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THE EFFECT OF HYPOXIA, HYPERCAPNIA AND HYPOTENSION UPON CAROTID BODY BLOOD FLOW AND OXYGEN CONSUMPTION IN THE CAT

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SUMMARY

1. Carotid body blood flow (c.b.f.), the arterio–venous oxygen (A–V O_2) difference and oxygen consumption were measured in forty-seven cats, anaesthetized with pentobarbitone, paralysed with gallamine and ventilated artificially. Carotid sinus and cervical sympathetic nerves were intact throughout.

2. A system for perfusing the carotid body artificially with blood is described and evidence is given which shows that similar results were obtained whether the carotid body was naturally or artificially perfused.

3. With arterial pressure, blood gas tensions and pH within physiological limits, c.b.f. varied between 33 and 68 μl./min, average 41·5; A–V O_2 difference between 0·21 and 0·46 ml./100 ml., average 0·34, and calculated oxygen consumption between 0·115 and 0·195 μl. O_2/min, average 0·147.

4. With constant mean arterial pressure, hypoxia (30–40 mm Hg P_{a,O_2}) or hypercapnia (> 50 mm Hg P_{a,CO_2}) resulted in a small increase of c.b.f., up to 14 μl./min above control; an average fall of A–V O_2 difference by 49 % of control and an average fall of oxygen consumption by 36 % of control.

5. Carotid body blood flow fell linearly with mean arterial pressure over the range 100–170 mm Hg, the slope of the curve varying between 0·78 and 1·22 μl. min^{-1}. mm Hg^{-1}. M.A.P. A–V O_2 difference was unaffected so that oxygen consumption fell in proportion to c.b.f.

6. It is concluded that the unique response of the carotid body to these stimuli is a fall in oxygen consumption and that this bears a closer relation to the known pattern of chemoreceptor discharge than do changes in total blood flow.

INTRODUCTION

The carotid body is known to receive a volume of blood flow and to have a metabolic rate for oxygen which, on a weight basis, is among the highest for any organ in the body so far studied (Daly, Lambertsen & Schweitzer, 1954). How these properties of the carotid body are involved in the excitation of the chemoreceptors is not known.

In this paper, the results are given of experiments in which the blood flow responses to three common physiological stimuli, hypoxia, hypercapnia and hypotension, were measured. In addition, the carotid artery–carotid body vein oxygen difference was measured so that oxygen consumption could be calculated. From these studies, it also appeared that activity mediated by the cervical sympathetic nerves regulated carotid body oxygen consumption and a further series of experiments was carried out to study this more fully. The results are given in the accompanying paper (Purves, 1970).

Some of these results have been presented to the Physiological Society (Purves, 1968, 1969).

METHODS

Forty-seven cats weighing between 2·5 and 5·2 kg were anaesthetized with pentobarbitone sodium, 30 mg/kg, given intraperitoneally. Further pentobarbitone at a rate of approximately 4 mg/kg.hr was given through a femoral vein cannula throughout the experiment. The trachea was cannulated with an angled Portex cannula which enabled mucus to be aspirated with minimal disturbance. The trachea and oesophagus were divided and reflected in the mid line to expose the carotid bifurcations. Generally the left sinus region was dissected.

The method used to isolate the sinus region and to collect carotid body venous blood was developed from that described by Daly *et al.* (1954). The commonest arrangement of vessels was that shown in Fig. 1*A*, as seen from the medial side. The carotid body vein originated from a plexus of veins overlying the carotid body and ran parallel to the axis of the superior cervical ganglion to join the transverse pharyngeal vein at an acute angle. It was easily identified by its bright red colour and, in many cats, this blood could be seen to stream separately for some distance along the transverse pharyngeal vein.

In thirty-seven out of the forty-seven cats, the carotid body vein was joined by a branch from the superior cervical ganglion and by smaller branches draining fat and connective tissue. These were tied. In the remaining cats, the vein from the superior cervical ganglion drained separately into the transverse pharyngeal vein. The transverse pharyngeal vein originated from two or more tributaries draining pharyngeal muscles and in addition to the carotid body and superior cervical ganglion veins, was joined by other small tributaries which drained, in particular, the prevertebral muscles. The initial problem was to isolate a segment of transverse pharyngeal vein for subsequent cannulation into which only the carotid body vein ran. When the carotid body was drained only as shown in Fig. 1*A*, it was fairly easy to tie off all the other branches of the transverse pharyngeal vein and place two loose ligatures around the vein, one at its origin from the pharyngeal muscles and one some 2–3 mm distal to the point of entry of the carotid body vein. However, in twenty-three out of forty-

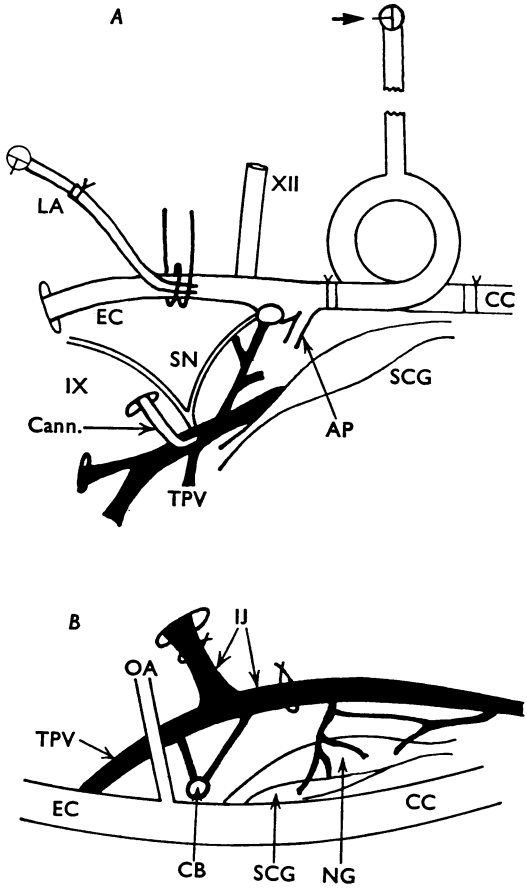

Fig. 1. (*A*). The left carotid sinus region of the cat viewed from the medial side. CC, common carotid artery with loop inserted, the arrow indicating the pressure inlet via the stopcock; SCG, superior cervical ganglion; AP, ascending pharyngeal artery; XII, hypoglossal nerve; TPV, proximal part of the transverse pharyngeal vein with cannula in position; SN, sinus nerve; IX, glossopharyngeal nerve; EC, external carotid artery; LA, lingual artery with cannula and stopcock. The snare is shown in position around the lingual artery catheter. (*B*). The left carotid sinus region viewed from the lateral side with the external carotid, EC and common carotid, CC arteries retracted medially. The distal part of the transverse pharyngeal vein TPV is seen to emerge from behind these arteries to join the internal jugular vein, IJ; CB, carotid body showing the two commonest courses of the second carotid body vein; SCG and NG, superior cervical and nodose ganglia showing a commonly observed pattern of venous drainage. Loose ligatures are shown in position around two parts of the internal jugular vein. When tightened, they isolate part of the internal jugular vein with the transverse pharyngeal vein to include one or other of the points of entry of the second carotid body vein.

seven cats, a second vein was found to drain the posterior pole of the carotid body and the two commonest courses for this vein are shown in Fig. 1*B*. On seventeen occasions, this vein joined the transverse pharyngeal vein some way distal to the first carotid body vein near the point where the transverse pharyngeal vein joined the internal jugular vein. On the remaining occasions, the second carotid body vein drained directly into the internal jugular vein. Isolation of a segment of transverse pharyngeal vein was more difficult in these cases. If the point of entry of the second carotid body vein allowed it, a second ligature was placed around the transverse pharyngeal vein prior to its junction with the internal jugular vein. If this was not possible or if the carotid body vein drained into the internal jugular vein, then two ligatures were placed as indicated in Fig. 1*B* around the internal jugular vein and this additional segment was included with the whole transverse pharyngeal vein for isolation. This arrangement could only be seen from the lateral side of the carotid artery and the artery was therefore retracted medially. In addition, it was also possible to observe a further series of veins which drained the superior cervical and nodose ganglia. These formed a plexus of veins which either joined the internal jugular vein directly or else ran parallel to it to join it at a variable distance distally in the neck. It was therefore possible in all cats to separate the venous drainage of the carotid body from that of the superior cervical and nodose ganglia.

When provision had been made for isolation of the segment of transverse pharyngeal vein joined only by the carotid body vein(s), the occipital, ascending pharyngeal and where present, the internal carotid arteries together with other small arteries originating from the carotid sinus and proximal part of the external carotid artery were tied as far distally as possible to avoid the possibility of retrograde clotting. The cats were given heparin (Pularin, Evans), 15 mg/kg, intravenously. The venous segment was then isolated by tightening the ligatures and cannulated as shown in Fig. 1*A*. The cannula consisted of an 8 cm length of nylon tube (Portex 55) of outside diameter 0·95 mm and inside diameter, 0·74 mm. The cannula was angled near its tip and led via a stainless-steel cannula through the cat's cheek so that the catheter tip was accessible and could be lightly clamped to be approximately 1 cm below the level of the carotid body. A short length of polyethylene tubing connected the catheter tip to the needle (i.d. 0·86 mm) of a 50 μl. Hamilton gas-tight syringe calibrated in μl. Blood flow was measured by timing the advance of the column of blood over 30–40 μl. to the nearest 0·2 sec. In the majority of experiments, blood flow was measured in duplicate.

Isolation of the carotid sinus. In thirty-four cats following the venous cannulation, a 15 cm length of polyethylene tubing was inserted into the common carotid artery to form a loop which included a T-piece and a sidearm consisting of a further 10 cm of polyethylene tubing closed with a three-way stopcock. Next the lingual artery was cannulated retrogradely so that the cannula tip lay approximately 8 mm above the carotid sinus and pressure within the carotid sinus was measured continuously with a Statham 23AC transducer. The external carotid artery could be closed intermittently by tightening a linen thread snare around the lingual artery catheter. The carotid sinus could be completely isolated by clamping the cardiac side of the loop and any desired pressure could be applied within the arterial segment by means of a sphygmomanometer attached to the stopcock on the sidearm. When a reading at constant pressure was to be made, the sidearm was filled with carotid artery blood, the carotid sinus segment isolated by snare and clamp and pressure instantly applied. The carotid loop and arterial segment contained approximately 1 ml. of blood. The completeness of the arterial segment isolation was tested by applying up to 160 mm Hg pressure: the pressure in the segment had to hold to within 2 mm Hg for 1 min and inflow had to exceed outflow by not more than 5 μl./min. In practice, a flow reading and filling the Hamilton syringe rarely took more than 90 sec.

Blood gas analysis. Femoral or carotid arterial oxygen (P_{a,O_2}) or CO_2 (P_{a,CO_2}) tensions were measured immediately from 0·4 ml. samples at 37° C with appropriate Radiometer electrodes which were regularly calibrated with O_2 in N_2 and CO_2 in air mixtures of accurately known composition. Arterial pH was measured with an E.I.L. capillary electrode and Vibron electrometer calibrated with standard phosphate buffers. Arterial and carotid body venous haematocrit were measured routinely from 40 μl. aliquots with a small (Hawkesley) centrifuge at 5000 rev/min for 5 min.

The oxygen content of 50 μl. samples of carotid arterial and carotid body venous blood was measured with a Beckman GC2A gas chromatograph and blood gas accessory. A single firebrick and molecular sieve column was used and, at maximum

TABLE 1. Reproducibility of measurement of oxygen content of blood using gas chromatography

Sample ...	1	2	3	4	5	6	7	8	9
	7·28	9·45	11·55	13·25	14·35	15·95	16·80	19·08	19·45
	7·30	9·45	11·50	13·20	14·35	16·00	16·75	19·12	19·40
	7·35	9·50	11·50	13·15	14·30	15·90	16·65	19·05	19·52
	7·30	9·47	11·50	13·15	14·27	15·85	16·72	19·00	19·35
	7·30	9·45	—	13·15	14·25	15·90	16·70	19·05	19·40
	7·35	9·50	—	13·20	14·20	—	16·80	19·10	19·40
	7·27	—	—	13·20	—	—	16·70	18·95	—
	—	—	—	13·25	—	—	16·65	—	—
	—	—	—	13·30	—	—	—	—	—
Mean	7·31	9·48	11·51	13·20	14·28	15·92	16·72	19·06	19·42
S.D. ±	0·028	0·040	0·023	0·038	0·072	0·057	0·059	0·045	0·059
C.V.(%)	0·38	0·42	0·18	0·28	0·51	0·36	0·35	0·23	0·30

attenuation, the oxygen content of 50 μl. blood containing 15·0 ml. O_2/100 ml. (7·5 μl. O_2) gave a peak of 8 in (20·5 cm) on the Honeywell slow recorder. The accuracy of measurements was estimated from repeated measurements of the oxygen content of samples of blood over the range 7·3–19·4 ml. O_2/100 ml. and the results are given in Table 1. This shows that the mean coefficient of variation (c.v.) was 0·33 %, range 0·18–0·51 %

Procedures. After cannulation of vessels was complete, the cats were paralysed with gallamine triethiodide (Flaxedil, May and Baker), 20 mg/kg, given intravenously and ventilated mechanically. The stroke volume of the pump was adjusted until end-tidal CO_2, continuously monitored with an LB1 infra-red analyser, was between 28 and 32 mm Hg. When arterial pressure, the mean of which was obtained electronically, and blood gas tensions were steady, one or two control measurements of c.b.f and the A–V O_2 difference were made.

In the first group of experiments, the effect of altering mean arterial pressure upon carotid body flow was measured when the carotid body was perfused naturally, or artificially by isolating the carotid sinus segment and applying pressure within it. Systemic arterial blood pressure was altered in random steps by bleeding and replacing blood. When arterial pressure was lowered below *ca.* 70–90 mm Hg, there was invariably a fall in P_{a,CO_2} and variable changes in P_{a,O_2} which presumably reflected changes in the ventilation/perfusion ratio of the lungs. Blood gas tensions were therefore adjusted to within 2 mm Hg of the control levels in the physiological range of mean arterial pressure by altering the inspired gas mixtures.

In the second group of experiments, either P_{a,O_2} or P_{a,CO_2} was altered, the other being held constant by giving various O_2 in N_2 or CO_2 in air mixtures, while mean arterial pressure was kept to within ± 2 mm Hg of control by means of a compensator (Roberts, 1921) inserted into a femoral artery. With the isolated carotid sinus perfusion system, changes in arterial gas tensions were made, a steady state confirmed, the sidearm of the carotid loop filled, the arterial segment isolated and pressure applied. It was thus possible to obtain blood gas tension/flow curves at more than one level of arterial pressure, or pressure/flow curves at more than one level of P_{a,O_2} or P_{a,CO_2}. When P_{a,CO_2} was altered, no attempt was made to control arterial pH.

In the experiments reported in this paper, the sinus nerves and pre- and post-ganglionic fibres of the cervical sympathetic nerve were left intact. Since it was clearly possible that these nerves or their blood supply could have been damaged during dissection, the integrity of afferent carotid body chemoreceptor and baroreceptor afferent pathways was tested in each experiment after initial dissection and again towards the end of the experiment. The first test consisted of raising pressure within the carotid sinus by 20–30 mm Hg above systemic arterial pressure for up to 60 sec. This invariably caused a fall of femoral artery pressure of between 10 and 20 mm Hg: that it was not greater may have been due to the fact that the other groups of baroreceptors were intact and responding to the normal pressure. In other tests of this type, one of which is illustrated in Fig. 2*A*, the external carotid artery was cannulated and the cannula, which was clamped externally, formed part of an arterial/femoral vein loop. Initially, pressure within the carotid sinus was low and thus the fall in femoral artery pressure when sinus pressure was raised may in addition have been due to the fact that carotid body chemoreceptor activity fell and, with it, some measure of peripheral vasoconstrictor activity. The second test consisted of perfusing up to 20 μg NaCN into the common carotid artery loop at constant pressure (Fig. 2*B*) and the response consisted of a transient rise in systemic pressure. Under the same experimental conditions, i.e. artificial respiration and paralysis, Comroe & Mortimer (1964) found that NaCN caused bradycardia and hypotension in the majority of experiments in the dog: the different results may reflect a species difference. In the third test, NaCN was infused as described above some 10–15 sec after the respiratory pump had been turned off and in all twelve of such tests, this caused bradycardia and hypotension: this response was similar to that found by Comroe & Mortimer (1964). The integrity of the pre-ganglionic sympathetic pathway and superior cervical ganglion transmission was tested by electrical stimulation and observing pupillary dilatation. At the end of each experiment, the post-ganglionic fibres of the superior ganglion running to the carotid body were cut and, if carotid body blood flow increased as is shown in the accompanying paper (Purves, 1970), this provided further evidence that, during the experiment, these fibres had been intact.

Tests of the method

Constancy of arterial pressure and carotid body flow. The results reported here were obtained from forty-seven cats in which there was no evidence of deterioration, e.g. spontaneously falling arterial pressure or c.b.f. over a period of 4–5 hr. The results from an additional sixteen cats were discarded, in seven because c.b.f. started to fall for no obvious reason but which was most probably due to defective dissection; in two because arterial pressure fell to low levels, 60 mm Hg; and in seven because of the presence of small clots in the venous cannula which made flow measurements unreliable.

Adequacy of vascular isolation. When the various procedures to isolate the carotid sinus segment had been completed and when the cannula had been inserted to collect carotid body venous blood, further tests were carried out in each experiment.

First, the carotid sinus was isolated without application of pressure. If all the vessels originating from the carotid sinus had been adequately tied, pressure within the carotid sinus fell within 1–2 sec and blood flow within the venous cannula ceased within 10–20 sec. This indicated that the blood perfusing the carotid body was derived from the isolated segment of carotid artery only. Secondly, when arterial pressure and blood gas tensions were stable following paralysis of the cat and the

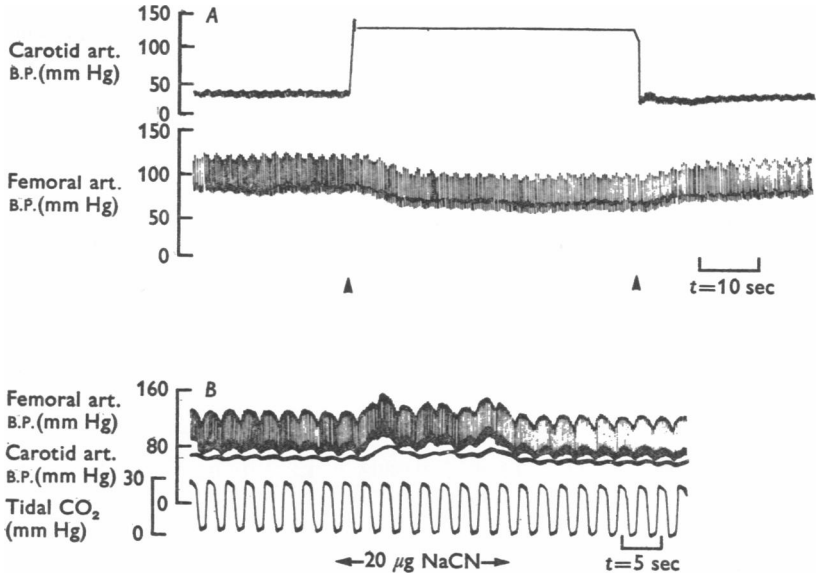

Fig. 2. (*A*). The effect of raising pressure within the carotid sinus segment upon femoral artery pressure. Upper trace, carotid sinus pressure showing the period for which pressure was raised; lower trace, femoral artery pressure. Cat, paralysed with gallamine and mechanically ventilated. (*B*). The effect of perfusing 20 μg NaCN in 0·5 ml. saline into blood flowing through the common carotid artery loop over the period indicated by arrows. From above down, femoral artery pressure, mean carotid artery pressure and tidal CO_2. Cat, paralysed with gallamine and mechanically ventilated. In both traces (*A*, *B*), the external carotid artery was cannulated and a loop formed to the femoral vein. Instead of snaring the lingual artery catheter, the external carotid artery catheter was closed by a tap as pressure within the carotid sinus segment was applied. For this reason, mean carotid sinus pressure, under control conditions, was lower than femoral artery pressure.

start of artificial ventilation, control measurements of c.b.f. and A–V O_2 difference were made. It was found that, under these conditions, the A–V O_2 difference exceeded 0·4 ml./100 ml. on eleven occasions in forty-seven experiments and in eight, a further search revealed a small tributary of the carotid body vein which did not originate in the carotid body and which was tied. In the other three cats, no such contaminating source was found and the A–V O_2 difference was assumed to be valid.

Reproducibility of measurements. The reproducibility of blood flow and other measurements was assessed in two ways. First, carotid body flow and the A–V O_2

difference were measured on five successive occasions under two different but steady sets of conditions. The results are shown in Table 2. With blood gas tensions within the physiological range and with mean arterial pressure at 65 and at 160 mm Hg, the coefficient of variation for c.b.f. measurements was 11·3 and 3·9 % respectively. The coefficient of variation for measurements of carotid body oxygen consumption at the lower pressure was 14·4 % and 5·3 % at the higher pressure.

Secondly, reproducibility of blood flow and oxygen consumption measurements was assessed from values obtained under the same conditions with respect to blood gas tensions and mean arterial pressure but which were separated by between 1 and 2½ hr. An example is given in Fig. 8*A*, measurements 1 and 8. Fourteen pairs of measurements fulfilled these requirements and in ten pairs, the A–V O_2 difference was also measured and oxygen consumption calculated. The results are given in Table 3.

TABLE 2. Reproducibility of measurements of carotid body blood flow, oxygen content of carotid arterial (C_{a,O_2}), carotid body venous (C_{v,O_2}) blood samples and calculated oxygen consumption of the carotid body ($\dot{V}_{O_2}$). Artificially perfused carotid body

	P_{a,O_2} 95 mm Hg, P_{a,CO_2} 31 mm Hg, M.A.P. 65 mm Hg				P_{a,O_2} 97 mm Hg, P_{a,CO_2} 29 mm Hg, M.A.P. 160 mm Hg			
	c.b.f. (μl./min)	C_{a,O_2}* (ml./100 ml.)	C_{v,O_2}* (ml./100 ml.)	$\dot{V}_{O_2}$* (μl./min)	c.b.f. (μl./min)	C_{a,O_2} (ml./100 ml.)	C_{v,O_2} (ml./100 ml.)	$\dot{V}_{O_2}$ (μl./min)
	16	15·03	14·90	0·0208	42	16·65	16·33	0·1344
	15	14·97	14·85	0·0180	42	16·60	16·26	0·1428
	16	15·07	14·92	0·0240	39	16·57	16·17	0·1560
	14	15·09	14·90	0·0266	45	16·67	16·35	0·1440
	12	15·04	14·86	0·0216	40	16·55	16·19	0·1440
Mean	14·6	15·00	14·88	0·0222	41·6	16·26	16·60	0·1442
S.D. ±	1·65	0·045	0·03	0·0032	1·6	0·08	0·05	0·0077
C.V. (%)	11·3	0·3	0·24	14·4	3·9	0·49	0·31	5·3

* C_{a,O_2}, Oxygen content of carotid arterial blood; C_{v,O_2}, oxygen content of carotid body venous blood; $\dot{V}_{O_2}$, volume of oxygen consumed/min.

Resistance to venous outflow. The resistance offered to blood flow by the catheter in the transverse pharyngeal vein, needle and syringe was measured *in vitro* over the flow range 30–150 μl./min. The increase in resistance was virtually linear over this range: at a blood flow of 30 μl./min, pressure at the proximal end of the catheter was 1·2 cm H_2O; at 70 μl./min, 1·5 cm H_2O; at 110 μl./min, 2·0 cm H_2O and at 150 μl./min, 2·7 cm H_2O. Thus within the range of blood flow encountered *in vivo*, the catheter system must have caused pressures within the vein which can have been only slightly greater than those expected in the intact vessel. Further evidence that resistance was small was that blood flowed at a constant rate along the syringe and there was no ballooning of the segment when the needle and syringe were attached. On the other hand, ballooning of the segment was easily seen and invariably occurred when there were small clots in the cannula.

Temperature changes during perfusion. In six cats, a calibrated thermistor mounted in a fine polyethylene cannula was placed alongside the lingual artery catheter so that it lay in the carotid sinus and the changes in temperature associated with the normal sequence of filling the sidearm of the carotid artery loop with blood, isolating

the carotid sinus segment and applying a pressure of 130–160 mm Hg for 60–90 sec were measured. With natural carotid blood flow, the temperature of the blood was between 0·5 and 1·2° C less than that of the saline irrigating the wound. The temperature of the carotid sinus was unaffected by filling the sidearm or by isolation of the arterial segment, and the maximum fall in temperature observed during the perfusion period was 0·4° C. That some cooling of the blood occurred in the rest of the carotid loop was shown by the fall in temperature of 1·5–2·3° C which lasted for 30–40 sec when the clamp was removed and natural flow was resumed.

TABLE 3. Reproducibility of measurements of carotid body blood flow and oxygen consumption

c.b.f. (μl./min)				Carotid body O_2 consumption (μl./min)			
1st measurement	2nd measurement	Change (%)		1st measurement	2nd measurement	Change (%)	
68	61	−7	10·2	0·085	0·072	0·013	15·2
43	43	0	0	0·021	0·024	0·003	12·5
40	40	0	0	0·115	0·125	0·010	8·6
36	32	−4	11	0·148	0·133	0·015	10·1
54	58	+4	7·4	0·163	0·153	0·010	6·1
20	17	−3	15	0·108	0·093	0·015	13·8
18	18	0	0	0·135	0·147	0·012	8·8
27	32	+5	18	0·140	0·153	0·013	9·2
12	12	0	0	0·088	0·103	0·015	17·0
31	34	+3	9·6	0·132	0·144	0·012	9·0
42	42	0	0	—	—	—	—
14	17	+3	14	—	—	—	—
50	55	+5	10	—	—	—	—
35	35	0	0	—	—	—	—
Average		2·7				0·011	

Effect of changes in haematocrit. Fig. 3*A* shows the relation between c.b.f. and haematocrit when various blood and saline mixtures equilibrated at P_{O_2} 95 mm Hg and P_{CO_2} 30 mm Hg were perfused at 130 mm Hg and at 38° C. Perfusion with saline gave rates of flow which are similar to those reported by Joels & Neil (1968) who used Krebs–Henseleit solutions. In the experiments reported in this paper, the value for arterial haematocrit varied between 29 and 41 %.

Effect of changes in temperature and P_{O_2} of the irrigating saline. Fig. 3*B* shows the relation between c.b.f. and the temperature of the saline used to irrigate the wound and which surrounded the carotid sinus region. In this and in two other tests, blood was perfused at constant blood gas tensions and pressure while the temperature of the irrigating saline was altered in steps by changing its flow rate.

In two cats, blood was perfused through the carotid body at constant blood gas tensions and pressure while the saline which was normally used for irrigation and which had a P_{O_2} of between 130 and 155 mm Hg was replaced by saline at the same temperature but which had been equilibrated for 40 min with either 100 % O_2 or 100 % N_2. On none of the fifteen occasions on which the different types of saline were altered was any significant change in c.b.f observed.

Rate of change of blood flow in response to changes in perfusion pressure or blood gas tensions. Before steady blood flow could be assumed, it was necessary to know the

rate at which blood flow altered in response to various stimuli. Fig. 4*A* illustrates a response typical of that seen in twelve tests in three cats in which pressure within the carotid sinus segment was abruptly raised, all nerves being intact. The greatest increase in blood flow occurred on each occasion within the first 5 sec after the pressure change and, in each test, an overshoot was observed over the next 10–15 sec after which c.b.f. settled at a new steady level. With comparable changes in pressure the overshoot, defined as the difference between the maximum and subsequent steady rates of flow, varied between 1 and 3 μl./5 sec period or the equivalent of some 10–30 μl./min. When perfusion pressure was abruptly lowered, the changes in c.b.f. were more variable. Blood flow fell gradually over the first 10–15 sec and in seven out of thirteen tests, an undershoot of up to 3 μl./5 sec period was seen before the flow became steady.

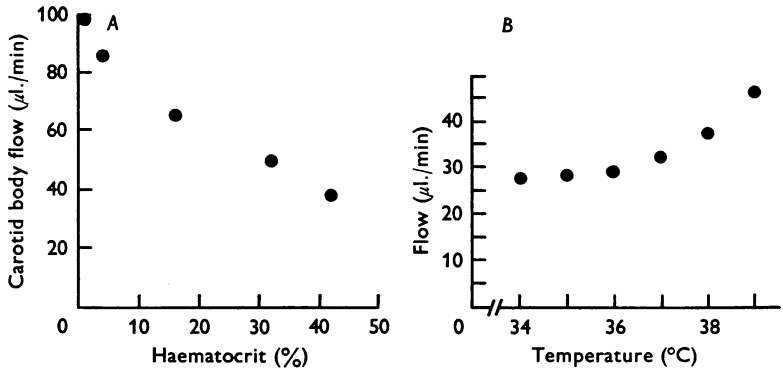

Fig. 3. (*A*). The relation between carotid body venous flow and arterial haematocrit. Mixtures of blood and saline equilibrated at P_{O_2} 95 mm Hg and P_{CO_2} 30 mm Hg were perfused at constant pressure of 130 mm Hg. (*B*). The relation between carotid body venous flow and the temperature of the saline irrigating the carotid sinus region. Changes in temperature, measured with a calibrated thermistor, were brought about by altering the rate of saline flow.

Fig. 4*B* shows the response seen on one of three occasions when P_{a,O_2}, monitored with a rapidly responding electrode placed in the carotid artery loop, was reduced. P_{a,O_2} reached a new steady level some 25–35 sec after first starting to change in the carotid artery. Carotid body flow, however, changed more slowly, reaching a steady level in 1½–2 min. Similar rates of change in c.b.f. were observed in three cats when P_{a,O_2} was raised from normal levels to > 400 mm Hg or when P_{a,CO_2} was raised from 28 to 50–58 mm Hg.

Dead space of venous blood collecting system. Measurement of the A–V O_2 difference requires that the blood sampled and analysed shall be representative of that proximal and distal to the region of gas exchange and although it was clear that the transit time for blood through the carotid body was rapid, i.e. up to 4 sec, the oxygen content of blood in the Hamilton syringe following a change in arterial gas tensions or pressure could only be considered representative of that in the carotid body vein when the dead space of cannula and syringe needle had been cleared. In six experiments, the time taken to clear the dead space was measured by replacing the blood perfusing the carotid body by saline and the saline by blood at four pressures between 80 and 160 mm Hg. The time taken for the saline column to be replaced by blood

from the proximal part of the carotid body vein to the tip of the cannula varied between 9 sec at high pressure to 24 sec at low pressure. This interval was therefore taken into account before the constancy of venous blood could be assumed and measurements of the oxygen content of venous blood made.

Separation of red blood cells and plasma during perfusion. The possibility that separation of blood occurred, particularly when natural blood flow was interrupted

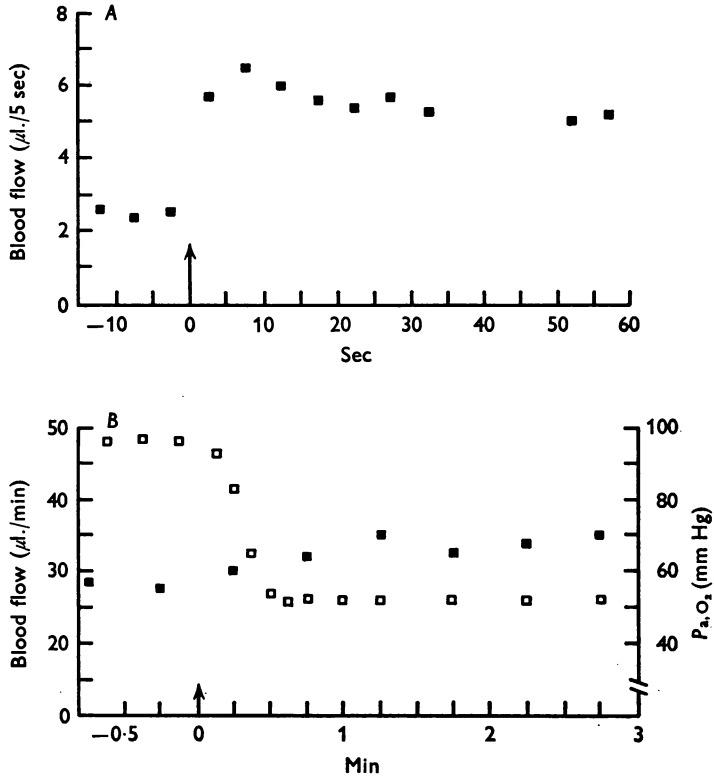

Fig. 4. (*A*). The time course of changes in carotid body venous flow (expressed as the volume of flow in μl./5 sec measuring period) following an abrupt increase in perfusion pressure from 100 to 140 mm Hg at $t = 0$. (*B*). The time course of changes in carotid body blood flow (μl./min) following the administration of a low O_2 in N_2 mixture at the arrow. P_{a,CO_2} 29 mm Hg, M.A.P. 140 mm Hg. Filled squares, c.b.f.; open squares, P_{a,O_2} (mm Hg) measured with an electrode placed in the carotid loop.

during the period of artificial perfusion, was real in view of the high rate of erythrocyte sedimentation of heparinized cat's blood. This was tested by measuring venous and arterial haematocrit under varying conditions of blood flow and blood gas tensions. Out of sixty-seven parallel measurements, no difference greater than 4% was observed in fifty-four: on the remaining occasions, venous haematocrit was between 6 and 11% higher than arterial and on all these occasions blood flow was < 15 μl./min. No difference was noted in this respect between artificially and naturally perfused preparations.

RESULTS

Normal values. These are given in Table 4, and were measured when blood gas tensions, pH and arterial pressure were within physiological limits, i.e. respectively, P_{a,O_2} 95–105 mm Hg, P_{a,CO_2} 28–33 mm Hg, pH 7·38–7·41 units, M.A.P. 130–160 mm Hg. Of the sixty-eight occasions on which measurements were made, the carotid body was perfused naturally on twenty-seven, artificially with blood on forty-one. The difference between mean values for c.b.f., A–V O_2 difference and oyxgen consumption of these two groups was not significant, $P > 0{\cdot}5$ in each case.

TABLE 4. Normal values for carotid body blood flow, A–V O_2 difference and oxygen consumption. Forty-two cats, sixty-eight observations

	c.b.f. (μl./min)	A–V O_2 diff. (ml./100 ml.)	O_2 consumption (μl. O_2/min)
Range	33–68	0·21–0·46	0·115–0·195
Average	41·5	0·34	0·147
S.D. ±	3·94	0·037	0·032

Effect of altering mean arterial pressure

(*a*) *Upon carotid body blood flow.* Mean arterial pressure was altered over the range 60–160 mm Hg in twelve cats and, in seven, c.b.f. was measured with the carotid body naturally perfused and artificially perfused at the same pressure. A typical response is shown in Fig. 5. In this and in the other experiments, the pressure/flow relation was linear over the range 100–160 mm Hg M.A.P. and the slope of this relation varied between 0·78 and 1·22 μl. min^{-1}. mm Hg^{-1} M.A.P., average 0·93. The slope of the curves obtained with artificial perfusion did not differ from those with natural perfusion, $t = 0{\cdot}158$, $P > 0{\cdot}5$. In five out of the eight experiments in which M.A.P. was altered by bleeding, the flow/pressure curves were displaced to the right of control during blood replacement by the equivalent of 8 μl./min. In the other three cats, the curves were identical. When perfusion pressure was altered in the isolated preparation alone, the sequence of pressures was chosen randomly and, as is illustrated in Fig. 5, there was no evidence of displacement of the curve. It has therefore been concluded that this displacement was due to the method of altering pressure by bleeding and blood replacement rather than deterioration with time.

In seven cats, the flow/pressure curve tended to flatten as M.A.P. was reduced below 90–100 mm Hg and the measurement of blood flow became increasingly inaccurate. Thus no estimates of the intercept of the flow/pressure curves with the pressure axis were possible.

The effect of high P_{a,CO_2} (42 mm Hg) or low P_{a,O_2} (55–60 mm Hg) upon the flow/pressure curve was tested in five cats. In all cats the curve was displaced to the left of control by between the equivalent of 7 and 11 μl./min c.b.f. These displacements were significant, $t = 4{\cdot}54$, $P < 0{\cdot}025$.

(*b*) *Upon A–V O_2 difference and oxygen consumption.* This was measured in eleven experiments and a typical response is shown in Fig. 6 (upper and middle panels). This indicates that the A–V O_2 difference was not affected

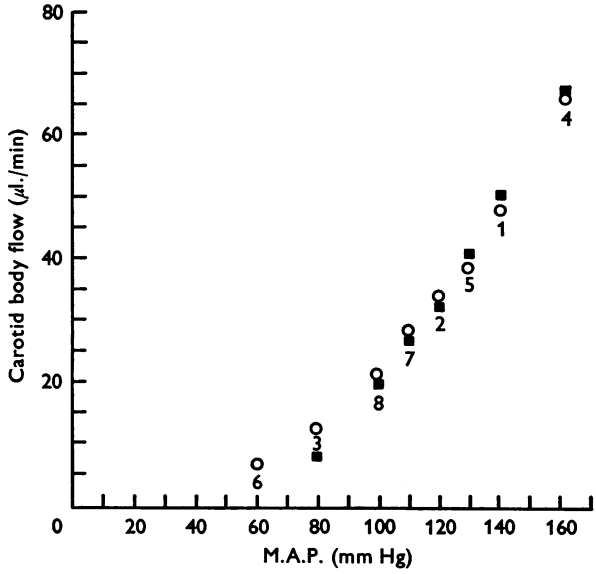

Fig. 5. The relation between carotid body blood flow (μl./min) and M.A.P. (mm Hg). P_{a,CO_2} maintained at 28–30 mm Hg, P_{a,O_2} at 95–97 mm Hg throughout. Filled squares, measurements obtained during bleeding and replacement of blood; open circles, parallel measurements of blood flow made with the artificially perfused preparation in the order indicated by the figures. Sinus and sympathetic nerves intact.

by changes in M.A.P. over the range 80–170 mm Hg. Carotid body oxygen consumption thus fell in proportion to blood flow. The results for this group of experiments are summarized in Table 5. They confirm that hypotension was associated with an average 59 % fall in c.b.f., a 6 % rise in A–V O_2 difference and a 79 % reduction in oxygen consumption.

Effect of altering P_{a,O_2}

(*a*) *Upon carotid body blood flow.* P_{a,O_2} was altered over the range 30–500 mm Hg in nineteen cats while P_{a,CO_2} and M.A.P. were kept constant at control levels. The results were the same whether the perfusion was natural or artificial. Typical changes in c.b.f. are shown for one cat in

Fig. 7 and the results from this group of experiments are summarized in Table 6. Although hypoxia was associated with a small rise in c.b.f. and hyperoxia with a fall when compared with control, these changes were either within or barely exceeded the experimental error: it is therefore

TABLE 5. Effect of altering M.A.P. upon carotid body blood flow, A–V O_2 difference and oxygen consumption

		c.b.f (μl./min)	A–V O_2 difference (ml./100 ml.)	O_2 consumption (μl. O_2/min.)
Control*; average		43	0·32	0·153
S.D. ±		3·8	0·03	0·02
M.A.P. 70–90; average		17	0·34	0·031
S.D. ±		4·2	0·03	0·03
Difference between means	t	3·08	0·15	3·89
	P	< 0·01	> 0·5	< 0·005

* Physiological limits for blood gas tensions, pH and M.A.P. as defined in text.

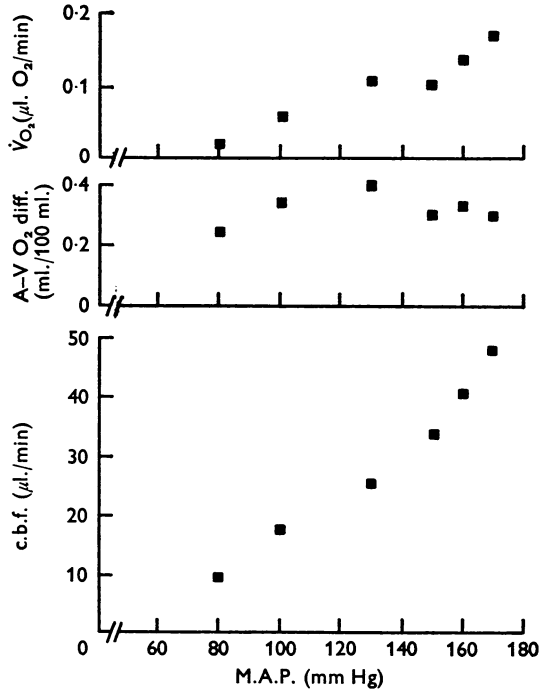

Fig. 6. The relation between calculated oxygen consumption of the carotid body (upper panel), A–V O_2 difference (middle panel) and c.b.f. (lower panel) and M.A.P. P_{a,O_2} 96–98 mm Hg, P_{a,CO_2} 30 mm Hg throughout. Artificial perfusion of the carotid body. Sinus and sympathetic nerves intact.

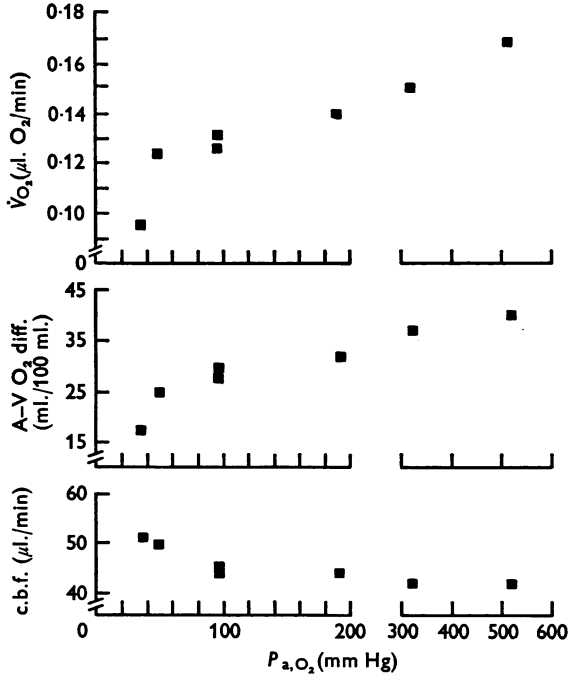

Fig. 7. The relation between calculated oxygen consumption of the carotid body (upper panel), A–V O_2 difference (middle panel) and c.b.f. (lower panel) and P_{a,O_2}. The scale of the P_{a,O_2} axis is reduced at high P_{a,O_2}. P_{a,CO_2} 29 mm Hg, M.A.P. 130 mm Hg throughout. Artificial perfusion of the carotid body, sinus and sympathetic nerves intact.

TABLE 6. Effect of altering P_{a,O_2} upon carotid body blood flow, A–V O_2 difference and oxygen consumption at constant P_{a,CO_2} and M.A.P.

	c.b.f. (µl./min)	A–V O_2 difference (ml./100 ml.)	O_2 consumption (µl. O_2/min)
(A) P_{a,O_2} 500 mm Hg			
average	37·1	0·43	0·160
S.D. ±	1·85	0·07	0·018
(B) P_{a,O_2} 95–103 mm Hg			
average	40·1	0·28	0·128
S.D. ±	1·9	0·04	0·012
(C) P_{a,O_2} 43 mm Hg			
average	50·1	0·14	0·082
S.D. ±	2·8	0·06	0·015
A:B	t = 1·8	4·3	5·6
	P > 0·1	< 0·01	< 0·01
B:C	t = 7·7	3·3	4·5
	P < 0·01	< 0·01	< 0·01

impossible to say whether the rise in blood flow with hypoxia was linear or otherwise.

In three cats, a second curve was obtained at the same M.A.P. as control but with P_{a,CO_2} 10 mm Hg above control. This response is shown in Fig. 8*A* (open circles). Carotid body flow in this and the other experiments was uniformly 5–10 μl./min higher than control over the range of P_{a,O_2} tested. There was thus no evidence that CO_2 potentiated the flow response to hypoxia.

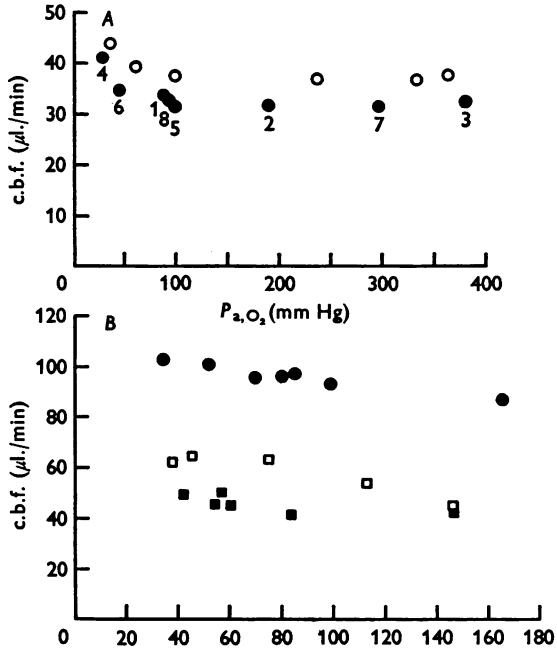

Fig. 8. (*A*). The relation between c.b.f. and P_{a,O_2} when the carotid body was artificially perfused at constant pressure 140 mm Hg. Filled circles, P_{a,CO_2} 31 mm Hg; open circles, P_{a,CO_2} 44 mm Hg. The figures indicate the order in which measurements were made at the lower P_{a,CO_2}. (*B*). The relation between c.b.f. and P_{a,O_2}, artificial perfusion of the carotid body, P_{a,CO_2} 28–30 mm Hg throughout. Filled circles, M.A.P. 160 mm Hg; open squares, M.A.P. 115 mm Hg; filled squares, M.A.P. 95 mm Hg. Sinus and sympathetic nerves intact.

In three cats, the relation between c.b.f. and P_{a,O_2} was tested at more than one level of M.A.P.: in two cats at control levels (140–160 mm Hg) and at one lower level (120–125 mm Hg) of M.A.P. and in one cat at two lower levels, 115 and 95 mm Hg. P_{a,CO_2} was maintained at control levels throughout. The results from the third cat are given in Fig. 8*B*. This shows that the c.b.f./P_{a,O_2} curve was depressed at the lower pressures so that over the range of P_{a,O_2} tested, c.b.f. was lower than control by 27–32 μl./min at

M.A.P. 115 mm Hg and by 37–40 μl./min at M.A.P. 95 mm Hg. These reductions in flow are consistent with the relation between c.b.f. and M.A.P. (see Fig. 5). There was thus no evidence in this or the other two experiments that the shape of the c.b.f./P_{a,O_2} curves at lower pressures was significantly different from control.

(*b*) *Upon A–V O_2 difference and oxygen consumption.* A–V O_2 difference was measured in seven cats and oxygen consumption calculated. The results from one cat are shown in Fig. 7 (upper and middle panels) and the results

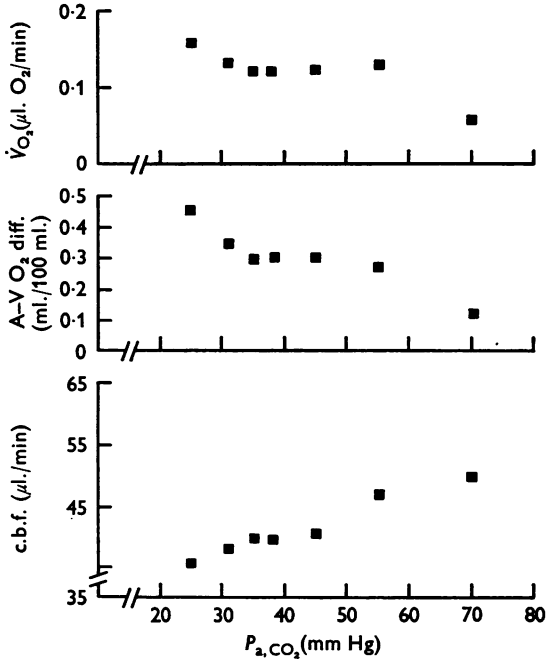

Fig. 9. The relation between calculated oxygen consumption of the carotid body (upper panel), A–V O_2 difference (middle panel) and c.b.f. (lower panel) and P_{a,CO_2}. P_{a,O_2} 95 mm Hg and M.A.P. 130 mm Hg throughout. Artificial perfusion of the carotid body, sinus and sympathetic nerves intact.

for this group of experiments are summarized in Table 6. In all experiments, hypoxia at constant P_{a,CO_2} and M.A.P. caused a fall in A–V O_2 difference, average 50 % reduction of control. Hyperoxia (> 500 mm Hg) caused an average increase of 53 % above control. The changes in oxygen consumption therefore largely reflected changes in A–V O_2 difference, the changes in c.b.f. being relatively small. There was no evidence that either A–V O_2 difference or oxygen consumption had reached an upper limit at high P_{a,O_2} since the mean values for each were significantly higher than

those at intermediate $P_{a\ O_2}$ (220–350 mm Hg), réspectively $P < 0{\cdot}025$ and $< 0{\cdot}01$.

The effect of altering P_{a,CO_2}

(*a*) *Upon carotid body blood flow.* P_{a,CO_2} was altered over the range 28–70 mm Hg in thirteen cats while P_{a,O_2} and M.A.P. were maintained constant at control levels. Arterial pH was not controlled and varied between 7·18 and 7·43 units. A typical response is shown in Fig. 9 (lower

TABLE 7. Effect of altering P_{a,CO_2} upon carotid body blood flow, A–V O_2 difference and oxygen consumption. Nine cats

	c.b.f. (μl./min)	A–V O_2 difference (ml./100 ml.)	O_2 consumption (μl. O_2/min)
Control. P_{a,CO_2} 28–32 mm Hg			
Average	39·8	0·274	0·128
S.D. ±	1·52	0·024	0·008
P_{a,CO_2} 55 mm Hg			
Average	49·9	0·145	0·076
S.D. ±	3·54	0·022	0·008
Comparison of means	$t = 6{\cdot}06$	3·79	7·32
	$P < 0{\cdot}01$	$< 0{\cdot}01$	$< 0{\cdot}01$

panel). Over the range tested, c.b.f. rose with increasing P_{a,CO_2} though as with increases in response to hypoxia, the rise in c.b.f. was small. In four cats, c.b.f. increased more markedly at high P_{a,CO_2} (> 55 mm Hg), the average increase being + 19 μl./min above control. The results are summarized in Table 7.

In two cats, the response was repeated at the same M.A.P. but at high $P_{a,O_2} > 450$ mm Hg. Carotid body flow was uniformly 7–9 μl./min below the curve at control P_{a,O_2}. In three cats, the results from one of which is shown in Fig. 10, the response was repeated at low P_{a,O_2}, 47–56 mm Hg. Over the range P_{a,CO_2} 28–55 mm Hg, c.b.f. was on average 5·2 μl./min higher than control; at $P_{a,CO_2} > 55$ mm Hg, c.b.f. was on average 7·7 μl./min above control.

(*b*) *Upon A–V* O_2 *difference and oxygen consumption.* This was measured in nine cats. A representative response in given in Fig. 9 and the results summarized in Table 7. In all cats, the response to high CO_2 consisted of a fall in A–V O_2 difference and a less pronounced fall in oxygen consumption because of the associated rise in c.b.f.

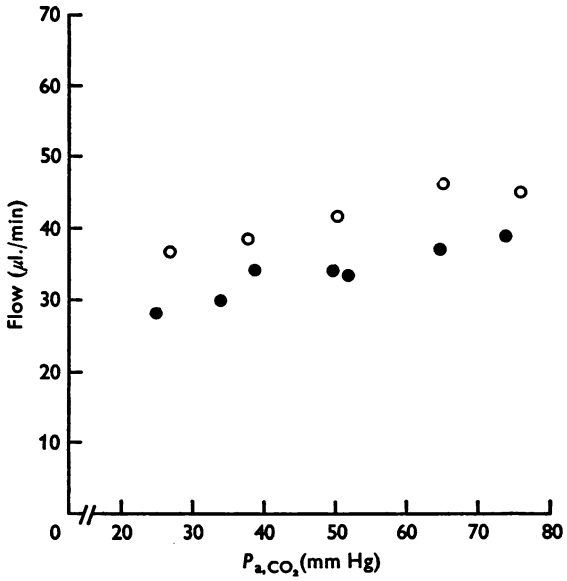

Fig. 10. The relation between c.b.f. and $P_{a.CO_2}$ at two levels of $P_{a.O_2}$. Filled circles, 95 mm Hg; open circles, 52–54 mm Hg. M.A.P. 150 mm Hg throughout. Artificial perfusion of the carotid body, sinus and sympathetic nerves intact.

DISCUSSION

The methods used and the normal values obtained are similar to those reported by Daly *et al.* (1954). It has been possible to confirm that the blood collected is derived from the carotid sinus and carotid body only, that separation of blood does not occur whether the carotid body is naturally or artificially perfused with blood except at low rates of blood flow, and that the dissection involved did not affect the chemoreceptor or baroreceptor pathways. Further and more direct evidence on this point has been given by Biscoe, Bradley & Purves (1970) and Biscoe, Purves & Sampson (1970) who showed that the quantitative chemoreceptor response to various stimuli was similar whether the carotid body was naturally perfused or was artificially perfused using this preparation.

The system of artificial perfusion with blood developed here was necessary for two reasons. First, it was found that the method of altering arterial blood pressure by bleeding and replacement of blood was cumbersome and time-consuming and made precise regulation of blood gas tensions difficult. It was also clear that, in a proportion of experiments, it led to deterioration of the preparation. Secondly, although perfusion of the carotid body with Krebs–Henseleit or other solutions has been carried out

previously (Joels, Neil & Vaughan Hudson, 1961), it has been shown more recently that the chemoreceptor response to various stimuli is altered considerably when blood is perfused (Joels & Neil, 1968). In general, the tests of reproducibility have shown that the method of artificial perfusion of the carotid body is satisfactory. As a result of these, it is considered that only changes in carotid body blood flow greater than 3 μl./min, in A–V O_2 difference of greater than 0·05 ml./100 ml. and in oxygen consumption greater than 0·08 μl./min are of significance.

Normal values. Such differences as there are between the present results and those of Daly *et al.* (1954) can almost certainly be explained when it is remembered that Daly *et al.* (1954) derived their values from six experiments in which the arterial pressure varied between 100 and 150 mm Hg and that, in their experiments, the sympathetic supply to the carotid body was cut before measurements were made. In the present series of experiments, all nerves were intact. The importance of this has been emphasized by the recent findings that efferent as well as afferent activity exists in the sinus nerve (Biscoe & Sampson, 1968) and that the efferent activity affects carotid body blood flow (Neil & O'Regan, 1969).

Responses to hypoxia, hypercapnia and hypotension. The common feature of these responses was a fall in oxygen consumption of the carotid body. With hypoxia and hypercapnia, this was due to a fall in the A–V O_2 difference; with hypotension, it was due to a fall in blood flow. The mechanism of these changes remains unclear. A possibility is that, with respect to the response to hypoxia and hypercapnia, blood is diverted into A–V anastomoses or otherwise takes a smaller part in tissue gas exchange so that the venous blood approximates to arterial. The total volume of flow is, however, unaffected. Whether this is a reflex response or due to the local effect of hypoxia or raised CO_2 is not certain. The sympathetic supply to the carotid body is involved in the response and this is considered in the next paper (Purves, 1970).

The response obtained with hypotension differs markedly from that observed by Daly *et al.* (1954). When they lowered arterial pressure, blood flow fell, A–V O_2 difference increased and oxygen consumption remained relatively unchanged. Indeed, they used this device to obtain more easily measurable A–V O_2 differences. It would therefore seem that, as in this experiment, an intact sympathetic supply is involved in regulating the distribution of blood flow so that the A–V O_2 difference remains constant with differing perfusion pressure. This question also is considered in more detail in the accompanying paper.

The most obvious conclusion to be drawn from the present series of experiments is that there is remarkably little relation between the volume of carotid body blood flow and the known pattern of chemoreceptor

discharge (Hornbein, Griffo & Roos, 1961; Biscoe, Sampson & Purves, 1967). A closer but inverse relation would appear to exist between chemoreceptor discharge and oxygen consumption. Two points may be made. First, this finding is consistent with the observations that substances which interfere with the oxidative metabolic chain and the synthesis of ATP provoke an increase in chemoreceptor discharge (Krylov & Anichkov, 1968; Joels & Neil, 1968). Secondly, the possibility arises that the chemoreceptors do not directly respond to changes in the chemical composition of blood. They respond rather to some function of the change in metabolic rate of the carotid body. It would not therefore be necessary to suppose that there were chemoreceptors which respond separately to changes in P_{a,O_2}, P_{a,CO_2} or pH_a. But precisely how these changes in oxygen metabolism are brought about remains unknown. Some possible mechanisms are considered in the accompanying paper (Purves, 1970).

I am grateful to Miss D. L. Patterson for skilled technical assistance.

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