Physiology. *"On the reaction course of physiological buffermixtures, examined by direct registration of the P_H changes"*. By Prof. F. J. J. BUYTENDIJK and R. BRINKMAN. (Communicated by Prof. H. ZWAARDEMAKER).

(Communicated at the meeting of February 27, 1926).

In the field of study of the biological neutrality regulation, in spite of the steady increase in detail, the most important problem is still the properties of the buffer-system as formed by the carbonic acid and its salts, the phosphates, the oxy-haemoglobin and perhaps by other weak acids. These systems have been so exhaustively treated in the well-known researches of L. HENDERSON, SPIRO, SÖRENSEN, HASSELBACH, MICHAELIS, WARBURG, VAN SLYKE and many others, and their study has given us such a deep insight into the mechanism of the biological regulation of the acidity, that we have an impression of the comparative completeness of our knowledge. The reaction of the respiratory centrum, of the function of the kidneys and other P_H regulating organs on the change in concentration of the buffer components may still be frequently less clear, but there is evidently little to add to our physico-chemical knowledge of the central buffer-systems, at least of the carbonic acid-bicarbonate and of the phosphate system.

The aim of this communication is to draw attention to the changes which *occur in a bicarbonate and phosphate system before an equilibrium is attained.*

It was obvious that the chemical examination of the neutrality regulation, as a typical application of the law of mass-action with ion-reactions, showed only the equilibrium conditions but not the course of the reaction. And yet there is, e.g. in the properties of the carbonic acid certainly a reason to examine this course of reaction more in detail, since the non-dissociated H_2CO_3, according to THIEL and STROHECKER, may be present for a short time after its formation as the strong oxy-formic acid, and in that time it may penetrate into cells by its very strong solubility and permeability. Thus for these reasons, besides by their difference in buffering action, the systems

$$\frac{[H_2CO_3]}{[Na\,HCO_3]} \text{ and } \frac{2\,[H_2CO_3]}{2\,[Na\,HCO]_3}$$

are by no means equal in their biological effect.

Thus, although the system $HCO'_3 + H \rightarrow H_2CO_3 \rightleftarrows CO_2 + H_2O$. or perhaps $HCO'_3 \leftrightarrows CO_2 + OH'$ is exactly known in its state of equilibrium, the time required for the accomplishment of this equilibrium is so long

that, for instance, the strong momentary H_2CO_3 might have a great biological influence. Of the duration of its existence, however, very little is known.

On the other hand, COLLINGWOOD[1]) has pointed out the phenomenon that when a current of gaseous CO_2 be passed 2—3 seconds through a "weak alkaline" solution and all the CO_2 above the solution carefully blown off, it may take as long as 30 seconds before the addition of phenol red as indicator will show a change to a more acid reaction. He concluded that if CO_2, formed in the tissues, were to be transported by the blood as HCO'_3 the formation time for this ion to be produced ($CO_2 + OH' = HCO'_3$) is much to long to ensure a sufficiently rapid transport of CO_2, so that there must be still unknown factors in this mechanism.

An indication that in the formation of the bicarbonate-carbonic acid equilibrium intermediate reactions may occur, whereby the time factor biologically becomes important in this equilibrium, is found in a simple experiment, part of which is described by MICHAELIS: Add to a solution of 0.1 N Na HCO_3 a little neutral red, which is yellow in alkali-sol.; add enough 0.1 N HCl to turn the colour red after mixing. *After a few seconds* the colour will change back to yellow; if more HCl be added the change to yellow-red-yellow will ensue.

Loss of CO_2 is not the cause of this phenomenon which, according to THIEL and STROHECKER, depends upon the relatively smaller velocity of the reaction $H_2CO_3 \rightarrow H_2O + CO_2$.

There is, however, a similar experiment of which we find no mention. If we take again 0.1 N $NaHCO_3$, add to it phenol red as indicator and then a little 0.1 N HCl, *the colour change from red to yellow will begin a few seconds after the mixing*, and not suddenly but gradually. And this colour does not change back to the alkaline red tint.

The field of change of phenol red lies between P_H 8.4—7.4 of neutral red between P_H 8.7.

From this simple experiment it may already be assumed that the increase of the hydrogen ion concentration in a bicarbonate sol. to which acid has been added, would, in the vicinity of the neutral reaction, not take place so regularly as might be expected. That the difference in the manner of change is not a peculiarity of both indicators themselves is evident from the fact that in the phosphate solutions of analogous reaction, buffer capacity and molarity, the change takes place *in both always* immediately and definitely.

A further examination of the process of buffer reactions was only possible by a method which directly indicated the momentary [H] with as little latency as possible.

[1]) Proc. Physiol. Soc. July 5th, 1925, Journ. Physiol. 59, XXII, 1924—25.

For this, an ordinary electrometric P_H determination with compensation connections as zero-method is not practicable, but we must be able to follow direct the strenght of the E. M. F. produced between the solution examined and a standard sol., and by preference be able to register it. A galvano-meter method with a lead-off by common Pt. electrodes is not possible owing to polarisation phenomena.

TREADWELL and WEISS [1]) have indeed indicated an H electrode which is nearly non-polarisable and which can sustain a permanent very slight lead-off of current, but this requires a passage of gaseous hydrogen, is very complicated, and acts for our purpose too slowly.

A purely electro-metric method with a binant or quadrant electro-meter as measuring instrument likewise appeared less well adapted, owing to the instrument adjusting slowly, whereby, moreover, it is impossible to register a rapid change of the E. M. F. to be measured in two directions.

Very suitable, however, appeared to us a triode-lamp connection, whereby the E. M. F. as the voltage on the grid influences the discharge current between the filament and the plate, as has been indicated by GOODE [2]). This system is unpolarisable, and the variation of voltage anode involves a change of current which may be multiplied and registered.

Moreover, in order to trace directly the P_H to be measured, a hydrogen-electrode, which was in equilibrium with the free gas, could not be used as electrode, so we employed the homogenous system with a constant hydrogen pressure by the equilibrium chinon $+ H_2 =$ hydrochinon (so-called chinhydronelectrode), and in which the electrode adjusts itself almost instantaneously.

When using the beaker electrode, after MISLOWITZER [3]), in which the conducting and comparison electrodes are combined, blank platinum electrodes are used, and all connecting pieces are avoided. The electrometric determination of [H] is by this method very simple, while in the range of the H-ion concentration, which we will examine, the chinhydronelectrode yields results as exact as the classic H-electrode.

As galvanometer the string-galvanometer was the proper instrument, with which every variation in current could be instantaneously registrated.

Schematically our method was as follows:

The buffer-system to be examined was in the inmost beaker of the chinhydronelectrode. Very vigorous stirring with the Pt-electrode itself ensures as rapid as possible a mixing of the added acid. The E.M.F. to be measured is conducted through a potentiometer where it can be exactly compensated and measured. From this potentiometer the

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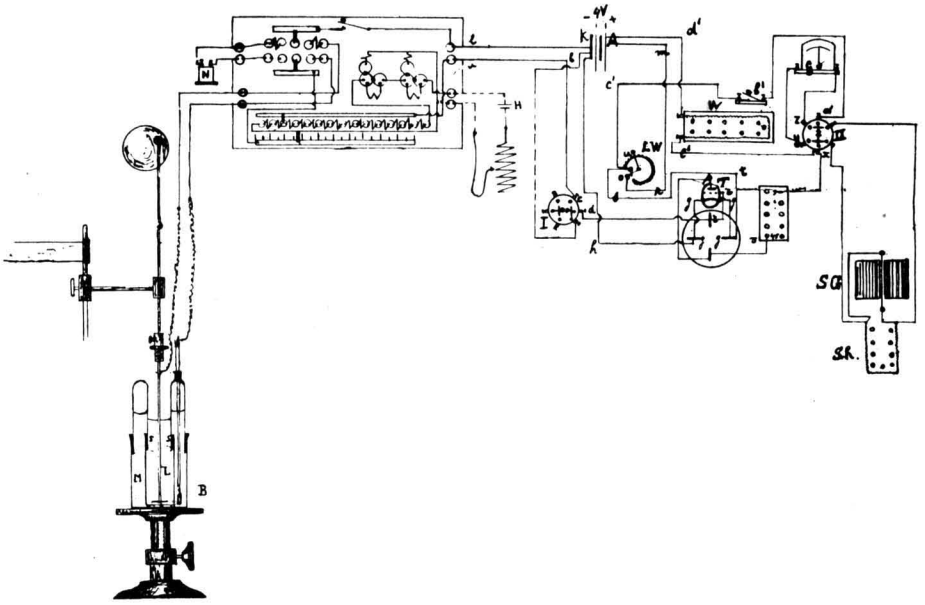
[1]) Helv. Chim. Acta 1, 410 (1919).

[2]) Journ. Amer. Chem. Soc. 1922.

[3]) Biochem. Zeitschr. **159** 58 (1925).

tension arrives at the grid of a triode-lamp, while the variation of the compensated anode-current of this lamp is registered with the string galvanometer.

For the exact description see diagram below:



B is a chinhydron electrode vessel model I, after MISLOWITZER [1]), in which a ground surface S wetted with saturated KCL forms the conductive connection between the standard buffer solution in the outermost beaker (M) and the unknown solution in the inner beaker (L). The outer solution (in M) is a mixture of equal parts of saturated KCL and a standard acetate solution (100 cc. N. NaOH + 200 cc. N. CH_3COOH + + 700 aq. $P_H = 4.62$). Both liquids are saturated with chinhydron.

For a rapid registration of the effect of an acid addition on the P_H vigorous stirring is of great importance. We obtained the best results with a disk electrode measuring $^5/_6$ diameter of the inner beaker lumen and moving rapidly up and down, over the whole length of the fluid column with a frequency of about 10 per sec.

The E.M.F. to be measured can be compensated by potentiometer, drawn schematically in the figure above. The compensation was accurate up to $^1/_4$ millivolt. (N standard cell, H additional battery.)

The E.M.F. reaches through the circuit *a b c d r g h k l* the grid of the triode-lamp.

The lamp is a miniwatt lamp B 406, with a plate potential of 36 V. A small Weston needle galvanometer with a sensibility of 22 micro-amp. serves as zero-instrument. After compensation the discharge current could be directed by the POHL's reverser II to the stringgalvanometer SG (with shunt Sh).[2])

The sensitivity of this apparatus easily exceeds 0.02 P_H for a displacement of 1 mM of the string shadow, and might even be increased by using more than one triode lamp.

[1]) l. c.

[2]) Our thanks are due to Mr. HUIZING for his valued explanation.

In the first place now we examined how the P_H varied in the time period immediately after the addition of diluted HCl to a 0.02 N. solution of $NaHCO_3$ and Na_2HPO_4.

The effect of adding separately 1 c.c. 0.04 N. HCl to 40 c.c. buffer mixture (i. e. each time 0.001 N. HCl) proved to be as follows:

With the phosphate solution the calculated P_H was reached immediately, and this P_H remains constant. The speed of the adjustment is entirely dependent upon the speed of stirring, and the phenomenon remains the same in the entire P_H range examined (8.5—6.5). Only the buffer capacity is naturally greatest near the neutral reaction.

Fig. 1 gives an example.

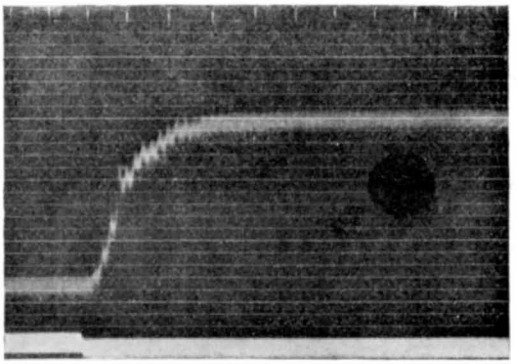


Fig. 1. Addition of diluted HCl to 0.02 N. $Na_2HPO_4 + NaH_2PO_4$. P_H changes from 7.93 to 7.35.
1. Time in secs.
2. String shadow in which stirring is visible.
3. Signal for the acid to be added.

The process in a bicarbonate system was entirely different, as can be seen in Fig. 2.

The variation of P_H thus actually took place as the indicator reactions had led us to suppose; from P_H 8.3 to P_H 7.7 added acid is at once completely buffered, and not until some tenths of seconds later does the reaction reach its equilibrium value.

Between P_H 7.7—7.5 the rise becomes steeper, and with P_H 7.4 a typical „acid summit" is seen, which is very probably caused by the existence of H_2CO_3 during 1—2 seconds.

It is very interesting to note that this variation in the reaction should just occur in the range of blood reaction, where one may expect the one type as well as the other.

We see from this observation thus that the short duration of the strong H_2CO_3 in vivo must certainly be taken into account.

We cannot in this communication go further into this question, but

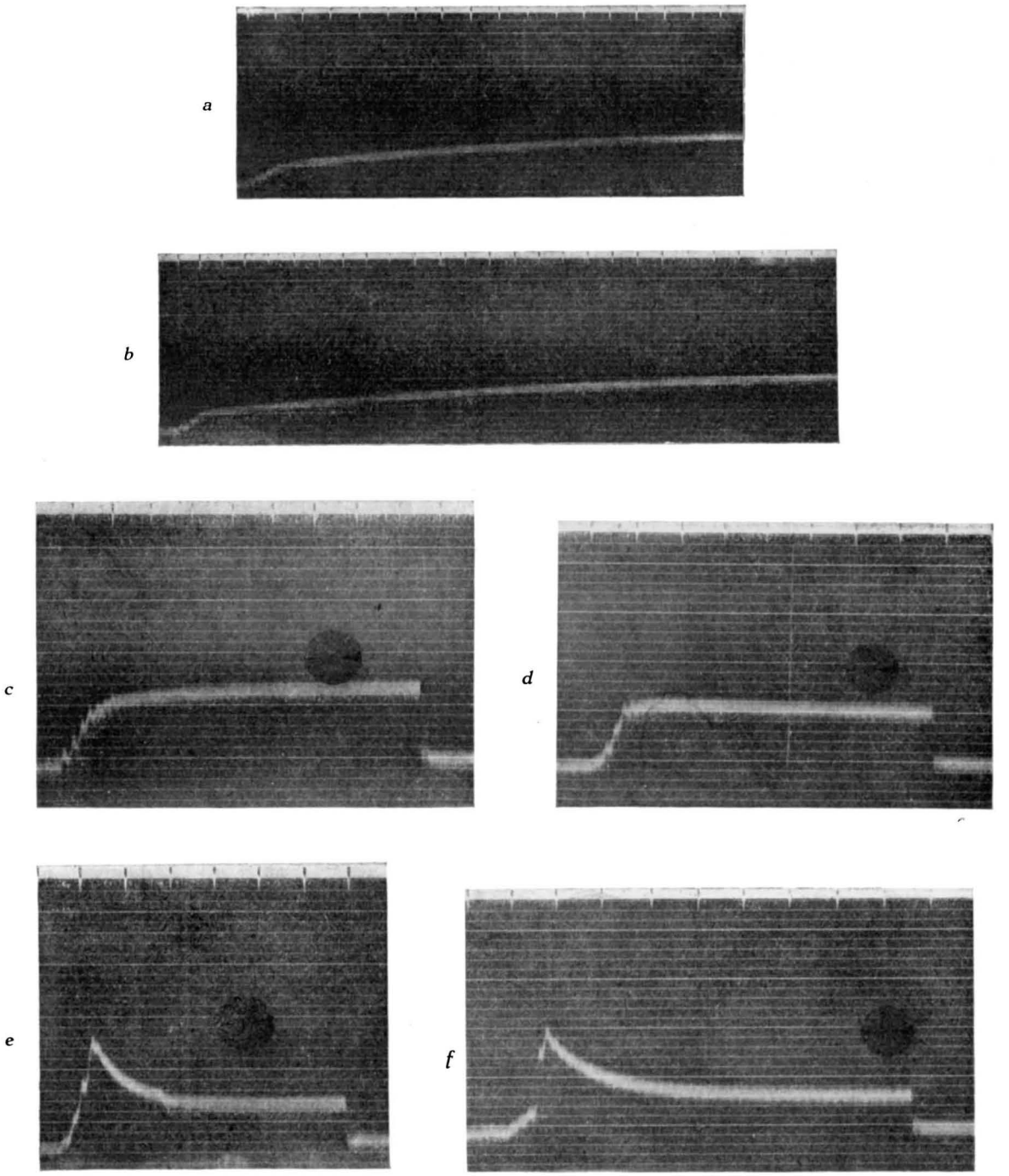


Fig. 2. Successive adding of 0.001 N. HCl to 0.02 N. $NaHCO_3$
Time in seconds.

d 0.004 N. HCl, P_H of 7.58—7.45
e 0.005 N. HCl, P_H of 7.42—7.36
f 0.006 N. HCl, P_H of 7.36—7.28
a 0.001 N. HCl, P_H of 8.31—8.03
b 0.002 N: HCl, P_H of 8.03—7.77
c 0.003 N. HCl, P_H of 7.77—7.58

wish to point out again that with a continued production of acid the H_2CO_3 may be permanently present in a larger concentration if the rapidity of the formation of this acid is at all comparable to the slowness of the CO_2 decomposition.

The actual P_H in an acidotic state can thus not be determined in the usual way, but must be examined in the living blood in the way we have indicated. Then it is possible that a more acid reaction may be found than has hitherto been observed.

Besides in the investigations as to the velocity of the buffer reactions, and in particular as to the significance of free H_2CO_3, we suppose that the method of registration above described may be useful in the study of different problems to which we hope to return later. We have here in view especially the direct registration of blood and tissue-fluid reactions in vivo, the measurement of biological membrane potentials and their change under nerve stimulation, and the direct registration of the carbonic acid percentage of the air exhaled.

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Groningen, February 1926.