A PHARMACEUTICAL STUDY OF p_H.*

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FORMULATION OF p_H.

Applying the law of mass action to an acid, HA, which ionizes according to the equation $HA \rightleftarrows [H^+] + [A^-]$, we find that an equilibrium exists according to the equation

$$\frac{[H^+][A^-]}{[HA]} = Ka,$$

in which Ka = the dissociation constant of the acid. The dissociation constant of a base is given by the corresponding equation.

Water dissociates to an extremely small extent according to the equation: $H_2O \rightleftarrows [H^+] + [OH^-]$. Applying the law of mass action to this dissociation, we obtain

$$\frac{[H^+][OH^-]}{[H_2O]} = Kw.$$

The concentrations of H^+ and OH^- in pure water or in dilute aqueous solutions are so small compared with the concentration of undissociated water molecules, that the concentration of the latter, $[H_2O]$, may be considered as constant during the dissociation. The equation then becomes $[H^+] \times [OH^-] = Kw$. The value of Kw at 25° C. has been found to be 1.0×10^{-14} (5), (6), (7), (8), (21), (31). In pure water, the concentrations of the H-ions and the OH-ions must remain equal. Therefore,

$$[H^+] \times [OH^-] = [H^+]^2 = [OH^-]^2 = 1 \times 10^{-14}.$$

Then, $[H^+] = [OH^-] = \sqrt{1 \times 10^{-14}} = 1 \times 10^{-7}$. This equation means that pure water or a neutral aqueous solution at 25° C. contains 1×10^{-7} moles of H-ions and 1×10^{-7} moles of OH^- ions per liter and is a 1/10,000,000 normal solution of both H- and OH-ions.

For practical reasons, hydrogen-ion concentrations are expressed as the logarithms of their reciprocals. A common logarithm is an exponent to the base 10. Thus the symbol p_H signifies "hydrogen exponent." The definition of p_H may be given mathematically:

$$p_H = -\log [H^+] = \log \frac{1}{[H^+]}.$$

Since the hydrogen-ion concentration varies in a definite reciprocal manner with hydroxyl-ion concentration, the p_H scale may be used to express degrees of alkalinity as well as degrees of acidity, as in the following synopsis.

From this data it is seen that, while the p_H values vary according to arithmetic progression, the corresponding hydrogen-ion concentrations vary according to geometric progression.

Acidity.	Concentration of H- or OH-Ions.	Alkalinity.
p_H 7 (neutral)	N/10,000,000	p_H 7 (neutral)
p_H 6	N/1,000,000	p_H 8
p_H 5	N/100,000	p_H 9
p_H 4	N/10,000	p_H 10
p_H 3	N/1,000	p_H 11
p_H 2	N/100	p_H 12
p_H 1	N/10	p_H 13
p_H 0	N/1	p_H 14

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FORMULATION OF BUFFER CAPACITY.

It would be of little value to discuss in this paper the action of buffers; however, since there are many references in the pharmaceutical literature to a very valuable formulation of buffer effect, this one expression will be explained here. Van Slyke (53) has adopted a unit for the buffer capacity of solutions. This unit is the differential ratio $\frac{dB}{d(p_H)}$ expressing the relationship between the increment (in gram equivalents per liter) of a strong base B added to a buffered solution and the resultant increment in p_H. An increment of strong acid is equivalent to a negative increment of base, or $-dB$. In these terms, a solution has a buffer capacity of 1 when a liter will take up 1-Gm. equivalent of strong acid or alkali per unit change in p_H. If a base is added, the p_H is increased, so that both dB and $d(p_H)$ are positive. If acid is added, both dB and $d(p_H)$ are negative. The ratio $\frac{dB}{d(p_H)}$ is, therefore, always a positive numerical value. This is called Van Slyke's ratio and is often designated by B. When this ratio is applied, for instance, to tinctures and fluidextracts, and when the buffer capacity is plotted with the quantities of acid as ordinates and the corresponding increments in p_H as abscissæ, the curve is usually a straight line within reasonable limits. From such a graph we obtain the ratio $\frac{\Delta B}{\Delta(p_H)}$ where each of these values is a measurable increment. Within the limits of the straight line curve, the buffer capacity obtained using the measurable increments is identical with that of the differential ratio $\frac{dB}{d(p_H)}$. Van Slyke's ratio is much used in adjusting pharmaceutical preparations to specified p_H values.

FORMULATION OF ELECTRODE POTENTIALS.

The most direct formulation of an electrode potential is as follows: If a metal is put in pure water, some of the metal dissolves forming positively charged ions, and consequently, the remaining metal assumes a negative charge. Three principal forces are present—the solution pressure of the metal, the electrostatic force of the charged ions, and the osmotic pressure of the solution. An equilibrium of the forces is soon reached. Assume an electrode, such that 1-Gm. mole of ions of charge n, carrying nF faradays of electricity (a faraday is the number of coulombs carried by a gram equivalent of an ion) pass from the electrode to the solution, increasing the partial osmotic pressure by dp. Thus n is equivalent to the valence of the metallic ions. The change in potential difference between the electrode and the solution will be dE. The electrical work expended will be nFdE and the work taken up by the system will be Vdp. Therefore, $nFdE = Vdp$.

According to the laws of an ideal gas, $V = \frac{RT}{p}$ in which p = pressure in atmospheres, V = volume in liters, R = the gas constant, and T = the absolute temperature. Thus, by eliminating V, $dE = \frac{RT}{nF}\frac{dp}{p}$. Integrating gives $E = \frac{RT}{nF}\log_e p + c$, where $\log_e$ is the natural logarithm to the base e, and c is some integration constant. In dilute solutions, p is equivalent to the activity of the ions. By introducing known values for R and F (R = 8.315 international joules and F = 96,500 coulombs) and, by transposing the natural logarithm into the common logarithm by multiplying by 2.3026, $E = 0.0001984\frac{T}{n}\log_{10} c\ ion + C$. Thus, at 25° C. $E = \frac{0.0591}{n}\log_{10} c\ ion + C$. C may be eliminated by equating E against ϵ_0 (normal potential of the metal, that is, the difference in potential between it and the normal hydrogen electrode in a solution whose ion activity is equal to 1). The normal hydrogen electrode is defined by international agreement according to the following specifications:

> "The potential at a hydrogen electrode under one atmosphere pressure of hydrogen in a solution of unit hydrogen activity shall be considered zero at all temperatures."

By eliminating the integration constant C, we arrive at the fundamental equation for converting electrode potentials to hydrogen-ion concentrations: $E = \epsilon_0 + \frac{0.0591}{n} \log_{10} c_{ion}$ (25° C.).

HISTORY OF METHODS.

The electrolytic dissociation theory of Arrhenius (2) and the application of the law of mass action to ionic equilibria marked the beginning of hydrogen-ion control. The dissociation constant of water was determined in various ways by Ostwald (5), Wijs (6), Nernst (7), Kohlrausch and Heydweiller (8), Sorensen (21) and Lewis, Brighton and Sebastian (31). These steps furnished the basis for the mathematical conception of hydrogen-ion concentration. Sorensen (21) suggested that the numerical values of the negative exponents of hydrogen-ion concentration values be adopted as the basis of an acidity scale and recommended the symbol $P_H{}^+$. Clark (110) introduced the simpler symbol, p_H, which is now used more extensively.

Pertaining to the potentiometric method of determining p_H, Nernst (3), Peters (10) and Crotogino (12), using the ideal gas laws (59) and Faraday's laws of electrolysis, have shown the logical derivation of a common expression for all electrode potentials. The hydrogen electrode was first used by Bugarszky and Liebermann (9) who applied it to a biochemical problem. Later, a thorough treatise on the hydrogen electrode was published by Hildebrand (25). The calomel electrode was developed through the early efforts of G. N. Lewis and his colleagues (20), (31), (59). Our fundamental information regarding the quinhydrone electrode is largely due to Biilmann (39), (40), (56). The development of the glass electrode dates back to Helmholtz (1), who, in 1881, first constructed a glass electrode. The further development of this electrode was carried on by Haber and Klemensiewicz (19) and Kerridge (76).

The first survey of indicators suitable for hydrogen-ion determinations was performed in Nernst's laboratory in 1904 by Salessky (15). The results of this and many other surveys were summarized in Salm's famous table (16). Then came the classic work of Sorensen (21) in which he eliminated a vast number of faulty indicators and organized a series of indicators suitable for biological work. Clark and Lubs (29) also selected a series of indicators which was composed mostly of sulphonphthaleins.

HYDROGEN-ION CATALYSIS.

Most drugs can be classified into two groups on the basis of optimum p_H stability: those having maximum stability at p_H 7 and those having maximum stability very near p_H 5. This suggests two distinct factors affecting the relation between hydrogen-ion concentration and stability of drugs. Stability in the absence of acidity or alkalinity is easily understood but stability at p_H 5 must be explained on the basis of hydrogen-ion catalysis.

The majority of reactions in solution appear to be catalyzed by hydrogen ions and hydroxyl ions. The experiments of Arrhenius (4) on the inversion of sucrose by weak acids in the presence of the corresponding salt gave results which showed that the catalytic activity of acids in aqueous solution is approximately proportional to the degree of dissociation as determined by electrolytic measurements. Later Arrhenius (11) found that the rate of inversion of sucrose by weak acids was greatly augmented by the addition of the neutral salts of strong acids; for example, KCl increased the catalytic activity of CH_3COOH. Arrhenius, therefore, postulated that dissolved salts increase the dissociation constants of weak acids in solution. (Taketomi and Horikoshi (295) have recently shown that while KCl and NaCl increase the rate of sucrose inversion by acids, K_2SO_4 and Na_2SO_4 decrease the rate of inversion.) In 1900, Euler (13) proposed a theory describing the catalytic effect of hydrogen ions and hydroxyl ions as an instantaneous salt formation. This conformed to the view commonly held (but certainly not justified) that ionic reactions are more rapid than reactions between electrically neutral molecules. In 1913, Stieglitz (26) proposed a theory agreeing with that of Euler.

The view that the catalytic effect represents the result of two simultaneous changes involving a hydration of the hydrogen ion and a change in the non-ionized acid was developed by a number of workers (17), (18), (22), (24), (27). This became known as the dual theory of acid catalysis and served as the first conception of the addition products formed between hydrogen ions and other substances. Kendall (43) developed this theory and postulated that the number of catalytically active particles is much greater than is ordinarily supposed. He recognized several

types of hydrogen ions, such as H^+, $[H.H_2O]^+$, $[H.CH_3COOCH_3]^+$, $[HCl.H_2O]$, $[H.CH_3COOH]^+$, etc., existing in a complex series of equilibria and each possessing a different catalytic activity. Rice (61), in 1923, recognized such equilibria and ascribed the catalytic effect of hydrogen ions to the free ions and correspondingly to the free or unhydrated hydroxyl ions. This leads to the conclusion that stoichiometrically neutral water is distinctly alkaline catalytically, and it is not until the hydrogen-ion concentration has a value of about p_H 5 that the concentrations of the unhydrated ions become equal and the catalytic activity is at a minimum.

The views which are most in favor to-day were put forward by Bronsted (109), in 1928. Bronsted first contended that the law of mass action cannot be applied in its classical form to ionic reactions. He developed a new mathematical conception of hydrogen-ion catalysis and a new definition of acids and bases. The new definition is represented by:

$$\underset{\text{(acid)}}{A} \rightleftharpoons \underset{\text{(base)}}{B} + H^+.$$

The mechanism of hydrogen-ion catalysis can then be visualized as being a transfer of a proton from catalyst to substrate (acid catalysis) or from substrate to catalyst (basic catalysis). The success of Bronsted's hypothesis consists in its ability to predict the changes in reaction velocity due to the addition of other molecules and ions by calculating the change in concentration of the complex between the reacting substances. Bronsted's work has been verified by later investigators.

The existence of various hydrogen-ion complexes is associated with the so-called "activities" of hydrogen ions. The term, hydrogen-ion activity, will be retained in this thesis.

Olivier and his co-workers (321), (322), (323) have recently published data concerning the relation of the nature of the substrate to its sensitiveness to hydrogen-ion and hydroxyl-ion catalysis.

EFFECT OF ELECTROLYTES ON p_H.

Electrolytes have considerable influence upon both the p_H and the stability of solutions. The fundamental effect of electrolytes is by no means understood. Some of the most valuable data concerning the effects of electrolytes will be cited.

Thomas and Baldwin (34) claimed that electrolytes increase the acidity of acid solutions in the following order: $MgCl_2 > LiCl > NaCl > KCl = NH_4Cl > BaCl_2 > MgSO_4 > Na_2SO_4 > (NH_4)_2SO_4$. The sulphates decrease the acidity. Lepper and Martin (87) stated that the influence upon p_H of the cations of univalent alkali metals varies inversely as their atomic weights. They found that NaCl reduces p_H and reduces the activity of anions which results in an increased dissociation of any acids which are present. To account for their results they assumed that NaCl increases the dielectric constant of water. Loeb (33) advanced a theory which assumes that neutral salts with univalent or bivalent cations assume a charge which is negative with reference to that of water particles and that neutral or acid salts with trivalent or tetravalent cations assume a charge which is positive with reference to that of water particles. Taft and Malm (228) found that in alkaline gum arabic solutions, the presence of neutral salts increases the p_H values. Northrop (45) found that neutral salts decrease the p_H of acid gelatin and increase the rate of its hydrolysis. Britton and Dodd (257) found that NaCl in a concentration as high as tenth normal does not affect the p_H of alkaline sodium hypochlorite solution. Taketomi and Horikoshi (295) stated that the rate of inversion of sugar by acids is increased by NaCl and KCl, but is decreased by Na_2SO_4 and K_2SO_4. Schmidt (176) claimed, with regard to the flocculation of diphtheria toxin and antitoxin, that there is a range of electrolyte concentration in which no flocculation occurs. Above or below this range flocculation does occur.

PHYSIOLOGICAL p_H.

Reaction of Distilled Water.—It is well known that distilled water of p_H 7.0 is seldom obtained. Water is considerably acid after one distillation and soon becomes alkaline during storage in ordinary glass. Acree and Fawcett (142) found that the solids and carbon dioxide in once-distilled waters gave them p_H values of about 5.0–5.5. Only by repeated distillations in tin apparatus and in filtered and carbon dioxide-free air can water be obtained of p_H about 6.9. By this method the authors obtained their so-called "super-pure water" having a specific conductivity

of 0.04×10^{-6} at 18° C. Acree and Fawcett included tables which showed the errors in p_H caused by the dilution of buffers with ordinary p_H 5.0 distilled water and with p_H 5.7 air-carbon dioxide equilibrium water. Williams and Swett (55) obtained fresh, distilled water of p_H 6.8; after 48 hours in contact with air the p_H became 5.23. Stock glucose solutions had a p_H of less than 5.0. Bruck (193) has observed the change in p_H during storage of water not in contact with air. Freshly distilled samples had a p_H of 5.65; when stored in ordinary corked glass the p_H reached 9.00 in 20 days. After standing 12 days more exposed to the air, the p_H was 7.80. Freshly distilled water was sterilized in Hageda ampuls and, in 5 weeks, the p_H changed from 5.55 to 7.5–8.0. Distilled water was placed in corked flasks of Jena glass and, within 4 weeks, the p_H remained between 5.4 and 5.5. Mattheus (217) reported that water which was sterilized and stored in washed Jena ampuls for 2 years increased in p_H from 6.2 to 6.4–7.0, and increased in total solids by 0.0048 Gm. per liter. Klobusitzky (273) has devised a method for testing glass ampuls to determine if their reactions are suitable.

Adjustment to Blood p_H.—Human blood has a p_H range from 7.3 to 7.5, being fairly constant at p_H 7.4. p_H values for blood or tissues above or below these figures are not compatible with life. A patient becomes comatose if a p_H of 7.1 is reached, or convulsive with a blood p_H above 7.5. Jacobs and Parpart (210) reported that hemolysis by hypotonic solutions was measurably increased by p_H changes of as little as 0.01 unit. It is important, therefore, that the p_H of solutions for injection be as close as possible to 7.4 and that the p_H values of these solutions remain stable during sterilization.

Physiological salt solution, when prepared with ordinary distilled water, has been found by Williams and Swett (54) to be dangerously acid. Fleisch (49) has criticized Ringer's and Tyrode's solutions, claiming the first to be too acid and the other too alkaline. He reported the following method for preparing a stable, sterilizable nutritive solution of physiological p_H: To 10.5 Gm. NaCl, 0.5 Gm. KCl, 0.3 Gm. $CaCl_2$, 0.1 Gm. $MgCl_2$ and 5 cc. *N* H_3PO_4, add H_2O *q. s.* to 58.7 cc. Filter, add 50 cc. of this to 1 liter of water and sterilize. Then saturate with oxygen and add 5 cc. of sterile *N* Na_2CO_3. Hansen, Schou and Tonnesen (268) found that invert sugar solutions have a p_H of about 3.0 when the inversion occurs during sterilization in sealed ampuls. Williams and Swett (54) reported that 10% glucose solutions of p_H 8.58 became as acid as p_H 4.9 when boiled or autoclaved. In a later publication (55), they stated that when these solutions were buffered to a p_H of 7.4 with KH_2PO_4 and K_2HPO_4 there were no unpleasant reactions following their free use.

In the same article they reported the p_H values of various sterilized commercial solutions. They found that a sodium citrate solution used in a transfusion where a violent reaction followed had a p_H of 10.25. Solutions of dyes for intravenous and intramuscular injection had a p_H of 5.0. When properly buffered, 10–40% more of these dyes were excreted. Solutions of arsphenamine, tetanus antitoxin and antipneumococcus serum were all too alkaline.

The sterilization of cocaine hydrochloride is always accompanied by increased acidity. Roy (78) reported p_H values of cocaine hydrochloride solutions, as follows: 1% solution, 5.20; 2% solution, 4.50; 3% solution, 3.75. The p_H of the 1% solution, sterilized for 30 minutes at 100° C., became 4.60. Regnier and David (285), (286) observed the p_H change of cocaine hydrochloride solutions during and after sterilization. The p_H values dropped from 5.9–6.0 to as low as 3.4 after 80 days' storage following sterilization. The authors pointed out the dangers of the pharmacological use of such acid solutions. In a later publication (287) the authors found that when the cocaine hydrochloride is buffered to a weakly acid reaction by Na_2CO_3 and NaH_2PO_4 or NaH_2PO_4 and Na_2HPO_4, the p_H remains stable during sterilization.

Dietzel and Huss (111) have found that alkaline or soluble glass causes considerable p_H change during the sterilization of morphine hydrochloride solutions. The instability caused by the hydroxyl ions was found to be catalytical. They recommended the use of Jena or quartz glass. Roy (78) found that novocaine solutions become dangerously acid (p_H 4.2–4.3) during sterilization. Later, Rae (223) suggested that the only safe way of sterilizing novocaine solutions is by filtering through a Chamberland filter fitted with Jena glass. Goldberg (266) stated that the p_H of procaine hydrochloride, mixed with epinephrine, can be stabilized between p_H 5.7 and neutrality by a water solution of Na_2HPO_4 and NaH_2PO_4.

Roy (78) claimed that the hydrogen-ion concentrations of all hydrochlorides of amine alcohols increase considerably during sterilization, producing small quantities of benzoic acid.

Besides cocaine and novocaine, he considered in his article stovaine and atropine sulphate. During sterilization the p_H of stovaine dropped from 5.40 to 4.0 and that of atropine sulphate changed from 6.45 to 5.70. Macht and Shohl (37) have noted that benzyl alcohol solutions, when sterilized or stored in soft glass containers, become dangerously alkaline and deteriorate rapidly. They recommended that the solutions be highly buffered to p_H 7.0–6.8 and sealed in hard glass containers.

Levy and Cullen (36) found that strophanthin solutions, when autoclaved in commercial glass ampuls, changed in reaction from p_H 6.0 to p_H 9.0. They reported that solutions of strophanthin in 0.02 molar phosphate solution adjusted to p_H 7 and autoclaved in hard glass ampuls retained a stable p_H after 5 months.

Mulford and Greenbaum (132) have reported the change in p_H by sterilization of many pharmaceutical products, as follows: dextrose, more acid; glycerophosphate compound, more acid; iron cacodylate, less acid; magnesium sulphate, more acid; mercurochrome, more alkaline; physiological salt solution, more acid; procaine, more acid; procaine and epinephrine, more acid; sodium cacodylate, no change; sodium iodide and salicylates with colchicine, no change; sodium salicylate, more alkaline.

A general method for maintaining stable p_H during sterilization was suggested by Robertson, Woo and Sia (62). Water was twice distilled and rendered CO_2-free by passing CO_2-free air through it for 24 hours. Solutions of 7.5 molar H_3PO_4 and 7.5 molar NaOH were boiled and kept in bottles stoppered with soda-lime traps. The solute (various drugs were used) was added to the water, the H_3PO_4 was added, 1 cc. per 100 cc., and the solution brought to the desired p_H by the addition of the NaOH solution. When autoclaved, the solutions were only 0.1–0.2 p_H lower and the p_H values remained constant for 2–3 weeks. Benda (143) stated that by adding acid-binding substances, such as caustic alkalies or soluble alkaline carbonates, phosphates or acetates, to solutions of salts of *p*-dialkylaminoarylphosphinous acids, sterilizable solutions of stable p_H are obtained.

Adjustment to Ophthalmic p_H.—The adjusting of p_H is of considerable importance in ophthalmic therapy. Gifford and Denton (265) have recently conducted a detailed investigation of this problem and their results are summarized below. The reaction of the lacrimal secretions is about p_H 8.0. An acid buffer solution, suitable for ophthalmic drugs soluble only in acid media, such as zinc salts, phenacaine, cocaine and epinephrine, is made from the following formula: 6.2 Gm. boric acid, 7.4 Gm. potassium chloride and distilled water to 1000 cc. The p_H of this solution is 5.5. An alkaline buffer solution is less irritating and is a suitable medium for atropine, homatropine, physostigmine and pilocarpine. It is prepared by adding 1 cc. of a 0.20 molar sodium carbonate solution to 50 cc. of the acid buffer solution. The p_H of this solution is 7.6. The alkaline solution is, of course, a less irritating collyrium than the acid solution.

The authors stated that phenacaine is soluble in the acid solution only if the p_H is as low as 4.8. Atropine, homatropine, physostigmine and pilocarpine are soluble in both solutions but are more effective in the alkaline solution. Zinc salts precipitate in the tears unless the medium is as acid as p_H 5.5. Sodium fluorescein dissolves only at p_H 9.0 or above. Metaphen is soluble only at p_H 8.0 or above. This is the reaction of the 1:500 solution and the p_H remains unchanged when it is diluted to 1:2500. Butyn is insoluble in either buffer solution but is soluble in distilled water.

Gifford and Smith determined the following p_H values for ophthalmic preparations:

	p_H.		p_H.
1.0% atropine sulphate	5.8	4.0% procaine hydrochloride	4.6
2.0% homatropine hydrobromide	5.5	1:1000 epinephrine	4.6
2.0% cocaine hydrochloride	4.6	2.0% butyn sulphate	5.4
1.0% pilocarpine nitrate	4.8	0.9% sodium chloride	7.0
0.2% physostigmine sulphate	6.6	0.2% zinc chloride	5.0
1:1000 nupercain	4.4	Saturated boric acid	4.2

When these are dissolved in saturated boric acid their reactions vary from p_H 4.2 to 5.0.

p_H AND TOXICITY.

Alkaloids.—In 1921, Crane (41) pointed out that the toxicity of alkaloidal salts is dependent upon the degree of hydrolysis. He stated that it is not the salt or ion which is toxic and that

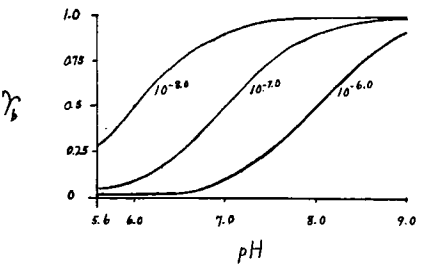
variations in the hydrogen-ion concentration affect the toxicity of alkaloids by changing the proportion of free undissociated base in the solution, rather than by direct action upon the cell. He found that with most alkaloidal salts there was 100 times as much undissociated base at p_H 8.0 as at p_H 6.0. Mayeda (116) later derived a mathematical expression for the degree of dissociation of alkaloidal salts as a function of p_H. Designating with Υ_b the degree of hydrolysis of a salt composed of a strong acid and a weak base (alkaloidal salt) with a dissociation constant K_b, and with Kw equal to the dissociation constant of water, it was shown that

$$\Upsilon_b = \frac{Kw}{Kw + K_b [H^+]} \text{ or } \Upsilon_b = \frac{Kw}{Kw + K_b . 10^{-pH}}.$$

This equation shows that Υ_b is independent of the concentration of the alkaloidal salt but is dependent upon the magnitude of the dissociation constant K_b the base or free alkaloid playing the rôle of a parameter. Mayeda plotted these functions (cf. Fig. 1) with the degrees of hydrolysis as ordinates and the p_H values as abscissas. Referring to the graph, it is noted that each curve has a turning point at which the degree of hydrolysis has the value 0.5. If pK_b = 7.0, the hydrolysis varies as follows:

p_H	5.5	6.0	7.0	8.0	8.5
% of hydrolysis	3.2	9.1	50.0	90.9	96.9

Assuming that the free alkaloid alone is biologically active, the activity is almost twice as great at p_H 8.0 than at p_H 7.0. Using cinchona alkaloids, Mayeda experimentally verified his mathematical results. He concluded the following: 1. The biological action of cinchona alkaloids is dependent entirely upon the amount of alkaloidal base freed by hydrolytic dissociation which, in turn, is a function of the p_H. 2. Only the free quinine base is the bearer of biological action.



Hydrolysis Curves of Alkaloidal Salts with Dissociation Constant $K_b = 10^{-6}, 10^{-7}, 10^{-8}$

Fig. 1.

Gerlough (204) obtained the following results pertaining to the effect of p_H on the actions of local anesthetics as measured by the rabbit cornea method. The duration of anesthesia produced by procaine hydrochloride, procaine borate and butyn increased with increasing p_H. Duration of anesthesia by butesin picrate was unaffected by changes in p_H. Butyn and procaine hydrochloride in buffered solutions had greater action than in unbuffered solutions of the same p_H. Fosdick, Hansen and Dragstedt (151) also reported that an increase in alkalinity augmented the anesthetic properties of procaine and cocaine hydrochloride solutions. Their investigations showed that the efficiency of procaine borate and hydrochloride is practically the same at p_H 8.4, but that the borate is more efficient at higher p_H values and the hydrochloride is more efficient at p_H values below 8.4. In general, these results with local anesthetic also point to increased biological activity when a greater amount of alkaloidal base is freed by dissociation.

Cosmetics.—Eggerth (80) has published the results of a detailed investigation concerning the effect of p_H on the germicidal action of soaps. Eggerth found for all soaps an acid and an alkaline range in which the soaps were most germicidal. Potassium butyrate was non-germicidal in a concentration of tenth-normal at all p_H values within the limits of 3.8 to 10.5. He stated that solutions of soaps having 12 or more carbon atoms in the molecule were alkaline in reaction, and that increasing the number of carbon atoms in the chain increased the alkalinity of the solution due to increased hydrolysis. With increasing molecular weight of the soap, the germicidal titer increased to a maximum and then diminished, the point of inflection varying with the p_H and the organisms. Thus, with B. typhosus at p_H 5.5 the titer rose with increasing molecular weights up to capric acid and then diminished. With most organisms, the maximum titer for the acid range was reached with lauric and tridecylic acids. In the alkaline range the germicidal action increased

with molecular weight to the palmitate and then diminished. The lower members of the saturated series of soaps were found to be most germicidal in an acid reaction. For instance, the titer for potassium caprate was 1000 times as great at p_H 4.4 to 4.7 as it was at p_H 9.0 to 10.0.

Eggerth proposed the following explanations concerning the relation between p_H and the germicidal action of soaps. 1. Hydrogen ions and hydroxyl ions may affect the bacterium rather than the soap. 2. An acid reaction may decrease the surface tension of the soap and thus increase its concentration at the surface of the bacterium. 3. The p_H effect may be due to alterations of the solubility of soap in the aqueous phase or in the bacterial protoplasm. 4. The fatty acid may have a greater germicidal action because the acid is less dissociated than the soap, and there is evidence that undissociated molecules penetrate more readily into protoplasm than do ions.

Janistyn (211) called attention to the value of certain acids (anisic, formic, benzoic, butyric, citric, acetic, gallic, tannic, glycerophosphoric, camphoric, etc.) in concentrations giving a therapeutic skin p_H of 3.0 to 5.0 in soaps, skin, mouth and hair preparations and other cosmetics. Janistyn stated that the disinfecting action of buffered acids is greater than that of unbuffered acids because of the higher acid-ion concentration. An anonymous article in the *Drug and Cosmetic Industry* (302) stated that the normal p_H of skin is between 4.5–5.0 and that any cosmetic which has a strong alkaline reaction is detrimental to the skin. This article stated that the p_H range of blood is from 7.0 to 7.4 and that face creams whose p_H values deviate from this range cause irritation to the epidermis. The author also pointed out the existence of acid and alkaline ranges wherein an optimum effect can be obtained from antiseptic lotions. Mayer (320) has shown the value of p_H control in the manufacture of shampoos and has stated that a properly designed soapless shampoo base should have a p_H of about 7.0 to 7.5.

Preservatives and Antiseptics.—The preservative action of acids and bases is, in a large measure, a function of hydrogen- and hydroxyl-ion activity, and also, specific effects which were previously suspected of certain acids and bases have now been clearly demonstrated by the use of hydrogen-ion methods. The effects of these ions are greatly complicated by various conditions associated with the colloidal phases and degrees of permeability of cell membranes. The alternation of the colloidal phase conditions often depends upon the inorganic ions which are present. Thus, calcium favors the permeation of oil-soluble substances and sodium of water-soluble substances. An interesting feature of permeability is that weak acids or bases penetrate cells readily, whereas strong ones fail to do so. The direct action of hydrogen-ion concentration upon cells must be kept distinguished from its control of the effective state of a toxic compound. The relationship between p_H and toxicity to microörganisms is discussed below in three phases: The preservation of foods, the preservation of drugs and the effect of antiseptics in therapy.

In 1929, Cruess and Richert (124) found that the concentrations of sodium benzoate required to prevent the growth of yeasts, molds and bacteria were greatly affected by the p_H values of the medium. In a later publication (195), Cruess, Richert and Irish stated that the concentration of sodium benzoate necessary to give the same preservative action at the neutral point as at p_H 3.0 was 200 times as great. Still later, Cruess (236) observed that for the destruction of fermentation organisms, 4% of sodium benzoate was required at p_H 7.0, 0.06–0.1% was required at p_H 3.5–4.0, and 0.02–0.03% was required at p_H 2.3–2.4. He stated that some molds grew profusely in a 10% sodium benzoate solution of p_H near neutrality. In asparagus juice, Clostridium botulinum grew and formed toxin at p_H 7.4 with 0.8 Gm. of sodium benzoate per 100 cc. The organism did not grow at p_H 4.7 with 0.1 Gm. of the preservative per 100 cc. In regard to other salts of weak acids, it was stated that in the p_H range 5.0 to 9.0 the necessary concentrations of sodium salicylate, sodium sulphite and potassium acetate were considerably higher than in the p_H range 2.0 to 4.5. From these observations Cruess concluded that the undissociated weak acids, rather than their ions, are the preservative agents. This conclusion is in direct accordance with that of Eggerth (80) who stated that undissociated molecules penetrate more readily into protoplasm than do ions. Cruess' observation that lowering the p_H did only slightly enhance the preservative actions of sodium chloride and formaldehyde also supports the conclusion of Eggerth.

In 1932, Back (233) reviewed the use of preservatives in pharmaceutical preparations. He stated that concentrations of vinegar, equivalent to 2.5–3.5% of acetic acid, are sufficient to prevent fermentation in sauces and similar preparations. Lactic acid is satisfactory as a preservative but there is evidence that citric acid in equivalent strength is less effective. He stated that in

addition to the p_H value, the nature of the anion affects the preservative properties. If sugar is used, the concentration must be 66% or above. He also stated that, in general, a concentration of 8–15% of salt inhibits bacterial growth but that many yeasts grow in a 25% salt solution; even concentrated brine will not kill spores. Walbum (297) found that the resistance of earth spores to destruction was greatest at p_H 8–9 and quickly decreased on the acid and alkaline sides.

The effect of insulin solutions (20, 32 and 40 units per cc.), without preservatives, and of a solution of 40 units per cc. with 0.3% tricresol on the viability of Staphylococcus albus, was studied by Hartley (208). Solutions of p_H 3–4 had a germicidal action with no preservatives present. When the normal acidity of a solution containing no preservative was even slightly reduced, staphylococci were not killed and considerable growth occurred at p_H 6.0. When p_H 8.5 was reached, the solutions, in most cases, again did not allow growth of staphylococci. It seems that the insulin itself has a toxic effect on microörganisms because, on ordinary culture media, staphylococci develop most favorably in a slightly alkaline reaction and growth is not inhibited by p_H changes from 5–9.

With regard to the British Liquor Arsenicalis, Milne and Rattray (280) found that when organic matter was completely excluded during preparation, very little growth of molds occurred at temperatures suitable for their rapid development and that the growth was most at p_H 7.0.

Joachimoglu (57) has investigated the influence of p_H upon the antiseptic effect of mercuric chloride solutions buffered with glycocoll, NaOH and HCl. The final dilutions of mercuric chloride were 1:672,000. There was strong antisepsis at p_H 3.3–4.0, no effect at p_H 7.8–10.1, a slight effect at p_H 10.5, and strong antisepsis at p_H 11–12, corresponding to that between p_H 3.3–4.0. When buffered with secondary sodium citrate and sodium hydroxide, positive antiseptic action was obtained between p_H 5.0–6.6.

Daniels and Lyons (196) have studied the effect of p_H upon the antiseptic actions of phenyl substituted acids from benzoic to ϵ-phenyl caproic. They could find no definite relation between p_H and antiseptic effect but did note that there was a gradual rise in p_H as the series ascended. They found the antiseptic actions of certain phenols to be strongest in an acid range below p_H 4.5 and in an alkaline range above p_H 10. They also stated that the undissociated acid molecule is the active portion in disinfection by means of acids, whereby the antiseptic action would be inversely proportional to the hydrogen-ion concentration. Janistyn (211) claimed that the disinfecting action of acids is greater when they are buffered because of the higher acid-ion concentration. He cited experimental evidence to show that lactic acid and sodium lactate of p_H 3.7 in a concentration of 1:15 is equivalent in disinfecting power to 65% ethyl alcohol.

Kunzmann (275) has stated that hydrogen-ion concentrations between p_H 6.5–7.5 had no effect upon the disinfecting properties of potassium iodide and sodium iodide solutions. He found that potassium iodide was more effective than sodium iodide but that the effect of both was decreased when they were mixed.

p_H AND STABILITY OF DISPERSED PHASES.

Before entering into the discussion of the relation between p_H and the stability of drugs, it will be of value to consider the work performed by the Swiss pharmacists, Tschirch and Fluck (119), (125), on instability caused by the presence of acacia. They have found that acacia contained one or more oxidizing enzymes and that these enzymes attacked most alkaloids and glucosides. The manner of action on morphine indicated that the oxidases attacked especially the free hydroxyl groups. Acacia, which had been inactivated, did not oxidize morphine. It was found that the oxidases of gum arabic were able to act in a pill mass with a moisture content of 2% or above. Tschirch and Fluck found that the acacia could be inactivated, as shown by the guaiac and benzidine test for oxidase, by boiling a mucilage to dryness, or by precipitating the gum with boiling neutral alcohol. The authors pointed out that precipitation of the gum by acid alcohol produced an inactive powder which, after completely washing out the acid, would not dissolve in distilled water but which required an alkaline condition for solution.

Emulsions.—The preparation of stable emulsions has been for many years a disconcerting problem. When emulsions were conceived as a fine dispersion of one liquid in another, the size of the dispersed particles was considered to be the only important factor controlling the stability. Later investigations have shown that a purely mechanical conception is not sufficient and that emulsion formation is affected by factors similar to those affecting colloid formation.

Different emulsifying agents have been found to cause opposite phase dispersions and to cause different ranges of stability along the p_H scale. Harkins (30) has stated that sodium oleate and similar compounds produce emulsions of the oil-in-water type, whereas calcium and magnesium oleates and other compounds in which 2 hydrocarbon radicals are attached to 1 metallic atom produce water-in-oil emulsions. Harkins found that emulsions produced by magnesium oleate disintegrated when the p_H of the aqueous phase became as low as 2.5. He attributed this disintegration to the decomposition of the emulsifying agent, which must be in contact with the dispersed phase. Krantz and Gordon (113) substantiated Harkin's results and stated that with magnesium oleate emulsions the most stable p_H range of the internal phase was between 11–12.5. The same authors (130) found that in magnesium oleate emulsions, the size of the dispersed particles varied between 17–30 microns. They also stated that with this type of emulsion, p_H did not significantly affect the surface tension, that there was a decided drop in viscosity on the acid and alkaline sides of the p_H range 0.9–13.0, that the emulsions in mineral oil were unaffected by the addition of sodium chloride, but that the emulsions in olive oil were made less stable by the addition of sodium chloride. The authors concluded their discussion of magnesium oleate emulsions by stating that the most important factor in the stability of these emulsions is the influence of the hydrogen-ion concentration of the dispersed phase upon the magnesium oleate.

Krantz and Carr (242) recommended the use of magnesium oleate in the preparation of Ointment of Rose Water because it produced a neutral, stable and compatible water-in-oil emulsion. They stated that all salts producing an hydroxyl-ion concentration represented by p_H 9.17 or above produced satisfactory ointments.

Krantz and Gordon (86), (113) found that oil-in-water emulsions produced by acacia had a wide stability range within p_H 1.6–10.35, with greatest stability between p_H 4.11–4.28. The results were the same whether the oil was vegetable or mineral, or whether the acid used was hydrochloric or sulphuric. Addition of sodium chloride showed that the chlorine ion did not influence stability. The surface tension varied slightly with p_H, being a maximum at p_H 7.3 and decreasing by about 30% at the acid and alkaline extremes. Viscosity was unaffected by p_H. The size of the particles was smallest near p_H 7 and increased considerably with increasing p_H values and increased very little with decreasing p_H.

The same authors found that oil-in-water emulsions produced by tragacanth had a range of greatest stability within the p_H limits of 1.9–2.3 and had another, more narrow, and less stable range, at about p_H 6.3. Tragacanth emulsions differed also from acacia emulsions in that their viscosity decreased with increasing p_H. In considering gels of tragacanth, the authors found that only those between p_H 0.4–2.1 remained free from the separation of water at the surface. The most stable range of p_H for tragacanth emulsions lies within this scale. The authors claimed that this supports the hydrate theory of emulsions[1] which posulates that oil is most thoroughly emulsified in a hydrophile colloid when just a sufficient amount of water is present to form a hydrate. With tragacanth, according to Krantz and Gordon, this amount of water is evidently a function of its hydrogen-ion concentration. Therefore, at the p_H value where tragacanth possesses the highest degree of hydratability, this value is the most stable point for emulsions prepared with tragacanth.

Krantz (129) has investigated the buffer actions of both acacia and tragacanth. The buffer capacity $\frac{\Delta B}{\Delta p_H}$ of acacia was found to be 0.034 and to extend over a wide range. Since acacia consists of the K, Ca and Mg salts of a weak acid, its buffer capacity is more effective in the neutralization of acids than of alkalies. This was shown by Krantz when he plotted the buffer capacity (cf. Fig. 2). At p_H 10.5 the curve became very abrupt, but below 2.5 the curve descends as a gradient. This accounts for the extreme instability beginning on the alkaline side of the p_H stability range. Tragacanth exerted considerable buffer capacity between p_H 3.0–10.0. In view of the wide buffer capacities of these two emulsifying agents, Krantz (165) attempted to increase their stability ranges by buffering the external phases. With acacia, no increase in stability was observed between p_H 2–10.5, but with tragacanth, the stable range of 1.9–2.3 was increased to p_H 1.9–5.0.

On account of the sharpness of the single break in the titration curve of acacia, Taft and Malm (228) have claimed that arabic acid is evidently a strong monobasic acid. They explained

[1] Fischer, "Fats and Fatty Degeneration," page 5 (1917).

the viscosities, densities, freezing points and conductances of acacia solutions of widely variable concentrations by assuming that acacia acts as a strong organic electrolyte rather than as a colloidal phase when in contact with water. They attributed the protective action of acacia solutions to their high viscosity and to the probable high adsorption of the acacia molecules.

According to Friedman and Evans (203), the stability of gelatin emulsions is dependent upon the p_H, and, to a small degree, the concentration of the gelatin. The stability range, in general, was between p_H 3–6, with a few emulsions becoming very unstable near p_H 3 when the gelatin concentration was as low as 0.25%. When the p_H of the gelatin solution was near the isoelectric point (about p_H 4.95), the most stable emulsions were formed. Stability sharply decreased at p_H of about 3.0, there was another point of increased stability near p_H 2.5, and a sharp drop in stability when the p_H was further decreased. Addition of alkali resulted in a marked decrease in stability above p_H 6.0, with another range of stability near p_H 8–10, and another decrease when the p_H rose above 11. A summary of these results shows that there are two p_H ranges for stability, one from p_H 3–6, and the other in the vicinity of p_H 8–10. This is in accordance with the results of Kraemer (85), who reported finding two isoelectric points for gelatin, one at p_H 4.95, and the other near p_H 8.

Enz and Jordan (239) reported the following results after investigating the ease of emulsification of alkaloid-containing preparations. Fluidextract of Belladonna Leaves showed least emulsification at the neutral point; Tincture of Stramonium and Fluidextract of Cinchona showed least in acid solution, and Fluidextract of Hydrastis and Tincture of Nux Vomica showed no uniformity. According to the authors:

> "The results indicate that the statement, 'emulsions are less apt to form in strongly acid or alkaline solutions than in those which are neutral,' is true only in specific instances."

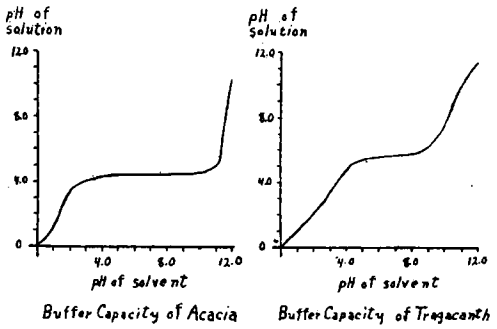


Fig. 2.

According to some experimental work reported by Weeks (185), at p_H values less than 7.0, the area of a water-spread emulsion for a given pressure increased with time without reaching an equilibrium. At p_H values above 7.0, the area finally became constant with time but the values were large and were dependent upon the p_H. At p_H 7.0, the water-spread emulsion showed a constant area with time for a given pressure and quickly reached the equilibrium.

Colloids.—Unless conditions are favorable, a colloidal solution may be a very transient system. There are many pharmaceutical colloids representing and acting as transition cases between the two practical classes of colloidal solutions. Thus, gelatin, acacia, albumen and mercury ointments represent the class of reversible or lyophile colloids, and metallic suspensions and the two silver protein preparations represent the irreversible or lyophobe colloids. A consideration of the stability of colloidal solutions reveals two important stabilizing agencies, solvation and electric double layers, the former being typical of lyophiles, the latter of lyophobes.

Considerable work has been done concerning the effect of acid-base equilibria on the stability of colloids. Those colloids of the lyophile class, representing mostly the effects of macromolecular solvation, are less affected by ionic phenomena than are those of the lyophobe class. Electric double layers are responsible for the stability of most hydrosols of inorganic substances in the absence of protective colloids and these layers are sensitive to the charges of electrolytes. According to theory, as two particles carrying electric double layers approach each other, the outer layers are deformed or polarized and an electrical repulsion results. The electrokinetic potential necessary to prevent adhesion is considered to be about 15–25 millivolts. Since the potential of non-ionic and sparingly soluble ionic particles in water is not so large, stability usually requires the presence of a definite amount of an electrolyte, one ion of which is adsorbed by the particle.

In 1922, Bogue (46) showed that various physical properties of colloids, including the viscosity, jelly strength, melting point and joining strength, were a minimum at a p_H corresponding to the isoelectric point. As the acidity or alkalinity increased from this point, these properties rose in value. In 1925, Johnston and Peard (75) determined the isoelectric point of gelatin as p_H 4.7. The surface tension was a maximum at this point and a minimum at p_H 3.8–4.0, and in the neighborhood of p_H 9.0. A second maximum was found at p_H 2.8–3.0, below which the surface tension steadily decreased. Addition of electrolytes also lowered the surface tension. A year later, Kraemer (85), using the Tyndall effect to designate the isoelectric point of gelatin, determined the point as p_H 4.9; the Tyndall effect indicating a maximum tendency to precipitate at this point. He suggested a second isoelectric point near p_H 8 where there was a distinct decrease in light dispersion. The maximum gel strength appeared on either side and very near to the optimum precipitation point, or isoelectric point, illustrating that gel formation takes place under conditions which are just adjacent to those leading to readiest precipitation or coagulation. Kraemer stated that, contrary to the usual assumption, the gel formation was very small within a narrow range at the isoelectric point.

Recent literature reflects considerable interest concerning the effects of hydrogen ions and other ions on the swelling of gelatin. This phenomenon has nothing to do with the decomposition of gelatin or with the destruction of the colloidal state, but rather it is caused by the production of a higher osmotic pressure within the separate particles of gelatin, due to a Donnan equilibrium set up between the particles. Concerning this subject the reader is referred to J. Loeb[1] and Miller and Pleass (131), (173).

Many workers have studied the influence of hydrogen ions on the coagulation of colloidal solutions. Ghosh and Dhar (82) have explained the coagulation by electrolytes of solutions of ferric hydroxide, chromium hydroxide, mastic and gamboge, according to the following principles: 1. Adsorption of ions carrying the same charge as the sol. 2. Hydrolysis of the sols and consequent generation of acids which stabilize the sols. Addition of acids renders the sols unstable by checking their hydrolysis, but addition of alkalies increases the stability by increasing the hydrolysis of the sols. 3. Hydrolysis of the coagulating electrolytes.

The explanation of coagulation which has been offered by Hazel and Sorum (209) is similar but more readily acceptable. They claim that for a positive sol (there are both positive and negative sols), the anion of the added electrolyte is the effective agent in producing coagulation. Hence the flocculation values for monovalent ions are higher than for bivalent or trivalent ions. The authors determined the effects of the following groups of electrolytes upon ferric oxide solutions:

1. Univalent salts.
2. Bivalent salts represented by $BaCl_2$.
3. Trivalent salts represented by $FeCl_3$ and $AlCl_3$.
4. K_2SO_4, $(NH_4)_2SO_4$, $CaSO_4$.
5. K_2CrO_4 and $K_2Cr_2O_7$.
6. $K_3Fe(CN)_6$ and KH_2PO_4.
7. $K_4Fe(CN)_6$.

The flocculation values decreased for Groups 1, 2 and 3, respectively. In Groups 1, 2, 5 and 6, the flocculation values decreased with increasing hydrogen-ion concentrations. The effect of electrolytes of Group 3 did not vary with p_H, both electrolytes being salts of a strong acid and weak base, whereby their hydrolyses produced a constant p_H. The flocculation values of the electrolytes in Group 4 increased with increasing hydrogen-ion concentrations. Bedford, Keller and Gabbard (256) have also reported that the presence of sulphates caused the stability of ferric oxide solutions to vary inversely with the hydrogen-ion concentration.

Morton (220) has performed electrometric studies which have revealed the complex formations and colloidal conditions of pharmaceutically important iron compounds. Electrometric titrations were performed to determine the cause of the non-precipitability by volatile alkalies of solutions of iron "citrochloride." This was found to be due to the formation of the basic colloid-complex, $FeC_6H_5O_7.2Fe(OH)_3$. In the absence of complex formation, that is, without the addition of citric acid or alkali-metal citrates, the red color of basic iron salts was exhibited at p_H 2.2, and

[1] "Proteins and the Theory of Colloidal Behavior" (1924).

at p_H 2.36 the solution became colloidal. Coagulation occurred at p_H 6.48. This indicated that only in strongly acid solutions can the ferric ion exist in an appreciable concentration.

Morton reported that Fehling's solution consists of a basic colloid-complex, $3CuC_4H_4O_6.5Cu(OH)_2$, peptized by excess of tartrate ion. He explained that the cataphoretic behavior of the hydrosol is due, not to the presence of complex anions, but to the negative colloid-complex.

In a later work, Morton (221) reported that in pharmaceutical mixtures of the ferric chloride-sodium salicylate-bicarbonate type the iron is present partly as a violet crystalloid complex, $Fe(OH)_2(C_6H_4OHCOO)$, and partly as a red basic hydrosol, the proportion of the metal present in each form depending on the degree of acidity or alkalinity. In acid solutions, the hydroxy-acid complexes of iron were found to be present mainly as true electrolytes, but in alkaline solutions they were decomposed to varying extents with the formation of basic hydrosols. Their apparent stability in alkaline solution was assumed to be due to the peptizing and protecting properties of the hydroxy-acid anion.

p_H AND STABILITY OF COMPLEX PRODUCTS OF BIOLOGICAL ORIGIN.

Proteins.—The vast amount of investigation dealing with the structure of proteins have given rise to much information concerning the effect of p_H upon the decomposition of proteins. Although most proteins do not require stabilization, the general principles concerning their decomposition are of wide scientific interest and can be applied to the problem of stabilizing toxins, antitoxins and insulin.

Northrop (45) has studied the decomposition of gelatin and has reported that in acid solutions of p_H less than 2.0, the velocity of hydrolysis was directly proportional to the hydrogen-ion concentration, and that in alkaline solutions of p_H greater than 10.0, the velocity was directly proportional to the hydroxyl-ion concentration. Between p_H 2.0 and 10.0 the rate of hydrolysis was approximately constant and was much greater than would be calculated from the hydrogen and hydroxyl-ion concentrations.

In 1930, Svedberg (181) published some generalized results related to the p_H stability regions of proteins. He found a number of proteins to be monodisperse, that is, homogeneous as to molecular weight. They were divided into two groups; the first containing those with molecular weights from 34,500 to 208,000, the second containing those with molecular weights in the millions. Those of the first group fell into four classes, with molecular weights 1, 2, 3 and 6 times 34,500. The monodisperse proteins had a wide stability region, usually between p_H 3–11, and including the isoelectric point of the protein. This point was never in the middle of the stability region but was always shifted in the direction of the low p_H values. The protein molecules containing more than one group of weight 34,500, split into molecules of $1/2$, $1/3$ and $1/6$ of the original as the p_H value was raised. At sufficiently high alkalinity all proteins had the molecular weight 34,500. The same results were obtained by lowering the p_H. This decomposition was reversible in many cases. When the mixture was restored to a p_H within the stability range, molecules of the original weight were built up out of the fragments.

Sjogren and Svedberg (178) found that egg albumen was stable with regard to molecular weight in the p_H range 4–9. The decomposition was measured by the velocity of sedimentation caused by ultracentrifuging. At p_H values below 3 the sedimentation increased, indicating the formation of aggregates of denatured proteins; at p_H values above 9 the sedimentation decreased, indicating the breaking up of the molecules. The molecular weight was determined as 34,200.

Svedberg and Heyroth (137) studied the p_H stability of hemocyanin, which has molecular weights of 5,000,000. The molecular weight was constant between p_H 4.5–7.4. As these limits were approached, the protein molecules became hydrated, and, as the limits were exceeded, they rapidly disintegrated into smaller particles of undetermined magnitude. The acid disintegration was reversible in its earliest stages but the disintegration of the products first formed continued slowly and was irreversible in its later stages.

Nasset and Greenberg (133) have studied the rate of hydrolysis of casein in acid solutions. They claimed that the effect of acids was catalytic in nature and reported that the hydrolysis was proportional to the hydrogen-ion activity of the acids. The velocity constant for the three acids used decreased in the following order: HCl, H_2SO_4, H_3PO_4. The rate of hydrolysis conformed to the equation for a reaction of the second order.

In 1925, Brownlee (73) generalized his observations concerning the stability of toxins and

lysins by stating that the optimum p_H for the stability of these substances varied from p_H 6–7.5. His observations showed that the decomposition at ordinary temperatures was slower on the alkaline side than on the acid side. Schmidt (176) reported that diphtheria toxin was stable at 18° C. between p_H 6.5–9.0, while at p_H 5.4 or 9.9 the antigenic power was slowly destroyed, even when the temperature was 0° C. At 37° C. and p_H 7.0 the antigenic power was destroyed. Flocculation by mixtures of toxin and antitoxin was prevented when the mixtures were freed from electrolytes or when the electrolyte content exceeded certain limits which varied greatly with different concentrations. The flocculating power of the antitoxin decreased with increasing temperature and alkalinity. The condition was reversed by acidifying. Schmidt stated that diphtheria antitoxin was much more stable to the destructive action of salts than was the toxin, and that salts of oxidizing and reducing acids and of certain aromatic acids completely destroyed both the toxin and antitoxin. Neutral salts had almost no influence.

With regard to tetanolysin, a component of tetanus toxin, Schrek (292) stated that acidification decreased the rate of heat inactivation of the lysin and that alkalization accelerated the heat inactivation. Acidification of the lysin to p_H 4.0 decreased the rate of oxidation, and acidification below p_H 4.0 increased the rate of oxidation. Acidifying to p_H 3.0 caused considerable inactivation in the absence of heat or oxidation. This indicates maximum stability of tetanus lysin near p_H 4.0.

Much work of a similar nature has been done concerning the decomposition and precipitation of serum proteins (81), (100), (156), (180).

Remesow (174) has achieved some interesting results bearing on the coagulation of l cithins and cholesterins. The solutions of both were lyophobe colloidal solutions of suspensoid character. The coagulation limit for cholesterins was p_H 4.0; for cholesterin esters, p_H 2.0; and for lecithins, between p_H 6.2–5.6. The flocculation optimum for lecithin solutions lay between p_H 2.8–2.0. A large number of substances, the most important biologically, such as egg white, hydrocarbons, alkaloids, ferments, etc., were found to sensitize the lecithins and cholesterins in such a manner that their coagulation values were displaced to different p_H values. Tables were included which showed the precipitation or lack of precipitation of cholesterins, cholesterin esters, lecithins and hydrolecithins at various p_H values and under the sensitizing actions of more than 50 physiologically important substances and drugs. This so-called sensitizing action was proved to be one not between the substance and the sensitizer as in ordinary precipitation.

Hormones.—Krogh and Hemminsen (114), in 1928, reported that the optimum p_H range for insulin stability was between p_H 2.0–4.0. This optimum range has been modified later. The authors reported a distinct alkaline shift during decomposition and stated that the solutions became opalescent above p_H 4. Gaddum (152) also found the stable range for insulin to be from p_H 2.0–4.0 and reported exceedingly rapid decomposition above p_H 7.0 and below p_H 1.0. In 1931, Sjorgren and Svedberg applied ultracentrifugal methods for measuring the decomposition of insulin and thereby determined the p_H range for optimum stability to be between p_H 4.5–7.0. Near the borders of this region the decomposition was reversible. By ultracentrifugal means, the authors determined the molecular weight (35,100), sedimentation constant, molar friction constant and molecular radius. The values were very nearly identical with those for egg albumen. Mogilskii (218) has reported that the isoelectric point of insulin was between p_H 4.3 and 5.7, depending upon the purity of the solution.

Freudenberg and Eyer (201) have investigated the destructive action of acids and alkalies on insulin. The extreme instability to alkalies was evidently due to the cleavage of 2 molecules of NH_3. One molecule of NH_3 was attacked by oxidizing agents and the other by reducing agents. Acids caused separation of NH_3 by hydrolysis. Wintersteiner (298) found that by varying the p_H between 6.0–8.0, and using cysteine, thioglycollic acid and alpha thiolactic acid as reducing agents, with all three compounds the rate of inactivation of insulin increased with increasing p_H.

The control of p_H is of special importance in the preparation of pituitary extracts free from animal protein. Whenever the protein is precipitated, a certain amount of the pituitary principles is destroyed. It is desirable to obtain a p_H value whereby the best possible compromise can be attained between the amount of protein destroyed and the amount of active principles remaining. The original work was done by Dudley (32), Abel and Nagayama (35), and Stasiak (90) who pointed out the acid lability and the extreme alkaline lability of both the oxytocic and vasopressor

constituents of pituitary. Abel and Nagayama observed that the acid destruction of the extracts caused the formation of histamine which produced a lowering of arterial pressure instead of the desired increase. Gaddum (152) stated that for the oxytocic principle the effect of variation of the p_H was a minimum between p_H 7–8, but that the absolute optimum p_H for stability was 3.0. The presence of salts caused a very small increase in destruction. Gaddum summarized his results in a table which gives the amount of destruction of the oxytocic principle to be expected, when a solution of any p_H is heated at any given temperature for any given time.

Gerlough (153) carefully measured the amounts of inert protein which were precipitated by various reagents and at various p_H values when the pituitary extracts were placed in boiling water for 12–14 minutes. These results are summarized in Fig. 3 and can be compared with the p_H stability curve for oxytocin formed from Gaddum's data for the same temperature and same duration of time. The tungstic acid curve shows the greatest precipitation of protein but this reagent must be excluded because only 30–40% of the oxytocic principle remains after treatment. A comparison of the two graphs shows that although the maximum stability occurs at p_H 3.0–3.4, the maximum precipitation of inert protein which allows only a reasonable decomposition of the active principle can be obtained when the extract is adjusted to p_H 4.4 with acetic acid.

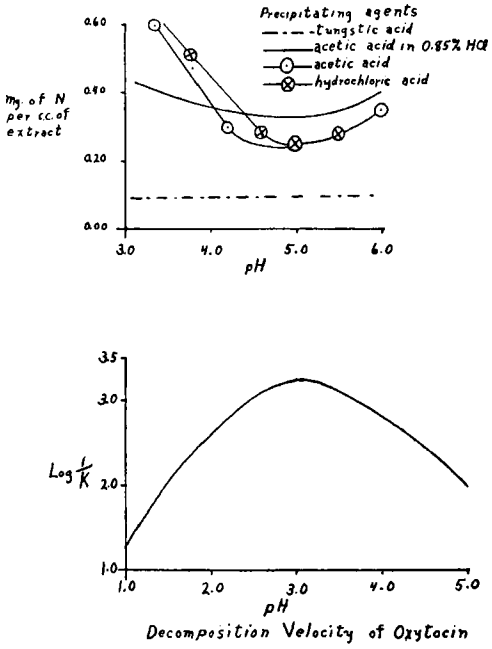


Fig. 3.

The hydrolysis of acetyl choline was investigated when a relation was suspected between it and the oxytocic pituitary principle. Gaddum (152) found the optimum p_H for its stability to be 3.9. Hofmann (157) found that the hydrolysis of acetyl choline chloride solutions produced an acid reaction which gradually checked the hydrolysis. The least hydrolysis occurred at p_H 3.9 with considerable hydrolysis occurring at p_H 5.0. The cleavage of acetyl choline was considerably depressed by the addition of non-electrolytes, such as antipyrine and acetamide. The effect of acid upon the racemization of *l*-epinephrine has been investigated by Haddock (267). In 10% hydrochloric acid, a solution of *l*-epinephrine was completely racemized in 12 hours, but in normal hydrochloric acid (3.6%), racemization was only 58.5% in 216 hours. In the latter case, 10% was destroyed by oxidation. In HCl solutions of p_H 1.4, the racemization of 1% epinephrine was negligible, even with exposure to ultraviolet light. Barker, Eastland and Evers (234) found that the oxidation of epinephrine by potassium persulphate was most rapid at p_H 5.6, the rate of oxidation being one-fourth as great at p_H 4.7 and 7.1. The presence of small quantities of copper greatly increased the rate of oxidation and iron showed a slight inhibitory effect.

Marshall (278) stated that the *p*-factor of the gonadotropic hormones lost 50% of its activity in 17 days when adjusted to p_H 7.0 and preserved with 0.5% phenol.

Vitamins.—The experimental work concerned with the stability of vitamins can best be summarized in the following table. The experiment represented by the first tabulation for the B_2 factor, in which no decomposition occurred, was performed upon a liver concentrate.

Enzymes.—A discussion of the relation between p_H and the activity of enzymes is far beyond the scope of this paper. It is sufficient to say that each enzyme has an optimum p_H for action and that changes in enzyme activity with changes in p_H are evidently related to the state of ionization of the substrate and the enzyme. References are: (21), (23), (51), (67), (73), (101), (104), (117), (207), (232), (283).

Vitamin Factor.	Temperature.	Time in Hours.	p_H.	% Decomposition.	Reference.
B_1	100°	24	1.0	0	126
	120°	1	6.0	0	135
	100°	1	5.2	10	89
	100°	1	7.9	30–40	89
	100°	24	5.0	50	126
	100°	1	9.2	60–70	89
	100°	1	10.9	90–100	89
	125°	30 min.	9.0	100	205
	120°	1	9.0	100	135
B_2	125°	3	9.0	0	205
	120°	1	9.0	0	135
	90–100°	2	5.0	0	145
	25°	24	0.05–0.10	0	170
	119°	4	2.5	0–10	146
	25°	10 days	9.5–10.0	30	145
	98–100°	2	8.3	50	145
	123°	4–5	5.0	50	145
	123°	4–5	3.0	50	145
	122–125°	4–5	8.3–10	75–100	145
	120–125°	4	10.0	100	147
	119°	4	9.6–10.3	100	146
B_3	20°	1–5 days	Alkaline	10–40	150
	120°	1	6.0	50	135
	60°	1–5 days	Neutral	100	150
B_4	120°	1	9.0	100	135
y	120–125°	4	9.0–10.0	0	147
C	25°	14 months	1.6–2.2	0	186

Northrop (38) found that pepsin in solution at 38° C. attained an optimum stability at p_H 5.0 with slow destruction occurring at lower p_H values and very rapid destruction at higher p_H values. The inactivation of the pepsin was not reversible. Loughlin (277) claimed that the rate of heat-inactivation of pepsin was a minimum and was almost constant within the p_H range 3–4.5. Vahlteich (93) stated that Elixir of Pepsin of the N. F. formula deteriorated 100% during 2 years, but when no hydrochloric acid was added the elixir deteriorated only 36% in the same period of time. The author stated that the p_H of the elixirs which lost their entire activity was close to the optimum p_H for peptic digestion (p_H 1.2–1.6).

Pace (172) reported the optimum p_H for trypsin stability as p_H 6.5. The optimum p_H for trypsin activity is 7.5–8.3. McGillivray (166) reported the optimum p_H for the thermal stability of pancreatic lipase as p_H 6.0. The optimum p_H for lipase activity varies from 5.0–8 5, depending on the source.

(*To be continued.*)

THE CHEMISTRY OF THE HORMONES.

Under above title the *Journal of the American Medical Association* (March 30th) states:

"Although the question of the chemical nature of the hormones has been a subject of interest for nearly two decades, progress in this field has been exceedingly difficult because of the lack of endocrine preparations of sufficient purity for accurate chemical studies. Recent extensive investigation, however, has led to vastly improved methods of preparation and ultimately to the isolation in pure crystalline form of a number of the hormones. Thus these heretofore inaccessible substances have been brought within the scope of attack by the chemist. Indeed, at present several hormones have been prepared in crystalline form and the chemical structure and method of synthesis of two of these have been definitely established. Also a number of other hormones have been prepared in a highly purified although noncrystalline state, and some information regarding their chemical properties has been obtained."