THE NEUTRALITY OF THE BLOOD.

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IN the course of certain experiments made for the purpose of finding out whether the passage of fixed acid into the blood was capable of producing a state of progressively falling blood-pressure, analogous to that of wound-shock, it was necessary to obtain determinations of the hydrogen-ion concentration of the arterial blood as it actually was in the body when the sample was taken. Since this depends on the amount of carbon dioxide dissolved, that is, on the alveolar tension of carbon dioxide at the time, it is clear that if the hydrogen-electrode is to be used for the purpose, the gaseous atmosphere with which the blood is brought into equilibrium must contain carbon dioxide at the alveolar tension in question. This implies a measurement of the alveolar carbon dioxide tension, not a simple matter in animal experiments. I therefore turned to the use of indicators and was able to devise a simple method capable, with practice, of as accurate results as the electrical method. Sørensen has shown the possibility of using indicators in this way and from his results it appeared that neutral red would be an appropriate one for the present object. This indicator has a regular series of changes in tint from crimson to yellow, through red and orange, being crimson at a hydrogen-ion concentration of 10^{-5} and bright yellow at 10^{-11} with a regular series of perceptible differences in tint all the way between these limits. Changes outside these limits are impossible in the blood of the living animal. Phenolsulphone-phthalein is a favourite indicator in the United States for a similar range and would doubtless serve as well as neutral red. I find myself, however, that I can detect the changes in neutral red more easily. Miss Homer (1917) and Van Slyke (1919) recommend it for work with serum and it is not sensitive to the presence of salts or proteins. In general it is a valuable indicator for living cells, being non-toxic and readily taken up by the cells.

Method. The method of using it for blood is as follows: A conical centrifuge tube holding 15 c.c. is supplied with a small amount of

powdered potassium oxalate, as for the Van Slyke method of determining "alkali-reserve." 3 or 4 c.c. of liquid paraffin are poured into the tube. From a cannula in an artery of the animal, blood is allowed to flow into the tube until about 10 c.c. are collected. Liquid paraffin is added until the tube is nearly full. It is then inverted a few times, with the thumb placed over the top, in order to dissolve the oxalate and is then centrifuged. There results a layer of paraffin at the top, which protects for some time from loss of carbon dioxide to the atmosphere, a mass of corpuscles at the bottom, and a layer of plasma between the two. A drop of 0·1 p.c. neutral red is added to the upper part of the plasma layer by means of a fine pipette inserted through the paraffin. The indicator is mixed with the upper part of the plasma by stirring with the pipette and the colour noted and compared with a similar sample taken after various treatment of the animal.

In the experiments for which the method was first used, it was merely desired to see whether the addition of acid or alkali to the blood during the life of the animal changed the reaction, so that it was unnecessary to compare the tint with standards. It was found that however slowly the acid was run into the vein, some degree of hæmolysis resulted. The plasma in the subsequent samples was therefore stained slightly red, so that a trace of hæmoglobin was added to the original sample until the colours matched before the addition of the indicator. The hæmolysis can be reduced to some extent by making the acid hypertonic by the addition of 2 p.c. sodium chloride.

If the actual hydrogen-ion concentration was required, the colour was compared with that of neutral red in a series of phosphate solutions of known H-ion concentration in the manner of Sørensen. In this case, the colour of the standards must previously be made to match the plasma by the addition of some dye. Dr C. J. Martin has used caramel and flavine. I find a trace of hæmoglobin of assistance. But various other appropriate dyes would serve. The method used by Walpole and Cole with the comparison in separate superposed tubes could also be used, but would require a larger supply of the plasma. As regards the standards, they may be made by mixing the acid and alkaline phosphates in various proportions. But I prefer Prideaux's method (1911, 1916) of mixing a standard phosphoric acid with sodium hydroxide in various proportions, as shown by the Table below. The only difficulty is the preparation of the molar phosphoric acid. The concentration may be estimated by precipitation with ammonium molybdate and dissolving the precipitate in sodium hydroxide, as in

the method known as Neumann's, or more easily by titrating with sodium hydroxide to phenol-phthalein as indicator, which becomes rose-coloured when all the phosphoric acid has been converted into the di-sodium-hydrogen phosphate. Both methods gave the same value with my solution.

The method above-described might be modified for use with venous blood for clinical purposes, where a simultaneous sample of alveolar air could be collected, say, in a rubber bag, or better, in a glass collecting tube. No paraffin need be placed in the centrifuge tube and probably less than 10 c.c. of blood would suffice. This may be collected in a syringe containing a little powdered oxalate, immediately transferred to the centrifuge tube and the plasma separated. A sample of the latter is drawn off with a pipette and run into a stoppered bottle. A drop of neutral red is added. The colour seen is not indicative of the H-ion concentration in the arterial blood of the patient because it was venous when taken and has also been exposed to the air. But if the bottle is filled with alveolar air of the patient and rotated several times, the H-ion concentration of the plasma becomes that of the arterial blood. It is well to replace the air by a second supply and if the colour changes, by a third supply.

c.c. NaOH	H-ion	Colour to neutral red	Colour to other indicators
10·0	10^{-4}	Crimson	Red to methyl-orange
10·6	10^{-5}	Crimson	Orange to methyl-orange
			Red to methyl-red
11·5	10^{-6}	Trace of red	Orange to methyl-red
13·0	$10^{-6 \cdot 4}$	$\frac{3}{4}$ crimson, $\frac{1}{4}$ red	Yellow-orange to methyl-red
15·5	$10^{-6 \cdot 7}$	$\frac{1}{2}$ crimson, $\frac{1}{2}$ red	Yellow to methyl-red
16·0	$10^{-6 \cdot 8}$	$\frac{1}{4}$ crimson, $\frac{3}{4}$ red	
16·5	$10^{-6 \cdot 9}$	Red	
17·2	10^{-7}	Trace of orange	
17·7	$10^{-7 \cdot 07}$	$\frac{1}{4}$ orange, $\frac{3}{4}$ red	
18·0	$10^{-7 \cdot 2}$	$\frac{1}{2}$ orange, $\frac{1}{2}$ red	
18·5	$10^{-7 \cdot 4}$	$\frac{3}{4}$ orange, $\frac{1}{4}$ red	
19·2	$10^{-7 \cdot 7}$	Orange	
19·6	10^{-8}	Trace more yellow	
20·0	10^{-9}	$\frac{3}{4}$ orange, $\frac{1}{4}$ yellow	Colourless to phenol-phthalein
20·4	10^{-10}	$\frac{1}{2}$ orange, $\frac{1}{2}$ yellow	Faint red to phenol-phthalein
21·0	$10^{-10 \cdot 5}$	$\frac{1}{4}$ orange, $\frac{3}{4}$ yellow	Red to phenol-phthalein
			Colourless to thymol-phthalein
22·6	10^{-11}	Yellow	Blue to thymol-phthalein
			Yellow to tropoeolin O
28·5	10^{-12}	Yellow	Orange to tropoeolin O

The first column gives the number of c.c. of normal sodium hydroxide to be added to 10 c.c. of the molar phosphoric acid in order to make a solution of the H-ion concentration given in the second column when diluted to 100 c.c. The colour assumed by neutral red in each solution is that stated in the third column, but it is to be understood that the finer shades can only be distinguished by comparison with those on each side. The object of the fourth column is to check the results at the extreme ends of the series, where neutral red is less sensitive than in the middle region. The effect of the colour of plasma or of urine is to shift the tint towards the yellow. Thus: neutral red in a phosphate solution of 10^{-7} coloured to match plasma has the colour of the watery solution of $10^{-7 \cdot 7}$.

The H-ion concentration of the laboratory distilled water was 10^{-6}: if boiled out in a Jena flask, it fell to $10^{-6 \cdot 4}$. The tap water was $10^{-9 \cdot 5}$.

The blood of the normal cat was found by the method described to have the hydrogen-ion concentration of the standard known to be $10^{-7.4}$ normal, the colour being reddish-orange; that is, slightly on the alkaline side of neutrality ($10^{-7.07}$), in accordance with previous observers. Experiments made by Dale and Richards and by myself had shown that comparatively large amounts of hydrochloric acid could be introduced into the circulation without harm and the question arose whether under such circumstances there was any actual increase in the acidity of the blood. It is clear that the term "acidosis" suggests that there is an increase in the H-ion concentration. But this state of acidosis is defined by Van Slyke and Cullen (1917) as consisting in a reduction of the bicarbonate concentration of the plasma, owing to combination of part of it with fixed acids.

In the present series of experiments, sufficient acid was injected intravenously to reduce the bicarbonate to one-third of its normal value, a severe degree of "acidosis." This required 10·4 c.c. of half-normal acid per kilogram of body-weight. Twenty to thirty minutes later, a second sample of blood was taken. Comparing this with one taken before the acid injection, the H-ion concentration was found to be *unaltered*, although the bicarbonate concentration was reduced to one-third. This behaviour is, in point of fact, what would be expected from the work of Haldane and Priestley (1905) and of L. J. Henderson (1908) and is merely a new form of a result already known. But the fact itself and its explanation are apt to be forgotten in discussions on acidosis, so that some further remarks are desirable. Assuming for the moment that the plasma contains sodium bicarbonate, a question to be returned to later, together with dissolved carbon dioxide, we may regard the H-ion concentration as determined by the relative proportion of the two, as shown by L. J. Henderson. The bicarbonate is a source of OH′ ions, that is, of alkalinity, because, as a salt of a weak acid with a strong base it is hydrolytically dissociated to a certain degree into NaOH and H_2CO_3. The former is strongly dissociated electrolytically, producing OH′, the latter comparatively little, but giving H˙ ions. The addition of more CO_2 raises the concentration of the latter ions. The reaction of the plasma might in theory vary between that of a pure sodium bicarbonate solution and that of a similar solution saturated with carbon dioxide at 760 mm. pressure. The latter value is readily obtained and is $10^{-6.8}$. The former is practically impossible to obtain because sodium bicarbonate dissociates in solution into sodium carbonate and carbon dioxide if the tension of the latter to which it is

subjected falls below 2·25 mm. of mercury or 0·3 p.c. by volume (Bohr; 1892. Buckmaster; 1918). But according to Clark (1913, p. 86), a 0·01 p.c. solution of bicarbonate in equilibrium with a tension of carbon dioxide of 7·6 mm. of mercury has a H-ion concentration of 10^{-7}. Blood plasma contains 0·25 p.c. and I find that a solution of this concentration in equilibrium with 0·38 p.c. carbon dioxide at 38° has a H-ion concentration of 10^{-10}. It is clear then that if the bicarbonate is diminished in concentration and thus the alkalinity decreased, the deficiency can be compensated by a corresponding reduction in carbon dioxide, which brings about a reduction in the H-ion and makes the ratio between the $H^{\cdot}$ and OH' ions the same as before.

A simple method by which small quantities of solution can be brought into equilibrium with various gaseous mixtures was used in the experiments described in this paper. A spherical flask of about one litre in capacity is provided with a perforated india-rubber stopper into which a glass stopcock is fitted. The capacity of the flask is measured. Part of the air is withdrawn by means of a filter-pump and the tap closed. A known volume of the solution is run in by connecting a pipette to the tap by a short bit of rubber tubing and then opening the tap for a moment. A gas collecting tube of the required capacity with a stopcock at both ends is filled with the gas and attached to the rubber tube in place of the pipette. The two stopcocks, that of the flask and the nearest one on the gas tube, are first opened and then the outer one. The rush of air carries the gas into the flask and is followed by air up to atmospheric pressure. The flask is then closed and the gas tube removed. By rotation of the liquid over the sides of the flask, equilibrium is rapidly attained. The solution can then be poured into a test-tube under liquid paraffin and compared with the standard phosphates. If the gas is taken up in large amount by the solution, it would be necessary to repeat the process with the solution already in approximate equilibrium.

When a fixed, non-volatile acid is added to the blood, a part of the bicarbonate is converted into the salt of the acid, while carbon dioxide is given off. This carbon dioxide raises the H-ion concentration of the plasma and at the same time the OH' ion is reduced because of a removal of part of the bicarbonate. The increased H-ion concentration excites the respiratory centre to increase the ventilation of the lungs, the carbon dioxide tension in the alveoli continues to be lowered until the H-ion concentration of the blood is brought back to a point at which the respiratory centre ceases to be abnormally stimulated. At this point, the free carbon dioxide in the blood has been reduced in proportion to the reduction of the bicarbonate. The increased ventilation at the first introduction of acid into the circulation is obvious, but it might appear that continued hyperpnœa would be needed in order to maintain the alveolar tension of the carbon dioxide at the required point. Presumably the sensibility of the centre is great enough to respond to so

slight an increase in the carbon dioxide tension in the blood that the necessary permanent increase in ventilation is so small as to require special means to detect it.

The reason why the hydrogen-ion concentration of the plasma remains the same after the injection of acid is therefore because less carbon dioxide is present. That this is the case is easily shown by taking samples of the plasma before and after injection of acid and bringing them both into equilibrium with normal alveolar air. Using my own alveolar air, I found that the tint of the former sample was unaltered, while that of the latter, of the same tint as withdrawn, became red, indicating that its previous carbon dioxide content was lower than that of normal alveolar air. The difference was still more marked if a further reduction in the bicarbonate of both was produced by adding sufficient acid to neutralise the remainder of the bicarbonate in the second sample.

These facts show the inappropriateness of the name "acidosis" as used to express a state in which all that is known is that the bicarbonate reserve is lowered. A reduction of the bicarbonate reserve is a fact in itself of no importance; it may be associated with an increase, a decrease, or no change in the H-ion concentration. "Acidosis" ought to be limited to the first case, but since its use has led to so many conflicting statements and confusion of thought, it would be better to avoid the use of it altogether. The name "alkalosis" is equally to be deprecated.

A further confirmation of the regulating action of the respiratory centre was afforded by depressing the excitability of this centre by the injection of morphine until the rate of respiration was reduced to about 8 per minute. In this state, the introduction of acid produced very little hyperpnœa and a sample of the blood showed that its H-ion concentration was actually raised.

It seemed of interest to test the behaviour to an increase in the concentration of bicarbonate in the blood, especially since 4 p.c. solutions have been frequently used for intravenous injection. I found some difference in the reaction of individual cats. An amount of the salt sufficient to add one-quarter to the original concentration was usually injected. Sometimes the H-ion was unaltered; but, in other cases, there was a long-lasting decrease in this value, even in cats which responded normally to acid injection. It appears that the necessary increase in the alveolar carbon dioxide tension to compensate for the increase in alkali is less readily brought about than the decrease required for acid. The initial effect of the bicarbonate is, as well known, a reduction in pulmonary ventilation and probably the reason why this does not

continue, so as to allow the alveolar carbon dioxide to rise, is because it would be necessarily associated with a deleterious diminution in oxygen supply. The latter would, by the production of acid substances in the respiratory centre, lead to an increase in pulmonary ventilation. The respiratory mode of reaction is, therefore, less effective against alkali than against acid, whereas a few experiments suggest that the renal reaction plays the greater part in the latter case.

These experiments, although incomplete, are of some interest. In one of them, an amount of hydrochloric acid sufficient to combine with half the bicarbonate of the blood was injected during 37 minutes. The urine collected during this period and the subsequent 40 minutes was just perceptibly more acid than that before the injection, but was still alkaline to methyl red, as the cat's urine normally is. Human urine is acid to methyl red. 5·2 c.c. of half-normal sodium carbonate per kilo. body-weight were then injected. The urine now became alkaline, not only to methyl red but to neutral red, whereas it was previously acid to this indicator.

Milroy (1917) has pointed out that sodium bicarbonate is rapidly eliminated by the kidney. It is obvious that if the H-ion concentration of the blood is quickly brought to normal, by loss of carbon dioxide from the lungs, when acid is injected, there is no stimulus to the kidney to excrete acid; whereas, when alkalies are injected, the H-ion concentration is not readily increased by restricted respiration, the blood becomes more alkaline and the kidney excretes the excess. In an experiment in which a large quantity of acid sodium phosphate was injected, no increase in the acidity of the urine could be detected.

In the former of the above experiments, the third known means of neutralising acid was tested, that is, the increased excretion of ammonia. There was, in fact, a slight increase after acid injection, but not great. The urine was aerated after addition of sodium carbonate, as in the urease method of determining urea, and the respective amounts of deci-normal acid required to neutralise one hour's urine before and after the acid were 6·4 and 7·8 c.c. respectively, a small difference compared with the amount of acid injected, which would have required 130 c.c. of deci-normal alkali to neutralise it completely. But the observation only lasted for 77 minutes after the injection and the effect might have been more marked if a longer time had been allowed.

If the increase in ammonia is due to a restriction of the conversion of the ammonia resulting from deamination of amino-acids, into urea, it would be expected that a decrease of carbonate in the blood would result by mass action in a decrease of the formation of

urea, since ammonia is converted into the carbonate before it is changed to urea. The urea was estimated in the experiment in question by the urease method and found to have practically the same ratio to ammonia before and after acid; so that the experiment, as far as it goes, does not support the interpretation given. This ratio was less than is the case in man, being about 8 to 1. The corresponding neutralisation of excess alkali by the production of lactic acid described by Macleod (1918) was not investigated in my experiments.

The question next arises as to whether there is actually free bicarbonate in the blood and if so, whether it is sufficient to account for the whole of the carbon dioxide given off when sulphuric acid is added to the plasma, as in Van Slyke's method.

When serum is dialysed against 0·9 p.c. sodium chloride, the dialysate behaves as if it contained sodium bicarbonate. That is, the H-ion concentration is found to vary between about 10^{-10} to 10^{-6} as the tension of the carbon dioxide with which it is in equilibrium rises from zero to 760 mm. It gives off gas in the Van Slyke apparatus, and the gas was absorbed by sodium hydroxide. Further, when a known volume of cat's serum is allowed to dialyse into equilibrium with an equal volume of 0·9 p.c. sodium chloride, and the dialysate titrated with acid to neutrality to methyl-orange, it is found that the value obtained, assuming equal partition of the bicarbonate between the serum and the salt solution, corresponds with the value for cat's serum obtained by Van Slyke's method, that is, a concentration of 0·023 molar or 49 volumes of carbon dioxide per cent. So far as this experiment goes, it would seem that all the carbon dioxide that comes off from plasma on addition of acid comes from bicarbonate and that no indiffusible constituent, such as protein, plays a part.

It remains to enquire whether possible variations in the alveolar carbon dioxide tension are sufficient to compensate for a reduction of the bicarbonate concentration in the blood to such a degree as occurs in "acidosis."

Two bicarbonate solutions were taken, one of 0·03 molar, the other of half this concentration. In equilibrium with alveolar air, the latter was obviously more acid than the former, having a H-ion concentration of $10^{-7\cdot1}$ as against the former of the usual $10^{-7\cdot4}$. The more acid solution was then brought into equilibrium with a carbon dioxide tension of 25 mm. of mercury. The H-ion concentration was brought down to $10^{-7\cdot4}$, that is, as alkaline as the more concentrated bicarbonate in equilibrium with 40 mm. carbon dioxide tension. On the other side, the more concentrated bicarbonate solution, when exposed to a carbon dioxide tension of 79 mm., became nearly equal in H-ion concentration to the more dilute one, namely $10^{-7\cdot15}$. With 62 mm. tension, it was $10^{-7\cdot2}$.

Such changes in carbon dioxide tension of alveolar air are well within the capacity of pulmonary ventilation. Yandell Henderson

and Haggard (1918, p. 336) point out that to compensate for a reduction of the bicarbonate by one-third only 50 p.c. more ventilation is required and if the bicarbonate rises by one-third, 25 p.c. less ventilation is necessary. Haldane and Priestley (1905) showed that a rise in the alveolar tension of carbon dioxide from 40 to 41·6 mm. of mercury (or 0·2 p.c. increase in the volume of carbon dioxide present) is sufficient to double respiratory ventilation.

On account of the statements made by Moore, McQueen and Webster (1919) that serum possesses ten times the capacity of neutralising acid, or regulating reaction, of a sodium bicarbonate solution of concentration equal to that present in serum and that this is due to the proteins contained in serum, I have devoted some experimental work to the question. No doubt, if serum, after dialysis against repeated changes of 0·9 p.c. sodium chloride in order to remove all the bicarbonate, is titrated with methyl-orange as indicator, a comparatively large amount of acid is required to change the colour of the indicator, indeed a volume of deci-normal hydrochloric acid nearly equal to the serum. But the H-ion concentration at which this indicator changes its colour is 10^{-4} and we are clearly beyond the limits within which it varies in the living organism. According to L. J. Henderson, an increase beyond 10^{-7}, or a decrease below 10^{-8}, is incompatible with life. The most severe cases of "acidosis" reported do not show a diminution of alkaline reserve to less than about one-third of the amount. A bicarbonate solution containing one-quarter of that in serum, even in equilibrium with normal alveolar air, has a H-ion concentration of 10^{-7}. It is necessary, therefore, to test whether proteins are capable of combination with acid or alkali in the region immediately in the neighbourhood of neutrality. In other words, whether they play any part in the neutralisation of the small amounts of acid produced in tissues, as they slowly pass into the blood. Similarly, if serum or a protein solution is titrated with alkali to the reaction indicated by phenol-phthalein (*i.e.* 10^{-10}), it combines with a notable amount of deci-normal sodium hydroxide. But here again the limits are beyond those possible in the organism. The excised heart is greatly weakened or even stopped at this degree of alkalinity.

An indicator such as neutral red, which changes tint at regions in close proximity to the neutrality of distilled water, must be used. The problem may be attacked in several ways. We may dialyse serum against 0·9 p.c. sodium chloride until it is deprived of diffusible alkali and then compare the behaviour of this serum with that of its final

dialysate. Or we may dialyse it against a solution of sodium bicarbonate of equal concentration and compare the two solutions after equilibrium is reached. Or again, we may test whether serum deprived of bicarbonate has any power of changing the reaction of a bicarbonate solution.

In my first experiments, I allowed dialysis to take place under a pressure of about 40 mm. of mercury applied to the contents of the dialyser, in order to avoid dilution by endosmosis, but the dilution was found later to be very small when left open to the air, and no difference could be detected between the behaviour of the solutions in the two cases. It was soon found necessary to ensure the presence of an excess of carbon dioxide during the whole course of the dialysis, or as long as any sodium bicarbonate was present. This was done by occasionally passing a current of carbon dioxide from a Kipp generator through the contents of the dialyser or the outer liquid. The reason why this is necessary is because the tension of this gas in the atmosphere is below the dissociation tension of sodium bicarbonate in solution, so that the carbonate is produced. The alkalinity thus brought about is sufficient to enable combination between the protein and sodium. In fact, if a current of air be passed through a 0·03 molar solution of the bicarbonate, this is found to become alkaline not only to phenol-phthalein, but to thymol-phthalein, that is, its H-ion concentration is reduced to 10^{-11}. It has already been pointed out that a sodium bicarbonate cannot exist in the presence of a lower tension of carbon dioxide than 2·25 mm. of mercury, or 0·3 p.c. by volume. This fact must be remembered in the use of saline perfusion fluids containing sodium bicarbonate, which are liable to be more alkaline than supposed.

Taking first serum that had been dialysed against 0·9 p.c. sodium chloride until all the bicarbonate had been removed, on comparing the effect of adding acid to the neutral protein solution, on the one hand, and to the dialysate, on the other, it was found that the addition of one drop of a 0·03 normal hydrochloric acid to 5 c.c. made both acid to neutral red and that the tint was identical in both. The amount of acid added would make the solution 0·0004 normal. Thus the protein present in serum will not combine with even this small amount of acid at or near the neutral point. Put in other words, a solution of serum proteins behaves like 0·9 p.c. sodium chloride towards acids when neutral red is used as indicator. Egg albumin in 6 p.c. solution behaved in the same way.

Taking next serum brought into equilibrium with sodium bicarbonate solution, acid was added to the serum and to the outer solution in sufficient quantity to combine with one-half of the bicarbonate present and then both were brought into equilibrium with alveolar air in order to ensure equal tension of carbon dioxide. The colour change of neutral red was identical in both, namely from orange to orange-red. In the experiments with serum, the colour of the dialysate was matched with that of the serum by the addition of caramel, flavine and hæmoglobin if necessary, but the tint was usually so slight in the dialysed serum

that it was difficult to detect any difference when neutral red was added without the precaution mentioned. In one experiment acid was added to both until all the bicarbonate was decomposed and the solution became $10^{-5.7}$ in H-ion, but no difference in tint could be detected. This is of course merely another form of the former experiments. Serum in equilibrium with bicarbonate behaves like the bicarbonate solution devoid of protein, within the limits referred to.

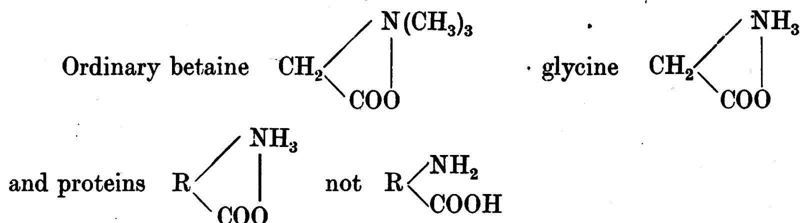
If proteins are able to combine with bicarbonate, that is, if they behave as stronger acids than carbonic acid, the addition of dialysed serum or egg albumin to a bicarbonate solution should change its reaction. If both solutions are neutral, the colour of neutral red in the bicarbonate solution should be changed to the acid side. In point of fact, it is unchanged. If, however, the bicarbonate solution is first aerated and thus converted into carbonate, its alkalinity is sufficient to ensure combination with protein and if neutral dialysed serum be added to such a carbonate solution, which is red to phenol-phthalein, the red colour disappears, owing to formation of the sodium salt of the protein, which is less dissociated than the carbonate.

This is, no doubt, the explanation of the fact to which Moore, McQueen and Webster (1919) call attention, namely, that all the carbon dioxide can be removed from serum by bubbling air freed from carbon dioxide through it. The proteins at the H-ion concentration of 10^{-11} combine with the sodium hydroxide provided by the hydrolytic dissociation of the carbonate and more sodium hydroxide continues to be produced until all the carbonate is decomposed. But it is incorrect to describe the phenomenon, as is often done, as a driving off of carbon dioxide by the proteins acting as acids. The carbon dioxide is removed by the air and the alkalinity resulting is great enough to enable combination between the $Na^{\cdot}$ ions and the protein to take place. It also accounts for the fact to which Buckmaster (1917) calls attention, namely, that carbon dioxide is not given off more rapidly from a bicarbonate solution *in vacuo* when blood is added to it. The proteins do not begin to play a part until the H-ion concentration is reduced to 10^{-10} by production of carbonate.

The following experiment shows that H_2CO_3 is a stronger acid than proteins. Horse serum was aerated by air free from carbon dioxide until its H-ion concentration was $10^{-10.5}$, the proteins then becoming combined with sodium. After exposure to carbon dioxide again, this serum behaved towards different tensions of the gas just as it did originally and like a solution of sodium bicarbonate. It became orange to neutral red with alveolar air, for example. Thus the sodium proteinate was decomposed by carbon dioxide and sodium bicarbonate formed again.

Proteins do not combine with weak acids or bases, as they are also unable to combine with neutral salts. Although some statements have been made, especially by Pfeiffer and Modelski (1912), that amino-acids form definite compounds with calcium and lithium chlorides, I have, in a repetition of their experiments, been unable to confirm the statement. Mixed crystals of all kinds of numerical proportion between the constituents are formed and if particular experiments are selected any proportion desired can be obtained. These experiments will be published elsewhere.

The explanation usually given of this different behaviour of proteins to weak and strong acids is that in neutral solution the acidic (COOH) and basic (NH_2) groups are not free, but combined in a ring formation in some way, and that until the hydrogen-ion or hydroxyl-ion concentration has risen to a certain height the ring remains unbroken and the protein unable to act either as acid or base. The most probable mode of connection seems to be that of an internal ammonium salt similar to the betaines. Thus,



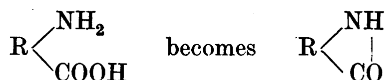
The following experiment shows that glycine itself has a very small capacity of neutralising acid near the neutral point.

The colour of methyl-red in a deci-molar solution of Kahlbaum's glycine was the same as that of the boiled out distilled water with which the solution was made, namely orange. If one drop of 0·03 normal hydrochloric acid was added to 5 c.c. of the glycine solution the colour was changed to red, whereas that of the distilled water became crimson. That is, the increase in H-ion concentration was slightly greater in water than in the glycine solution. But the capacity of glycine at deci-molar concentration is very much less than that of even 0·006 molar bicarbonate. The latter solution is yellow-orange to methyl-red, but by adding a trace of neutral red it can be made to match the glycine when both are brought into equilibrium with alveolar air. The addition of one drop of 0·03 normal HCl changes the colour of the glycine to crimson-red, but has no perceptible effect on the bicarbonate.

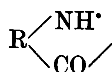
According to Van Slyke and Birchard (1914), the free amino-groups of proteins are represented by half those of the lysine contained. Hartley (1914) finds that serum proteins contain 13·5 p.c. of their nitrogen in the form of lysine, so that 6·75 p.c. is present as free amino-groups. Taking the protein content of serum as 6 p.c. and the nitrogen

content of protein as 15 p.c., the nitrogen as free NH_2 groups works out as about 0·06 or 0·05 p.c. molar, a lower value than that of the glycine used in the above experiment and corresponding with that found by titration with hydrochloric acid to the change of tint of methyl-orange. On the basis of the osmotic pressure of the proteins in serum, its concentration is 0·002 molar; hence each molecule would possess about 25 free amino-groups. The word "free" means, of course, potentially free, that is, not combined as NH—CO.

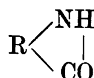
There is another possibility of explaining the behaviour of proteins. An internal anhydride may be formed by removal of water, so that



with a "peptide linkage" similar to that between the constituent amino-acids of the protein, or to that of the amphoteric ion of Bredig and Walker:



in which the opposite charges unite to



Against this latter view is the fact that proteins are not electrolytically dissociated in neutral solution. Pauli was able to dialyze serum until its conductivity was practically that of water. Moreover, it requires the powerful action of strong acid to split this linkage by hydrolysis. On the other hand, the former mode of ring-formation might seem to be too easily broken, but the acidity with which we are concerned is very small, only N 10,000 or N 100,000. Thus, a H- or OH-ion concentration below 10^{-4} is unable to break the linkage and no neutralization of the acid or alkali occurs.

It is well to be clear on the question as to what happens when dilute acid, say N 100 HCl, is added to a neutral solution of a protein until the change of tint of methyl-orange occurs. This indicator tells us nothing until a H-ion concentration of 10^{-4} is reached. At this point, the ring is broken and the free amino groups combine with the acid. That no combination takes place until sufficient acid has been added to change the colour of methyl orange is shown by the behaviour to neutral red, which changes colour as soon as the first trace of acid is added. Owing

to this absence of combination, very little acid is needed to bring the H-ion concentration to 10^{-4}, 1 c.c. of N 100 to 100 c.c. of the solution.

The application of the word "buffer," or more correctly, "tampon," to substances present in blood is apt to suggest more complexity than is actually the case. Practically, the only substance of any importance physiologically is sodium bicarbonate and the mode in which it acts is simple.

Since then there is no evidence that weak acid or alkali can combine with proteins, it seems unlikely that they should be able to carry carbon dioxide from the tissues to the lungs.

When serum dialysed free from bicarbonate is compared with water as regards the change in reaction produced by alveolar air, it is found that the increase in H-ion concentration is just as great in the case of serum as in that of water. It is scarcely possible that any combination could take place without a change in this value.

Of course, if the tension of carbon dioxide to which blood is exposed falls below 2·25 mm. of mercury, the sodium carbonate formed has an alkalinity great enough to combine with proteins and thus lead indirectly to a giving off of carbon dioxide. But the alveolar tension can never be reduced to this value.

The possibility of the formation of protein compounds with carbon dioxide of the nature of Siegfried's "carbamino-acids," in which the NH_2 group is converted into NHCOOH, must be taken into consideration. The experiments given by this investigator (1905), in which calcium hydroxide was added and then carbon dioxide led through, while the changes in electrical conductivity were determined, are not convincing; indeed, the investigator himself seems to have some doubt about them. He does not give any experiments similar to those with glycine and alanine in which an increase of conductivity occurred when carbon dioxide was led through the pure acid. This increase was due to the carbamino-acid being dicarboxylic and a stronger acid than glycine, etc. I was able to confirm this (1907, p. 251), but obtained only a very small and somewhat doubtful effect with leucine, so that a result with protein seems improbable.

The compound of carbon dioxide with glycine is reversible according to the tension of carbon dioxide. Thus, a deci-molar solution of glycine becomes red-orange to methyl-red when in equilibrium with alveolar air. The original orange tint returns when exposed to atmospheric air. If a carbamino-acid were formed, the solution of glycine should become more acid than water does when in equilibrium with alveolar air, because this acid is stronger than carbonic acid. I have been unable to detect any difference. Possibly this tension of carbon dioxide is below the dissociation tension of the carbamino-acid.

Nevertheless, according to Moore, McQueen and Webster (1919), serum between 10 and 80 mm. of mercury has three times as great a carbon dioxide capacity as a solution of bicarbonate of the same concentration as that in the serum. And serum is said to give out or take up more carbon dioxide between 60 and 33 mm. tension than an equivalent solution of bicarbonate does. Their results, however, do not show a very great difference. The bicarbonate may be taken to dissolve the same amount as water does; that is, 4·3 c.c. at 33 mm. and 7·9 at 60 mm., a difference of 3·6 c.c. Serum is stated to have a corresponding difference of 5·7 c.c. p.c. leaving 2·1 c.c. in its favour. This is a very small part of that given out or taken up by whole blood between these values of carbon dioxide tension. According to the curve of Christiansen, Douglas and Haldane (1914), the corresponding value for whole blood would be about 20 c.c. The fact confirms the view taken by Bohr (1887) and by Buckmaster (1917) that carbon dioxide is carried by hæmoglobin. It is possible that the small amount carried by serum in excess of that by sodium bicarbonate may be due to the formation of carbamino-protein, acting as a stronger acid than carbonic acid and driving out part of the carbon dioxide from the bicarbonate. But it is difficult to see how such a compound is afterwards decomposed in the lungs with formation of bicarbonate again and we shall see reason later for doubting the formation of carbamino-protein. Another possibility is that carbon dioxide is adsorbed by the colloidal proteins to a slight extent and this would be reversible. Findlay (1908), however, was unable to detect any influence of egg-white on the solubility of carbon dioxide in water; gelatin slightly increased it.

I have made an experiment of the kind referred to. Serum was dialysed against 0·03 molar sodium bicarbonate solution, taking care that free carbon dioxide was always present. Samples of 10 c.c. of each solution were brought into equilibrium with 10·4 p.c. and with 3·3 p.c. carbon dioxide atmospheres, and the total carbon dioxide driven out in a Van Slyke apparatus by addition of sulphuric acid. The values obtained were:

	Serum	Bicarbonate
10·4 p.c. (79 mm. Hg.)	4·5	4·8
3·3 p.c. (25 mm. Hg.)	3·6	4·2
Difference	0·9	0·6

The original bicarbonate solution gave off, using the same method, 6·1 c.c. of carbon dioxide, so that some dilution had occurred in dialysis.

The difference between bicarbonate and serum is quite unimportant. The smaller absolute amounts given off by serum suggest that the volume occupied by the dissolved protein plays a part in decreasing the volume of the bicarbonate solution available. The somewhat

greater relative amount taken up by the serum at the higher tension suggests adsorption, but the data are not of sufficient accuracy to warrant conclusions on this point. The only explanation that I can offer for the divergence of my results from those of Prof. Moore is that, if his serum had been exposed to the air and the amount of carbon dioxide then taken up by it estimated, it is probable that it contained sodium proteinate, which would take up considerably more carbon dioxide than if sodium bicarbonate alone were present in the serum. But, as already pointed out, the conditions for formation of sodium proteinate are not present in the blood during life.

The result of the preceding experiment seems to exclude the serum proteins from acting as acids in competition with CO_2 for possession of sodium, according to the view put forward by Parsons (1919). Hæmoglobin cannot be used in such experiments, because it takes up CO_2 in absence of sodium salt. But the experiments showing this fact require repetition with solutions dialyzed in presence of CO_2. Bohr's (1887, p. 170) results were indeed obtained with recrystallized hæmoglobin, free from alkali.

A further experiment was made with the same serum after repeated dialysis against 0·9 p.c. sodium chloride. The serum and the last dialysate were saturated with carbon dioxide of 10·4 p.c. In the Van Slyke apparatus, the saline solution gave off 0·8 c.c. while the serum gave off 0·9 c.c. This result shows that no appreciable amount of carb-amino-protein was formed at this tension of carbon dioxide.

Brief reference should be made to the results obtained by Hamburger (1894) when he exposed blood *in vitro* to excess of carbon dioxide. A large increase in the sodium bicarbonate present in the serum occurred. As might be expected, a similar result is to be obtained when a fixed acid is added to blood, so that a great part of the original bicarbonate is neutralised. The acid added merely drives off carbon dioxide, in fact.

Two samples of 25 c.c. each of oxalated cat's blood were taken. One of these was centrifuged at once. To the other, 1 c.c. of 0·3 normal HCl was added before centrifuging. The plasma from both was collected and to the first a volume of acid equal to that already in the second was added. They were thus similar except that in the second the acid had produced the Hamburger effect on the corpuscles before centrifuging. Neutral red was added to both and they were exposed to alveolar air. In the second the colour was orange-red; in the first, crimson, indicating a marked increase in the alkali of the second.

It is clear that if this effect occurs in the body, it must play an important part in neutralising fixed acids, since an additional inflow of bicarbonate into the plasma would be provided. In some experiments which I made in conjunction with Prof. Cannon, we were unable

to obtain any significant increase in the bicarbonate reserve when cats were made to breathe excess of carbon dioxide without decreasing oxygen. *In vitro*, we obtained a marked increase, sometimes the bicarbonate was apparently nearly doubled by the action of carbon dioxide. Although these experiments need repetition, some doubt is thrown on the actual occurrence of the Hamburger effect *in vivo*. Indeed, the effect in general is not satisfactorily explained.

The conclusion to be drawn from the experiments of the present paper is that the only function of the sodium bicarbonate of the blood is to regulate the hydrogen-ion concentration. Since the serum-proteins play no part in this nor, to any appreciable extent, in the transport of carbon dioxide, and other evidence shows that they do not serve as food for the tissues, their only function, in normal conditions, must be to give a colloidal osmotic pressure to the blood, so that it does not lose liquid to the tissues with excessive rapidity, and to contribute to some extent to the viscosity of the blood, which enables a high arterial pressure to be maintained, although the corpuscles play the chief part in this viscosity. When the blood vessels are injured, certain proteins serve to form a clot and they are apparently the source of the anti-bodies to bacterial toxins.

The properties of bicarbonate solutions require consideration in respect to the addition of the salt to liquids used for perfusion. Ringer himself calls attention to the advantage of adding a small amount of bicarbonate in order to neutralise acid produced in the contractions of the frog's heart. Moore and Whitley (1919) point out that if bicarbonate in concentration equal to that in the blood is added to the perfusion fluid used for the mammalian heart, the beats are diminished and may stop, especially if air or oxygen is passed through the solution. Although they are doubtless well aware of the reason for this, Moore and Whitley do not mention it. Since bicarbonate can only exist in the presence of free carbon dioxide, aeration of a solution rapidly converts it to carbonate, and, as we saw previously, the H-ion concentration may fall to 10^{-11}. Mines (1913, p. 20) showed that the optimal H-ion concentration for the frog's heart is $10^{-7 \cdot 9}$. If therefore we desire to use a concentration of sodium bicarbonate as high as it is in the blood, the air or oxygen used for aeration must contain 4 p.c. of carbon dioxide in order to make it equivalent to alveolar air. For purposes of regulation of neutrality it might be of value to use the higher bicarbonate content. Moore and Whitley find that breathing through the perfusion fluid greatly reduces its toxic action. The presence of proteins is only of use

in order to decrease the œdema due to absence of a colloid possessing an adequate osmotic pressure; but gum acacia serves the same purpose.

Summary.

Intravenous injection of acid in sufficient quantity to neutralise half the bicarbonate in the blood does not increase the hydrogen-ion concentration of the plasma. The chief mode of compensation is by increase in pulmonary ventilation and consequent decrease of the carbon dioxide in the blood. Renal excretion of acid, and ammonia production in the liver, do not appreciably come into play in short experiments.

Injection of alkali is not so readily neutralised. The chief agent in this case appears to be excretion of alkaline urine.

The proteins of the plasma play no perceptible part in the maintenance of neutrality between the limits of hydrogen-ion concentration possible in the living organism, namely, below 10^{-4} or above 10^{-10} normal.

No evidence was obtained that either sodium bicarbonate, serum-proteins or both together convey carbon dioxide from the tissues to the lungs.

Thus the only function of the sodium bicarbonate in the blood is to regulate the hydrogen-ion concentration, while, under normal conditions, the only function of the proteins is to give a colloidal osmotic pressure and a moderate degree of viscosity to the plasma.

A simple method of determining the hydrogen-ion concentration of blood by means of indicators is described.

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