CIX. VARIATIONS IN THE p_H AND BICARBONATE OF THE PLASMA AND OF THE ALVEOLAR CO_2 DURING FORCED BREATHING.

BY ELIZABETH HERDMAN LEPPER AND MARJORIE MARTLAND.

*From the Departments of Experimental Pathology and Biochemistry,
Lister Institute, London.*

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INTRODUCTION.

THE production of a rise in the p_H of the blood in voluntary hyperpnoea has been inferred by Davies, Haldane and Kennaway [1920] and by Collip and Backus [1920] as the result of observations made on the blood and urine during forced breathing. These observers found that the titratable acidity of the urine was decreased, and thus confirmed the experimental work of Leathes [1919].

Collip and Backus also estimated the alveolar CO_2 and the bicarbonate of the plasma, which they found were always reduced, and calculated the p_H of the blood from Hasselbalch's formula. They found that the hydrogen ion concentration of the blood was lowered.

Haldane investigated the CO_2 -absorption curve of blood drawn at the end of an hour's forced breathing, and found that it was unaltered. The changes produced in the partition of the available base between the plasma and corpuscles by a lowered tension of CO_2 must therefore be transient and depend only on the alveolar CO_2 tension. He calculated that the p_H of his blood had been raised 0.38 in the course of the experiment.

Grant and Goldman [1920] using the dialysis method of Levy and Rowntree, and Peters and his colleagues [1926] using Cullen's [1922] colorimetric method, made direct observations on the p_H of the blood during hyperpnoea, and found an increase of 0.1 to 0.2 p_H above the normal.

Observations at frequent intervals have not however been carried out. The micro-method of Martin and Lepper [1926, 1] furnished us with a technique whereby we could take successive readings at intervals of a few minutes, and so study in detail the changes which take place as a result of reducing the tension of CO_2 in the alveolar air.

In some preliminary experiments on forced breathing, observations were confined to the rate of rise and the return to normal of the p_H of the blood, samples being taken at intervals of 3 minutes. Estimation of the percentage of CO_2 in the alveolar air at different rates of respiration, combined with observations on the changes which occurred in the p_H of the blood, were then

made. These experiments showed us the most effective rate of respiration for the subject concerned. At the same time we found out certain precautions that had to be taken in the collection of approximately simultaneous samples of blood and of alveolar air respectively. The limits of endurance of the experimental subject (E. H. L.), who had to be responsible for the collection of blood, was also ascertained. The symptoms experienced were a feeling of chilliness and slight mental confusion, which came on within a few minutes. Increased salivation was accompanied by a troublesome cough towards the end of the experiments. Tetanic spasms were never experienced, slight rhombism was nearly always present, and in long experiments, twitching of the muscles of the face accompanied by shakiness of the hands. During recovery the longest period of apnoea noted was $1\frac{1}{2}$ minutes. The rapid rate at which the mental symptoms came on was a constant surprise. By the end of 2 minutes it was difficult to concentrate sufficiently to count the respirations with a stop-watch. To the onlooker the effect was comparable with the early stages of alcoholic intoxication. Irritability, unreasonableness with a tendency to argue, and a lowering of the critical faculty were marked. Nevertheless, no difficulty in collecting the samples of blood was experienced so long as the routine was not departed from. The rate of respiration, once established, became almost automatic. We therefore arranged all the details of an experiment beforehand, and the subject took samples of blood only when ordered to do so and was not responsible for timekeeping. We found that we could be certain of carrying on an experiment for 25 to 30 minutes, but that after that time symptoms such as cough or shakiness of the hands might interfere with the collection of samples.

METHODS EMPLOYED.

The p_H of the blood was estimated by the Martin-Lepper [1926, 1] method. Capillary blood was collected from the finger. The saline with which the blood was diluted was adjusted to p_H 7.60 for the normal observations and to 7.75 for the forced breathing experiments.

The alveolar CO_2 was estimated with a small Haldane-Priestley apparatus. Owing to the limited time at our disposal we could only make single observations. The sample of alveolar air was always taken at the end of a forced expiration.

The bicarbonate of the plasma was estimated by the method of Van Slyke [Van Slyke, Stillman and Cullen, 1919; Van Slyke, 1922] in which excess of acid is added to a measured amount of plasma and the mixture titrated back to the discovered p_H of the blood as drawn.

The plasma was collected by pricking the ear and running the blood down a capillary tube into a glass capsule containing liquid paraffin with a small amount of solid potassium oxalate to prevent clotting. The corpuscles were spun down as soon as possible and the plasma pipetted off into a tube coated with solid paraffin. Some preliminary experiments with rabbits' blood showed

us that the titration value of the bicarbonate was the same whether the cells were separated at 38° in the hot room or the blood was allowed to cool for a few minutes and then centrifuged at room temperature. We had to rely on the presence of oxalate to prevent glycolysis between the withdrawal of the blood and the separation of the cells from the plasma, it being impossible to use un-neutralised solid sodium fluoride without lowering the alkali content of the blood. In view of the rapid glycolysis of blood in which the CO₂ tension has been reduced [Evans, 1922] we were doubtful as to whether the antiglycolytic action of oxalate were sufficient for our purpose, but a control experiment failed to detect any error from this cause over a period such as was used in the experiments.

The bicarbonate of the urine was estimated by Haldane's method [Davies, Haldane and Kennaway, 1920] in which excess of acid is added to 10 cc. of urine, the CO₂ shaken out and the mixture titrated to p_H 7.60.

The conditions of the experiment.

The forced breathing was carried out from $\frac{1}{2}$ to $2\frac{1}{2}$ hours after meals. The majority of the experiments took place about an hour after lunch. Half an hour after that meal the subject took two samples of blood for the p_H estimations and then gave a sample of alveolar air into the Haldane pipette. It is important to observe this sequence, as it is difficult when apnoeic to avoid cessation of respiration whilst blocking the mouthpiece of the sampling tube with the tongue. Blood was then collected for the bicarbonate estimation. The results of these observations gave the values for the subject when breathing naturally.

The experiment was then started. The respirations were maintained at 40 to 44 per minute, forced inspiration and expiration being carried out. In those experiments in which the p_H and bicarbonate of the plasma and the CO₂ of the alveolar air were all estimated, the following order was observed. After a period of 5 minutes' forced breathing, blood was taken for the p_H estimations, then a sample of alveolar air, then blood for bicarbonate, and further samples in the same order as soon as the gas analysis had been completed.

RESULTS.

Normal conditions.

The values obtained for p_H , alveolar CO₂ and plasma bicarbonate under normal conditions are given in Table I. p_{K_1} has been calculated from Hasselbalch's formula, after subtracting 0.22 from the p_H observed at 18° to obtain the value for the p_H of plasma at 38° [Martin and Lepper, 1926, 2]:

$$p_{K_1} = p_H^{38^\circ} - 38^\circ \log \frac{\text{plasma bicarbonate}}{\text{CO}_2 \text{ dissolved in plasma at } 38^\circ}.$$

Table I.

Date (1927)	p_H (mean)	Alveolar CO_2 %	CO_2 dis- solved in plasma, 38° vols. %	Plasma bicarbonate vols. % CO_2	p_{K_1}
21. iii.	7.61	6.00	3.25	51	6.195
21. iii.	7.605	6.15	3.35	48	6.229
24. iii.	7.66	5.97	3.25	54.4	6.217
24. iii.	7.68	6.03	3.3	56	6.231
24. iii.	7.678	6.03	3.3	52	6.260
26. iii.	7.667	5.90	3.2	51	6.245
26. iii.	7.678	5.85	3.17	56	6.211
7. iv.	7.65	5.95	3.23	48	6.258
10. iv.	7.607	6.05	3.3	49.75	6.209
15. iv.	7.635	5.87	3.2	45.5	6.263
24. iv.	7.624	6.11	3.35	46	6.264
25. iv.	7.629	6.04	3.3	41	6.315
					Mean = 6.241

Observations during forced breathing.

1. The p_H of the blood showed a rapid rise reaching in 10 to 15 minutes a maximum of 0.2 to 0.24 above the initial value, at which level it was maintained as long as observations could be carried out. As soon as the forced breathing was stopped the p_H fell rapidly.

2. The percentage of alveolar CO_2 was halved at the end of 5 minutes, this rapid fall being followed by a further gradual decrease. The return to normal is shown in Figs. 2 and 3.

Fig. 1. Showing the rise in the p_H of plasma during forced breathing and the return to normal.

• — • — • p_H of blood.

3. The bicarbonate of the plasma was reduced. The titration of the plasma after forced breathing afforded results corresponding to 10 to 12 volumes % less total CO_2 than before the experiment was started. This drop was caused in two ways. The diminished CO_2 tension in the alveoli of the lungs accounts in

different experiments for 5 to 8 vols. %. The balance, 4 to 5 vols. %, is due to the higher p_H to which the plasma had to be titrated, sometimes as high as 7.85. More than the normal amount of sodium was in combination with the blood proteins, for it was found that, for the blood used, the titration value

Fig. 2. Showing the rise in the p_H of the plasma and the fall in alveolar CO_2 during forced breathing. The somewhat low values for p_H at 4 and 10 minutes are due to the sample of alveolar air having been taken immediately before the p_H estimation.

• — • — • p_H of blood.
 ○ — ○ — ○ Percentage CO_2 in alveolar air.

Fig. 3. Showing the rapid return to normal of the alveolar CO_2 when forced breathing is discontinued.

• — • — • p_H of blood.
 ○ — ○ — ○ Percentage of CO_2 in alveolar air.

of the plasma bicarbonate corresponded to 4 vols. % less CO_2 at p_{H} 7.80 than at p_{H} 7.60.

4. *Changes in the urine* were studied in two experiments. In both cases the urine became alkaline, containing 0.019 *M* and 0.036 *M* bicarbonate respectively. In the course of an experiment which lasted for 1 hour 0.357 g. of bicarbonate was excreted.

Fig. 4 gives a résumé of a typical experiment. It will be seen from Fig. 4 that the p_{H} of the blood and the alveolar CO_2 are changing continuously throughout the period of the observations. The values of p_{K_1} have therefore been calculated by interpolation from charts of the experiments, as it was impossible to estimate all three factors simultaneously. The results of a number of experiments are shown in Table II.

Table II.

Date (1927)	Duration of experi- ment (mins.)	p_{H} (mean)	Alveolar CO_2 %	CO_2 , vols. % dissolved	Plasma bicarbonate vols. % CO_2	p_{K_1}
7. iv.	14	7.845	2.65	1.45	36	6.231
10. iv.	20	7.830	2.48	1.37	40	6.150
15. iv.	10	7.771	2.80	1.55	35	6.200
15. iv.	15	7.815	2.60	1.42	34.5	6.210
15. iv.	20	7.860	2.40	1.30	34	6.223
15. iv.	23	7.862	2.24	1.23	34	6.200
15. iv.	10	7.775	3.00	1.65	35	6.229
15. iv.	15	7.790	2.75	1.50	35	6.202
						Mean = 6.213

DISCUSSION OF RESULTS.

The experimental error with micro-methods is necessarily high, and when a constant depends on the value of three different micro-estimations, the errors of which may be cumulative, the results cannot agree very closely. The experimental error on the p_{H} observations was probably not more than ± 0.02 , on the alveolar $\text{CO}_2 \pm 2.5$ %, on the bicarbonate titration ± 1.5 %. If in any experiment a summation of errors occurred, the variation of the constant might be ± 0.08 on p_{K_1} . The mean of a number of experiments should, however, give a fair approximation to that constant. The value 6.24 obtained for p_{K_1} at 38° under normal conditions is higher than the values obtained for human blood by Warburg, 6.18, or by Parsons, 6.19 [Warburg, 1922]. These observers did not, however, add any fluoride to prevent glycolysis, and their experiments frequently lasted for 24 hours or more.

On the other hand, our bicarbonate estimations are probably slightly too low, as the solid potassium oxalate used raised the tonicity of the plasma and abstracted water from the corpuscles. The recent experiments of Eisenman [1927] would suggest that the error from this cause was not more than 1 %. Further, owing to the conditions of our experiments we could take only one sample of alveolar air, and we chose the expiratory sample.

Fig. 4. The p_H and bicarbonate of the plasma and the alveolar CO_2 during forced breathing.

• — • — • p_H of blood. ○ — — — — ○ Percentage of CO_2 in alveolar air.
— — — — Bicarbonate of plasma.

Moreover, the correction factor of 0.22, which we have used for calculating the p_H at 38° from the observed value at 18° may be too low. Marrack and Thacker [1926], with a number of different bloods at the two temperatures, found a mean value of 0.247 for this correction factor.

These four considerations would all have the effect of making our value for p_{K_1} higher than that of previous observers.

The constants we obtained under normal conditions and during forced breathing are, however, comparable, and do not differ more than can be accounted for by the experimental error. We conclude therefore that in hyperpnoea carried out within 2 hours after a meal the rise in the p_H of the blood corresponds to the alteration found in the concentration of bicarbonate in the plasma and in the tension of CO_2 in the alveoli respectively.

The true nature of the "alkalosis" produced in forced breathing has been pointed out by Grant and Goldman [1920] who drew attention to the fact that, although the p_H of the blood was raised, the bicarbonate of the plasma was considerably reduced, their experiments showing an average reduction of 14 % less total CO_2 . It would appear that the rise in p_H protects the body from loss of bicarbonate.

A slight definite diminution in the amount of base in the body does, however, occur during forced breathing, as evidenced by the amount of bicarbonate excreted in the urine. In Haldane's experiments this was 0.557 g., in ours 0.357 g. The loss of this quantity of alkali from the whole body could not, however, be detected by Haldane in the CO_2 -absorption curve of the blood, nor by us in the titration value of the bicarbonate of the plasma.

SUMMARY.

1. Repeated observations on the hydrogen ion concentration of the blood during forced breathing have shown that the p_H rises, rapidly reaching a maximal increase of 0.20 to 0.24 in 10 to 15 minutes. When forced breathing is stopped the p_H falls even more rapidly, returning to normal within 5 minutes.

2. Simultaneous observations on the p_H and the bicarbonate of the plasma and on the alveolar CO_2 under normal conditions and during forced breathing have been made, and show that the rise in the p_H during forced breathing corresponds to the alteration found in the concentration of the bicarbonate of the plasma and the tension of CO_2 in the alveoli respectively.

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