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SYSTEMIC ARTERIAL BARORECEPTORS IN DUCKS AND THE CONSEQUENCES OF THEIR DENERVATION ON SOME CARDIOVASCULAR RESPONSES TO DIVING

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SUMMARY

1. In the duck systemic arterial baroreceptors which cause bradycardia in response to induced hypertension are located in the walls of the ascending aorta, innervated by the depressor nerves.

2. The location of the baroreceptors was confirmed both histologically and by recording activity from the depressor nerve. Stimulation of the central cut end of a depressor nerve caused transient bradycardia and a fall in blood pressure which was maintained throughout the period of stimulation.

3. Cardiovascular adjustments to submergence of 2 min duration were monitored in intact, sham-operated and denervated ducks. The sham-operated and denervated ducks were used in the experiments some 20–50 days post-operation. The denervations were checked at post-mortem.

4. In the first series of experiments on young ducks mean arterial pressure during a 2 min dive fell by 30 % in intact, 17·5 % in sham-operated, and 48 % in denervated ducks. In all ducks heart rate was reduced by 84–85 %.

5. In a second series of experiments on older ducks sciatic artery blood flow was also recorded and mean arterial blood pressure fell by 9·2 % in intact and by 53 % in denervated animals, although there were no significant differences in heart rate during the 2 min dives. In normal animals sciatic vascular resistance increased after 2 min submergence by $7{\cdot}86 \pm 1{\cdot}7$ times, whereas in denervated ducks it increased by only $2{\cdot}32 \pm 0{\cdot}5$ times.

6. The role of systemic arterial baroreceptors in generation of the cardiovascular responses to submergence in ducks is discussed in terms of the input supplied by the baroreceptors to the central nervous system.

INTRODUCTION

During submergence of diving birds and mammals blood pressure is maintained despite the pronounced bradycardia (Irving, Scholander &

Grinnell, 1942; Butler & Jones, 1971). A possible role of baroreceptors in the generation of diving bradycardia was discounted by Hollenberg & Uvnäs (1963) and Johansen & Aakhus (1963), although Andersen (1966) counselled against this dismissal. Furthermore, Holm & Sørensen (1971) attributed lack of diving bradycardia in carotid body denervated ducks to a new circulatory state caused by baroreceptor denervation. On the other hand, in a later paper (Holm & Sørensen, 1972) they concluded that the lack of bradycardia was due to chemoreceptor denervation, a view compatible with that of Jones & Purves (1970) and Angell James & Daly (1972*a*). However, even if baroreceptors have only a secondary role in the development of bradycardia (Kobinger & Oda, 1969) their effect on the integrated cardiovascular response to diving may still be profound. Recently, Angell James & Daly (1972*a*) have suggested that if resetting of baroreceptor-cardiac and -vasomotor reflexes occurs during diving then baroreceptors must obviously play an important part in the diving response. In the present series of experiments it was proposed to denervate the baroreceptors in ducks and, after their recovery from the operation, to compare their diving responses with those of normal animals.

Unfortunately the precise location of the systemic arterial baroreceptors of birds has never been conclusively proved either morphologically or physiologically, although there is no doubt that the reflex cardiac chronotropic effects of either hypo- or hypertension confirm their existence (Durfee, 1963; Djojosugito, Folkow & Kovach, 1968). Since birds lack a carotid sinus (Muratori, 1934; Nonidez, 1935) it is unlikely that the location of systemic arterial baroreceptors in birds will follow the mammalian scheme of arterial reflexogenic zones: nevertheless, there is some neurophysiological evidence (Jones, 1969) and good histological agreement that baroreceptors are present in the tunica adventitia of the aortic trunk (Heymans & Neil, 1958; Ábrahám, 1969). The situation with regard to the carotid reflexogenic zone is less clear. Histologically Ábrahám (1969) describes nerve terminations in this region as a plexus, and denies the presence of specialized afferent endings in the form of endknots or rings as described previously by Nonidez (1935) in the chick. Physiologically the evidence is equally contradictory with Van der Linden (1934), Ara (1934), and Muratori (1934) claiming this area to be a pressure reflexogenic zone in strict contrast to the later studies of Durfee (1964) and McGinnis & Ringer (1966). Consequently before studies could be made on the role of the baroreceptors in diving it was necessary to identify their location in the arterial system both anatomically and physiologically.

METHODS

The experiments were performed on fifty-five Khaki Campbell ducks (*Anas platyrhynchos* var.) 5–14 weeks of age and weighing from 0·8 to 2·2 kg. In the present account 'left' and 'right' refer respectively to the left and right sides of the animal. The word 'control' describes the response of any group of animals before submergence. Mean arterial blood pressure (M.A.P.) was calculated from the formula M.A.P. = diastolic pressure + ($f \times$ pulse pressure). The factor, f, was not a constant but varied from 0·45 to 0·51 with heart rate. The relationship between f and heart rate was established by comparison with measurements of mean pressure obtained by the 'area under the curve' method (Butler & Jones, 1971). Numerical values, when referring to determinations of variables in a group of animals, are given as means ± S.E. of the mean. Statistical analysis of the results was done by Fisher's t test and 5% ($P < 0·05$) was considered the fiducial limit of significance. All experiments were carried out at room temperature (22–24° C).

(*a*) *Location of systemic arterial baroreceptors*

A polyethylene cannula (external diameter 0·125 cm; length 30 cm) filled with heparinized avian saline (40 i.u./ml.) was inserted into the brachial vein and advanced until the tip was close to the right atrium. The cannulation was performed under local anaesthesia (2% Xylocaine, Astra Pharmaceutical Div., Mississauga, Ontario). The duck was then anaesthetized by intravenous injection of urethane 1 g/kg or 'Ketalar' 20–30 mg/kg (Ketamine Sulphate, Parke, Davis and Co., Brockville, Ontario). The animal was opened ventrally from the mid-cervical region to the posterior end of the sternum as described by Jones & Purves (1970). The sternum was held open by means of a retractor, and then the cervical vagi, carotid arteries proximal and distal to the carotid bodies, and depressor nerves to the aorta (Terni, 1931; Nonidez, 1935) were freed from adjoining tissues.

An heparinized saline-filled polyethylene catheter (external diameter 0·15 cm; length 15 cm) was inserted into one brachiocephalic artery and blood pressure was recorded using a Hewlett Packard 267 BC pressure transducer. Heart rate was recorded from the e.c.g., using bipolar copper wire electrodes (Butler & Jones, 1968) and after amplification the signal was fed into an instantaneous heart rate-meter to give pulse frequency. Blood pressure and instantaneous heart rate were displayed on a heated stylus pen recorder writing on rectilinear co-ordinates. Reversible cold block of the vagi was achieved by laying the nerve trunks on a brass plate through the interior of which an ice-salt water solution was circulated. The temperature of the brass plate was between −1·0 and 0·0° C. Bipolar silver electrodes were used for nerve recording or stimulation. For stimulation the electrodes were connected to a Grass S4 stimulator (Grass Instruments, Quincy, Mass., U.S.A.). For recording the nerve was laid on an earthed back plate and desheathed, and small slips dissected from the nerve were placed on the electrodes. The signal was amplified in a conventional manner, displayed on a dual beam oscilloscope, and stored on an FM Tape System (Hewlett-Packard 3907 C, H.P. Ltd Waltham, Mass., U.S.A.). The events were replayed on the oscilloscope and recorded by a moving film camera. Adrenaline or noradrenaline (5 μg/kg) was injected I.V. through the polyethylene cannula in the brachial vein.

(*b*) *Denervation of systemic arterial baroreceptors*

As experiments required that the systemic arterial baroreceptors be denervated and that the animals should survive the effects of the anaesthetic and operation,

young ducks (5–8 weeks old) were chosen since in ducks of this age the sternum is still cartilaginous (Jones & Purves, 1970). Animals were surgically anaesthetized by I.V. injection of 'urethane' (1 g/kg) or 'Ketalar' (20–30 mg/kg) and the skin over the sternum and anterior air sac was reflected and the air sac opened. The furculum and sternum were split in the mid line to expose the pericardium. The vagi were located on each side caudad to the thyroid gland. The depressor nerves, arising from the lower pole of the nodose ganglion, were identified on each side and were both sectioned approximately midway in their course from the nodose ganglion to the aortic root. The wound was closed and a mixture of streptomycin and tetracycline powder instilled (Jones & Purves, 1970). The ducks were given penicillin (60,000 i.u.) I.M. for 6 days after operation. A total of twelve ducks were operated on and the denervations were checked at post-mortems. At post-mortem complete denervation was confirmed in only ten of the twelve animals.

Twelve ducks were operated on as controls using anaesthetic and operative procedures outlined above, except that the depressor nerves were identified but not cut; these are referred to as 'sham-operated' ducks. The denervated and 'sham-operated' ducks were typically used as experimental subjects some 20–30 days after operation when they had exhibited a weight gain of from 8–15% of their pre-operative weight, a gain that was consistent with the weight increase of normal (unoperated) ducks kept under the same conditions. However, three 'sham-operated' ducks were used only 5 days post-operation when their average body weight had decreased by 8%.

(c) *Determination of cardiovascular adjustments to diving*

Heart rate, central venous pressure, and arterial blood pressure were measured in four unoperated, twelve 'sham-operated' and six denervated ducks before, during and after submergence of 2 min duration. In a second series of experiments sciatic blood flow was recorded simultaneously with the above variables in two intact, two sham-operated, and four denervated ducks. During experiments the birds were restrained horizontally ventral side down. All operative procedures were of a minor nature and were performed under local anaesthesia as described in detail previously (Butler & Jones, 1971). Periods of 24–48 hr were allowed to elapse after any operation before the start of the experiment. All animals that had undergone operations were killed at the end of the experiment by an overdose of urethane or pentobarbitone sodium.

A polyethylene cannula (external diameter 0·15 cm, length 30 cm) was inserted into a brachial vein and advanced some 10 cm towards the heart to allow recording of central venous pressure. Arterial blood pressure was recorded from one sciatic artery after cannulation with polyethylene tubing (external diameter 0·125 cm, length 10 cm), and blood flow from the other using an implanted cuff-type flow probe. A pneumatic flow occluder was sited downstream of the flow probe to allow determination of zero flow. The operative techniques and the instruments used to record pressure and flow, including their static and dynamic calibrations, were identical to those described in detail by Butler & Jones (1971) except that the phase lag of the flow-meter system was 3·2°/Hz. All cardiovascular recordings were displayed on a Techni-Rite TR888 (Techni-Rite Electronics, Inc., Warwick, Rhode-Island, U.S.A.) pen recorder writing on rectilinear coordinates. Adrenaline or noradrenaline (5 μg/kg) was injected through the cannula inserted in the brachial vein before and at the completion of the series of dives on each animal.

In the present account the terminology regarding the diving manoeuvres is the same as that used previously (Butler & Jones, 1968) and signifies that only the head of the duck was involved in these procedures. Three to six dives of 2 min duration were performed on each duck, with rest periods of 1 hr between each dive.

A series of briefer introductory dives, whose duration was gradually increased, enabled the birds to endure the experimental dives with little sign of discomfort or stress.

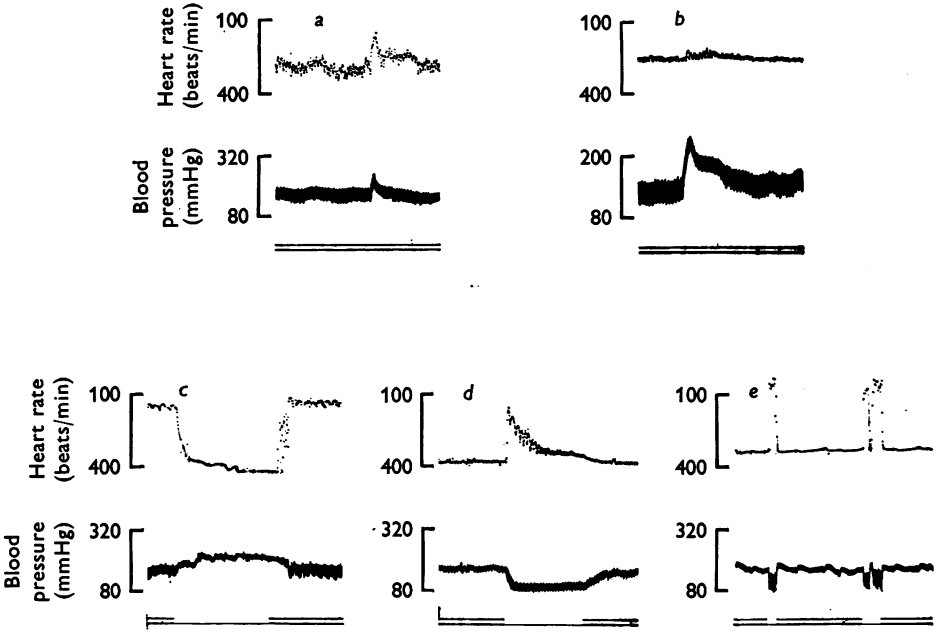
RESULTS

(*a*) *Location and innervation of systemic arterial baroreceptors*

Obviously at the inception of these experiments it was necessary to define a cardiovascular response which could reasonably be attributed to the activity of systemic arterial baroreceptors. From analogy with mammals, the cardiac chronotropic response to changes in systemic arterial blood pressure was chosen. In the majority of cases the change in blood pressure was induced by injection of adrenaline (5 μg/kg) or noradrenaline, although at the same dose level the former was more potent than the latter and was consequently used more frequently. A preliminary series of experiments was performed on five ducks comprising drug injection, blood vessel occlusion, and nerve stimulation and section in order to assist determination of the site and innervation of the systemic arterial baroreceptors.

The later experiments were performed on ten ducks and typical results are illustrated in Text-fig. 1. Following the operation to expose the heart and major blood vessels the cardiac chronotropic response to injection of adrenaline (5 μg/kg) or noradrenaline was determined. The increase in mean blood pressure following drug injecton was of the order of 50–100 mmHg and heart rate fell by 50–150 beats/min (Text-fig. 1*a*). The largest fall in heart rate was generally associated with those animals which displayed the higher rates before drug injection. The cervical vagi were then placed in turn on the cooling plate and the drug injections repeated. In four of the ducks cooling one vagus produced marked increases in resting heart rate (Text-fig. 1*c*), whereas cooling the other caused no significant change. This finding has been reported and discussed previously by Butler & Jones (1968). Consequently only one vagus was active in cardiac chronotropic control at the time of the experiment and this was the left in two ducks and the right in the other two. In the three other ducks tested cooling either vagus produced similar small increases in heart rate. In the ducks with only one 'active' vagus the cardiac chronotropic response to increased blood pressure was eliminated when the 'active' vagus was cooled cephalad to the origin of the depressor nerve but was unchanged from normal when the 'inactive' vagus was cooled. As would be predicted from this result, in ducks in which both vagi were active the induced hypertensive bradycardia was relatively unaffected by cooling either vagus singly. Some 30 min after completion of the cooling tests a control drug injection was performed and all ducks showed a normal cardiac chronotropic re-

sponse to the induced hypertension. One vagus was then sectioned high in the neck in each duck cephalad to the origin of the depressor nerve (the 'inactive' one in four ducks and left or right, at random, in the others) and the drug injections were repeated. All animals displayed prompt bradycardia when the mean blood pressure rose. Cooling the remaining intact vagus high in the neck destroyed the heart rate response to the drug-induced hypertension.



Text-fig. 1. Records from experiments designed to trace the location of the systemic arterial baroreceptors in ducks. In all records, upper trace = instantaneous heart rate (beats/min); middle trace = arterial blood pressure (mmHg); lower trace = time (sec) and event marker. *a*, Normal duck, response to injection of adrenaline 5 μg/kg; *b*, after mid-cervical section of left vagus and right depressor nerve, response to injection of adrenaline 5 μg/kg; *c*, cooling the active vagus, other vagus intact; *d*, stimulation of the cut central end of the depressor nerve (150 Hz, 1 msec duration, 0·7 V); *e*, stimulation of the peripheral end of the cut vagus nerve (150 Hz, 1 msec duration, 0·5 V (first mark) and 0·25 V (second mark)). Records *a* and *b* are from one duck, and *c*, *d*, and *e* are from one duck. See text for further details.

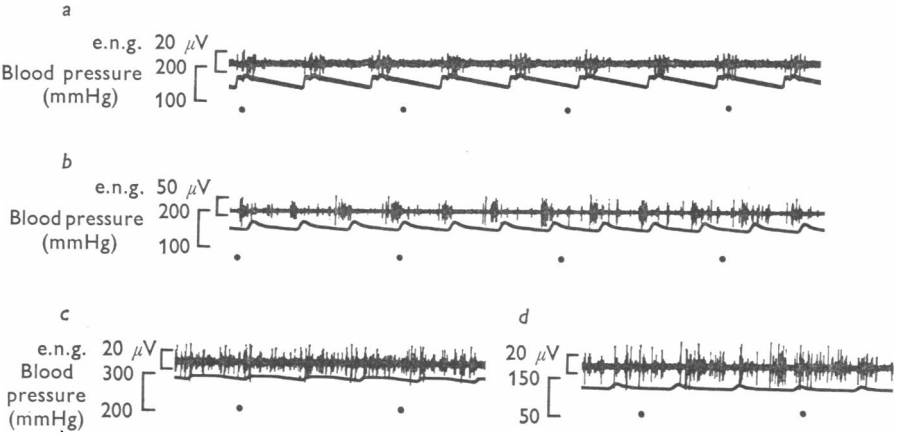
These results show that afferent receptors, stimulated by the rise in blood pressure and causing reflex bradycardia, are innervated by the vagus and that only one vagus is necessary for the complete response; furthermore the reflexogenic zone must be located caudad to the mid-cervical

region. Since neither atrial nor ventricular sensory receptor structures have been found histologically in the avian heart (Ábrahám, 1969) the only probable sites anterior to the heart carrying vagal innervation are the carotid body area and the central great arteries (Nonidez, 1935; Jones & Purves, 1970). The carotid body's role in the response was investigated in those animals in which one vagus had been previously sectioned cephalad to the origin of the depressor nerve. The cardiac chronotropic response to drug injection was compared before and after the carotid artery was occluded by haemostats, anteriorly and posteriorly to the carotid body area, on the side of the animal in which the vagus was intact. This procedure caused no change in resting heart rate and blood pressure and time courses of the changes in both heart rate and blood pressure following drug injection were identical before and after occlusion. Similar procedures performed on the side on which the vagus was sectioned gave similar results. The anterior occlusion of the carotid artery is necessary in birds since both carotids are in open connexion in the intrasphenoid segment (Baumel & Gerchman, 1968); accordingly pressure changes occurring in the patent carotid artery following drug injection would also affect the other carotid if the vessel was clamped only on the side nearest the heart. Finally the depressor nerve, which had previously been traced anatomically to supply the walls of the ascending aorta (see diagram in Jones & Johansen, 1972), was sectioned on the side of the animal where the vagus was intact and the adrenaline induced rise in blood pressure was now in the range of 100–175 mmHg while heart rate did not change significantly (Text-fig. 1*b*). Consequently these experiments confirmed that the carotid area was not involved in the cardiac chronotropic response to induced hypertension.

Central stimulation of the cut depressor nerve (0·2–0·7 V, 1 msec duration, 50–200 Hz) caused an immediate fall (of from 100–175 beats/min) in heart rate and of from 100 to 150 mmHg (Text-fig. 1*d*) in blood pressure. However, as can be seen from Text-fig. 1*d* the heart rate returned to normal levels during stimulation while the fall in blood pressure was maintained for the duration of stimulation. Section of the remaining intact vagus in the mid-cervical region destroyed the response to central stimulation of the cut depressor nerve; in fact no response was observed even when intensities in excess of 10 V were used. However, stimulation of the cut peripheral end of this vagus (0·5 V, 1 msec duration, 150 Hz) caused bradycardia and hypotension (Text-fig. 1*e*) indicating that the efferent nervous innervation of the heart had been intact. Stimulation (0·2–0·7 V, 1 msec duration, 50–200 Hz) of the peripheral end of the cut depressor nerve had no effect on heart rate or blood pressure.

Afferent activity in the left depressor nerve was recorded in seven

animals while blood pressure was recorded simultaneously from either the brachiocephalic (Text-fig. 2*a*) or sciatic arteries (Fig. 2*b–d*), using implanted polyethylene cannulae (length 15 cm, external diameter 0·15 cm). The cut peripheral end of the nerve was split into small slips using fine dissecting forceps until a small number of identifiable units was obtained.



Text-fig. 2. Electroneurograms from the peripheral end of the cut depressor nerve. Upper trace = e.n.g.; middle trace = arterial blood pressure (mmHg); lower trace = time (sec). *a*, Relationship between baroreceptor discharge and brachiocephalic blood pressure; *b*, relationship between baroreceptor discharge and sciatic artery blood pressure; *c*, discharge following injection of adrenaline 1·5 μg/kg, blood pressure trace limited at 300 mmHg; *d*, discharge during punctate stimulation of the ascending aorta.

The actively discharging units showed a constant relationship with the systolic pressure rise, falling silent during diastole (Text-fig. 2*a*). During an elevation of blood pressure, caused by injection of adrenaline 1–2 μg/kg, this relationship was lost and the units fired throughout the cardiac cycle (compare Text-fig. 2*b*, *c*). Punctate stimulation of the ascending aorta (before division of the vessel into the systemic and brachiocephalic vessels) caused a marked increase in nerve discharge and the relationship with the systolic portion of the blood pressure change disappeared (compare Text-fig. 2*b*, *d*). This electrophysiological evidence confirms the presence of baroreceptors located in the ascending aorta and innervated by the depressor nerves. Histological sections of the ascending aorta, stained by Bielschowsky–Ábrahám's method, confirmed that typical baroreceptor end plates are present in the tunica adventitia and outer regions of the tunica media of the aortic wall close to the heart (Pl. 1). These characteristic receptor structures have not yet been identified in the wall of other

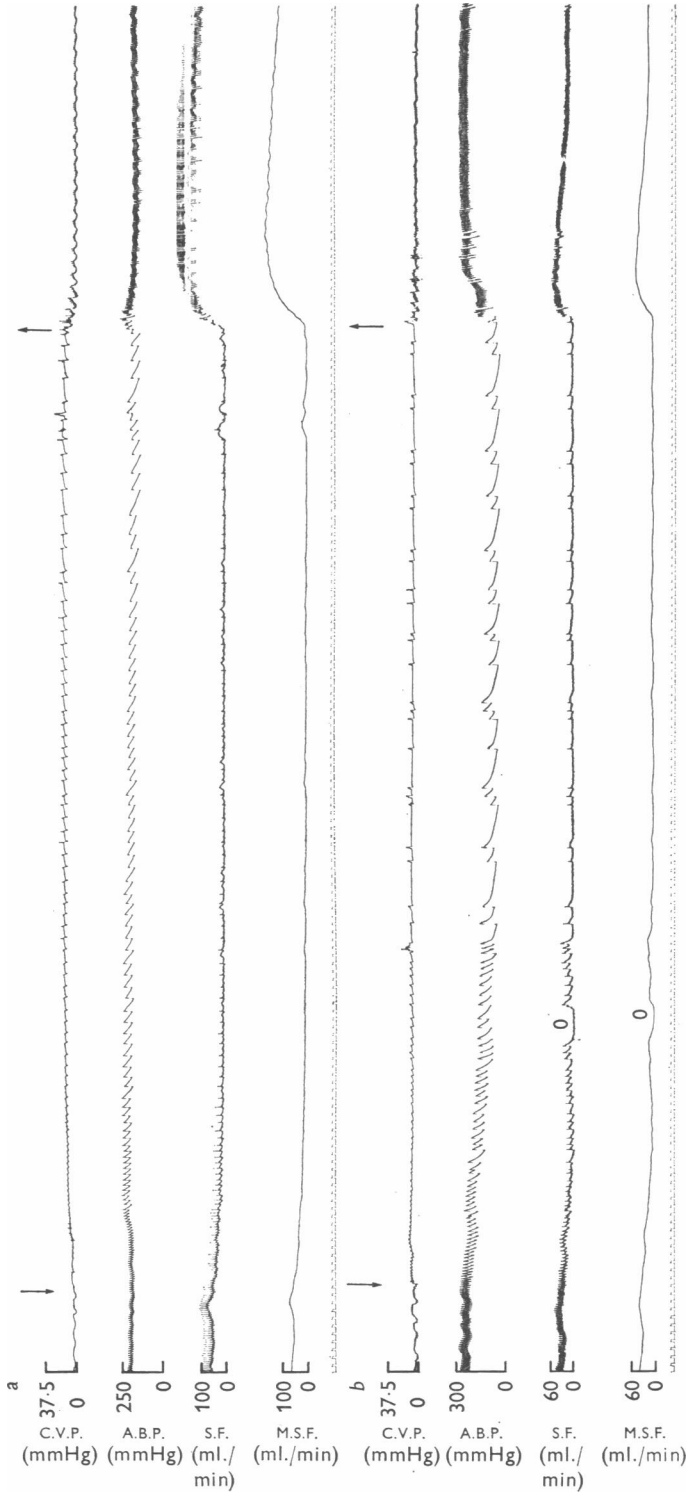
parts of the aorta. Furthermore, in the sections investigated, no tissues were observed which resembled those to which a chemoreceptor function is ascribed (De Kock, 1954, 1958, 1959; Jones & Purves, 1970).

(*b*) *Changes in heart rate and blood pressure during diving in intact, sham-operated and denervated ducks*

In resting animals venous pressure was similar for all, but heart rate and systolic and diastolic arterial blood pressures of the intact ducks were lower than in sham-operated or denervated ducks (Table 1) although well above those found in older intact ducks examined previously (Butler & Jones, 1971). An obvious difference between intact and sham-operated animals and their denervated counterparts was not apparent until 60 sec into the dive when systolic and diastolic pressures in the denervated animals were significantly below those in the others, although heart rates were more or less the same (Table 1). Also at this time venous pressure in the denervated ducks was significantly lower than in either the intact or sham-operated ducks (Table 1). This situation held for the remainder of the dive. Systolic arterial pressure in the sham-operated ducks was significantly higher than that in the intact animals after 60 sec submergence. In all three groups of animals heart rate fell by 84–85 % of the resting value after submergence of 2 min duration whereas mean arterial pressure (M.A.P. = diastolic pressure + ($f \times$ pulse pressure) where $f\alpha$ heart rate) fell by 17·5 % in the sham-operated, 30 % in intact and 48 % in denervated ducks. The fall in M.A.P. in the intact animals was about twice the fall recorded in older ducks examined previously (Butler & Jones, 1971). On emergence heart rate increased and within 10 sec was in the control range as were systolic and diastolic arterial pressure and venous pressure for all three groups of animals. However, in all three groups systolic and diastolic pressures then declined somewhat but were only significantly lower than the control values, 60 and 120 sec after emergence, in the denervated ducks (Table 1).

Although all animals exhibited the same mean reduction in heart rate during the dive, in the denervated ducks long periods of time frequently occurred between heart beats, during which blood pressure fell markedly, followed by a series of rapid heart beats during which blood pressure rose. The average of the longest cardiac intervals per dive in denervated ducks was $4{\cdot}8 \pm 0{\cdot}3$ sec (thirty-two dives), significantly longer than in either the normal ($3{\cdot}2 \pm 0{\cdot}32$ sec, thirteen dives) or sham-operated ducks ($3{\cdot}5 \pm 0{\cdot}26$ sec, forty-seven dives).

In order to test physiologically the efficacy of denervation, each animal was injected with adrenaline (5 μg/kg I.V.) before and after the series of dives. Both intact and sham-operated ducks displayed a significant fall



Text-fig. 3. Representative traces of the cardiovascular adjustment to diving in ducks, intact *a* and denervated *b*. Traces in *a* and *b* from top to bottom; 1, central venous blood pressure (mmHg); 2, arterial blood pressure (mmHg); 3, sciatic artery blood flow (ml./min); 4, mean sciatic artery blood flow (ml./min); 5, time (sec). Submersion and emersion at the downward and upward pointing arrows respectively.

TABLE 1. Cardiovascular responses in intact and denervated ducks before, during, and after submergence of 2 min duration. Mean values (± S.E. of mean) of thirteen dives on four intact ducks, forty-seven dives on twelve sham-operated ducks, and thirty-two dives on six denervated ducks

	Heart rate (beats/min)			Venous pressure (mmHg)			Systolic arterial pressure (mmHg)			Diastolic arterial pressure (mmHg)		
	Intact	Sham	Denervated	Intact	Sham	Denervated	Intact	Sham	Denervated	Intact	Sham	Denervated
Control	275·6 ± 19·67	320·9 ± 7·78	348·7 ± 10·94	3·68 ± 0·38	4·895 ± 0·68	3·82 ± 0·35	203 ± 5·05	231·7 ± 8·87	210·7 ± 4·61	171·6 ± 4·66	181·8 ± 5·58	188·6 ± 5·11
Dive												
30 sec	85·85 ± 6·67	93·36 ± 5·96	83·63 ± 6·77	10·28 ± 0·56	11·79 ± 0·61	8·64 ± 0·44	189·7 ± 6·26	221·5 ± 10·98	187·0 ± 7·5	146·7 ± 6·18	159·69 ± 9·41	148·3 ± 8·08
60 sec	55·85 ± 6·49	63·13 ± 4·19	58·94 ± 3·27	12·15 ± 0·53	13·88 ± 0·59	9·04 ± 0·53	173·5 ± 4·62	215·4 ± 12·0	127·99 ± 3·86	116·3 ± 6·34	140·0 ± 9·01	91·86 ± 4·7
120 sec	42·26 ± 3·21	49·57 ± 4·30	53·44 ± 4·77	12·58 ± 0·68	12·92 ± 0·49	9·26 ± 0·55	162·8 ± 3·77	209·8 ± 12·1	123·45 ± 5·58	103·2 ± 3·85	136·6 ± 8·62	87·96 ± 5·97
Recovery												
10 sec	324·9 ± 34·8	302·9 ± 14·5	366·5 ± 18·7	5·42 ± 0·57	7·26 ± 1·22	5·81 ± 1·22	200·34 ± 9·48	244·1 ± 13·2	204·7 ± 5·94	154·99 ± 8·07	182·95 ± 10·15	186·1 ± 6·58
60 sec	302·8 ± 26·6	306·7 ± 12·3	344·9 ± 13·5	4·52 ± 0·54	4·45 ± 0·57	5·40 ± 1·22	193·98 ± 4·46	230·3 ± 11·19	159·9 ± 4·47	151·5 ± 4·33	164·2 ± 7·94	124·4 ± 5·7
120 sec	301·9 ± 22·15	315·4 ± 10·42	360·4 ± 12·1	3·94 ± 0·51	4·37 ± 0·61	4·71 ± 0·79	190·1 ± 3·9	228·5 ± 10·12	179·1 ± 6·04	155·7 ± 6·38	156·11 ± 5·01	143·4 ± 6·66

in heart rate of some 50%, 5–10 sec after the adrenaline injection when blood pressure was highest. During this period the change in heart rate of denervated animals was at maximum 2%, a value well within the range of normal variation.

(c) *Changes in heart rate, blood pressure, and sciatic vascular resistance during diving in intact and denervated ducks*

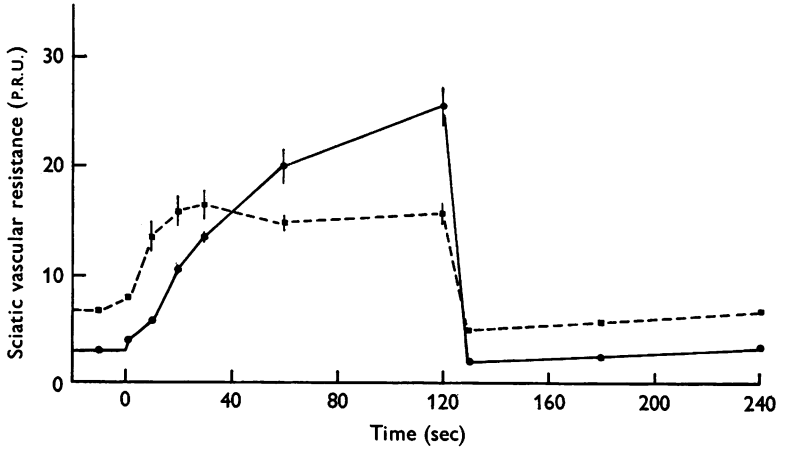
In the above experiments, although conclusively demonstrating the role of the systemic arterial baroreceptors in blood-pressure maintenance during diving, the blood-pressure response of normal animals was inferior to that of animals examined previously (Butler & Jones, 1971). This may have been due to the difference in ages of the ducks examined, and so a further group of experiments were performed on ducks which were up to 14 weeks of age and up to 2·2 kg in weight. In this series six ducks were denervated and experimented on some 30–50 days after the operation. One duck died 14 days after operation, and denervation in one duck could not be confirmed at post-mortem. Consequently only data from four animals is included in the denervated group. The two sham-operated ducks behaved identically to the two unoperated animals and have been included with them in the intact group.

At rest, mean sciatic flow in denervated ducks was significantly lower than in intact animals, while systolic and diastolic arterial pressures and venous pressure were significantly above those of intact animals (Table 2, Text-fig. 3). Heart rate of denervated ducks was higher than in intact animals but not significantly so. During diving there were no significant differences in heart rate between intact and denervated ducks at any time; both groups showed reductions in heart rate of 87% of control after 2 min submergence (Table 2). In both groups venous pressure rose markedly; after five seconds submergence both denervated and intact ducks showed a significant elevation above control (Table 2; Fig. 3). However, the significant difference between venous pressures in intact and denervated ducks existing at rest was maintained until 1 min submergence (Table 2). In intact animals systolic arterial pressure although falling by 9·2% was never significantly below control, whereas in denervated animals this pressure was significantly below control after 20 sec and significantly below that in intact ducks after 30 sec, thereby reversing the situation seen at rest (Table 2; Fig. 3). After 2 min submergence systolic pressure in denervated ducks was reduced to 47% of control pressure (Table 2). The same pattern of change held for the diastolic arterial pressure between intact and denervated animals. At 20 sec submergence diastolic pressure in denervated animals was significantly below control and also that in intact ducks. However diastolic pressure in intact animals

TABLE 2. Cardiovascular responses in intact and denervated ducks before, during, and after submergence of 2 min duration. Mean values (± S.E. of mean) of twenty-one dives on four intact ducks and twenty-three dives on four denervated ducks

	Heart rate (beats/min)		Venous pressure (mmHg)		Systolic arterial pressure (mmHg)		Diastolic arterial pressure (mmHg)		Mean sciatic artery flow (ml./min)	
	Intact	Denervated	Intact	Denervated	Intact	Denervated	Intact	Denervated	Intact	Denervated
Control	322 ± 19·2	380·7 ± 17·6	2·61 ± 0·45	6·4 ± 0·88	191 ± 5·13	257 ± 8·15	154 ± 4·6	202 ± 7·0	55·3 ± 2·8	36·3 ± 2·2
Dive										
5 sec	192 ± 8·3	213·9 ± 11·2	6·04 ± 0·62	13·6 ± 1·02	204 ± 6·6	267 ± 5·7	162 ± 5·4	203 ± 4·1	48·2 ± 3·5	31·5 ± 2·4
10 sec	151 ± 6·4	153·4 ± 6·3	7·52 ± 0·57	13·8 ± 1·12	207 ± 7·2	246 ± 6·2	160 ± 7·6	180 ± 4·5	35·4 ± 2·9	21 ± 1·9
20 sec	94·3 ± 5·7	101·5 ± 5·2	9·32 ± 0·47	15·2 ± 1·24	206 ± 5·5	197 ± 5·7	158 ± 6·9	124 ± 6·2	22·1 ± 3·1	13·1 ± 1·6
30 sec	72 ± 4·0	75·9 ± 5·3	10·8 ± 0·53	15·5 ± 1·18	197 ± 5·9	169 ± 6·0	145 ± 7·8	88·3 ± 6·5	15·0 ± 1·8	9·6 ± 1·2
60 sec	54·9 ± 3·9	63·6 ± 7·4	12·6 ± 0·63	16·0 ± 1·54	183 ± 5·3	131 ± 6·6	130 ± 5·6	66·1 ± 3·9	9·95 ± 1·32	6·17 ± 0·75
120 sec	40 ± 3·3	50 ± 4·5	11·9 ± 0·77	13·9 ± 1·44	176 ± 6·3	122 ± 5·0	126 ± 7·3	65 ± 3·8	4·69 ± 1·12	6·7 ± 0·85
Recovery										
10 sec	403 ± 15·3	427 ± 17·5	3·19 ± 0·59	7·91 ± 1·18	170 ± 5·5	201 ± 9·9	123 ± 6·0	139 ± 9·7	82·5 ± 8·0	35·4 ± 2·0
60 sec	348 ± 15·8	360 ± 12·7	1·96 ± 0·51	5·31 ± 1·05	176 ± 4·3	199 ± 8·7	134 ± 4·5	132 ± 7·9	65·6 ± 5·7	31·3 ± 2·5
120 sec	333 ± 17·4	359 ± 12·5	1·94 ± 0·52	5·72 ± 1·07	184 ± 4·4	222 ± 6·9	146 ± 4·3	167 ± 8·0	51·7 ± 3·4	31·1 ± 2·1

was also significantly below control at 1 and 2 min submergence. In intact animals the fall was to only 87 % of control whereas in denervated animals diastolic pressure fell to 32 % of control (Table 2). Sciatic flow fell markedly in both groups of ducks during the first 20 sec of the dive (Text-fig. 3) and, at this stage, both flows were significantly below control although the significant difference in flow between groups was maintained until 30 sec (Table 2).



Text-fig. 4. Mean changes in sciatic vascular resistance (P.R.U.S) during 2 min dives in four intact (twenty-one dives) and four denervated (twenty-three dives) ducks. Submergence and emersion at time 0 and 120 sec respectively. ●——●, Intact animals; ■– –■, denervated animals.

Peripheral resistance before and during the dives was calculated for the sciatic vascular bed using the formula:

$$\text{Vascular resistance (P.R.U.S)} = \frac{\text{Mean arterial pressure (mmHg)} - \text{mean venous pressure (mmHg)}}{\text{Mean sciatic flow (ml./min)}}$$

where M.A.P. = diastolic pressure $+(f \times$ pulse pressure$)$, $f \propto$ heart rate. The control values were significantly different, vascular resistance in the denervated ducks being more than twice that in intact animals (Text-fig. 4) Vascular resistance increased in more or less the same proportion in both groups of ducks; in both it was significantly above control after 20 sec submergence (Text-fig. 4). However, in intact ducks vascular resistance continued to increase reaching a value $7{\cdot}86 \pm 1{\cdot}7$ times control whereas in denervated ducks there was little change from 20 sec submergence on; at 2 min vascular resistance had only increased by $2{\cdot}32 \pm 0{\cdot}5$ times control (Text-fig. 4).

During recovery a significant tachycardia was displayed after 10 sec

emergence, but only by intact ducks (Text-fig. 3). Venous pressure fell to the normal range in both groups within 10 sec recovery (Table 2, Text-fig. 3). In denervated ducks systolic and diastolic blood pressures rose on emergence, but the former remained significantly below control until 2 min after recovery while the latter did not attain control levels in the monitored recovery period. Systolic and diastolic pressures attained control levels in intact ducks within 60 sec recovery (Table 2). Sciatic flow increased quickly in both groups and after 10 sec was significantly above control in intact ducks (Text-fig. 3): after 60 sec recovery flow in both groups was at the control level. The large increase in sciatic flow in intact ducks was associated with a significant reduction in vascular resistance 10 sec after emergence (Text-fig. 4).

In response to injection of noradrenaline (5 μg/kg) intact animals displayed a marked reduction in heart rate as blood pressure rose but, as before, this was not observed in denervated ducks. A point of interest is that, although in intact ducks sciatic flow was significantly higher than in denervated animals, after drug injection flow fell to the same level in both groups.

DISCUSSION

The results have shown conclusively that receptors causing reflex bradycardia in response to induced hypertension are located in the wall of the ascending aorta before the division of that vessel into the systemic aorta and brachiocephalic arteries. Essentially this paper confirms the view of Durfee (1964) and McGinnis & Ringer (1966) that the carotid body area is not a pressor reflexogenic zone in birds. Furthermore the region of the aorta in which typical baroreceptor endings have been identified, both histologically and electrophysiologically, is not the area described by Nonidez (1935) as innervated by the left depressor nerve in the chick. Nonidez (1935) also reports that the right depressor nerve is absent in the chick, unlike the duck where both right and left are present. It may be that this is a species difference although Ábrahám (1969) has recently suggested that nerve terminations described by Nonidez (1935) are more typically chemoreceptor than baroceptor. In this case Nonidez (1935) may have been describing the histology and innervation of the analogue of the aortic chemoreceptors of mammals.

In the closed-loop situation the baroreceptor blood pressure control system of birds shows many similarities to that of mammals. For instance, a rapid change in blood pressure causes the vagal effectors to respond rapidly as in mammals (Katona, Barnett & Jackson, 1967; Thames & Kontos, 1970); heart rate can return to normal levels during electrical stimulation of the depressor nerve while blood pressure remains depressed, implying that the response of sympathetic constrictor and cardiac sym-

pathetic nerves is slower, although in the long term more effective in blood pressure regulation. It has frequently been shown in mammals that the peak response of the sympathetic effector limb is reached 10–20 sec after a step change in pressure (Hatakeyama, 1967; Levison, Barnett & Jackson, 1966; Martin, Levy & Zieske, 1969; Zingher & Grodins, 1964), and that the major effector limb of aortic barostatic reflex control is systemic arterial resistance rather than reflex control on cardiac pumping (Allison, Sagawa & Kumada, 1969; Corcondilas, Donald & Shepherd, 1964; Kumada & Iriuchijima, 1965).

In ducks, denervation of systemic arterial baroreceptors resulted in a more rapid development of diving bradycardia, although later on in the dive heart rates of both normal and denervated animals were nearly identical. The most striking difference between intact and denervated ducks was in their ability to adjust systemic vascular resistance after 20 sec of submergence. In normal animals resistance continued to increase with the developing bradycardia but sciatic vascular resistance was, in the case of denervated animals, more or less unchanged from the 20 sec value after 2 min submergence and consequently blood pressure fell markedly. However, the fall in systemic blood pressure in denervated animals may not have been caused solely by the failure to increase vascular resistance as stroke volume may also have been depressed more than in normals. Folkow, Nilsson & Yonce (1967) suggested that in normal ducks stroke volume decreased during a dive due to the negative inotropic effect of vagal efferent activity (Folkow & Yonce, 1967). This finding has also been confirmed in mammalian divers, e.g. coypu (Ferrante, Browner & Opdyke, 1968; Ferrante & Opdyke, 1969; Folkow, Lisander & Öberg, 1971). However, Jones & Holeton (1971) found on average a slight increase in stroke volume in lightly restrained unanaesthetized ducks during diving, two animals showing increases as large as 175 % of pre-dive values. Furthermore, calculations based on the present data show that an argument that stroke volume falls more in denervated animals than in normal ducks cannot be supported.

Although there are differences in some properties of aortic and carotid baroreceptors in mammals there is no doubt that both groups of receptors respond to rate of change of pressure as well as absolute pressure (Adrian, 1926; Bronk & Stella, 1932; Landgren, 1952; Ead, Green & Neil, 1952; Angell James, 1971). This also appears to be true of the aortic baroreceptors in ducks so, since heart rate decreases during a dive, the central nervous system will be presented with less information even though mean blood pressure may not change. This decrease in activity will be interpreted as a fall in blood pressure (Ead *et al.* 1952; Neil, 1952). Consequently in the early stages of the dive (when heart rate is controlled by

interaction of pulmonary stretch receptor and any reflexes set in train by immersion *per se* (Andersen, 1963; Jones & Purves, 1970; Angell James & Daly, 1972*a*, *b*) the initial response to the change in input to the central nervous system will be to oppose the developing bradycardia by means of the sympathetic cardiac and vasomotor effects, a process that will take 10–120 sec for final equilibrium (Hatakeyama, 1967; Levison *et al.* 1966). This conclusion agrees well with the experimental data as regards heart rate changes in intact and denervated ducks in the first 30 sec of the dive. As the dive is prolonged the bradycardia becomes more intense (now under the major controlling influence of chemoreceptor drive (Jones & Purves, 1970; Angell James & Daly, 1972*a*)), and information supplied by the baroreceptors will be further reduced. This in turn will tend to enhance the vasomotor response. In fact the present results confirm that arterial pressure in denervated ducks falls due to the duck's inability to increase peripheral resistance in the later stages of the dive. However, the observed stability of blood pressure will only be achieved if reference is now being made to a new set point which coincides with the current information from the baroreceptors. The necessary reduction in set point for the arterial barostatic control system could be caused by the increased chemoreceptor drive in this period of the dive (Korner, 1971).

Upon surfacing heart rate increases rapidly when breathing movements commence (Angell James & Daly, 1969, 1972*a*; Butler & Jones, 1968, 1971) before any significant change in arterial oxygen tension occurs (Jones & Purves, 1970). Consequently the information supplied to the central nervous system by the baroreceptors will increase, but not inordinately as pulse pressure is usually reduced in this period. However, this factor may be offset by the fact that the set point will still be reduced by the chemoreceptor drive. Theoretically the control system will respond by reducing heart rate and vasodilatation. Without doubt a conspicuous vasodilatation was exhibited in normal ducks 10 sec after emergence although there were no significant differences in heart rate between intact and denervated animals at this time.

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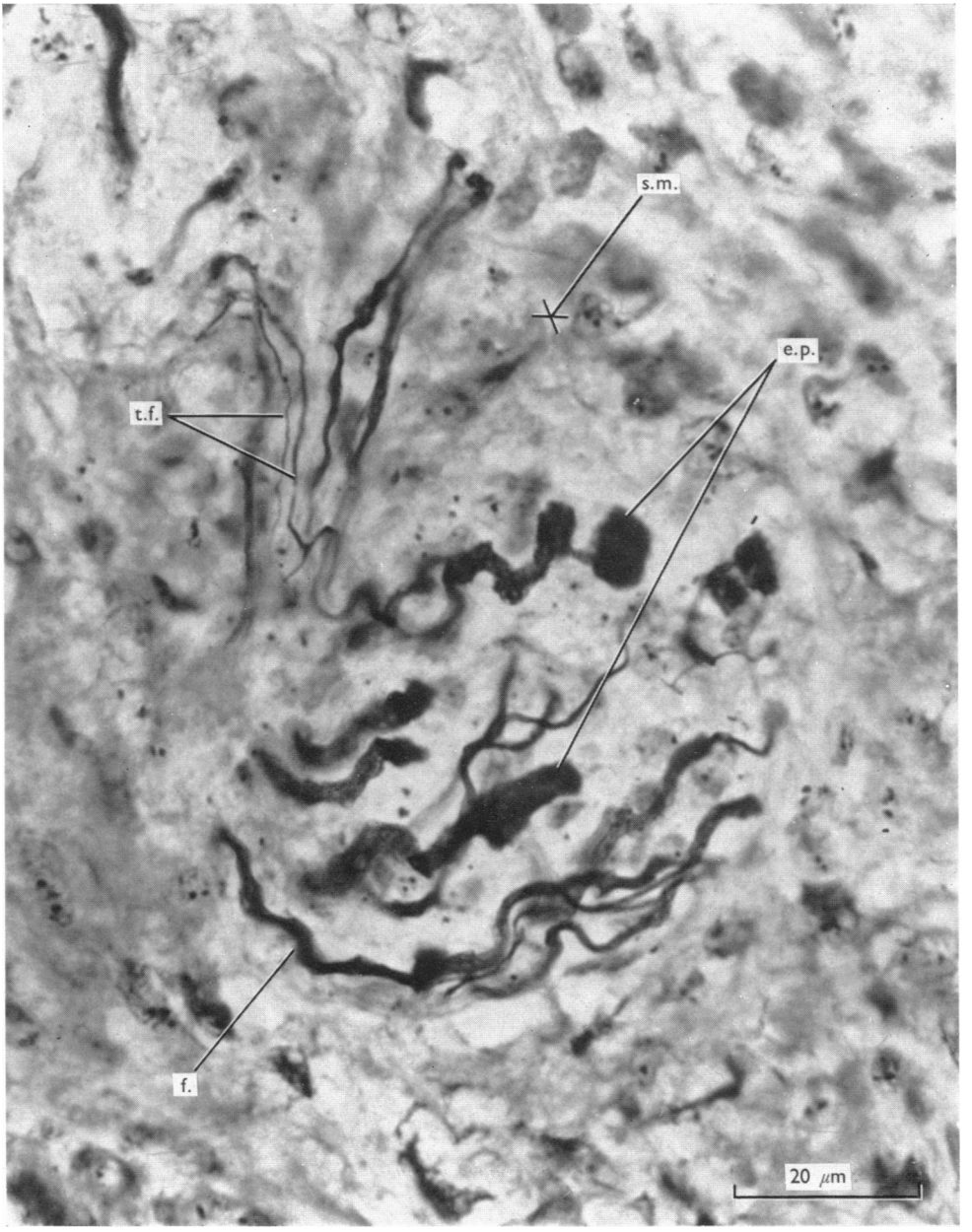
EXPLANATION OF PLATE

Detail from a section of a block of the ascending aorta showing typical baroreceptor nerve-end plates (e.p.) in the smooth muscle (s.m.) of the outer region of the *tunica media*. The section also shows a number of thin terminal fibres (t.f.) as well as some thicker nerve fibres (f.). In this block the majority of end-plates were located in the *tunica adventitia* and confined to a length of 1·8 mm of the ascending aorta. Bielschowsky-Ábrahám's method, × 1000. The bar represents 20 μm.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