J. Physiol. (1960), **153**, *pp.* 413–422
With 4 text-figures
Printed in Great Britain

CHANGES IN pH OF THE PERFUSATE DURING HYPOXIA IN ISOLATED PERFUSED CAT LUNGS

BY HELEN N. DUKE, THE LATE ESTHER M. KILLICK AND JEAN V. MARCHANT

From the Department of Physiology, Royal Free Hospital School of Medicine, London, W.C. 1

(*Received for publication:* 17 *February* 1960)

Hypoxia has been shown to produce an increased pulmonary arterial pressure in the anaesthetized cat (von Euler & Liljestrand, 1946; Logaras, 1947). An increase in pulmonary vascular resistance also occurs in isolated perfused cat lungs (Nisell, 1948; Duke, 1951, 1954, 1957; Duke & Killick, 1952) and in the perfused left lung preparation of the anaesthetized cat (Duke, 1954, 1957) in response to substituting a gas mixture of low oxygen content for air. The experiments now reported were performed in order to test the hypothesis (Liljestrand, 1958) that hypoxia acts on the pulmonary blood vessels by liberation of lactic acid and thereby may cause a local regulation of the relation between blood flow and ventilation.

METHODS

Isolated cat lungs were set up and perfused through the pulmonary artery at constant-volume inflow as previously described (Duke, 1951). Gas mixtures used for ventilation were made by the British Oxygen Company and were contained in Douglas bags which were attached to the pump. The lungs were ventilated either with air or with N_2. The period during which the lungs were exposed to hypoxia varied between 3 and 25 min but was usually 10–15 min. In one experiment 5 % O_2 in N_2 was used instead of N_2. In three experiments 7 % CO_2 in air was used instead of air and the change was made to 7 % CO_2 in N_2; in the remaining five experiments the change was made from air to N_2.

The initial volume of the perfusate varied between 104 and 160 ml. In order to obtain enough perfusate to allow for taking numerous blood samples additions had to be made to the blood collected from the animal. In two experiments heparinized blood from a donor cat was used, in two other experiments Ringer–Locke solution 25 ml. was added to 79 or 98 ml. blood. In three experiments 30 ml. Dextran (Intradex, Glaxo) was added to the blood (110–120 ml.) before starting the experiments. The pump output varied from 44 to 83 ml./kg body wt./min in different experiments and the total volume of the perfusate would have made a complete circuit at times varying from 30 sec to 1 min 30 sec in different experiments. This time is slightly underestimated because no allowance could be made for the initial volume of blood in the lungs.

Mean pulmonary arterial pressure was measured with a manometer filled with 0·9 % NaCl solution, the zero of which was arranged to be approximately 1 cm posterior to the mitral valve. The pressure was recorded with a small-capacity tambour. Changes in lung blood volume were measured by recording changes in the volume of blood in the venous reservoir.

Under the conditions of these experiments this record shows inverse changes in lung blood volume (Daly, 1938).

Samples of blood were drawn anaerobically from the tubing leading from the left auricle. Lactate, pH, O_2 content, and haemoglobin were determined in these samples with as little delay as possible.

Lactate was determined by the method of Barker & Summerson (1941); ten determinations by this method on a single sample of blood showed a standard deviation of 1·0 mg from the mean percentage value. Total haemoglobin was determined spectrophotometrically; 0·05 ml. blood was mixed with 10 ml. 0·1 % Na_2CO_3 solution, and moist coal gas was bubbled through the mixture. The percentage transmission of this solution of carboxyhaemoglobin was read at a wave-length of 569 mμ (Hunter, 1951). The O_2 content of the blood was determined by the micro-method of Scholander & Roughton (1943).

Blood pH was determined at 37° C with the micro-electrode described by Joels & MacNaughton (1957), using either a Marconi pH meter, or a direct reading meter made by Electronic Instruments. The determination was usually made within 5 min of taking the sample, but during one experiment this was not possible owing to the necessity of checking drift on the pH meter; the maximum delay caused was 30 min and the samples were kept at room temperature during this time. The change in pH under these conditions was negligible; pH determinations made on a sample of heparinized cat blood at 10 min intervals confirmed that the earliest detectable change occurred 40 min after the sample was taken. It is difficult to assess the error of the method of pH determination on blood samples, but from repeated determinations of the pH of standard buffer solutions the error appeared to be $\pm 0{\cdot}02$.

Blood oxygen tension was calculated from O_2 content, O_2 capacity and O_2 saturation of blood samples by using dissociation curves for cat's blood (Duke & Stedeford, 1959).

Sodium bicarbonate and lactic acid solution were injected into the tubing between the left auricle and venous reservoir.

RESULTS

Response to hypoxia

Pulmonary arterial pressure. In twenty-one tests out of twenty-two the pulmonary arterial pressure (PAP), increased when the lungs were ventilated with N_2 instead of air or 7 % CO_2 in N_2 instead of 7 % CO_2 in air. In one test the PAP was unchanged (Fig. 1). The maximum pressure rise was 75 % of the initial value but increases of about 30 % were the most usual. In most, but not all, of the tests the pressure had reached a new stable level before the end of the hypoxic period. It was noticed that in most experiments the magnitude of the pressor response to hypoxia increased with time during the experiment. The one test in which no response occurred was the first test of an experiment in which pressor responses of average size were later obtained. In six tests the pressor responses to hypoxia occurred in the absence of changes in tidal air overflow volume. In the other tests (one of which is shown in Fig. 2) changes of from $+3{\cdot}0$ ml. to $-2{\cdot}5$ ml. were observed.

Blood pH. In fifteen tests out of twenty-two the pH of the left auricular blood was increased during hypoxia, in six of the tests the change in pH was within the limits of error of the determination ($\pm 0{\cdot}02$) and in one it decreased (see Fig. 1). There was apparently no relationship between the

magnitude of the pressor response in different preparations and the change in blood pH. Nor was there any clear indication that in the same preparation the percentage change in pressure was related to the degree of change in blood pH.

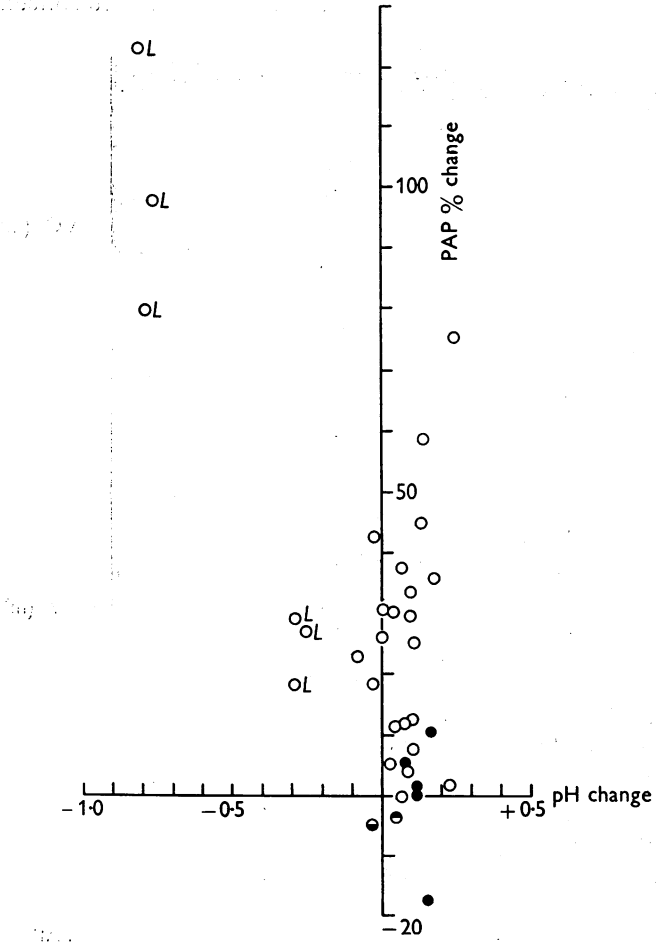

Fig. 1. The relationship between percentage change in pulmonary arterial pressure (ordinate) and change in pH of the left auricular blood (abscissa). Changes produced ○ at the end of a period of hypoxia; ○L after addition of lactic acid; ● after addition of sodium bicarbonate; ◒ during inhalation of 100 % CO instead of air; ◓ during inhalation of 20 % CO instead of air.

There was also no apparent relationship between the initial pH of the left auricular blood and the magnitude of the pressor response in different preparations (Fig. 3). There was no constant relationship between the pO_2 of the left auricular blood during hypoxia and the change in pH, but in three of the six tests in which there was no significant change in pH the

pO_2 of the perfusate remained relatively high (above 40 mm Hg compared with the usual 10–25 mm Hg).

Lactate. Blood lactate was measured about 1 min before changing from air to N_2 ventilation, just before the end of N_2 ventilation, and again after changing back to air. In a total of nineteen tests the lactate concentration

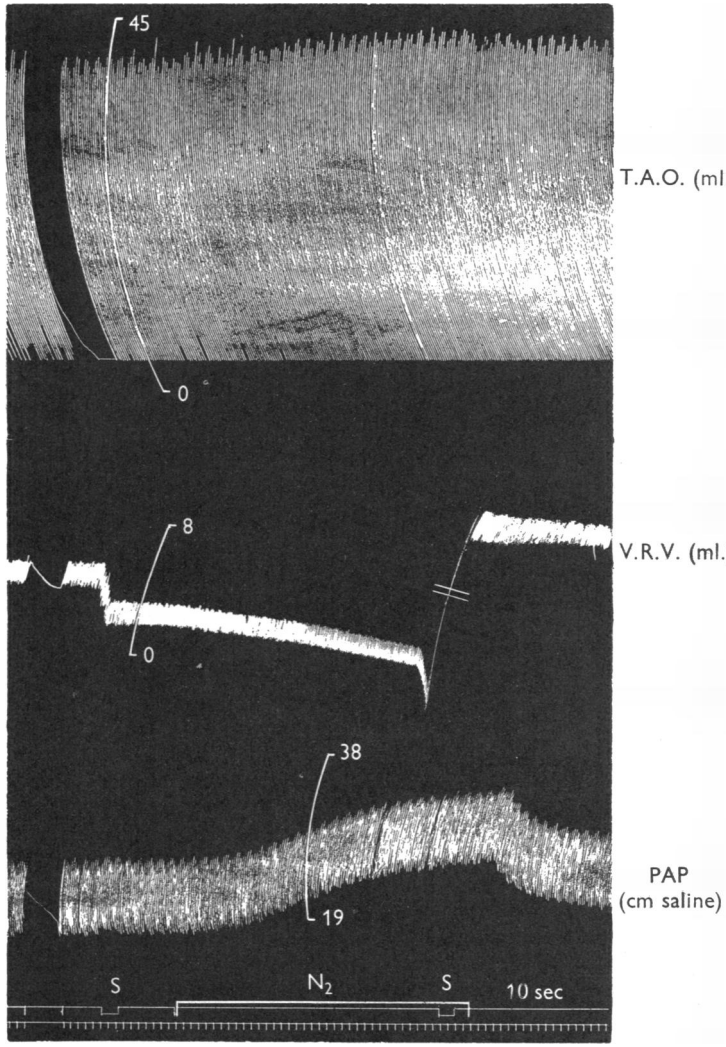

Fig. 2. The effects of changing the ventilating gas mixture from air to N_2. Cat 3·2 kg. Perfusion started at 11.05 a.m. (240 ml./min). During signal (12.22 to 12.29 p.m.) the lungs were ventilated with N_2 instead of air. At S approx. 5 ml. blood taken as sample. T.A.O. = tidal air overflow volume at 7 cm H_2O; V.R.V. = change in venous reservoir volume; PAP = pulmonary arterial pressure. Time marker, 10 sec.

was increased in eight, decreased in five and unchanged in six. The maximum increase in rate of change of lactate concentration during hypoxia compared with the control was 1·6 mg/100 ml./min and the maximum decrease was 1·9 mg/100 ml./min.

It was clear that there was a progressive increase of the blood lactate concentration during each experiment and that any changes due to hypoxia were superimposed on this. This increase in blood lactate was usually rapid in the early stage of perfusion, and became slower as the duration of

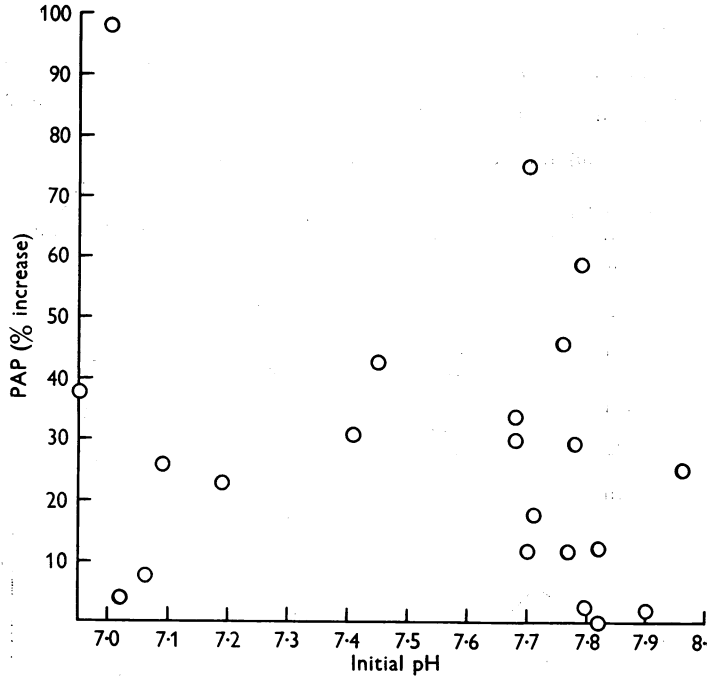

Fig. 3. The effect of the initial pH of the left auricular blood on the percentage increase in pulmonary arterial pressure in response to hypoxia.

perfusion increased. In several experiments it was necessary to add Dextran or Ringer–Locke solution to the perfusate to maintain a sufficient total volume. In other experiments lactic acid was added to the perfusate. Only three experiments provided a long period of perfusion without additions to the perfusate, and the mean rate of lactate increase is shown for these experiments in Table 1 (*a*).

The results for a typical experiment are shown in Fig. 4 in which mean PAP, blood lactate and blood pH are plotted against time. The increasing pulmonary arterial pressor response with time, and the progressive rise in blood lactate can be seen. In this experiment there was an increase of blood pH and a slight increase of blood lactate with hypoxia.

Blood oxygen tension. Immediately before the end of the hypoxic period the pO_2 of the blood from the left auricle was usually 10–25 mm Hg. Since the total volume of fluid in the system was perfused through the

TABLE 1. (*a*) Lactate formation in blood during perfusion through lungs

Perfusate	Amount (ml.)	Duration of perfusion (min)	Lactate (mg/100 ml. blood) Initial value	Final value	Increase (mg/100 ml./min)
Blood + dextran	115 30	144	14·1	50·2	0·25
Blood + donor blood	83 77	191	18·4	61·0	0·22
Blood + donor blood	85 70	116	33·5	92·0	0·50

(*b*) Lactate formation in blood *in vitro* at 37° C

Tonometer gas	Time (min)	Lactate (mg/100 ml. blood)	Lactate increase (mg/100 ml./min)
Air	0	7·5	0·29
	120	42·3	
N_2	146	46·0	0·44
	202	70·5	
Air	207	72·0	0·20
	260	82·7	
Air	0	5·4	0·32
	125	45·2	
N_2	141	46·5	0·24
	197	60·2	
Air	202	60·2	0·21
	231	67·5	

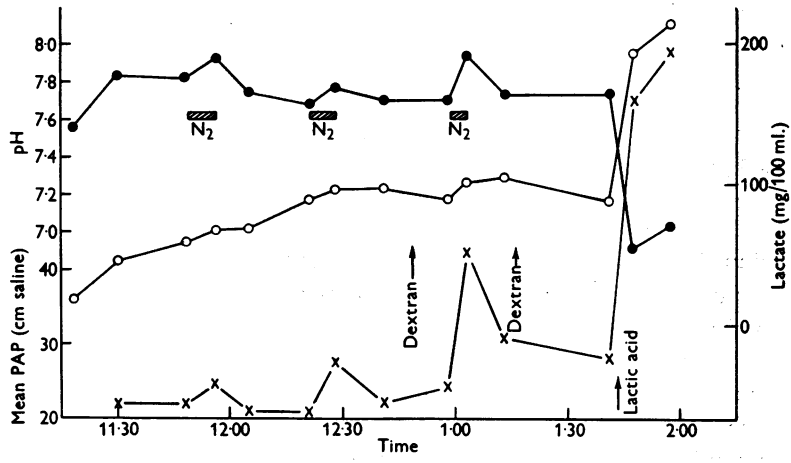

Fig. 4. The effects of hypoxia in isolated perfused cat lungs. Cat 2·5 kg. Blood flow 180 ml./min. Perfusion begun at 11.00 a.m. Positive pressure respiration with air. Periods of N_2 ventilation are indicated. At first two arrows addition of 20 ml. dextran to perfusate; at third arrow addition of 1 ml. N lactic acid to perfusate. ● pH of perfusate; ○ lactate content of perfusate; × mean pulmonary arterial pressure.

lungs in a time varying between 30 sec and 1 min 30 sec in different preparations, it would be expected that the pO_2 of the pulmonary arterial blood would decrease *pari passu* with that of the alveolar air and the left auricular blood. Studies made on other preparations perfused in similar circumstances (H. N. Duke, unpublished observations) have shown that the pO_2 of the pulmonary arterial blood differs little from that of the left auricular blood. There was no apparent relationship between the size of the pressor response to hypoxia and the change in blood pO_2 either in the same preparation at different times or in different preparations.

In twelve tests in which the pO_2 of the left auricular blood was between 10 and 25 mm Hg the percentage increase in PAP varied from 4 to 75; in six tests in which the pO_2 was between 25 and 50 mm Hg, the percentage increase in PAP varied from 12 to 59.

Addition of lactic acid and sodium bicarbonate. In six tests 0·25–1·0 ml. N lactic acid was added to the perfusate in the venous reservoir. This caused a rise in PAP and a reduction of left auricular blood pH (Fig. 1). The increase in PAP was more sustained than during the hypoxic tests, probably because the stimulus could not be withdrawn.

In five tests sodium bicarbonate was added in solution to the venous reservoir in amounts varying from 50 to 100 mg. In three tests the pulmonary arterial pressure was slightly increased, in one test it was decreased and in one test it was unchanged. In all the tests the blood pH was increased (Fig. 1).

Carbon monoxide. In two tests CO was used to ventilate the lungs instead of air. In both tests the PAP was decreased (Fig. 1). With 20 % CO in air the blood pH was increased and with 100 % CO the blood pH was decreased, although the changes in pH are of doubtful significance, since they are very near to the limits of error of the method.

Lactate accumulation in blood in vitro. In view of the gradual increase in the lactate content of blood during lung perfusion it was decided to investigate changes in blood lactate during rotation of blood in a tonometer at 37° C. Cat blood was used, drawn and heparinized in the same way as for the lung perfusion. Lactate was determined before placing the blood in the tonometer, and subsequent samples were withdrawn for lactate determination at intervals of approximately 30 min. After 2 hr the air in the tonometer was replaced by N_2 and the lactate determinations continued. After another hour the N_2 was replaced with air and lactate determinations again continued. The saturation of the blood with O_2 fell to about 10% when the tonometer contained N_2.

The lactate in the blood increased under these conditions (Table 1 *b*). The change from air to N_2 was associated with an increased rate of rise of lactate in one experiment; in the second experiment it seemed to have

little effect. The rate of increase of lactate *in vitro* was of the same order of magnitude as the rate during lung perfusion.

DISCUSSION

Liljestrand (1958) found that the pulmonary arterial pressure might show either a rise or a fall in response to hypoxia in isolated lungs of cats perfused through the pulmonary artery at constant volume inflow. The former effect was associated with a decrease in pH of the left auricular blood and the latter effect with an increase in blood pH. The results were unaffected by the addition of 5% CO_2 to both gas mixtures. The results now reported do not confirm this dual response and this accords with previous observations in similar preparations (Duke, 1951, 1954, 1957; Duke & Killick, 1952). Ventilation of the lungs with N_2 or with mixtures containing less than 10% O_2 caused a rise in pulmonary arterial pressure whether the pulmonary arterial blood was fully saturated with O_2 or whether the pO_2 in this blood was reduced concomitantly with that of the alveolar air by recirculation of the blood (Duke & Killick, 1952; Duke, 1954). The almost invariable finding in the present series of experiments has been an increase of pulmonary arterial pressure in response to hypoxia. This was usually associated with an increase of the left auricular blood pH but the pressor response did not appear to depend either on the initial blood pH or on the extent or direction of the change in blood pH. The extent of the pressor response appeared unrelated to the pO_2 in the left auricular blood, which under the conditions of perfusion differed little from the pO_2 of the pulmonary arterial blood. Addition of 7% CO_2 to the air or N_2 which was used to ventilate the lungs did not appear to influence the results.

In the present series of experiments the blood used for perfusion was diluted with blood from a donor animal, dextran or Ringer–Locke solution. The latter solution was used by Liljestrand (1958). No difference was noted in the results in consequence of these varying procedures.

The increase in lactate concentration of the perfusate during the course of each experiment was a constant and striking feature of the present experiments. Equilibration of cats' blood with air or N_2 at 37° C for several hours also led to an increase in the concentration of lactate in the blood and the final blood lactate concentration was similar to the concentration reached in the perfusate of isolated lungs after a similar time interval. In one of the tests the concentration of lactate in the blood increased more rapidly when the blood was exposed to N_2 than when it was exposed to air, but in the other test the increase in blood lactate occurred at a similar rate with air and N_2. This is in agreement with the findings in the perfused lungs in that hypoxia of the perfused lungs did not invariably lead to a

rise in the concentration of lactate in the perfusate. The tension of CO_2 in these *in vitro* experiments was not measured but experience with cats' blood in similar circumstances would lead one to believe that the CO_2 tension was 2–4 mm Hg (Duke & Stedeford, 1959).

The rise in blood pH found in the present series of experiments as a result of hypoxia may be associated with the change of oxyhaemoglobin to reduced haemoglobin in the perfusate. Values for the change in pH produced by this are not available for cat blood, but those for horse haemoglobin given by German & Wyman (1937) make this a reasonable explanation. Dill, Daly & Forbes (1937) found the effects of oxygenation of haemoglobin on pK′ to be of the same order of magnitude for ox and for human haemoglobin, and the few values they give for dog haemoglobin are similar. It would seem, therefore, that species differences in this respect are not large. It is of interest that the percentage of reduced haemoglobin in the perfusate in the tests accompanied by an increase in pH was usually high (mean 81·4 %), while it was unusually low (23–43 %) in three of the six tests unaccompanied by a change in pH.

SUMMARY

1. Ventilation of isolated perfused cat lungs with N_2 or 7 % CO_2 in N_2 instead of air or 7 % CO_2 in air causes a rise in pulmonary arterial pressure in preparations perfused at constant volume inflow.

2. The most usual effect of hypoxia was to produce a small increase of pH in the perfusate.

3. The pressor response did not appear to be dependent on changes in pH or lactate concentration of the perfusate.

The authors wish to thank Dr R. D. Stedeford and Mrs M. W. Duckworth, who assisted in some of the experiments. Mrs Marion Nixon gave invaluable technical help. Part of the apparatus was bought with a grant (to H.N.D.) from the Central Research Fund of London University.

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