

heart rate is recorded (Figure 1). When facing a certain direction, the animal gets a weak electrical shock. If the bird is able to determine this direction, its heart rate increases in anticipation of the shock, and there is a maximum at this angle even if no shock at all is applied (Figure 2). By this means it is possible to show whether a certain stimulus situation is appropriate to establish a conditioned reaction of this kind (Figure 3). It is assumed

that this method can easily be adapted to investigate other problems of perception and learning.

H. G. WALLRAFF und N. KLEBER

*Max-Planck-Institut für Verhaltensphysiologie,
Abt. Mittelstaedt, 8131 Seewiesen über Starnberg
(Deutschland), 20. Oktober 1966.*

Acid-Base Equilibrium in the Heart-Lung Preparation¹

The Starling heart-lung preparation has frequently been used by physiologists and pharmacologists, who have considered it an adequate means for studying diverse parameters of the isolated heart^{2,3} and the relationship between acid-base variations and myocardial function^{4,5}. However, few studies concerning the blood acid-base state of the preparation have been undertaken when it is performed with the usual technique⁶⁻⁸. When controls of the acid-base situation were carried out in our laboratory, the blood of the preparation presented a respiratory alkalosis together with a metabolic acidosis. It was evident that the ventilation employed was excessive, leading to a relative hyperventilation and to hypocapnia. The reasons for the development of the metabolic acidosis were much less evident, and the following sources of metabolic acidosis were then analysed: (1) hyperventilation of the animal to be used in the preparation prior to the isolation of the heart; (2) bleeding of the donor dogs; and (3) changes in the collected blood during the time elapsed between the bleeding of the donor dogs and the set-up of the preparation.

Methods. The Starling heart-lung preparation was performed following the classic technique⁷. Blood used in the preparation was obtained from donor dogs, which were bled through a cannula placed in the femoral artery. In 12 experiments donor animals were bled to death; a sample of arterial blood was obtained prior to the start of bleeding and another from the total amount of the blood withdrawn. In 4 of these experiments additional samples were taken during hemorrhage in order to follow variations in base excess (BE) value. 3 additional experiments were performed in which several donor animals were used to obtain the necessary blood volume. These animals were not bled to death, but a quantity of blood equivalent to 1.5% of body weight was withdrawn from each and then analysed.

In all cases, heparin was used as anticoagulant and the blood was stored under aerobic conditions at a constant temperature of 40°C for approximately 1 h. A sample of arterial blood was obtained immediately prior to thoracotomy of the dog to be utilized in the preparation, followed by ventilation of the dog with air or pure oxygen. Another sample was obtained just prior to the isolation of the heart; the difference observed between these two samples was considered as being representative of the changes that took place in the 10 min period of hyperventilation prior to the isolation of the heart.

In 5 experiments, the preparation was performed under the usual conditions; but after a 10 min period, the acid-base equilibrium was corrected by the addition of sodium

bicarbonate to the blood and by ventilation with a mixture of CO₂ and O₂. Blood samples were obtained at 10, 20 and 30 min after the correction. The hemodynamic parameters (mean aortic pressure, left atrial pressure, cardiac output and coronary blood flow) were determined with mercury and water manometers and by timed collection.

In all the arterial blood samples, CO₂ and O₂ content was determined by the manometric method of VAN SLYKE and NEILL⁹. Arterial blood pH was determined anaerobically at 37°C with a model G Beckman pH meter.

Partial pressure of CO₂ in arterial blood (PCO₂) was computed from the Henderson-Hasselbach equation. These values were then used for the determination of BE by means of the SIGAARD ANDERSEN alignment nomogram¹⁰. Lactate determinations were performed by the method of BARKER and SUMMERSON¹¹. Results are expressed as the mean $\pm$ S.E. of the mean.

Results and discussion. In 8 experiments with massive bleeding of the donor animals and ventilation with air or oxygen, the acid-base equilibrium of the circuit blood presented the following values: pH 7.68 ± 0.058 ; PCO₂ 7 ± 1.5 mm Hg; BE -10 ± 0.9 mEq/l, showing the respiratory alkalosis already mentioned together with a metabolic acidosis.

Several possible mechanisms could account for the striking rise in acid metabolites in our preparations. The first to be investigated was the influence of the respiratory alkalosis of the dog ventilated at positive pressure with air or oxygen for an average time of 10 min prior to the isolation of the heart. It has been proved that respiratory alkalosis may originate a compensatory metabolic acidosis¹² due mainly to lactic acid production, even though no significant differences were found. We have thus to consider the possibility that the time of hyperventilation necessary to evoke the metabolic compensation is longer than that used in our experiments.

¹ This work has been supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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A second possible cause of the negative BE values was the bleeding to death of the donor dogs. The mean blood BE values of donor animals before being bled (-3 ± 0.5) is significantly different from that of the sample representative of the total volume of the blood withdrawn (-7 ± 1 ; $P < 0.01$; Table I). The Figure shows a continuous increment of the negative BE value as bleeding progresses. On the other hand, partial bleeding not greater than 1.5% of the body weight was carried out in 3 experiments resulting in blood BE values within normal range (Table II).

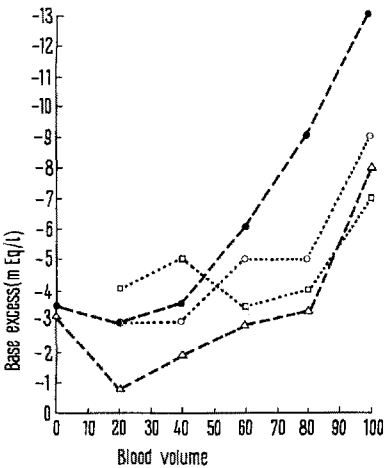
It is evident that bleeding causes a release of acid metabolites to the blood, possibly through an epinephrine release evoked by hemorrhage, producing muscle glycogen breakdown with formation of lactic and piruvic acids¹³⁻¹⁵. Greater production of these acids would be further enhanced by anoxia due to bleeding. In 2 additional experiments, the variation in arterial blood lactate concentration was determined as hemorrhage progressed; a continuous increase was seen reaching a maximum value of 6 mM/l in the last sample.

The last possibility to be evaluated was the influence of the time elapsed between the withdrawal of blood from the donor dog and its use in the preparation^{16,17}. Blood BE value varied significantly during this period. An initial BE value of -7 ± 1.0 and a final one of -9 ± 1.1 showed an increase in acid metabolites obviously due to spontaneous glycolysis taking place (Table I, 1 and 2).

These findings show that a way to obtain a preparation with blood acid-base conditions approaching physiological ones is to avoid the bleeding to death of the donor dogs, and to decrease the time lapse between the blood collection and the set-up of the preparation. Thus, in 3 experiments in which blood was obtained by partial bleeding of several donor dogs, the conservation period

was decreased to less than 30 min and ventilation was always maintained with 95.5% O₂ and 4.5% CO₂, it was possible to obtain a normal preparation as regards the acid-base equilibrium.

It was also possible to obtain a preparation with acid-base conditions approaching physiological ones by the addition of an adequate quantity of sodium bicarbonate and ventilation with 95.5% O₂ and 4.5% CO₂. This correction was performed in 5 experiments in which the following mean values were obtained: pH 7.80 ± 0.02 ; PCO₂ 3 ± 0.2 mm Hg; BE -11 ± 1.0 mEq/l before the correction, and 7.4 ± 0.03 , 38 ± 2.7 and -1 ± 1.3 mEq/l respectively afterwards. Normalization by this method was followed by minimal and transitory hemodynamic changes, either in cardiac output, left atrial pressure or coronary blood flow.



In 4 animals bled to death, the volume of blood withdrawn is represented on the abscissa as % of the total. Base excess (BE) values are represented on the ordinate. A continuous increase of the BE values may be seen, especially in the last 3 samples.

Table I. Blood base excess values* during the different experimental steps

Experi- ment No.	1	2	3	4
1	-4	-6	-12	-13
2	-3	-8	-9	-11
3	-1	-2	-5	-6
4	-2	-4	-7	-8
5	-5	-9	-10	-14
6	-2	-7	-10	-8
7	-4	-10	-14	-8
8	-3	-9	-6	-11
Mean $\pm$ S.E.	-3 ± 0.5	-7 ± 1.0	-9 ± 1.1	-10 ± 0.9

* Expressed as mEq/l. (1) Blood of the donor animal before bleeding. (2) Blood of the donor animal immediately after bleeding. (3) Blood of the donor animal after 1 h at 40°C. (4) During functioning of the preparation.

Table II. Blood base excess values after withdrawing blood to the value of 1.5% of the body weight per animal

Experi- ment No.	Dog No.					Mean
	1	2	3	4	5	
1	- 4	0	- 1	+ 3	- 1	- 1
2	- 3	- 1	- 2	- 3	- 2	- 2
3	0	+ 1	- 3	- 8	- 2	- 2

Résumé. Etude de l'équilibre acide-basique de la préparation cœur-poumon de l'étourneau, réalisée avec la technique habituelle et les moyens de corriger les altérations constatées. La recherche des causes de ces altérations a permis de conclure que l'alcalose respiratoire est due à la ventilation dont on se sert habituellement, tandis que l'acidose métabolique est due à une production de métabolites acides déclanchés surtout par la saignée des animaux donneurs et par la glucolyse qui se produit à partir de l'extraction du sang et jusqu'au moment de son emploi dans la préparation.

MARÍA N. F. DE LEW, H. CINGOLANI,
N. GONZALEZ, E. S. BLESÁ
and J. C. MARSIGLIA

Instituto de Fisiología, Universidad Nacional de La Plata (Argentina), 3rd October 1966.

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