# THE REACTION REGULATOR MECHANISM OF THE BLOOD BEFORE AND AFTER HÆMORRHAGE.

By T. H. MILROY.

*(From the Physiological Laboratory, Queen's University of Belfast.)*

CONTENTS.



RECENTLY attention has been directed to the value of various substances as ingredients of fluids suitable for intravenous infusion after loss of blood. Thus Bayliss(1) has shown the value of maintaining the viscosity of the blood by the addition of suitable substances to infusion fluids, and others have adopted the method of direct transfusion of blood so as to restore to the circulation all the constituents which have been lost. As the maintenance of a fairly constant reaction is one of the most important functions of the blood, it is necessary to gain some idea of the nature and degree of the alterations in the reaction of the blood produced by hæmorrhage and also the most suitable means of counteracting such disturbances. Before referring, however, to the effects produced by hæmorrhage, it is necessary to consider somewhat fully the reaction regulator mechanism possessed by the normal blood.

As a ·8–·9 % sodium chloride solution is often employed to replace blood which has been lost, one might consider in the first place how the reaction of such a fluid is altered when it is in equilibrium with carbonic acid at such pressures as it might be exposed to in the circulatory system.

It is better however to consider how such an albuminous fluid as the blood plasma would behave under these conditions, if the plasma contained no substance which could chemically combine with the carbonic acid. The reaction of such a fluid may be stated in terms of the hydrion concentration and the value of the latter may be determined from the following equation of the first dissociation constant of carbonic acid

$$k_1 = \frac{[H^{\cdot}] \times [HCO_3']}{[H_2CO_3]} = \frac{[H^{\cdot}]^2}{[H_2CO_3]} \text{ as } [H^{\cdot}] = [HCO_3'], \text{ or } [H^{\cdot}] = \sqrt{k_1 \times [H_2CO_3]}$$

the $[H_2CO_3]$ being the total $CO_2 + H_2CO_3$ concentration

(where the square brackets denote molar or gram ionic concentrations of the enclosed substances). $k_1$ may be taken at body temperature as having the value $4{\cdot}125 \times 10^{-7}$, as calculated from Kendall's(2) recent determinations of this constant. The average carbonic acid pressure to which the blood is exposed may be taken as 30 mm. From Bohr's values for the absorption coefficient of blood plasma at body temperature and considered simply as a solution containing physically dissolved $CO_2$, one may state that at this pressure the molar concentration of $CO_2$ per litre should be approximately $1 \times 10^{-3}$.

Then $$[H^{\cdot}] = \sqrt{(4{\cdot}125 \times 10^{-7})(1 \times 10^{-3})} = 2{\cdot}031 \times 10^{-5} \text{ N.}$$

or as frequently stated in hydrogen exponent form

$$10^{-4{\cdot}69} \text{ i.e. } p_{H^{\cdot}} = 4{\cdot}69.$$

Under the same conditions of temperature and pressure the true blood plasma has approximately the hydrion concentration of $4{\cdot}5 \times 10^{-8}$ N. or a $p_{H^{\cdot}}$ of 7·34. It is therefore evident that even the very partial replacement of blood by a physiological sodium chloride or a solution of similar concentration containing no reserve alkali for the fixation of carbonic acid must disturb to a serious extent the reaction regulator mechanism of the blood. As the normal blood therefore maintains a fairly constant reaction, always on the alkaline side of neutrality within the range of carbonic acid pressures to which it may be exposed, it must possess some reserve mechanism for the neutralisation of added $H^{\cdot}$ or $OH'$. An ideal solution as regards neutralising power for both acids and alkalies, constituting therefore a protective mechanism against additions of both, would be one which contained a mixture of a weak acid with its salt (or weak base with its salt) the hydrion concentration of which was the same as the ionisation constant of the weak acid. If we consider the mechanism as consisting of the salt of a weak acid BA along with the weak acid HA, we find that the dissociation of the former

into $B^{\cdot}$ and $A'$ takes place to a large extent while that of the acid HA is very much less in degree. There exist therefore a large number of $A^{\cdot}$ from the salt and a small number from the HA so that the undissociated HA is proportionately large to the dissociated. If hydrogen ions be added to such a solution, they combine with the $A'$ to form undissociated HA and if hydroxyl ions be added these unite with the acid to form water and free $A'$. Thus $A' + H^{\cdot} = HA$ and $HA + OH' = H_2O + A^{\cdot}$. It is therefore clear that in order to possess an equal power of neutralising $H^{\cdot}$ and $OH'$ the [HA] and the $[A^{\cdot}]$ must be equal. According to the law of mass action the equation is

$$\frac{[H^{\cdot}][A']}{[HA]} = k \text{ (acid) and therefore } [H^{\cdot}] = \frac{[HA]}{[A']} k.$$

If [HA] and [A'], that is the concentration of the undissociated acid and the anion respectively, be equal to one another, the hydrion concentration of the fluid is equal to the ionisation constant value of the weak acid. It is evident that the $[H^{\cdot}]$ varies directly as the [HA] and inversely as the [A'] so that the addition of $A'$ without raising the HA, which can be conveniently carried out by the addition of an easily dissociated salt of the acid, will give rise to a decrease in the degree of dissociation of the weak acid and hence a fall in the $[H^{\cdot}]$.

The blood plasma reacts in the main as a sodium bicarbonate-carbonic acid system with very small quantities of other salts and proteins which might possibly act as regulators of the reaction. It is necessary therefore to consider the equilibrium conditions in the first place of carbonate, bicarbonate and carbonic acid mixtures. These have recently been dealt with by Auerbach and Pick(3) and Seyler and Lloyd(4).

As carbonic acid in its hydrated form is a dibasic acid, it shows ionisation in two stages, the dissociation of the first $H^{\cdot}$ being much greater than that of the second, and it is with the first constant that one is mainly dealing in the case of the blood. Following the law of mass action, the position of equilibrium is characterised as fulfilling the following conditions

$$\frac{[H^{\cdot}][HCO_3']}{[H_2CO_3]} = k_1, \text{ and } \frac{[H^{\cdot}][CO_3'']}{[HCO_3']} = k_2.$$

These constants $k_1$ and $k_2$ are independent of the concentrations of the participants and are dependent only on the temperature. Both dissociations must lead in a particular solution to the same value of $[H^{\cdot}]$ thus

$$[H^{\cdot}] = k_1 \frac{[H_2CO_3]}{[HCO_3']} \text{ and also } [H^{\cdot}] = k_2 \frac{[HCO_3']}{[CO_3'']}.$$

The former equation is the more important one as regards the blood, where the solution is one which contains free carbonic acid along with bicarbonate. If the carbonic acid pressure be kept constant, an addition of bicarbonate ($HCO_3'$) must give rise to a diminution in the [H'] in order to maintain a constant value of $k_1$. As the blood is continually being exposed to slight variations in carbonic acid pressure, these will give rise to corresponding variations in the concentration of the dissolved carbonic acid, and the disturbances in the hydrion concentration throughout the range of variation in the concentration of $H_2CO_3$ will depend upon the $Na(HCO'_3)$ concentration of the plasma. As has already been stated the ideal regulator mixture is one where the hydrion concentration of the fluid is equal to the dissociation constant of the weak acid, in this case the first dissociation constant of $H_2CO_3$. What relationship then exists between the [H'] of the blood and the value of this constant? It is important to arrive at this knowledge in order to gauge the reserve alkali-acid value of the blood. The average [H'] of the "whole" blood at body temperature may be taken as $4{\cdot}5 \times 10^{-8}$ gm. ion per litre. As regards the first ionisation constant of $H_2CO_3$ at body temperature, one may arrive at a very close approximation from Kendall's recent determinations at 0°, 18° and 25°. As these values lie nearly on a straight line, $3{\cdot}75 \times 10^{-7}$ may be taken as the value at 30° and $4{\cdot}25 \times 10^{-7}$ at 40°, and ·05 per degree within that range. If one then takes the [H'] of the blood as $4{\cdot}5 \times 10^{-8}$ at 37·5°, or the log. of this $\bar{8}{\cdot}6532$ ($p_{H'}$ 7·34), and the first ionisation constant of carbonic acid at this temperature as $4{\cdot}125 \times 10^{-7}$ ($\bar{7}{\cdot}6154$), it is evident that the hydrion concentration value of the blood is only approximately one-tenth of the first ionisation constant value. If the $NaHCO_3$ of the blood were completely dissociated, this would give a ratio of $1H_2CO_3$ to $10HCO'_3$ but as the dissociation even at body temperature and at the comparatively low concentration of the $NaHCO_3$ in the blood is only somewhat over 80 %(5), one may take the ratio of $[H_2CO_3]$ to $[HCO_3']$ as approximately 1 : 12.

If one were dealing with a mixture of carbonate and bi-carbonate, the hydrion concentration of such a mixture, which would furnish an ideal regulator mechanism, might be obtained from the equation of the second ionisation constant,

$$[H'] = k_2 \frac{[HCO_3']}{[CO_3'']}.$$

As the second ionisation constant has an approximate value of $6 \times 10^{-11}$, the [H'] of the mixture would also require to have this

value, in order that the concentrations of the $HCO'_3$ and the $CO_3''$ should be the same, and so furnish a mechanism equally capable of neutralising added hydrion or hydroxyl ion.

Such a "buffer" mixture would of course be much more alkaline than the blood.

## I. *The reaction of bicarbonate solutions.*

Before studying the reaction disturbances produced in the blood by exposing it to gas mixtures with varying pressures of carbonic acid, it was considered advisable to expose bicarbonate solutions of different concentrations to a similar series of gas mixtures and to experimentally determine the disturbances produced in the hydrion concentration. As a certain experimental procedure was adopted in the study of the effects produced by various carbonic acid pressures on the reaction of the blood, a similar method was employed in the case of the bicarbonate mixtures, even although the method might not really be the most satisfactory for pure bicarbonate solutions. For example it might be better in the case of the latter to keep a particular hydrogen carbonic acid mixture bubbling through the bicarbonate solution while the electrometric determination was being actually carried out, but such a procedure is unsatisfactory in the case of blood owing to the inconvenience arising from the frothing of the viscous fluid in a small electrode vessel. Hence the bicarbonate solutions were introduced into a tonometer similar to the one employed by Barcroft(6) and Peters(7) and there exposed at body temperature to the series of hydrogen carbonic acid gas mixtures. 10 c.c. bicarbonate solution were taken in the tonometer filled with the gas mixture, the capacity of the tonometer being 160 c.c. The tonometer was rotated in a large water bath at 38° for fifteen minutes, then a hydrogen electrode was attached to the tonometer, so that the former might be filled with the gas mixture and finally the bicarbonate solution was passed in until rather more than the half of the platinised electrode was immersed in the fluid. A specimen of the gas mixture was taken from the tonometer after the rotation in the bath and the carbonic acid percentage determined. From this, after the necessary corrections for temperature and pressure, the $CO_2$ pressure was calculated.

The electrometric determinations were made at a temperature varying from 37°–38°, as a rule at 37·5°, an oil thermostat with toluene regulator being employed.

A decinormal calomel electrode was employed and a 3·5 normal

KCl solution as the contact fluid. From the electrometric determination the hydrion concentration was calculated, after making the necessary temperature correction for the calomel electrode, the pressure correction for the gas in the hydrogen electrode and the temperature correction for the gas constant (Sörensen)(8).

In Table I the hydrion concentrations of four bicarbonate solutions, after exposure to a series of various carbonic acid pressures, are given. A solution of ·2 molar $NaHCO_3$ (16·802 grams Kahlbaum's sodium bicarbonate puriss. per litre) was made using conductivity water of good quality. From this solution dilutions were obtained by the addition of ·2 molar sodium chloride. The mixtures therefore possessed approximately the same $Na^{\cdot}$ concentration and therefore the $NaHCO_3$ may be regarded as having throughout a more or less constant degree of dissociation. The hydrion concentration is given directly in mol./litre and the carbonic acid in mm. pressure. In Fig. 1, the former is plotted as a function of the latter.

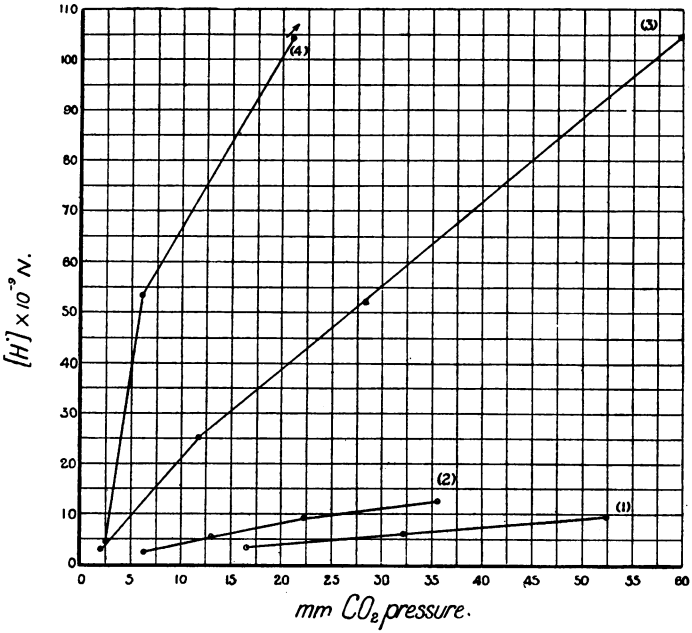


Fig. 1. $NaHCO_3$ solutions. (1) ·2 molar, (2) ·1 molar, (3) ·02 molar, (4) ·01 molar.

It is evident that in the case of both ·2 and ·1 molar $NaHCO_3$ the variations in hydrion concentration produced by raising the $H_2CO_3$

concentration of the fluid are comparatively slight compared to those which occur in the ·02 and ·01 solutions.

TABLE I. *Bicarbonate solutions exposed to various* $CO_2$ *pressures.*

| | Composition of solution | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NaHCO_3$ 0·2 mol. c.c. | NaCl 0·2 mol. c.c. | $NaHCO_3$ mol./litre | $[H^+]\times10^{-9}$ N. | $CO_2$ mm. | $[H^+]\times10^{-9}$ N. | $CO_2$ mm. | $[H^+]\times10^{-9}$ N. | $CO_2$ mm. | $[H^+]\times10^{-9}$ N. | $CO_2$ mm. |
| (1) | 10 | 0 | ·2 | — | — | 3·71 | 16·6 | 5·89 | 32·3 | 9·33 | 52·4 |
| (2) | 5 | 5 | ·1 | 2·50 | 6·2 | 5·50 | 13·2 | 8·91 | 22·2 | 12·50 | 35·6 |
| (3) | 1 | 9 | ·02 | 3·72 | 2·0? | 25·12 | 11·6 | 51·29 | 28·2 | 104·7 | 59·6 |
| (4) | ·5 | 9·5 | ·01 | 4·26 | 2·5? | 53·50 | 5·8 | 104·5 | 20·8 | 240 | 57·4 |

When blood plasma is studied in the same way it is found to behave in a similar manner to ·02 molar $NaHCO_3$, as will be seen from the plasma determinations to be described later.

In the study of the reaction regulator mechanism of the blood by the method employed for the bicarbonate solutions, it is important to treat the plasma and cellular elements separately, because, as will be shown, the former behaves in a different manner from the latter and any disintegration of the red blood corpuscles, such as is found to occur in the treatment of the whole blood at body temperature with the various gas mixtures, gives rise to disturbances in the reaction of the fluid apart altogether from those produced by variations in the concentration of the dissolved carbonic acid.

The blood plasma will therefore be dealt with in the first place, then a fluid similar to the plasma but only containing traces of protein, namely cerebro-spinal fluid, and in the last place the laked cellular deposit from the blood.

## II. *The blood plasma before hæmorrhage.*

In each case the blood was received directly into centrifuge tubes containing sufficient hirudin to prevent coagulation. This addition does not affect the reaction of the fluid. The blood was then centrifugalised and the plasma removed. With as little delay as possible specimens of the plasma were placed in tonometers, evacuated and exposed to pure hydrogen, and then to three mixtures of hydrogen and carbonic acid, the latter varying from about 10–60 mm. pressure. The capacity of the tonometers was 160 c.c. and the volume of plasma 10 c.c. The tonometers were kept rotating at 38° in the water bath

for about fifteen minutes and then the hydrion concentrations determined in the usual way. Practically constant readings of the E.M.F. of the electrode system were obtained usually about half an hour after being placed in the thermostat.

Determinations were made in the blood of man, rabbit, cat and dog, but as the disturbances produced by hæmorrhage were only studied in the dog and cat, the values obtained for these alone will be given in this communication.

Table II gives the results obtained in the case of a dog, in which the disturbances produced by hæmorrhage were also studied; the hydrion concentrations are also given in logarithmic form ($p_{H^\cdot}$).

TABLE II. *Dog's blood plasma.*

| | [H·]×10⁻⁸ N. | $p_{H^\cdot}$ | mm. $CO_2$ pressure in tonometer |
|---|---|---|---|
| 1. Exposed to hydrogen ... ... | 1·05 | 7·98 | 4·5 |
| 2. Exposed to 1st $CO_2$–$H_2$ gas mixture | 3·09 | 7·51 | 15·73 |
| 3. Exposed to 2nd gas mixture ... | 5·25 | 7·28 | 25·0 |
| 4. Exposed to 3rd gas mixture ... ... | 10·23 | 6·99 | 61·87 |

In Fig. 2, the [H·] is plotted as a function of the carbonic acid pressure.

On comparing these results with the values obtained for ·02 molar $NaHCO_3$, one may readily derive from the graph (Fig. 1) the hydrion concentrations of the bicarbonate solution (·02 molar) at the $CO_2$ pressures to which the blood plasma was exposed. I shall take the three $H_2$ and $CO_2$ gas mixtures, placing alongside for comparison the $NaHCO_3$ and blood plasma [H·] values.

TABLE III. *Comparison between plasma and* ·02 *molar* $NaHCO_3$.

| | [H·]×10⁻⁸ N. | |
|---|---|---|
| $CO_2$ pressure | Blood plasma | $NaHCO_3$ ·02 mol. |
| 15·73 mm. | 3·09 | 3·25 |
| 25·0 | 5·25 | 4·9 |
| 61·87 | 10·23 | 10·8 |

At very low $CO_2$ pressures (see Table I) hydrolysis is greater in the case of the bicarbonate solution than in the blood, but within the range of pressures to which the blood is normally exposed the two fluids behave practically in the same way.

The blood plasma of the cat was treated in the same way and the hydrion concentration displacements produced by variations in the carbonic acid pressure determined (Table IV).

TABLE IV.

| | $[H^{\cdot}]\times10^{-8}$ N. | $p_{H^{\cdot}}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| *Blood plasma of cat* (A). | | | |
| 1. Exposed to hydrogen ... | 1·66 | 7·78 | 4·35 mm. |
| 2. Exposed to 1st gas mixture | 6·16 | 7·21 | 26·84 |
| 3. Exposed to 2nd gas mixture | 11·22 | 6·95 | 49·64 |
| 4. Exposed to 3rd gas mixture | 13·80 | 6·86 | 63·84 |
| *Blood plasma of cat* (B). | | | |
| 1. Exposed to hydrogen ... | 0·74 | 8·13 | 4·5 mm. |
| 2. Exposed to 1st gas mixture | 3·02 | 7·52 | 15·17 |
| 3. Exposed to 2nd gas mixture | 5·58 | 7·23 | 27·12 |
| 4. Exposed to 3rd gas mixture | 13·80 | 6·86 | 69·57 |

These two specimens of the blood plasma of the cat show greater displacements in hydrion concentrations with increasing carbonic acid pressure than the corresponding plasma of the dog (Fig. 2). That is to say the $HCO'_3$ concentration of the dog's blood plasma has been higher than in the other two specimens.

Although blood plasma behaves in much the same way as a ·02 molar aqueous $NaHCO_3$ solution, there are undoubted differences between the two, as for example the greater hydrolysis of the latter at low $CO_2$ pressures. When we compare the chemical composition of the two fluids it is most probable that the high protein content of the plasma constitutes the most important differentiating factor. It was therefore deemed advisable to study the behaviour of one of the body fluids possessing an extremely low protein content, namely cerebro-spinal fluid.

I obtained therefore a small specimen of normal cerebro-spinal fluid of man, and tested it in the same way as the bicarbonate solutions and the plasma.

### III. *The cerebro-spinal fluid.*

Hurwitz and Tranter(9) have recently determined the hydrion concentration of cerebro-spinal fluid obtained by lumbar puncture. They made use of the colorimetric method, sulphophenolphthalein being the indicator. They found the $p_H$ to vary from 8·15 to 8·3 and therefore regarded it as distinctly more alkaline than blood. As will be shown this is undoubtedly true at low carbonic acid pressures.

The specimen which I examined contained only traces of protein, less than ·15 %. The results are given in Table V.

TABLE V. *Cerebro-spinal fluid (human).*

| | [H·]×10⁻⁸ N. | $p_{H·}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| 1. Exposed to hydrogen ... | 0·46 | 8·33 | 2·26 mm. |
| 2. Exposed to 1st gas mixture | 5·75 | 7·24 | 29·32 |
| 3. Exposed to 2nd gas mixture | 10·23 | 6·99 | 53·4 |

On comparing the graph of the hydrion displacements of cerebro-spinal fluid with those obtained for the plasma of the dog and cat, it is evident that this fluid lies about midway between the dog's and cat's plasma (Fig. 2). The hydrion concentration of the fluid, when exposed to hydrogen, corresponds very closely to that possessed by a pure bicarbonate solution as the hydrolysis is greater than in the case of the plasma specimens. This is most probably due to the extremely low protein content of the cerebro-spinal fluid. The $p_{H·}$ at low $CO_2$ pressures is therefore higher, in other words the alkalinity is greater, than that of blood plasma.

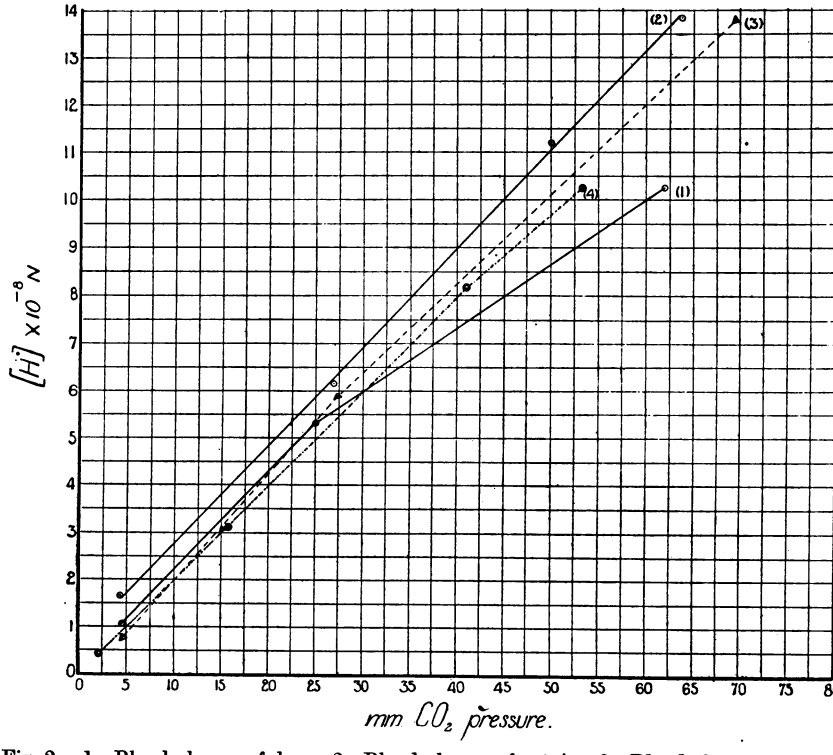


Fig. 2. 1. Blood plasma of dog. 2. Blood plasma of cat A. 3. Blood plasma of cat B. 4. Cerebro-spinal fluid of man.

## IV. *The cellular elements of the blood.*

It has been shown that the blood plasma behaves as a reaction regulator mechanism in a similar way to a ·02 molar bicarbonate solution, and one is now led to consider the behaviour of the contents of the cellular elements when treated in the same way as the plasma to varying $CO_2$ pressures. To the cellular deposit obtained after centrifugalisation of the dog's blood distilled water containing a small quantity of ether was added until the volume was that of the original blood specimen from which the deposit was obtained. The mixture was thoroughly shaken, kept in the ice-chest until the following day and then passed through two or three layers of fine muslin. The solution contained also finely suspended material and had a protein content of about 11·8 %.

The disturbances produced in the hydrion concentration of the blood cells of the dog and cat by increasing carbonic acid pressures are shown in Table VI and Fig. 6.

TABLE VI. *The laked blood corpuscles.*

| | [H·]×10⁻⁷ N. | $p_{H}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| *Dog's blood corpuscles.* | | | |
| 1. Exposed to hydrogen ... | 1·17 | 6·93 | 6·08 mm. |
| 2. Exposed to 1st gas mixture | 1·74 | 6·76 | 13·77 |
| 3. Exposed to 2nd gas mixture | 2·45 | 6·61 | 22·23 |
| 4. Exposed to 3rd gas mixture | 2·51 | 6·60 | 49·01 |
| *Cat's blood corpuscles.* | | | |
| 1. Exposed to hydrogen ... | 1·41 | 6·85 | 0·7 |
| 2. Exposed to 1st gas mixture | 2·51 | 6·60 | 22·86 |
| 3. Exposed to 2nd gas mixture | 3·31 | 6·48 | 55·93 |

It is in the first place quite evident that the reaction of the cellular elements of the blood is very different from that of the plasma. Even when exposed to pure hydrogen the reaction is practically neutral. Attention has already been directed to the difference in reaction of the tissue constituents from that of the blood by Hasselbalch(10), Michaelis and Dawidoff(11), Konikoff(12) and most recently by Michaelis and Kramaztyk(13). In the first three of these communications attention was directed to the effects of partial laking of the red cells on the reaction of the "whole" blood. A more or less distinct decrease in alkalinity being the constant accompaniment of hæmolysis. In the last mentioned communication the reaction of voluntary and cardiac muscle, liver, kidneys and pancreas was determined electrometrically and was found to range from $p_{H}$ 6·78 to

6·40 in the fresh condition and from 7·04 to 6·82 on extraction of the tissues with boiling water. It is also evident that the cellular constituents, when exposed to varying carbonic acid pressures behave in

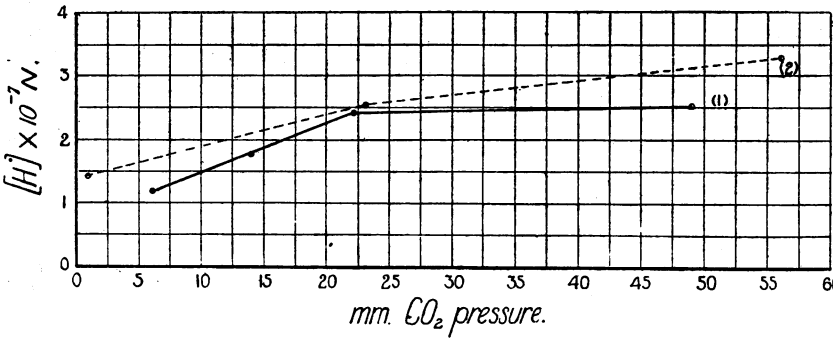


Fig. 3. 1. Laked cellular deposit of dog. 2. Laked cellular deposit of cat.

a different way from the blood plasma. The variations in hydrion concentration at the higher carbonic acid pressures are comparatively slight (Fig. 3).

At the seat therefore of the formation of the acid products of tissue metabolism, the reaction of the medium is much less alkaline than that of the transport medium, the blood plasma, and the changes in reaction produced by an increase in the $H_2CO_3$ concentration are less evident than in the case of the latter.

## V. *The plasma and corpuscles after dialysis.*

It is evident that the reaction of the plasma is dependent upon the bicarbonate-carbonic acid ratio, but it is of interest to determine the reaction of plasma and blood cells after removal of the dialysable salts. The dog's blood plasma was subjected to dialysis for 48 hours in celloidin tubes*, made according to the directions of Walpole(14). To the dialysed plasma sodium chloride solution was added to dissolve the separated proteins. The sodium chloride concentration of the plasma was ·1 molar and the protein approximately 2 %. To prevent decomposition during dialysis a small quantity of toluene was added. In making the electrometric determinations of the dialysed plasma, it was soon evident that the series required to be examined without delay

* I beg to express my sincere thanks to Dr Dale for kindly supplying me with the celloidin solution.

as there were distinct changes in reaction on standing, while increasing carbonic acid concentrations produced only very slight disturbances in the hydrion concentration. The dialysed cat's plasma prepared in the same way was also examined.

TABLE VII. *The dialysed blood plasma.*

| | [H·]×10⁻⁶ N. | $p_{H·}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| *Dialysed dog's plasma.* | | | |
| 1. Exposed to hydrogen ... | 1·32 | 5·88 | 0 mm. |
| 2. Exposed to 1st gas mixture | 1·41 | 5·85 | 16·5 |
| 3. Exposed to 2nd gas mixture | 1·41 | 5·85 | 25·7 |
| 4. Exposed to 3rd gas mixture | 1·44 | 5·84 | 57·9 |
| *Dialysed cat's plasma.* | | | |
| 1. Exposed to hydrogen ... | 3·38 | 5·47 | 0 |
| 2. Exposed to 1st gas mixture | 3·98 | 5·40 | 31·6 |
| 3. Exposed to 2nd gas mixture | 5·01 | 5·30 | 55·5 |

It is evident that a sodium chloride solution of the dialysed proteins of blood plasma has a distinctly acid reaction and therefore differs greatly from the normal plasma. The alterations in hydrion concentration produced by variations in the $CO_2$ pressure are very slight. On the other hand when the laked solution of the corpuscles is dialysed and examined it is found to differ only very slightly from the undialysed material. It is only necessary to give one example of the dialysed cellular deposit and so I shall take that obtained from the dog's blood. The cellular deposit was the same one as had already been examined before dialysis. Dialysis was continued for a week, sodium chloride being added in small quantities to keep the proteins in solution. The protein content of the solution was 10·8 %, but a portion of the nitrogen was in the form of finely suspended material in the fluid.

TABLE VIII. *Dialysed dog's blood corpuscles.*

| | [H·]×10⁻⁷ N. | $p_{H·}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| 1 Exposed to hydrogen ... | 1·17 | 6·93 | 6·60 mm. |
| 2. Exposed to 1st gas mixture | 1·38 | 6·86 | 13·98 |
| 3. Exposed to 2nd gas mixture | 1·66 | 6·78 | 22·37 |
| 4. Exposed to 3rd gas mixture | 2·24 | 6·65 | 47·6 |

It is evident in the first place that the reaction of the dialysed cell contents is practically neutral and therefore they may be regarded as almost identical with the undialysed cell contents. When exposed to the series of carbonic acid gas mixtures, they show also much the same

degree of disturbance in the hydrion concentration. It appears therefore probable that the regulation of the reaction of the cell contents is mainly dependent upon the proteins in whatever form of combination these may exist within the cell.

Before leaving the subject of the reaction of the dialysed plasma and cells, it is of interest to consider the effect produced upon the reaction of the dialysed plasma by the addition of $NaHCO_3$. To the dialysed plasma $NaHCO_3$ and NaCl were added, the concentration of the former in the plasma being ·02 molar and of the latter ·1 molar. The protein content was 1·98 %. This fluid therefore differed from ordinary blood plasma in the low protein content and in the absence of dialysable salts other than sodium bicarbonate and sodium chloride.

TABLE IX.

| *Dialysed dog's plasma with* $NaHCO_3$ *added (conc.* $NaHCO_3$ = ·02 *molar)* | $[H^{\cdot}]\times10^{-8}$ N. | $p_{H^{\cdot}}$ | $CO_2$ pressure in tonometer |
|---|---|---|---|
| 1. Exposed to hydrogen ... | 2·69 | 7·57 | 11·5 mm. |
| 2. Exposed to 1st gas mixture | 3·38 | 7·47 | 15·4 |
| 3. Exposed to 2nd gas mixture | 4·90 | 7·31 | 27·0 |
| 4. Exposed to 3rd gas mixture | 10·23 | 6·99 | 55·0 |
| *Dialysed cat's plasma with* $NaHCO_3$ *added (conc.* $NaHCO_3$ = ·02 *molar)* | | | |
| 1. Exposed to hydrogen ... | 1·04 | 7·98 | 5 |
| 2. Exposed to 1st gas mixture | 4·26 | 7·37 | 18·2 |
| 3. Exposed to 2nd gas mixture | 6·31 | 7·20 | 30·4 |
| 4. Exposed to 3rd gas mixture | 10·72 | 6·97 | 54·0 |

In Fig. 4. the hydrion values of these two specimens are plotted as functions of the $CO_2$ pressure and for comparison the graphs of the ·02 molar $NaHCO_3$ and of the normal dog's plasma before dialysis are also given.

It is evident that the dialysed blood plasma, regarded as a reaction regulator mechanism, can be brought back approximately to its original condition by raising the bicarbonate concentration to ·02 molar. One may conclude therefore that the regulating power possessed by the plasma is mainly due to the bicarbonate concentration of that fluid.

## VI. *The reaction of the blood after hæmorrhage.*

After the loss of one third or even more of the total blood volume, the animal organism within a few hours usually is able to bring about a restoration of the circulating fluid to the original volume. This restoration takes place at the expense of the tissue fluids and evidently occurs very rapidly, if one may judge from the lowering in the specific

gravity of the blood which is to be observed during the course of a fairly rapid hæmorrhage, the later specimens of blood obtained during the

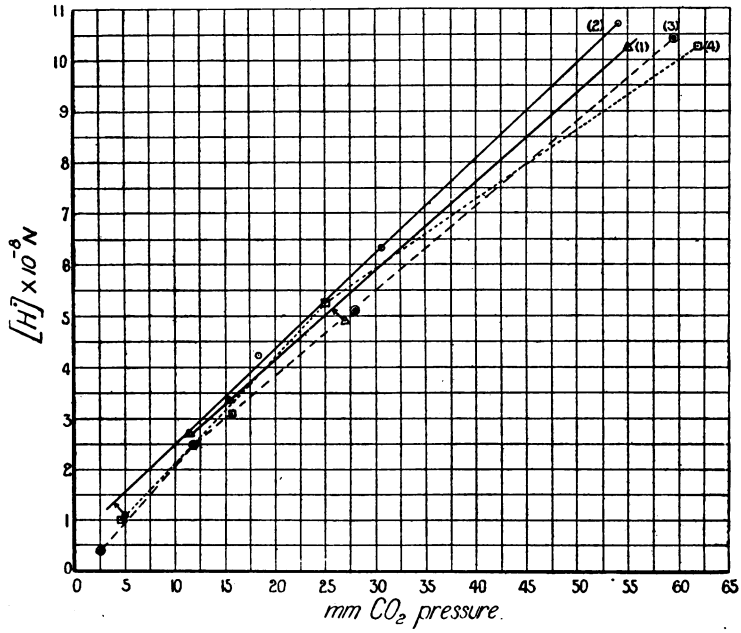


Fig. 4. 1. Dialysed plasma of dog in ·02 molar $NaHCO_3$. 2. Dialysed plasma of cat in ·02 molar $NaHCO_3$. 3. $NaHCO_3$ ·02 molar. 4. Dog's plasma before dialysis.

period of bleeding possessing a distinctly lower specific gravity than the earlier ones. The tissue fluid therefore which takes the place of the lost plasma has a lower saline concentration than the latter. Physiological saline on intravenous injection behaves more or less like a foreign substance and is fairly rapidly removed from the circulation. Evidently when the same salt solution is given by mouth or rectum, restoration and maintenance of the blood volume is more readily brought about so that, during the process of absorption, material is conveyed into the circulation which renders the fluid entering by those channels of absorption a more suitable one for admixture with the blood constituents. There are however many factors to be considered in this regulation of the blood volume, for example, alterations in the capacity of the vascular system, the viscosity and the oxygen and carbonic acid carrying powers of the circulating fluid. The importance of the restoration of the normal blood-pressure and of the regeneration of the

oxygen carrier after hæmorrhage is self-evident, but attention also requires to be directed to the subject of the reaction of the blood after hæmorrhage as the regulation of tissue metabolism and therefore vital processes generally is dependent very largely upon the reaction of the medium. Reference is occasionally made in books dealing with the blood or with pathological chemistry to a diminution in the fixed alkali content of the blood after hæmorrhage. No attention has been directed however to the study of the alteration in the reaction of the blood produced by hæmorrhage by investigators employing modern methods for the determination of disturbances in alkalinity.

The following experiments deal with the reaction of the blood after hæmorrhage and also the effects produced on the reaction by the introduction directly or indirectly into the blood of sodium chloride and sodium bicarbonate solutions.

As previously stated the dog's blood plasma referred to in Table II was obtained from an animal in which the effects of hæmorrhage were also studied. The weight of the animal was 7½ kilos. and from it 200 c.c. or approximately one third of the total blood volume, was removed from the carotid artery. About three-quarters of an hour later, a specimen of blood (hirudinised) was taken and placed in the centrifuge. The plasma from this specimen, about 10 c.c., was placed in a tonometer of 160 c.c. capacity, evacuated and exposed to the series of gas mixtures, commencing with pure hydrogen and ending with a mixture of hydrogen and carbonic acid in which the partial pressure of the latter was slightly over 60 mm. Table X gives the hydrion concentration values in the blood plasma of the same animal before and after hæmorrhage.

TABLE X. *Dog's blood plasma.*

| | Exposed to hydrogen | | | No. 1 gas | | | No. 2 gas | | | No. 3 gas | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $[H^{\cdot}]\times10^{-8}$ N. | $p_H$. | $CO_2$ mm. | $[H^{\cdot}]\times10^{-8}$ N. | $p_H$. | $CO_2$ | $[H^{\cdot}]\times10^{-8}$ N. | $p_H$. | $CO_2$ | $[H^{\cdot}]$ | $p_H$. | $CO_2$ |
| Before hæmorrhage | 1·05 | 7·98 | 4·5 | 3·09 | 7·51 | 15·7 | 5·25 | 7·28 | 25 | 10·23 | 6·99 | 61·8 |
| After hæmorrhage | 1·44 | 7·84 | 3·9 | 5·01 | 7·30 | 14·9 | 7·08 | 7·15 | 25 | 15·50 | 6·81 | 61·4 |

Table X and Fig. 5 illustrate clearly the very evident difference in regulating power of the two specimens, the blood after hæmorrhage showing undoubted diminution in reserve alkali.

It is interesting to note that the blood plasma obtained after severe hæmorrhage is often slightly stained with hæmoglobin due evidently

to the hypotonic character of the fluid which has been supplied by the tissues to replace the blood which has been lost.

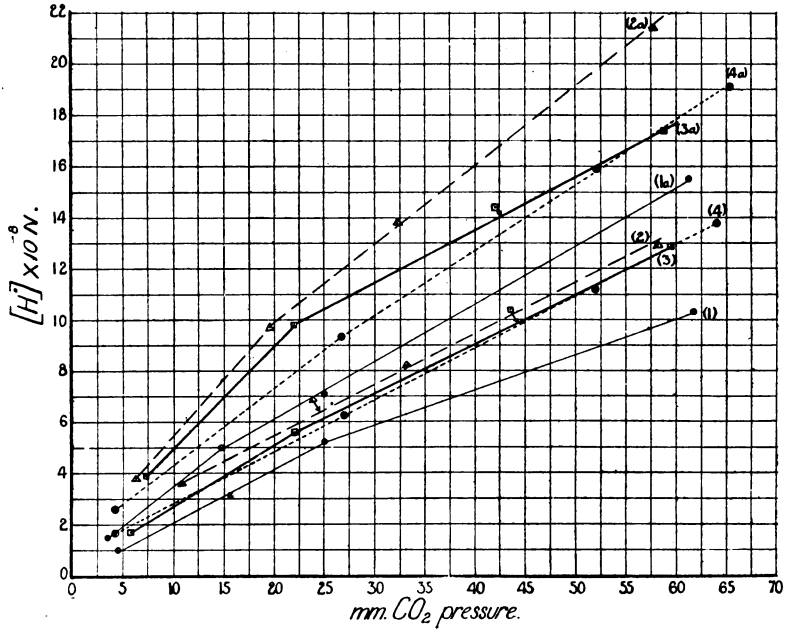


Fig. 5. 1. Dog's plasma before H. 1*a*. After H. 2. Cat's plasma A before H. 2*a*. After H. 3. Cat's plasma B before H. 3*a*. After H. 4. Cat's plasma C before H. 4*a*. After H.

The effects of hæmorrhage on the reaction of the blood were also studied in the cat. Three examples illustrating the nature and degree of the disturbance in the hydrion values will be given.

From an animal weighing 2·4 kilos., a specimen of 20 c.c. arterial blood was received into a tube containing a small quantity of hirudin. Immediately afterwards 50 c.c. were withdrawn and after an interval of 15 minutes a second specimen of 20 c.c. was taken for the determination of the reaction after hæmorrhage. The short interval of fifteen minutes was chosen in order to determine the rapidity with which the tissues parted with their fluid to compensate for the blood loss. The blood specimens were immediately centrifugalised and then kept at a low temperature until required for examination. The blood plasma only was examined in each case. Neither specimen showed any coloration with hæmoglobin.

TABLE XI. *Cat's blood plasma* (A).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before hæmorrhage | $[H^{\cdot}] \times 10^{-8}$ N. | 3·46 | 6·92 | 8·13 | 12·88 |
| | $p_{H^{\cdot}}$ | 7·46 | 7·16 | 7·09 | 6·89 |
| | $CO_2$ mm. | 10·57 | 23·88 | 33·15 | 58·22 |
| After hæmorrhage | $[H] \times 10^{-8}$ | 3·80 | 9·77 | 13·80 | 21·38 |
| | $p_H$ | 7·42 | 7·01 | 6·86 | 6·67 |
| | $CO_2$ mm. | 6·19 | 19·88 | 32·22 | 57·65 |

As in the case of the dog's plasma here also the blood after hæmorrhage shows evident signs of serious impoverishment in its store of bicarbonate, so that when brought into equilibrium with a gas mixture in which the carbonic acid partial pressure was approximately 58 mm. the hydrion concentration was raised from about $13 \times 10^{-8}$ (the value before hæmorrhage) to slightly over $21 \times 10^{-8}$ gm. ion per litre (Fig. 5). This change had taken place within the very short period of fifteen minutes after the withdrawal of approximately one third of the total volume of blood, so that the compensatory passage of the tissue fluid into the circulation occurred very rapidly, and the fluid which first entered the circulation was extremely poor in reserve alkali.

In the case of the second animal which weighed 3·18 kilos. practically the same procedure was adopted. After a specimen of 20 c.c. blood had been taken, 50 c.c. were immediately withdrawn. A period of twenty minutes was then allowed to elapse before taking the second specimen of 20 c.c. blood. The blood plasma obtained from the two specimens was then examined in the usual way.

TABLE XII. *Cat's blood plasma* (B).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before hæmorrhage | $[H^{\cdot}] \times 10^{-8}$ N. | 1·15 | 5·62 | 10·47 | 12·88 |
| | $p_{H^{\cdot}}$ | 7·94 | 7·25 | 6·98 | 6·89 |
| | $CO_2$ mm. | 6·10 | 22·01 | 43·50 | 59·40 |
| After hæmorrhage | $[H^{\cdot}] \times 10^{-8}$ N. | 3·89 | 9·77 | 14·45 | 17·38 |
| | $p_{H^{\cdot}}$ | 7·41 | 7·01 | 6·84 | 6·76 |
| | $CO_2$ mm. | 7·24 | 22·08 | 42·00 | 59·00 |

In this case the disturbances are of the same general character as in the other cat. It is evident from the graphs in Fig. 5 that although hydrolysis at the lower carbonic acid pressures is similar in cats A and B after hæmorrhage, at the higher pressures it is less marked in cat B. In all probability this difference is due to a higher protein content of the plasma in cat B.

From the third cat (C) weighing 2·4 kilos., after taking the first specimen of 20 c.c., 60 c.c. blood were withdrawn. After half an hour's interval, the second sample of 20 c.c. was taken.

TABLE XIII. *Cat's blood plasma* (C).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before hæmorrhage | $[H^{\cdot}] \times 10^{-8}$ N. | 1·66 | 6·16 | 11·22 | 13·80 |
| | $p_{H^{\cdot}}$ | 7·78 | 7·21 | 6·95 | 6·86 |
| | $CO_2$ mm. | 4·35 | 26·84 | 52·00 | 63·84 |
| After hæmorrhage | $[H^{\cdot}] \times 10^{-8}$ N. | 2·63 | 9·33 | 15·85 | 19·05 |
| | $p_{H^{\cdot}}$ | 7·58 | 7·03 | 6·80 | 6·72 |
| | $CO_2$ mm. | 4·40 | 26·78 | 52·14 | 65·23 |

The displacements in hydrion concentration in this case are evidently due to the same alteration in the blood after hæmorrhage as was observed in the other two cases.

The graphs in Fig. 5 demonstrate clearly the degree of the disturbance. It appears as if hydrolysis were less marked at the lower pressures, but very slight errors in the determination of the latter may account for the apparent difference. It is clear however that in all cases, the blood after hæmorrhage shows a serious impoverishment in its reserve alkali. As the maintenance of the normal alkalinity of the blood is essential in order that so many vital processes may be carried out under the best conditions, one may conclude that the organism, to some extent deprived of the important regulator of its reaction, must suffer as regards general tissue oxidation processes, and the regulation of the respiratory and cardiac mechanisms, to mention only a few of the more important disturbances.

One was therefore led to consider the best means of restoring to the organism the regulator constituent or constituents which had been lost during hæmorrhage. If, from the evidence at our disposal, we are led to conclude that it is simply a loss of alkali which constitutes the disturbing factor, the natural procedure would be to replace this alkali either by direct or indirect introduction into the blood. Before referring however to such experiments, I wish in the first place to refer briefly to the effects produced on the reaction of the blood after hæmorrhage by the intravenous injection of a sodium chloride solution.

## VII. *Sodium chloride injections after hæmorrhage.*

These effects were studied in cat C. Immediately after taking the second sample of blood, which on examination showed a distinct

decrease in reserve alkali, an intravenous injection of 60 c.c. ·2 molar NaCl, warmed to 38°, was given, and a quarter of an hour later a third sample of blood was taken for investigation. The results are shown below. For comparison the hydrion concentrations of the same animal's blood just before the saline injection (showing therefore the effects of hæmorrhage) are repeated.

TABLE XIV. *Cat's blood* (C).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before injection | $[H^{\cdot}] \times 10^{-8}$ N. | 2·63 | 9·33 | 15·85 | 19·05 |
| | $p_{H^{\cdot}}$ | 7·58 | 7·03 | 6·80 | 6·72 |
| | $CO_2$ mm. | 4·40 | 26·78 | 52·14 | 65·23 |
| After injection of ·2 molar NaCl. | $[H^{\cdot}] \times 10^{-8}$ N. | 3·31 | 11·75 | 20·42 | 22·90 |
| | $p_{H^{\cdot}}$ | 7·48 | 6·93 | 6·69 | 6·64 |
| | $CO_2$ mm. | 4·71 | 26·78 | 51·18 | 63·40 |

It is evident from Table XIV and Fig. 6 (Graphs 4*a*, 4*b*) that the saline injection has led to an exaggeration of the condition produced by the hæmorrhage, as was to be expected from the lowered $HCO'_3$ concentration of the plasma.

## VIII. *Intravenous injections of bicarbonate after hæmorrhage.*

The alkaline solution might be introduced directly into the circulation or indirectly by rectal or other injections when the intravenous method might under special circumstances be inconvenient. I shall therefore in the first place give the results of intravenous injection, and in conclusion very briefly refer to the results obtained by rectal injection of sodium bicarbonate.

As in cat A the post-hæmorrhagic disturbances in hydrion concentration produced by the series of gas mixtures were extremely well marked, the effects of bicarbonate injections may be most satisfactorily studied in the case of this animal. Immediately after the withdrawal of the specimen of the blood which was taken after hæmorrhage and which showed the disturbances in reaction given in Table XI and Fig. 5, an intravenous injection of 50 c.c. ·2 molar sodium bicarbonate solution was given. After an interval of half an hour, a specimen of blood (20 c.c.) was taken and the plasma investigated by the same method which was employed in the case of the other specimens. There was no sign of hæmolysis in the specimen obtained after the bicarbonate

injection. For comparison the hydrion values of the plasma after hæmorrhage are also given.

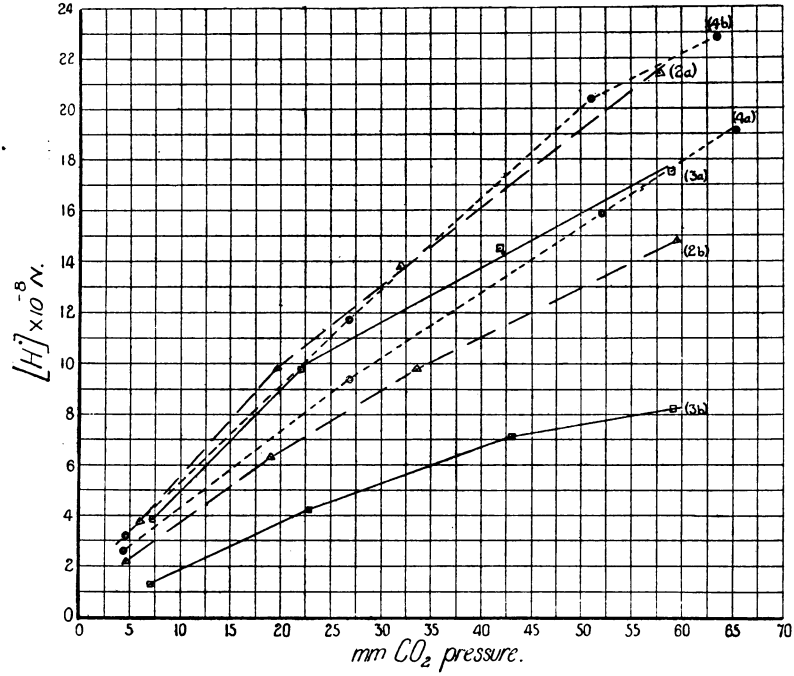


Fig. 6. Injection of various fluids after hæmorrhage. 2*a*. Cat's plasma A after H. 2*b*. Cat's plasma A after H. and then $NaHCO_3$ (intravenous injection). 3*a*. Cat's plasma B after H. 3*b*. Cat's plasma B after H. and then $NaHCO_3$ (rectal injection). 4*a*. Cat's plasma C after H. 4*b*. Cat's plasma C after H. and then NaCl (intravenous injection).

TABLE XV. *Cat's blood plasma* (A).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before injection | $[H^{\cdot}] \times 10^{-8}$ N. | 3·80 | 9·77 | 13·80 | 21·38 |
| | $p_{H^{\cdot}}$ | 7·42 | 7·01 | 6·86 | 6·67 |
| | $CO_2$ mm. | 6·19 | 19·88 | 32·22 | 57·65 |
| After injection of $NaHCO_3$ ·2 molar | $[H^{\cdot}] \times 10^{-8}$ N. | 2·24 | 6·31 | 9·77 | 14·79 |
| | $p_{H^{\cdot}}$ | 7·65 | 7·20 | 7·01 | 6·83 |
| | $CO_2$ mm. | 4·71 | 19·00 | 33·57 | 59·51 |

As will be evident from an examination of Tables XI and XV and especially of the graphs in Fig. 6, the bicarbonate injection has brought back the hydrion displacement graph nearly to the same position as that occupied by the graph of the blood plasma before hæmorrhage.

The fact however that it has not effected a complete restoration of the reserve alkali of the blood is of interest when we bear in mind that the 90 c.c. blood which had been removed were replaced by 50 c.c. ·2 molar $NaHCO_3$. If we take the original blood volume in this animal as 240 c.c., then, even on the highly improbable supposition that the original blood volume had been restored in the interval which had elapsed since the hæmorrhage, the bicarbonate injection (containing ·84 grm. $NaHCO_3$) ought to have raised the bicarbonate concentration of the plasma to a ·04 molar value, even if the blood prior to the injection had contained no bicarbonate, which was of course by no means the case. As the concentration had not even reached the ·02 molar or normal value, the bicarbonate which was injected must have been largely removed. As regards the volume and protein content of the tissue fluid entering the circulation after hæmorrhage the paper by Scott in this *Journal* may be consulted(15). It would have been of interest to determine the reaction of the urine and the effect upon the alveolar carbonic acid pressure before and after the injection but it was impossible with the time at my disposal.

A small bicarbonate injection was given in the case of cat C subsequent to the sodium chloride injection. Immediately after taking the third specimen of 20 c.c. blood from this animal, an intravenous injection of 20 c.c. ·2 molar $NaHCO_3$ was given, and fifteen minutes later the fourth 20 c.c. specimen of blood was removed for examination. For comparison the hydrion concentration values of the plasma after the sodium chloride injection are given along with those obtained after the bicarbonate.

TABLE XVI. *Cat's blood plasma* (C).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before injection | $[H^{\cdot}] \times 10^{-8}$ N. | 3·31 | 11·75 | 20·42 | 22·90 |
| | $p_{H^{\cdot}}$ | 7·48 | 6·93 | 6·69 | 6·64 |
| | $CO_2$ mm. | 4·71 | 26·78 | 51·18 | 63·40 |
| After injection of ·2 molar $NaHCO_3$ | $[H^{\cdot}] \times 10^{-8}$ N. | 3·39 | 9·55 | 16·98 | 20·42 |
| | $p_{H^{\cdot}}$ | 7·47 | 7·01 | 6·77 | 6·69 |
| | $CO_2$ mm. | 6·47 | 27·06 | 50·26 | 63·34 |

As was to be expected the effect of the bicarbonate injection was to bring back the reaction of the blood towards the normal, but the plasma reaction still remained slightly less alkaline than the blood examined after the first hæmorrhage, although more alkaline than the blood after the subsequent sodium chloride injection.

IX. *Rectal injection of bicarbonate after hæmorrhage.*

In the case of cat B (weight 3·18 kilos.) after the removal of 90 c.c. blood a solution of ·4 molar $NaHCO_3$ was given per rectum after the bowel had been well washed out. It is difficult to state how much of this solution was actually absorbed but possibly from 70 to 100 c.c. The solution, warmed to 38°, was given slowly, the injection being spread over a period of about one hour, at the conclusion of which a sample of blood was withdrawn and the plasma examined in the usual way. For comparison the hydrion values of the plasma after hæmorrhage are given along with those obtained one hour after the rectal bicarbonate injection had commenced.

TABLE XVII. *Cat's blood plasma* (B).

| | | Exposed to hydrogen | No. 1 gas | No. 2 gas | No. 3 gas |
|---|---|---|---|---|---|
| Before injection ... | $[H^{\cdot}] \times 10^{-8}$ N. | 3·89 | 9·77 | 14·45 | 17·38 |
| | $p_{H^{\cdot}}$ | 7·41 | 7·01 | 6·84 | 6·76 |
| | $CO_2$ mm. | 7·24 | 22·08 | 42·00 | 59·00 |
| After rectal injection of $NaHCO_3$ ·4 molar | $[H^{\cdot}] \times 10^{-8}$ N. | 1·23 | 4·17 | 7·08 | 8·13 |
| | $p_{H^{\cdot}}$ | 7·91 | 7·38 | 7·15 | 7·09 |
| | $CO_2$ mm. | 7·02 | 22·64 | 42·80 | 59·20 |

It is evident that the rectal injection of bicarbonate has led to a much higher $HCO'_3$ concentration of the plasma than the intravenous injections. It is true that the solution injected per rectum was ·4 instead of ·2 molar, but the important point is that by rectal administration of sodium bicarbonate, the reserve alkali value of the blood can be raised even above the normal. As the rectal administration can be given under conditions when it might be most inconvenient to inject directly into the blood, it offers a ready means of restoring alkali to the circulation during a period when the altered reaction of the blood might otherwise produce harmful effects upon the cardiac and respiratory mechanisms.

SUMMARY.

1. The disturbances produced in the hydrion concentration of bicarbonate solutions by variations in the carbonic acid concentration were studied. It was found that a ·02 molar $NaHCO_3$ solution behaved in much the same way as blood plasma.

2. The normal blood plasma of the dog showed a rise in $[H^{\cdot}]$ from about $1 \times 10^{-8}$ N. to $10 \times 10^{-8}$ N. when the $CO_2$ pressure was raised from 4 to 60 mm. (approximate values).

3. The normal blood plasma of the cat under the same range of pressures showed a rise from about $1 \times 10^{-8}$ N. to $14 \times 10^{-8}$ N.

4. Human cerebro-spinal fluid behaved like ·02 molar $NaHCO_3$ solution.

5. The laked blood cells of dog and cat gave [H·] values which ranged from about $1 \times 10^{-7}$ N. up to slightly over $3 \times 10^{-7}$ N. when the $CO_2$ pressure was raised from a very low value to 50–55 mm.

6. The blood plasma after dialysis gave, when exposed to the various $CO_2$ pressures, [H·] values ranging from $1{\cdot}3 \times 10^{-6}$ N. to $1{\cdot}45 \times 10^{-6}$ (dog) or from $3{\cdot}4 \times 10^{-6}$ to $5 \times 10^{-6}$ N. (cat).

7. The dialysed blood cells reacted in much the same way as the undialysed.

8. On making up the dialysed plasma to ·02 molar concentration $NaHCO_3$, it behaved similarly to the original plasma.

9. After hæmorrhage there was a loss of reserve alkali which resulted in greater [H·] disturbances under rising $CO_2$ pressures than in the case of normal plasma, for example in the dog from $1{\cdot}4 \times 10^{-8}$ to $15{\cdot}5 \times 10^{-8}$ instead of up to $10 \times 10^{-8}$ and in the cat from $3{\cdot}8 \times 10^{-8}$ N. up to $21 \times 10^{-8}$ N. instead of a rise from $3{\cdot}4 \times 10^{-8}$ N. up to about $13 \times 10^{-8}$ N. as occurred in normal blood plasma.

10. The injection of ·2 molar sodium chloride solution exaggerated the condition, while intravenous or rectal administration of sodium hydrogen carbonate brought the plasma back towards the normal as regards response of the latter to varying carbonic acid pressures.

In conclusion I wish to express my sincere thanks to Professor Langley for granting me permission to work, unfortunately only for a brief period, in his laboratory and to Mr Barcroft for many valuable suggestions which were of great assistance to me in the prosecution of my work.

REFERENCES.

(1) Bayliss. Proc. Roy. Soc. Ser. B. **89**. p. 380. 1916
(2) Kendall. Journ. Amer. Chem. Soc. **38**. p. 1480. 1916.
(3) Auerbach and Pick. Arbeit. a. d. k. Gesundheitsamte, **38**. p. 274. 1911.
(4) Seyler and Lloyd. Journ. Chem. Soc. **111. 112**. p. 138. 1917.
(5) Seyler and Lloyd. Ibid.
(6) Barcroft. The Respiratory Function of the Blood, p. 304. 1914.
(7) Peters. Physiol. Soc. Proceed. Jan. 1914. (This Journal, **48**.)
(8) Sörensen. Ergebn. d. Physiol. **12**. pp. 415–418. 1912.
(9) Hurwitz and Tranter. Arch. Int. Med. Chicago, **17**. p. 828. 1916.
(10) Hasselbalch. Biochem. Ztsch. **30**. p. 317. 1911.
(11) Michaelis and Dawidoff. Ibid. **46**. p. 131. 1912.
(12) Konikoff. Ibid. **51**. p. 200. 1913.
(13) Michaelis and Kramaztyk. Ibid. **62**. p. 180. 1914.
(14) Walpole. Bioch. Journ. **9**. p. 284. 1915.
(15) Scott. This Journal, **50**. p. 157. 1916.