venous endocardium: first, a syncitial network of fine fibres and cells, forming the terminal nervous network, as described by Meyling (1953) and others; and, secondly, thick nerve fibres terminating in apparent end structures (Pl. 1, fig. 5).

The thick fibres emerged from the deeper layers of the wall and followed a sinuous course in the endocardial tissue (Pl. 1, fig. 4), often giving off several branches. These then divided into finer terminal filaments which radiated out from the parent fibres, forming a structure occupying appreciable depth as well as area. These fine branches had a slightly beaded appearance and were closely associated with cellular elements which also stained deeply. The whole formed a conspicuous and localized structure, and appeared to be a true nervous ending (Pl. 1, fig. 6).

Bearing in mind the fact that the silver-impregnated sections were only 15μ in thickness, while the methylene blue-stained material was viewed intact from the endocardial surface, the close similarity in structure and disposition of the thick nerve fibres and their circumscribed endings in the two types of preparation leaves little doubt of their correspondence. The essential similarity of structure revealed by the different techniques can be seen by a comparison of Pl. 1, figs. 2 and 6. It must be emphasized again that these formations were the only constant and discrete ones found in the 'functionally' localized receptor areas.

Viewed from the endocardial surface the size of each end formation varied from approximately 40×50 to $200 \times 350\mu$; the diameter of the fibres running into them was generally between 3 and 10μ, while that of the fibres constituting the terminal network was always smaller. The larger end formations occurred at the endings of the larger diameter fibres.

Endocardial tissue from all parts of the atria and veins was examined and it was found that the two types of nervous structure had a different distribution. The terminal network, while more prominent on the posterior atrial walls, extended towards the atrio-ventricular orifices, into the veins and the auricular appendages and on to the anterior atrial wall. The thick fibres and their endings, however, were limited to the atrio-venous junctional regions, to the proximal parts of the veins and to the adjacent parts of the atrial wall and interatrial septum.

The endings tended to occur in groups and sometimes ten or more, arising

from several main fibres, lay within an area of 2 or 3 mm across, separated from another group by variable distances of up to 10 mm. Single endings were seen, and the distribution pattern almost certainly varies in different dogs. The left side appeared to be more richly innervated than the right and the total number of typical endings from both sides was in the region of 150, although again variations might be expected.

Two distinctive endocardial nervous structures have so far been described. However, in some fields, smaller and less well marked end formations arose from a complex mesh formed from several entering fibres, similar to those described by Nettleship (1936) in the cat. Furthermore, a few localized areas of the terminal nervous network were more richly cellular than the rest, giving individual fibres a superficial resemblance to those in the circumscribed endings. But on close examination the features of each type of structure were quite distinctive: neither formation could be an artifact resulting from incomplete staining of the other.

A further point of interest was that, in their course through the endocardium, the thick fibres passing to branched nerve endings occasionally appeared to give fine branches to join the nerve net. Some of these were clearly filaments of the net running for a distance closely applied to the thicker fibres, while others appeared to be true branches. In well stained preparations, it was often possible to see the terminal network extending superficially over circumscribed endings with no communication; but, here again, occasional terminal filaments of the endings appeared to run on into the general net, and the possibility of communications between the two structures could not be entirely discounted.

DISCUSSION

Receptors which can be stimulated by distension of the atria and the pulmonary and caval veins, or by localized pressure, and which appear to be situated in the junctional tissues between the veins and the atria in the cat and in the dog heart have been described previously by many workers. The purpose of the present investigation was to identify these receptors histologically. For this, the receptor areas had to be located precisely and therefore the technique and difficulties of localization have been emphasized in detail.

Although most of the previous work on the properties of atrial receptors and their fibres has been done on the cat heart (summarized by Dawes, 1952; Paintal, 1953*a*) it seems difficult because of the size and rate of the heart in this animal to do more than limit the site of a receptor to a particular chamber or vessel. In general, our experimental findings about the situation of the receptors agree with those already reported. Jarisch & Zotterman (1948) stated that impulses could be readily elicited from both atria in the cat by touching the walls around and between the caval orifices on the right side and the pulmonary veins on the left side. They emphasized the ease with which

similar impulses could be elicited by pulling on other neighbouring structures showing that exact localization was difficult. Our results from the dog heart differ from theirs in that no sensitive points were found in the interatrial septum and that precise localization in the superior vena cava and pulmonary veins was obtained. In the cat, Paintal (1953*a*) found that receptors were located in the posterior part of the atria—some near the venous openings; he found none near the interatrial septum or in the auricular appendage. The few observations on vagal impulses from atrial receptors in the dog heart already reported (Dawes & Widdicombe, 1953; Henry & Pearce, 1956) have not been specially concerned with localization although Henry & Pearce thought it likely that stretch receptors were situated between the roots of the lungs and the left atrium.

In the present work a correlation has been established between electrophysiological and histological observations. In every case, when the regions which had been shown to be sensitive to pressure were examined histologically, the silver-impregnated sections showed thick nerve fibres, lying deeply in the endocardium, and one or more branching end formations which were in some cases demonstrably connected with thick fibres. These endings, examined under the highest magnifications, could be seen to be terminal structures, the fibres branching down to fine fibrillae which ended in relation to cells.

A comparison of these localized endocardial endings with those described previously shows great similarity. Among more recent workers, Nonidez (1937, 1941), using a silver impregnation technique, demonstrated nerve endings in the atria and large veins of young rabbits, cats and dogs. These endings were discrete, being formed by the branching of thick nerve fibres which had penetrated the muscle layer to reach the subendothelial connective tissue. Pannier (1940), using a similar method, described endings in the superior vena cava and pulmonary veins of the adult cat. Tcheng (1951) described sensory endings in the hearts of three-day-old puppies, although these did not correspond in appearance to those of Nonidez. Recently Sato (1954), again using silver impregnation methods, demonstrated branched nerve endings lying deep to the endothelium in the adult dog heart. Allowing for slight differences in appearance arising from variations in technique, we think that the circumscribed end formations described here correspond to those demonstrated by the above workers.

However, the mode of ending of afferent nerves in the heart and great vessels is a topic on which varying opinions are held (Mitchell, 1956). More recent workers in this field, such as Meyling (1953), have not favoured the idea of discrete nerve endings, but consider that a fine-meshed subendocardial plexus is the only structure present.

Nevertheless, it seems, for several reasons, that the circumscribed end formations are not artifacts. Examination of serial sections made it clear

that these apparent endings were not nerve bundles cut obliquely. Furthermore, striking confirmation that circumscribed endings exist in the dog atria and veins was obtained from the study of whole thickness methylene blue preparations. In the junctional regions of the great veins and atria, thick fibres, branching and ending in specific end structures, could be followed in continuity.

Again, other investigators employing methylene blue techniques have also described such endings. Smirnow (1895) and Dogiel (1898) observed them in the dog and cat; Woollard (1926) described a subendothelial nerve plexus in dog atria and also referred to occasional 'endings'; and Nettleship (1936), while stating that in the cat heart the endocardial nerve fibres were mostly plexiform, also described and reproduced occasional branched and arborizing endings which he found most frequently in the region of insertion of the great veins, around the atrio-ventricular orifices, and at the base of the interatrial septum. Hence, in view of the similarity in appearance and distribution of the discrete endings in both the silver impregnation and methylene blue preparations, there seemed little doubt that the circumscribed end formations were clearly actual structures.

In addition to the thick nerves and their discrete end formations, the terminal nerve net with its wider distribution and distinctive structure was also demonstrated in both the silver impregnation and methylene blue preparations. On examination, it was clear that each type of nervous structure had quite characteristic features and that neither was an artifact resulting from incomplete staining of the other. Though the circumscribed endings were the only ones constantly found in the localized silver-impregnated specimens, the generalized terminal network could be seen ramifying throughout the whole thickness methylene blue preparations; consequently it must be assumed that both formations were present in the endocardium of the localized areas.

Although it is impossible, on the evidence presented, to state with certainty that any single end formation was responsible for a given set of recorded impulses, initiated either by distension of the veins or chambers of the heart with blood, or by punctate pressure, yet it seems very probable that the large end formations rather than the terminal network were the mediators of the recorded impulses.

These end formations have been found in all the portions of tissue which responded to punctate stimulation and from which impulses showing characteristic rhythms of atrial activity had previously been recorded. That they are the receptors responding to stimulation is suggested by a comparison of the respective sizes of the nerve fibres to the circumscribed endings and the generalized nerve net. The diameter of the thick fibres running into the circumscribed nerve endings was generally between 3 and 10 μ, while that of

the fibres constituting the general network was always much smaller. Estimates of the size of afferent atrial fibres have been made by several workers who have studied impulses in the vagus: Jarisch & Zotterman (1948) said that the atrial discharges occurred in myelinated fibres with a diameter of 2·8–7 μ; Dickinson (1950*b*) concluded that the venous fibres had a diameter of 4–6 μ; while Whitteridge (1952) tentatively ascribed to them a fibre diameter of 4–7 μ. These estimates were based on observations made in the cat, and though, as far as is known, there is no corresponding information for the dog, they do provide some indirect support for the view put forward. Nettleship (1936) showed by degeneration experiments that the large endocardial fibres with a diameter of about 6 μ were probably afferent. More recently Daly & Evans (1953), on the basis of experiments which involved studies of degeneration after division and the observation of the effects produced by electrical stimulation of the vagus before and after degeneration, concluded that small myelinated fibres of the 2–4 μ diameter group carried efferent impulses to the heart while afferent impulses travelled in myelinated fibres of 1–14 μ diameter (the majority of the fibres being between 4 and 6 μ) and in non-myelinated fibres. The diameter of the largest efferent fibres to the heart was stated by Dickinson (1950*b*) to be 2–3 μ.

A further point is that when a sensitive region had been definitely located the variation in the response to light pressure according to the placing of the glass probe was very striking. A lateral movement of 1–2 mm was sufficient to cause large changes in frequency of discharge. This seems to imply that the receptor structure is either a single end formation or a group of branching, closely spaced end formations. This would be in accord with the histological observations that what appear to be receptors are not interconnected through a network but are in direct continuity with individual nerve fibres, either singly or in small groups. Though there may be communications between the thick fibres with their circumscribed endings and the terminal network, these communications are small and are not present in relation to many of the large endings, even in preparations where the whole terminal network in the area was sharply stained.

Finally, in regard to the distribution of the receptors the experimental evidence and the histological findings agreed. The complete histological survey of the atria and the veins showed that the circumscribed endings were concentrated particularly in the regions from which the impulses had been shown to originate, in contrast to the much more widely distributed terminal network. Similarly, Nonidez (1937, 1941) found that the localized endocardial endings were restricted to the proximal portions of the veins which had an extension of myocardium, to the atrio-venous junctions, and to a lesser extent to the adjacent parts of the atria.

Of the seventeen receptors found in the atrio-venous regions, six were

finally located precisely in the veins, and in only one case was a receptor shown unequivocally to be situated in what appeared in the intact heart to be atrium proper. However, it is probably unwise to emphasize too strongly this apparent concentration of receptors in the veins. On histological examination typical receptors were found in the atrial walls adjacent to the venous orifices. Furthermore, there was histologically no sharp division between vein and atrium in these areas. Perhaps it is better, therefore, to regard these receptors as occurring most frequently in the transitional regions where the intrapericardial parts of the veins, invested by a prolongation of myocardium, merge into the atria proper.

There is little information as to the function of these receptors. Nonidez (1937, 1941) claimed that they were responsible for the afferent side of the Bainbridge reflex, but there is, at present, little experimental evidence to support this view. In experiments in which the veins and atria have been stretched to produce reflex effects on heart rate and breathing, it has been claimed that the maximal effects were produced when the stimulus was close to the orifices of the veins (Sassa & Miyazaki, 1920; Megibow, Katz & Feinstein, 1943), although Ballin & Katz (1941) using a mechanical device could not elicit cardiac or vascular responses by distension of the superior vena cava. Recently Henry & Pearce (1956) have presented evidence which, they claim, supports the view that the atrial receptors serve as one sensory mechanism in a reflex regulation of blood volume by control of urine output. However, it seems that the function of the atrial receptors must remain obscure until the problem of their effective stimulus has been clarified.

SUMMARY

1. An attempt has been made to localize and to identify histologically in the dog heart the receptors which give rise to vagal impulses with an atrial rhythm.

2. The position of the receptors was first found approximately by occluding vessels and inducing local changes of blood pressure in the chambers of the heart and in the great vessels which altered the discharge in a single vagal fibre. Subsequently the position of the receptor was defined accurately by punctate stimulation of the endocardial surface after the heart had been opened. Receptors were found in the atrio-venous tissues on the right and left sides of the heart.

3. Portions of tissue in which receptors had been located were taken for histological examination and in all cases characteristic end formations were found in them.

4. The end formations were situated in the endocardium and were formed by the branching of myelinated fibres 3–10μ diameter. The branches formed a flattened plate parallel to the endocardial surface.

5. A histological survey of the heart showed a close correspondence between the distribution of these end formations and the receptor points which were located by electro-physiological methods.

6. The end formations and the points which could be stimulated were situated, with very few exceptions, in the junctional tissues of the venae cavae and right atrium and the pulmonary veins and left atrium. A few end formations were seen in the posterior part of the interatrial septum but no receptors were located there by punctate stimulation.

Part of the expenses of this research were defrayed by a grant to A.H. from the Medical Research Council.

REFERENCES

ADRIAN, E. D. (1933). Afferent impulses in the vagus and their effect on respiration. *J. Physiol.* **79**, 332–358.

AMANN, A. & SCHAEFER, H. (1943). Über sensible Impulse im Herzenerven. *Pflüg. Arch. ges. Physiol.* **246**, 757–789.

BALLIN, I. R. & KATZ, L. N. (1941). Observations on the localization of the receptor area of the Bainbridge reflex. *Amer. J. Physiol.* **135**, 202–213.

COLERIDGE, J. C. G., HEMINGWAY, A., HOLMES, R. L. & LINDEN, R. J. (1956). Atrial receptors in the dog. *J. Physiol.* **132**, 68–69 *P.*

COLERIDGE, J. C. G. & LINDEN, R. J. (1954). The measurement of effective atrial pressure. *J. Physiol.* **126**, 304–318.

COLERIDGE, J. C. G. & LINDEN, R. J. (1955*a*). The effect of intravenous infusions upon the heart rate of the anaesthetized dog. *J. Physiol.* **128**, 310–319.

COLERIDGE, J. C. G. & LINDEN, R. J. (1955*b*). The effect upon the heart rate of increasing the venous return by opening an arterio-venous fistula in the anaesthetized dog. *J. Physiol.* **130**, 674–702.

DALY, M. DE BURGH & EVANS, D. H. L. (1953). Functional and histological changes in the vagus nerve of the cat after degenerative section at various levels. *J. Physiol.* **120**, 579–595.

DAWES, G. S. (1952). Reflexes from the heart and lungs. *Brit. med. Bull.* **8**, 324–330.

DAWES, G. S. & WIDDICOMBE, J. G. (1953). The afferent pathway of the Bezold reflex: the left vagal branches in dogs. *Brit. J. Pharmacol.* **8**, 395–398.

DICKINSON, C. J. (1950*a*). Afferent nerves from the heart region. *J. Physiol.* **111**, 399–407.

DICKINSON, C. J. (1950*b*). *The properties of afferent nerve fibres from the venous regions.* D.Phil. Thesis, University of Oxford.

DOGIEL, A. S. (1898). Die sensiblen Nervendigungen im Herzen und in den Blutgefässen der Säugethiere. *Arch. mikr. Anat.* **52**, 44–70.

HENRY, J. P. & PEARCE, J. W. (1956). The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. *J. Physiol.* **131**, 572–585.

HOLMES, R. L. (1956*a*). Receptor nerve endings in the adult dog heart. *J. Anat., Lond.*, **90**, 588.

HOLMES, R. L. (1956*b*). Further observations on the nerve endings in the adult dog heart. *J. Anat., Lond.*, **90**, 600.

HOLMES, W. (1947). The peripheral nerve biopsy. In *Recent Advances in Clinical Pathology*, ed. Dyke, S. C. London: Churchill.

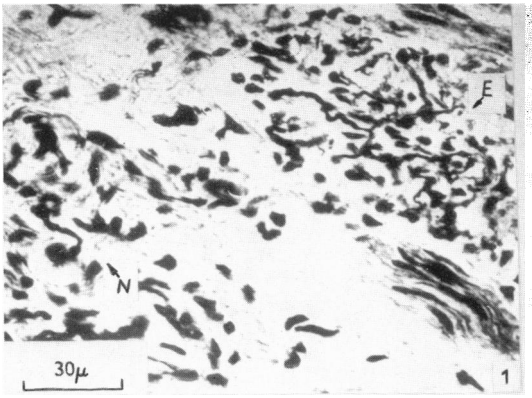
JARISCH, A. & ZOTTERMAN, Y. (1948). Depressor reflexes from the heart. *Acta physiol. scand.* **16**, 31–51.

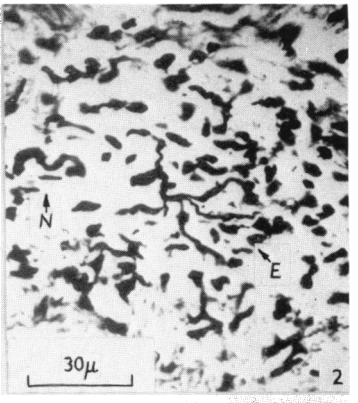
LEVY, S. E. & BLALOCK, A. (1937). Fractionation of the output of the heart and of the oxygen consumption of normal unanesthetized dogs. *Amer. J. Physiol.* **118**, 368–371.

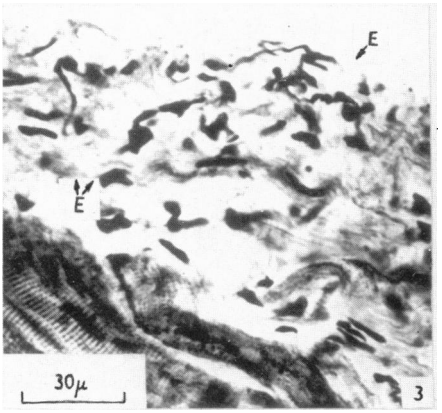
MEGIBOW, R. S., KATZ, L. N. & FEINSTEIN, M. (1943). Kinetics of respiration in experimental pulmonary embolism. *Arch. intern. Med.* **71**, 536–546.

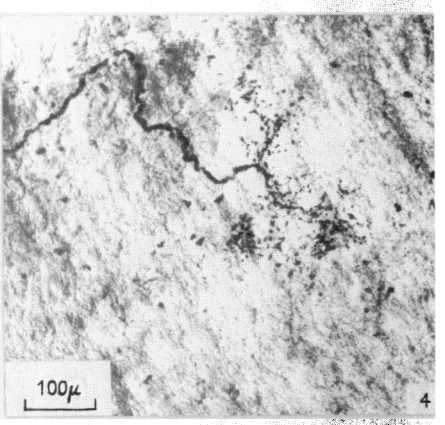
MEYLING, H. A. (1953). Structure and significance of the peripheral extension of the autonomic nervous system. *J. comp. Neurol.* **99**, 495–543.

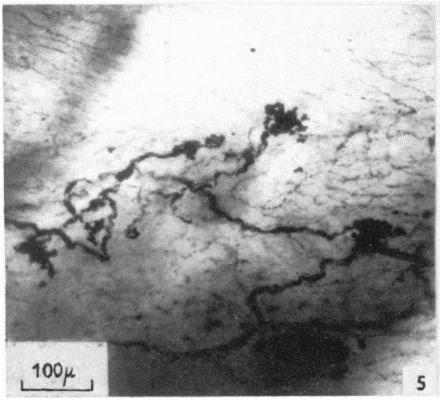
MITCHELL, G. A. G. (1953). Visceral nerves demonstrated by combined intravital and supravital techniques. *Acta Anat.* **18**, 81–86.

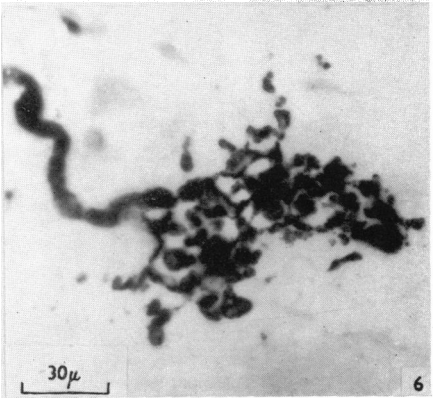












(*Facing p.* 197)

MITCHELL, G. A. G. (1956). *Cardiovascular Innervation.* Edinburgh: Livingstone.

NEIL, E. & ZOTTERMAN, Y. (1950). Cardiac vagal afferent fibres in the cat and the frog. *Acta physiol. scand.* **20**, 160–165.

NETTLESHIP, W. A. (1936). Experimental studies on the afferent innervation of the cat's heart. *J. comp. Neurol.* **64**, 115–133.

NONIDEZ, J. F. (1937). Identification of the receptor areas in the venae cavae and pulmonary veins which initiate reflex cardiac acceleration (Bainbridge's reflex). *Amer. J. Anat.* **61**, 203–231.

NONIDEZ, J. F. (1941). Studies on the innervation of the heart. II. Afferent nerve endings in the large arteries and veins. *Amer. J. Anat.* **68**, 151–189.

PAINTAL, A. S. (1953*a*). A study of right and left atrial receptors. *J. Physiol.* **120**, 596–610.

PAINTAL, A. S. (1953*b*) The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.* **121**, 341–359.

PANNIER, R. (1940). Contribution à l'étude de l'innervation presso- et chemo-sensible des oreillettes et des vaisseaux de la base du cœur. *Arch. int. Pharmacodyn.* **64**, 476–484.

PEARCE, J. W. & WHITTERIDGE, D. (1951). The relation of pulmonary arterial pressure variations to the activity of afferent pulmonary vascular fibres. *Quart. J. exp. Physiol.* **36**, 177–188.

SASSA, K. & MIYAZAKI, H. (1920). The influence of venous pressure upon the heart-rate. *J. Physiol.* **54**, 203–212.

SATO, H. (1954). Innervation of heart in dog. *Tohoku J. exp. Med.* **59**, 343–356.

SMIRNOW, A. (1895). Ueber die sensiblen Nervendigungen im Herzen bei Amphibien und Säugetieren. *Anat. Anz.* **10**, 737–749.

TCHENG, K. T. (1951). Innervation of the dog's heart. *Amer. Heart J.* **41**, 512–524.

WHITTERIDGE, D. (1948). Afferent nerve fibres from the heart and lungs in the cervical vagus. *J. Physiol.* **107**, 496–512.

WHITTERIDGE, D. (1952). Afferent paths of cardiovascular reflexes. In *Visceral Circulation*, ed. Wolstenholme, G. E. W. Ciba Foundation Symposium. London: Churchill.

WOOLLARD, H. H. (1926). The innervation of the heart. *J. Anat., Lond.*, **60**, 345–373.

EXPLANATION OF PLATE

Figs. 1–3 are sections taken from physiologically localized receptor areas of the atrio-venous wall of the dog. Figs. 4–6 are taken from whole thickness methylene blue preparations. All photomicrographs are untouched.

Fig. 1. Tangential section through endocardium of pulmonary vein. The localization of this receptor area was achieved in the experiment depicted in Text-fig. 10. To the left, a thick nerve fibre (*N*) is cut across several times; to the right, the fine branched nerve fibres and associated nuclei of a characteristic end formation (*E*). 12 μ section; silver impregnation.

Fig. 2. Tangential section through endocardium of pulmonary vein. Again, a thick nerve fibre (*N*) and a typical end formation (*E*) are present. 12 μ section; silver impregnation.

Fig. 3. Transverse section through right atrial endocardium. The site of this receptor area is shown in Text-fig. 12*A*; and the potentials recorded from the fibre arising from this area are shown in Text-fig. 2. In the endocardial connective tissue, branched nerve fibres (*E*) and nuclei can be seen. 12 μ section; silver impregnation.

Fig. 4. A thick nerve fibre in the atrial endocardium running to three end formations. Whole thickness methylene blue preparation, viewed from the endocardial surface.

Fig. 5. Thick nerve fibres, ending as terminal expansions, in the posterior wall of the left atrium. In the background, the fine plexiform fibres of the terminal nervous network can be seen. Whole thickness methylene blue preparation.

Fig. 6. High-power photomicrograph of a typical end formation. A thick nerve fibre terminates by branching into finer fibres which are partly obscured by deeply stained associated cellular elements. Note the essential similarity between this structure, seen in a whole thickness preparation, and the ending shown in the silver impregnated section in fig. 2.