SCIENCE

FRIDAY, MARCH 14, 1913

CONTENTS

MSS. intended for publication and books, etc., intended for review should be sent to Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

THE REGULATION OF NEUTRALITY IN THE ANIMAL BODY[1]

IT was a favorite figure of Cuvier's, recurring again and again in his works,[2] to compare life with a vortex into which molecules continually enter, from which they continually depart; meantime the vortex remains, and thus the form of a living thing appears to be more important than the substance. Cuvier's analogy, though almost forgotten, is quite as valid to-day as a century ago, but I suspect that the modern physiologist will be disposed to see in such a view a justification of the study of *conditions* rather than a claim for morphology. What, indeed, is the importance of the anatomy of a whirlpool in comparison with the dynamics thereof?

Now it is the study of conditions within the organism which physical chemistry has contributed to physiology—solution, surface tension, the colloidal state, osmotic pressure, ionization, alkalinity or neutrality—and these are dynamical equilibria rather than in any sense morphological elements. To such conditions Cuvier's figure exactly applies, and provides, moreover, the very best means for their systematic investigation; while the conditions, in turn, most fully reveal that which was partly made clear to Cuvier by the imagination of genius, and, in spite of it, quite certainly in part unknown to him.

The right working of physiological proc-

---

[1] Read in the joint meeting of Section K of the American Association, the American Physiological Society and the American Society of Biological Chemists at Cleveland.

[2] See Merz, "A History of European Thought in the Nineteenth Century," Vol. I., p. 129.

esses depends, then, upon accurate adjustment and preservation of physico-chemical conditions within the organism. Such conditions as temperature, molecular concentration and neutrality are now known to be nicely adjusted and maintained; adjusted by processes going on in the body, maintained by exchanges with the environment. This paper is concerned with those physiological processes whereby the normal reaction of the body fluids is permanently preserved.[3]

Throughout the human body, while life exists, there occurs a regular formation of acid substances, excretory products of metabolism. As they form, these various matters, carbonic acid, sulphuric acid and phosphoric acid in the main, immediately combine, but only partially, according to their several avidities, with the basic constituents of protoplasm and blood. In pathological conditions great quantities of acetoacetic acid and β-oxybutyric acid may be produced and claim their share of base. At irregular intervals varying quantities of acids and bases pour in with the food. Thus, through resulting changes in equilibria between bases and acids, normal metabolism steadily operates to lower the unvarying alkaline reaction (almost neutrality[4]) of the body. This tendency to acidity is held sharply in check by special protective mechanisms, acting coordinately, in cooperation and regular succession.

The chemical reactions whereby such material is first neutralized, the chemical substances which aid in neutralization, the shares of more important substances in the process, and their efficiency, the changes in chemical equilibria, including resulting changes in hydrogen and hydroxyl ion concentrations, all, *so far as they concern true solution,* are known with a fair approach to certainty. Principally this work of neutralization is done by salts of phosphoric and carbonic acids, with aid from the amphoteric proteins. In simplified form the process may be represented by the two reactions,

$$M_2\mathrm{HPO}_4 + \mathrm{H}A = MA + M\mathrm{H}_2\mathrm{PO}_4;$$

$$M\mathrm{HCO}_3 + \mathrm{H}A = MA + \mathrm{H}_2\mathrm{CO}_3,$$

where M stands for any basic radical, A for any acid radical. Other less important simultaneous reactions are of the same type, except perhaps the union of the weak acids with basic proteins like globine, and the union of bases with more acid proteins. Through the remarkable circumstance that phosphates and carbonates possess, among all known chemical substances, the highest power to preserve neutrality in solution,[5] this function is so well performed that the alkaline reaction of the body scarcely varies, even when the load upon the mechanism is heavy.

But, however efficient such an arrangement may be, it is of its very nature only the first stage in the process of the excretion of acid, and wholly dependent upon constant support by the kidney, and of course upon a supply of alkali in the food. Regularly, as they form, the acid bodies must be afforded alkali by blood and protoplasm, for every molecule of carbonic acid about 0.93 molecule of alkali, for every molecule of phosphoric acid 1.89 molecules of alkali, and for every molecule of sulphuric acid 2 molecules of alkali, in accordance with chemical laws and the normal reaction of the body. Clearly, therefore,

---

[3] See L. J. Henderson, "The Theory of Neutrality Regulation in the Animal Organism," *American Journal of Physiology,* XXI., 427, 1908, and "A Critical Study of the Process of Acid Excretion," *Journal of Biological Chemistry,* IX., 403, 1911.

[4] The terms may be used interchangeably for an alkalinity which is so slight.

[5] L. J. Henderson, *American Journal of Physiology,* XXI., 173, 1908.

this neutralization must rest upon physiological processes which serve to reestablish the original conditions, for if such great amounts of alkali were discharged from the body with the acid excretory substances, the organism would lose its protection and acidity would speedily ensue throughout the system. Thus an imperative necessity arises for the retention of a part of the alkali which serves as a carrier in the process of removing acid from the body. Of course the necessary magnitude of such alkali retention by the kidney varies with the net amount of alkali ingested and with the acid formation of the body.

The conditions in man are closely paralleled by those in other higher animals, and there is reason to believe that constancy of alkalinity is quite the earliest and most universal physico-chemical regulation of active protoplasm. In fact, as the investigations of Palitzsch[6] show, the ocean itself is likewise quite constant in its alkalinity. It is worthy of note that this is due to the simultaneous presence of carbonic acid and bicarbonates in the sea water, a fact which lends support to Macallum's ideas about the derivation of the body fluids. Thus active protoplasm everywhere, as well as that which surrounds it—the environment and the *milieu intérieur*—appear to be and to have been always of stable reaction.

According to the modern theory of solution water itself, like the dissolved electrolytes, is dissociated into ions, though only to a very slight degree. The reaction is expressed thus:

$$H_2O = \overset{+}{H} + O\overset{-}{H}.$$

If the water be pure the concentrations of hydrogen and hydroxyl ions are necessarily equal, for water is electrically neutral. A variety of independent methods of estimation have shown that at 25° this concentration amounts almost precisely to N/10,000,000 in the ordinary units. This corresponds to 0.0000001 gram of ionized hydrogen and 0.0000017 gram of ionized hydroxyl in 1,000 grams of water. Further, the theory of solution explains acidity in water by the occurrence of hydrogen ions, formed from dissolved electrolytes, in excess of hydroxyl ions; and alkalinity by a similar excess of hydroxyl over hydrogen ions. Neutrality is, accordingly, the condition when, as in pure water, the two concentrations are equal. In short, expressing the concentration of ionized hydrogen by $(\overset{+}{H})$ and of ionized hydroxyl by $(O\overset{-}{H})$, if

$$(\overset{+}{H}) = \frac{N}{10,000,000} = (O\overset{-}{H})$$

the solution is neutral. If

$$(\overset{+}{H}) > \frac{N}{10,000,000} > (O\overset{-}{H})$$

the solution is acid. If

$$(\overset{+}{H}) < \frac{N}{10,000,000} < (O\overset{-}{H})$$

the solution is alkaline.

It remains to point out that implicit in these definitions is the well-founded hypothesis that in water the concentrations of hydrogen and hydroxyl ions vary inversely, so that, with constant temperature, under all circumstances their product is constant:

$$(\overset{+}{H}) \times (O\overset{-}{H}) = K.$$

Thus the nature of acidity and alkalinity may readily be represented by a straight line with the neutral point at its center, acidity increasing in one direction, and alkalinity in the other.

Whenever a weak acid is present in aqueous solution in company with such bases as sodium, potassium, calcium, magnesium, etc., which are invariable constitu-

---

[6] *Comptes-rendus des travaux du Laboratoire de Carlsberg*, X., 85, 1911.

				Acidity				|			Alkalinity				
$(\overset{+}{H})$	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}	10^{14}
$(\overset{-}{OH})$	10^{-14}	10^{-13}	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1 −

ents of the ocean, blood, protoplasm, etc., *provided the acid be in excess,* it is a simple matter to determine the reaction, which can best be measured by the values of $(\overset{+}{H})$ and $(\overset{-}{OH})$, following the considerations above.

For this we possess a host of reliable data and a tried and well-seasoned theory —the mass law. Now there is, in connection with the application of the mass law to ionization, a certain characteristic property of an acid, its ionization constant, k, which measures its tendency to dissociate in aqueous solution, thereby to produce hydrogen ions, and hence to increase the intensity of acidity. Strong acids have ionization constants which are of the order of magnitude of 1.0, weak acids of the order of magnitude of 0.0001, the weakest acids, 0.00000001, or less.

TABLE OF IONIZATION CONSTANTS

HCl, HNO_3, etc.	1
H_3PO_4	0.011
H_3AsO_4	0.005
HNO_2	0.0005
H_2CO_3	0.0000003
NaH_2PO_4	0.0000002
H_2S	0.000000091
H_3BO_3	0.0000000007
Na_2HPO_4	0.00000000000036

It has been discovered that in the general case above discussed of weak acid and salt, the concentration of ionized hydrogen is always almost exactly proportional to the ratio of free acid to salt, and is equal, in very close approximation, to the product of this ratio by a number slightly greater than the ionization constant of the acid. That is to say, representing free acid by HA and salt by BA.

$$(H) = k \times \frac{HA}{BA},$$

whence, if $k = (\overset{+}{H})$

$$\frac{HA}{BA} = 1.$$

From this relationship therefore follows the conclusion, fully established by experiment, that whenever in such a solution the excess of acid, HA, is chemically equivalent to the quantity of salt, BA, the hydrogen ion concentration is almost exactly equal to the ionization constant of the acid, and this is one of the very best methods quickly to detect and characterize an acid. But the ionization constant of carbonic acid (first hydrogen atom) at room temperature is 0.0000003. Hence, in a solution containing exactly equivalent quantities of free carbonic acid, for example, sodium bicarbonate, the hydrogen ion concentration must be approximately 0.0000003 N. Further, since

$$\frac{HA}{BA} = \frac{(\overset{+}{H})}{k},$$

if the amount of acid be ten times the amount of salt

$$\left(\frac{HA}{BA} = 10\right)$$

the hydrogen ion concentration must be about 0.000003 N, and if the reverse be the case

$$\left(\frac{HA}{BA} = \frac{1}{10}\right)$$

the value must be nearly 0.00000003 N.

The range of variation of concentration of hydrogen ions in the usual solutions of the chemical laboratory considerably surpasses the limits of 1.0 N and 0.00000000000001 N. In comparison with such enormous differences those between 0.000003 N and 0.00000003 N are almost negligible (1/100: 1/100,000,000,000,000). Hence ordinarily it is quite accurate enough to speak of any solution containing both free carbonic acid and a bicarbonate, when the disparity between the concentrations of the two substances is not very great, as of constant reaction. For, obviously, the neutral point, which at a temperature of 25° amounts to a concentration of hydrogen and hydroxyl ions 0.00000001 N, falls well within the narrow range of reaction of such solutions, being characterized by a ratio of carbonic acid to bicarbonate of about 1:3.

Thus carbonic acid, like the almost equally weak acid, phosphoric acid (after its first hydrogen has been neutralized by base), has the remarkable property of preserving a neutral reaction whenever it exists in solution with its salts, provided there be an excess of acid. All acids whose strength is even a little either greater or less than carbonic acid lack the property. There is nothing mysterious about this fact; any other weak acid will hold constant the reaction in its own range of reaction; thus acetic acid in the neighborhood of a hydrogen ion concentration N/100,000, etc.

This characteristic of carbonic acid is of the utmost significance, first by regulating one of the most fundamental of physico-chemical conditions, and secondly, by preserving throughout nature the characteristic chemical inactivity of water, which disappears whenever the reaction becomes either appreciably acid or appreciably alkaline. Almost the only case of important geological action due to acidity or alkalinity of water is the action of fresh water, containing carbonic acid itself, to weather the rocks. This process is however self-limited, for the dissolved material forms bicarbonates, and thus at once provides permanently balanced solutions.[7]

Elsewhere, within and without the organism, carbonic acid is almost always accompanied by bicarbonates, and a close approach to neutrality is the result. In the organism the variation in ratio of phosphates is similar to the case of the carbonates, as may readily be illustrated by experiment. Thus a solution consisting of equal parts of monosodium phosphate and disodium phosphate will be found to give a neutral reaction with both methyl orange and phenol phthalein, and the neutrality, thus indicated, will not be disturbed by the addition of relatively large amounts of either acid or alkali.

We may next consider the equilibrium within the organism, where the concentration of ionized hydrogen can undoubtedly vary between 5 N/100,000,000 and N/10,000,000, but during life probably not much more widely, in the body at large. At body temperature the most probable values of the ionization constants of the acids in question yield the equations:

$$(\overset{+}{H}) = 6.9 \times 10^{-7} \times \frac{H_2CO_3}{NaHCO_3},$$

$$(\overset{+}{H}) = 2.1 \times 10^{-7} \times \frac{NaH_2PO_4}{Na_2HPO_4}.$$

If

$$(\overset{+}{H}) = 0.5 \times 10^{-7}\ N$$

$$\frac{H_2CO_3}{NaHCO_3} = \frac{1}{13.8}, \quad \frac{NaH_2PO_4}{Na_2HPO_4} = \frac{1}{4.2},$$

and if

$$(\overset{+}{H}) = 1.0 \times 10^{-7}\ N$$

$$\frac{H_2CO_3}{NaHCO_3} = \frac{1}{6.9}, \quad \frac{NaH_2PO_4}{Na_2HPO_4} = \frac{1}{2.1}.$$

In short, in order to bring about this seemingly insignificant change in reaction, the relative quantities of acid and base in the body must undergo very great changes; or, otherwise stated, until very large quantitative changes in the amount of acid or base in the body have come about, there can be no appreciable change in the reaction.

---

[7] L. J. Henderson, "The Fitness of the Environment," Chapters IV. and V. New York, The Macmillan Company, 1913.

In the case of carbonic acid the equilibrium is further complicated by the activity of the lung in excreting the free acid and regulating the concentration of that substance, which is kept nearly constant. Thus, when acid reacts with bicarbonate in the body, it diminishes the latter substance without increasing the amount of the acid. In this manner through the escape of carbonic acid, the efficiency of the equilibrium in the preservation of neutrality is further greatly increased. Thus it is that even in extreme acid intoxication, as for instance diabetic coma, almost the only chemical change that can be detected, as a result of the action of enormous quantities of acid through long periods of time, is a large diminution in the bicarbonates of the blood; in the instances above calculated this would amount to a decrease of about 50 per cent. in the total carbonic acid. Meantime about 20 per cent. of the phosphoric acid of the body will probably be changed from alkaline to acid phosphate, and the proteins will have given up a portion of the alkali with which they are combined.

The recognition of the fact that diminution of bicarbonates is the principal effect of acid intoxication upon the blood, involves important consequences. On the one hand it has become clear that the therapeutic use of sodium bicarbonate is desirable in a large variety of pathological conditions and, on the other hand, it seems to be certain that the evil effects of acidosis largely depend upon interference with the transport of carbonic acid and its excretion from the body. In truth this equilibrium is intimately associated with the respiratory function, and with a great number of other fundamental physiological activities, and with the osmotic pressure of the cell.

Further the profound influence of hydrogen and hydroxyl ions upon many enzymatic processes, and upon colloids in general has been established, and it is gradually becoming clear that all the physicochemical conditions in protoplasm—alkalinity, osmotic pressure, colloidal swelling, chemical equilibrium, temperature—are interdependent, and that carbonic acid and the acid-base equilibrium are among all these things probably the most important variables.

The reason why it may be asserted that carbonic and phosphoric acids and the proteins are the only important substances which are involved in the physiological regulation of neutrality is that, for the body as a whole, in the narrow range of reaction which can actually occur, these substances can neutralize about 30 liters of 0.1 N acid, and nothing else except other substances of like ionization constants, in equal concentration, even with the advantage of the escape of acid, can do as much.

It can not be too strongly emphasized that this conclusion applies only to the true aqueous solutions of the body. Of the colloidal phase we have no knowledge, but it is evident that they may act as reservoirs of supply and as vehicles of escape.

It is also evident that, if enough acid be produced locally, for instance, lactic acid in the muscle, the protective mechanism may be overthrown, and true acidity result. There is an important connection between this consideration and theories of fatigue and muscular contraction.

But, as for the assertions themselves, they rest upon one of the immutable properties of matter. Phosphoric and carbonic acids in solution everywhere possess this characteristic, independent of the presence of everything else, just as they everywhere possess their characteristic chemical composition.

Thus it is that the regulation of reaction of blood and protoplasm manifests the very highest stability.[8]

In exactly the same way that neutrality is favored by the ionization constant of phosphoric acid, the excretion of acid is facilitated. By variation of the relative amounts of acid and alkaline phosphates the relative amounts of acid and basic constituents of the urine may be varied in the highest degree, accompanied by the very smallest possible variations of hydrogen ion concentration. Thus the presence of phosphoric acid makes of the urine an ideal regulatory apparatus for the preservation of the normal ratio of acid to base in the blood.

Such are the more striking aspects of this subject. Neutrality is quite as definite, quite as fundamental and quite as important a characteristic of the organism as its temperature, or osmotic pressure, or in fact anything else that we know. And it turns out to possess those remarkable characteristics of stability that have been revealed by the researches of Rubner and others in the case of temperature, only in far higher degree.

Within wide limits of amount any acid or base may be poured into the organism, and the reaction will not vary; nor will it vary if such be produced by the organism, and this constancy will protect all enzymatic processes, the function of respiration and the whole distribution of material throughout the body.

Let us return to Cuvier's vortex. Into it let us pour anything, for example hydrochloric acid. No sooner has it entered than it is neutralized, and neutralized it remains until, on leaving the body, it appears as sodium chloride, ammonium chloride and a slightly heightened excess of acid phosphate over alkaline phosphate in the urine.

The urine is variable, the ingesta are variable, even the products of metabolism are variable; but, while life endures, the dynamical equilibrium of hydrogen and hydroxyl ionizations persists.

L. J. HENDERSON
HARVARD UNIVERSITY

THE PHYSIOLOGICAL SIGNIFICANCE OF SOME SUBSTANCES USED IN THE PRESERVATION OF FOOD[1]

FOOD preservatives are those substances which delay or prevent the decomposition of food as a result of the action of bacteria or other ferment-producing organisms. Other substances, sometimes classed as preservatives, and in the popular mind associated with them, are not so much preservatives as agents for the conservation of some special property of the food in question, as, for example, the use of copper sulphate in the fixation of the color of green vegetables. A large number of bodies may be included under the head of preservatives, but our interest to-day centers in the so-called "artificial" or "chemical" preservatives, because of the question of the permissibility of using them. Some of these bodies have been condemned largely because of their origin, because of their artificial character, which basis of condemnation can not be regarded as sufficient or scientific.

Their merits or faults must be decided on the basis of physiological behavior, essentially, and from this point of view I wish to speak of several substances concerning which the discussions have been

---

[8] For a larger survey of this whole subject two articles in the *Ergebnisse der Physiologie*, VIII., 254, 1909 (the author) and XII., 393, 1912 (Sörensen) may be consulted.

[1] A paper read before the Fifteenth International Congress on Hygiene and Demography, Washington, September 23, 1912.