J. Physiol. (1979), **287**, *pp.* 149–161
With 5 text-figures
Printed in Great Britain

THE EFFECT OF CARBON DIOXIDE UPON MYOCARDIAL CONTRACTILE PERFORMANCE, BLOOD FLOW AND OXYGEN CONSUMPTION

BY G. C. VAN DEN BOS*, ANGELA J. DRAKE AND M. I. M. NOBLE
From the Midhurst Medical Research Institute, Midhurst, Sussex GU29 0BL

(*Received 15 May 1978*)

SUMMARY

1. Mongrel dogs were anaesthetized with chloralose, paralysed, ventilated and vagotomized and given a β-blocking drug, sotalol, in sufficient doses to block the effects of 5 μg of adrenaline.

2. Changes in inspired CO_2 concentration were produced, causing increases of arterial P_{CO_2} up to 120 mmHg. The effects on myocardial blood flow were measured with radioactive microspheres. Coronary sinus and arterial blood was sampled.

3. In the absence of β-blockade, an increase in arterial P_{CO_2} produced variable effects. In some dogs coronary blood flow increased, while in others there was no change. There was a mean increase in coronary blood flow at arterial P_{CO_2} values above 85 mmHg which was abolished by β-blockade.

4. In the presence of β-blockade, an increase of arterial P_{CO_2} produced depression of left ventricular performance, i.e. a fall of maximum rate of rise of left ventricular pressure and a rise of left ventricular end-diastolic pressure.

5. In the presence of β-blockade, there were no consistent changes in myocardial blood flow, left ventricular pressure or cardiac output.

6. In the absence of β-blockade, coronary arterial minus venous oxygen content was reduced by hypercapnia. In the presence of β-blockade, the changes were small and not statistically significant. The direct coronary vasodilator effect was therefore negligible.

7. It is concluded that the previously reported hypercapnic vasodilatation was mainly an effect of sympatho-adrenergic stimulation by hypercapnia.

8. In the presence of β-blockade, coronary sinus P_{O_2} increased markedly, with little change in coronary sinus oxygen content; this was consistent with a shift to the right of the oxy-haemoglobin dissociation curve. Under circumstances of hypercapnia, a rise in coronary sinus (and presumably tissue) P_{O_2} failed to produce vasoconstriction.

9. It is argued that the vasodilator effect of hydrogen ions and the vasoconstrictor effect of oxygen probably cancel one another when the arterial P_{CO_2} is raised.

* Present address: Laboratory for Physiology, Free University of Amsterdam, Amsterdam, The Netherlands.

INTRODUCTION

The effect of an increase in arterial P_{CO_2} on the mechanical performance of the heart has not been established beyond doubt; reports in the literature are conflicting (Feinberg, Gerola & Katz, 1960; Ledingham, McBride, Parratt & Vance, 1970; Alexander & Liv, 1976; Hilton & Eichholtz, 1925; Jerusalem & Starling, 1910; Noble, Trenchard & Guz, 1967). These apparent discrepancies may be due to the difference in animal preparation and the duration and intensity of the change in P_{CO_2}. The study by Noble *et al.* (1967) in conscious dogs showed that CO_2 inhalation produced an initial transient depression followed by a stimulation of performance. The authors interpreted this stimulation as being caused by the sympatho-adrenal response to hypercapnia (Morris & Millar, 1962). They concluded that the initial cardiac depression was the true direct effect of CO_2 on heart muscle, which is in accordance with results in isolated papillary muscle experiments. One of the purposes of the present investigation was to test this conclusion by studying the effects of changing arterial P_{CO_2} during β-adrenergic blockade.

The effect of changes in arterial P_{CO_2} on coronary blood flow is also unclear. The majority of reports state that CO_2 is a coronary vasodilator (Alexander & Liv, 1976; Ledingham *et al.* 1970; Feinberg *et al.* 1960). One paper goes so far as to suggest that myocardial P_{CO_2} is the primary agent controlling coronary flow (Case, Greenberg & Moskowitz, 1975). However, if the true effect of hypercapnia on contraction is masked by the sympatho-adrenal stimulation, the true effect on the coronary vasculature may also have been masked in previous studies. Therefore, we studied the effects of changing P_{CO_2} on coronary blood flow during β-adrenergic blockade.

METHODS

Prior preparation of animals. Twelve dogs weighing 12·0–22·0 kg were anaesthetized with I.V. thiopentone sodium followed by inhalation of halothane in nitrous oxide and oxygen. The lungs were ventilated by an intermittent positive pressure ventilator through a cuffed endotracheal tube. A left thoractomy was made through the fourth intercostal space. The pericardium was split to expose the atria, and the left atrial catheter (silastic filled with heparin) implanted in the left atrial appendage. Two pacing electrodes were sewn on the right atrial appendage. In some dogs a Konigsberg P 22 pressure transducer was implanted into the left ventricular cavity through an incision in the apex and secured with a purse-string suture. The leads were brought out at the back of the neck and then buried under the skin in an oiled silk pouch, to be exteriorized at the time of the studies. The left chest was closed leaving a drainage tube *in situ*.

The experiments were carried out one week later. The prior preparation of animals described above enabled us to carry out experiments in closed chest animals. Preliminary pilot experiments in open chest anaesthetized dogs had revealed problems with this preparation, e.g. need for deep anaesthesia, steady deterioration of cardiac function, metabolic acidosis and cardiac alternans. These problems were avoided to a large extent in the closed chest preparation.

Protocol. The dogs were anaesthetized with I.V. methohexitone sodium followed by a standard dose of chloralose (100 mg.kg^{-1}) (Arfors, Arturson & Malmburg, 1971). A cannula was inserted into a branch of the femoral artery and connected to a three-way tap. Sampling for the microsphere measurement was carried out at a pre-set flow rate using a peristaltic pump (BYO 800. Norris Industries Rushenden Ltd). The flow rate was checked by timed collections of blood into weighed vials. Left ventricular pressure and maximum left ventricular dP/dt were unchanged during the sampling and during the microsphere injection.

The left atrial catheter and pacing electrodes were exteriorized at the back of the neck. The left atrial catheter was used for injection of the microspheres and sampling of arterial blood.

In four dogs the heart was paced electrically throughout, to ensure that any effects were not the result of heart rate changes. A Digitimer type 3290, triggering a Devices isolated stimulator type 2533, was used. In those dogs in which a pressure transducer was not implanted in the left ventricle, a Millar or Gael-Tec catheter tip manometer was inserted into the left ventricle in a retrograde fashion from a femoral artery under fluoroscopic control.

The left jugular vein was exposed through a small incision and a cardiac catheter inserted. The catheter was advanced and positioned in the coronary sinus by fluoroscopic control. The position of the catheter was checked by visualizing the coronary sinus with a bolus injection of 10 ml. Urografin. With this method of coronary sinus catheterization there is a risk of contamination with right atrial blood (Gregg, Khouri, Donald, Lowensohn & Pasyk, 1972). Therefore we advanced the catheter more than 15 mm inside the coronary ostium, as recommended by Koberstein, Pitman & Klocke (1969). In addition, the sampling rate through the coronary sinus was kept to 2 ml. per min or less.

The vagus nerves were sectioned in the neck. Gallamine (120 mg) was given to paralyse respiration and intermittent positive pressure ventilation was maintained with a Harvard respirator. Arterial P_{CO_2} was changed by adding varying flow rates of CO_2 to the input port of the ventilator. The concentration of inspired CO_2 was controlled with the aid of a CO_2 meter monitoring the airway gas. The Beckman LB 2 and Hartmann and Braun URAS 4 I.R. CO_2 analysers were used for this purpose. By this means, the range 3–10% inspired CO_2 concentration was explored.

In the first series of experiments (five dogs) β-adrenergic blockade was induced in all dogs. Sotalol (20 mg) was used; this is an adrenergic blocking drug without direct effects on the heart (Stanton, Kirchgessner & Parmenter, 1965). The effectiveness of the blockade was checked with test doses of 5 μg adrenaline. Blockade was considered adequate if there was no change in heart rate or maximum rate of rise of left ventricular pressure following the test dose of adrenaline. Additional doses of sotalol were given if subsequent test doses of adrenaline produced any effects.

This procedure does not necessarily ensure total β-blockade. We did not stimulate the stellate ganglia to ensure that the chronotropic and inotropic responses to activity in sympathetic nerves were completely abolished; this would have involved considerable additional surgery which we wished to avoid. The procedure adopted above appeared adequate to exclude most of the β-adrenergic responses.

In the second series of experiments (five dogs) the effects of CO_2 were studied in the absence of sotalol; the CO_2 administration was repeated after β-blockade; four measurements (two before and two after sotalol) were made by using an extra isotope and counting twice (see below). The same criteria for β-blockade were used as in the first series. An additional two dogs were studied only in the absence of sotalol.

Myocardial blood flow

Myocardial blood flow was measured by the method of Domenech, Hoffmann, Noble, Saunders, Henson & Subijanto (1969) using 15 μm 3 M radioactive carbonised microspheres. Sonication was used to break up any aggregates in the injection vial. An injection of the microspheres was made directly into the left atrium via the implanted catheter by flushing the injection vial (used to keep the microspheres in suspension) with 15 ml. of isotonic saline over a period of 10–15 sec. The microspheres then mix thoroughly in the left ventricle before being distributed to the myocardium and all organs of the body, where they are trapped in the capillary bed. The distribution of the microspheres between organs is in direct proportion to the blood flow reaching those organs. An accurate reference blood sample was required for the method. This was carried out as a pre-set flow rate, using the peristaltic pump, from the femoral artery cannula into weighed counting vials for 66 sec. This optimum timing had been determined in previous experiments as follows: sequential sampling beyond 1 min was carried out, and it was established that less than 5% of the microspheres appear after 60 sec. Mixing of the microspheres had been tested in these studies by withdrawing reference samples simultaneously from the carotid, brachial and femoral arteries. This yields the same reference flow/reference counts ratio.

At the end of each study the heart was excised and any fat or adherent lung tissue was removed before weighing. The right and left ventricle and atria were weighed separately, and then fixed in 10% formalin. After fixation the tissue was cut into small, full thickness pieces,

each weighing in the region of 3 g. Each piece was then placed in a glass counting vial, the atria, right and left ventricles being kept separate. The entire heart was counted. As the reference flow samples were counted at the same time as the tissue, decay corrections were not necessary. Each sample was counted for 2×10^4 counts or 1000 sec to conform with counting statistics. The pooled left ventricular counts were derived from at least 12 600 spheres, well in excess of the 400 required for accurate measurements (Buckberg, Luck, Payne, Hoffmann, Archie & Fixler, 1971). In addition, each piece contained more than 400 microspheres. The coefficient of variation of the measurement of left ventricular blood flow is less than 2%. This figure was obtained by dividing the standard deviation by the mean flow for duplicates in the same whole heart.

Myocardial blood flow (m.b.f.) was calculated from the formula:

$$\text{M.b.f.} = \text{myocardial counts} \times \frac{\text{reference flow}}{\text{reference sample counts}}.$$

Separation of the labels injected (^{141}cerium (^{141}Ce), ^{85}strontium (^{85}Sr), ^{95}niobium (^{95}Nb)) was made in a Nuclear Enterprises automatic spectrometer, model 8312, by use of their differing energy spectra. By adjusting the channel window settings, the counter could be set so that there was no overlap of the ^{141}Ce spectrum in either the ^{85}Sr or ^{95}Nb channels, though both ^{85}Sr and ^{95}Nb contributed to the counts in the ^{141}Ce channel, and the ^{95}Nb to the counts in the ^{85}Sr channel. The counts were corrected by calculation of the interference of ^{85}Sr and ^{95}Nb in the ^{141}Ce channel and of ^{95}Nb in the ^{85}Sr channel from the reference flow counts, which contained each isotope alone. In five dogs an additional measurement was made with ^{125}I microspheres by recounting the heart and correcting for the interference of the other three isotopes.

Left ventricular pressure

Left ventricular pressure was measured with the implanted Konigsberg P 22 transducer, the Millar or the Gael-Tec catheter tip manometer. The Konigsberg or Gael-Tec transducers were used in conjunction with a Hewlett–Packard 8805B carrier amplifier. The Millar transducer was used with a Millar DC bridge control unit, and the output signal amplified with a Hewlett–Packard 8802A DC amplifier.

The rate of change of left ventricular pressure (maximum left ventricular dP/dt) was obtained using a Hewlett–Packard 8814A derivative computer. Changes in left ventricular end-diastolic pressure were measured by amplifying the left ventricular pressure signal via a Hewlett–Packard 8802A DC amplifier. Reference zero values were determined at the end of each experiment by carrying out a thoracotomy and opening the left ventricular cavity to the atmosphere; temperature changes were avoided.

An e.c.g. was obtained from a Hewlett–Packard 8811A Bioelectric amplifier. Variables were recorded on a Gould Brush 480 pen recorder.

Cardiac output measurement

Cardiac output was measured using the microsphere technique with a different microsphere–isotope combination that avoided interference with the myocardial blood flow measurements. The microspheres used were 3 M human albumin microspheres for lung imaging (Riker Laboratories, Loughborough). These microspheres have a diameter range of 15–30 μm, and are trapped by the lungs when injected intravenously and by the systemic bed when injected into the left atrium. Prior to injection into the left atrium, microspheres were labelled with ^{99m}Technichium (^{99m}Tc) and the total activity of the injectate determined. A reference sample was withdrawn at a known flow from the arterial cannula in the same way as for the carbonized microspheres (see above). The residual activity in the injection vial was determined and subtracted from the pre-injection counts after the latter had been corrected for radioactive decay. This gave a value for the injected counts. The reference sample was also counted at the same time as the injection vial, and any decay corrections necessary were made. Cardiac output was calculated from the following formula:

$$\text{Cardiac output} = \text{injected counts} \times \frac{\text{reference flow}}{\text{reference counts}}.$$

Since ^{99m}Tc has a short half-life (6·05 hr), all activity had decayed to zero by the time the heart had been excised, fixed and counted for the myocardial blood flow measurement with carbonized microspheres.

The albumin microsphere technique for cardiac output was checked in another set of dogs against a direct measurement with an electromagnetic flowmeter (Drake, 1976). Flow transducers of the design and calibration of Dennis & Wyatt (1969) were used. Phasic aortic flow was measured with an SEM 275 flowmeter. Base line drift elimination, integration and analogue computation of cardiac output were carried out with the integrator described by Dijkema & Elzinga (1973). Phasic aortic flow and cardiac output were displayed on a Brush 480 pen recorder. The relationship between ^{99m}Tc albumin microsphere cardiac output (Y) and the electromagnetic cardiac output (X) was given by the equation:

$$Y = 0{\cdot}16275 + 0{\cdot}9962X; \quad r = 0{\cdot}9215.$$

In the second series of experiments, cardiac output was measured using the same carbonized microspheres as for the coronary blood flow measurement. A second injection was made using approximately one tenth of the dose, thus allowing counting of the injectate without flooding the sodium iodide crystal of the gamma counter. Two reference samples were collected for the two injections and pooled for calculation of coronary blood flow.

Oxygen content

Oxygen saturation was measured spectrophotometrically using a blood cell counting chamber as a microcuvette (0·1 mm light path) (SigaardAnderson, Jorgenson & Naerra, 1962) with the modification that 5% saponin solution was used as the haemolytic agent, rather than rapid freezing and thawing of the blood. Analysis was carried out as soon as possible after sampling, and all measurements were made in triplicate. P_{O_2} was measured from a sample taken immediately after the saturation sample, using a Corning–Eel pH blood gas analyser. Haemoglobin was measured using the cyanmethaemoglobin method, using a Unicam SP 1700 ultraviolet spectrophotometer. Optical densities were read at 546 nm. The oxygen capacity per g haemoglobin for dog blood was taken as 1·37, as reported by Dijkhuizen, Buursma, Fongero, Gerding, Oeseberg & Zijlstra (1977) for human blood. The oxygen content was then calculated from the following formula:

$$O_2 \text{ content (ml. } O_2 \text{.ml.}^{-1} \text{ blood)} = \frac{0{\cdot}137 \times \text{Hb} \times \%\text{ sat.}/100 + (0{\cdot}0031 \times P_{O_2})}{100},$$

where Hb is in g.l.$^{1-}$, P_{O_2} in mmHg. Coefficient of variation 1·2%.

In the second series of dogs, O_2 content measurements were made with a Lex–O_2–CON, TL. A comparison of the two methods was carried out at five different oxygen saturations, and the measurements made in triplicate. Agreement between the two methods was found to be very satisfactory; variance about the line of identity was 0·00053 ml.ml.$^{-1}$.

Oxygen consumption was calculated from the formula:

$$MV_{O_2} = \text{M.b.f.} \times (\text{arterial } O_2 \text{ content} - \text{coronary venous } O_2 \text{ content})$$
$$(\text{units: ml.}O_2\text{.min}^{-1}\text{.g}^{-1}).$$

Blood gas tensions

P_{O_2}, P_{CO_2} and pH were measured in all samples using a Corning–Eel pH/blood gas analyser.

Statistical analysis

Statistical evaluation of results was carried out according to Snedecor & Cochran (1973). The probability of differences in paired studies being due to chance was then calculated by the Sign test (Dixon & Mood, 1946) with a correction for continuity (Snedecor & Cochran, 1973). Linear regression analysis of pooled data was carried out according to Snedecor & Cochran (1973).

RESULTS

Effect of CO_2 on mechanical performance after β-blockade

A recording of mechanical variables is shown in Fig. 1. In this experiment an electromagnetic flowmeter on the ascending aorta was used to illustrate the beat to

beat changes in stroke volume (Noble *et al.* 1967). There was a depression of peak aortic flow, stroke volume, cardiac output and the maximum rate of rise of left ventricular pressure and an increase in left ventricular end-diastolic pressure.

The results for the dogs in the first series are shown in Fig. 2. In all dogs of both series (total of ten) maximum left ventricular dP/dt fell with increasing arterial

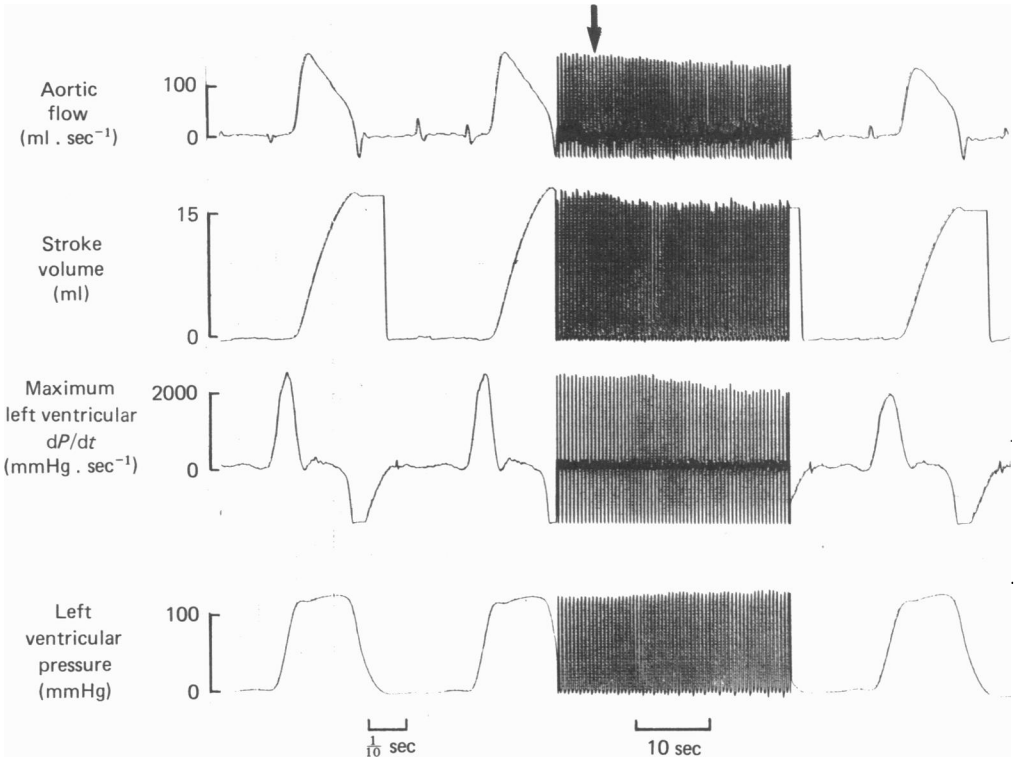

Fig. 1. Chart recording of changes in haemodynamic variables following the start of administration of 5% CO_2 at the time indicated by the arrow.

P_{CO_2} over the entire range studied (Fig. 2*A*). The probability of this result being due to chance was $< 0{\cdot}005$, i.e. statistically significant (s). The changes in the other mechanical variables for series 1 are given in Fig. 2*B*. The probability values for the various changes were: left ventricular end-diastolic pressure increased, $0{\cdot}05 < P < 0{\cdot}1$; cardiac output did not change, $0{\cdot}75 < P < 0{\cdot}5$ (not significant; n.s.); stroke volume was also unchanged, $0{\cdot}5 < P < 0{\cdot}25$ (n.s.). Table 1 presents mean data (± 1 S.D.) for ten dogs after β-blockade. Probability values are given for each of the ten data pairs (Sign test).

Effect of CO_2 on myocardial blood flow and oxygen consumption after β-blockade

The results for the first series are shown in Fig. 3, and the total results in Table 1. The changes in both variables were inconsistent. The probabilities of these changes being due to chance were $P < 0{\cdot}9$ for myocardial blood flow (n.s.) and $0{\cdot}1 < P < 0{\cdot}25$ for myocardial oxygen consumption (n.s.).

Effect of β-blockade on the response to CO_2

In the dogs of the second series, the response to hypercapnia in the absence of β-blockade was variable. Some dogs did not show a response to CO_2. The mean values for several dogs are shown in Fig. 4. The elevation of myocardial blood flow in the absence of β-blockade is of the same order of magnitude as that reported by

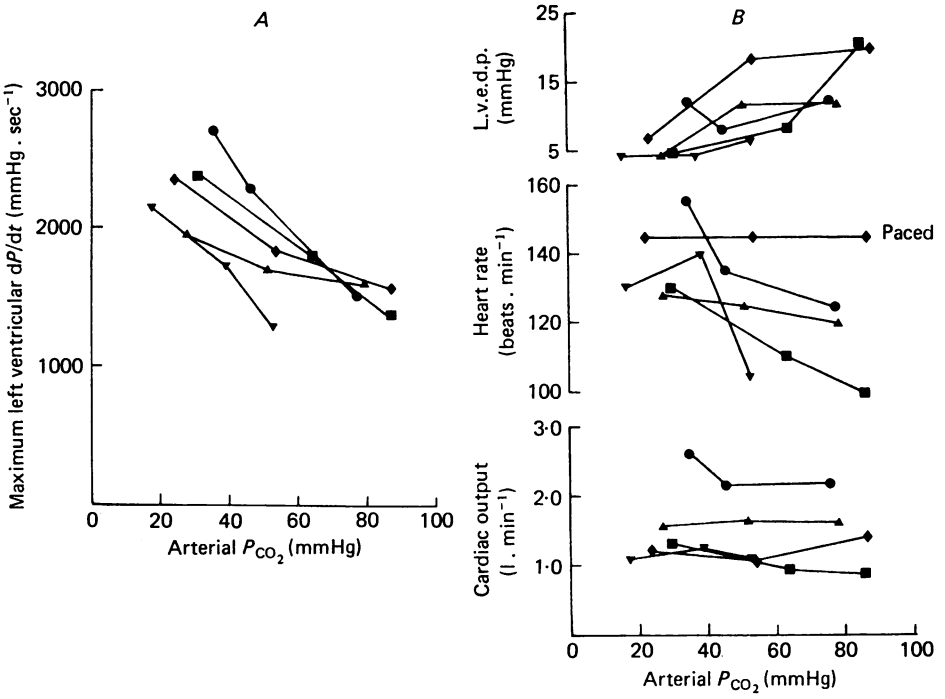

Fig. 2. *A*, maximum rate of rise of left ventricular pressure after β-blockade; each symbol represents one dog; note decrease with increasing arterial P_{CO_2}. *B*, left ventricular end-diastolic pressure (l.v.e.d.p.), heart rate and cardiac output plotted against arterial P_{CO_2}; same symbols as in *A*; β-blockade.

TABLE 1. Mean values (± 1 S.D.) for ten dogs studied with β-blockade. The probability values are calculated on the basis of ten pairs of data, by the Sign test (Dixon & Mood, 1946)

	P_{a,CO_2} 30–60 mmHg	P_{a,CO_2} 60–110 mmHg	Probability of no difference
Arterial P_{CO_2} (mmHg)	39·01 ± 8·07	87·4 ± 11·25	$P < 0.005$
Heart rate (beats.min^{-1})	143·0 ± 28·0	135·7 ± 29·0	$0.1 < P < 0.25$ (n.s.)
Left ventricular systolic pressure (mmHg)	129·7 ± 11·5	131·3 ± 15·9	$P = 0.75$ (n.s.)
Cardiac output (l.min^{-1})	1·37 ± 0·44	1·28 ± 0·49	$0.25 < P < 0.5$ (n.s.)
Left ventricular coronary blood flow (ml.min^{-1}.g^{-1})	1·03 ± 0·47	1·01 ± 0·46	$P = 0.75$ (n.s.)
Arterial venous O_2 difference (ml.ml.$^{-1}$)	0·1432 ± 0·0203	0·1315 ± 0·0254	$P = 0.75$ (n.s.)
Coronary sinus P_{O_2} (mmHg)	19·58 ± 3·382	33·06 ± 5·77	$P < 0.005$
Arterial O_2 saturation (%)	96·3 ± 2·57	93·4 ± 3·46	$0.01 < P < 0.025$

Ledingham *et al.* (1970) at an arterial P_{CO_2} of 100 mmHg. This contrasted with the lack of effect with β-blockade. In five dogs, the myocardial blood flow during hypercapnia was measured before and after β-blockade; it was always lower after β-blockade ($P = 0{\cdot}05$). However, the role of the adrenergic response was best demonstrated in one of these dogs which showed a large decrease in $A\text{-}V$ O_2 difference

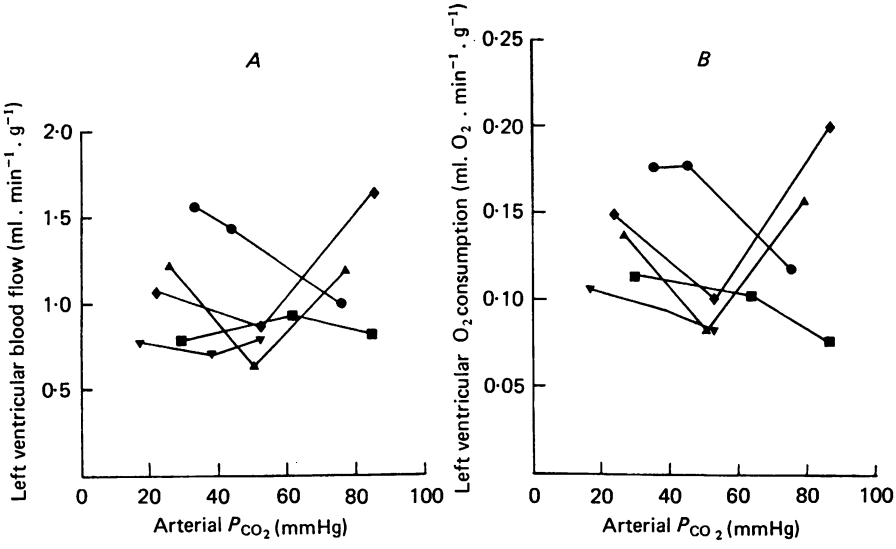

Fig. 3. Left ventricular coronary blood flow (*A*) and oxygen consumption (*B*) plotted against arterial P_{CO_2}; β-blockade. Same symbols as in Fig. 2.

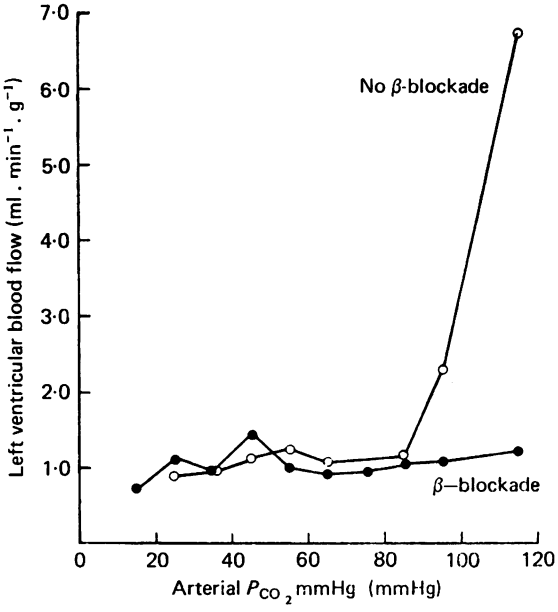

Fig. 4. Mean values for left ventricular coronary blood flow in seven dogs in the absence of β-blockade (open circles). Closed circles are mean values in dogs with β-blockade.

before β-blockade which was completely abolished after β-blockade. Coronary arterio-venous oxygen content difference was reduced from 0·121 ml./ml. ± 0·035 (1 S.D.) to 0·096 ml./ml. ± 0·049 (1 S.D.) by hypercapnia in seven dogs in the absence of β-blockade. Only one of these dogs failed to show this response. The corresponding values in the presence of β-blockade (ten dogs) were: normocapnia 0·143 ml./ml. ± 0·0250 (1 S.D.) hypercapnia 0·132 ml./ml. ± 0·025 (1 S.D.). Values for the individual dogs in the first series are shown in Fig. 5*A*.

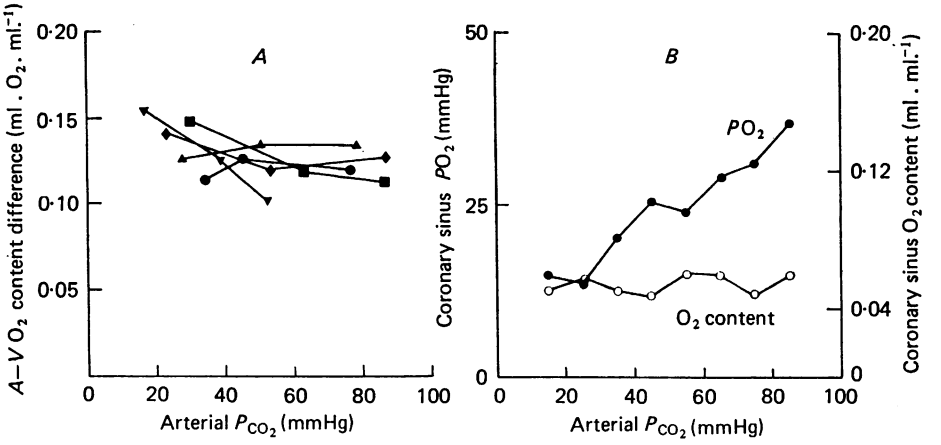

Fig. 5. *A*, arterial – coronary sinus oxygen content difference plotted against arterial P_{CO_2}. Same symbols as in Figs. 2 and 3; β-blockade. *B*, mean values for coronary sinus oxygen content (open circles) and coronary sinus P_{O_2} (closed circles) plotted against arterial P_{CO_2}; β-blockade.

Effect of CO_2 on arterial and coronary sinus oxygen content and P_{O_2} after β-blockade

The results for the *A–V* difference of oxygen content in the first series are shown in Fig. 5*A*. The changes were not statistically significant, $0{\cdot}75 < P < 0{\cdot}5$ (n.s.) There was no change in coronary sinus oxygen content with increasing arterial P_{CO_2} (Fig. 5*B*). Coronary sinus P_{O_2} rose with increasing arterial P_{CO_2}. Linear correlation of coronary sinus P_{O_2} on arterial P_{CO_2} yielded the relationship:

$$\text{Coronary sinus } P_{O_2} = 0{\cdot}33 \text{ arterial } P_{CO_2} + 7{\cdot}377; \quad r = 0{\cdot}896.$$

A summary of the results for the 'normocapnic' and hypercapnic ranges of arterial P_{CO_2}, are given in Table 1.

DISCUSSION

The results show that when the adrenergic effects are blocked, an increase in arterial P_{CO_2} causes a depression of mechanical performance of the heart, with little effect on coronary blood flow or myocardial oxygen consumption, but a rise in coronary sinus P_{O_2}.

The effects on mechanical performance appear to be straightforward, i.e. a consistent fall of maximum left ventricular dP/dt and a rise of left ventricular end-diastolic pressure with increasing arterial P_{CO_2} (Fig. 2*B*). This supports the view of Noble *et al.* (1967), that the initial transient depression of the heart with increasing CO_2 is a direct effect, and the later stimulation is an adrenergic response.

CO_2 has a direct negative inotropic effect on isolated papillary muscles (Cingolani,

Blesa, Gonzalez & Mattiazzi, 1969, 1970; Gonzalez, Cingolani, Mattiazi, Marsiglia & Serur, 1971; Johannsson & Nilsson, 1974; Mattiazzi, Cingolani & Graham, 1977) and isolated perfused hearts (Williamson, Safer, Rich, Schaffer & Kobayashi, 1975). CO_2 probably crosses the membrane and causes an intracellular acidosis. Heart muscle is relatively insensitive to extracellular acidosis (Noble *et al.* 1967; Williamson *et al.* 1975; Smith, 1926; Pannier & Leusen, 1968), and intracellular pH is affected less by extracellular acidosis than by changes in CO_2 (Ellis & Thomas, 1976). Johannsson & Nilsson (1975) increased P_{CO_2} at constant extracellular pH by adding HCO_3^- and found a negative inotropic effect and an abbreviation of the action potential; they thought that intracellular acidosis caused by CO_2 could reduce the release of activator calcium ion and/or affect the mechanical response of the contractile system at any given calcium ion concentration. There is recent evidence from skinned cardiac fibres that both mechanisms are likely (Fabiato & Fabiato, 1978).

The confusion concerning the effects of CO_2 on coronary blood flow is more easily understood when considering those of our experiments in which a β-blocker was not given. In some experiments coronary blood flow increased, whereas in others there was no effect. Why is the response so variable? If one takes an experiment in which the coronary blood flow was greatly increased, no effect was observed in the same dog after giving sotalol in a dose that was sufficient to block the effects of 5 μg of adrenaline. It seems reasonable to suppose that the dogs showing an increase of coronary blood flow have a greater sympatho-adrenergic response to CO_2 and/or slower rate of metabolic breakdown of catecholamines than those showing no response.

When the entire study was carried out under β-blockade, myocardial oxygen consumption or coronary blood flow did not seem to change in any consistent direction with increasing arterial CO_2. There may be other factors tending to modify the energy requirements of the heart in these experiments which contribute to the variability of the O_2 consumption. Since the coronary blood flow is primarily determined by the metabolic demands, any variations in O_2 consumption will be accompanied by concomittant changes in coronary blood flow (Mohrman & Feigl, 1978). In order to normalize coronary blood flow for changes in metabolic demands, it seems reasonable to divide by oxygen consumption. This however, gives the arterial – coronary sinus oxygen content difference which is directly measured in the experiments. Thus under the circumstances of these experiments, one might expect the $A\text{-}V$ O_2 difference to show less scatter and this is indeed the case (Fig. 5). The changes in this index were not statistically significant and do not indicate a vasodilator effect of CO_2, as one would expect from the direct relaxing effect of CO_2 on vascular smooth muscle, (Radawski, Dabney, Dangherty, Haddy & Scott, 1972; MacLellan, Pickard & Spurway, 1974). This result is in contrast to that in previous studies in which either adrenergic blockade was not used or, if used, not tested for completeness (Alexander & Liv, 1976; Case *et al.* 1975; Feinberg *et al.* 1960; Hilton & Eichholtz, 1925; Ledingham *et al.* 1970; Scheur, 1968; Tarnow, Bruckner, Eberlein, Gethman, Hess, Patschke & Wilde, 1975). We therefore suggest that most of the coronary vasodilatation previously observed was due to an adrenergic effect and not to a direct effect of CO_2.

We have considered the possibility that other influences mask a vasodilator action

of CO_2 in these experiments. (1) A fall in the pressure load of the left ventricle could cause a fall in oxygen consumption (Sarnoff, Braunwald, Welch, Case, Stainsby & Maeruz, 1958; Weber & Janicki, 1977), and therefore of coronary blood flow which would counteract increased flow caused by CO_2. However, there are no significant changes in left ventricular systolic pressure (Table 1). (2) CO_2 tends to cause a fall in heart rate which in turn would tend to reduce coronary blood flow (Laurent, Bolene-Williams, Williams & Katz, 1956; Berglund, Borst, Duff & Schreiner, 1958). However, the over-all changes in heart rate were small and not significant (Table 1). The results in the paced experiments were indistinguishable from the others. The approach of Mohrman & Feigl (1978) discussed above includes any changes in metabolic demands such as those caused by changes in left ventricular pressure and heart rate. (3) Replacement of O_2 with CO_2 in the inspired gas causes a fall in arterial P_{O_2} and saturation (Table 1). However the arterial oxygen saturation during hypercapnia remained over 90% because the inspired gas was enriched with oxygen. Thus no important change in oxygen delivery to the myocardium took place. (4) The sympatho-adrenergic response to CO_2 includes α- as well as β-adrenergic effects, including α-adrenergic coronary vasoconstriction (Feigl, 1967; Pitt, Elliot & Gregg, 1967). These effects were not blocked by sotalol. Could coronary vasoconstriction from this cause be masking vasodilatation due to CO_2? This possibility cannot be excluded by the present experiments and would require to be tested in a preparation with denervated heart because α-adrenergic blockade causes generalized systemic hypotension.

Although CO_2 had little effect on coronary sinus oxygen content, there was a clear-cut increase in coronary sinus P_{O_2}. This was in accordance with the expected shift to the right of the oxy-haemoglobin dissociation curve with acidosis (Bartels & Harms, 1958–9), the Bohr effect. Therefore the negligible changes in coronary vascular resistance are associated with a rise in coronary sinus P_{O_2} and therefore tissue P_{O_2}. This assumes that changes in tissue P_{O_2} are reflected by changes in venous P_{O_2} in the same direction. It is generally believed that coronary vascular resistance is controlled by tissue P_{O_2} or by a myocardial metabolite, the concentration of which depends on the level of tissue oxygenation. Thus lower tissue oxygenation is a vasodilator and higher tissue oxygenation is a vasoconstrictor. Why then, was there no vasoconstriction in our experiments in which tissue P_{O_2} rose? Is the coronary vascular bed regulated by venous oxygen content? The latter possibility seems unlikely since haemoglobin does not leave the vessels. The most reasonable explanation for this discrepancy is that the direct dilator effect of CO_2 on the blood vessels is sufficiently strong to counteract the constrictor effect of increased P_{O_2}. CO_2 presumably acts by increasing the hydrogen ions to which the vascular smooth muscle is exposed. That hydrogen ions cause active coronary arteriolar dilation was shown by Molnar, Scott, Frohlich & Haddy (1962). By infusing neutralized sodium acetate, citrate and nitrite they produced an intracellular acidosis which caused a marked fall of coronary perfusion pressure at constant flow.

A recent study by Case, Felix, Wachter, Kyriakidis & Castellana (1978) comes to a similar conclusion to that outlined above. They perfused the coronary vascular bed in such a way as to control coronary arterial blood gases with no change in systemic arterial blood gases; the adrenergic response to CO_2 was therefore pre-

sumably avoided. By lowering the coronary arterial P_{O_2}, they were able to compare hypercapnia with normocapnia at the same coronary sinus P_{O_2}. Under these circumstances, a clear cut though small vasodilatation was observed.

We conclude that the vasodilatory effect of CO_2 mediated through intracellular pH, is masked by the rise in tissue P_{O_2} caused by the altered affinity for oxygen of haemoglobin. Thus in the absence of secondary effects of sympatho-adrenal stimulation by CO_2, the effect of CO_2 on coronary vascular resistance is negligible.

REFERENCES

ALEXANDER, C. S. & LIV, S.-M. (1976). Effect of hypercapnia and hypocapnia on myocardial blood flow and performance in anaesthetized dogs. *Cardiovasc. Res.* **10**, 341–348.

ARFORS, K. E., ARTURSON, G. & MALMBURG, P. (1971). Effect of prolonged anaesthesia on acid-base balance and cardiovascular function in dogs. *Acta physiol. scand.* **81**, 47–53.

BARTELS, H. & HARMS, H. (1958–9). Sauerstoftdissoziationskurven des Blutes von Saugetieren (Mensth, Kaninchen, Meerschweinchen, Hund, Katze, Schwein, Rind und Schaf). *Pflügers Arch. ges. Physiol.* **268**, 334–365.

BERGLUND, E., BORST, H. G., DUFF, F. & SCHREINER, G. L. (1958). Effect of heart rate on cardiac work, myocardial oxygen consumption and coronary blood flow in the dog. *Acta physiol. scand.* **42**, 185–198.

BUCKBERG, G. D., LUCK, H. C., PAYNE, D. B., HOFFMAN, J. I. E., ARCHIE, J. P. & FIXLER, D. E. (1971). Some sources of error in measuring regional blood flow with radioactive microspheres. *J. appl. Physiol.* **31**, 598–604.

CASE, R. B., FELIX, A., WACHTER, M., KYRIAKIDIS, G. & CASTELLANA, F. (1978). Relative effect of CO_2 on canine coronary vascular resistance. *Circulation Res.* **42**, 410–418.

CASE, R. B., GREENBURG, M. & MOSKOWITZ, R. (1975). Alterations in coronary sinus P_{O_2} and O_2 saturation resulting from P_{CO_2} changes. *Cardiovasc. Res.* **9**, 167–177.

CINGOLANI, H. E., BLESA, E. S., GONZALEZ, N. C. & MATTIAZZI, A. R. (1969). Extracellular vs intracellular pH as a determinant of myocardial contractility. *Life Sci.* **8**, 775–781.

CINGOLANI, H. E., MATTIAZZI, A. R., BLESA, E. S. & GONZALEZ, N. C. (1970). Contractility in isolated mammalian heart muscle after acid-base changes. *Circulation Res.* **26**, 269–278.

DENNIS, J., WYATT, D. G. (1969). Effects of haematocrit value upon electromagnetic flowmeter. *Circulation Res.* **24**, 875–886.

DIJKEMA, F. K. & ELZINGA, G. (1973). Integrator for aortic and pulmonary flow signals, with automatic zero adjustment and beat to beat flow computation. *Cardiovasc. Res.* **7**, 572–576.

DIJKHUIZEN, P., BUURSMA, A., FONGERS, T. M. E., GERDING, A. M., OESEBURG, B. & ZIJLSTRA, W. G. (1977). The oxygen binding capacity of human haemoglobin, Hufner's factor redetermined. *Pflügers Arch.* **369**, 223–231.

DIXON, W. J. & MOOD, A. M. (1946). The statistical sign test. *J. Am. statist. Ass.* **41**, 557–566.

DOMENECH, R. J., HOFFMAN, J. I. E., NOBLE, M. I. M., SAUNDERS, K. B., HENSON, J. R. & SUBIJANTO, S. (1969). Total and regional coronary blood flow measured by radioactive microspheres in conscious and anaesthetized dogs. *Circulation Res.* **25**, 581–596.

DRAKE, A. J. (1976). Measurement of cardiac output by ^{99m}Tc albumin microspheres. *Proceedings of the European Conference on Microcirculation. Biblioth. Anat.* 15/16. Basel: S. Karger.

ELLIS, D. & THOMAS, R. C. (1976). Direct measurement of intracellular pH of mammalian cardiac muscle. *J. Physiol.* **262**, 755–771.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscle. *J. Physiol.* **276**, 233–255.

FEIGL, E. O. (1967). Sympathetic control of coronary circulation. *Circulation Res.* **20**, 262–271.

FEINBERG, H., GEROLA, A., KATZ, L. N. (1960). Effect of changes in blood carbon dioxide level on coronary flow and oxygen consumption. *Am. J. Physiol.* **199**, 349–354.

GONZALEZ, N. C., CINGOLANI, H. E., MATTIAZZI, A. R., MARSIGLIA, J. C. & SERUR, J. R. (1971). Mechanism of action of Tris-(hydroxymethyl) aminomethane on the negative inotropic effect of carbon dioxide. *Circulation Res.* **78**, 74–83.

GREGG, D. E., KHOURI, E. M., DONALD, D. E., LOWENSOHN, H. S. & PASYK, S. (1972). Coronary circulation in the conscious dog with cardiac neural ablation. *Circulation Res.* **31**, 129–144.

HILTON, R. & EICHHOLTZ, F. (1925). The influence of chemical factors in the coronary circulation. *J. Physiol.* **26**, 413–425.

JERUSALEM, E. & STARLING, E. H. (1910). On the significance of carbon dioxide for the heart beat. *J. Physiol.* **40**, 279–294.

JOHANNSSON, M. & NILSSON, E. (1975). Acid-base changes and excitation contraction coupling in rabbit myocardium. I. Effects on isometric tension development at different contraction frequencies. II. Effects on resting membrane potential, action potential characteristics and propagation velocity. *Acta physiol. scand.* **93**, 295–317.

KOBERSTEIN, R. C., PITMAN, D. E. & KLOCKE, F. J. (1969). Right atrial admixture in coronary venous blood. *Am. J. Physiol.* **216**, 531–535.

LAURENT, D., BOLENE-WILLIAMS, C., WILLIAMS, F. L. & KATZ, L. (1956). Effects of heart rate on coronary flow and oxygen consumption. *Am. J. Physiol.* **185**, 355–364.

LEDINGHAM, I. MCA., MCBRIDE, T. I., PARRATT, J. R. & VANCE, J. P. (1970). The effect of hypercapnia on myocardial blood flow and metabolism. *J. Physiol.* **210**, 87–105.

MCLELLAN, D. G., PICKARD, J. D. & SPURWAY, N. C. (1974). A contribution by anions to the pH-dependence of tone in a perfused artery preparation. *J. Physiol.* **242**, 97–98*P*.

MATTIAZZI, A. R., CINGOLANI, H. E. & GRAHAM, T. P. (1977). Lack of effect of hypercapnic acidosis on elasticity of cat papillary muscles. *Cardiovasc. Res.* **11**, 559–567.

MOHRMAN, D. E. & FEIGL, E. O. (1978). Competition between sympathetic vasoconstriction and metabolic vasodilation in the canine coronary circulation. *Circulation Res.* **42**, 79–91.

MOLNAR, J. I., SCOTT, J. B., FROHLICH, E. D. & HADDY, F. J. (1962). Local effects of various anions and H^+ on dog limb and coronary vascular resistances. *Am. J. Physiol.* **203**, 125–132.

MORRIS, M. E. & MILLAR, R. A. (1962). Blood pH/plasma catecholamine relationships: respiratory acidosis. *Br. J. Anaesth.* **34**, 672–681.

NOBLE, M. I. M., TRENCHARD, D. & GUZ, A. (1967). Effect of changes in P_{a,CO_2} and P_{a,O_2} on cardiac performance in conscious dogs. *J. appl. Physiol.* **22**, 147–152.

PANNIER, J. L. & LEUSEN, I. (1968). Contraction characteristics of papillary muscle during changes in acid-base composition of the bathing fluid. *Archs int. Physiol.* **76**, 624–634.

PITT, B., ELLIOTT, E. C. & GREGG, D. E. (1967). Adrenergic receptor activity in the coronary arteries of the unaesthetized dog. *Circulation Res.* **21**, 75–84.

RADAWSKI, D., DABNEY, J. M., DAUGHERTY, R. M., MADDY, F. J. & SCOTT, J. B. (1972). Local effects of CO_2 on vascular resistances and weight of the dog forelimb. *Am. J. Physiol.* **222**, 439–443.

SARNOFF, S. J., BRAUNWALD, E., WELCH, G. H., CASE, R. B., STAINSBY, W. N. & MACRUZ, R. (1958). Haemodynamic determinants of oxygen consumption of the heart with special reference to the tension-time index. *Am. J. Physiol.* **192**, 148–156.

SCHEUR, J. (1968). The effects of respiratory and metabolic alkalosis on coronary flow, haemodynamics and myocardial carbohydrate metabolism. *Cardiologia* **52**, 275–286.

SIGAARRDANDERSON, O., JORGENSON, K. & NAERRA, N. (1962). Spectrophotometric determination of oxygen saturation in capillary blood. *Scand. J. clin. Lab. Invest.* **14**, 298–302.

SMITH, H. (1962). The action of acids on turtle heart muscle with reference to the penetration of anions. *Am. J. Physiol.* **76**, 411–447.

SNEDECOR, G. W. & COCHRAN, W. G. (1973). *Statistical Methods.* Iowa State University Press.

STANTON, H. C., KIRCHGESSNER, T. & PARMENTER, K. (1965). Cardiovascular pharmacology of two new β-adrenergic receptor antagonists. *J. Pharmac. exp. Ther.* **149**, 174–182.

TARNOW, J., BRUCKNER, J. B., EBERLIN, H. J., GETHMAN, J. W., HESS, W., PATSCHKE, D. & WILDE, J. (1975). Blood pH and Pa_{CO_2} as chemical factors in myocardial blood flow control. *Basic Res. in Cardiol.* **70**, 685–696.

WEBER, K. T. & JANICKI, J. S. (1977). Myocardial oxygen consumption: the role of wall force and shortening. *Am. J. Physiol.* **233**, *H*421–430.

WILLIAMSON, J. R., SAFER, B., RICH, T., SCHAFFER, S. & KOBAYASHI, K. (1975). Effects of acidosis on myocardial contractility and metabolism. *Acta med. scand.* Suppl. 587, 95–112.