

## APNEIC OXYGENATION IN ADRENALECTOMIZED DOGS

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SYMPATHO-ADRENAL stimulation, direct or compensatory, is a characteristic feature of apneic oxygenation<sup>1</sup> ("diffusion respiration") and to some degree counteracts the direct depressant effect of carbon dioxide on heart and circulation.<sup>2</sup> In the later stages of apneic oxygenation, when arterial  $P_{CO_2}$  is grossly elevated and oxygen saturation reduced moderately, plasma epinephrine is greatly increased. Within the first fifteen minutes of apnea, however, while arterial  $P_{CO_2}$  is increasing and when oxygen saturation is within normal limits, the rise in plasma catecholamine concentration involves norepinephrine predominantly.<sup>3</sup>

In the present study, some effects of apneic oxygenation in adrenalectomized dogs are compared with those already observed in intact animals,<sup>3</sup> and an attempt is made to determine the extent to which release of norepinephrine (and epinephrine) from areas outside the adrenal medulla contributes to the increased plasma catecholamine concentrations during apneic oxygenation.

### METHOD

In six experiments, dogs were anesthetized with intravenous thiopental, their tracheas were intubated with a No. 9 or 10 Magill cuffed tube, and intermittent positive pressure ventilation (+ 10 to 15 cm.  $H_2O$ ) was begun, using 100 per cent oxygen and the Bird respirator (Marks 4 and 8), with a Ruben non-rebreathing valve. An intravenous infusion of succinylcholine chloride (0.1 per cent) was given at a slow rate for the duration of each experiment. Light anesthesia was maintained with small increments of thiopental.

The right and left adrenal glands were then removed through bilateral loin incisions, care being taken to ensure complete excision with

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a minimum of blood loss. When considered necessary (in 2 experiments), dextran (6 per cent in saline) was given intravenously to replace blood lost. Pulmonary ventilation with 100 per cent oxygen was continued for 30–45 minutes after adrenalectomy, and in this period a femoral artery was cannulated for removal of blood samples and recording of blood pressure, by means of a Satham transducer Model P 23A, and Sanborn recorder.

Control blood samples were then withdrawn and the period of apneic oxygenation was begun, the endotracheal tube being connected through a T-piece and loose expiratory valve to a 5-liter rubber bag which was kept partly filled with a low flow of 100 per cent oxygen. Blood samples were withdrawn at intervals of 15, 30 and 60 minutes, whereupon the period of apnea was terminated by ventilation of the lungs with 100 per cent oxygen for approximately 15 minutes, after which another blood sample was taken. The endotracheal tube was then disconnected from the oxygen supply and a second period of apnea was begun, with the airway open to air. A final blood sample was taken, after an average of 7 minutes (range 4 to 10), just before hypotension and cardiac arrest occurred from acute asphyxia.

Blood for assay of norepinephrine and epinephrine (35 ml.) was withdrawn into tubes containing a few drops of heparin (Connaught Labs., 1000 units/ml.). After centrifuging, the plasma was aspirated and applied to alumina columns, the extraction procedure being completed within the next few hours. Epinephrine and norepinephrine in the plasma eluates were determined by the trihydroxyindole method<sup>4</sup> essentially as described previously,<sup>5</sup> but with the addition of 0.5 ml. of 1 per cent disodium ethylene diamine tetraacetate to the final mixture before measurement of fluorescence. Errors within  $\pm 25$  per cent are involved in the differential estimation of epinephrine and norepinephrine in single plasma samples, and the values, which refer to micrograms of free base/liter of plasma, are

TABLE 1  
AVERAGE RESULTS IN 6 ADRENALECTOMIZED DOGS SUBJECTED TO ONE HOUR OF APNEIC OXYGENATION  
TERMINATED BY VENTILATION WITH 100 PER CENT OXYGEN

Time (minutes)	pH	P <sub>CO<sub>2</sub></sub> (mm. Hg)	Standard HCO <sub>3</sub> <sup>-</sup> Separated Plasma (mM/L)	Epinephrine (μg./l.)	Norepinephrine (μg./l.)	Arterial Blood Pressure		Heart Rate (beats, minute)
						Systolic	Diastolic	
						(mm. Hg)		
Control—	7.44	29	19	0.10	0.25	171	112	165
A.O. +15	6.92	142	23	0.25	1.1	171	80	141
A.O. +30	6.72	248	24	0.30	2.4	199	89	157
A.O. +60	6.57	356	25	0.31	3.2	83	43	147
Vent. O <sub>2</sub> +15	7.28	42	18	0.20	1.1	114	74	166
Asphyxia	6.85	153	24	0.39	4.1	143	59	125

Each experiment ended with a period of "apnea in air." A.O. = apneic oxygenation.

uncorrected for losses in recovery up to 30 per cent.

Blood for pH, P<sub>CO<sub>2</sub></sub> and "standard" bicarbonate estimations (8 ml.) was withdrawn anaerobically into syringes moistened with heparin. Whole blood pH, and the pH of separated plasma equilibrated at 38 C. with known concentrations of carbon dioxide<sup>6</sup> were determined by means of the Radiometer pH meter Model 22 and Astrup apparatus.<sup>7</sup> From these two pH determinations the "standard" bicarbonate in separated plasma, and the

plasma CO<sub>2</sub> tension were calculated as described by Astrup.<sup>8</sup> In three experiments arterial oxygen saturations were determined spectrophotometrically.<sup>9</sup>

## RESULTS

The average data from the six experiments are shown in table 1. The progressive fall in arterial pH and increase in P<sub>CO<sub>2</sub></sub>, with a moderate rise in "standard" bicarbonate (separated plasma) reflect the severe respiratory acidosis induced by apneic oxygenation. Table 2 shows that with the experimental technique employed there was some variation in the degree to which oxygenation was maintained throughout apnea, although in our experience<sup>3, 20</sup> it is rare to encounter as pronounced a fall in arterial oxygen saturation as was noted in one experiment of the present study.

Figure 1 shows the average values for arterial plasma norepinephrine, epinephrine and

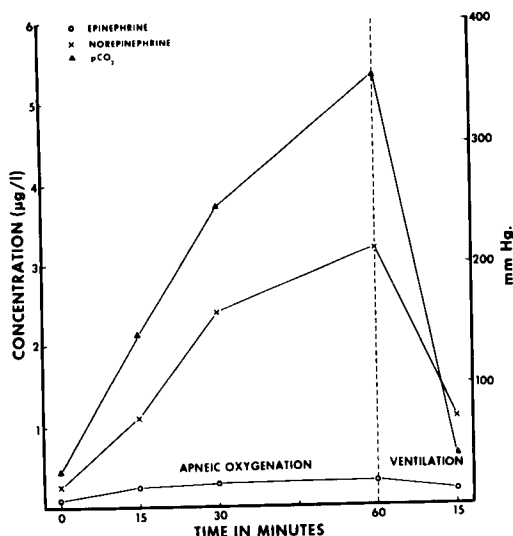


FIG. 1. Average changes in arterial plasma norepinephrine (X), and epinephrine (O), and P<sub>CO<sub>2</sub></sub> (▲) levels in six adrenalectomized dogs during one hour of apneic oxygenation, followed by ventilation with 100 per cent oxygen for 15 minutes.

TABLE 2  
ARTERIAL OXYGEN SATURATION (PER CENT) IN 3  
ADRENALECTOMIZED DOGS DURING ONE  
HOUR OF APNEIC OXYGENATION AND  
AFTER VENTILATION WITH 100  
PER CENT OXYGEN

Dogs	Control	Apneic Oxygenation			Vent'n 100 per cent O <sub>2</sub>
		+15'	+30'	+60'	
1	98	—	91	83	99
2	99	—	88	44	98
3	98	100	99	98	100

TABLE 3  
ANALYSIS OF VARIANCE, PLASMA NOREPINEPHRINE AND EPINEPHRINE CONCENTRATIONS,  
IN SIX DOGS SUBJECTED TO ONE HOUR OF APNEIC OXYGENATION

	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Norepinephrine	Total	83.24	23			
	Between treatments	31.86	3	10.62	5.71	<0.01
	Between dogs	23.54	5	4.72	2.53	
	Error	27.84	15	1.86		
Epinephrine	Total	1.55	23			
	Between treatments	0.17	3	0.06	3.0	<0.01
	Between dogs	1.12	5	0.22	11.0	
	Error	0.26	15	0.02		

P<sub>CO<sub>2</sub></sub> in the 6 adrenalectomized dogs. From a mean control level (following adrenalectomy) of 0.25 µg./l. (range 0.00–1.0), plasma norepinephrine increased to 1.1 µg./l. after 15 minutes (range 0.23–2.0), to 2.4 µg./l. after 30 minutes (range 0.00–6.9), and to 3.2 µg./l. after 60 minutes (range 0.87–4.7). By analysis of variance (table 3) these increases in plasma norepinephrine were highly significant (P < 0.01).

Figure 2 compares the average plasma nor-

epinephrine levels measured in the present study with those determined previously under the same experimental conditions in 5 intact dogs,<sup>3</sup> and shows that after 15 minutes of apneic oxygenation the norepinephrine concentrations measured in intact and adrenalectomized animals were almost identical, 1.2 and 1.1 µg./l., respectively. This suggests that as P<sub>CO<sub>2</sub></sub> increases during the early period of apnea, norepinephrine release occurs from areas outside the adrenal glands. Thereafter,

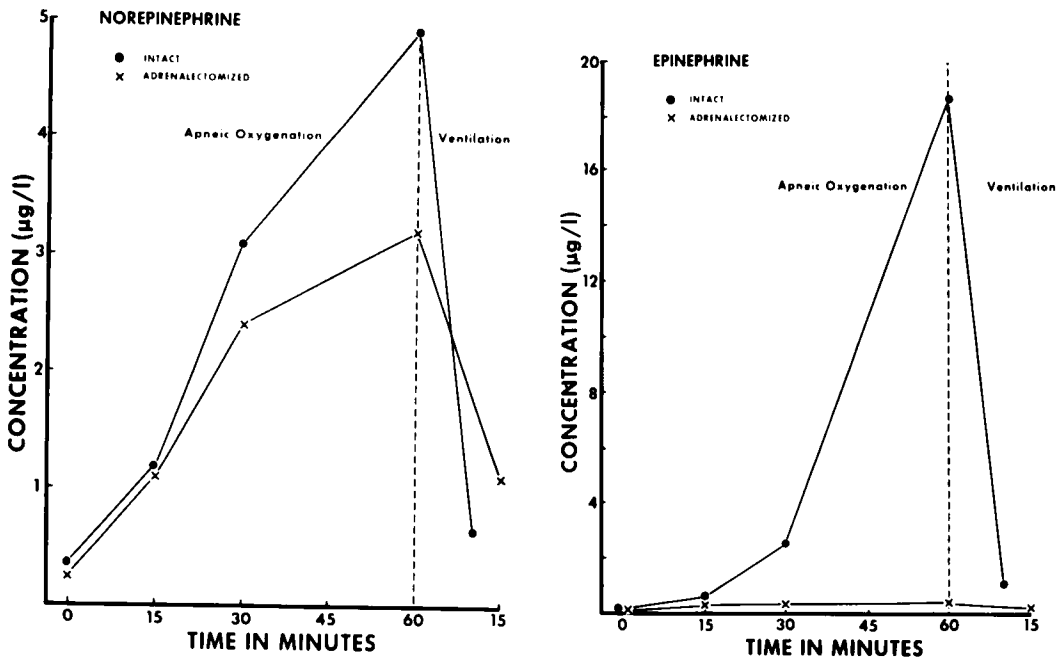


FIG. 2. Average plasma norepinephrine and epinephrine levels in five intact (●) and 6 adrenalectomized (×) dogs during one hour of apneic oxygenation, and after a subsequent period of ventilation with 100 per cent oxygen.

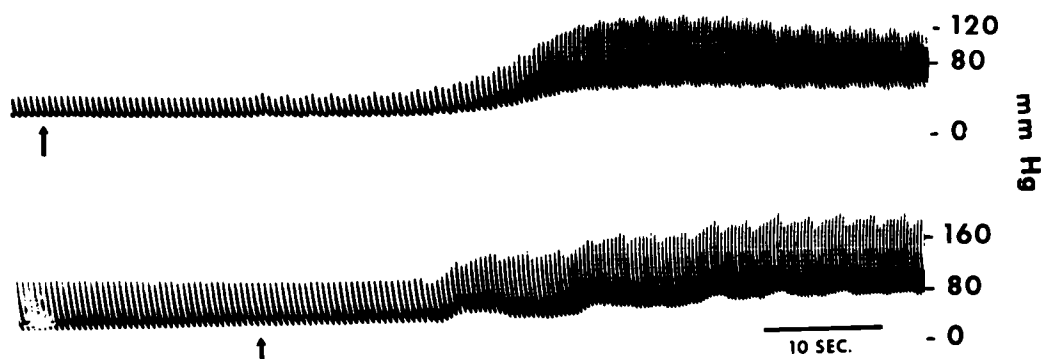


FIG. 3. The increase in arterial blood pressure induced within seconds of starting ventilation with 100 per cent oxygen after 60 minutes of apneic oxygenation in adrenalectomized dogs.

a greater rise in plasma norepinephrine in the intact dogs demonstrates the reinforcing role of the adrenal medulla.

Although small increases in plasma epinephrine were measured during apneic oxygenation, these were usually too small for accurate assessment, and in five studies the highest level reached was only  $0.37 \mu\text{g./l.}$  In the sixth experiment the rises were greater, from a control of  $0.39 \mu\text{g./l.}$  to  $0.65$ ,  $1.1$ , and  $0.57 \mu\text{g./l.}$  after 15, 30 and 60 minutes of apnea, respectively. The over-all increases in plasma epinephrine in the six experiments were not significant, by analysis of variance, although there was a highly significant variation between dogs (table 3). The negligible average changes in plasma epinephrine measured in the adrenalectomized animals subjected to apneic oxygenation are contrasted in figure 2 with the gross increases previously demonstrated in intact animals.<sup>3</sup>

The elevated plasma norepinephrine concentrations induced by 60 minutes of apneic oxygenation (average  $3.2 \mu\text{g./l.}$ ) were reduced significantly ( $P < 0.05$ ) by ventilation with 100 per cent oxygen for approximately 15 minutes (table 1). The average level regained,  $1.1 \mu\text{g./l.}$  (range  $0.14$ – $2.5$ ), did not differ significantly from control (pre-apnea) levels, although it was considerably higher. When asphyxia was induced by subjecting the animals to "apnea in air," plasma norepinephrine again increased significantly ( $P < 0.01$ ) to an average maximum of  $4.1 \mu\text{g./l.}$  (range  $2.7$ – $7.6$ ) just prior to terminal hypotension and cardiac arrest.

Plasma epinephrine concentrations were not

significantly reduced by ventilation with oxygen and did not show consistent or significant increases during the subsequent period of "apnea in air."

Changes in arterial blood pressure followed closely those already described in intact dogs<sup>3</sup>—an early diastolic fall, then an increase mainly in systolic pressure, followed by progressive hypotension. When the period of apnea was ended by ventilating with 100 per cent oxygen, blood pressure increased (fig. 3), the abrupt response being very similar to that previously described in intact animals.<sup>1, 3</sup> Transient cardiac arrhythmias were noted on the blood pressure tracing in only one experiment of the six during reduction of the elevated arterial  $P_{\text{CO}_2}$  levels by pulmonary ventilation.

A metabolic acidosis develops during apneic oxygenation in intact dogs.<sup>1, 10</sup> In the present study "standard" bicarbonate (separated plasma) in control samples averaged  $19 \text{ mM/l.}$  (range  $13$ – $23$ ), at an average  $P_{\text{CO}_2}$  of  $29 \text{ mm. of mercury}$  (range  $17$ – $41$ ) (table 1); the lower values resulted from overventilation (respiratory alkalosis) in the period before apneic oxygenation. During apnea "standard" bicarbonate increased by an average maximum of  $6 \text{ mM/l.}$  (range  $4$ – $12$ ), a greater rise than that previously noted in intact dogs,<sup>3</sup> which suggests that in the adrenalectomized animals a less severe degree of metabolic acidosis occurred during apneic oxygenation.

The average values for  $p\text{H}$ ,  $P_{\text{CO}_2}$ , and "standard" bicarbonate, in samples withdrawn after apnea had been terminated by ventilation with 100 per cent oxygen, show that a moder-

ate nonrespiratory acidosis existed after one hour of apneic oxygenation (table 1). At this time arterial  $P_{CO_2}$  averaged 42 mm. of mercury (range 23–72), while "standard" bicarbonate was in the range 12–22, with an average of 18 mM/l., which did not differ significantly from the levels measured in control samples. In intact animals, by comparison, it was previously found that "standard" bicarbonate was significantly lower following apnea, at a higher average  $P_{CO_2}$ , than in pre-apnea samples.<sup>3</sup> In four experiments of the present study the average  $P_{CO_2}$  and "standard" bicarbonate prior to apnea were 27 mm. of mercury and 19 mM/l., whereas after apnea, the values were 32 and 16 respectively. In the other two experiments a respiratory acidosis still existed after ventilation, "standard" bicarbonate levels of 21 and 22 mM/l. being associated with  $CO_2$  tensions as high as 72 and 54 mm. of mercury, respectively. Figure 4 presents the data in the form of  $P_{CO_2}/pH$  diagrams (semi-logarithmic scale). In intact dogs the plots

for samples withdrawn after apneic oxygenation lie to the left of those for pre-apnea samples<sup>3</sup>; in the adrenalectomized animals the difference is less marked. Thus a nonrespiratory acidosis is present after one hour of apneic oxygenation in adrenalectomized dogs, but it is less severe than in intact animals.

DISCUSSION

From these experiments it is concluded that during apneic oxygenation release of norepinephrine occurs from sympathetic nerves and other extra-adrenal areas or organs containing this amine. A decreased rate of destruction could also be partly responsible for the increased plasma norepinephrine concentrations measured at low levels of blood pH. It should be noted that the condition of apneic oxygenation usually results in a slow fall in arterial oxygen saturation so that when apnea is prolonged beyond about 30 minutes a state of asphyxia is gradually approached. In the first 15 to 30 minutes, however, oxygen saturation

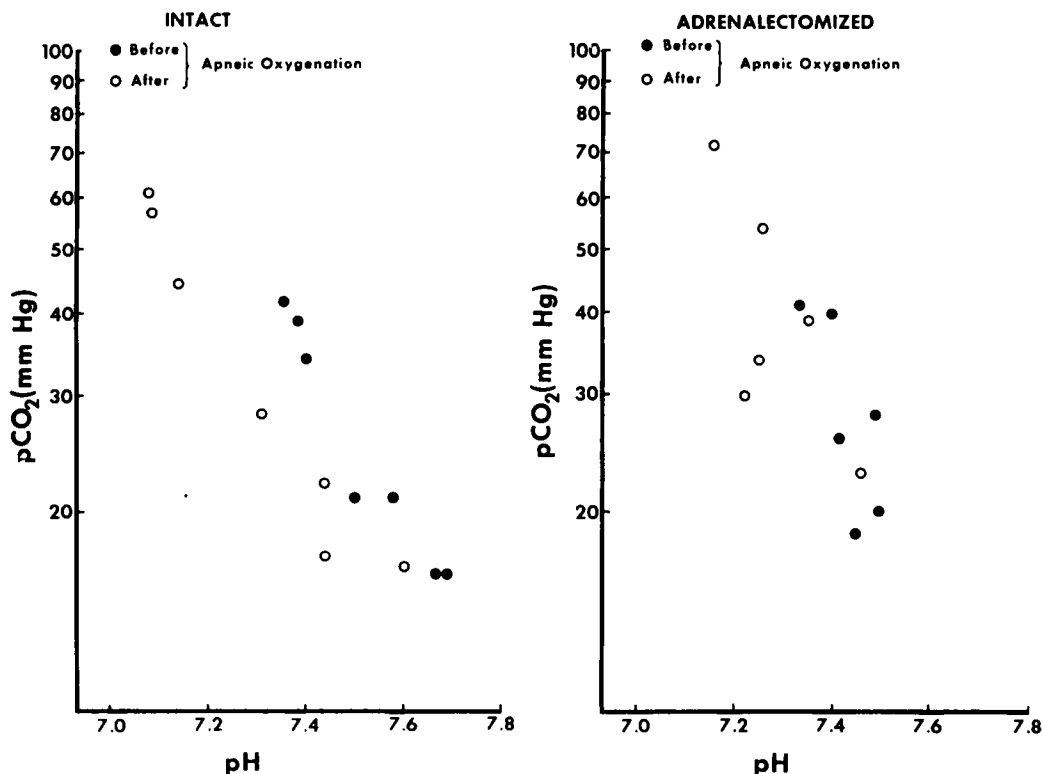


FIG. 4.  $P_{CO_2}/pH$  plots in 7 intact and 6 adrenalectomized dogs, for separated plasma samples withdrawn before (●) and after (○) one hour of apneic oxygenation (semi-logarithmic scale).

is well maintained, while increases in plasma norepinephrine consistently accompany the rise in arterial  $P_{CO_2}$ , so that it is reasonable to believe that respiratory acidosis *per se* leads to norepinephrine release. This conclusion has been substantiated by recent experiments in which a rise in plasma norepinephrine was induced by ventilation of adrenalectomized dogs with 20 per cent carbon dioxide in oxygen.<sup>11</sup> Increases in plasma norepinephrine apparently occur, therefore, as an early response to respiratory acidosis.

In spite of the unphysiological nature of apneic oxygenation, it is noteworthy that pronounced increases in circulating norepinephrine were measured at arterial  $P_{CO_2}$  levels which have been reached in anesthetized patients<sup>12, 13, 14</sup>—the highest plasma concentration measured in any experiment of the present study was 6.9  $\mu\text{g./l.}$  at a  $P_{CO_2}$  of 193 mm. of mercury.

The data suggest that a milder and more variable degree of metabolic acidosis occurs in the absence of adrenal medullary secretion. In intact animals the metabolic acidosis of apneic oxygenation is associated with lactic acidemia<sup>10</sup>; pyruvic and citric acids are probably also involved.<sup>15</sup> Since an increase in blood lactate concentration is more readily induced by epinephrine than by norepinephrine,<sup>16</sup> a less pronounced metabolic acidosis might be expected to occur during apneic oxygenation in adrenalectomized animals. In addition to a probable effect from increased levels of circulating norepinephrine, a nonrespiratory acidosis could result from deficient tissue oxygenation secondary to a reduction in arterial oxygen saturation, without catecholamine mediation; however, Holmdahl<sup>1</sup> reported a metabolic acidosis within ten minutes of starting apneic oxygenation.

An adequate circulation can apparently be maintained in dogs for at least one hour of apneic oxygenation, without reinforcement of pressor activity by adrenal medullary secretion (under the conditions described here, with the period of apnea following shortly after bilateral adrenalectomy). The characteristic changes in arterial blood pressure noted in intact dogs occur in the absence of the adrenal glands. A less pronounced rise in systolic pressure after 30 minutes of apnea, and a

greater degree of hypertension after one hour, were the only differences observed in the adrenalectomized animals. While a much greater physiological effect might have been expected to result from the grossly increased plasma epinephrine levels measured in intact dogs, the pressor response to circulating epinephrine may be diminished at reduced levels of blood pH in dogs.<sup>17, 18</sup>

The early fall in diastolic pressure noted consistently in both intact and adrenalectomized dogs can bear little relation to changes in plasma epinephrine, but may result from a direct action of carbon dioxide on blood vessel musculature. The association at this time of a reduced diastolic pressure and an increased plasma norepinephrine concentration deserves further study, since it might conceivably represent a direct action of carbon dioxide at sympathetic receptor sites.

During apneic oxygenation in dogs cardiac rhythm appears to be remarkably stable, an observation which is supported by evidence that ventricular arrhythmias initiated by epinephrine are less frequent at very low levels of blood pH.<sup>19</sup> Furthermore, in this and other studies<sup>3, 10, 20</sup> we have been unable to substantiate the findings of Brown and Miller,<sup>21</sup> who reported a high incidence of cardiovascular collapse during rapid reduction of elevated arterial carbon dioxide tensions in dogs made to breathe air after breathing 30–40 per cent carbon dioxide in oxygen for 3–4 hours. In our experience circulatory difficulties are very infrequent when the respiratory acidosis of apneic oxygenation is reversed by ventilation with 100 per cent oxygen (thus avoiding the possibility of a carbon dioxide induced "diffusion anoxia" similar to that described with nitrous oxide by Fink<sup>22</sup>).

#### SUMMARY

In six adrenalectomized dogs subjected to one hour of apneic oxygenation, average plasma norepinephrine concentration increased progressively from a control of 0.25  $\mu\text{g./l.}$  to 3.2  $\mu\text{g./l.}$ , as arterial  $P_{CO_2}$  rose to a maximum of 356 mm. of mercury. Plasma epinephrine showed small and variable increases, to an average maximum of 0.31  $\mu\text{g./l.}$  Ventilation with 100 per cent oxygen reduced the elevated

plasma norepinephrine concentrations, which were subsequently increased by severe asphyxia to an average of 4.1  $\mu\text{g./l.}$

Apneic oxygenation induced a nonrespiratory acidosis which was less pronounced and consistent than that previously observed in intact dogs. Blood pressure changes were similar to those noted in intact animals. Transient cardiac arrhythmias occurred in only one experiment as the elevated arterial  $\text{P}_{\text{CO}_2}$  was lowered by pulmonary ventilation. Acutely adrenalectomized dogs can withstand at least one hour of apneic oxygenation, and the circulatory changes which result are not greatly different from those induced in intact animals.

It is concluded that during apneic oxygenation substantial amounts of norepinephrine are released from sympathetic nerves and other extra-adrenal areas and organs containing this amine. A rise in plasma norepinephrine appears to be an early response to respiratory acidosis.

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#### REFERENCES

- Holmdahl, M. H.: Pulmonary uptake of oxygen, acid-base metabolism and circulation during prolonged apnea, *Acta chir. scand.* (suppl.) **212**: 1, 1956.
- Boniface, K. J., and Brown, J. M.: Effect of carbon dioxide excess on contractile force of heart, in situ, *Amer. J. Physiol.* **172**: 752, 1953.
- Millar, R. A.: Plasma adrenaline and noradrenaline during diffusion respiration, *J. Physiol.* **150**: 79, 1960.
- Euler, U. S. v., and Floding, I.: Fluorimetric micromethod for differential estimation of adrenaline and noradrenaline, *Acta physiol. scand.* (Suppl. 118) **33**: 46, 1955.
- Millar, R. A., Keener, E. B., and Benfey, B. G.: Plasma adrenaline and noradrenaline after phenoxybenzamine administration and during haemorrhagic hypotension, in normal and adrenalectomized dogs, *Brit. J. Pharmacol.* **14**: 9, 1959.
- Nunn, J. F.: Accuracy of measurement of blood  $\text{pCO}_2$  by interpolation technique, *A Symposium on pH and Blood Gas Measurement* (edited by R. F. Woolmer). London, J. & A. Churchill Ltd., 1959.
- Astrup, P., and Schröder, S.: Apparatus for anaerobic determination of the pH of blood at 38 degrees Centigrade, *Scand. J. Clin. Lab. Invest.* **8**: 30, 1956.
- Astrup, P.: Simple electrometric technique for determination of carbon dioxide tension in blood and plasma, total content of carbon dioxide in plasma, and bicarbonate content in "separated" plasma at fixed carbon dioxide tension (40 mm. Hg), *Scand. J. Clin. Lab. Invest.* **8**: 33, 1956.
- Nahas, G. G.: Spectrophotometric determination of hemoglobin, and oxyhemoglobin in whole hemolyzed blood, *Science* **113**: 723, 1951.
- Joels, N., and Samueloff, M.: Metabolic acidosis in diffusion respiration, *J. Physiol.* **133**: 347, 1956.
- Millar, R. A., and Morris, M. E.: Norepinephrine release during respiratory acidosis in adrenalectomized dogs, *ANESTHESIOLOGY* **22**: 62, 1961.
- Dripps, R. D., and Severinghaus, J. W.: General anesthesia and respiration, *Physiol. Rev.* **35**: 741, 1955.
- Wynn, V.: The clinical significance of blood pH and blood gas measurement, *A Symposium on pH and Blood Gas Measurement* (edited by R. F. Woolmer). London, J. & A. Churchill, Ltd., 1959.
- Gray, T. C.: A clinical note on difficulty of maintaining adequate pulmonary ventilation during hypothermia, *A Symposium on Pulmonary Ventilation* (edited by R. P. Harbord and R. F. Woolmer). Altrincham, John Sherratt & Son, 1958.
- Ellis, S.: Metabolic effects of epinephrine and related amines, *Pharmacol. Rev.* **8**: 485, 1956.
- Brewster, W. R., Isaacs, J. P., Osgood, P. F., and King, T. L.: Hemodynamic and metabolic interrelationships in activity of epinephrine, norepinephrine and thyroid hormones, *Circulation* **13**: 1, 1956.
- Page, I. H., and Olmsted, F.: Influence of respiratory gas mixtures on arterial pressure and vascular reactivity, *Circulation* **3**: 801, 1951.
- Nahas, G. G., Ligou, J. C., and Mehlman, B.: Effects of pH changes on  $\text{O}_2$  uptake and plasma catecholamines levels in dog, *Amer. J. Physiol.* **198**: 60, 1960.
- Houle, D. B., Weil, M. H., Brown, E. B., Jr., and Campbell, G. S.: Influence of respiratory acidosis on electrocardiogram and pressor responses to epinephrine, norepinephrine and metaraminol, *Proc. Soc. Exp. Biol. Med.* **94**: 561, 1957.
- Millar, R. A., Brindle, G. F., Gilbert, R. G. B.: Studies with organic buffer (T.H.A.M.) during apnoeic oxygenation in dogs, *Brit. J. Anaesth.* **32**: 248, 1960.
- Brown, E. B., and Miller, F.: Ventricular fibrillation following rapid fall in alveolar carbon dioxide concentration, *Amer. J. Physiol.* **169**: 56, 1952.
- Fink, B. R.: Diffusion anoxia, *ANESTHESIOLOGY* **16**: 511, 1955.